VNIVERSITAT DE VALÈNCIA

FACULTAT DE QUÍMICA
DEPARTAMENT DE QUÍMICA ANALÍTICA

# ANALYTICAL APPLICATIONS OF POROUS CYCLODEXTRIN-BASED MATERIALS: A VERSATILE STRATEGY

Memoria para alcanzar el Grado de Doctora en Química
dentro del Programa de Doctorado en Química (RD 1999/2011)
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CERTIFICAN

Que la presente memoria, que lleva por título "*Analytical applications of porous cyclodextrin-based materials: a versatile strategy*", constituye la Tesis Doctoral de Dña. CAROLINA BELENGUER SAPIÑA.

Asimismo, certifican haber dirigido y supervisado los distintos aspectos del trabajo, así como su redacción.

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*„If we assume we've arrived, we stop searching.*
*We stop developing.“*

Jocelyn Bell Burnell

*A los míos*

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## ABBREVIATIONS

| | |
|---|---|
| **0D** | Zero-dimensional |
| **1D** | One-dimensional |
| **2D** | Two-dimensional |
| **3D** | Three-dimensional |
| **2,4-D** | 2,4-dichlorophenoxyacetic |
| **β-MCD** | Methyl-β-cyclodextrin |
| **β-HPCD** | 2-hydroxypropyl-β-cyclodextrin |
| **ACN** | Acetonitrile |
| **AIBN** | Azo-bisisobutyronitrile |
| **Allyl-β-CD** | (6A-N-allylamino-6A-deoxy)-β-cyclodextrin |
| **Allyl-γ-CD** | 6I-O-allyl-γ-cyclodextrin |
| **BTEX** | Benzene, toluene, ethybenzene, and xylene |
| **Bodipy-Me** | difluoro{2-[1-(3,5-dimethyl-2H-pyrrol-2-ylidene-N)ethyl]3,5-dimethyl-1H-pyrrolato-N} boron |
| **CD** | Cyclodextrin |
| **CF** | Concentration factor |
| **COF** | Covalent organic framework |
| **CTAB** | Cetyl trimethylammonium bromide |
| **CV** | Coefficient of variation |
| **DLLE** | Dispersive liquid-liquid extraction |
| **DMF** | N,N-dimethylformamide |
| **DSPE** | Dispersive solid-phase extraction |
| **EDC** | Endocrine-disrupting chemical |
| **EDMA** | Ethylene dimethacrylate |
| **EPA** | Environmental Protection Agency |
| **EUROPOL** | European Police Office |
| **FAO** | Food and Agricultural Organization |

| | |
|---|---|
| **FT-IR** | Fourier transform infrared spectroscopy |
| **FQ** | Fluoroquinolone |
| **GC** | Gas chromatography |
| **GC-ECD** | Gas chromatography coupled to electron capture detection |
| **GC-FID** | Gas chromatography coupled to flame ionization detection |
| **GC-FPD** | Gas chromatography coupled to flame photometric detection |
| **GC-MS** | Gas chromatography coupled to mass spectrometry |
| **GC-NPD** | Gas chromatography coupled to nitrogen phosphorus detection |
| **GMA** | Glycidyl methacrylate |
| **HPLC** | High-performance liquid chromatography |
| **HPLC-DAD** | High-performance liquid chromatography coupled to diode-array detection |
| **HPLC-FLD** | High-performance liquid chromatography coupled to fluorescence detection |
| **HPLC-MS** | High-performance liquid chromatography coupled to mass spectrometry |
| **HPLC-UV** | High-performance liquid chromatography coupled to ultraviolet detection |
| **HRMS** | High-resolution mass spectrometry |
| **IMS** | Ion mobility spectrometry |
| **IR** | Infrared spectroscopy |
| **IR-ATR** | Fourier transform infrared spectroscopy with attenuated total reflectance |
| **IUPAC** | International Union of Pure and Applied Chemistry |
| **LC** | Liquid chromatography |
| **LLE** | Liquid-liquid extraction |
| **LPME** | Liquid-phase microextraction |

| | |
|---|---|
| **LOD** | Limit of detection |
| **LOQ** | Limit of quantification |
| **MALDI** | Matrix-assisted laser desorption/ionization |
| **MALDI-TOF** | Matrix-assisted laser desorption/ionization coupled to time of flight mass spectrometry |
| **MAS NMR** | Magic-angle spinning nuclear magnetic resonance |
| **MeOH** | Methanol |
| **MIP** | Molecularly imprinted polymer |
| **MOF** | Metal-organic framework |
| **MSPE** | Magnetic solid-phase extraction |
| **NMR** | Nuclear magnetic resonance |
| **NP** | Nanoparticle |
| **NPS** | New psychoactive substances |
| **NS** | Nanosponge |
| **PAH** | Polycyclic aromatic hydrocarbon |
| **PC** | Polycarbonate |
| **PCB** | Polychlorinated biphenyls |
| **PE** | Polyethylene |
| **PET** | Polyethylene terephthalate |
| **POP** | Persistent organic pollutant |
| **PUF** | Polyurethane foam |
| **PVC** | Polyvinyl chloride |
| **REACH** | Registration, Evaluation, Authorization, and Restriction of Chemicals |
| **RP-HPLC** | Reversed-phase high-performance liquid chromatography |
| **SBSE** | Stir-bar sorptive extraction |
| **SDME** | Single-drop microextraction |
| **SEM** | Scanning electron microscopy |
| **SPE** | Solid-phase extraction |

| | |
|---|---|
| **SPME** | Solid-phase microextraction |
| **TCEP** | Tris(2-carboxyethyl)phosphine |
| **TEA** | Triethanolamine |
| **TEM** | Transmission electron microscopy |
| **TEOS** | Tetraethyl orthosilicate |
| **TGA** | Thermogravimetric analysis |
| **Ts-β-CD** | 6A-O-p-toluenesulfonyl-β-cyclodextrin |
| **UPLC** | Ultra-performance liquid chromatography |
| **UPLC-MS/MS** | Ultra-performance liquid chromatography coupled to triple quadrupole mass spectrometry |
| **VOC** | Volatile organic compound |
| **WHO** | World Health Organization |
| **WPAC** | Working Party on Analytical Chemistry |
| **XRD** | X-ray diffraction |

**Phenolic compounds**

| | |
|---|---|
| **Ph** | Phenol |
| **o-C** | o-cresol |
| **m-C** | m-cresol |
| **p-C** | p-cresol |
| **G** | Guaiacol |
| **E** | Eugenol |
| **4-EP** | 4-ethylphenol |
| **4-EG** | 4-ethylguaiacol |
| **VPh** | 4-vinylphenol |
| **MVPh** | 2-methoxy-4-vinylphenol |

### Polycyclic aromatic hydrocarbons

| | |
|---|---|
| **Naph** | Naphthalene |
| **Ace** | Acenaphthene |
| **Fl** | Fluorene |
| **Phe** | Phenanthrene |
| **Flt** | Fluoranthene |
| **Pyr** | Pyrene |
| **B(a)A** | Benzo(a)anthracene |
| **Chrys** | Chrysene |
| **B(b)F** | Benzo(b)fluoranthene |
| **B(k)F** | Benzo(k)fluoranthene |
| **B(a)P** | Benzo(a)pyrene |
| **DiB(a,h)A** | Dibenzo(a,h)anthracene |
| **B(g,h,i)P** | Benzo(g,h,i)perylene |
| **I(1,2,3)P** | Indeno(1,2,3)pyrene |

### Endocrine-disrupting chemicals

| | |
|---|---|
| **BPA** | Bisphenol A |
| **BPAP** | Bisphenol AP |
| **BPC** | Bisphenol C |
| **MP** | Methylparaben |
| **EP** | Ethylparaben |
| **PP** | Propylparaben |

# RESUMEN

De acuerdo con la normativa sobre depósito, evaluación y defensa de la Tesis Doctoral de la Universidad de Valencia aprobada por Consejo de Gobierno el 29 de noviembre de 2011 y con última modificación el 31 de octubre de 2017, se presenta a continuación un resumen global de la temática, objetivos, metodología y principales resultados del trabajo.

## Objetivo

La presente Tesis Doctoral contempla dos grandes líneas de actuación estrechamente relacionadas entre sí. La primera de ellas consiste en la síntesis, caracterización y modificación de materiales porosos basados en ciclodextrinas para su uso como fases sólidas adsorbentes en técnicas de extracción y muestreo ambiental de compuestos que puedan ser de interés en los campos de la seguridad alimentaria, clínico o medioambiental. La segunda línea de actuación consiste en la puesta a punto de procedimientos analíticos completos con capacidades mejoradas respecto a los ya existentes para la determinación de estos analitos que utilicen como fases sólidas extractantes los materiales desarrollados con anterioridad.

La etapa de tratamiento de muestras con diversos orígenes constituye una tarea complicada y a la vez fundamental entre todas las etapas de un análisis químico debido al amplio espectro de interferentes que se pueden presentar en las mismas. Por tanto, el aislamiento, separación y preconcentración de los analitos de interés pueden verse comprometidos por las posibles interacciones de éstos con la matriz de la muestra, dificultando así su extracción y posterior análisis y pudiendo dar lugar de este modo a resultados tanto subestimados como sobreestimados. En este sentido, existe una gran variedad de métodos de adsorción y extracción que hoy en día ayudan al analista en tal fin. Asimismo, una diversidad de fases sólidas se encuentra comercialmente disponibles para llevar a cabo esta tarea, aunque muchas de las tradicionalmente distribuidas son poco selectivas, lo que contribuye en parte a un deterioro de los resultados analíticos obtenidos.

Por este motivo, hoy en día se desarrollan constantemente metodologías de extracción, así como nuevos materiales adsorbentes y tratamientos de muestra que refuercen las técnicas instrumentales utilizadas posteriormente. De entre las características de los materiales utilizados que pueden ser moldeadas, la mejora de la selectividad en la adsorción mediante variaciones estructurales de los materiales utilizados se está desarrollando cada vez con más fuerza. Concretamente, el uso de la formación de complejos de inclusión reversibles entre los analitos de interés y las fases sólidas utilizadas es un campo en constante investigación.

Las ciclodextrinas son una familia de oligosacáridos cíclicos obtenidas por la unión de monómeros de glucosa unidos por enlaces glicosídicos. Su forma tridimensional se asemeja a la de un cono truncado debido a la asimetría constitucional de los anillos de glucopiranosa, lo que las dota de una cavidad interna hidrofóbica capaz de capturar analitos con propiedades físicas y químicas que los hagan aptos para entrar en la misma con elevada afinidad y una cavidad externa hidrofílica que les facilita su modificación y enlace a distintos tipos de soportes sólidos. Debido a su capacidad de reconocimiento molecular, se han utilizado como nanoportadores en campos tan diversos como las industrias farmacéutica y alimentaria para mejorar las propiedades de los productos fabricados, así como también en medicina para suavizar la intensidad de ciertos tratamientos, focalizando su actuación en las partes del cuerpo humano que sean problemáticas en cada caso. Aunque su estructura y propiedades han sido ampliamente estudiadas desde hace algunas décadas, no ha sido hasta comienzos del siglo XXI cuando su uso en química analítica como parte de materiales adsorbentes para el tratamiento de muestra se empezó a popularizar.

Este trabajo se centra por tanto en el estudio de nuevos soportes sólidos conteniendo ciclodextrinas para su aplicación en el campo de la química analítica. En este sentido, tanto la naturaleza del soporte como la de la ciclodextrina utilizada, así como también el tipo de muestra tratada, son los puntos centrales de la investigación llevada a cabo. Las individualidades de cada una y las posibles interacciones de todas estas variaciones entre ellas hacen que las conclusiones extraídas constituyan una contribución al campo de estudio. Concretamente, se han establecido tres objetivos principales en relación con la temática expuesta:

a) Sintetizar y evaluar materiales cuya estructura se basa en xerogeles porosos de sílice conteniendo ciclodextrina para la retención de contaminantes en muestras de origen ambiental y clínico.

b) Sintetizar y evaluar materiales cuya estructura se basa en fases sólidas mesoporosas de sílice conteniendo ciclodextrina para la evaluación de la seguridad de muestras de alimentos.

c) Sintetizar y evaluar materiales cuya estructura se basa en fases sólidas poliméricas conteniendo ciclodextrina para la retención de contaminantes en muestras de origen medioambiental y alimentario.

## Estructura

La presente memoria se estructura así en cinco grandes partes.

La primera parte constituye la introducción del trabajo realizado, donde se muestra una visión general del estado del arte de la temática expuesta. Concretamente, se trata la problemática actual en el campo de la química analítica en lo que respecta al tratamiento de muestra para, a continuación, realizar una revisión bibliográfica sobre las ciclodextrinas y las fases sólidas conteniendo ciclodextrina reportadas en la bibliografía en los últimos años. El bloque de la *Introducción* va seguido de la declaración de los objetivos y la estructura del trabajo en el bloque titulado *Objetivos y Plan de trabajo*.

La segunda parte, que ya se adentra en la descripción de la metodología y la discusión de los resultados obtenidos, se encuentra constituida por la *Sección A: Aplicación de xerogeles porosos de sílice con ciclodextrina*. Este bloque, compuesto por seis capítulos, trata del diseño, la síntesis y la caracterización de este tipo de materiales, así como de su posterior aplicación analítica. Concretamente, el *Capítulo 1* se centra exclusivamente en la síntesis y caracterización de los mismos, mencionándose sus posibles aplicaciones. Se desarrollan diferentes tipos de materiales, esto es, con la ciclodextrina simplemente incluida en la estructura y con la ciclodextrina anclada covalentemente a la misma, así como distintos tipos de ciclodextrinas atendiendo a las características diferentes que puedan mostrar los analitos. A continuación, los capítulos 2 al 5 se centran en su evaluación analítica. El *Capítulo 2* describe su uso para el muestreo ambiental de compuestos orgánicos volátiles, concretamente, de compuestos fenólicos. Seguidamente, el *Capítulo 3* describe su aplicación para la extracción en fase sólida de hidrocarburos aromáticos policíclicos en muestras de agua ambientales. El *Capítulo 4* continúa la misma temática y describe su uso como adsorbentes para la captura de bifenilos policlorados también en muestras de agua. Por el contrario, el *Capítulo 5* se centra en su uso para el aislamiento y posterior análisis de canabinoides sintéticos en saliva. Por último, el *Capítulo 6* muestra una visión global de las principales conclusiones extraídas en base a los resultados obtenidos para esta primera sección.

La tercera parte está constituida por la *Sección B: Aplicación de fases sólidas mesoporosas de sílice con ciclodextina*. Dentro de este bloque podemos encontrar cuatro capítulos, que tratan también del diseño, síntesis y caracterización de este tipo de materiales y de su posterior aplicación analítica. Siguiendo el esquema del bloque anterior, el *Capítulo 1* se centra exclusivamente en la síntesis y caracterización de los materiales, mencionándose sus posibilidades en el campo de la química analítica en base a las características establecidas para los mismos. A continuación, el *Capítulo 2* describe su uso para la adsorción de disruptores endocrinos en zumos. El *Capítulo 3* trata de su aplicación para la extracción en fase

sólida de antibióticos que puedan estar presentes en muestras de leche por transferencia desde los animales productores. Por último, el *Capítulo 4* es una visión general de las principales conclusiones extraídas en base a los resultados obtenidos.

La cuarta parte se corresponde con la *Sección C: Aplicación de materiales poliméricos basados en ciclodextrina*. Este bloque se compone también de cuatro capítulos. El primer capítulo, el *Capítulo 1*, trata también de la síntesis y caracterización de los materiales mencionados, mencionándose sus posibles aplicaciones para el tratamiento de muestra en el campo de la química analítica. Seguidamente, el *Capítulo 2* describe su uso para la extracción en fase sólida de compuestos fenólicos en tés listos para su consumo. En el *Capítulo 3*, estos mismos materiales se utilizan para la adsorción de antibióticos y su análisis en muestras de agua residuales, pudiendo constituir una alternativa para la evaluación indirecta del consumo de este tipo de fármacos por parte de la población. Para finalizar, el *Capítulo 4* muestra una visión global de las principales conclusiones extraídas a partir de los resultados obtenidos en esta tercera sección.

La quinta parte constituye el resumen y las conclusiones globales extraídas en base a los resultados obtenidos durante el desarrollo del trabajo presentado en esta memoria, y se puede encontrar en el bloque titulado *Resumen y Conclusiones*. Por último, se muestran las contribuciones en forma de publicaciones de los resultados obtenidos durante el desarrollo de esta Tesis Doctoral por parte de la autora, así como las referencias bibliográficas citadas.

## Metodología y resultados

A continuación, se describen los principales resultados obtenidos y conclusiones derivadas del trabajo realizado como parte de la presente Tesis Doctoral. Los resultados se muestran en correspondencia con las tres secciones principales en lo referente a la metodología y resultados mencionadas anteriormente. Se indica de forma desglosada por capítulos la metodología adoptada en cada uno de ellos para alcanzar los objetivos planteados, así como los resultados y conclusiones más relevantes obtenidos en los mismos.

### A. Aplicación de xerogeles porosos de sílice con ciclodextrina

#### A.1. Síntesis y caracterización de los xerogeles

En esta parte del trabajo, se diseñaron y sintetizaron xerogeles silíceos con unidades de ciclodextrinas para su posterior aplicación en la adsorción selectiva de distintos tipos de contaminantes. La caracterización de los materiales se llevó a

cabo mediante diferentes técnicas, entre las que se pueden destacar la microscopía electrónica de transmisión, la microscopía electrónica de barrido, y el analizador de tamaño de partículas para su caracterización morfológica. Asimismo, se llevó a cabo la obtención de isotermas de adsorción-desorción de nitrógeno, agua y dióxido de carbono para la caracterización de su porosidad. Por último, la resonancia magnética nuclear y la difracción de rayos X, así como el análisis termogravimétrico y el análisis elemental, se utilizaron para conocer la estructura de las fases sólidas y su composición química.

Por una parte, se desarrollaron fases sólidas en las que la ciclodextrina se podía encontrar meramente incluida en la estructura del material. Tras su completa caracterización, se llegó a la conclusión de que la capacidad de adsorción de las ciclodextrinas no se veía alterada o modificada tras su inclusión en la estructura silícea seleccionada. Sin embargo, aunque su eficiencia se preserva en diferentes condiciones, se podría esperar una pérdida en su capacidad cuando se utilicen en condiciones de humedad elevada, especialmente cuando se trabaja con muestras acuosas.

Por otra parte, se desarrollaron las mismas fases sólidas, pero encontrándose la ciclodextrina esta vez anclada covalentemente a la estructura. En este sentido, se esperaría una mejora en las características analíticas de estos materiales debido a su enlace covalente, lo que los haría aptos para su uso en un mayor rango de condiciones en comparación con el uso de fases sólidas conteniendo la ciclodextrina incluida, incluyendo no solo su uso con muestras de aire sino también en el tratamiento de muestras acuosas.

En general, las buenas características observadas para estos materiales pueden deberse a un diseño adecuado de la fase sólida. Los materiales combinan una superficie de sílice hidrofílica con sitios hidrofóbicos adsorbentes bien distribuidos a lo largo de la misma que se corresponden con la presencia de ciclodextrinas. Así, su estructura se expande desde la escala nanométrica, con un sistema microporoso para la difusión de los analitos bajo estudio, hasta el nivel molecular, preservando en todo momento la estructura nativa de las ciclodextrinas que permiten la adsorción con la selectividad mejorada para distintos tipos de analitos.

### A.2. Determinación de compuestos fenólicos en aire

En este capítulo, se evaluó el uso de los xerogeles de sílice conteniendo ciclodextrina tanto incluida como anclada descritos con anterioridad para el muestreo de compuestos fenólicos volátiles en muestras de aire. Para ello, dos procedimientos distintos de muestreo y extracción se desarrollaron de acuerdo con

las diferentes posibilidades que cada tipo de fase sólida ofrecía para posteriormente compararlos entre ellos en base a los parámetros analíticos establecidos y su eficiencia en el análisis de muestras reales en comparación con los resultados obtenidos utilizando métodos de análisis de referencia. Los analitos seleccionados fueron fenol guaiacol, los isómeros del cresol, 4-vinilfenol, 2-metoxi-4-vinilfenol, etilfenol, etilguaiacol y eugenol.

En el caso del método de muestreo y análisis desarrollado para el uso de los xerogeles conteniendo la ciclodextrina en forma incluida en la red de sílice, el mismo constituyó una buena alternativa a otros métodos descritos en la bibliografía con el mismo fin. El método demostró dar buenos resultados para el análisis de compuestos fenólicos en entornos laborales, así como también en los vapores de ambientadores y aceites esenciales. Sin embargo, entre las desventajas encontradas se pueden mencionar que no se consiguieron buenas recuperaciones en el caso del 2-metoxi-4-vinilfenol ni tampoco para el 4-vinilfenol debido tanto a la pérdida de ciclodextrina en la fase de desorción por su solubilidad como a la incompleta extracción de los dos analitos mencionados. Además, se requirió desorción térmica de los analitos bajo estudio.

Por el contrario, el método de muestreo y análisis desarrollado para el uso de los xerogeles conteniendo la ciclodextrina anclada a la red de sílice mejoró los resultados obtenidos con anterioridad. Aunque las recuperaciones calculadas fueron similares al caso anterior, se consiguió una mejora significativa de los valores medidos para el 2-metoxi-4-vinilfenol y el 4-vinilfenol. Los resultados fueron repetibles y no se requirió desorción térmica. Además, se evitó la pérdida de ciclodextrina durante la etapa de desorción observada con anterioridad, lo que mejoró a su vez los parámetros analíticos obtenidos.

### A.3. Extracción de hidrocarburos aromáticos policíclicos en agua

El trabajo experimental incluido en este capítulo tuvo un doble propósito. Por una parte, se pretendió demostrar también la mejora que se obtenía en los resultados para la extracción en fase sólida de hidrocarburos aromáticos policíclicos en muestras de agua al utilizar como fase sólida los xerogeles conteniendo la ciclodextrina anclada covalentemente a la red de sílice en lugar de los materiales con la ciclodextrina incluida. Por otra parte, se pretendió además demostrar que el tipo de ciclodextrina seleccionada para formar parte de la fase sólida tiene mucho que ver en la mejora de los resultados obtenidos, ya que según las propiedades fisicoquímicas de los analitos puede resultar más o menos beneficioso utilizar ciclodextrinas con mayor o menor tamaño, que serán por tanto capaces de

encapsular más eficientemente a los compuestos de interés. En este caso, los analitos seleccionados fueron benzo(a)antraceno, criseno, benzo(b)fluoranteno, benzo(k)fluoranteno, benzo(a)pireno, dibenzo(a,h)antraceno, benzo(g,h,i)perileno e indeno(1,2,3)pireno. En cada caso, se optimizó un método de extracción, se establecieron sus parámetros analíticos y se analizaron muestras de agua reales.

En el caso del uso de xerogeles conteniendo la ciclodextrina incluida, se pudo concluir que estas fases sólidas pueden ser una alternativa a otros adsorbentes reportados en la bibliografía. El método analítico desarrollado fue capaz de cuantificar hidrocarburos aromáticos policíclicos en cumplimiento con las Directivas Europeas 2013/39/UE y 98/83/UE relativas a la calidad de las aguas. Sin embargo, las pérdidas de ciclodextrina al trabajar con muestras acuosas fueron evidentes, lo que puede llevar a largo plazo a resultados poco repetibles y una mayor dificultad a la hora de reutilizar las fases sólidas.

En cambio, el uso de xerogeles conteniendo la β-ciclodextrina anclada covalentemente ayudó a superar algunos de estos inconvenientes. Del mismo modo que en el caso anterior, el método analítico desarrollado constituyó una alternativa a otros métodos reportados en la bibliografía utilizando fases sólidas diferentes. Sin embargo, los parámetros analíticos establecidos mejoraron considerablemente respecto al caso anterior. Teniendo en cuenta el tamaño medio de los hidrocarburos aromáticos policíclicos bajo estudio, se consideró la posibilidad de anclar covalentemente la γ-ciclodextrina a la red de sílice en lugar de la hasta ahora utilizada β-ciclodextrina, lo que podría dar lugar a una mejora de los resultados.

Efectivamente, el anclaje de la γ-ciclodextrina comportó una mejora significativa de las recuperaciones obtenidas para los analitos, probablemente debido a que los mismos caben mejor dentro de la cavidad interna hidrofóbica de estas moléculas, lo que facilitó la formación de los complejos de inclusión y por tanto mejoro la extracción de los compuestos de interés.

En general, se consiguió una doble mejora de los resultados obtenidos. En primer lugar, de los xerogeles con ciclodextrina incluida a los xerogeles con ciclodextrinas anclada, evitando sus pérdidas. En segundo lugar, de las fases sólidas conteniendo β-ciclodextrina a las fases sólidas conteniendo γ-ciclodextrina para la captura de este tipo de analitos.

### A.4. Extracción de bifenilos policlorados en agua

Debido a los buenos resultados obtenidos con anterioridad para el análisis de compuestos contaminantes en muestras de agua, se consideró en este capítulo la posibilidad de reafirmar las posibilidades de los xerogeles conteniendo la

ciclodextrina anclada para la captura de distintos tipos de analitos. En concreto, se seleccionaron los bifenilos policlorados (PCB28, PCB52, PCB101, PCB138, PCB153 y PCB180) para tal fin. Tras una comparación inicial entre los distintos xerogeles desarrollados conteniendo no sólo distintos tipos de ciclodextrina (β-ciclodextrina y γ-ciclodextrina) sino también distintas cantidades de las mismas para evaluar su rendimiento en este caso, se establecieron también los principales parámetros analíticos para el método desarrollado y se analizaron muestras de agua reales, comparándose los resultados con aquellos obtenidos utilizando un método de análisis de referencia.

Como era de esperar, y basándonos en el tamaño medio de las moléculas de bifenilos policlorados bajo estudio, la fase que mejor funcionaba era la que contenía β-ciclodextrina debido, una vez más, a un mejor acople entre la misma y los compuestos contaminantes bajo estudio. Además, se observó una mejora de las recuperaciones calculadas al aumentar la cantidad de ciclodextrina contenida en la fase sólida.

En lo que respecta al método analítico desarrollado, la cantidad y toxicidad de los disolventes orgánicos utilizados, así como el tiempo de análisis utilizado fueron las mayores ventajas observadas respecto a otros de los métodos reportados. Debido a la probada capacidad de los xerogeles conteniendo ciclodextrina anclada para aplicarse en la determinación analítica de contaminantes en muestras ambientales, se planteó la posibilidad de aplicarlos en otros campos de análisis diferentes como, por ejemplo, en el análisis de matrices de muestra biológicas.

### A.5. Determinación de canabinoides sintéticos en fluidos orales

Este capítulo trató de demostrar el uso potencial de los xerogeles conteniendo ciclodextrina incluida para la extracción en fase sólida de canabinoides sintéticos de muestras de fluidos orales humanos. Aunque su aplicación para el análisis de muestras medioambientales ya había sido probada mediante la preconcentración de analitos a nivel de trazas en las mismas, su uso con el propósito de limpiar matrices de muestra complejas por encima de la preconcentración de los analitos todavía quedaba poco explorado. Los canabinoides seleccionados para llevar a cabo el estudio fueron ADB-CHMICA, MMB-CHMICA y MDMB-CHMZCA. Igual que en los casos anteriores, se desarrolló un método analítico de extracción completo, se establecieron sus parámetros analíticos y el mismo se aplicó al análisis de las sustancias bajo estudio en muestras de saliva reales.

Los resultados obtenidos durante el proceso de optimización demostraron buena sensibilidad y selectividad de los analitos bajo estudio. Los xerogeles demostraron

contribuir eficientemente a la limpieza de la matriz de muestra. Así, la mayor novedad del trabajo experimental expuesto en este capítulo fue el uso de una fase sólida adsorbente para el análisis de los canabinoides sintéticos seleccionados en contraposición a los, mayoritariamente, métodos de *screening* descritos en la bibliografía hasta la fecha, que pueden dar lugar a identificaciones y cuantificaciones incorrectas en el control de rutina

## B. Aplicación de fases sólidas mesoporosas de sílice con ciclodextrina

### B.1. Síntesis y caracterización de los materiales mesoporosos

Esta segunda parte de las tres principales establecidas en cuanto a metodología y resultados obtenidos se centró en el diseño, síntesis y caracterización de materiales silíceos con estructura mesoporosa conteniendo ciclodextrina para su posterior aplicación en la adsorción selectiva de contaminantes en muestras alimentarias. La caracterización de los materiales se llevó a cabo mediante distintas técnicas, entre las que se pueden mencionar la microscopía electrónica de transmisión, la resonancia magnética nuclear, la difracción de rayos X, el análisis elemental, el análisis termogravimétrico y los estudios de porosidad mediante las isotermas de adsorción-desorción de nitrógeno por parte de las fases sólidas obtenidas.

De este modo, se pudo concluir que una nueva variación de las sílices mesoporosas tipo UVM-7 ya existentes se obtuvo mediante su modificación superficial con ciclodextrinas accesibles. Las elevadas áreas superficiales con porosidad interconectada que presentan este tipo de materiales supusieron sin duda alguna una importante ventaja. Además, la presencia de ciclodextrinas, igual que en el caso anterior, contribuiría con mejoras en la sensibilidad y selectividad de los métodos analíticos que se pudieran desarrollar mediante su uso como fases sólidas adsorbentes. La presencia de unidades de ciclodextrina ancladas a la red de sílice y no meramente incluidas expande también considerablemente las condiciones en la que estas fases sólidas pueden aplicarse, pudiendo funcionar correctamente no únicamente con muestras de aire sino también con muestras acuosas mientras mantienen su buen rendimiento, así como también su capacidad de ser reutilizadas en diversas ocasiones.

### B.2. Determinación de disruptores endocrinos en zumo

En este capítulo, se evaluó el uso de los materiales mesoporosos descritos anteriormente conteniendo ciclodextrina como adsorbentes para la extracción en fase sólida de disruptores endocrinos que pudieran haber migrado a zumos de fruta desde sus respectivos envases contenedores. Los analitos seleccionados fueron el

bisfenol A, bisfenol AP, bisfenol C, metilparabeno, etilparabeno y propilparabeno. Paralelamente a cómo se actuó en los casos anteriores, se desarrolló un método analítico completo y se establecieron sus principales parámetros analíticos. Asimismo, se analizaron muestras de zumo reales una vez optimizado el método y establecido el procedimiento experimental completo, y los resultados se compararon con aquellos obtenidos utilizando un método analítico de referencia.

Los resultados mostraron un mejor funcionamiento de la fase sólida conteniendo β-ciclodextrina debido a una mejor correspondencia del tamaño de su cavidad con el tamaño de los analitos bajo estudio. Además, se consiguió buena sensibilidad y repetibilidad de los resultados obtenidos, lo que podría dar lugar a una aplicación del método desarrollado para analizar sistemáticamente disruptores endocrinos migrantes en zumos de fruta.

### B.3. Determinación de fluoroquinolonas en leche

En este caso, se buscó el uso de analitos de mayor tamaño para reforzar la tesis establecida con anterioridad sobre la posibilidad de adaptar el tamaño de la ciclodextrina utilizada a las propiedades fisicoquímicas de los analitos bajo estudio para optimizar los resultados obtenidos. Por tanto, dado que las fluoroquinolonas constituyen una familia de analitos de tamaño relativamente grande en comparación con otros de los utilizados hasta el momento, se evaluó el uso de materiales mesoporosos tipo UVM-7 conteniendo γ-ciclodextrina para su extracción de muestras de leche. Concretamente, los analitos seleccionados fueron ofloxacino, norfloxacino y ciprofloxacino. El buen funcionamiento del método analítico desarrollado fue comprobado mediante el establecimiento de sus principales parámetros analíticos y el análisis de muestras reales en comparación con un método de análisis de referencia seleccionado.

La mayor ventaja del método de análisis desarrollado en este caso respecto a otros existentes en la bibliografía fue su elevado grado de limpieza de una matriz tan compleja como pueda ser la leche. Mientras que el extracto obtenido utilizando el método de extracción de referencia dio lugar a cromatogramas donde la presencia de otros compuestos interferentes era patente, los cromatogramas obtenidos en la fase de cuantificación mediante el método de extracción desarrollado utilizado los materiales mesoporosos conteniendo ciclodextrina no mostró una presencia excesiva de interferentes, lo que refuerza una vez más la idea de una mejora de la selectividad en los resultados obtenidos en presencia de ciclodextrinas como parte de las fases sólidas adsorbentes utilizadas.

### C. Aplicación de materiales poliméricos basados en ciclodextrina

#### C.1. Síntesis y caracterización de los materiales poliméricos

La última parte del desarrollo de materiales adsorbentes se centró en el diseño, síntesis y caracterización de materiales poliméricos con estructura porosa conteniendo ciclodextrina para su posterior aplicación en la adsorción selectiva de contaminantes en distintos tipos de muestras. Concretamente, los materiales se sintetizaron utilizando glicidil metacrilato y dimetacrilato de etilenglicol como monómeros, y su superficie fue más tarde modificada mediante reacciones de tipo *click-chemistry* para anclar las moléculas de ciclodextrina a la misma. En este caso, la caracterización de los materiales se llevó a cabo mediante distintas técnicas, entre las que se pueden destacar la resonancia magnética nuclear, la ionización asistida por láser, la microscopía electrónica de barrido, el análisis termogravimétrico, la espectroscopia infrarroja, la espectroscopia Raman, la fluorimetría, el análisis microscópico confocal y las isotermas de adsorción-desorción obtenidas para el gas nitrógeno.

En este caso, aunque aparentemente los macroporos de este tipo de fase sólida dan lugar a materiales con una baja área superficial, al contrario de lo que ocurría con los materiales silíceos mesoporosos, los mismos presentan una serie de ventajas distintas que les hacen también válidos para el propósito establecido. La posibilidad de modificar selectivamente su superficie, en este caso con moléculas de ciclodextrina, abre la posibilidad a una gran variedad de aplicaciones entre las que la adsorción con selectividad mejorada de distintos tipos de analitos juega un papel fundamental.

La caracterización completa a la que los materiales sintetizados fueron sometidos permitió comprobar que la obtención de los mismos se llevó a cabo con éxito, y que tanto la β-ciclodextrina como la γ-ciclodextrina se anclaron a su superficie correctamente para su posterior evaluación en el campo analítico.

#### C.2. Determinación de compuestos fenólicos en te

Siguiendo los resultados obtenidos con anterioridad, en los que la β-ciclodextrina era aquella que mejor funcionaba para encapsular reversiblemente los compuestos fenólicos debido al tamaño de estas moléculas, se decidió aplicar los materiales poliméricos conteniendo β-ciclodextrina a la extracción en fase sólida de estos analitos en muestras de té. Concretamente, los analitos seleccionados fueron fenol, guaiacol, los isómeros del cresol, 4-vinilfenol, etilfenol, etilguaiacol, 2-metoxi-4-vinilfenol y eugenol. Siguiendo el esquema anterior, se establecieron los principales

parámetros analíticos del método de extracción desarrollado y el mismo se utilizó para el análisis de los analitos de interés en muestras reales.

Los beneficios obtenidos en este tipo de extracción para los compuestos fenólicos fueron similares a los obtenidos utilizando xerogeles basados en sílice conteniendo β-ciclodextrina, lo que respalda el hecho de que el punto clave de las fases sólidas bajo estudio es el contenido en ciclodextrina y no la naturaleza del soporte sólido utilizado que, aunque tiene un papel importante, no es fundamental.

Por último, se decidió comprobar la influencia del tipo de ciclodextrina en los resultados obtenidos dependiendo del tipo de analito seleccionado, para lo que se decidió poner a prueba los materiales poliméricos conteniendo γ-ciclodextrina para el análisis de analitos de mayor tamaño.

### C.3. Extracción de fluoroquinolonas en agua

Como se ha mencionado, el último capítulo de la sección experimental de este trabajo se centró en la aplicación de los materiales poliméricos conteniendo la más grande γ-ciclodextrina para la extracción de antibióticos de muestras de agua residual, lo que podría contribuir a un análisis indirecto del consumo de este tipo de medicamentos por parte de la población. En este caso, los antibióticos seleccionados para llevar a cabo el estudio fueron enoxacino, ofloxacino, norfloxacino, ciprofloxacino y sparfloxacino. Previamente a la aplicación de la metodología desarrollada para la determinación de antibióticos en muestras reales, sus parámetros analíticos fueron establecidos. Fue entonces cuando se llevó a cabo el análisis de muestras de agua reales y se compararon los resultados obtenidos con aquellos calculados utilizando un método de análisis de referencia.

El método desarrollado resultó ser una alternativa a aquellos métodos descritos con la misma finalidad en la bibliografía. Paralelamente a los casos anteriores, y tal y como se esperaba, la selectividad del método resultó aumentada debido a la presencia de moléculas de ciclodextrina accesibles en la fase sólida, concretamente de la γ-ciclodextrina.

# Table of contents

# List of Tables

## INTRODUCTION



## METHODS AND RESULTS

### SECTION A. Application of porous cyclodextrin-silica xerogels

# List of Figures

## INTRODUCTION

METHODS AND RESULTS

SECTION A. Application of porous cyclodextrin-silica xerogels

## SECTION B. Application of mesoporous cyclodextrin-silica solid phases

# INTRODUCTION

## 1. Analytical chemistry: the main challenges

Back in 1894, the German chemist and philosopher Wilhelm Ostwald defined analytical chemistry as "the art of recognizing different substances and determining their constituents" (Schwedt, 2020). Later, the Working Party on Analytical Chemistry (WPAC), held in Edinburg in 1993, stated that analytical chemistry is "the scientific discipline that develops and applies methods, tools, and strategies to obtain information on the composition and nature of matter in space and time" (Karst, 2004; Valcárcel, 2012).

Currently, these definitions are not at all out of date. Analytical chemistry is considered a measuring science focused on investigating the composition and structure of matter, as well as its chemical constituents, contents, distribution, and interaction. In this way, it contributes to revealing matter changes through the existing space-time rules (Ju, 2013). Although often merely considered as a support to other disciplines, it plays an important role and usually contributes with principles, theories, and tools to the development of a variety of areas such as biology, geology, environmental sciences, agricultural chemistry, physics, engineering, medicine, materials science, social sciences and, of course, chemistry itself (Bergquist & Turner, 2018; Locatelli, Mandrioli, Samanidou, & Bocklitz, 2020). Its significance has specifically been shown in different fields such as new drug development, disease diagnosis, and early warning, life process study, food and environmental safety, quality control, economics, trade, or forensic medicine, among others.

In general, the demands of scientific research on this discipline include accurate, sensitive, selective, stable, fast, automated, high-throughput, and even *in situ* analytical methods and protocols (Ju, 2013). A classical analytical method often refers to: (i) a first step focused on the treatment of the sample under study, and (ii) a second step including the detection and quantification stages carried out with specific instrumentation. Consequently, constant innovation is mandatory both in sample pre-treatment procedures and in instrumental techniques to obtain reliable, true, and reproducible information of analysis (Merone et al., 2020). This phenomenon often leads to a synergistic effect of the advantages of the single procedures, obtaining a combined analysis that shows greater analytical performances.

Analytical chemistry has experienced perspective changes over time. Early on, analytical technologies and instruments were mainly studied to carry out elemental analysis and speciation. Oppositely, instrumental development, which is now driven by instrument manufacturers, has nowadays gone into the

background to let research in analytical chemistry migrate to method development and novel applications (Bergquist & Turner, 2018).

Thus, some general rules regarding the sampling and extraction procedures applied such as a reduction of sample handling in order to reduce analytes loss, the application of greener procedures that allow obtaining high enrichment factor values, and the use of preparative methods as selective as possible with respect to the analyte of interest may be enormous advantages. Therefore, the leading goals of analytical chemistry with regard to sample preparation include the development of new materials with improved properties (Abbasi & Ammar Haeri, 2021) that also possess the ability to be reused, as well as the validation of novel strategies and procedures to meet the growing need for methods of analysis with the mentioned characteristics. In this context, the possibility of having both sampling and sample clean-up procedures available, but also robust and reproducible instrumental configurations for detection and quantification is increasingly important.

However, since analytical chemistry is established in multidisciplinary applications, the fundamental difficulty of this science is the challenge with which analytical chemists are faced when isolating the target compounds from different types of complex matrices or variable compositions where qualitative or quantitative analysis is necessary. Nowadays, life science is a prior objective of scientific research, while human development may be also decided by environmental and food safety, in addition to quality controls on a variety of trade articles (Bergquist & Turner, 2018). Analytical chemistry is increasingly focusing on these fields to care for the new needs in life science research to develop screening strategies and ultrahigh sensitive detection and imaging technologies, following at the same time the social demands in urgent events, environmental pollution, accidents, and deleterious food to provide new tools for obtaining information on these fields. Thus, this science should introduce new concepts and technologies to combine computer, nanotechnology, and biotechnology to create new analytical principles, design-automated detection procedures, and specific detection methods, as well as new measurement instruments (Ju, 2013).

## 1.1. The environmental problem

The reduction of environmental pollution is one of the highest challenges worldwide for global ecological preservation. Since industry and transport have become an essential part of modern society, waste production is an unavoidable outcome of human developmental activities (Landy, Mallard, Ponchel, Monflier, & Fourmentin, 2012). In recent years, the release of various non-regulated harmful

legacy compounds into the environment has attracted great attention due to their toxicity and widespread use (Pyrzynska, 2008). The pollutants emitted from different sources contaminate air, water, and soil environments, as well as food crops and, in general, biota, with an impact on both human health and the ecological system from it. The more frequently detected pollutants cover such a broad range of organic and inorganic compounds (Ligang Chen, Wang, & Tong, 2011) as trace metals, polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), pesticides, dyes, pharmaceutical residues, and other emerging pollutants whose specific effects are in the majority of cases still poorly known. Indeed, the large number of pollutants of potential environmental concern poses a challenge for regulatory agencies (León et al., 2020).

Over the years, different regulations have established a legislative framework for the presence of pollutants all around. In Europe, there exist directives and recommendations regarding the quality of water (2013/39/EU, 2013; 98/83/EC, 1998), air (2004/107/EC, 2004; 2008/50/EC, 2008), or soil (2008/98/EC, 2008). Some of them establish specific concentration limits for harmful compounds in the respective samples. Since the effects that some of these pollutants may have are increasingly better known, the concentration limits recognized are lower every time, frequently reaching trace levels.

The exposed trouble makes the role of chemistry important here. On the one hand, the growing demand for using the available natural resources has prompted rapid development in waste management by introducing cleaning, recycling, and reuse. Recycling human-affected natural sources requires competent methods for the removal of habitual and emerging pollutants from them (Rego, Kuriya, Kurkuri, & Kigga, 2021). Over the last decades, there have been significant research and engineering advances in remediation. In this sense, treatment technologies include extraction, transformation, or degradation of pollutants, as well as sequestration and immobilization by sorption, either used individually or in a combined way (T. Zhang et al., 2019). On the other hand, it is mandatory to develop analytical methods that allow detection of such low concentrations of the compounds of interest to monitor them and implement the appropriate corrective measures if necessary. Indeed, considerable efforts have been made in recent decades towards the identification and quantification of the more relevant contaminants of emerging concern in the environment.

## 1.2. The importance in food safety

The Food and Agricultural Organization (FAO) established in the World Food Summit that took place in 1996 that food safety exists when all people have access

to enough safe and nutritious food to satisfy their needs in order to lead an active and healthy life (Chammem, Issaoui, De Almeida, & Delgado, 2018). In developed countries, the safety and nutritional quality of food take on special relevance when faced with the taken-for-granted access to foodstuff, being the policies created to defend consumers' health and rights remarkable.

Problems related to food safety can be traced back to the origins of humanity and are linked to the risk posed by the consumption of toxic, altered, or contaminated food (El-Shibiny, El-Sahhar, & Adel, 2017). There are many factors to cause a food to be problematic because of illness or intoxication provoked in the consumer. These are the so-called food hazards, which are defined as any biological, chemical, or physical condition present or inherent to nourishments that may have a harmful effect on health.

Chemical hazards include a great variety of substances of different origins (Figure 1), whether they are natural components of foodstuff itself or come to it through contamination. The main chemical compounds included in this group are substances of natural origin (Inés Molina et al., 2022), toxins produced by microorganisms (H. Xie et al., 2022), marine biotoxins (Vale, 2020), environmental pollutants (C. Wang et al., 2021), pollutants developed during food processing (Zastrow, Speer, Schwind, & Jira, 2021), unauthorized food additives (X. Meng et al., 2020), veterinary drug residues (L. Zhang, Shi, He, & Li, 2021), as well as different types of allergens (Jia & Evans, 2021).


**Figure 1. Summary of the main types of contamination found in food products.**

Some of these dangers can be or have already been the origin of major food crises that took place all around the world in relatively recent times (Bernard et al., 2002) such as the dioxin incident or the widely known as the "mad cow disease". These crises have generated significant mistrust in citizens to the point of changing their eating habits in addition to posing a risk to public health.

Therefore, the best way to prevent this type of emergencies is through implementing improvements in food safety systems by public administrations. Among them, there can be mentioned:

a) Adequate legislation that regulates aspects related to the safety of the food consumed (1881/2006/EC, 2006; 2017/2158/EU, 2017; 315/93/EC, 1993).
b) Official inspection and control systems that allow verifying compliance with regulations throughout the food chain.
c) Self-control systems for food operators that allow obtaining safe food.
d) Crisis management systems in the event that the above elements are not enough and the safety of nourishment is hindered (Chammem et al., 2018).

Thus, analytical chemistry must participate in guaranteeing high food safety through controls that allow its hazards to be detected with a high confidence level to find the best solutions to them. Monitoring the abovementioned dangers is needed for many purposes, which include food safety, regulatory enforcement, risk assessment, international trade, label claims, environmental protection, industry needs, academic research, and consumer confidence (Lehotay & Chen, 2018).

## 1.3. Application in bioanalysis

Human biomonitoring refers to the detection of substances or elements in biological samples, including the establishment of identity, concentrations, and the profiles of other related or metabolized species that can provide valuable information about the exposure level to drugs or contaminants, physiological processes taking place, and the general state of health of the body (e.g., elemental profiling, understanding the correlation with a disease, classification of diseases, and disease diagnosis or prognosis). Thus, it constitutes another important field related to analytical chemistry (Lum, Chan, & Leung, 2021).

It is widely known that humans are continuously exposed to a variety of environmental pollutants. Since essential elements play an important role in human health, abnormal levels of them in the body may be related to different diseases and environmental exposure (Ye, Qiu, Li, Jiang, & Jing, 2018), which makes the scientific interest focus on the development of methods for this purpose. However, it is important to highlight that the analytical methods applied in this

field do not need to be limited to toxic elements or substances. As many essential elements are involved in functions of the human body, monitoring them may also provide useful information about general health conditions, being any change in their content useful as an indicator (Stojsavljević et al., 2019). Indeed, routine clinical analyses include not only major elements such as sodium or magnesium but also others equally essential such as copper or bromine, although in minor concentrations. Therefore, establishing reference levels for different biomarkers is important in supporting exposure monitoring and disease evaluation.

In this context, drug abuse constitutes nowadays a major concern in society. As reflected in government studies (European Monitoring Centre for Drugs and Drug Addiction, 2021), it is highly prevalent in every country around the world and poses serious health and social risks. Among the most common drugs consumed with this end, amphetamines, hallucinogens, cocaine, cannabinoids, heroin, and psychotropic drugs can be mentioned. Besides, an additional problem to trafficking and consumption of drugs of abuse is found in the emergence of new psychoactive substances (Bade et al., 2021), synthesized from known, widely used, legislated drugs with minimal structural changes to mimicking and even enhancing their effects without these being recognized as illegal substances by the authorities (Sumnall, Evans-Brown, & Mcveigh, 2011).

In general, sensitive, precise, and accurate analytical methods for chemical analysis are fundamental tools in biochemical research and assessment (Augusto & Luiz Pires Valente, 2002). However, the development of new procedures is a major challenge faced by analytical chemistry due to the high variability of biological samples as complex mixtures in which the analytes of interest can be present in reduced amounts. Among the different types of biological samples used, blood is the most widely adopted sample choice. However, blood can only reflect short-term exposure and requires invasive sampling, so other non-invasive specimens such as hair, nails, or urine may be feasible alternatives. Less common samples include breast milk, teeth, meconium, and saliva, as well as tear fluid, exhaled breath, and sweat (Lum et al., 2021).

Additional analytical difficulties arise from the intrinsic dynamic behavior of living biological systems. It should be noted that the detection times of a substance in different biological matrices are affected by numerous variables such as the dose administered, the route of administration, or the pH of the specimen under analysis, as each subject possesses its own metabolism (Verstraete, 2004).

Thus, analyses of live biological samples typically may require state-of-the-art techniques for sampling and sample preparation, analyte separation, detection, and quantification (Augusto & Luiz Pires Valente, 2002).

## 2. An outline on analytical approaches

As mentioned, an analytical method is mainly composed of two steps. The first one is referred to sample preparation techniques, this is, the process of isolating the target compounds from the matrix and treating them through concentration or derivatization procedures (Hyötyläinen, 2009), among others, while the second stage includes the identification and quantification often carried out with specific instrumentation (Z. Guo, Huang, Wang, & Feng, 2020).

However, analyses need sometimes to be conducted in a variety of samples presenting a wide chemical diversity of analytes, making their simultaneous occurrence them show a need for rapid multi-analyte methods suited for different matrices that are sensitive and selective enough to detect the analytes of interest at least under the legal limits imposed in different areas. Since the extraction and concentration of samples are usually time-consuming and complex processes, screening methods have been presented as a fast and cheaper alternative to them. They usually provide rapid detection, are easy to use, cost-effective, and require minimal sample treatment, thus they can be used by non-specialists and under field conditions as well (Cigić & Prosen, 2009). Their benefits contrast though with their cross-reactivity, which is one of their major drawbacks.

In any case, the most important criterion to choose the analytical approach to make a measurement should be the fit-for-purpose principle. Therefore, although non-targeted analysis is commonly used in complex analyses that require a rapid response, the obtained positive results must be preferably confirmed with classical targeted two-step methods to avoid misinterpretations.

### 2.1. Screening methods

From an etymological point of view, there is no difference between "conducting an analysis" and "screening". However, screening methods seem to present a discriminatory action (Muñoz-Olivas, 2004) reflected in the definition established for them from the analytical point of view. Thus, screening methods are those consisting of fast acquisition of semi-quantitative data about all components of a sample that are able to indicate whether target analytes are present above or below a defined threshold. In general, screening methods tend to have qualitative rather than quantitative emphasis, involve little or no sample treatment, are rapid for immediate decision-making, and sometimes require confirmation by conventional alternatives of analysis.

It is important to establish guidelines and standardize criteria for non-targeted screening analysis and to determine its applicability in the goal fields (Z. Guo et

al., 2020). The scarce quantitative information needed to establish the reliability of a screening method includes the limit of detection of the technique used and its uncertainties, the threshold imposed by the client or by legislation (e.g., regulatory or risk assessment needs), and the cut-off level taken by the analyst. Usually, the error coming from a binary response system defined by false negative or false positive results is taken into account.

A good example of the recent advances in non-targeted screening methods are high-resolution mass spectrometry technologies (HRMS), which provide great advantages to the analyses carried out (Huérfano Barco, España Amórtegui, & Guerrero Dallos, 2022). However, other numerous screening methods can be classified according to different criteria (Table 1).

**Table 1. Overview of the main types of screening methods of analysis.**

| Criterion | Type | Reference |
|---|---|---|
| Sample treatment | Extensive preparation | (Wei Liu et al., 2020) |
| | Little preparation | (Tisler, Liang, Carvalho, & Bester, 2021) |
| Place of analysis | *In situ* | (Karapinar, Kaynar, Hayirli, & Kom, 2013) |
| | Laboratory | (Caballero-Casero et al., 2021) |
| Response | Unknown screening | (Blum et al., 2017) |
| | Suspect screening | (Wei Liu et al., 2020) |
| Complexity of the instrument | Simple instruments | (Nogueira, Alves, Matias, & Veras, 2021) |
| | Complex instruments | (N. Yu et al., 2018) |
| | Commercial kits | (Karapinar et al., 2013) |
| Sample origin | Environmental | (Blum et al., 2017) |
| | Food | (Liang et al., 2022) |
| | Clinical | (Caballero-Casero et al., 2021) |

In short, although screening methods are frequently applied, the usually insufficient method validation and the absence of benchmarking in comparison with conventional methods are noticed (Tsagkaris et al., 2019). Therefore, confirmatory analysis plays a key role in analytical chemistry. However, the typically long and complicated sample preparation mentioned remains a challenge that has to be faced in the near future.

## 2.2. Confirmatory analytical methods

### 2.2.1. Analytical techniques

Chromatographic and other related techniques are usually used in confirmatory analysis methods due to their greater reliability and robustness in quantitatively determining the content of analytes under study in any type of matrix after sample treatment. Depending on the nature of the analytes of interest, liquid chromatography and gas chromatography can be mentioned, although it is also worth mentioning other derived techniques such as electrophoresis (Ben Hassine, Hammami, Touil, & Driss, 2015). All of them can be coupled to a variety of detectors.

First, gas chromatography (GC) is one of the preferred techniques when it comes to the analysis of volatile or semi-volatile compounds. Nevertheless, the analysis of mostly non-volatile compounds by GC can be also carried out by prior derivatization, usually conducted by silylation, esterification, or methylation procedures, thus making them suitable for their correct separation with adequate sensitivity. Numerous studies carried out have described the development of rapid methods with the ability to determine simultaneously more than one analyte with this technique, although in most of them a previous extraction stage was essential to reduce the matrix effect, as well as the use of compatible solvents. Among the most common detectors, the electron capture detector (GC-ECD) and the flame ionization detector (GC-FID) have been implemented in some reports (P. de Morais, Stoichev, Basto, Carvalho, & Vasconcelos, 2011; Romero, Manero, & Laso, 2007). On the one hand, it has been shown that GC-FID lacks sufficient sensitivity and selectivity for certain compounds. On the other hand, GC-ECD can be only adequately sensitive and selective with analytes containing the halogens bromine and chlorine. Other detectors such as the nitrogen-phosphorus detector (GC-NPD) and the flame photometric detector (GC-FPD) can be also highlighted.

Liquid chromatography (LC), usually used as high-performance liquid chromatography (HPLC), is a well-known technique in which low-volatility analytes are separated on the basis of their different solubility between a mobile phase and a liquid-solid stationary phase. Thus, solutes establish in this case interactions such as dipole attraction or proton donation-acceptance between both phases. Therefore, the factors that can be modified to achieve separation are more numerous than in GC. With regard to the detection modes, ultraviolet detection (HPLC-UV) or diode-array detection (HPLC-DAD) are the most simple and popular ways (Ben Hassine et al., 2015) to quantify compounds containing

functional groups that are capable of absorbing radiation in this range. However, they also suffer from a lack of sensitivity and selectivity (Nikolin, Imamovic, Medanhodzic-Vuk, & Sober, 2004). In this context, using fluorimetric detection (HPLC-FLD) is helpful when it is necessary to determine compounds with fluorescent properties reaching very low detection limits (L. Yang et al., 2022), as is usually the case with environmental or clinical samples.

Mass spectrometry has been used as a detection manner in both types of chromatography (GC-MS and HPLC-MS). In mass spectrometry, the energy communicated to the analyte molecules is able to cause their ionization and separation in two or more fragments, which can be both ionic and uncharged. Thus, the mass spectrum shows the quantity or abundance of the ionized molecule and ionic fragments collected at increasing values of the ratio mass/charge. This type of detection has a high capacity for identification, being currently the detection technique providing the greatest reliability to identify the presence of a certain molecule or atom in a sample and an excellent tool for quantifying analytes (Šimek, Lemr, Hermannová, & Havlíček, 2020). It is capable of handling the analysis of very complex samples, and its capacity is greatly increased when coupled to a previous chromatographic system.

Finally, electrophoretic techniques can be defined as the set of analytical techniques in which the separation takes place usually in a capillary column with the application of an electric field (Saar-Reismaa et al., 2019). Normally, the separations are efficient and fast, being able to separate analytes of any nature both in the form of ions and in the form of uncharged molecules.

### 2.2.2. Sample preparation

The choice of an appropriate sample preparation method is a critical factor in the success of analyses because a complete analytical protocol may fail if an inadequate sample treatment is conducted (Hyötyläinen, 2009).

Sample preparation is a very active research area in analytical chemistry. Major incentives for this are to increase selectivity and sensitivity, clean up, enrichment, high compatibility with analytical instrumentation, speed, simplicity, automation, reduced consumption of reagents, and application of soft extraction conditions (Ju, 2013). Although significant technological advances emerged in analytical instrumentation in the last years, traditional extraction and separation processes must be frequently used to overcome interferences of the samples under analysis (Payanan, Leepipatpiboon, & Varanusupakul, 2013; Pyrzynska, 2008). Some of them may still present several shortcomings such as low enrichment factor, low extraction efficiencies, and poor selectivity.

Liquid-liquid extraction (LLE) has been one of the most used procedures in analytical chemistry for the treatment of samples with different origins (Karimpour Zahraei, Salemi, & Schmidt, 2021). This sample preparation technique requires the use of a solvent, mostly organic, which is immiscible with water. Thus, the partition of the analyte is carried out between the organic phase and the immiscible aqueous phase where the analyte is presumably present. However, when analytes are present at the trace level, it is necessary to carry out several consecutive extractions, which leads to high solvent consumption, low extraction yields, and commonly low precision. A significant variation of LLE is represented by dispersive liquid-liquid extraction (DLLE), which incorporates the use of a solvent that is miscible in both the organic and the aqueous phase, being a smaller volume of extracting solvent required due to its dispersion in the formed emulsion (Cortada, Vidal, & Canals, 2011). Another variation based on liquid extraction methodologies uses ionic liquids instead of organic solvents while maintaining the same experimental methodology (Cumplido, Cháfer, Guayazan-Jaimes, de la Torre, & Badia, 2022).

In the last decades, solid-phase extraction (SPE) has progressively replaced LLE. This technique uses a solid material that acts as sorbent for the analytes on its surface, according to an existing affinity sorbent-analyte (Ben Hassine et al., 2015). There exists a great variety of sorbents. Among them, polymeric phases, molecularly imprinted polymers (MIPs), metal-organic frameworks (MOFs), resins, or compact silica materials introduced into extraction cartridges stand out. Within these techniques, dispersive solid-phase extraction (DSPE) can be mentioned. In this method, the solid phase is unsupported and added directly to the sample, which, after stirring, transfers the analytes of interest to the sorbent. Subsequently, the solid phase is separated by mechanical processes such as centrifugation or filtration, later using organic solvents for its elution and subsequent quantification. Another promising alternative is magnetic solid-phase extraction (MSPE), where modified magnetic particles that allow the separation of the solid from the sample due to its magnetic properties are used, then following the traditional SPE procedure (Farajzadeh, Niazi, & Sattari Dabbagh, 2021). Other methodologies in this wide group may include stir-bar sorptive extraction (SBSE), which is based on a magnetic stirrer coated with the solid extractant (Benanou, Acobas, Deroubin, David, & Sandra, 2003). Then, two possibilities to carry out the extraction of the analytes arise: conducting it by thermal desorption or by solvent elution as a usual SPE procedure.

Finally, microextraction technologies include different approaches such as solid-phase microextraction (SPME) (Maggi, Zalacain, Mazzoleni, Alonso, &

Salinas, 2008) and liquid-phase microextraction (LPME), and they are an example of an ongoing process of miniaturization. Besides, single-drop microextraction (SDME) is based on the suspension of a drop of an organic solvent that will accumulate the analyte through passive diffusion until equilibrium is achieved within an aqueous sample. Subsequently, this drop will be quantified into the selected analytical instrument. In short, these methods are similar to classical SPE and LLE, but the extraction phase is downscaled in this case, thus applying to extraction methods the main principles of green chemistry.

As shown, new types of extraction methodologies, as well as novel extraction sorbents and chemistries are currently being developed and assessed (Ju, 2013). These include new types of solid sorbents and new types of liquids as ionic liquids and deep eutectic solvents. Among them, SPE has been increasingly applied for the pre-concentration and clean-up of different samples due to its advantages over LLE (Tsagkaris et al., 2019) and other techniques. Thus, developing new types of sorbents is nowadays a major challenge.

## 3. Sorbent materials in extraction techniques

Several solid phases are commercially accessible for the adsorption of analytes in different-origin matrices. Undoubtedly, the most widely used sorbent is $C_{18}$ (Payanan et al., 2013) due to its high availability, affordable price, and the good results it usually offers in diverse scenarios. Other commercially available solid phases showing high request for analytical purposes are hydrophilic-lipophilic balance cartridges, carbon-based materials such as activated carbon or carbon molecular sieves (Dettmer & Engewald, 2002; T. Li, Wang, Xu, & Chakraborty, 2020), or ionic exchange columns, among others. Different criteria have been established to guarantee accurate cleaning and high specificity towards the target analytes. Among them, proper enrichment, fast adsorption-desorption processes, a homogeneous and inert surface to avoid artifact formation, low affinity to water, low competition with other interfering constituents of the sample, high stability, and the possibility of using them on multiple occasions can be mentioned (Dettmer & Engewald, 2002).

However, the lack of selectivity and the competition of analytes with water in aqueous matrices are some drawbacks presented by several commercially available solid phases (Jung et al., 2013). For this reason, the investigation and development of new materials with enhanced properties for their application in adsorption processes is a challenge that must be overcome.

In this sense, improving selectivity through directed structural variations of the sorbents used is recently being developed with increasing force (Motia,

Bouchikhi, Llobet, & El Bari, 2020). Moreover, the formation of inclusion complexes between adequate molecules in the solid phase and the analytes of interest is also an area of ongoing research.

## 3.1. Cyclodextrins: host-guest adsorption

Inclusion complexes, or host-guest complexes, are non-covalent reversible structures of two or more molecules with physicochemical properties that are superior to those exhibited by the molecules individually (Teyssandier et al., 2016). Although historically developed in solution, there exists increasing interest in implementing these principles to systems assembling solid surfaces since the presence of a firm surface not only ensures a high degree of crystallinity in the host network, thus enabling efficient capture of guests, but also provides additional stability to the resultant host-guest complex via molecule-surface interactions. In this sense, host-guest interactions are already being exploited for the reversible adsorption of analytes in solid materials as they can improve some features of the analytical methods employed (Szente & Szemán, 2013). To this end, several compounds that are able to act as host molecules have been synthesized and used in sorbents, including crown ethers, cryptands, carcerands, cucurbiturils, paracyclophanes, calixarenes, and cyclodextrins.

**Table 2. Three-dimensional form and size of native cyclodextrins (Z. Li et al., 2007; Tsuchido, Fujiwara, Hashimoto, & Hayashita, 2017).**

| Molecule | Glucose units | Average cavity volume (Å$^3$) | Average cavity diameter (nm) | Average molecular diameter (nm) |
|---|---|---|---|---|
| α-CD | 6 | 174 | 0.45 – 0.55 | 1.50 |
| β-CD | 7 | 262 | 0.65 – 0.70 | 1.65 |
| γ-CD | 8 | 427 | 0.80 – 0.90 | 1.80 |

Cyclodextrins (CDs) are a family of cyclic oligosaccharides obtained from the union of glucose monomers linked by α-1,4 glycosidic bonds. The natural occurrence of CDs can be classified into α-, β-, and γ-cyclodextrin that are composed of 6, 7, or 8 glucose units, respectively (Figure 2). Thus, their diameters increase with the number of glucose units in their structure (Table 2). They are shaped as truncated cones due to the constitutional asymmetry of the glucopyranose rings. On the one hand, the hydroxyl groups are oriented to the outer space flanking the upper and lower rims, with the primary hydroxyl groups towards the narrow edge of the cone and the secondary ones towards the wider edge (Fourmentin, Crini, & Lichtfouse, 2018). This provides hydrophilic

properties to the external area. On the other hand, the central cavity of the cone is lined with the skeletal carbons and ethereal oxygen of the glucose residues, thus producing hydrophobicity. Therefore, they exhibit the ability to trap guest molecules with appropriate physicochemical properties inside them through the formation of host-guest complexes (Kfoury, Landy, & Fourmentin, 2018). This feature has positioned CDs as promising nanoscale carriers.

Among their benefits, their use can lead to advantageous changes in the chemical and physical properties of the guest molecules. Some examples are stabilization of light- or oxygen-sensitive substances, modification of the chemical reactivity, fixation of volatile substances, improvement of solubility, masking of smell and taste, masking of pigments and colors, and modification of the catalytic activity. In short, they are capable of improving stability while decreasing the reactivity of the guest compound. For all these reasons, they have been used in a variety of industries such as cosmetics, personal care and toiletry, food and flavors, pharmaceutics, agriculture, or as a part of adhesives and coatings (Jug, Yoon, & Jackman, 2021; Yaowen Liu et al., 2022; Votava & Ravoo, 2021).

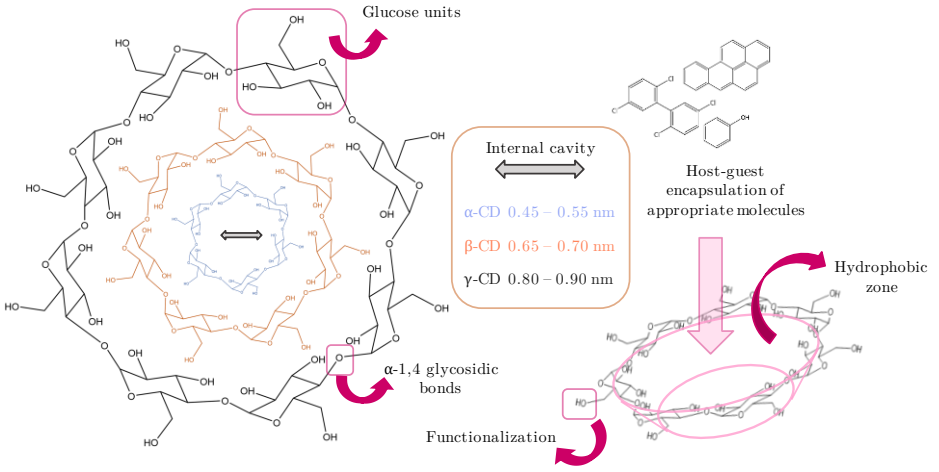



**Figure 2. Schematic representation of the native α-, β-, and γ-cyclodextrins. Their structure, shape, and other functions they present are also mentioned.**

Apart from the naturally occurring CDs, different derivatives can be obtained to suit the application by, for example, amination, esterification, or etherification (Del Valle, 2004). The most frequent ways to reach this purpose are: (a) the electrophilic attack to the OH- groups of the hydrophilic zone, or (b) the nucleophilic attack to C-OH bonds. This possibility has conducted to the study of new synthetic methods for producing cyclodextrin-based materials, since using the well-known host-guest chemistry of natural CDs is a stepping-stone to form more complex solid phases.

Wide research on CDs as a part of extracting materials to allow the adsorption of different compounds from urine, water, soil, or food samples has been reported. Thus, an increase in the use of CDs as everyday commodities in separation sciences is evident for some years, when we have assisted to a revival in the interest shown by the scientific community towards them through a progressive increase in the number of inventions related to cyclodextrin-based solid supports. As a matter of fact, they are extensively used as reversible sorbents in analytical chemistry, not only in the form of cross-linked cyclodextrins (Alsbaiee et al., 2016), but also for the functionalization of several supporting structures (Lee D. Wilson et al., 2010).

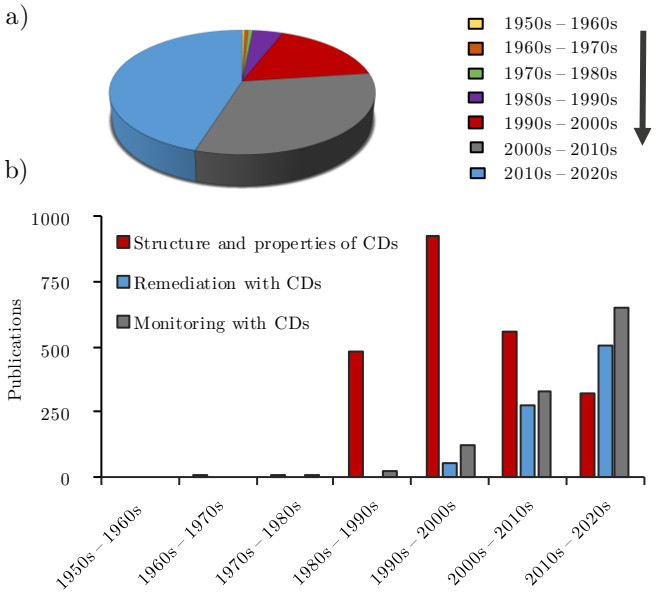



**Figure 3. Summary of the tendency in the number of works on CDs reported over the years in the literature: (a) publications talking about CDs, whatever the focus is, and (b) evolution in the number of publications on the basic structure and properties of CDs compared to their increasing use in remediation and monitoring.**

As shown in Figure 3, chemical aspects such as their structure or their intercalation were already studied some years ago. Their properties were then extensively used in different fields such as the pharmaceutical and food industry to improve the availability of poorly soluble or biodegradable compounds. However, the structural aspects of CDs that enable an improvement of separation and an enhancement of sensitivity and accuracy in analytical methods with monitoring purposes have been nowadays more deeply discussed (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gándara, 2009; Crini, 2014). Among their novel applications, using materials based on CDs in environmental

sciences to ease remediation technologies by selectively trapping pollutants in different matrices (Landy et al., 2012) can be also mentioned. Thus, the integration of cyclodextrin molecules and their chemical derivatives in supporting structures is being more widely studied each time. This includes the preparation, characterization, and finding of their potential applications as the most important issues of research (Morin-Crini, Fourmentin, Fourmentin, Torri, & Crini, 2019).

### 3.2. Materials containing cyclodextrin

The synthesis of cyclodextrin-containing materials can be carried out mainly in two ways. On the one hand, by chemical bonding through grafting or coating reactions using previously modified CDs. On the other hand, by inclusion through sol-gel or self-assembly processes, hereafter referred to as hybrid cyclodextrin materials, by using either native or previously modified CDs.

It is well known that CDs present certain limitations as individual sorbents (Gentili, 2020) or simply included as a part of hybrid materials. Their solubility in water makes their losses during analyte-caption procedures significant when working with aqueous samples, reflecting this in a lower precision of analytical methods and a decrease in the loading capacity of the solid phases. For example, non-supported CDs were used as sorbents to perform SPE of pesticides from water and juice samples (Peng et al., 2017). Additionally, hybrid cyclodextrin materials were tested to extract VOCs from air samples (Mauri-Aucejo, Llobat-Estellés, Egea, Guillem, & Amorós, 2012). These studies showed the benefits of CDs, but also the mentioned limitations. In this sense, the key to making CDs suitable for extraction purposes is to increase their insolubility by chemically connecting them to water-insoluble supports.

These supports can be of a varied chemical nature: inorganic, organic, or also mixed-nature solids. Regardless of their composition, they all must provide a fundamental feature: a good dispersion and accessibility of CDs, thus maximizing the possible interactions with analytes to be retained. Indeed, the irregular distribution of CDs and the frequent low loading of these phases can limit their adsorption capability (Morin-Crini et al., 2019). For this reason, the materials used as supporting agents must provide high surface areas, which are achieved through a porous structure or by reducing particle sizes. Therefore, microporous ($< 2$ nm), mesoporous ($2 - 50$ nm), and macroporous ($> 50$ nm) (nano)materials (McCusker, Liebau, & Engelhardt, 2003) are commonly synthesized.

Since a large variety of materials, nanomaterials, and nanoparticles with cyclodextrin units on their structures have been reported, accurate classification of the developed solid phases is difficult to carry out because, in many cases, the

advantages offered by different types of supporting sorbents are used in a combined way.

Among the polymeric-natured ones, various materials with attached CDs, as well as cross-linked CDs, can be found. They have been used in quite heterogeneous contexts due to their insoluble nature. Compared to the use of polymeric materials, other inorganic ones such as those derived from silica have some advantages. Their physical robustness, their enhanced chemical inertness, or their larger surface areas (Lambert, Liu, Boyne, Zhang, & Yin, 2003) must be highlighted. In fact, the use of silica as a support for CDs has spread due to the variety of siliceous structures that can be obtained with fine control of the reactions through their low reactivity and their ease to incorporate new groups (Walcarius & Collinson, 2009).

Anyway, all the supporting materials reported have both advantages and disadvantages, which makes an in-depth analysis of their obtaining and application convenient. Concretely, the variety of supports used for the inclusion of CDs can be mainly divided into silica-based materials, polymeric-based materials, and other nanomaterials or metallic nanoparticles such as carbon-based supports and solid phases with magnetic properties, among others.

### 3.2.1. Cyclodextrin-silica materials

Silica-based supports offer a variety of structures (Figure 4) with a wide perspective of functionalities that can be enhanced when cyclodextrin units are added to the structure of the solid phase.

A first approach can broadly divide and classify silica phases into commercial and non-commercial products.

Commercial amorphous silica can be wet- or dry-type silica. On the one hand, silica gel is a granular, porous form of $SiO_2$ manufactured from sodium silicate in aqueous alkaline media. The relatively high inter-particle condensation leads to void formation, thus resulting in solids with a high porosity and surface areas up to 800 $m^2\ g^{-1}$. On the other hand, fumed silica is known as pyrogenic silica, because it is produced in a flame. It consists of nano or micrometric primary particles of amorphous silica fused into branched chain-like aggregated at the submicron scale. Its particle size is established in 5 – 50 nm and the grains are non-porous, with surface areas in the 50 – 600 $m^2\ g^{-1}$ range. From the structural point of view, the main difference between both types lies in the aggregation level of the primary particles, with greater compactness in the case of wet-type silica gel when compared to fumed silica due to the preparative method used. Thus, the ratio of silanol groups ($Q^2$ and $Q^3$) is much higher in the silica gel, which converts it into

highly hydrophilic, as well as an appropriate candidate for the functionalization or anchoring of modified CDs to it. Contrary, the condensed Si species ($Q^4$) dominate in the fumed silica, which provides them with a marked hydrophobicity. Additionally, it is possible to use already shaped siliceous supports such as commercial capillary silica (untreated or later modified), which are normally obtained by pyrolysis and can be therefore classified as fumed silica.

Oppositely, sol-gel chemistry strategies using different silica sources such as tetraethyl orthosilicate (TEOS) or other modified alkoxides have made it possible to synthesize a great variety of silica gels (pure or hybrid) to obtain silica or organo-silica xerogels (Brinker & Scherer, 1990) after extraction of the solvent. As opposed to pure silica, hybrid silica would contain CD units for analytical applications. Therefore, depending on the preparative parameters such as pH, temperature, reaction media, the ratio of TEOS compared to the other silanes with organic groups, size and nature of the organic groups, etc., a wide variety of porous silica-based xerogels are available in a range of sizes (from microporous to macroporous). A decisive step when designing the desired porosity is to achieve fine control of the hydrolysis and condensation processes (highly pH-dependent) of siliceous species (Walcarius & Collinson, 2009). Xerogels prepared at a pH around the silica isoelectric point are microporous. As the pH increases, an evolution through mesoscale to macroscale pores occurs. Along with the aging time of the gel, the extraction mode of the liquid component constitutes an important step that can modify its textural properties. The most common way is through a mild heat treatment that usually induces moderated collapse of the structure. However, when the liquid component of the gel is replaced with a gas (supercritical drying or freeze-drying), a lower collapse occurs. The result is a solid with extremely low density called aerogel (Pierre & Pajonk, 2002), which usually shows larger pores in comparison with xerogels.

Lastly, a revolution in porous materials occurred in 1992 when scientists from the Mobil Company published the synthesis and characterization of a material called MCM-41, the first ordered mesoporous silica (C.T. Kresge, Leonowicz, Roth, Vartuli, & Beck, 1992). This solid, as well as many others synthesized to date, are obtained by taking advantage of the template effect generated by the surfactant micelles (Charles T. Kresge & Roth, 2013). The condensation of the inorganic component in the intermicellar space of the surfactant-silica assembly leads to solids that can be considered as mineral replicas of liquid crystal phases when the surfactant removal generates mesopores (Sábio, Meneguin, Martins dos Santos, Monteiro, & Chorilli, 2021). Thus, controlling the size of the micelles is crucial to modulate the dimensions of the mesopores. The possibility of using

different surfactants (ionic, anionic, or neutral), as well as the use of swelling agents, makes it possible to regulate pore sizes of ca. 2 – 50 nm. These solids reach surface areas around 1000 $m^2$ $g^{-1}$. Thus, a variety of materials has been described (Wan & Zhao, 2007), not only with different pore sizes but also with different mesopore arrays (hexagonal or cubic symmetry). The most common ones are solids MCM-41, MSU-H, SBA-15, MCM-48, and SBA-1. The differences in their symmetry can affect the degree of accessibility of analytes to active centers such as CDs, with cubic arrays being in principle more favorable due to the interconnection of mesopores in 3D. Regardless of the symmetry and unlike xerogels (which normally generate cage-like pores), the mesopores generated thanks to the surfactant micelles are cylindrical and with very homogeneous sizes.

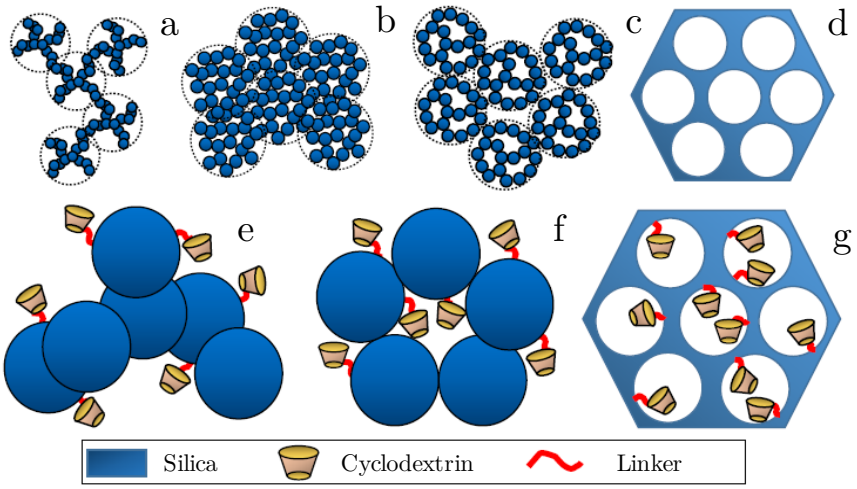



**Figure 4. Schematic representation of the possible structures for: (a) fumed silica, (b) silica xerogels, (c) silica aerogels, (d) mesoporous silica, (e) CD-modified fumed silica, (f) CD-modified xerogels or aerogels, and (g) CD-modified mesoporous silica.**

Following the path of silica-based materials, there already exist several studies on the synthesis and applications of cyclodextrin-based silica materials in separation technologies (Morin-Crini et al., 2019). They can also be divided into those using commercial silica during their synthesis and those whose source of silica is not commercial, but it is obtained from the co-reaction of silica-based substances.

Some examples use minimally modified commercial silica. These include the incorporation of vinyl groups on the surface of silica gel or the immobilization of polymerizable derivatives of CD onto silica gel (Akiyama, Hishiya, Asanuma, & Komiyama, 2001), among others. The synthesis and application for analytical

purposes of this type of solid phases had greater success in the first decade of this century. For example, Fan et al. used commercial silica gel (20 – 30 µm) to bond β-CD and then used it as a selective SPE sorbent for extracting 4-nitrophenol from lake-coming water samples (Fan, Feng, & Da, 2003). Moreover, commercial fused silica fibers subsequently coated with β-CD were also reported to extract and quantify phenolic compounds from water samples through SPME (Y. Hu, Zheng, & Li, 2004). Faraji et al. quantified phenolic compounds in water with the help of β-CD-bonded silica synthesized from purchased irregular silica gel. They optimized the extraction of these compounds, first using SPE (Faraji, 2005), then SBSE (Faraji, Husain, & Helalizadeh, 2011), and finally LPME (Faraji, Husain, & Helalizadeh, 2012) while maintaining the synthesis procedure and thus the basic properties of the sorbent used. More recently, a silica sorbent (40 – 63 µm) containing β-CD was developed and used for the separation and purification of epigallocatechin gallate from green tea extracts (Lai, Gu, Huang, Chang, & Lee, 2012). In this case, the batch adsorption experiments conducted demonstrated that the sorbent possessed enhanced selectivity towards this compound compared to other tea catechins and caffeine.

Other authors have used non-commercial silica for the synthesis of the solid phases, being them a feasible alternative to the previous ones. Sawicki et al. obtained a mesoporous silica solid phase with attached CD in a two-step process, and then applied it in remediation of pesticides (Sawicki & Mercier, 2006). As it is known, pesticide traces can reach drinking or superficial waters and affect human health since they show carcinogenic and mutagenic effects. That is the reason why they must be controlled and monitored according to water guidelines. Another alternative is presented in the form of a one-pot synthetic process for the obtaining of CD-silica xerogels applied to the sampling of VOCs (Mauri-Aucejo et al., 2012). Additionally, silica-coated magnetic graphene oxide materials functionalized with β-CD ($Fe_3O_4$@$SiO_2$@GO-β-CD) using TEOS as the silica source were used for the entrapment of tetracycline, oxytetracycline, and doxycycline coming from bovine milk samples (Al-Afy, Sereshti, Hijazi, & Rashidi Nodeh, 2018). These compounds are broad-spectrum antibiotics widely used in human and veterinary medicine. The environmental problem they represent resides in their presence in animal-origin food, which poses a serious threat to consumer health (allergic reactions, chronic toxicity, and antimicrobial resistance). In this case, the use of TEOS allowed for greater flexibility when designing the material, which in turn can supply a better extraction performance.

Another possible classification of cyclodextrin-silica materials is by paying attention to their physical parameters such as the pore size or the order that the

supporting structure presents. Among them, non-ordered hybrid materials and mesoporous silica sorbents can be mentioned.

Due to their large internal surface area, high porosity, and high amount of silanol groups, mesoporous materials have attracted considerable attention for application in catalysis, filtration and separation, sorption, and storage of gases. A large number of studies have been carried out concerning the use of surfactants as a template to obtain ordered silica structures. The synthesis of mesoporous oxides through the called "atrane route" (W. M. Liu, Cao, Fan, & Chen, 2011; Valtchev, Mintova, & Tsapatsis, 2009) is an example. In this case, cyclodextrins are linked into the well-constructed cavities of the mesopores and have demonstrated to be effective as sorbents in both aqueous and gaseous media (Trofymchuk, Roik, & Belyakova, 2016). Besides, the X-ray diffraction measurements are consistent with the preservation of an ordered mesophase, as expected (Mahmud & Wilson, 2016; L.D. Wilson & Mahmud, 2015). Xu et al. applied imprinting technology to mesoporous silica materials with SBA-15 as support and linked β-CD to it through molecule templates during the synthetic procedure. Specifically, they used cholesterol as a template (Z. Xu et al., 2016), and observed that the binding amount of these molecules was enhanced in chromatography and for SPE of water. In addition, 2,4-dichlorophenoxyacetic (2,4-D) served as a template in cyclodextrin-silica mesoporous hybrid materials (Z. Xu, Deng, Li, Tang, & Cui, 2019). In this case, the molecularly imprinted material was prepared by using 2,4-D as the template molecule, alkyne-modified β-CD, propargyl amine as the combinatorial monomers, and SBA-15 as the support. Results of the equilibrium binding experiments and selectivity tests demonstrated that the material had binding affinity and high specificity for a group of analytes with similar size and shape than those of the template, and the binding kinetic experiments showed an enhancement of the mass transfer rate through the imprinting approach described. Moreover, SPE recoveries for this compound in aqueous samples were around 80%. However, the mesoporous order has still been combined with the presence of CDs in certainly few studies. Moreover, their analytical use has also been barely validated.

Despite the interest that mesoporous solids have aroused, there exists some controversy regarding the virtues associated with their order. In short, an ordered mesoporous structure does not present great advantages over other types of materials in some specific applications such as catalysis, remediation, or analytical determination. For this reason, high interest in preparative alternatives for porous materials in the absence of surfactants, which also implies additional costs, has also been observed (Chunqing Liu, Lambert, & Fu, 2004). In some cases, it is

necessary to go back to classical sol-gel synthesis ideas, which made it possible to prepare porous materials such as xerogels and aerogels in the absence of surfactants. Indeed, a variety of synthetic strategies with analytical applications has been described to obtain silica gels with no structural order. The versatility of sol-gel chemistry allows the synthesis of a great variety of siliceous and organosiliceous materials with controlled structure, composition, and porosity, with simple procedures and at low temperatures. These silica-based sol-gel derivatives have reached a priority place in several research areas since it is greatly versatile in controlling the porosity, the hydrophobic-hydrophilic balance, and its reactivity. Fan et al. (Fan, Feng, Da, & Wang, 2005) used sol-gel chemistry to link CD to commercial capillary silica, and then used the phase for in-tube SPME of non-steroidal anti-inflammatory drugs from urine samples. A year later, Zhou et al. proposed the sol-gel technology to obtain a novel fiber from hydroxyl-terminated silicone oil coated with CD. This fiber was used to extract ephedrine and methamphetamine in human urine (Zhou & Zeng, 2006) and polybrominated diphenyl ethers from soil (Zhou, Yang, Cha, Zeng, & Xu, 2007) through headspace SPME. Furthermore, Zhang et al. described the development of β-CD-modified silica for SPE of methyl jasmonate in aqueous and plant samples (Wenpeng Zhang, Du, Su, Zhu, & Chen, 2013), and Chen et al. reported the analysis of pesticides in fruits and vegetables by surface-enhanced Raman spectroscopy after SPE with a 3,5-dimethyl phenyl carbamoylated β-CD-bonded silica gel (X. Chen, Yan, Xiao, & Li, 2016). Also, a study on different hydrophobic-hydrophilic natures of xerogels and aerogels to understand the dominant adsorption interactions of phenolic compounds with silica-based sorbents was carried out. The functionalization of aerogels with cyclodextrin was compared with the previously cited solid phases (Matias et al., 2015). As the authors describe, the sol-gel synthesis followed a one-step catalyzed procedure and the subsequent drying of the gels was accomplished in this case by supercritical fluid drying and extraction with $CO_2$ to obtain aerogels, and evaporative drying to produce xerogels. Newly, Chen et al. obtained an acryloyl β-CD-silica hybrid monolithic column by applying a sol-gel polymerization method in their synthesis. These materials were useful for pipette-tip SPE of parathion and fenthion (Ling Chen, Dang, Ai, & Chen, 2018). The determination of carbendazim and carbaryl in leafy vegetables was also carried out with the same material through SPME (Ling Chen, Li, et al., 2018), with limits of detection of 1.0 μg $kg^{-1}$ for carbendazim and 1.5 μg $kg^{-1}$ for carbaryl, respectively. In addition, recoveries ranged from 93% to 110%.

Finally, other approaches have been also reported regarding an improvement of the analytical performance of supports based on cyclodextrin-containing silica

structures. As an example, attapulgite modified with functionalized β-CD and glycidoxypropyltrimethoxysilane was effective to adsorb fluoroquinolones from honey through dispersive SPE (X. Cui et al., 2015) with high extraction efficiency and selectivity. At the same time, Gao et al. functionalized silica gel modified with cyclodextrin and vinyl groups to obtain surface molecularly imprinted materials. They were used in the selective determination of (-)-epigallocatechin gallate by applying a SPE methodology in toothpaste samples (Gao, Hu, & Yao, 2015). The work reported a promising approach for the purification of complex samples. Additionally, functionalized β-CD was grafted with silica gel in the presence of salicylamide for the adsorption of $UO_2^{2+}$. Uranium plays an important role in the modern energy industry. For this reason, large amounts of wastewater containing uranium have been discharged into the environment, which has resulted in widespread environmental pollution and can contribute to severe damage to health. In this study, the developed material demonstrated to be effective in the presence of interfering ions (H. J. Liu, Jing, Liu, Du, & Sun, 2016). Besides, silica was the main support used to synthesize novel solid phases based on β-CD coupled to graphene oxide to determine isoflavones in soy-based juice (Da Silva & Lanças, 2020) and PAHs in fried food (N. Wang, Lu, & Cui, 2021).

### 3.2.2. Polymer-based supports with cyclodextrin

The variety of chemical reactions involved in the formation of polymeric materials is much wider than in the case of silica supports. Polymeric supports are an extensive and common family used for different applications, including those related to industry and analytical chemistry (Figure 5).

For them, two synthetic strategies can be differentiated in a simple approach: two-pot-type syntheses, involving a post-modification step of an already formed support, and one-pot-type syntheses, where CDs or other modifying agents can be incorporated simultaneously to the formation of the polymeric structure.

First, it is possible to use fibers or layers as a supporting phase where the polymer is deposited to increase the surface area. Polymers such as poly(dimethylsiloxane) can be used. When the polymeric layer presents a minor thickness (< 30 µm), the material obtained does not usually show porosity so only the CDs on the surface would be exposed to the analytes. When the thickness of the layer increases (< 50 µm), a certain porosity can be generated, which is associated with the globular growth of the polymer. In this case, the pores formed are in the macropore range due to the micrometric size of the polymeric globules.

In other cases, an additional substrate is not necessary. In the two-pot processes, it is possible to first synthesize the polymer and then carry out a process

of post-functionalization. Thus, polymers derived from methacrylate, such as poly(glycidyl-*co*-ethylene dimethacrylate) (Horák, Labský, Bleha, Pelzbauer, & Svec, 1993), can be fabricated from glycidyl methacrylate to obtain modifiable solids. Different alternatives are possible to connect modifiers on it, including click-chemistry reactions. In this case, the globular growth of the polymer allows the formation of large pores that would also be dominant in polymeric materials containing CDs. Oppositely, one-pot strategies are the most used for the incorporation of CDs. In some cases, materials similar to those prepared in two different stages are obtained. In those cases, the morphology and porosity of the final solid containing the CD may be similar to that of the pure polymer, being the result a macroporous polymer with bonded cyclodextrin molecules.

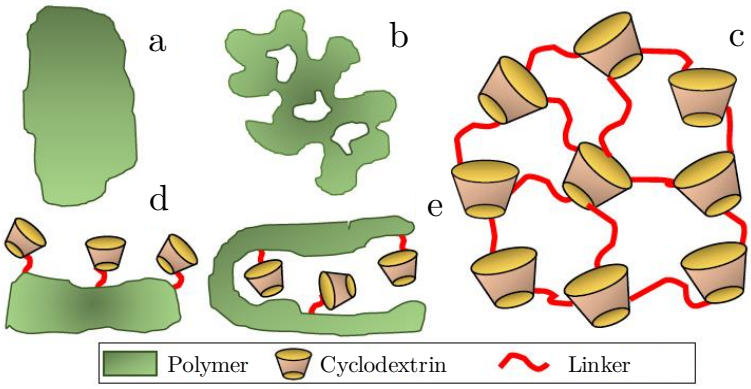



**Figure 5. Schematic representation of the possible structures for: (a) a non-porous polymer, (b) a porous polymer, (c) nanosponges, (d) a CD-functionalized non-porous polymer, and (d) a CD-functionalized porous polymer.**

Additionally, nanosponges (NSs) are an extensive family of nanomaterials synthesized using one-pot methods. The term was first used in 1999 (D. Q. Li & Ma, 1999) to refer to novel nanoporous polymers made up of CDs connected with diisocyanate linkers. However, the origin of cross-linked insoluble CD polymers dates back to 1965, when the preparation of networks made up of cross-linked CDs using epichlorohydrin was described (Solms & Egli, 1965). Now, the term nanosponge refers to a class of insoluble materials with distinctive nanometric porosity that can be synthesized using either organic or inorganic compounds. A recent review on the subject classified nanosponges into four categories (Caldera, Tannous, Cavalli, Zanetti, & Trotta, 2017). The first generation comprises urethane, carbonate, ether, and ester-based nanosponges synthesized by reacting CDs with a cross-linking agent. The addition of specific functionalities to the first-generation nanosponges allowed them to extend their field of application and gave

rise to the second generation. In this sense, three strategies can be used to incorporate the functional groups of interest: functionalization by post-cross-linking, pre-cross-linking modification of CDs, or addition of the functionalizing agent simultaneously to the cross-linking step. The third generation contains, therefore, stimuli-response NSs, whose behavior can be modified according to changes in the environment. Finally, the fourth generation includes molecularly imprinted nanosponges with high selectivity towards specific guest molecules. The synthesis of MIPs is based on the incorporation of a template molecule during the polymerization process (Belbruno, 2019). Contrary to what occurs in siliceous and simpler polymeric materials, where the functionalizing agents are not an intrinsic part of the essential backbone of the support, these groups are essential to nanosponges. In the case of CDs, they may be a fundamental part of the structure in addition to the improved features they provide the support with. Taking into account the size of the CD monomers and the common linking agents used, the resulting materials are normally in the range of micropores and small mesopores.

Perhaps, covalent organic frameworks (COFs) are one of the newest families of porous materials (X. Li et al., 2020). They represent an emerging class of crystalline solids entirely composed of light elements and connected by covalent bonds in 2D and 3D (Figure 6). These materials were first described in 2005 (Coté et al., 2005) and combine diverse interesting properties such as a high surface area and low framework density, homogeneous pore size distribution, and stable structures that give them applicability in a wide range of fields. Pre-designable topologies and tunable pore sizes, usually in the range of micro and small mesopores, can be achieved by selecting adequate experimental conditions, including the nature and size of the linking units used (Díaz de Greñu et al., 2021; Yusen Li, Chen, Xing, Jiang, & Chen, 2020). To date, more than twenty different linkages have been described. Among them, boronic esters, triazines, and imines can be mentioned.

Although they bear certain similarities with nanosponges since the whole skeleton has organic nature and the pores are in the same size domain, they also present important differences. While polymeric nanosponges are unordered materials, COFs are crystalline. Moreover, while nanosponges necessarily require modified CDs for their preparation, in the case of COFs they are only an option.

Recently, COFs containing CDs in their crystalline structure have been described. The CD molecules can be incorporated through one-pot strategies, thus taking part in the crystalline COF skeleton, or through post-functionalization of two-pot synthesis procedures (Yuan et al., 2019).

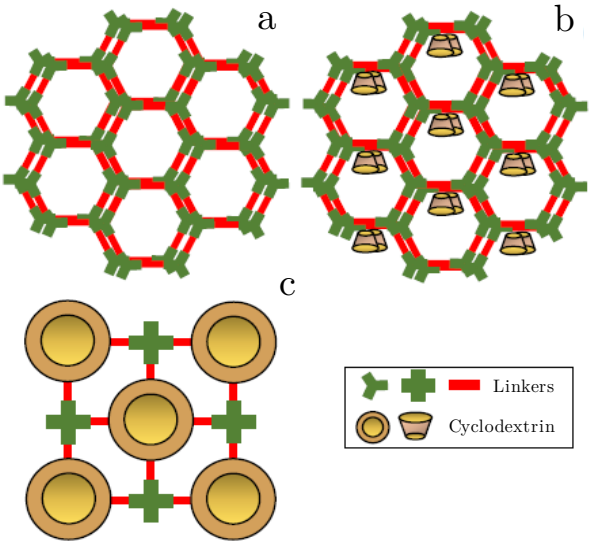



**Figure 6. Schematic representation of the possible structures for: (a) COFs, (b) a CD-functionalized COF, and (c) a CD-containing COF.**

Thus, polymeric supports containing cyclodextrin have been widely used as drug delivery systems, although in recent times many of them have found application in SPE and other extraction methods with analytical purposes (Gentili, 2020).

On the one hand, polymeric solid phases with longer synthetic procedures can be mentioned. In this case, cyclodextrin molecules, which should be previously functionalized, are anchored to an already existing polymer-based support (frequently fibers or columns, but also batch materials) using an appropriate binding agent. A poly(dimethylsiloxane)/β-CD coating was prepared in the form of a membrane to extract phenolic compounds and PAHs from water (Y. Hu, Yang, Huang, & Li, 2005) and in the form of a fiber to reversibly adsorb phenolic compounds and amines from aqueous samples (Fu, Hu, Zheng, & Li, 2006) by SPME. The coating demonstrated to have a porous structure that provided the material with high surface areas and allowed for high extraction efficiency in both cases, together with a low cost of preparation. Another example is the synthesis of an acryloyl-β-CD monolithic column for the SPME of carbofuran and carbaryl in rice. These pesticides have manifested to be hazardous for humans and animals due to their accumulation and potentially toxic effects on living organisms, which involves food safety as a part of an environmental problem. An advantage of this work is revealed by its one-step polymerization method (Sun et al., 2017). Recently, Liu et al. reported a SPME procedure with cyclodextrin molecularly

imprinted fibers of polymeric nature for the selective recognition of polychlorophenols in water (Yuanchen Liu, Liu, Liu, Hu, & Xu, 2018).

On the other hand, some other works described cross-linked cyclodextrin units in the form of polymers for the adsorption of a diversity of analytes. Epichlorohydrin has been frequently used as a linker to this end. For example, Yu et al. described a β-CD epichlorohydrin copolymer as a SPE sorbent for aromatic compounds in water (J. C. Yu, Jiang, Liu, Yu, & Zhang, 2003), and Zhu et al. used a β-CD cross-linked polymer as SPE material for the separation of trace $Cu^{2+}$ (Zhu, Wu, Sun, & Zhang, 2008) and $Co^{2+}$ (Zhu, Wu, & Gu, 2009). As it is known, metal contamination in the water stream from industries is a major problem since the effects of acute poisoning in humans and plants are very serious, potentially leading to liver damage with prolonged exposure. For this reason, the determination of trace metals in the environment constitutes a contribution to the field. Moreover, cyclodextrin-cross-linked copolymers were examined in terms of the sorption towards p-nitrophenol and methyl chloride, two model agrochemical pollutants (Lee D. Wilson et al., 2010). Other linkers reported in the literature are bifunctional isocyanate linkers (Mhlanga, Mamba, Krause, & Malefetse, 2007) and 1,4-phenylenediisocyanate (Mohamed, Wilson, Headley, & Peru, 2008), both used to obtain cyclodextrin-based polymeric materials as supramolecular sorbents for environmental remediation. Recently, Li et al. reported a tetrafluoroterephthalonitrile-cross-linked β-CD as a binding agent of diffusive gradients in thin films for sampling endocrine-disrupting chemicals in water (H. Li et al., 2021)

Moreover, SPE of pollutants such as diphenylphthalate, phenolic compounds, glycyrrhizic acid, and pyrethroids was achieved by using molecularly imprinted polymers of allyl-β-cyclodextrin and methacrylic acid (Yongfeng, Wuping, Yan, Junxia, & Jing, 2012), β-CD functionalized ionic liquid polymers (Raoov, Mohamad, Bin Abas, & Surikumaran, 2014), molecularly imprinted polymers with bismethacryloyl-β-cyclodextrin and methacrylic acid as double functional monomers (Tang et al., 2017), and a namely hyperbranched polymer functionalized with cyclodextrin (Mi et al., 2019). Ibuprofen is a drug of environmental concern since it has been found that pharmaceutical substances are commonly found in the environment and cause negative effects on aquatic life. In this sense, Shang et al. developed an immobilized poly(vinylalcohol)/cyclodextrin eco-sorbent, which has also been described for the removal of ibuprofen from pharmaceutical sewage (Shang, Chiu, & Jiang, 2017) in the form of a transparent and easy-handle film, with entrapment efficiencies of around 90%.

As mentioned, a group of special interest when using cross-linking agents is cyclodextrin-based nanosponges. They can comprise inorganic and organic materials and, subsequently, are not only limited to polymeric-natured solid phases, although they constitute a majority inside the groups. NSs are insoluble materials that, despite being micro- or macro-sized objects, have been classified as nanomaterials by virtue of their internal cavities, pores, or voids in the nanometer range (Gentili, 2020). A good illustration is the β-CD-polyurethane polymer used as SPE material for the analysis of carcinogenic aromatic amines in water described by Bhaskar et al. (Bhaskar, Aruna, Jeevan, & Radhakrishnan, 2004), or in the β-cyclodextrin polymers for the extraction of steroidal compounds from urine (Moon, Jung, Moon, Chung, & Choi, 2008) and BTEX from aqueous solutions (Nojavan & Yazdanpanah, 2017), also based on the use of epichlorohydrin as cross-linker. Important is to mention the work by Alsbaiee et al. (Alsbaiee et al., 2016), where a porous β-CD polymeric network for remediation of micropollutants in water samples was described. Specifically, β-CD units were cross-linked with rigid aromatic groups, providing thus a high surface area, which supposed an advantage in comparison to other nanosponge-type materials reported. The mesoporous polymer of β-CD showed to be able to sequester a variety of organic micropollutants with adsorption rate constants greater than those of non-porous β-CD sorbents. Moreover, the reusability of the material permitted the rapid removal of a complex mixture of organic micropollutants at environmentally relevant concentrations several times. This material gained such a lot of attention that it was described afterward for different applications such as the dispersive SPE of quinolones from water (Jingyi Zhang et al., 2017) or the SPE of bisphenols in water and orange juice (Yarong Li, Lu, Cheng, Zhu, et al., 2018; Yarong Li, Lu, Cheng, Wang, & He, 2018).

Finally, the COFs new family of organic-based supports has been combined with CDs to improve their properties. However, the analytical applications of these novel materials remain mostly unexplored, and the works reported in this sense are still limited to date. A β-CD covalent organic framework has been described as a chiral stationary phase for the separation of antibiotics (Yuying Wang, Zhuo, Hou, Li, & Ji, 2019) as a proof of concept. Although not applied in extraction techniques, the interest of this work resides in the proven capability of cyclodextrins in COFs to encapsulate analytes of environmental interest. In this sense, the described material can be applied in the future to the extraction of the same trace pollutants from complex matrices. Additionally, Yang et al. (Y. Yang et al., 2020) reported a β-CD-AuNPs-functionalized COF as a magnetic sorbent for the SPE of sulfonamides, reaching limits of detention in the range of 0.8 – 1.6 μg $kg^{-1}$ and recoveries from 19% to 112%.

### 3.2.3. Other materials and particles based on cyclodextrins

Along with COFs, MOFs also constitute an extensive family of porous materials, in which a great variety of metal ions or clusters take part together with organic ligands. The discovery and development of MOFs occurred in the 1990s thanks to several pioneering groups led by Robson, Moore, Yaghi, Kitagawa, and Ferey (Q. Wang & Astruc, 2020). MOFs are nanoporous materials, also referred to as porous coordination polymers, that show 1D, 2D, or 3D structures. Among the possible metallic species used in their structures, alkaline and alkaline earth metals, lanthanides, and actinides can be mentioned. Similarly, a variety of organic linkers such as carboxylate, phosphonate, sulfonate, pyridyl, imidazole, and azolate functional groups have been used to incorporate other molecules of interest into their structure. The number of coordination compounds that can be considered as MOFs is enormous and some authors indicated that it is around one million (J. Liu & Wöll, 2017). Therefore, diversity turns out to be a label for MOFs. They can reach surface areas in the 1000 to 10000 $m^2 g^{-1}$ range, which are much higher than those reached by other porous materials.

To date, CDs have also been included in MOFs' structures based on alkaline or alkaline earth metals as inorganic counterparts through different synthetic strategies such as hydrothermal or solvothermal methods, vapor diffusion, or microwave irradiation (Rajkumar, Kukkar, Kim, Sohn, & Deep, 2019). Regardless of the specific method used for their obtaining, the CD incorporation takes place during the MOF formation without additional functionalization treatments, which constitutes a great benefit.

The use of nanoparticles as supporting objects is also a versatile strategy to enhance the active surface where CDs must be located. Moreover, in order to favor and ease the separation, the designed composites can incorporate magnetic nanoparticles, usually $Fe_3O_4$.

There exist different well-established protocols for the isolation and stabilization of magnetite nanoparticles with fine control of the size and shape, which is usually spherical (J. C. Yang, Lee, Hong, & Park, 2020). However, these particles cannot be used for many applications without a protective layer due to their chemical reactivity. Then, it is necessary to cover the $Fe_3O_4$ nanoparticles with more stable and less reactive materials such as silica or polymers. The resulting core-shell nanoparticles (Ghosh Chaudhuri & Paria, 2012) preserve the magnetic properties and can incorporate foreign molecules such as CDs in the external shell (Figure 7). The silica shell can be massive or porous depending on the preparative conditions. In the case of polymeric shells, the previously

mentioned preparative strategies for polymeric supports can be adapted to the anchoring of CDs, including the use of MIPs.

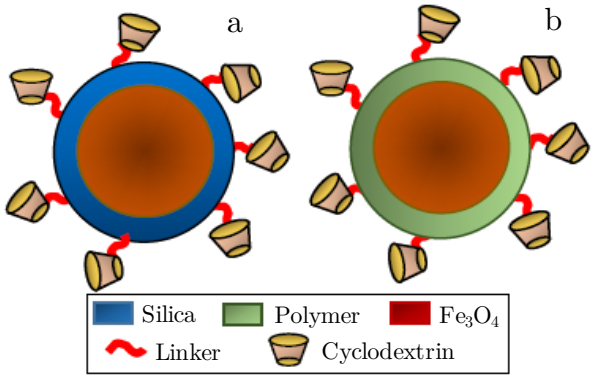



**Figure 7. Schematic representation of the structures for CD-modified core-shell nanoparticles: (a) silica-coated magnetic nanoparticles, and (b) polymer-coated magnetic nanoparticles.**

Nanomaterials and nanoparticles present some advantages in comparison with supports based on micro-sized materials. In general, they present a superior extraction capability and selectivity due to a higher surface-to-volume ratio and easily modifiable surface functionality. Among them, magnetic nanoparticles ($Fe_3O_4$, $Fe_2O_3$, etc.), metallic nanoparticles ($Al_2O_3$, MnO, etc.), or carbonaceous nanomaterials (graphene, carbon nanoparticles, etc.) are the focus of a great number of the studies (Azzouz et al., 2018; Khajeh, Laurent, & Dastafkan, 2013). Based on their dimensionality, nanomaterials are classified as 0D (nanoparticles), 1D (nanotubes), and 2D (nanowalls, nanodiscs, etc.). Numerous nanomaterials have been combined with cyclodextrins to obtain composites with improved sorbent properties for analytical uses due to the benefits there can be obtained. For example, an enhanced extraction capability and selectivity are attributable to the heterogeneity of the composites and so to the different interactions carried out. Moreover, the influence of CDs may be essential when the analytes molecule size plays an important role.

One group to be mentioned is those nanomaterials or nanoparticles combining magnetic properties with the advantages of host-guest chemistry. In this sense, the liquid-solid separation is eased due to the magnetism of the material used, for example in the use of a magnetic SPE procedure. Ghosh et al. described magnetic $Fe_3O_4$ silica-coated nanoparticles grafted with carboxymethyl-β-cyclodextrin via carbodiimide activation (Ghosh, Badruddoza, Uddin, & Hidajat, 2011). Taking profit of the enantiomeric properties of CDs, these nanoparticles were used to adsorb chiral aromatic aminoacid enantiomers as a proof of concept of the adsorption advances they can provide. A similar procedure based on cyclodextrin

functionalization of magnetic nanoparticles with the participation of silica was described with remediation purposes for the removal of carcinogenic azo dyes from water (Arslan, Sayin, & Yilmaz, 2013) with favorable results regarding the sorption ability reached, which was reported around 98 – 99%. A different remediation achievement was carried out by Badruddoza et al. for the selective removal of $Pb^{2+}$, $Cd^{2+}$, and $Ni^{2+}$ from water by substituting the silica part with polymer participation in the synthesis procedure (Badruddoza, Shawon, Tay, Hidajat, & Uddin, 2013). Specifically, epichlorohydrin-cross-linked carboxymethyl-β-CD was used to coat magnetic iron nanoparticles, and the adsorption process was found to be dependent on pH, ionic strength, and temperature. From 2014 onwards, the studies describing the analytical applications of these magnetic-CD approaches are more frequent each time. In this way, there can be mentioned the magnetic $Fe_3O_4$ nanoparticles coated with silica as support of cyclodextrin molecules for the SPE of 5-hydroxy-3-indole acid from urine (Gaber Ahmed, Badía Laíño, García Calzón, & Díaz García, 2014). Carboxymethyl-hydroxypropyl-β-CD and carboxymethyl-β-CD were also used to modify magnetite nanoparticles with the help of polymer modification (Gong, Ping, Wang, & Zhu, 2014) and amino groups (Jiabin Zhang, Pan, Gan, Cao, & Wu, 2014) for the adsorption of rutin from plants and PCBs from the soil. Karimnezhaz et al. reported the use of magnetic chitosan nanoparticles grafted with β-CD for the dispersive SPE of $Zn^{2+}$ and $Co^{2+}$ from water followed by quantification by adsorption spectrometry (Karimnezhad & Moghimi, 2014; Moghimi, 2014). In both cases, the loading capacity of the sorbent demonstrated to be quite good. Over time, the presence of silica as a facilitator for the anchoring of CD can be seen in the work of Wang et al. (Manlin Wang et al., 2015), who described a new approach of $Fe_3O_4$@β-CD superparamagnetic composites for the host-guest adsorption of PCBs, and Chen et al. (J. Chen et al., 2018), who functionalized a graphene oxide network containing linked CD with magnetic nanoparticles modified with silica to obtain $Fe_3O_4$@$SiO_2$@GO/β-CD for the dispersive SPE of plant growth regulators through the formation of inclusion complexes with CD in plant residues. In this case, the merits of superparamagnetism were combined with antioxidation, high surface area, and high supramolecular recognition in an environmentally friendly methodology. The work has been recently modified with the same end (Cao et al., 2019). Other analytes with similar characteristics have been reported recently to extract methyl parathion and fenthion from lettuce samples (X. Wu et al., 2021).

The variety of works is so large that a wide selection of nanoparticles and structures based on nanomaterials for the adsorption of different types of analytes has been reported. Zhang et al. carried out the separation of erythromycin-A from

wastewater with imprinted magnetic nanoparticles containing β-CD (Y. Zhang et al., 2016), and Liu et al. reported the advantages of using an ionic liquid-coated CD-functionalized magnetic core dendrimer for the dispersive SPME of pyrethroids in juice samples (F. Liu et al., 2018). The importance of this achievement is found in the fact that pyrethroid residues are an important source of pollution in agriculture and a potential public health threat. Indeed, it has been proven that pyrethroid intoxication can alter the nerves' function. Additionally, the combination of the advantage of polymer-natured parts and silica for the obtaining of a namely magnetic cyclodextrin polymer ($Fe_3O_4$@$SiO_2$@P-CDP) was applied for the magnetic extraction of microcystins from environmental water samples, with limits of detection in the trace level and good extraction efficiencies (Wenmin Zhang et al., 2017). Microcystins have raised a concern, making their detection at trace level in drinking water necessary, because they are a family of monocyclic heptapeptide toxins produced by cyanobacteria. In this sense, they can produce acute poisoning and promote cancer through chronic exposure. Recently, Yazdanpanah et al. have reported the use of cyclodextrin onto iron oxide/silica core-shell nanoparticles obtained through a polydomamine-assisted synthesis procedure for the magnetic SPE of aromatic molecules from environmental samples (Yazdanpanah & Nojavan, 2019), and Moradi et al. have studied the simultaneous magnetic SPE of malachite green and crystal violet from aqueous samples with a poly(β-CD-ester) functionalized silica-coated magnetic nanoparticles (Moradi Shahrebabak, Saber-Tehrani, Faraji, Shabanian, & Aberoomand-Azar, 2020), reporting recoveries in the range 92 – 100%. Additionally, a MOF with functionalized β-CD, which is prepared by creating metal-organic framework layers on the surface of a $Fe_3O_4$-graphene oxide nanocomposite and binding them with β-CD molecules, was applied for the efficient extraction and determination of fungicides prochloraz and triazole in vegetable samples (Guangyang Liu et al., 2019). In this case, the functionality granted by the MOF is mainly related to the magnetic activity of the solid phase for an easier separation, but it is not due to its structural properties as porous support.

Making the difference from the rest of the reported works based on MOFs, the one presented by Wang et al. described an efficient γ-CD-MOF-$K^+$ for the adsorption of formaldehyde molecules from the air with thigh selectivity, speed, and capacity (L. Wang et al., 2018). The excellent properties shown by the material are due both to its porous structure and to a synergistic effect of hydrogen bonding and host-guest interactions. Since formaldehyde is a major indoor pollutant due to its use in adhesives for construction and furnishing and plays, therefore, a very important role in human health, the environmental

interest of this research seems notable. The same authors described recently a derived material for microwave adsorption applications (S. X. Zhang et al., 2021). In addition, two MOFs based on β-CD-MOF-$K^+$ and β-CD-MOF-$Cs^+$ were obtained and applied in the adsorption research of myricetin as a first approach to a future drug delivery system (Jiang et al., 2021).

Finally, other nanomaterials in the literature with carbon intervention in their structures as a remarkable point have been informed (Maciel et al., 2020). Song et al. described the application of a hollow fiber based on carbon nanotubes modified with β-CD for the efficient and environmentally friendly SPME of plant hormones to overcome the lack of selectivity of bare hollow fibers (X. Y. Song, Ha, Chen, & Shi, 2014). Moreover, a novel $U^{6+}$-imprinted graphitic carbon nitride composite for the selective and efficient removal of $U^{6+}$ from seawater was reported to break with the side effects of the future long-term development of nuclear energy in the world (Hao et al., 2018). The adsorption capacity was calculated to be 860 mg $g^{-1}$, and the selectivity factors were high enough to affirm the high selectivity of the material for the purpose. Finally, Tezerji et al. have newly described a facile one-pot green synthesis of a porous graphene nanohybrid decorated with cyclodextrin units as a highly efficient sorbent for extraction of aflatoxins from maize and animal feeds (Tezerji et al., 2020) through SPME. In this case, the large specific surface area of the porous graphene and high recognition and enrichment capability of CDs helped the nanohybrid to be an effective sorbent for integrated sample clean-up, extraction, and pre-concentration of aflatoxins, which are a current issue as secondary metabolites in a wide variety of agricultural products and plantations thus being a risk for both human and animal health.

## 3.3. Overview and motivation

Overall, the determining aspects for choosing one or another type of support, the type of cyclodextrin used, the way CDs are found in the support (this is, simply included as hybrid materials or chemically attached to it), as well as their high accessibility, are very varied. Every material, nanomaterial, or nanoparticle containing CD molecules from those described has shown great potential in analytical chemistry and remediation actions in the last decades.

First, the advantageous properties offered by the use of CDs deserve special attention. It has been observed that materials containing cyclodextrin units are very versatile platforms in the same way as CDs are. As long as they are accessible to the compounds to isolate, these molecules usually possess the leading role in the adsorption processes taking place.

Besides, the modification of sample preparation techniques has been in the spotlight for improving analytical features while implementing the principles of green chemistry in recent years. Thus, significant progress has been made to find more sustainable sorbents. In this sense, CDs especially contribute to this common goal. Given that cyclodextrins are starch derivatives, they can be easily obtained through green enzyme-directed procedures (Szente & Szemán, 2013). Thus, their sugar-based structure makes them ideal alternatives to other possible structures in sorbents, providing them with improved selectivity, due to their low toxicity and ease of degradation.

Important is to mention that cyclodextrins are also commonly recognized for their enantiomeric properties, being one of their most valuable applications resolving enantiomer compounds. Although it is difficult to establish a general mechanism of chiral recognition, as enantioselective retention mechanisms depend on the reaction conditions and the chemical microenvironment of the hydrophobic cavity, it is proven that no inclusion complexes analyte-CD govern enantiomeric separations. Instead, the enantio-recognition is due to the combination of different interactions such as H-bonding and dipolar and steric interactions between the hydroxyl groups of CDs and the target compounds (Armstrong et al., 1997).

However, the versatility of CDs in analytical chemistry is rather related to their unique structures and their capability of retaining their analyte-recognizing and entrapping properties under a relatively broad range of experimental conditions. In fact, the practical utilization of CDs in the mentioned discipline is almost exclusively related to their host-guest type molecular recognition processes, also known as supramolecular complex formation. In this way, some studies have shown the importance of the size of the CD cavity to encapsulate analytes (Szente & Szemán, 2013; Tijunelyte et al., 2017) since it can influence not only the capability of CDs to host the analyte molecules, depending on the size of the last ones, but also in the porosity of the supporting material. Thus, it may be that the analyte does not comfortably fit in the CD if it is too large, but it may also be that the CD cavity is too big for our target compound, being the directing apolar interactions of the host-guest complex formed more fragile. Therefore, the retention would not occur so strongly. Nevertheless, it is remarkable that the most commonly used CD is β-CD, probably due to its intermediate size, as well as its price, which makes it the most flexible of them.

Secondly, regarding the structuring material chosen, no significant differences are observed when using different supports for certain analytes or samples. Instead, a difference between supporting materials can be, for example, the price.

As an example, more affordable solid phases can be chosen for remediation actions since what really matters, in this case, is not specifically the structure or the functionalities of the material (e.g., high porosity, CD anchoring to the support), but mainly that it is capable of performing its function efficiently. Moreover, it has been observed that the chemical anchoring of the CD to the support is better for certain types of samples, but not decisive. Thus, in the case of air samples, the advantages between anchoring the CD (which implies besides an increase in the price and in the time invested) and not anchoring it are hard to find since the CD losses by lixiviation are not as clear as with aqueous samples.

Regarding the porosity of the structure, the type of solid phase we are looking for should be selected based on the application we want to give it.

In the case of porous silica, different strategies can be proposed to favor the accessibility of the analytes to the active centers by modulating both the size of the pores and their shape and organization. The cage-like pores of xerogels have to guarantee a pore window with enough size for the passage of pollutants. This aspect can be controlled with the final silica preparative method. For example, in ordered mesoporous materials, it is advisable to avoid mesopores blocking with the anchored CD units. Materials with larger pores, such as SBA-15 silica, may be more accessible than typical MCM-41 materials (Z. Xu et al., 2019). On the other hand, interconnected mesoporous systems (either ordered or disordered) such as MCM-48 may have advantages over 1D and non-interconnected pores (C.T. Kresge et al., 1992; Charles T. Kresge & Roth, 2013; Wan & Zhao, 2007). Hierarchical porous materials can provide certain accessibility advantages over unimodal pore systems (X. Y. Yang et al., 2017). An example of hierarchical systems is bimodal silica of UVM-7 type (El Haskouri et al., 2002, 2008) formed by aggregation of mesoporous nanoparticles. They combine the typical porosity of MCM-41 with excellent capabilities for concentrating several analytes. For these reasons, bimodal UVM-7-type silica may represent a field of great interest in combination with CD for its application in analysis and can constitute a new and attention-grabbing research field.

In the case of polymeric supports, larger CDs can also lead to a certain pore clogging, which prevents the accessibility of the analytes of interest to CDs, and the hydration of the material may be necessary. It is the case of some types of nanosponges reported, being their effectivity enhanced when they are applied with aqueous samples. Moreover, the use of rigid linkers for the nanosponges (Alsbaiee et al., 2016) should be valued since they allow greater accessibility of the CDs in the support with an adaptable porous system to different types of samples.

Thus, the selection of the base porous system is important. In general, there is greater accessibility in open or larger pore systems of interconnected ones. Additionally, those with cubic-shaped porosity tend to give better adsorption results than those with a hexagonal shape. There still exist novel porous systems showing diverse virtues in terms of structure and porosity, but whose combination with CD and application for analytical purposes remains relatively unexplored.

Finally, although a variety of promising alternatives to conventional commercial materials usually used have been described, additional efforts should be aimed at translating the achievements reported into practical applications, contributing in this sense further to the benefits of nanotechnology. For this reason, studies on the development of new CD-based reversible adsorbents for remediation and quantification in analytical methods may focus on the following areas: (i) greater sophistication of CD-based materials with more flexible and efficient synthetic methods of supporting materials, nanomaterials, and nanoparticles as carriers for CDs, (ii) improved distribution of CD units along the solid supports used to increase the accessibility to the analytes to be captured, (iii) development of faster, easier, more affordable, greener, and smarter separation techniques with better abilities for the isolation of the compounds of interest thanks to progress in the structure of the sorbents used, and (iv) use of more efficient analytical methodologies with better analytical performance, including higher extraction recoveries, selectivity, and sensitivity, especially in the case of methods to treat complex samples with analytes at trace level. Furthermore, while major progress has been accomplished in the creation of new opportunities in the field, the demonstration of the possibilities of the sorbents reported in their use on an industrial scale with a promising capacity of reusability remains still pending. Therefore, further research on developing and selecting the most promising types of CD-based materials is still necessary.

In this Doctoral Thesis, the study of new CD-based solid supports for their application in analytical chemistry is presented. Not only the nature of the sorbent or the size of the CD used with this regard but also the type of samples explored are the central points of the research. Based on these individualities and their possible interactions, different conclusions are extracted subsequently, constituting all of them a contribution to the field of study.

# OBJECTIVES AND WORKING PLAN

The main objective of this Doctoral Thesis is to design innovative solid phases based on cyclodextrins. Later, their potential application as sorbents for the retention and extraction of pollutants and other high-concern compounds is evaluated as a part of analytical methods for their determination in a variety of samples. In this sense, combining the promising features of cyclodextrins together with the advantages offered by the different supporting porous structures used in order to develop sorbents for the efficient capture of organic hazardous compounds represents a bright perspective.

As stated in the Introduction, the growing presence of contaminants all around us in our daily life makes it necessary to find new materials that allow an efficient pre-concentration step for a later reliable determination. Thus, this work addresses this important challenge in analytical chemistry through the use of cyclodextrins. The usage of these molecules for several applications, including the cosmetics, food, and pharmaceutics industries has been widely studied to date. As mentioned, they have also been used in separation sciences, mainly in chromatographic techniques, as a part of stationary and mobile phases. However, their manifold uses as sorbents for sample treatment remains not explored at all. Cyclodextrins can contribute to the matrix cleaning and concentration of analytes, thus increasing the sensitivity and the selectivity of methods based on the physicochemical properties of both the cyclodextrin chosen together with those of the target analytes.

Hence, the objectives of the thesis can be divided as follows:

**Objective 1**. To synthesize porous cyclodextrin-silica xerogels with suitable porosity for the retention of different pollutants in several types of samples. The porosity of the solid phases is expected to ease the accessibility of analytes to cyclodextrins to form selective host-guest complexes, which may improve the functionalities presented by the materials designed.

**1.1.** To establish the best strategy to carry out the synthesis process.

**1.2.** To characterize and define the main features of the cyclodextrin-silica xerogels prepared, especially those affecting their sorptive capabilities.

**1.3.** To develop analytical methods for the determination of polycyclic aromatic hydrocarbons, phenolic compounds, polychlorinated biphenyls, and synthetic cannabinoids using the cyclodextrin-silica xerogels proposed. In this sense, not only the environmental but also the biological field are explored.

**1.4.** To understand the role of the cyclodextrin-silica xerogels structure together in presence of cyclodextrin molecules for the retention of the

analytes selected through size-exclusion mechanisms and the possible interaction of the compounds of interest with the silica support.

**Objective 2**. To synthesize mesoporous type UVM-7 silica materials for the retention of pollutants in different samples. The bimodal porosity of the solid phases in comparison with the previous xerogels is expected to influence the sorption mechanism and the ways used by the analytes to reach the cyclodextrins to form host-guest complexes with enhanced selectivity.

**2.1.** To establish the best strategy to carry out the synthesis process.

**2.2.** To characterize and define the main features of the type UVM-7 mesoporous silica materials containing cyclodextrins prepared, especially those affecting their sorptive capabilities.

**2.3.** To develop analytical methods for the determination of antibiotics and endocrine-disrupting chemicals in foodstuff using UVM-7-based sorbents.

**2.4.** To understand the role of the type UVM-7 materials structure together with the presence of cyclodextrins in the retention of the target analytes carried out through size-exclusion mechanisms and the possible interaction of the compounds under study with the mesoporous silica support.

**Objective 3**. To synthesize polymeric cyclodextrin-based materials for the retention of pollutants in different samples. The presence of cyclodextrins onto the surface of the polymeric support, which eases their accessibility to analytes, is expected to influence their retention properties in comparison with the previous silica-based materials.

**3.1.** To establish the best strategy to carry out the synthesis process.

**3.2.** To characterize and define the main features of the polymeric cyclodextrin-based solid phases prepared, especially those affecting their sorptive capabilities.

**3.3.** To develop analytical methods for the determination of phenolic compounds and antibiotics in environmental samples using cyclodextrin-based polymeric materials.

**3.4.** To understand the role of the polymeric structure of the sorbents obtained together with the presence of cyclodextrins in the retention of the analytes under study through size-exclusion mechanisms and the possible interaction of analytes with the polymeric support.

To achieve these objectives, a general working plan was designed, consisting it of three differentiated parts that coincide with the main objectives established before. In each case, the synthesis process and the analytical application of the sorbents are described and assessed.

### Synthesis and characterization of the sorbents

First, the materials described were synthesized. The synthesis process included in each case either the bare supports or those already modified with cyclodextrins for their comparison. In the case of silica materials, the versatility of sol-gel processes employed during the concurrent synthesis steps allows the introduction of modifications in the solid structure. Oppositely, the two-pot synthesis carried out for the polymeric materials allows their later surface modification through click-chemistry reactions.

Several aspects were studied in each case:

- The best choice to conduct the synthesis process, this is, in a one-pot or two-pot procedure according to the final result desired.
- The incorporation of cyclodextrins either by inclusion or by covalent attachment to the supporting structure. For the second purpose, the cyclodextrins must be first functionalized with appropriate organic groups.
- The amount of cyclodextrins with respect to the quantity of solid support that is present in the structure.
- The type of cyclodextrin used was also considered. Taking into account the physicochemical characteristics of the target analytes, a choice between the different native cyclodextrins (α-, β- or γ-), as well as other already modified commercial cyclodextrins based on them was studied.

Once the solids were synthesized, their structure was properly characterized to assess the influence of the modifications introduced during the synthesis and understand thus their final structure and properties, evaluating, therefore, their potential abilities for the retention of the analytes. This characterization was carried out using several techniques such as microscopy, X-ray spectroscopy, porosimetry and $N_2$ adsorption-desorption, nuclear magnetic resonance, Raman spectroscopy, elemental analysis, or thermogravimetric analysis, among others.

### Application to the sorption of high-concern substances

Then, the solid phases designed and synthesized were evaluated as sorbents for the retention of several target compounds selected, and the best material was selected in each case to develop a complete analytical method. As mentioned,

their application to extraction methods was taken into account for a variety of fields such as the environmental (air and water), food, and biological ones.

For this purpose, different aspects were considered:

- For environmental air sampling methods, the optimization of the experimental procedure was carried out through contamination of the samplers containing the material designed, followed by air pumping through the solid phase to simulate a real sampling process.
- In the case of environmental water samples, synthetic samples were prepared for the optimization steps. In the beginning, spiked ultrapure water was used, while spiked real water matrices were considered once the optimum retention and extraction conditions are evaluated.
- Regarding food and biological samples, previous sample treatment steps were needed to help overcome the difficulties presented by the complex matrices under study. These steps were generally based on previous publications and reoptimized if necessary. Similarly as before, spiked ultrapure water was used in the first steps to optimize the conditions, while real matrices were used later.

In every case, the same general protocol was applied for method optimization and subsequent assessment:

a) Comparison among the developed materials (usually the bare support with respect to the material containing cyclodextrin or the materials containing different types of cyclodextrins between them). This comparison pretends to understand the interaction between the solid phase and the analytes, thus explaining the possible differences observed. The material that works best is selected in each case.

b) Optimization of the main analytical parameters of the protocol such as sorbent type and amount, solvent nature, and volume necessary for the desorption or elution processes, as well as other sampling and loading conditions. After the optimization of the methods, the main analytical conditions are described individually.

c) Description of the analytical figures of merit of the whole method developed regarding sensitivity, linearity, repeatability, extraction efficiency, recovery, and matrix effects.

d) Comparison of the main analytical parameters of the method with other similar methods reported in the literature. In this sense, the main

advantages and drawbacks of the analytical protocol designed are thus assessed.

e) Application of the developed method to the analysis of real samples, usually in comparison to a reference method, to confirm its feasibility. In the cases where no target compounds are detected in none of the analyzed samples, the analysis of synthetic samples prepared by spiking real matrices is considered.

Through this working plan, it is possible to respond to the objectives defined initially. The development of the research and the results obtained are described hereafter.

# METHODS AND RESULTS

# SECTION A
# Application of porous cyclodextrin-silica xerogels

# 1

# Synthesis and characterization of cyclodextrin-silica xerogels

## 1.1. Introduction

A large number of processes of interest take place in the interface between a liquid medium and a porous solid. To a large extent, the result obtained will depend on the characteristics of the porous solid chosen.

From an analytical point of view, a solid support should ideally include a convenient surface area, interconnected pores, the ability to be doped with other reagents, mechanical and chemical stability, and feasibility in terms of cost and producing time. As mentioned, developing innovative materials with analytical applications is a challenge that depends, therefore, on the design of preparative strategies capable of controlling within certain limits the surface properties of the solids, in addition to their composition and other preferences.

The use of silica as support has spread due to its thermal stability and the great variety of possible structures available. The precise control of the hydrolysis and condensation reactions taking place during the synthesis due to its low reactivity makes it able to incorporate different functional groups that may be of interest in monitoring, remediation, and separation techniques (Walcarius & Collinson, 2009). In this context, the versatility of sol-gel chemistry allows generating a great variety of silica-based materials with controlled structure, composition, morphology, and porosity. Their hosting and recognition properties, as well as their open structures that may contain accessible active sites, make them attractive for analytical purposes (E. C. Morais et al., 2012) and as drug delivery systems (L. F. W. Brum, dos Santos, Zimnoch Santos, & Brandelli, 2022).

Sol-gel processing of silica is, chemically, a simple process. It can be described as the growth of a 3D polymeric silica network, which may or may not be modified by insertion of other reagents (da Fonseca et al., 2021). Specifically, it is based on the development of primary particles produced by a sol phase, which later produce a macromolecular network of covalent siloxane bonds called gel (Walcarius & Collinson, 2009) in a process called sol-gel transition. The reaction rate is commonly influenced by the strength and concentration of acids and bases, while other parameters such as temperature and nature of solvents may present a secondary role. Since an evolution from mesoscale to macroscale pores occurs as the pH of the synthesis increases, it is common to use the acid-catalyzed route to obtain microporous xerogels prepared at a pH that is near to the silica isoelectric point (L. F. W. Brum et al., 2022).

Therefore, the use of silica supports for incorporating cyclodextrin units into the developed solid phases has become widespread due to the advantageous properties they contribute with.

Within the use of silica, there can be mentioned, on the one hand, those examples that use minimally modified commercial silicas, including the incorporation of vinyl groups or polymerizable derivatives of CDs onto a silica gel (Akiyama et al., 2001; Phan, Bacquet, Laureyns, & Morcellet, 1999). Oppositely, other synthetic routes use silicon alkoxide precursors such as TEOS to produce the silica network (Walcarius & Collinson, 2009) can be mentioned, highlighting among them the described xerogels. In this sense, the importance of the sol-gel process when incorporating CDs comes from the different properties obtained by the synthesis of mixed organic-siliceous solids, which are unique (da Fonseca et al., 2021). Thus, these materials are able to combine the host-guest chemical properties of CDs with the good properties shown by a silica matrix, exhibiting combined chemical and physical properties that are different from those they own individually. Their hydrophobic/hydrophilic balance and chemical reactivity have given this family of materials a priority place in several areas of research.

As stated above, CDs can be either included in a xerogel structure during the synthesis without actively participating in the hydrolysis/condensation processes taking place or bonded through a previous modification with different organic precursors. This bonding may improve the contribution of CDs in different analytical contexts, as well as the stability of the properties of interest shown by the material obtained (Faraji, 2005).

In this chapter, the design and characterization of different materials containing cyclodextrin obtained from a sol-gel-based synthesis are described and discussed. Different types of CDs, including the natives α-, β-, and γ-CD, as well

as other commercially available CD derivatives, have been used to change the possible benefits offered by the solid phases synthesized. Besides, the different ways in which CDs are found in the solid phases, this is, either included forming hybrids or chemically bonded, have also been tested. Finally, the advantages and disadvantages of the materials are exposed and their possibilities within the field of analytical chemistry are mentioned.

## 1.2. Experimental

### 1.2.1. Reagents, materials, and instrumentation

The cyclodextrins used in the synthesis processes described were α-CD, β-CD, γ-CD, methyl-β-CD (β-MCD), and 2-hydroxypropyl-β-CD (β-HPCD), all of them of reagent grade. They were acquired from CycloLab (Budapest, Hungary). Other chemicals used were 3-(triethoxysilyl)propylisocianate and dry pyridine ≥ 99.5% from Sigma-Aldrich (St. Louis, United States), TEOS from Honeywell Fluka (Fisher Scientific, Buchs, Switzerland), and NaOH (s) and HCl (aq.) 37% from Panreac AppliChem (Barcelona, Spain). Ultrapure water from an Adrona (Riga, Latvia) purification system was employed in every case.

When applicable, the previous drying of CDs was carried out using a LyoAlfa 10/15 Azbil Telstar laboratory freeze drier (Barcelona, Spain). After the sugar modification, the solvent removal was conducted with a rotary evaporator from Büchi (Flawil, Switzerland).

The characterization of the materials was executed using a variety of techniques. First, the external morphology of the solid phases was checked through transmission electron microscopy (TEM) using a JEOL JEM1010 microscope (Tokyo, Japan), scanning electron microscopy (SEM) using a Hitachi S-4800 microscope (Tokyo, Japan), and particle size analysis with a Panalytical Master-Sizer 2000 instrument (Malvern, United Kingdom). Then, the porosity was measured through gas adsorption-desorption experiments using a Micromeritics ASAP-2020 automated analyzer (Norcross, United States). In this case, $N_2$ isotherms were acquired at 77 K, whereas $CO_2$ isotherms at 273 K. All the samples were previously degasified at 120°C for 15 hours at a $10^{-6}$ Torr pressure and dried to constant weight under 0% relative humidity. The structure of the solid phases obtained was assessed with 40 kHz magic angle spinning nuclear magnetic resonance (MAS NMR) at 79.5 MHz ($^{29}Si$) and 128.3 MHz ($^{13}C$), respectively, with a Varian Unity 300 spectrometer from Agilent Technologies (California, United States). Besides, X-ray diffraction (XRD) experiments were carried out using a Bruker D8 Advance diffractometer (Massachusetts, United States), which operated at 40 kV and 40 mA in steps of 0.02° (2Θ) over the

angular range 1-10° for 25 s per step using Cu $K_\alpha$ radiation. For more details on the materials, thermogravimetric analysis (TGA) was done with a Setaram Setsys 16-18 Instrument (Caluire-et-Cuire, France) under a dynamic $O_2$ atmosphere at 10°C $min^{-1}$, and elemental CNH analysis was carried out with an elemental analyzer 1100 from CE Instruments (Hindley Green, United Kingdom).

### 1.2.2. Synthesis of included CD-silica hybrid xerogels

The role played by CDs on the silica structure seems to be highly dependent on the sugar concentration in the reaction media (Mauri-Aucejo et al., 2012). Thus, while at low CD concentrations a good molecular dispersion can be assumed in both the aqueous solution and the subsequent silica gel formed, certainly ordered aggregations of CDs might be expected when the CD concentration increases. Therefore, the pore system is generated only after thermal evolution of the embedded CD aggregates (Polarz, Smarsly, Bronstein, & Antonietti, 2001).

Since the goal of the synthetic process applied here was to obtain porous hybrid CD-silica composites presenting a significant microporosity without evolution of the trapped sugars, preserving in this way the active sites able to interact with the analytes of interest, CD concentrations lower than 18 wt% were used in every case to this end. Under these conditions, the porosity cannot be ascribed to any effect of the organic counterpart and can consequently only be due to the intrinsic nature of the silica gel. Besides, the presence of trapped CD units among the highly branched silica structure, hindering inorganic shrinkage, must be viewed as beneficial to favor porosity preservation.

In this case, different hybrid materials were obtained using α-, β-, and γ-CD, as well as β-MCD and β-HPCD. The synthesis process is as follows. Under stirring, 60 mL of an aqueous solution containing 0.9 mmol of each of the CDs tested and 48 mL of TEOS are mixed, previously adjusting the pH in the range 1.7 – 2.0 by adding small amounts of HCl. Under these conditions, hydrolysis and condensation of the silicon alkoxides are favored, and an evolution from an, in appearance, immiscible mixture to a homogeneous solution occurs after completing the reaction. Then, the ethanol generated through the hydrolysis processes is evaporated by letting the gel formed age and dry at 50°C. Finally, it is crushed and sieved to adjust the particle size in the 250 – 600 µm range in order to avoid excessive particle packing.

### 1.2.3. Synthesis of bonded CD-silica hybrid xerogels

The decision to carry out the chemical binding of CDs to the silica network was made taking into account the possible advantages that this type of materials

could possess from an analytical point of view. To allow the chemical bonding of CDs to silica, it is first necessary to modify them. For this reason, the first preparative step corresponds to the obtaining of silane derivatives of CDs. Hence, the reagent 3-(triethoxysilyl)propylisocianate was used to carry out this prior modification, reacting with the outer hydroxyl groups of CDs and provoking each molecule to be modified with functional groups containing terminal silanes. Subsequently, these species were used as precursors through their combination with TEOS in an aqueous acidic solution, parallel to how it was done in the case of the included xerogels, to generate the final silica derivatives containing CD molecules. The hydrolysis and condensation processes of the terminal silane groups of the modified CDs with the silicon alkoxides coming from TEOS allowed obtaining the desired solid phase that contains CD covalently anchored to a silica network.

**Table A1. Data for the synthesis of the different bonded CD-silica xerogels.**

| Type of CD | Material | Modified CD (mmol) | Water (mL) | TEOS (mL) | Si/CD molar ratio |
|---|---|---|---|---|---|
| β-CD | B1 | 0.9 | 60 | 11.4 | 57 |
| | B2 | 0.9 | | 5.7 | 28 |
| | B3 | 1.8 | | 5.7 | 14 |
| γ-CD | G1 | 0.9 | | 11.4 | 57 |
| | G2 | 0.9 | | 5.7 | 28 |
| | G3 | 1.8 | | 5.7 | 14 |

In this case, the bonded xerogels described were obtained using β- or γ-CD. The preparative protocol to functionalize both CDs was adapted from that previously reported to prepare a β-CD silane derivative (Mahmud & Wilson, 2016). The synthesis process was thus composed of two steps: (i) chemical modification of CDs, and (ii) synthesis of the final bonded CD-silica hybrid xerogel. In short, 9.4 mmol of each prior dried CD (10.7 g for β-CD and 12.2 g for γ-CD) are mixed with 9.3 mL of 3-(triethoxysilyl)propylisocianate in 130 mL of dry pyridine. The solution is stirred at 70°C under nitrogen gas for two days. Then, the solvent is removed under reduced pressure, obtaining the CD precursors in the form of a light yellow solid. After this, the synthesis of the two different solid phases is carried out using 0.9 mmol of each modified cyclodextrin in a 60 mL acidic aqueous solution (pH in the 1.7 – 2.0 range), and adding 11.4 mL of

TEOS. The reduction in the amount of TEOS with respect to included CD-silica materials was carried out with the aim of maintaining an acceptable amount of CD in the material, taking into account that the functionalized CD also contributes silanol groups to the silica network in this case. Thus, the reaction leads to the formation of a homogeneous gel, which is aged and dried at 50°C causing these processes the evaporation of the ethanol formed. Finally, the materials are crushed and sieved to adjust the particle size in the 250 – 600 µm range in order to avoid excessive particle packing.

It is important to emphasize that the phases obtained must be thoroughly washed with water before their use since the pH adjustment stage results in the formation of NaCl, which could clog its pores and reduce the adsorption capacity. To test that no NaCl is left, the ionic strength of the washing solution is measured and compared with the one of a reference solution.

Going one step further, a variation of the amounts of TEOS, as well as those of CDs, was tested to verify the benefits these materials may offer (Table A1).

## 1.3. Results and discussion

### 1.3.1. Characterization of included CD-silica hybrid xerogels

All solids obtained were analyzed by elemental CNH analysis and TGA. Then, surface area, and pore size and volume from $N_2$ adsorption-desorption isotherms were calculated. Additionally, the samples were also characterized by XRD, and $^{29}Si$ and $^{13}C$ MAS NMR. To study the morphology of the materials, TEM micrographs were acquired.

Taking into account the low aging temperature used, a significant proportion of water molecules and uncondensed silanol groups should be present in the solids synthesized. To evaluate their ratios, a CD-free silica xerogel, obtained following the same experimental procedure but without adding CD, was used as a reference. Thus, the TGA curve of the CD-free xerogel presented one first sharp weight loss between 40°C and 168°C (16.6 wt%) that may be attributed to the $H_2O$ molecules evolution, and a second weight loss expanding up to 950°C (6.1 wt%), which is undoubtedly associated with the formation of water through condensation of the remaining free silanol groups. Based on these data, a general formulation for the bare silica xerogel as $SiO_{1.5}(OH)_{0.5} \cdot 0.7H_2O$ can be proposed, being it consistent with the presence of a significant amount of uncondensed silanol sites according to MAS NMR data. It is more difficult to propose a general formulation for the included CD-silica hybrid xerogels from TGA since the CD degradation and the water evolution overlap. However, a general formula for the synthesized solids

can be proposed combining the TGA carried out and the elemental CNH analysis information. The formulation may be $(CD)_xSiO_{1.5}(OH)_{0.5} \cdot 0.7H_2O$ (x being 0.002, 0.0007, 0.0013, 0.0017, and 0.0016 for the solids containing α-CD, β-CD, β-HPCD, β-MCD, and γ-CD, respectively).

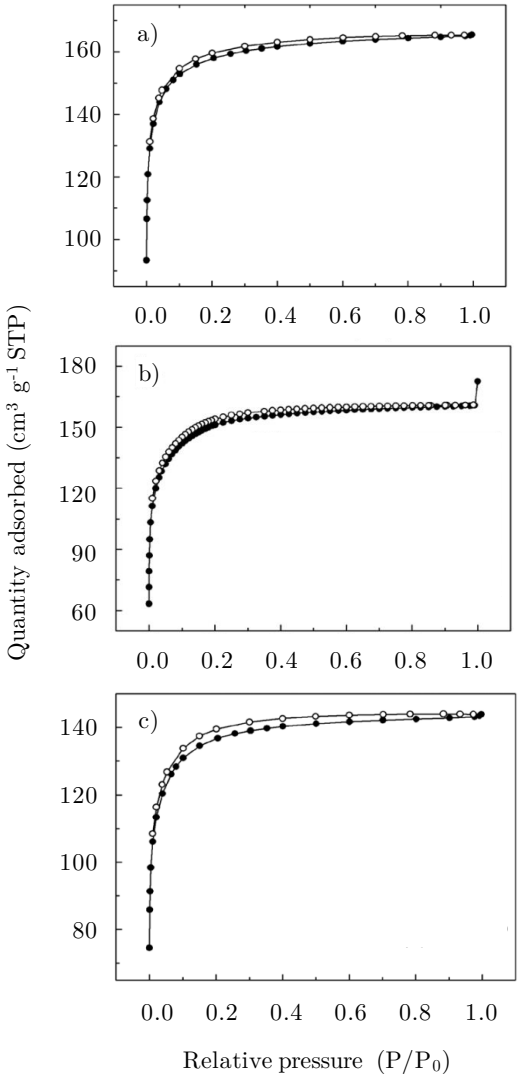



**Figure A1. Representative $N_2$ adsorption-desorption isotherms of included CD-silica hybrid xerogels: (a) bare silica xerogel, (b) $(\beta\text{-HPCD})_{0.0013}SiO_{1.5}(OH)_{0.5} \cdot 0.7H_2O$, and (c) $(\beta\text{-MCD})_{0.0017}SiO_{1.5}(OH)_{0.5} \cdot 0.7H_2O$.**

The $N_2$ adsorption-desorption isotherms acquired confirmed the existence of an expected porosity in the solid phases. Regardless of the CD used, the curves showed one well-defined step that is characteristic of Type I isotherms. This

should be due to the capillary condensation of nitrogen inside the pores (Figure A1). The isotherm type established, with adsorptions at low $P/P_0$ pressures, indicates that the dominant pores fall in the micropore range (< 2 nm) (McCusker et al., 2003). In general, high BET surface areas were achieved in the 350 – 550 $m^2\ g^{-1}$ range, with the exception being the solids containing α-CD and γ-CD (Table A2). These values must be considered as unusually high taking into account the wet nature of the samples. In all cases, a significant proportion of surface area must be attributed to the existing micropores, described according to Figure A1. The total pore volumes are also characteristic of microporous silica xerogels. Besides, medium pore sizes were calculated by DFT analysis. All DFT pore size distributions showed a main peak centered at ca. 1.2 nm and at least two clear shoulders at 1.5 and 1.9 nm, even in the case of the bare silica xerogel. Consequently, the porosity achieved must be attributed to the inorganic counterpart architecture. In general, the pore size values obtained are close or slightly lower than the molecular diameters of the CDs used (Table 2). This fact supports that CDs are trapped inside the silica network during the shrinkage step, and probably remain included inside cage-like interconnected micropores. In any case, they would remain accessible to the possible analytes interacting with them.

**Table A2. Physical and textural parameters of $(CD)_xSiO_{1.5}(OH)_{0.5} \cdot 0.7H_2O$.**

| Type of CD | Total area[a] ($m^2\ g^{-1}$) | Micropore area[b] ($m^2\ g^{-1}$) | Pore volume[c] ($cm^3\ g^{-1}$) | Pore size[d] (nm) |
|---|---|---|---|---|
| Bare silica | 530.5 | 422.9 | 0.26 | 1.19 – 1.86 |
| α-CD | 927.5 | 284.2 | 0.46 | 1.20 – 1.95 |
| β-CD | 352.2 | 307.5 | 0.16 | 1.18 – 1.50 |
| β-HPCD | 535.7 | 360.2 | 0.25 | 1.21 – 1.98 |
| β-MCD | 476.4 | 353.7 | 0.22 | 1.18 – 1.87 |
| γ-CD | 137.5 | 128.5 | 0.06 | 1.18 – 1.47 |

[a] Total area, micro- and mesopores were estimated through the BET model.
[b] Micropore area provided by the t-plot analysis.
[c] Single point adsorption pore volume.
[d] Pore sizes calculated by original DFT analysis.

Therefore, it can be concluded that the preserved pore sizes and pore volumes are larger enough for the typical target analytes hypothetically being trapped. Thus, TEM micrographs supported the existence of porosity. The images

captured confirm the existence of a disordered microporous system, probably with wormhole-like interconnected pore topology. The order absence is in accordance with XRD data measured, as expected. There, no diffraction peaks appeared at low nor high angle domains for all the solid phases under study.

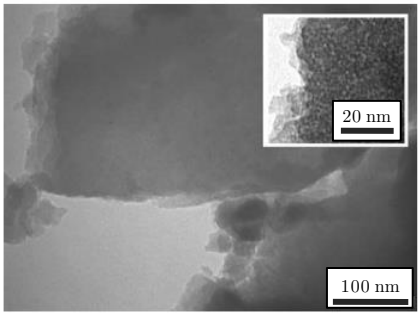



**Figure A2. Representative TEM image for $(\beta\text{-HPCD})_{0.0013}SiO_{1.5}(OH)_{0.5} \cdot 0.7H_2O$.**

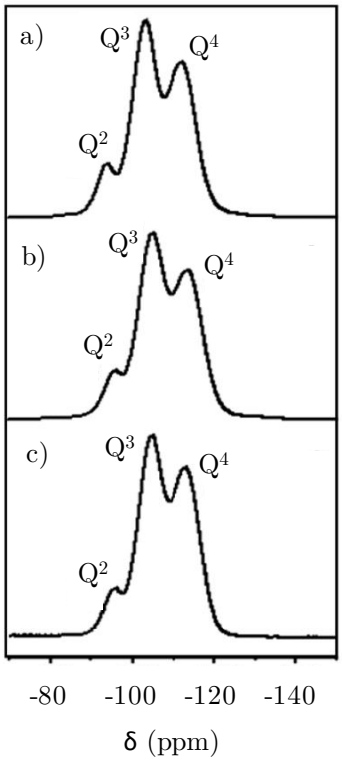



**Figure A3. Representative $^{29}Si$ MAS NMR spectra of included CD-silica hybrid xerogels: (a) bare silica xerogel, (b) $(\beta\text{-CD})_{0.0007}SiO_{1.5}(OH)_{0.5} \cdot 0.7H_2O$, and (c) $(\beta\text{-HPCD})_{0.0013}SiO_{1.5}(OH)_{0.5} \cdot 0.7H_2O$.**

Regarding the MAS NMR experiments, the deconvolution of the $^{29}Si$ MAS NMR spectra confirmed that hydroxylated silicon species ($Q^3$+$Q^4$) are the dominant silicon sites (Figure A3) in the solid phases. This fact is in accordance with the general formulation proposed from TGA and elemental CNH analysis above. The intensity and chemical shift seemed not to be affected by the presence of CD molecules when compared to the bare silica xerogel.

In addition, the $^{13}C$ MAS NMR spectra were also recorded for the included CD-silica hybrid xerogels and the corresponding pure CD. In this case, every spectra acquired were comparable, presenting all the same number of peaks and also extremely close chemical shift values and relative intensities. Therefore, it can be inferred that the original CD arrangement seems to be unaltered after trapping them inside the silica network.

From all these data, it can be affirmed that the natural adsorption capacity of CDs may not be altered or modified after inclusion inside the silica network. Thus, the success of these materials can be found in an adequate design of the solid phase. The included CD-silica hybrid xerogels described combine a hydrophilic silica surface with well-dispersed hydrophobic CD sites. The solid design expands from the nanoscale (providing an open and accessible micropore system for easy diffusion of the target analytes throughout) to the molecular level (preserving the CD conformation inside highly hydrophilic silica cage-like pores and permitting the formation of CD-analyte inclusion complexes).

Although the efficiency of these solid phases should be preserved in a range of conditions, a loss of capacity for the interaction with a variety of analytes when working in extremely humid conditions may be expected, especially when trapping analytes from aqueous samples. This justifies the experiments described in coming chapters and the synthesis of bonded CD-silica hybrid xerogels also carried out, whose characterization is described below.

### 1.3.2. Characterization of bonded CD-silica hybrid xerogels

In this case, not only the solids obtained but also the β- and γ-CD precursors (Figure A4) were analyzed by elemental CNH analysis and TGA to establish their chemical composition. Also, surface area and pore size, in addition to the sorption properties of the materials obtained, were established from the $N_2$, $CO_2$, and $H_2O$ adsorption-desorption isotherms measured. Other techniques such as XRD and MAS NMR ($^{29}Si$ and $^{13}C$) served for studying the structure of the solids. To observe the morphology, SEM and TEM micrographs were acquired. To end, the average particle size was analyzed as verification of the correct synthesis and processing of the solid phase carried out.

The chemical modification of CDs was first assessed. As mentioned, the composition of both functionalized CDs was determined by using CNH elemental analysis combined with TGA. In the TGA curve (Figure A5), a first weight loss up to a temperature of 120°C, which can be attributed to hydration water molecules, was observed. At higher temperatures and in a stepped way, the degradation and oxidation of CDs occurred (Christy, 2015), which was reflected in a weight loss of ca. 85%. Taking into account that the TGA experiments were carried out in an oxygen atmosphere, the final residue (T > 550°C) of ca. 15% may correspond to the final $SiO_2$ remaining. From these data, it was possible to estimate the stoichiometric relationship or number of silane groups coming from reagent 3-(triethoxysilyl)propylisocianate with which CDs functionalization took place. The obtained values were 3.9 for β-CD, and 3.8 in the case of γ-CD, and they were in good agreement with the percentages also determined through elemental CNH analysis. These similar values could be expected, as similar reaction conditions were used in both cases and the same amount was previously reported in the literature for β-CD (Mahmud & Wilson, 2016). Thus, it can be considered that every single CD molecule has four functionalizing organic arms to the silica network establishment (Figure A4).

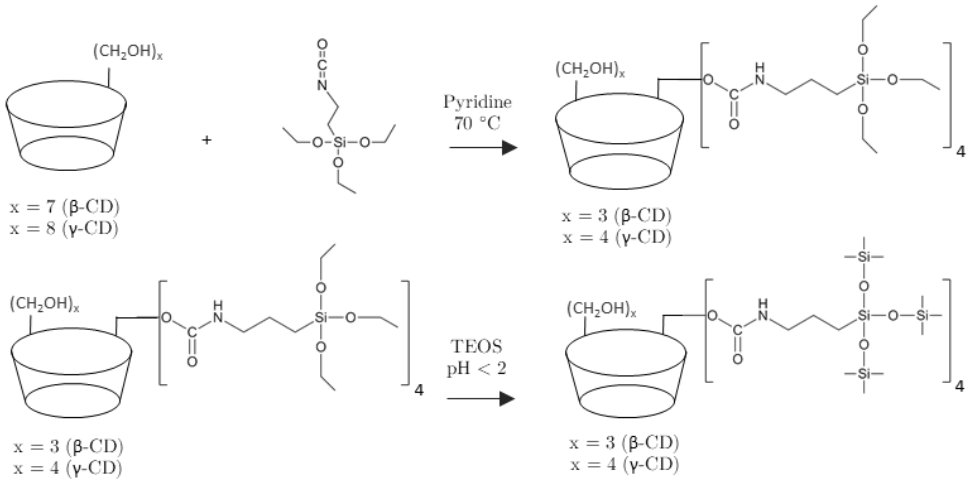



**Figure A4. Schematic representation of the preparation of bonded CD-silica hybrid xerogels.**

The characterization of the final bonded CD-silica hybrid xerogels was then conducted. An exhaustive characterization of the bonded β- and γ-CD xerogels was carried out exclusively with materials B1 and G1, which were initially those selected as representative. For the rest of the solid phases containing different Si/CD ratios (Table A1), some structural and morphological checks were conducted throughout the characterization process. However, the hypothetic

improvement of their properties due to their higher CD contents was tested through their specific analytical applications in the sample treatment stage, which are described later.

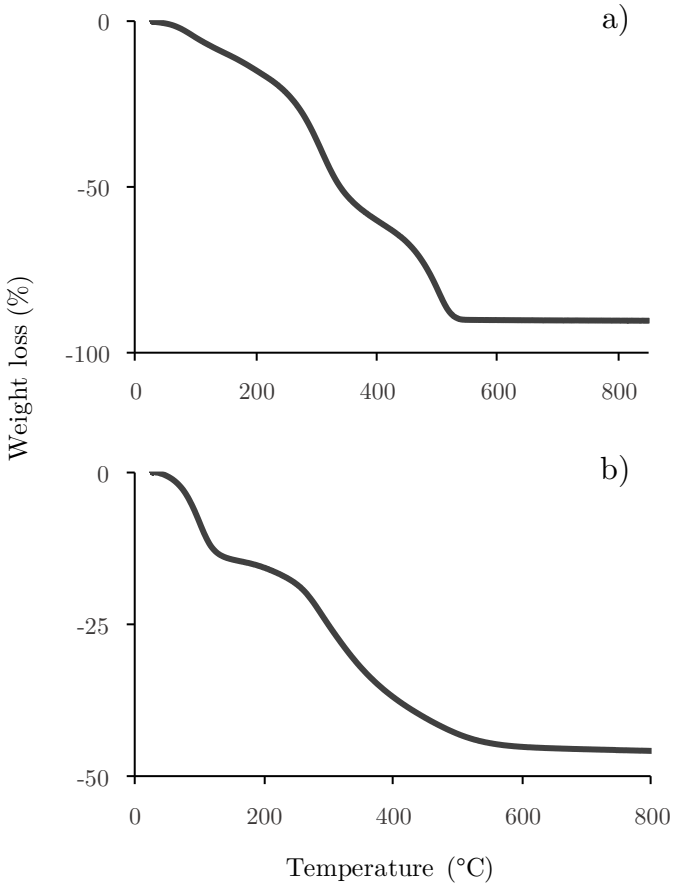



**Figure A5. Representative thermogravimetric analysis curves for: (a) CD precursors, and (b) the final bonded CD-silica hybrid xerogels.**

First, and parallel to the assessment of the CD precursors, the CNH elemental analysis combined with TGA were used to estimate their chemical composition. They supported the hypothesis of a hybrid silica material containing CD molecules. In comparison with the bare silica xerogel measured as a reference (95.9 wt% Si, 3.7 wt% O, and 0.4 wt% N), B1 and G1 solid phases possessed a significant amount of wt% C, which is consistent with the presence of CD in the silica network. These results are also in good agreement with TGA results, in which an initial weight loss of ca. 10 wt% ascribed to water molecules together with a second loss of ca. 40 wt% in the 200 – 700°C temperature range can be observed (Figure A5). This last sequential evolution can be attributed to the

pyrolysis of the covalently bonded CDs. Additionally, the results of the elemental CNH analysis permitted calculating a CD concentration of ca. 1 mmol $g^{-1}$ in the solids, taking into account that every CD entity possessed four functionalized arms to react with the silica network created from TEOS through their silanol groups.

Regarding the morphology of the xerogels, SEM and TEM images showed that, regardless of the type of CD selected, the size and morphology of the particles under study were similar in both cases. Non-aggregated particles of micrometric size without well-defined forms and order presenting a homogeneous distribution of a wormhole-like interconnected pore topology were observed in the case of the B1 material (Figure A6). This order absence was in accordance with XRD data. However, while in the β-CD material the microporosity was evident throughout the particles, this did not occur for the G1 material containing γ-CD, whose microporous structure was only observed at the grain limits. Besides, its porosity did not appear homogeneous throughout the structure.

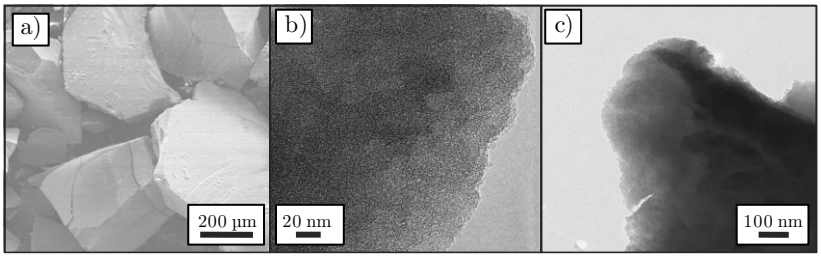



**Figure A6. (a) Representative SEM micrograph of the bonded CD-silica hybrid xerogels, (b) TEM micrograph of B1 CD-silica hybrid xerogel, and (c) TEM micrograph of G1 CD-silica hybrid xerogel.**

The XRD diffraction patterns obtained in both cases did not show any peak at low nor at high angles. These results are consistent with their intended amorphous nature and with the morphology observed in SEM and TEM micrographs (Figure A6). During the synthesis, the silica condensation is performed by obtaining a non-mesostructured framework in surfactant absence. For this reason, a broad signal was observed at angular values of ca. 21° (2Θ), which is typical of materials based on amorphous silica.

Figure A7 shows representative MAS NMR spectra measured not only for the CD precursors but also for the final B1 and G1 xerogels, which were comparable between them.

On the one hand, $^{29}$Si spectra of the CD precursor and the final xerogels need to be commented on. As it can be seen, the spectra of the modified CDs presented a very intense peak at a chemical shift value of -46.5 ppm and two additional

signals, of less intensity, at -60.0 ppm and -68.0 ppm. These signals can be associated with T-type Si-centres, in which each silicon atom is linked to a carbon atom. While the intense signal at -46.5 ppm can be assigned to the uncondensed CD precursor (centers of type $T^1$), the peaks of less intensity correspond to silicon sites that may be partially hydrolyzed and condensed ($T^2$ and $T^3$), in which some Si-O-Si siloxane bonds are present. This may be due to the high hydrolytic reactivity presented by the terminal alkoxidic groups that are able to react with each other simply in the presence of a certain humidity degree. In any case, the intensity of the signal at -46.5 ppm compared to the others indicated that the desired compound was clearly the dominant phase. For the final xerogels, two sets of signals were observed in the ranges of chemical shift between -90 ppm and -120 ppm, and between -50 ppm and -60 ppm, which correspond to sites Q and T, respectively. The three signals at -93.0 ppm, -102.5 ppm, and -119.9 ppm can be assigned to environments of type $Q^2$, $Q^3$, and $Q^4$, respectively. These silicon centers are generated by the hydrolysis and condensation processes from the TEOS reagent. Moreover, the lower intensity signals at -56.5 ppm and -65.8 ppm are associated with environments of type $T^2$ and $T^3$, respectively. As it can be observed, type T centers are a minority in this case, being their proportion of ca. 6% of the total silica content in the solid.

On the other hand, $^{13}C$ spectra measured also provided valuable information. In the case of the functionalized CD precursors, the spectrum presented a variety of signals that can be assigned. The peaks from 1 to 6 correspond to carbon atoms of the cyclodextrin molecules, while peaks from 7 to 11 are associated with the carbons of the organic arm used to bond the sugar to the silica structure. Finally, peaks 12 and 13 correspond to the carbon atoms of the terminal alkoxy groups of the mentioned arms. In contrast, although the $^{13}C$ spectrum for the final xerogels is very similar to that previously shown for the precursors, the incorporation of the CD to the silica network in an unaltered way could be confirmed. Specifically, all signals assigned to the sugar and the organic arm were also clearly observed (peaks 1 – 11). However, the absence of peaks 12 and 13 confirm that the hydrolysis and condensation processes occurred, as expected. In summary, these reactions gave rise to the final bonded CD-silica xerogel searched for.

The analysis of the particle size measured a distribution centered in average size in the range 300 – 400 μm, as expected.

To understand the apparent poor porosity for the bonded γ-CD-silica material observed before (Figure A6), an exhaustive porosity characterization protocol was conducted. With this aim, different adsorption experiments were carried out.

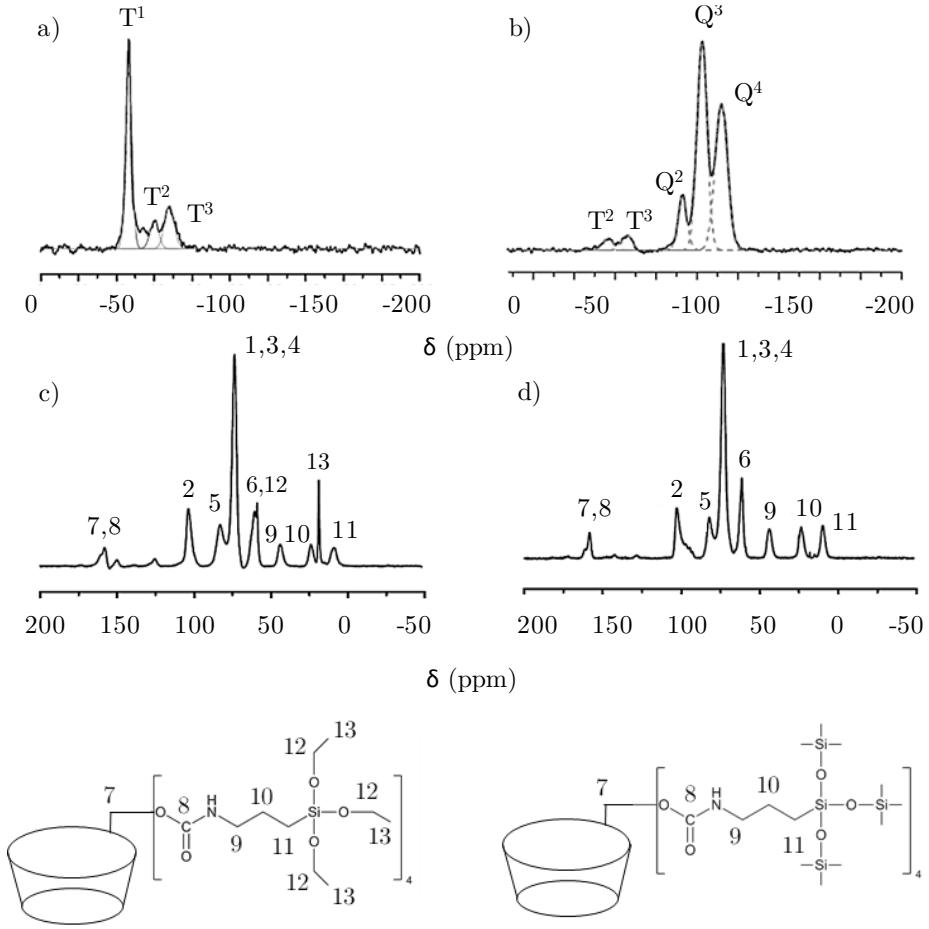



**Figure A7. Representative $^{29}$Si MAS NMR spectra of (a) the CD precursors and (b) the final bonded CD-silica hybrid xerogels. Representative $^{13}$C MAS NMR spectra of (c) the CD precursors and (d) the final bonded CD-silica hybrid xerogels.**

The $N_2$ adsorption-desorption isotherms were first recorded. Surprisingly, and in agreement with TEM micrographs, the adsorption of the bonded γ-CD-silica xerogel was extremely low when compared with the isotherm of the analogous bonded β-CD-silica composite (Figure A8). For this reason, a comparison of the surface area and pore volume determined from this experiment was also carried out for samples having similar CD content, as described, but changing several parameters. Not only the type of CD used (β- or γ-CD), but also the way with which CD interacted with silica, this is, the previous included CD-silica xerogels and the bonded CD-silica xerogels studied in this case (Table A3), were examined. From the data acquired, the following tendencies can be highlighted: (i) regardless of the CD-silica interaction, the CD incorporation reduces the BET area and

porosity with respect to the bare silica xerogel, (ii) in general, lower BET surface areas and pore volumes were detected for the phases containing the bigger γ-CD, and (iii) the covalent bonding of the CD leads to lower BET areas and porosity, being this effect especially notable for γ-CD.

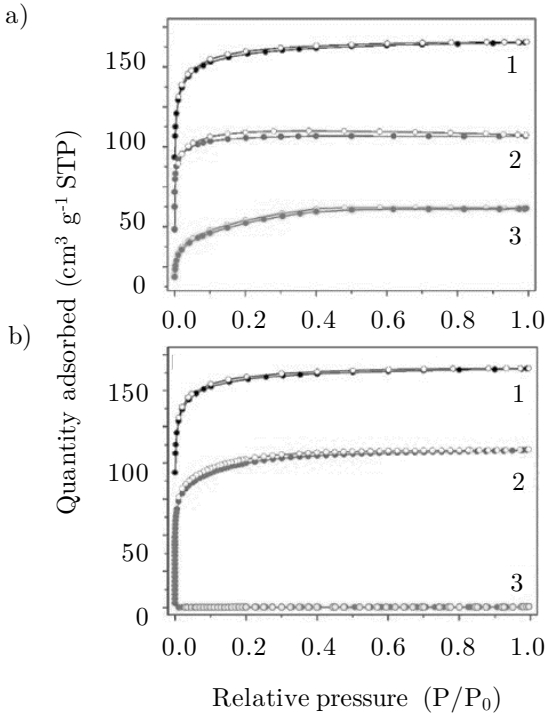



**Figure A8. $N_2$ adsorption-desorption isotherms of (a) included CD-silica xerogels, and (b) bonded CD-silica xerogels. For each one, (1) refers to the bare silica xerogel, (2) refers to β-CD-silica xerogels, and (3) refers to γ-CD-silica xerogels.**

The ability of pure CDs to adsorb relatively small gases depends, basically, on their internal cavity size. Then, it is the α-CD that has the greatest adsorption capacity due to a better size adaption when compared with the β- or γ-CD (Trotta et al., 2011). In fact, the adsorption capacity of free CD both for $N_2$ and $CO_2$ has been reported to be very limited (Ho, Howes, & Bhandari, 2014; Trotta et al., 2011). This low ability can be improved through ordering the CD molecular units, leading to 1D pore systems (Ho et al., 2014). To significantly enhance its adsorption degree, different strategies have been successfully applied to date, such as their combination with polymeric supports, their inclusion in MOFs, or their combination with silica-based materials (Gassensmith et al., 2011; B. Meng, Li, Mahurin, Liu, & Dai, 2016). When CD molecules are trapped inside supports, both their adsorption ability and their possible gas-selectivity seem to be more

related to the porosity and the whole structure of the material than with the CD type. Moreover, it must be taken into account that, in most cases, the CD contents are relatively low, so the contribution of the internal cavities of the CD molecules to the pore volume may be negligible. In this way, compared to the pure silica gel, a decrease in the specific surface area is usually observed after the chemical grafting or trapping of CD molecules. This trend can only be correlated with the covering of the adsorption sites by the CD molecules or their aggregates on the silica surface or due to a total or partial micropore blocking, which hinders the $N_2$ molecules' access to the binding sites. This agrees with previous results obtained on different kinds of bonded-to-silica CDs. In addition, the higher stiffness at the structural level imposed by the anchored CD could explain its lower adsorption capacity.

If these considerations were true, the removal of CD molecules would allow recovering, at least, certain porosity. Hence, in order to understand if the lower microporosity observed for solids containing γ-CD is due to an effect of the sugar or a different organization of the silica support, two solids having identical content of bonded β- and γ-CD were calcined. Interestingly, after CD removal, the BET surface area of both samples increased and they tended to equalize. This result suggests that both solids have probably a similar silica-based skeleton, with, *a priori*, a resembling pore system. Then, it seems to be the different sizes of the CDs the main responsible factor allowing well the preservation of a notable porosity (in the case of the lower sized β-CD) or the practically complete blocking of the micropores (in the case of the bigger γ-CD). Moreover, there is not only the sugar size but also the higher rigidity expected when the CD is covalently bonded, the two factors that cooperatively contribute to the apparent micropore clogging for $N_2$ at 77 K.

**Table A3. Physical and textural parameters of bonded CD-silica xerogels.**

| Type of CD | Total area[a] ($m^2\ g^{-1}$) | Surface[b] ($m^2\ g^{-1}$) | Pore volume[b] ($cm^3\ g^{-1}$) | $CO_2$ adsorption ($cm^3\ g^{-1}$ STP) |
|---|---|---|---|---|
| Bare silica | 530.5 | 422.9 | 0.26 | 72.3 |
| β-CD | 352.2 | 353.6 | 0.14 | 39.2 |
| γ-CD | 137.5 | 128.5 | 0.08 | 14.5 |

[a] Total surface area determined through the BET model.

[b] Surface and volume determined from the $CO_2$ adsorption curves by the Dubinin-Radushkevich model.

The main characteristics of $CO_2$ adsorption-desorption experiments and the lower size of this molecule with respect to $N_2$ may be enough changes to open the poor micropore system that seems to be present in the structure from the TEM images of the γ-CD-silica xerogel. Thus, the $CO_2$ adsorption-desorption isotherms for the xerogels containing bonded β- and γ-CD are shown in Figure A9. In both cases, typical isotherms of microporous materials can be observed with a moderately hysteresis loop. The measurements obtained suggested that the adsorption of carbon dioxide can occur in the inner hydrophobic cavity of the CD as well as in the silica micropores that are partially filled with CD molecules. As mentioned, the larger the size of the CD, the lower its ability to trap simple gases (Trotta et al., 2011). Moreover, and related to the CD size, in those sugar-occupied micropores, the larger the CD is, the smaller free space evidently results or even a certain pore blockage can occur. Both factors working cooperatively can explain the lower $CO_2$ adsorption observed for the γ-CD derivatives when compared to the rest of the xerogels measured.

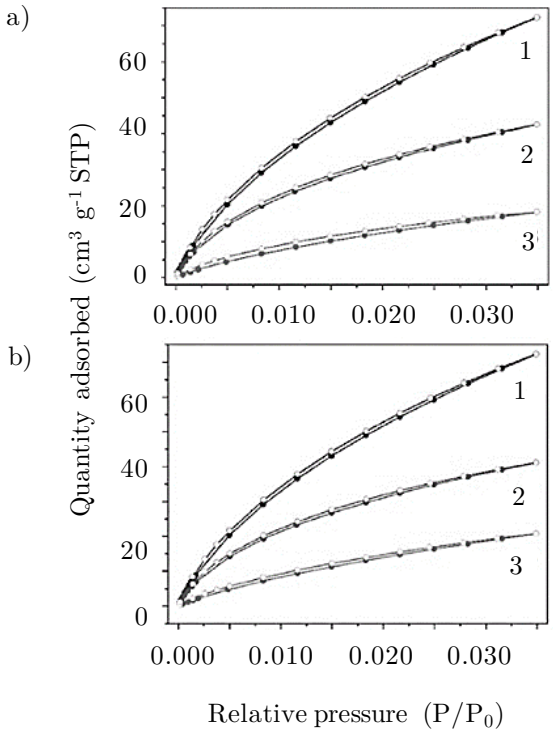



**Figure A9. $CO_2$ adsorption-desorption isotherms of (a) included CD-silica xerogels, and (b) bonded CD-silica xerogels. For each one, (1) refers to the bare silica xerogel, (2) refers to β-CD-silica xerogels, and (3) refers to γ-CD-silica xerogels.**

Additionally, the water vapor adsorption-desorption (Figure A10) was also studied. In both cases, and parallel to what occurs for the gas $CO_2$, both solids containing either β- or γ-CD molecules bonded to the silica network were able to adsorb water vapor. The adsorption of water molecules progresses with slightly different rates and profiles in both cases, so, in difference with $N_2$ and $CO_2$ adsorption-desorption experiments, the γ-CD material was now capable of adsorbing water, although a larger amount of water was adsorbed for the solid containing β-CD. These results agree with the previous studies reported in the literature (Christy, 2015; Tanada, Nakamura, Kawasaki, Kurihara, & Umemoto, 1996). This last tendency fits very well with previous data on CD-polymer composites that show a higher water affinity for those polymers containing β-CD. Although the covalent anchoring of the γ-CD diminishes the water vapor adsorption, its strong interaction with the silica support hinders the CD leaching. Therefore, this characteristic can be considered beneficial for the use of these materials for the sample treatment of aqueous specimens.

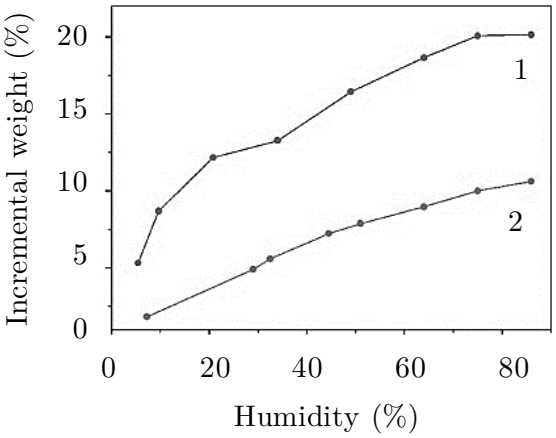



**Figure A10. Water adsorption-desorption curves of bonded CD-silica xerogels containing: (1) β-CD, and (2) γ-CD.**

In the same way as included CD-silica xerogels, the synthesis and complete characterization of bonded CD-silica xerogels have been described in this part. These are also microporous materials that combine the hydrophilic surface of silica with the hydrophobic contribution of CD units anchored to the silica network. Moreover, the solid is similarly designed in a way that permits the easy diffusion of the target analytes throughout an open and accessible micropore system created by the silica network to the formation of inclusion complexes with CDs at the molecular level. Besides, the synthesis procedure described is easy and inexpensive.

However, an improvement in the analytical performance of these materials in comparison with the included CD-silica ones may be expected, generating, in this case, new applications of silica-based materials containing CD with disordered structure. Based on the characterization results, it can be concluded that the suitable design of the solid phases obtained may allow their adaption to a wider range of conditions than in the previous case. On the one hand, the possibility of adapting the material to the type of analyte under study by taking into account the type and size of the CDs contained in the silica network may be an advantage (Szente & Szemán, 2013; Tijunelyte et al., 2017), as mentioned. On the other hand, the medium in which analytes are contained, air- or water-type, could be also changed while maintaining a proper analytical performance, as has been demonstrated through the studies on their adsorption-desorption capacities carried out.

## 1.4. Conclusion

For the reasons exposed, the experiments hereunder are focused on a series of assessments to measure the capacity for sample treatment of the different types of silica-based materials containing CD described, this is, with included or bonded CD, with different types of matrices, in this case, air-based or water-based ones, as well as with a variety of possible analytes based on their different physicochemical properties and, thus, their interaction with the type of CD selected in each case.

# 2

# Determination of phenolic compounds in air samples

## 2.1. Introduction

Most of the generally accepted classifications of volatile organic compounds (VOCs) are based on their physicochemical properties. The World Health Organization (WHO) suggested that the term "volatile organic compound" should cover those substances mostly vaporized at room temperature and easily adsorbed onto solid sorbents, whose boiling points lie between 50 and 260°C (World Health Organization, 1989). By contrast, the Environmental Protection Agency (EPA) definition of VOCs includes any polar or non-polar compound made of carbon, excluding carbon monoxide, carbon dioxide, carbonic acid, metallic carbides or carbonates, and ammonium carbonate, that participate in atmospheric photochemical reactions and whose vapor pressure at 25°C exceeds 13.33 Pa (Król, Zabiegała, & Namieśnik, 2010). VOCs include aliphatic and aromatic hydrocarbons, aldehydes, ketones, ethers, acids, or alcohols such as phenolic compounds. They can be classified in accordance with a number of their properties as the degree of volatility, ozone-forming potential, polarity, or their effects on particular ecosystems (Bandehali, Miri, Onyeaka, & Kumar, 2021).

The VOCs present in atmospheric air may have a natural or anthropogenic origin. The main natural sources of VOCs emissions are the growth procedures of plants, volcanic processes, combustion of organic matter and biomass, and forest fires (K. H. Chang, Chen, & Huang, 2005; D. K. W. Wang & Austin, 2006). Oppositely, anthropogenic VOC emissions to atmospheric air can be divided into stationary and mobile (Parra et al., 2006; P. Wang & Zhao, 2008). While large

amounts of VOCs in the atmosphere come from the different forms of transportation, other anthropogenic VOCs are emitted from stationary sources as a result of crude-oil refining and spills from tankers, combustion of fossil fuels in heat and power plants, use of solvents, and the production of synthetic materials. Thus, aerosols, cleaning agents, polishes, varnishes, paints, pressed-wood products, and pesticides are some of the VOCs sources at homes and offices.

Phenolic compounds, which are classified as VOCs, are similarly emitted in the atmosphere due to the activity of some plant and animal species, snuff smoke, the use of air fresheners and cleaning products, and industrial activities. Intensive agricultural activities can be a major source of phenolic pollution and bad odors for the environment, so these facilities are usually located in areas away from the population. In this regard, the literature describes the presence of high levels of phenol, xylene, 2-methyl-1-propanol, toluene, and 4-ethylphenol in the indoor and outdoor air of cattle and pig fattening farms (Feilberg, Liu, Adamsen, Hansen, & Jonassen, 2010). Besides, high levels of phenol, cresol, toluene, or xylene have also been found in rooms perfumed with incense (Chuang et al., 2011), as well as in places where tobacco smoke tends to accumulate.

Liquid and gas chromatography are the most commonly used analytical techniques to carry out the determination of phenolic compounds (Mauri-Aucejo, Llobat-Estellés, Escarti-Carrasco, & Marín-Saez, 2006). On the one hand, reversed-phase high-performance liquid chromatography (RP-HPLC) has been mainly used for the determination of phenols in different samples. On the other hand, gas chromatography implies a derivatization step and/or the use of a highly sensitive and selective detector (e.g., GC-ECD or GC-MS).

However, the evaluation of VOCs in air requires the use of a sampling technique to take a representative sample and avoid unexpected variations in real compositions (Król et al., 2010). Since the concentration of pollutants varies over time, small sample volumes are not considered representative and, accordingly, short sampling times are not recommended. Moreover, the low levels of pollutants in air make enrichment necessary (Aragón, Atienza, & Climent, 2000). This enrichment is determined by detector sensitivity and quantification requirements.

The main techniques for sampling analytes are dynamic techniques, passive techniques, and denuder techniques (Aragón et al., 2000). The main disadvantages of passive techniques are that the enrichment factor is dependent on ambient conditions and also that it is less effective than other sampling techniques. In addition, denuder techniques require laminar flow through the tube and the denuder preparation is laborious. However, dynamic techniques represent a worthy alternative. Although the collection of samples has a high cost in this

case, it encloses a very effective enrichment. Then, the use of solid sorbents and active sampling is usually recommended to evaluate workplace exposure.

A traditional method for sampling phenolic compounds is their retention as phenolates with impingers containing a basic aqueous solution. One of its drawbacks is the usually required pre-concentration of analytes prior to their quantification (Conde, Afonso, González, & Ayala, 2006). Alternatively, solid sorbents may be used to this end, being silica gel followed by solvent desorption the most described. However, the tendency of silica gel to adsorb water vapor and displace other collected components with lower polarity is its major disadvantage.

The literature describes the formation of inclusion complexes between CDs and some phenolic compounds. Specifically, Divakar et al. studied the interaction of guaiacol and eugenol with β-CD. These molecules exhibit identical orientations with the phenyl ring within the cavity and the hydroxyl and methoxyl groups projected outside (Divakar & Maheswaran, 1997). Therefore, their reactivity is decreased while their stability increases. For this reason, CDs may represent a feasible way for reversibly adsorbing phenolic compounds.

This chapter aims to assess the use of included and bonded CD-silica xerogels for the sampling of volatile phenolic compounds from air. To this, two different sampling and extraction procedures have been developed according to the features offered by each solid phase to later compare them. Different volatile alkylphenols have been selected to carry out this study, namely: phenol, guaiacol, cresol isomers, 4-vinylphenol, 2-methoxy-4-vinylphenol, ethylphenol, ethylguaiacol, and eugenol. Thus, the advantages and disadvantages of each of the methods designed are commented based on the analytical features established from the experiments carried out with synthetically-spiked samples. Finally, both methods are applied to the sampling and analysis of the target analytes in real air samples.

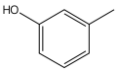
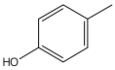
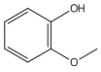
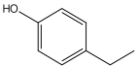
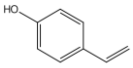
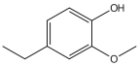
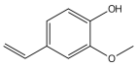
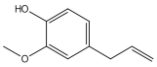
## 2.2. Experimental

### 2.2.1. Reagents, materials, and instrumentation

Stock standards of the target phenolic compounds were prepared from individual reagents ≥ 99.8%. Phenol, o-cresol, m-cresol, p-cresol, and guaiacol were acquired from Honeywell Fluka (Fischer Scientific, Buchs, Switzerland), while 4-ethylguaiacol, 4-ethylphenol, 4-vinylphenol, 2-methoxy-4-vinylphenol, and eugenol were purchased from Sigma-Aldrich (St. Louis, United States). The standard solutions were prepared in methanol (MeOH) and stored at -18°C in darkness. Additionally, all solvents used during the experimental procedure were HPLC grade quality, and they were purchased from Panreac AppliChem

(Barcelona, Spain). Ultrapure water from an Adrona purification system (Riga, Latvia) was employed during the procedure, and all samples were previously filtered with Nylon 0.45 µm filters from Sartorius Stedim Biotech (Göttingen, Germany).

**Table A4. Physicochemical properties of the studied phenolic compounds (Scifinder Scholar Database, 2021).**

| Compound | Structure (CAS) | Boiling point (°C) | Vapor pressure[a] (kPa) | logP[a] | Molecular size (nm) |
|---|---|---|---|---|---|
| Phenol |  (108-95-2) | 182 | 0.082 | 1.17 | 0.675 |
| o-cresol |  (95-48-7) | 191 | 0.051 | 1.96 | 0.714 |
| m-cresol |  (108-39-4) | 202 | 0.028 | 1.78 | 0.714 |
| p-cresol |  (106-44-5) | 202 | 0.028 | 2.07 | 0.714 |
| Guaiacol |  (90-05-1) | 205 | 0.024 | 1.34 | 0.734 |
| 4-ethylphenol |  (123-07-9) | 218 | 0.011 | 2.58 | 0.750 |
| 4-vinylphenol |  (2628-17-3) | 189 | 0.022 | 2.62 | 0.735 |
| 4-ethylguaiacol |  (2785-89-9) | 236 | 0.002 | 2.43 | 0.799 |
| 2-methoxy-4-vinylphenol |  (7786-61-0) | 224 | 0.003 | 2.57 | 0.786 |
| Eugenol |  (97-53-0) | 253 | 0.001 | 2.40 | 0.816 |

[a] Vapor pressure and logP measured at 25 °C.

Sampling tubes were prepared using glass tubes with a 0.4 cm internal and a 0.7 cm external diameter, 8 cm length. These glass tubes contained two sections separated by glass wool from Supelco (Sigma Aldrich, St. Louis, United States). The front part contained twice of solid phase as the back section in every case (NIOSH Manual of Analytical Methods, 1994).

Air samples were collected using a portable Buck-Genie CSS-5 pump from A.P. Buck (Orlando, United States) previously calibrated with a Multicon KS external flow calibrator from Dräger (Texas, United States) with flexible connection tubes. On the one hand, the collecting flow was set at 110 mL $min^{-1}$ for 3 hours for environmental samples. On the other hand, for the vapors generated by incense sticks or cones, a system that allowed the incense burning inside a glass ball with a hole to allow sample collection was designed (Figure A11). The bell was placed on 0.5 cm supports to enable the entry of air and thus combustion. In this case, the sampling was set at 200 mL $min^{-1}$, and samples were collected individually, from the burning start until 5 minutes after the incense ran out.

**Figure A11. Design of the system for collecting vapor from incense sticks or cones.**

The comparison of the results obtained with reference methods was carried out with sampling tubes of silica type NIOSH (140 mg/70 mg) from Dräger (Texas, United States) in the case of included CD-silica xerogels, and with tubes containing XAD-7 from Acros Organics (Fisher Scientific, Massachusetts, United States) in the case of bonded CD-silica xerogels (NIOSH Manual of Analytical Methods, 1994).

For the method developed using the included CD-silica xerogels, the analytes were separated and quantified using an L-7100 liquid chromatograph equipped with a F-1080 fluorescence detection system and an L-2300 column oven from Merck-Hitachi (Chiyoda, Japan). The analytical column was a β-CD phase Astec CYCLOBOND I 2000 (25 cm x 4.6 mm, 5 µm) from Supelco (Sigma Aldrich, St. Louis, United States). The temperature of the columns was thermostatically controlled at 25°C, and the sample injection volume was 2 µL. The flow rate was 1 mL $min^{-1}$ with a gradient-mode mobile phase composed of water buffered at pH

4 with acetic acid 0.005 M and acetonitrile (ACN). The gradient program used was 0-5 min ACN:$H_2O$ 0:100, 5-9 min ACN:$H_2O$ 2:98, 9-15 min ACN:$H_2O$ 2:98, and finally 15-25 min ACN:$H_2O$ 10:90. The fluorimetric detection was carried out at a $\lambda_{ex}$ of 275 nm and a $\lambda_{em}$ of 300 nm.

For the method developed using the bonded CD-silica xerogels, the analytes were separated and quantified using an LC-2000 Plus liquid chromatograph equipped with an FP-2020 Plus Intelligent Fluorescence Detector and a PU-2089 Plus Quaternary Gradient Pump with integrated degasser from Jasco (Madrid, Spain). The injection volume was 20 µL in a Supelco six-way injection valve (Sigma Aldrich, St. Louis, United States). The stationary phase was a $C_{18}$ ZORBAX column from Agilent Technologies (10 cm x 4.6 mm, 3.5 µm) and the separation was carried out at 1.7 mL $min^{-1}$ (California, United States). The gradient-mode program of the mobile phase composed of a mixture ACN:$H_2O$ containing acetic acid at 0.1 M was from 0 minutes (5:95 ACN:$H_2O$) to 25 minutes (40:60 ACN:$H_2O$).

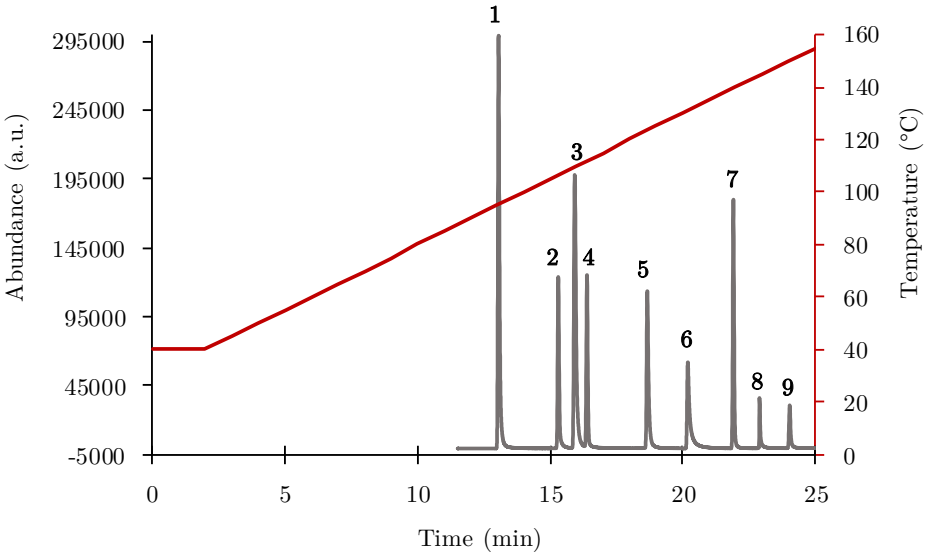



**Figure A12. GC-MS chromatographic profile of a multianalyte solution of the phenolic compounds under study in methanol: (1) phenol (2) o-cresol (3) m+p-cresol (4) guaiacol (5) 4-ethylphenol (6) 4-vinylphenol (7) 4-ethylguaiacol (8) 2-methoxy-4-vinylphenol (9) eugenol.**

Finally, phenolic compounds were also analyzed qualitatively to confirm their identity in real samples using an Agilent 5977A gas chromatograph (California, United States) coupled to a mass spectrometry detector, where qualitative and quantitative analyses were carried out simultaneously (Figure A12). The

stationary phase was an HP-5MS (5% phenyl methylpolysiloxane) 30 m/0.25 mm/0.25 µm. Helium was used as the carrier gas at a flow of 0.7 mL $min^{-1}$. The temperature was initially set at 40°C (2 minutes) and then heated to 280°C at 5°C $min^{-1}$. The mass selective detector operated in electron impact mode with a potential ionization of 70 eV and a source temperature of 250°C. The scan range used in SCAN mode was m/z 40 – 340, whereas ions m/z 94, 108, 124, 107, 120, 152, 150, and 164 were used for isolating the signal of phenol, cresol isomers, guaiacol, 4-ethylphenol, 4-vinylphenol, 4-ethylguaiacol, 2-methoxy-4-vinylphenol, and eugenol, respectively, in SIM mode. The interface temperature and the injector temperature were both set at 250°C. Besides, 1 µL of sample was injected in splitless mode with a solvent delay of 2 minutes.

### 2.2.2. Optimization and validation for included CD-silica xerogels

The optimization study was carried out using spiked sampling tubes prepared according to the recommended procedure of norm UNE-EN 1076:2010 for assessing workplace exposure through active sampling. For this purpose, 10 µL of the multianalyte stock solution of phenolic compounds containing 2 µg of each analyte were injected directly onto the sorbent. Subsequently, the tubes were allowed to equilibrate in air for several minutes with the help of a portable pump. After collection, tubes were closed with caps, sealed in plastic bags and stored at 4°C. The blank sample was prepared in the same way.

Desorption was carried out independently in the two sorbent sections. To do this, two sections were placed in separated extraction tubes and, after extraction, they were filtered and injected in the chromatographic system. The recovery was calculated as the ratio of the obtained and added amount of each compound.

The desorption procedure was optimized by varying one parameter at a time while keeping the others constant. The parameters studied were the nature of the solid phase, the nature and volume of the extracting solvent, and the time and temperature of desorption. Moreover, the influence of the amount of analytes and the sample storage time were also evaluated.

Regarding the analytical figures of merit of the sampling method developed, the repeatability study was carried out based on triplicate recoveries obtained for each one of the phenolic compounds tested using included β-CD-silica xerogels as solid phases. To that end, 10 µL of the multianalyte solution in acetonitrile were injected directly onto the front sorbent section. Subsequently, the tubes were allowed to equilibrate in air for several minutes. Then, the ends of each tube were capped and the tubes were endorsed to stand overnight at 4°C. The blank was prepared following the same procedure. Desorption was carried out following the

previously optimized experimental conditions. Also, the limit of detection (LOD) and limit of quantification (LOQ) of the method for each target analyte were calculated considering the appropriate volume of air sample.

Finally, the proposed procedure (Figure A13) was applied to the assessment of the occupational exposure of phenolic compounds in air samples collected at a farmhouse, a block, and a chicken coop. In addition, samples of the gases and vapors generated by lavender, rosemary, sandalwood, apple, and pine essential oils from sticks were also analyzed. Stick samples were also collected using NIOSH type silica tubes and analyzed by the reference method chosen (Instituto Nacional de Seguridad e Higiene en el Trabajo, 2015), carrying out the desorption with 1 mL of acetone and stirring occasionally for 30 minutes. In all cases, a blank was prepared using a sampler tube without passing air through it. Then, air samples were analyzed by HPLC-FLD and GC-MS. Before the quantification of phenolic compounds by HPLC-FLD, the peaks obtained from the mass spectra obtained were identified by using literature data.

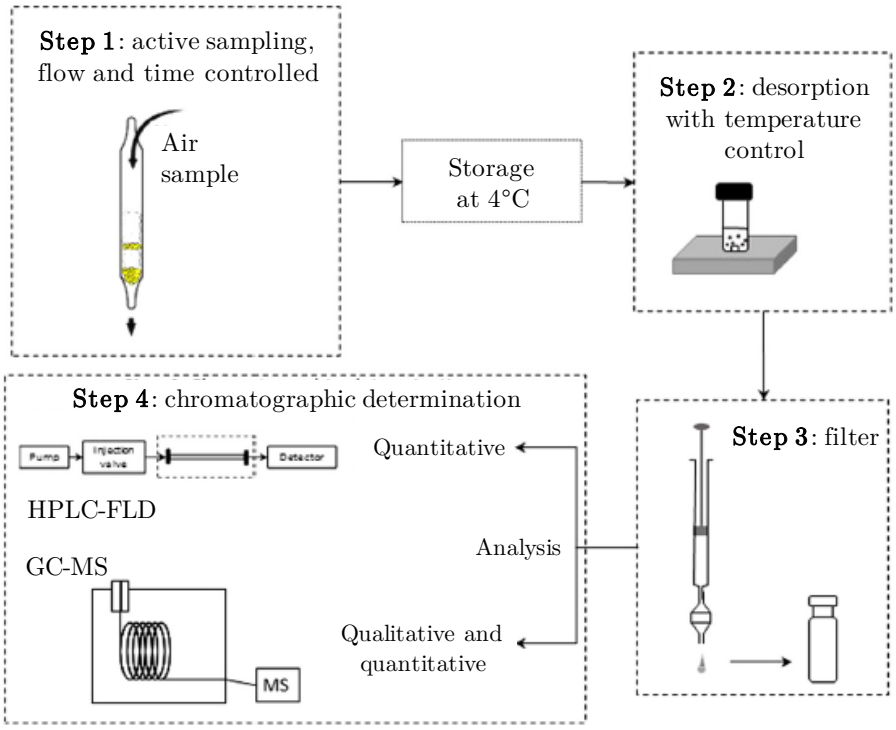



**Figure A13. Schematic representation of the experimental procedure followed for sampling and extracting phenolic compounds using included CD-silica xerogels.**

The final recommended procedure was as follows. Samplers containing included β-CD-silica xerogels as sorbents are prepared according to standard recommendations (NIOSH Manual of Analytical Methods, 1994). Then, air is allowed to pass through the materials with a flow rate of 110 mL $min^{-1}$ for 3 hours for environmental samples and of 200 mL $min^{-1}$ for a time established from the start and until 5 minutes after burning for incense sticks, cones, or essential oils. After storage, the desorption is carried out using a water bath and a magnetic stirrer inside tubes containing 2 mL of ACN at 70°C for 30 minutes. Then, the extracting solutions are filtered and injected into the chromatographic system to carry out the identification and quantification of the target phenolic compounds.

### 2.2.3. Optimization and validation for bonded CD-silica xerogels

Parameters affecting the retention and desorption processes of phenolic compounds from air samples were optimized. The optimization process was also carried out using spiked sampling tubes prepared according to the recommended procedure of norm UNE-EN 1076:2010 for active sampling previously mentioned. For this purpose, 10 µL of a multianalyte solution were injected directly onto the sorbent and air was then forced to pass through the sampling tubes to emulate the real sampling procedure. Finally, analytes were extracted in contact with an organic solvent, being the desorption step carried out independently in the two sorbent sections. The blank sample was prepared as described, too. After extraction, the solutions were filtered and injected in the chromatographic system. The recovery was calculated as the ratio of the obtained and added amount of each compound.

The extraction procedure was optimized by varying one parameter at a time while keeping the others constant. In this case, the parameters studied were those affecting the retention (type of solid phase, quantity of solid phase, volume of air sampled, and concentration of analytes) and desorption (nature and volume of the solvent, type and time of agitation). Besides, the sample conservation time and the possibility of reusing the material were also evaluated.

Regarding the analytical figures of merit of the sampling method developed, the repeatability study was carried out based on triplicate recoveries obtained for each one of the phenolic compounds tested using bonded β-CD-silica xerogels as solid phases. Also, the limit LOD and LOQ of the method for each target analyte were calculated considering the appropriate volume of air.

Finally, the developed method was applied to the analysis of phenolic compounds emitted from different aroma incense cones (orange blossom, white moss, and vanilla) following the sampling procedure designed. Results were

validated with those obtained by applying a reference method (NIOSH Manual of Analytical Methods, 1994) that used XAD-7 as solid phase with the same end. In all cases, samples were analyzed by HPLC-FLD and GC-MS. Before the quantification of phenolic compounds by HPLC-FLD, the peaks obtained from the mass spectra obtained were identified by using literature data.

The final recommended procedure was as follows. Samplers containing 150 mg of bonded β-CD-silica xerogels as sorbents in the front part are prepared according to standard recommendations (NIOSH Manual of Analytical Methods, 1994). Then, air is allowed to pass through the materials with a flow rate established at 200 mL $min^{-1}$ for a time measured from the cones burning start and until 5 minutes after the end. After storage, the desorption is carried out at room temperature using an oscillating shaker for 20 minutes with 4 mL of ACN as extracting solvent in closed tubes. Then, the extracting solutions are filtered and injected into the chromatographic system to carry out the identification and quantification of the target phenolic compounds.

## 2.3. Results and discussion

### 2.3.1. Use of included CD-silica xerogels

The evaluation of the nature of the solid phase used was conducted using 500 mg of all of the included CD-silica synthesized and described previously (Table A2), as well as the bare silica xerogel to prove the benefits of including CDs in the materials used for the sampling procedure. Specifically, the solid phases used were based on α-CD, β-CD, γ-CD, β-MCD, and β-HPCD. During the optimization study, HPLC-FLD was used as detection technique.

Since the presence of cyclodextrin in the solution can affect the fluorescence signal, calibration solutions were prepared in the presence of blank extracts obtained from the treatment of solid phases at 55°C for 30 minutes with methanol, ethanol, acetonitrile, and a 0.01 M sodium hydroxide solution (Conde et al., 2006) In the last case, the extracts obtained were neutralized with acetic acid to pH 4.5 before their injection in the chromatographic system.

First, results indicated that the fluorescence signals obtained for o-cresol, eugenol, 2-methoxy-4-vinylphenol, 4-vinylphenol, and guaiacol were strongly affected in alkali extracts. The variation of the fluorescence was lower when the extracts were obtained with acetonitrile, being this effect greater for the more soluble cyclodextrins. Specifically, changes in the fluorescence signal were greater when using the included α-CD-silica xerogel and lower when working with the included β-CD-silica xerogel (Saokham, Muankaew, Jansook, & Loftsson, 2018).

The fluorescence signal varied about a 30% for 2-methoxy-4-vinylphenol and 4-vinylphenol in the presence of blank extracts obtained after a desorption treatment using the included β-CD-silica xerogel and ACN as desorbing solvent, and 5% for all the other target phenolic compounds. Therefore, to quantify analytes, calibration solutions must be prepared from blank extracts obtained under the same conditions as the samples.

Based on the recoveries obtained and the influence on the fluorescence signals observed, the selected solid phase to carry out the experimental procedure designed was the included β-CD-silica xerogel. Moreover, acetonitrile was selected to carry out the desorption providing enhanced sensitivity and recovery.

The influence of the solvent volume, temperature, and time of desorption on recovery was also studied. To this, the procedure outlined above was followed by injecting 10 µL of a multianalyte solution containing 2 µg of each target analyte in the solid phase. Desorption was carried out using a water bath and a magnetic stirrer with 1, 2, and 3 mL of acetonitrile and 55, 65, and 75°C for 15, 30, and 45 minutes. The recovery increased between 55 and 65°C, remaining it constant with a temperature from 65 to 75°C. Also, recoveries increased when a greater heating time was used, remaining them constant after 30 minutes. Therefore, a stirring time of 30 minutes and a temperature between 65 and 75°C were selected to carry out the desorption process.

The loading capacity of the materials was assessed by injecting different amounts of phenolic compounds based on different molar ratios CD:pollutant. To this end, 10 µL of multianalyte solutions containing different concentrations of phenolic compounds were injected onto the sorbent. Following the complete sampling and desorption procedure, recoveries differed less than a 10% from the established values in all cases.

Finally, the desorption carried out after 44 days of sample storage showed no significant differences in recovery, so it was concluded that the maximum storage time at 4°C of the sampling tubes was, at least, the mentioned time.

Regarding the analytical figures of merit, the repeatability study was based on triplicate recoveries obtained for each phenolic compound following the recommended procedure. The extracts were analyzed not only by HPLC-FLD but also through GC-MS this time in order to confirm if the low recovery obtained for 4-vinylphenol and 2-methoxy-4-vinylphenol was due to an uncorrected matrix effect or to the non-retention or non-desorption of these analytes. The determination by GC-MS confirmed the lower recovery of both analytes (36 and 33%, respectively). Thus, these results suggested the non-retention or desorption of these compounds in the solid phase proposed, making it necessary to remove

them from the target analytes of the designed sampling method. Oppositely, results for phenol, guaiacol, cresol isomers, 4-ethylguaiacol, 4-ethylphenol, and eugenol indicated enhanced repeatability, with coefficients of variation (CV) lower than 6% in all cases and recoveries obtained that were higher than 90%. Results can be observed in Table A5.

**Table A5. Analytical figures of merit established for the sampling method of phenolic compounds using the included β-CD-silica xerogel.**

| Compound | Recovery (%) | CV (%) | LOD (µg $m^{-3}$) | | LOQ (µg $m^{-3}$) | |
|---|---|---|---|---|---|---|
| | | | HPLC-FLD | GC-MS | HPLC-FLD | GC-MS |
| Phenol | 109 | 4 | 4 | 20 | 12 | 60 |
| Guaiacol | 99 | 4 | 2 | 8 | 6 | 20 |
| o-cresol | 102 | 3 | 3 | 30 | 9 | 90 |
| m-cresol | 94 | 3 | 2 | 17 | 6 | 50 |
| p-cresol | 94 | 6 | 5 | 17 | 15 | 50 |
| 4-ethylguaiacol | 91 | 4 | 7 | 4 | 20 | 12 |
| Eugenol | 95 | 0.8 | 9 | 4 | 30 | 12 |
| 4-ethylphenol | 102 | 2 | 3 | 30 | 9 | 80 |

Compared to other methods described in the literature (Table A6), the proposed method provided similar recoveries, repeatability, and time of analysis to those others assessing not only occupational exposure of phenol and cresol isomers but also contents of the rest of the target phenolic compounds in several air samples. In this context, LODs were comparable, recoveries are higher than 75% and the variations are lower than 10% for eugenol, guaiacol, 4-ethylguaiacol, and 4-ethylphenol. Therefore, the method offered improved repeatability compared to other methods based on thermal desorption.

At this point, the sampling procedure was applied to the determination of phenolic compounds in the air samples described previously.

In the case of air samples collected from a farmhouse, a stable, and a chicken coop, the chromatograms obtained only indicated the presence of m-cresol, while the rest of phenolic compounds were all below the LODs established. Specifically, the concentration of m-cresol was of 8.7 µg $m^{-3}$ for the farmhouse, of 13.5 µg $m^{-3}$ for the stable, and of 12.4 µg $m^{-3}$ for the chicken coop. In this way, and assuming that the concentration remains constant over time, it was in all cases below the threshold limit value (22 mg $m^{-3}$), resulting in an exposure index lower than 0.1 (Gobierno de España, 2015).

**Table A6. Comparison of the developed method for phenolic compounds sampling using the included β-CD-silica xerogel with other methods in the literature.**

| Sorbent | Analytes[a] | Recovery (%) | CV (%) | Storage time | LOD | Time | Reference |
|---|---|---|---|---|---|---|---|
| Impingers | Ph, o-C, m-C, p-C | > 80 | < 20 | 48 hours | 1 – 5 mL $m^{-3}$ | Few minutes | (Environmental Protection Agency, 1986) |
| Impingers + SPME | Ph, G, o-C, m-C, p-C, E | > 80 | 7 – 18 | No data | 3.5 – 10 µg $m^{-3}$ | > 90 minutes | (Conde et al., 2006) |
| Silica | Ph, o-C, m-C, p-C | 83 – 89 | 2.4 – 6.2 | 48 hours | No data | 120 minutes | (Knecht & Nitsch, 1986) |
| XAD-7 | Ph, o-C, m-C, p-C | > 90 | 2.8 | > 30 days | 1 – 3 µg per sample | 30 minutes | (NIOSH Manual of Analytical Methods, 1994) |
| Tenax TA | p-C, 4-EP | 95 – 128 | 9.3 – 22.2 | > 120 hours | No data | 8 minutes | (Koziel et al., 2005) |
| Included CD-silica xerogels | Ph, G, o-C, m-C, p-C, 4-EG, E, 4-EP | 91 – 109 | 0.8 – 6 | > 44 days | 4 – 30[b] µg $m^{-3}$<br>1.7 – 8[c] µg $m^{-3}$ | 30 minutes | Proposed method |

[a] Phenol (Ph), o-cresol (o-C), m-cresol (m-C), p-cresol (p-C), guaiacol (G), Eugenol (E), 4-ethylphenol (4-EP), 4-ethylguaiacol (4-EG).
[b] GC-MS.
[c] HPLC-FLD.

Table A7. Analysis of phenolic compounds using the sampling method developed with included β-CD-silica xerogel (mg $m^{-3}$, $\bar{x} \pm s$) and its validation.

| Compound | Lavender sticks | | Rosemary sticks | | Pine sticks | | Sandalwood sticks | | Apple sticks | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NIOSH silica | Included β-CD-silica xerogel | NIOSH silica | Included β-CD-silica xerogel | NIOSH silica | Included β-CD-silica xerogel | NIOSH silica | Included β-CD-silica xerogel | NIOSH silica | Included β-CD-silica xerogel |
| Phenol | 1.16 ± 0.08 | 1.01 ± 0.10 | 0.78 ± 0.07 | 0.78 ± 0.07 | 1.1 ± 0.2 | 1.1 ± 0.2 | 1.49 ± 0.08 | 1.3 ± 0.3 | 0.95 ± 0.08 | 1.01 ± 0.15 |
| Guaiacol | 0.74 ± 0.16 | 0.75 ± 0.14 | 0.74 ± 0.06 | 0.9 ± 0.2 | 1.8 ± 0.3 | 1.8 ± 0.2 | 1.08 ± 0.16 | 1.03 ± 0.08 | 0.51 ± 0.02 | 0.69 ± 0.04 |
| o-cresol | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOD | < LOD |
| m+p-cresol | < LOQ | < LOQ | < LOQ | < LOQ | 0.19 ± 0.10 | 0.43 ± 0.06 | 0.15 ± 0.03 | 0.118 ± 0.012 | < LOD | < LOQ |
| 4-ethylguaiacol | < LOQ | < LOD | 0.047 ± 0.005 | 0.07 ± 0.03 | 0.06 ± 0.02 | 0.07 ± 0.02 | 0.12 ± 0.03 | 0.102 ± 0.012 | < LOQ | < LOQ |
| Eugenol | 0.8 ± 0.3 | 0.6 ± 0.3 | 0.08 ± 0.02 | 0.08 ± 0.04 | 0.215 ± 0.015 | 0.170 ± 0.010 | 0.10 ± 0.02 | 0.12 ± 0.02 | < LOD | < LOD |
| 4-ethylphenol | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |

In the case of air samples coming from the gases and vapors generated by fresheners, the chromatograms obtained showed the presence of different phenolic compounds in them. From the GC-MS chromatograms obtained, it can be mentioned that many of the compounds identified were terpenes such as linalool, borneol, camphor, or eucalyptol, which usually form part of essential oils. Indeed, some studies related dermatitis (An et al., 2005) with some typical compounds in fragrances such as cinnamaldehyde and eugenol in lavender, rosemary, pine, and sandalwood samples and γ-methylionone in sandalwood and oil samples. In this case, linalool was only detected in the lavender sample, borneol was detected in pine and oil samples and camphor and eucalyptol were only detected in rosemary samples. None of the terpenes indicated were detected in apple samples.

In addition, diethyl phthalate was detected in all samples. Other phthalates such as dibutyl phthalate in rosemary samples and sandalwood sticks or bis(methoxyethyl)phthalate and diisobutyl phthalate in rosemary sticks were also detected. No other phthalates were detected in the apple sample except diethyl phthalate. Phthalates provide flexibility and durability to plastic and are also used as solvents. They are commonly present in cosmetics, perfumes, toys, fresheners, etc. However, they can affect the hormonal and reproductive systems. For this reason, some of them are strictly regulated, including dibutyl phthalate, which was already banned in the manufacture of toys due to its effect on the reproductive system of male babies.

The complete results of the sample analysis can be seen in Table A7. As noted, the content per stick varied from 0.06 to 1.49 mg $m^{-3}$. Remarkably, all samples contained phenol and guaiacol in concentrations above the LOQs established. Oppositely, 4-ethylphenol was not detected in any of them. Regarding cresol isomers, only the quantification of m-cresol and p-cresol was possible in sandalwood and pine samples. Finally, lavender and apple sticks had the analyte 4-ethylguaicol in a concentration under the LOQs and LODs, and in none of the apple samplers was the presence of eugenol detected.

As indicated, incense sticks were also analyzed after the retention of the analytes using a reference method with silica tube samplers as solid phase. In order to know their environmental concentration, the recovery of the compounds was also calculated as in the case of the proposed tube samplers. The obtained recovery was 91, 81, 85, 75, 85, 73, and 55% for phenol, m+p-cresol, o-cresol, guaiacol, 4-ethylphenol, 4-ethylguaiacol, and eugenol, respectively.

The regression analysis of the results obtained with both methods indicated that, for a 95% of confidence level, the proposed method did not give a constant relative error and did not, thus, require a blank correction.

Based on the study carried out, it can be concluded that included CD-silica xerogels are suitable for the sampling of phenol, o-cresol, m-cresol, p-cresol, eugenol, guaiacol, 4-ethylguaiacol, and 4-ethylphenol from air samples. Specifically, included β-CD-silica xerogels were those that worked better as sorbents with these target analytes, probably due to a better analyte:CD adaption (Divakar & Maheswaran, 1997) that may be attributable to the predicted size of the analytes (Table A4) together with the average size of the CD hydrophobic cavity (Table 2).

Additionally, the proposed sampling method constitutes an alternative to other methods described in the literature for the same purpose. Therefore, in comparison with the reference method chosen (Instituto Nacional de Seguridad e Higiene en el Trabajo, 2015), the here designed method gives higher recoveries for several analytes, including guaiacol, 4-ethylguaiacol, and eugenol, making it suitable for their determination in air samples and working atmospheres. The qualitative analysis carried out with the vapors collected from sticks and essential oils indicated that these solid phases might be also used to determine terpenic compounds and phthalates from air samples.

However, some of the disadvantages found in the sampling must be acknowledged. For example, the method designed did not allow sufficient recoveries neither for 2-methoxy-4-vinylphenol nor for 4-vinylphenol. Based on the experimental procedure carried out, this might be attributed not only to the CD leaching when using an organic solvent, which affects the fluorescence signal, but also to the existent incomplete extraction of these analytes, which was proven through the GC-MS analysis. Besides, the thermal desorption of phenolic compounds proposed may also represent a disadvantage.

The drawbacks described justified the study described below, where the possibility of using bonded CD-silica xerogels for the same purpose was tested. The work aimed to improve not only the recovery of certain analytes but also the working conditions used.

### 2.3.2. Use of bonded CD-silica xerogels

The sampling and subsequent desorption of phenolic compounds from air through the use of bonded β- and γ-CD-silica xerogels were optimized similarly as described previously for included CD-silica xerogels. The optimization was carried out in both cases, although recovery trends kept similar every time. For this reason, the results mentioned correspond to the β-CD-silica xerogel.

First, the nature and volume of desorption solvent were evaluated. The solvents assessed were methanol, acetonitrile, a 0.1 M solution of acetic acid, a 1

M solution of acetic acid, a 1:1 mixture methanol:acetic acid 0.1 M, and a 1:1 mixture acetonitrile:acetic acid 0.1 M. From them, recoveries using acetonitrile were higher than those obtained using the other solvents proposed (99, 92, 94, 87, 85, 91, 91, 92, and 91% for phenol, guaiacol, m+p-cresol, o-cresol, 4-vinylphenol, 2-methoxy-4-vinylphenol, 4-ethylphenol, 4-ethylguaiacol, and eugenol, respectively). Additionally, the recoveries obtained did not vary hardly when increasing the volume of solvent from 2 mL to 4 mL. For this reason, it was finally decided to use 4 mL of solvent since this quantity allowed more comfortable handling.

The desorption procedure was designed by avoiding the previous thermal treatment. Thus, an oscillating shaker and an ultrasound equipment were evaluated as agitation modes to carry out the mentioned process. Recoveries obtained were similar in both cases for every phenolic compound. However, the use of the oscillating shaker was chosen since the ultrasound equipment might lead to the heating of the sample and, therefore, the loss of solvent and/or analytes during the extraction procedure, which would result in obtaining irreproducible and non-representative results. In this context, the recovery of the target analytes remained constant from 10 minutes of agitation except for some analytes such as 4-vinylphenol, which required higher times. Based on the results described, 20 minutes of agitation were chosen.

Regarding the amount of solid phase used for the sampling procedure, the recovery rates remained constant from 100 mg of solid phase onwards. For this reason, 150 mg were selected to continue with the optimization study.

For the volume of air used, a slight tendency to decrease the recovery as the volume of air samples increased was observed, as expected. The tendency was more pronounced for phenol and 2-methoxy-4-vinylphenol (recoveries of 98, 86, 95, 87, 88, 97, 83, 85, and 87% were obtained for 0 L of air, whereas recoveries of 92, 77, 91, 88, 80, 83, 86, 78, and 80% for phenol, guaiacol, m+p-cresol, o-cresol, 4-vinylphenol, 2-methoxy-4-vinylphenol, 4-ethylphenol, 4-ethylguaiacol, and eugenol, respectively, for 18 L of air). Therefore, these materials can be applied to the sampling of phenolic compounds in air; however, the use of calibration standards treated analogously to the sample may be necessary.

The concentration of analytes, from 0.8 µg of each individual phenolic compound to 20 µg, did not influence the recovery obtained for the chosen amount of solid phase.

The storage and conservation of sampling tubes at 4°C were also studied. From the results, it was concluded that there were no noteworthy losses to some analytes such as guaiacol and m+p-cresol even after a week, while in the case of

4-vinylphenol and 2-methoxy-4-vinylphenol these losses were already observed from the second day. Losses of analytes were significant enough from the second week of storage (recoveries were of 71, 68, 79, 78, 71, 80, 75, 93, and 77% for phenol, guaiacol, m+p-cresol, o-cresol, 4-vinylphenol, 2-methoxy-4-vinylphenol, 4-ethylphenol, 4-ethylguaiacol, and eugenol, respectively).

Finally, the reusability of the solid phases was studied. There were no important differences between the sampling and subsequent determination carried out with a newly synthesized material and a reused material. Thus, results clearly indicated that these materials were reusable at least once, as expected.

Once the working conditions were optimized, it was studied which of both cyclodextrins worked better. As can be seen in Figure A14, recoveries were greater in the case of the bonded β-CD-silica material (values between 10 and 20% higher). A probable explanation for this improvement in the extraction performance may be the size of the molecules of the phenolic compounds and the hydrophobic cavities of CDs. The approximate diameters of the analytes under study were around 0.75 nm (Table A4), which are of a similar size to the β-CD cavity (Table 2) and smaller than the γ-CD cavity. Thus, it seems logical to think that these compounds have relative ease, at least in terms of size, to enter the cavities of the two types of CDs, although the interactions occurring in the case of β-CD would be greater. When a stronger interaction occurs, greater retention of phenolic compounds in the cavity might be possible.

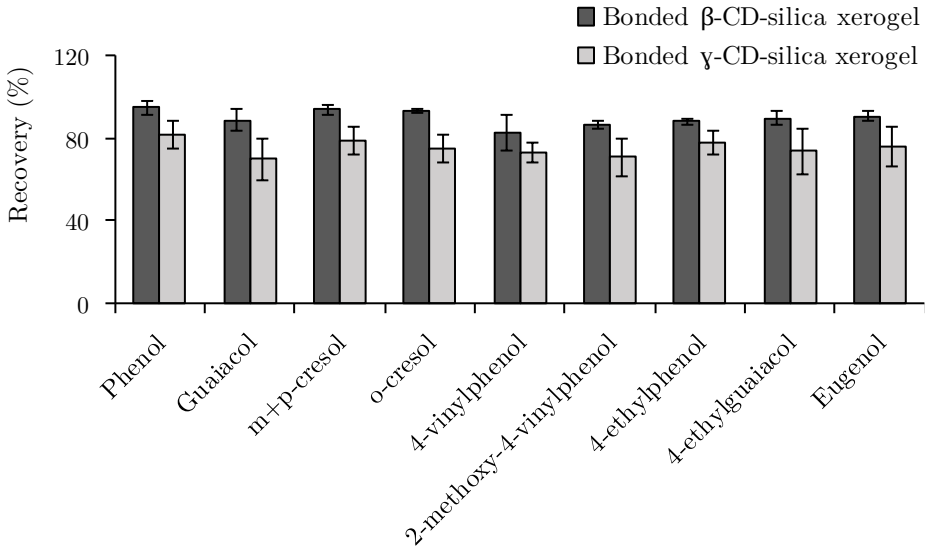



**Figure A14. Influence of the cyclodextrin type of the bonded CD-silica xerogel used as solid phase in the recovery of phenolic compounds from air samples.**

**Table A8. Analytical figures of merit established for the sampling method of phenolic compounds using the bonded β-CD-silica xerogel.**

| Compound | Recovery (%) | CV (%) | | Sensitivity ($\mu V\ L\ \mu g^{-1}$) | LOD[a] ($\mu g\ L^{-1}$) | LOQ[a] ($\mu g\ L^{-1}$) | Linearity[a] ($\mu g\ L^{-1}$) |
|---|---|---|---|---|---|---|---|
| | | Intra-day | Inter-day | | | | |
| Phenol | 95 ± 3 | 3 | 3 | 13.9 ± 0.3 | 20 | 60 | 20 – 2000 |
| Guaiacol | 89 ± 5 | 2 | 5 | 16.4 ± 0.8 | 16 | 50 | 16 – 1000 |
| m-cresol | 94 ± 2 | 3 | 2 | 20.0 ± 0.4 | 14 | 40 | 14 – 1000 |
| p-cresol | | | | 38.9 ± 0.8 | 7 | 20 | 7 – 1000 |
| o-cresol | 93.2 ± 1.1 | 2 | 1.1 | 19.8 ± 0.3 | 14 | 40 | 14 – 1000 |
| 4-vinylphenol | 83 ± 9 | 4 | 9 | 0.60 ± 0.02 | 400 | 1200 | 400 – 20000 |
| 2-methoxy-4-vinylphenol | 87 ± 2 | 2 | 2 | 33.6 ± 0.4 | 8 | 20 | 8 – 1000 |
| 4-ethylphenol | 88.4 ± 1.4 | 3 | 1.4 | 11.0 ± 0.3 | 20 | 60 | 20 – 1000 |
| 4-ethylguaiacol | 90 ± 3 | 3 | 3 | 12.1 ± 0.3 | 20 | 60 | 20 – 1000 |
| Eugenol | 91 ± 2 | 4 | 2 | 16.3 ± 0.33 | 17 | 50 | 17 – 1000 |

[a] LOD, LOQ, and linearity referred to the measuring solution.

**Table A9. Analysis of phenolic compounds using the sampling method developed with bonded β-CD-silica xerogel ($mg\ m^{-3}$, $\bar{x} \pm s$) and its validation.**

| Compound | Orange blossom | | White moss | | Vanilla | |
|---|---|---|---|---|---|---|
| | XAD-7 | Bonded β-CD-silica xerogel | XAD-7 | Bonded β-CD-silica xerogel | XAD-7 | Bonded β-CD-silica xerogel |
| Phenol | 2.1 ± 0.3 | 1.8 ± 0.4 | 1.3 ± 0.2 | 1.52 ± 0.16 | 1.5 ± 0.3 | 1.4 ± 0.3 |
| Guaiacol | 2.2 ± 0.4 | 1.6 ± 0.2 | 2.3 ± 0.5 | 1.66 ± 0.15 | 2.0 ± 0.4 | 1.5 ± 0.3 |
| m+p-cresol | 0.49 ± 0.03 | 0.43 ± 0.04 | < LOD | < LOD | < LOD | < LOD |
| o-cresol | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| 4-vinylphenol | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| 2-methoxy-4-vinylphenol | < LOD | < LOD | < LOD | < LOD | 0.79 ± 0.19 | 0.75 ± 0.11 |
| 4-ethylphenol | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| 4-ethylguaiacol | 0.50 ± 0.11 | 0.473 ± 0.03 | 0.35 ± 0.05 | 0.23 ± 0.08 | 0.38 ± 0.06 | 0.33 ± 0.06 |
| Eugenol | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |

**Table A10. Comparison of the developed method for phenolic compounds sampling using the bonded β-CD-silica xerogel with other methods in the literature.**

| Sorbent | Analytes[a] | Recovery (%) | CV (%) | Storage time | LOD | Reference |
|---|---|---|---|---|---|---|
| Impingers | Ph, o-C, m-C, p-C | > 80 | < 20 | 48 hours | 1 – 5 mL $m^{-3}$ | (Environmental Protection Agency, 1986) |
| Impingers + SPME | Ph, G, o-C, m-C, p-C, E | > 80 | 7 – 18 | No data | 3.5 – 10 µg $m^{-3}$ | (Conde et al., 2006) |
| Silica | Ph, o-C, m-C, p-C | 83 – 89 | 2.4 – 6.2 | 48 hours | No data | (Knecht & Nitsch, 1986) |
| XAD-7 | Ph, o-C, m-C, p-C | > 90 | 2.8 | > 30 days | 1 – 3 µg per sample | (NIOSH Manual of Analytical Methods, 1994) |
| Tenax TA | p-C, 4-EP | 95 – 128 | 9.3 – 22.2 | > 120 hours | No data | (Koziel et al., 2005) |
| Phenol-specific passive solid phases | Ph, o-C, m-C, p-C | No data | No data | No data | No data | (Sturaro, Rella, Parvoli, & Ferrara, 2010) |
| Bonded CD-silica xerogels | Ph, G, o-C, m-C, p-C, 4-EG, VPh, MVPh, E, 4-EP | 83 – 95 | 1.1 – 9 | 2 weeks | 13 – 40 µg $m^{-3}$ | Proposed method |

[a] Phenol (Ph), o-cresol (o-C), m-cresol (m-C), p-cresol (p-C), Guaiacol (G), Eugenol (E), 4-vinylphenol (VPh), 2-methoxy-4-vinylphenol (MVPh), 4-ethylphenol (4-EP), 4-ethylguaiacol (4-EG).

The analytical figures of merit established for the method designed are shown in Table A8. There, the intra-day and inter-day variation, the overall recovery of the analytes under study as well as the sensitivity, the LOD, LOQ, and linear range using the bonded β-CD-silica material as sorbent are shown. As can be seen, repeatability was satisfactory since CVs that did not exceed 4% on the same day and 9% between days were obtained. Moreover, recoveries calculated from results were also satisfactory since in all cases their values were above 80%. All phenolic compounds were detectable in concentrations at around 20 µg $L^{-1}$ in the measuring solution except 4-vinylphenol, which needed a more concentrated calibration due to its lower sensitivity in the chromatographic system. Thus, the LODs and LOQs would oscillate around 16 µg $m^{-3}$ and 40 µg $m^{-3}$, except for 4-vinylphenol, taking 6 L as the approximate volume of air sampled. It must be highlighted that these limits are comparable to those obtained in the previous experience using the included β-CD-silica xerogel as solid phase. However, as a different detector was used, the comparison was not decisive at all.

The method developed was also applied to the determination of phenolic compounds in real samples, specifically in different aroma incense cones. In the extracts obtained from the backup section of the tubes, phenolic compounds were not detected in any sample, this indicating that sampling was performed correctly. Results obtained from the extracts of the front section are shown in Table A9. As can be seen, those phenolic compounds that were present in all incense cones were phenol, guaiacol, and 4-ethylguaiacol. Compounds such as o-cresol, 4-vinylphenol, 4-ethylphenol, and eugenol were not detected in any of the cases. Regarding the validation of the developed method with a proposed reference method (NIOSH Manual of Analytical Methods, 1994), results obtained in both cases were comparable among them (comparison of variances by Fisher-Snedecor's test and comparison of means by Student's test, 95% of confidence level). This fact represents an advantage since for desorption of the analytes from the solid phase used, it was not necessary to use the ultrasound equipment as it was the case of the reference method. Thus, the danger of loss of analytes due to heating was reduced. Finally, it should be noted that, although some analytes were not detected, results obtained were in all cases above the LOQs established for the different phenolic compounds.

Besides, Table A10 compares the characteristic features of the method designed with other methods reported in the literature for the determination of phenolic compounds in air, parallel to how it was done in the previous study. Thus, the recovery values in this case and the LOQs in the sample are comparable to those found in the literature. Moreover, the sample treatment step followed

here is simpler than other treatments proposed such as thermal desorption. However, a drawback that can be highlighted in this case is the extraction solvent used, which is less green than others reported (Conde et al., 2006).

## 2.4. Conclusion

In this study, an improvement in the results obtained for volatile phenolic compounds sampling using included CD-silica xerogels has been achieved through the assessment of bonded CD-silica xerogels. The method designed resulted in repeatable results, being the desorption conditions softer and the time invested to carry out the analysis lower than those reached previously. It should be noted that the recoveries obtained with the bonded CD-silica xerogel were similar to those observed previously with the included CD-silica xerogel in the case of cresol isomers, phenol, guaiacol, eugenol, 4-ethylphenol, and 4-ethylguaiacol, being the standard deviations calculated also similar. In contrast, there was an improvement in the recoveries of 4-vinylphenol and 2-methoxy-4-vinylphenol in comparison with the included version of the solid phase, whose values are around 85% for the use of the bonded CD-silica solid phase. In the case of the included CD-silica xerogel, they were 36 and 33%, respectively.

The adsorption capacity of the bonded solid phase tested is justified by its adsorption-desorption isotherms presented previously (Figure A8), which demonstrated its porosity when working with air samples. Additionally, the improvement from using the included CD-silica xerogels to the bonded CD-silica xerogels was shown in the avoidance of CD leaching during the desorption procedure, which demonstrated to affect the fluorescence signal of some of the analytes and so the results of analysis obtained.

# 3

# Extraction of polycyclic aromatic hydrocarbons from water

## 3.1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are semi-volatile pollutants whose presence in the environment is widespread. They are mainly produced from the incomplete combustion of fossil fuels. Therefore, their origin is commonly anthropogenic (Garcia Londoño, Garcia, Scussel, & Resnik, 2013; Y. C. Lin, Lee, Chen, Chang-Chien, & Tsai, 2008), being the most common sources of PAHs waste incineration, incomplete combustion of carbon-containing materials, industrial processes, high-temperature cooking, traffic, heat generation, or tobacco (Son & Choi, 2020). However, some other natural sources such as volcanic eruptions and forest fires can also generate them (L. Guo & Lee, 2011).

Chemically, PAHs are non-polar substances composed of multiple aromatic rings. They are characterized for high thermodynamic stability, with boiling points over 200°C, and can thus remain in the atmosphere for a long time (Kueseng, Thammakhet, Thavarungkul, & Kanatharana, 2010). When their molecular mass increases, their polarity decreases and they tend to be more stable and toxic. Besides, PAHs are likewise known for suffering from photochemical decomposition (Rawa-Adkonis, Wolska, & Namieśnik, 2006).

Due to their described properties, PAHs are present in the atmosphere as gaseous phase or adsorbed in atmospheric particles, whereas in water samples the concentration of these organic pollutants is found at the µg $L^{-1}$ level due to their low solubility (Garcia Londoño et al., 2013; Kueseng et al., 2010). Several PAHs are considered carcinogenic and mutagenic compounds, and some of them are

considered priority substances by the EPA since people may be usually exposed to them (Son & Choi, 2020) by breathing contaminated air and drinking contaminated water or other derived liquids.

Some regulations regarding emissions and contents of PAHs in the environment have thus been established due to their harmful effects on health and the ecosystem. To control their presence in different atmospheres, benzo(a)pyrene has been chosen as the representative of the group, establishing its equivalent toxicity as 1. Therefore, contents of PAHs are often expressed based on the concentration of this compound, which is obtained as the sum of the equivalent concentrations for benzo(g,h,i)perylene, benzo(b)fluoranthene, benzo(k)fluoranthene, and indeno(1,2,3)pyrene. Legally, there is a PAHs limit of $1.4 \cdot 10^{-4}$ µg $L^{-1}$ expressed in annual average levels and of 0.27 µg $L^{-1}$ as the maximum permissible concentration in inland surface waters (2013/39/EU, 2013). There is also an upper limit of 0.1 µg $L^{-1}$ for the presence of these organic compounds in drinking water (98/83/EC, 1998) for European member states.

Thus, there is growing interest in monitoring trace PAHs in the environment (Schummer, Appenzeller, & Millet, 2014). The determination of PAHs in water requires previous enrichment. The main problem hindering this low-concentration determination is the usual lack of accuracy and precision of results, mainly derived from the sampling and sample preparation stages. For this reason, there is a need of developing analytical procedures with low detection and quantification limits.

Typically, PAHs are quantified using chromatography. Among them, GC-FID and GC-MS are normally used as gas-based chromatography techniques (Yingjie Li, Xian, & Li, 2017). Regarding liquid chromatography (Kumari, Chaturvedi, Ansari, Murthy, & Patel, 2012), the most common instruments are HPLC-UV, although they may present higher sensitivity when working with HPLC-FLD due to their notable quantum yield. Jjunju et al. also reported the analysis of PAHs using a portable mass spectrometer (Jjunju et al., 2015). Other analytical techniques described to this end include ion mobility spectrometry (IMS) (Son & Choi, 2019), which can separate ions in the gas phase at atmospheric pressure under the influence of an electric field according to their size and shapes.

Besides, there have been described several procedures for PAHs concentration and interferences elimination, presenting all of them advantages and disadvantages (Jung et al., 2013). For example, LLE uses toxic solvents (Ramirez, Wang, & Gardinali, 2014) and, for membrane-based extraction methods, the selection of the most appropriate membrane type is critical (Bortolato, Arancibia, & Escandar, 2008). Other extraction techniques such as SPME (Y. Hu et al., 2005), LPME (Xiao et al., 2012), and SBSE (Zuin, Montero, Bauer, & Popp,

2005) are highly subjected to matrix effects. SPE has enjoyed greater use and development and so larger research over time. For this reason, it constitutes a good alternative to other methods for PAHs extraction and enrichment (Jung et al., 2013) from water although being highly time-consuming. Therefore, developing new solid phases for application in SPE is a challenge to overcome.

Among different cavitated molecules, CDs have been extensively studied due to their ability to form host-guest complexes with hydrophobic molecules. Indeed, they have been described to their use in environmental pollution monitoring and remediation with hydrophobic PAHs (Tijunelyte et al., 2017), concluding that the size between these target substances and the cyclodextrin cavities strongly correlates with the ability to engage complexation.

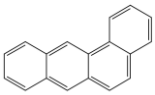
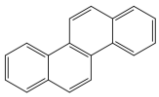
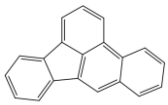
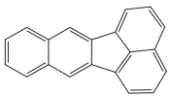
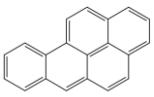
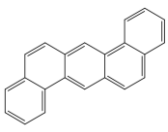
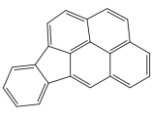
This chapter has a twofold purpose. On the one hand, it aims to assess the potential use of bonded β-CD-silica xerogels as fillers for SPE cartridges for the extraction and enrichment of PAHs from water samples in comparison with included β-CD-based silica solid phases. On the other hand, the higher efficiency expected in capturing PAHs for the bonded γ-CD-silica xerogels in comparison with the β-CD-silica materials due to the physicochemical properties of the target analytes is proven later. Different PAHs have been selected to carry this out, including benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, and indeno(1,2,3)pyrene. The efficiency of the different methods developed is commented based on the results obtained from experiments conducted with synthetically-spiked samples. Finally, the developed methods are applied to the analysis of PAHs in real environmental water samples.

## 3.2. Experimental

### 3.2.1. Reagents, materials, and instrumentation

The PAH standards were acquired from Supelco Analytical Products (Sigma Aldrich, St. Louis, United States) as a polycyclic aromatic hydrocarbons calibration mix of 10 mg $L^{-1}$ in acetonitrile. Stock standard solutions were prepared in methanol and stored at -18°C in darkness. Additionally, all solvents used during the procedure were HPLC grade quality, purchased from Panreac AppliChem (Barcelona, Spain). The comparison of the extraction of PAHs with a reference method was performed with Varian $C_{18}$ Bond Elut extraction cartridges from Agilent Technologies (California, United States). Finally, ultrapure water from an Adrona purification system (Riga, Latvia) was employed during the whole experimental procedure, and all samples were previously filtered with Nylon 0.45 µm filters from Sartorius Stedim Biotech (Göttingen, Germany).

**Table A11. Physicochemical properties of the studied PAHs (Scifinder Scholar Database, 2021).**

| Compound | Structure (CAS) | Boiling point (°C) | Vapor pressure[a] (kPa) | logP[a] | Molecular size (nm) |
|---|---|---|---|---|---|
| Benzo(a)anthracene |  (56-55-3) | 435 | $2.69 \cdot 10^{-8}$ | 5.73 | 0.896 |
| Chrysene |  (218-01-9) | 448 | $1.13 \cdot 10^{-8}$ | 5.73 | 0.896 |
| Benzo(b)fluoranthene |  (205-99-2) | 481 | $2.41 \cdot 10^{-9}$ | 6.19 | 0.916 |
| Benzo(k)fluoranthene |  (207-08-9) | 480 | $8.73 \cdot 10^{-10}$ | 6.19 | 0.916 |
| Benzo(a)pyrene |  (50-32-8) | 495 | $2.49 \cdot 10^{-10}$ | 6.19 | 0.917 |
| Dibenzo(a,h)anthracene |  (53-70-3) | 524 | $1.88 \cdot 10^{-11}$ | 6.91 | 0.953 |
| Benzo(g,h,i)perylene |  (191-24-2) | 550 | $1.49 \cdot 10^{-10}$ | 6.65 | 0.938 |
| Indeno(1,2,3)pyrene |  (193-39-5) | 536 | $2.08 \cdot 10^{-10}$ | 6.65 | 0.937 |

[a] Vapor pressure and logP measured at 25 °C.

A Vac Elut 20 connected to a vacuum pump model CKNF from Agilent Technologies (California, United States) and 3 mL polypropylene cartridges from Análisis Vínicos (Ciudad Real, Spain) were used during the SPE procedure. Besides, solvent evaporation was carried out with a miVac sample concentrator from SP Scientific (Warminster, United States).

To conduct the comparison between included and bonded CD-silica xerogels, analytes were separated and quantified using an L-7100 liquid chromatograph equipped with a F-1080 fluorescence detector and an L-2300 column oven from Merck-Hitachi (Chiyoda, Japan). To carry out the comparison between bonded β-CD and γ-CD-silica xerogels, an LC-2000 Plus liquid chromatograph equipped with an FP-2020 Plus Intelligent Fluorescence Detector and a PU-2089 Plus Quaternary Gradient Pump with integrated degasser from Jasco (Madrid, Spain) were used. In both cases, the analytical column was a SUPELCOSIL™ LC-PAH (25 cm x 4.6 mm, 5 µm) thermostatically controlled at 25.0 ± 0.5°C (Sigma Aldrich, St. Louis, United States). The detection step was conducted using an excitation and emission wavelength program (0–20 min $\lambda_{ex}/\lambda_{em}$ 280/330 nm and 20–40 min $\lambda_{ex}/\lambda_{em}$ 280/410 nm). The separation was carried out at a constant flow of 1.5 mL $min^{-1}$ with a mobile phase composed of a mixture of ACN and water, working with elution gradient (0–5 min ACN:$H_2O$ 40:60; 5–30 min ACN:$H_2O$ from 40:60 to 100:0; 30 –40 min ACN:$H_2O$ 100:0). The injection volume was 20 µL in a Supelco six-way injection valve (Sigma Aldrich, St. Louis, United States).

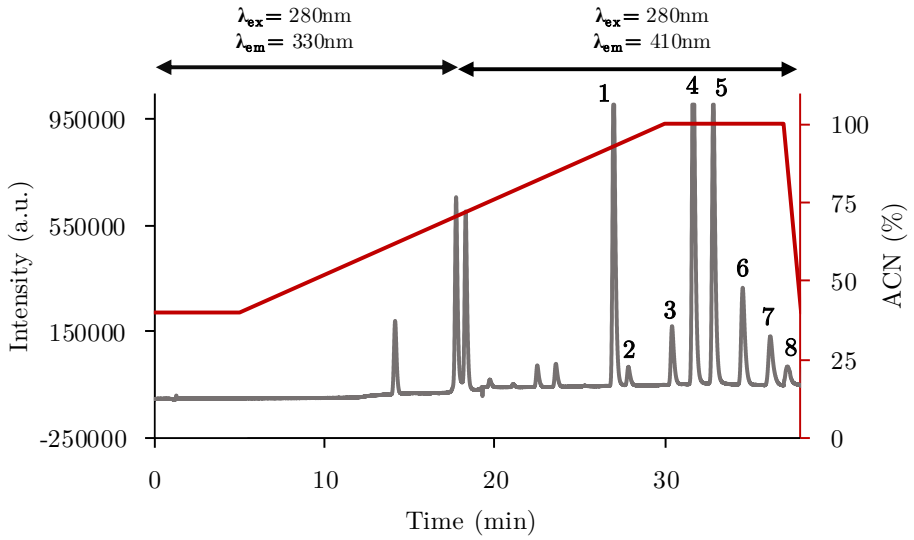



**Figure A15. HPLC-FLD chromatographic profile of a multianalyte solution of the PAHs under study in methanol: (1) benzo(a)anthracene (2) chrysene (3) benzo(b)fluoranthene (4) benzo(k)fluoranthene (5) benzo(a)pyrene (6) dibenzo(a,h)anthracene (7) benzo(g,h,i)perylene (8) indeno(1,2,3)pyrene.**

### 3.2.2. Optimization and validation for included CD-silica xerogels

The enrichment performance of the SPE procedure can be influenced by several parameters. Thus, those factors that may affect PAH retention in the material selected and their elution were studied and optimized. In this case, six PAHs were selected to carry out the study: benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene. Chrysene and indeno(1,2,3)pyrene were not included in the optimization study because they were harder to detect in several cases, thus being their results not reliable at all.

The external calibration solutions were prepared in acetonitrile with concentrations between 0 and 20 µg $L^{-1}$, and the recovery obtained from synthetically-spiked samples was calculated and used to extract the conclusions regarding the optimization of the extraction conditions. This recovery was calculated from the ratio between the calculated concentration and the theoretical concentration. Therefore, the procedure was optimized by varying one parameter at a time while keeping the other parameters constant. The studied parameters included the nature and amount of solid phase, the ionic strength, the pH, the sample volume, the loading capacity, and the nature of the elution solvent.

Moreover, the analytical figures of merit of the method were established. To this, the limit of detection was expressed as the concentration or mass flow of the substance of interest in the mobile phase giving a detector signal equal to twice the noise level (IUPAC, 1997). The noise level was calculated as the amplitude of the envelope of the baseline, which includes all random variations of the detector signal. In the case of concentration-sensitive detectors such as fluorescence detection, the sensitivity is calculated per unit of concentration in the mobile phase. In this way, the limit of quantification was expressed as 3.04 times the limit of detection (IUPAC, 1997). In addition, the linearity range was also considered.

Experimentally, the establishment of these analytical parameters was carried out using aqueous calibration solutions in the range of concentration between 0.1 and 0.8 µg $L^{-1}$. Following the experimental procedure designed, the recovery of the analytes was evaluated by analyzing different spiked samples. The considered samples were two tap water samples (0.1 and 0.6 µg $L^{-1}$, respectively), a water sample from a well (0.6 µg $L^{-1}$), a river sample (0.6 µg $L^{-1}$), and a salty water sample (0.6 µg $L^{-1}$). Besides, the repeatability of the extraction method was estimated based on the variation coefficient (CV), calculated as the ratio between the standard deviation and the recovery average of the results obtained from a triplicate extraction of each spiked water matrix (n=3). Furthermore, the

concentration factor for the SPE procedure was calculated as the ratio between the sample volume and the elution volume, while taking into account the recovery obtained for each of the studied analytes.

Since the various organic and inorganic components contained within natural water samples can greatly affect the recovery of PAHs measured by applying the described method, the matrix effect was also studied using the same previously indicated spiked samples. Based on the results obtained, it was concluded that PAH calibration solutions subjected to the same treatment as the sample must be used due to the existing matrix effect in the experimental procedure. Moreover, the analysis of a spiked sample should be carried out in parallel to provide the recovery of each analyte in each matrix.

Thus, the recommended procedure was as follows. An aliquot of 10 mL of sample or standard solution is placed in a beaker and NaCl is added to provide a 2 M salt concentration. Then, the sample is suctioned into the extraction cartridge containing 500 mg of solid phase by using vacuum, being the flow rate through the cartridges 3 mL $min^{-1}$. Next, the cartridge is washed with 2 mL of water and dried with air for 2 minutes. Finally, PAHs are eluted with 4 mL of methanol and the solvent is evaporated at 50 °C. Later, it is redissolved in 400 µL of methanol.

Finally, the experimental procedure developed was applied to determine PAHs in eight samples taken from the Valencia boat harbor, the Bellús reservoir (Vall d'Albaida, Valencia), the Canyoles river (Xàtiva, La Costera, Valencia), Lleó spring (Barxeta), the Xúquer river (La Rivera Baixa, Valencia), the Malvarrosa beach (Valencia), the Túria river (Chulilla, Valencia), and the Canyada del Corral well (Torrent, Valencia). To this, standard aqueous solutions containing between 0.1 and 0.8 µg $L^{-1}$ of PAHs were prepared, and the final optimized SPE procedure was followed to carry out the sample analysis. The conductivity of all samples was measured previously to prove whether it was necessary or not to adjust ionic strength. The concentrations for all analytes in samples were calculated by applying the concentration factor calculated for each analyte.

### 3.2.3. Optimization and validation for bonded β-CD-silica xerogels

In the same way as in the included CD-silica xerogels, the enrichment performance may also be influenced by several parameters this time. Thus, those factors affecting PAH retention in the solid phase and their elution were studied and optimized. Six PAHs were selected for the study: benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene. Neither chrysene nor indeno(1,2,3)pyrene were used during the whole optimization procedure since

indeno(1,2,3)pyrene could only be detected when evaporating the eluent after the extraction procedure and redissolving the analytes in a lower methanol volume, and chrysene could not be detected in most cases during the optimization process.

The optimization study was carried out based on the recovery obtained from spiked samples. In this case, the recovery was calculated from the ratio between the obtained concentration and the theoretical concentration. The procedure was optimized by varying one parameter at a time while keeping the other parameters constant. The studied parameters were the nature and amount of solid phase, the ionic strength, the nature and volume of elution solvent, the sample volume, and the loading capacity of the sorbent material.

Also, the recovery, concentration factor (CF), repeatability, sensitivity, and linearity of the method were studied. In the sensitivity case, the LOD was expressed as the concentration or mass flow of the substance of interest in the mobile phase giving a detector signal equal to twice the noise level (IUPAC, 1997). The LOQ was expressed as 3.04 times the LOD. In addition, the upper limit of linearity of the method was established as the concentration at which the deviation exceeds 5% whereas the LOQ was considered as the lower limit of linearity (IUPAC, 1997). The recovery of the analytes was evaluated by analyzing a triplicate of 10 mL of spiked water with 6 µg $L^{-1}$ of PAH in three different days using the optimized conditions, specifically three replicas each day (n=3). The repeatability of the extraction method was thus calculated from the variation coefficient of the obtained results. In this way, the concentration factor of the method was established taking into account the sample and the elution volume.

Additionally, standard aqueous solutions containing from 0 to 70 µg $L^{-1}$ of PAHs were prepared to perform the external calibration of the proposed analysis method. The general analytical procedure was as follows. An aliquot of 50 mL of sample or standard solution is placed in a beaker, and NaCl is added to provide a salt concentration of 2 M. Then, the sample is suctioned through the extraction cartridge with 200 mg of solid phase by using vacuum. The flow rate of the samples through the cartridges is 3 mL $min^{-1}$. Next, the cartridge is washed with 2 mL of water, dried with air for 3 minutes and, then, PAHs are eluted with 4 mL of hexane and 4 mL of methanol. Finally, the solvents are evaporated under vacuum at 60°C and the residue is redissolved in 500 µL of methanol. Concentrations for all the analytes in the samples are calculated by applying the method concentration factor, previously established.

The matrix-type influence in PAH recovery was studied with a river matrix, a well water matrix, a rain matrix, and a salty water matrix. For each case, three aliquots of 50 mL of spiked sample were determined with the previously

mentioned procedure. Then, PAHs were determined in four samples taken from the Túria River (Ribarroja del Túria, Valencia), the Canyada del Corral well (Torrent, Valencia), rain water (Picanya, Valencia), and the Malvarrosa beach (Valencia) to verify the correct application of the extraction method.

Finally, the recoveries obtained with the mentioned matrices were compared with those obtained with same-origin matrices but using the included CD-silica xerogel selected previously. The extraction method followed was identical in both cases to compare the influence of the sorbent. The objective was to verify that the bonded material improves the obtained results for the included material.

### 3.2.4. Optimization and validation for bonded γ-CD-silica xerogels

In this case, the conditions for the retention and the subsequent desorption of PAHs from the bonded γ-CD-silica xerogel were re-optimized based on those already studied for the bonded β-CD-silica material.

Six PAH were selected this time as representative of the group: benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, and dibenzo(a,h)anthracene. The optimization study was carried out based on the recovery obtained from synthetically-spiked water samples, and the experimental procedure was designed based on the previous studies conducted. Thus, the studied parameters were limited to the nature and amount of the solid phase, the loading capacity of the material, the sample volume, and the nature and volume of the solvent of extraction. In addition, the possibility of varying the pH of the water samples to increase analytes' retention was also assessed.

The analytical figures of merit of the re-optimized extraction method were established following the same criteria as described before (IUPAC, 1997).

The optimized procedure was as follows. An aliquot of 50 mL of sample or standard solution is placed in a beaker, and NaCl is added to provide a 2 M salt concentration in all solutions. Then, the samples are suctioned through the SPE cartridges containing 200 mg of bonded γ-CD-silica xerogel at 3 mL $min^{-1}$. Next, the cartridges are washed with 2 mL of water and dried for 2 minutes. PAHs are then eluted with 4 mL of ACN and 4 mL of hexane, being solvents evaporated under vacuum at 60°C and re-dissolved in 500 µL of methanol. To end, all the solutions are filtered and the analytes are quantified by chromatography.

Finally, the developed method was also applied to the pre-concentration of PAHs in samples belonging to the Malvarrosa beach (Valencia, Spain) and the Valencia boat harbor. This analysis was carried out through the standard addition calibration method due to the matrix influence previously proven, and the results

were validated with those obtained by applying a reference method (Bruzzoniti, Fungi, & Sarzanini, 2010) using $C_{18}$ as sorbent.

## 3.3. Results and discussion

### 3.3.1. Use of included CD-silica xerogels

The assessment of the nature of the solid phase used was conducted using three of the included CD-silica xerogels described previously (Table A2), as well as the bare silica xerogel, to prove the benefits of including CDs in the materials used for the extraction process. The physicochemical properties of these materials have been described above. To this, spiked samples containing 0.25 µg $L^{-1}$ of the target PAHs were prepared, and 25 mL of them were introduced into extraction cartridges containing 500 mg of solid phase each one. Analytes were eluted with 4 mL of acetonitrile and injected into the chromatographic system for the HPLC analysis. Results indicated that the included β-HPCD-silica xerogel provided better recoveries. As an example, the recovery obtained with this solid phase was 37% for benzo(b)fluoranthene (30 and 35% using included β-CD-silica and γ-CD-silica xerogels, respectively), 41% for dibenzo(a,h)anthracene (34 and 29% using included β-CD-silica and γ-CD-silica xerogels, respectively), and 27% for benzo(a)pyrene (24 and 26% using included β-CD-silica and γ-CD-silica xerogels, respectively). Moreover, results also indicated that the presence of CD in the solid phase helped to improve the recovery obtained when using the bare silica xerogel. In this case, the recoveries obtained rounded in all cases values of 15 ± 3% using the same conditions.

The amount of phase was then determined using the included β-HPCD-silica xerogel as the selected material. Cartridges containing 100, 250, and 500 mg of solid phase were prepared. Subsequently, 25 mL of a sample with 0.6 µg $L^{-1}$ of PAHs were introduced into the extraction cartridges, and the analytes were eluted with 4 mL of acetonitrile. The recoveries obtained using cartridges of 100 mg were significantly lower than results obtained for 250 and 500 mg, which did not differ largely between them. Specifically, recovery values for benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene were, respectively: 15, 34, 35, 13, 51, and 23% using 100 mg of solid phase; 31, 52, 52, 31, 65, and 27% using 250 mg of solid phase, and 30, 68, 55, 33, 61, and 44% using 500 mg of solid phase. For this reason, the selected amount of material to continue with the optimization study was 500 mg.

The recovery rates between pH 2 and pH 8 were not significantly affected. In fact, not only did there not exist any clear tendency in the recoveries obtained

but also the trends observed were disparate for the different PAHs. This is, some of the studied compounds improved their recovery for a certain pH value while others decreased it. For example, the recoveries obtained by adjusting the pH at 2, 5, and 9 were, respectively: 57, 40, and 49% for benzo(a)anthracene; 54, 36, and 42% for benzo(k)fluoranthene, and 60, 59, and 37% for benzo(a)pyrene. However, the ionic strength did influence the recovery.

The addition of salt to the sample can decrease the solubility of non-polar analytes in water and enhance the distribution coefficient of the solute to the extracting solid phases. Thus, the influence of the ionic strength was studied based on the recoveries obtained with a NaCl concentration of 1 M, 2 M, and 3 M. The results obtained proved that the peak area registered increased with NaCl 1 M to NaCl 2 M (reaching peak area increases of 100%), and remained constant from NaCl 2 M to 3 M, with peak area variations between -10% and 14%. For this reason, an ionic strength of 2 M was chosen to continue with the optimization.

Furthermore, the use of methanol as eluent was studied since it is more environmentally friendly and less costly than other organic solvents such as ACN. As can be seen in Table A12, the recoveries obtained using methanol as the eluent were slightly higher for most of the analytes under study than those obtained using acetonitrile. It is important to highlight the cases of benzo(g,h,i)perylene and dibenzo(a,h)anthracene, whose recoveries improved significantly when using methanol. Based on these results, methanol was selected as the elution solvent.

**Table A12. Influence of the nature of the elution solvent in the recoveries obtained for the SPE of PAHs using the included β-HPCD-silica xerogel.**

| Compound | Recovery (%) | |
|---|---|---|
| | ACN | MeOH |
| Benzo(a)anthracene | 36 ± 2 | 39 ± 3 |
| Benzo(b)fluoranthene | 44 ± 4 | 55.1 ± 1.7 |
| Benzo(k)fluoranthene | 48 ± 4 | 49 ± 6 |
| Benzo(a)pyrene | 39 ± 3 | 42 ± 3 |
| Dibenzo(a,h)anthracene | 47.0 ± 0.9 | 58 ± 4 |
| Benzo(g,h,i)perylene | 49 ± 5 | 73 ± 6 |

Subsequently, the possibility of increasing the sensitivity of the proposed procedure was assessed. To do so, after SPE, the 4 mL extracted of the elution solvent were evaporated at 50°C using a rotary evaporator, and the residue was

redissolved in 400 µL of methanol. Benzo(g,h,i)perylene, benzo(a)anthracene, dibenzo(a,h)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, and benzo(k)fluoranthene were all recovered at a 94 ± 13% with respect to the expected theoretical concentration. Therefore, the results obtained showed that no significant differences in the recovery were observed after evaporation of the extracting solvent and re-dissolution of the residue. This indicates the possibility of carrying out the method following this final procedure. Moreover, this step enabled the presence of indeno(1,2,3)pyrene to be detected.

Finally, the influence of the sample volume and the analyte concentration on recovery rates were evaluated. To this end, following the same procedures as described above, 10, 25, and 50 mL of samples containing 0.6 µg $L^{-1}$ of PAHs and NaCl 2 M were introduced into the cartridges. For benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene, the measured recoveries were 39, 55, 49, 42, 58, and 72%, respectively, when using 10 mL of sample, whereas sample volumes of 25 and 50 mL gave rise to lower recoveries each time. For example, recoveries obtained for the previously mentioned compounds were of 33, 42, 42, 29, 51, and 50% using 25 mL, and of 26, 31, 31, 29, 52, and 45% for 50 mL. In conclusion, the results for the study of the volume of the sample indicated that the recovery rates decreased while increasing the sample volume. Furthermore, the recoveries obtained for spiked samples containing 0.4 and 0.6 µg $L^{-1}$ of PAHs did not differ significantly, being the major recovery differences between 10% in the case of benzo(g,h,i)perylene and 5% in the case of benzo(b)fluoranthene.

**Table A13. Analytical figures of merit established for the PAH extraction method using the included β-HPCD-silica xerogel.**

| Compound | Linearity[a] (µg $L^{-1}$) | LOD[b] (ng $L^{-1}$) | LOQ[b] (ng $L^{-1}$) | CV (%) |
|---|---|---|---|---|
| Benzo(a)anthracene | 3 – 250 | 4 | 12 | 8 |
| Benzo(b)fluoranthene | 0.9 – 200 | 12 | 36 | 8 |
| Benzo(k)fluoranthene | 0.09 – 250 | 1.2 | 3.6 | 11 |
| Benzo(a)pyrene | 3 – 250 | 4 | 12 | 9 |
| Dibenzo(a,h)anthracene | 4.5 – 500 | 6 | 18 | 7 |
| Benzo(g,h,i)perylene | 0.9 – 200 | 12 | 36 | 6 |
| Indeno(1,2,3)pyrene | 2.9 – 200 | 38 | 115 | 15 |

[a] Linearity is referred to the measuring solution.
[b] LOD and LOQ are referred to the water sample.

The analytical figures of merit established for the optimized method are shown in Table A13. The detection and quantification limits are referred to the sample solutions, which were calculated by applying the concentration factor of the method, while the linearity range is referred to the measuring solution.

Results for the matrix effect indicated that recoveries were affected when using different matrices, as can be seen in Table A14. It seems important to highlight the case of the salty water matrix, whose recoveries were the most significantly affected. This may be due to the several interfering compounds contained in this type of water, which can result in a higher matrix effect. Moreover, the ionic strength is not as easy to control in salty water as in other types. Also, the matrix effect is lower in the fresh water case, taking as an example the recoveries for the river matrix. In contrast, in the tap water case, a variation in the results can be seen, which is higher when spiking with 0.1 µg $L^{-1}$, even though this is simply due to the lower concentration used. For all these reasons, the use of a standard addition calibration may be advisable for future applications of the described method.

As mentioned, the proposed procedure was also applied to determine PAHs in water samples. Results are given in Table A15. From them, it can be mentioned that the concentration of benzo(a)pyrene was below 270 ng $L^{-1}$, the maximum permissible concentration in inland surface water, in all samples where it could be quantified. However, it seems important to highlight the case of the well water, because its concentration is close to that allowed by legislation (2013/39/EU, 2013). In general, the results for the analyzed samples indicate that the total content of benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, and indeno(1,2,3)pyrene was below 100 ng $L^{-1}$ in all cases, which is in accordance with the minimum requirements for human drinking water established (98/83/EC, 1998). As can be seen, this is not the case for the well water.

Finally, the method developed was compared to other methods reported in the literature (Table A16). The LOQs informed are similar to those obtained with other procedures using the same analytical technique. Moreover, the volume of solvent used in the here proposed procedure is lower than the volume employed by most other methods since procedures based on SPE are intended to consume a higher solvent volume in comparison with other alternatives. In most cases, the solvent used to carry out the sample treatment is equal to or more toxic than the solvent proposed in this study. Finally, the time required to perform sample processing is significantly more convenient in this case than most other published procedures.

**Table A14. Influence of the matrix in the recovery of PAHs using the included β-HPCD-silica xerogel.**

| Compound | Recovery (%) | | | | |
|---|---|---|---|---|---|
| | Tap water 0.1 μg $L^{-1}$ | Tap water 0.6 μg $L^{-1}$ | Well water 0.6 μg $L^{-1}$ | River water 0.6 μg $L^{-1}$ | Salty water 0.6 μg $L^{-1}$ |
| Benzo(a)anthracene | 100 ± 10 | 112 ± 7 | 76 ± 6 | 83 ± 4 | 81 ± 9 |
| Benzo(b)fluoranthene | 77 ± 5 | 95 ± 8 | 100 ± 10 | 120 ± 120 | 116 ± 5 |
| Benzo(k)fluoranthene | 100 ± 9 | 120 ± 10 | 77.0 ± 0.6 | 104 ± 0.6 | 65 ± 6 |
| Benzo(a)pyrene | 100 ± 8 | 117 ± 8 | 87.3 ± 0.7 | 109 ± 67 | 57 ± 5 |
| Dibenzo(a,h)anthracene | 80 ± 5 | 93 ± 9 | 111 ±6 | 100 ± 20 | 70 ± 910 |
| Benzo(g,h,i)perylene | 89 ± 4 | 82 ± 5 | 117 ± 3 | 110 ± 20 | 78 ± 5 |
| Indeno(1,2,3)pyrene | < LOQ | 80 ± 4 | < LOQ | < LOQ | 88 ± 9 |

* Recovery values of 100% correspond to the concentration factor obtained for each analyte to the method developed. Results in this table show the recovery increase or decrease when working with different matrices.

**Table A15. Analysis of PAHs using the included β-HPCD-silica xerogel (ng $L^{-1}$, $\bar{x} \pm s$).**

| Compound | Valencia boat harbor | Bellús reservoir | Canyoles river | Lleó spring | Xúquer river | Malvarrosa beach | Túria river | Well water |
|---|---|---|---|---|---|---|---|---|
| Benzo(a)anthracene | < LOQ | < LOQ | < LOQ | 15 ± 3 | 16 ± 3 | 91 ± 22 | 87 ± 8 | 392 ± 3 |
| Benzo(b)fluoranthene | 69 ± 5 | 61 ± 4 | 53 ± 3 | 60 ± 5 | 60 ± 5 | < LOQ | < LOQ | 580 ± 40 |
| Benzo(k)fluoranthene | 7 ± 3 | 11 ± 4 | 7 ± 1 | 5 ± 3 | 5 ± 3 | < LOD | 32 ± 8 | 250 ± 20 |
| Benzo(a)pyrene | 14 ± 3 | 18 ± 1 | < LOQ | 15 ± 3 | 15 ± 3 | < LOD | 13 ± 7 | 210 ± 20 |
| Dibenzo(a,h)anthracene | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOD | < LOD | < LOD |
| Benzo(g,h,i)perylene | < LOD | 40 ± 10 | < LOQ | < LOQ | < LOQ | < LOQ | < LOD | < LOD |
| Indeno(1,2,3)pyrene | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |

**Table A16. Comparison of the developed method for PAHs extraction using the included β-HPCD-silica xerogel with other methods in the literature.**

| Analytes[a] | Analytical technique | Sample treatment | Solvent | Recovery (%) | LOQ[b] (ng $L^{-1}$) | Reference |
|---|---|---|---|---|---|---|
| Naph, Ace, Flr, Phe, Ant, Flt, Pyr, B(a)A, Chry, B(b)F, B(k)F, B(a)P, DiB(a,h)A, B(g,h,i)Pe, I(1,2,3)P | GC-MS | SPME<br>Carbon nanotubes | Acetone-ACN<br>0.1 mL | 72 – 98 | 20 – 160 | (L. Guo & Lee, 2011) |
| B(a)A, B(b)F, B(a)P | HPLC-FLD | SPE<br>Carbon nanotubes and cryogel composite | Hexane 15 mL<br>Propanol 15 mL<br>ACN 5 mL<br>Methanol 1 mL | 89 – 98 | 17 – 27 | (Kueseng et al., 2010) |
| Naph, Ace, Flr, Phe, Ant, Flt, Pyr, B(a)A, Chry, B(b)F, B(k)F, B(a)P, DiB(a,h)A, B(g,h,i)Pe, I(1,2,3)P | HPLC-FLD | LLE | Hexane 77 mL<br>ACN 1 mL | 81 – 106 | 0.11 – 0.43 | (D. M. Brum, Cassella, & Pereira Netto, 2008) |
| Naph, Ace, Flr, Phe, Ant, Flt, Pyr, Chry, B(a)P | HPLC-UV | SPE<br>Porous metal membranes | $CH_2Cl_2$ 22 mL<br>Methanol 21 mL | 83 – 112 | 100 – 270 | (S. M. Xie, Zhang, Wang, & Yuan, 2011) |
| Naph, Ace, Flr, Phe, Ant, Flt, Pyr, B(a)A, Chry, B(b)F, B(k)F, B(a)P, DiB(a,h)A, B(g,h,i)Pe, I(1,2,3)P | HPLC-FLD | SPE<br>$C_{18}$ | $CH_2Cl_2$ 8 mL<br>Methanol 8 mL<br>ACN > 0.5 mL | 20 – 92 | 0.04 – 1 | (Bruzzoniti et al., 2010) |
| Naph, Ace, Flr, Phe, Ant, Flt, Pyr, B(a)A, Chry, B(b)F, B(k)F, B(a)P, DiB(a,h)A, B(g,h,i)Pe, I(1,2,3)P | HPLC-FLD | SBSE | ACN 0.2 mL | 24 – 87 | 0.3 – 30 | (Xiao et al., 2012) |
| B(a)A, B(b)F, B(k)F, B(a)P, DiB(a,h)A, B(g,h,i)Pe, I(1,2,3)P | HPLC-FLD | SPE<br>Included CD-silica xerogel | ACN 4 mL | 80 – 117 | 4 – 115 | Proposed method |

[a] Naphthalene (Naph), Acenaphthene (Ace), Fluorene (Fl), Phenanthrene (Phe), Anthracene (Ant), Fluoranthene (Flt), Pyrene (Pyr), Benzo(a)anthracene (B(a)A), Chrysene (Chrys), Benzo(b)fluoranthene (B(b)F), Benzo(k)fluoranthene (B(k)F), Benzo(a)pyrene (B(a)P), Dibenzo(a,h)anthracene (DiB(a,h)A), Benzo(g,h,i)perylene (B(g,h,i)P), Indeno(1,2,3)Pyrene (I(1,2,3)P).

[b] LOQ referred to sample.

From the study reported here, it can be concluded that the included CD-silica xerogels proposed are feasible alternatives to other sorbents used for the SPE of the target PAHs. The proposed procedure can be used to determine PAHs in drinking and environmental water with good selectivity and sensitivity, accomplishing the quality control of water in accordance with European Directives 2013/39/EU and 98/83/EC. Moreover, the LOQs defined are similar to those obtained using other analytical procedures described in the literature. The method designed has the advantage of reducing the use and toxicity of organic solvents than those commonly used for the treatment of samples. Also, the time required to perform the sample processing is significantly lower than that of most procedures reported in the literature.

Regarding the solid phase chosen, its main disadvantage may be the lack of anchoring of the CDs to the xerogel silica network. This phenomenon has been observed by a worsening of the results while the sample volume is increased, probably attributable to the leaching of CDs when working with aqueous samples (Gentili, 2020). Indeed, a possible explanation for the β-HPCD-silica xerogel working better in this case may be the chemical modification of the β-HPCD. Although, *a priori*, it might be expected that the solid phase containing γ-CD worked better due to the molecular size of PAHs (Table A11) and the size of the CD cavity (Table 2), the chemical modification of β-HPCD may probably cause a CD anchoring to the silica network rather than the expected inclusion, thus causing no leaching of CDs and, in this way, an improvement in the results obtained when working with water samples.

All these observations justify the study of bonded CD-silica solid phases to the extraction of PAHs from water samples, including both bonded β-CD-silica and γ-CD-silica xerogels.

### 3.3.2. Use of bonded β-CD-silica xerogels

The study of bonded β-CD-silica xerogels was carried out using the B1 phase described previously (Table A1). The possibility of using bonded CD-silica xerogels containing β-CD was assessed because the previous study showed that included CD-silica xerogels containing derivatives from β-CD (with the same molecular size in terms of their cavity) worked better for the SPE of PAHs. Thus, it is possible to conduct a comparison at the end of this fragment between included and bonded β-CD-based silica xerogels. The evaluation of bonded γ-CD-silica xerogels was left pending as a future study.

This time, not only the recoveries obtained but also the collected previously passed-through-the-cartridge water were measured and taken into account to

verify the proper retention of analytes during the optimization study, since PAHs losses may arise when passing the sample through the SPE cartridges due to their incomplete retention. It was concluded that the non-retained PAHs fraction in the SPE cartridge was not significant in all cases because the signal was under the LOQ in every experiment checked.

First, the amount of solid phase was optimized. To this, cartridges containing 100 mg, 200 mg, and 300 mg of material were prepared. Subsequently, 10 mL of a sample spiked at 8 µg $L^{-1}$ were introduced into the extraction cartridges, and PAHs were eluted with 8 mL of ACN. Recoveries obtained using 100 mg of solid phase were of 29, 30, 25, 30, 32, and 41%; using 200 mg they were 32, 28, 35, 41, 53, and 44%, and using 300 mg they were 29, 20, 36, 41, 48, and 65% for benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene, respectively. Therefore, results using 200 mg of solid phase were significantly better than those using other amounts excepting the case of benzo(g,h,i)perylene. For this reason, this amount of solid phase was selected to continue with the optimization study.

Since the previous study showed the possibility of increasing the sensitivity of the experimental procedure by evaporating the solvent after SPE and redissolving the residue again, this opportunity was also evaluated this time. To this, the elution solvent was evaporated at 60°C using a vacuum concentrator and the residue was redissolved in 500 µL of MeOH. Recoveries obtained for all the target analytes were 112 ± 4%, showing that no significant differences in the recovery were obtained. This indicates the possibility of carrying out the described concentration step. Besides, this post-concentration stage enabled the presence of indeno(1,2,3)pyrene to be detected, whose quantification is usually important due to its importance in the water legislation (2013/39/EU, 2013; 98/83/EC, 1998).

Then, the use of ACN as eluent was studied since it did not provide such high recoveries as expected. Methanol was proposed as an alternative since it is more environmentally friendly than other organic solvents, and hexane was also proposed because of its apolar nature. Cartridges containing 200 mg of solid phase were prepared and 10 mL of spiked sample with 8 µg $L^{-1}$ were placed inside them. The best recoveries were obtained with hexane for all analytes with the exception being benzo(g,h,i)perylene following the complete extraction and concentration procedure. Specifically, recoveries registered were of 56, 50, 63, 61, 68, 5, and 45% for benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, and indeno(1,2,3)pyrene. Oppositely, benzo(g,h,i)perylene registered a recovery of 66% when using methanol as elution solvent. For this reason, it was decided to

carry out the elution with 4 mL of MeOH and 4 mL of hexane previously to the post-concentration step. The decision of using 4 mL of each solvent was taken based on their elution profile, which indicated that most analytes were eluted in the first 4 mL of each solvent.

As mentioned, the salt addition to aqueous solutions can decrease the solubility of non-polar analytes in water and enhance the distribution of solutes to the studied solid phase. The influence of the ionic strength was studied from the recoveries obtained with NaCl 0 M, 2 M, and 4 M, preparing to this 10 mL of sample spiked with 8 µg $L^{-1}$ of PAHs. Results proved that the peak area of PAHs increased from NaCl 0 M to 2 M, and remained constant from NaCl 2 M to 4 M. Specifically, recoveries obtained using an ionic strength of 2 M were 38, 37, 43, 34, 50, 53, and 38% for benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, and indeno(1,2,3)pyrene.

**Table A17. Repeatability and concentration factor for the PAHs extraction method using the bonded β-CD-silica xerogel.**

| Compound | CV (%) | Concentration factor |
|---|---|---|
| Benzo(a)anthracene | 5 | 47 ± 3 |
| Benzo(b)fluoranthene | 4 | 49 ± 2 |
| Benzo(k)fluoranthene | 4 | 57 ± 2 |
| Benzo(a)pyrene | 8 | 54 ± 3 |
| Dibenzo(a,h)anthracene | 4 | 63 ± 2 |
| Benzo(g,h,i)perylene | 6 | 60 ± 3 |
| Indeno(1,2,3)pyrene | 7 | 44 ± 4 |

Finally, the influence of the sample volume and the concentration of analytes on recovery rates was studied. To this end, following the same experimental procedure described, 10, 25, and 50 mL of samples containing 1.2, 2.4, and 6 µg $L^{-1}$ of PAHs, respectively, and NaCl 2 M were introduced into the extraction cartridges. This was designed with the objective of keeping the absolute amount of analytes passing through the cartridge constant. The differences observed in the recoveries for different sample volumes can be due to the dispersion of the results and not to the volume influence. For example, benzo(a)anthracene, benzo(k)fluoranthene, benzo(a)pyrene, and indeno(1,2,3)pyrene showed recoveries of 42, 58, 55, and 43% when using 10 mL; of 51, 50, 53, and 39% when using 25 mL, and of 60, 55, 49, and 43% when using 50 mL. For this reason, it

was decided to use 50 mL of sample in each case in order to increase the limits of detection of the analytes, although the sample preparation time may also increase. Furthermore, recoveries obtained from spiked samples containing 3, 6, 12, and 24 µg $L^{-1}$ of PAHs did not differ significantly, being the major recovery differences between 10% in the case of benzo(k)fluoranthene and 6% in the case of benzo(g,h,i)perylene.

The analytical figures of merit established can be observed in Table A17 and Table A18. Thus, results indicate good repeatability of the designed method, with CVs below 8% in all cases. Following the optimized method, the concentration factor was calculated with the final corresponding recoveries and considering 50 mL of sample volume and 0.5 mL of extracting volume.

Besides, the method sensitivity was determined from the calibration line slope, and then LODs and LOQs were calculated, obtaining fine results. Specifically, the LOQs were lower than 1 µg $L^{-1}$ of PAHs in all cases with the exception being indeno(1,2,3)pyrene, whose chromatographic sensitivity is lower. Finally, the linearity range is also indicated in the following table.

**Table A18. Analytical figures of merit established for the PAHs extraction method using the bonded β-CD-silica xerogel.**

| Compound | Sensitivity (µV mL $mg^{-1}$) | LOD[a] (µg $L^{-1}$) | LOQ[a] (µg $L^{-1}$) | Linearity[b] (µg $L^{-1}$) |
|---|---|---|---|---|
| Benzo(a)anthracene | 76 ± 2 | 0.05 | 0.2 | 1.7 – 70 |
| Benzo(b)fluoranthene | 13.1 ± 0.3 | 0.2 | 0.5 | 7.5 – 70 |
| Benzo(k)fluoranthene | 82 ± 2 | 0.03 | 0.09 | 1.3 – 70 |
| Benzo(a)pyrene | 82 ± 2 | 0.04 | 0.11 | 1.3 – 70 |
| Dibenzo(a,h)anthracene | 24.4 ± 0.7 | 0.09 | 0.3 | 3.6– 70 |
| Benzo(g,h,i)perylene | 14.0 ± 0.5 | 0.2 | 0.7 | 7.7 – 70 |
| Indeno(1,2,3)pyrene | 3.1 ± 0.3 | 0.8 | 2.4 | 34.5 – 70 |

[a] LOD and LOQ are referred to the water sample.
[b] Linearity is referred to the measuring solution.

Results for the study of the matrix effect shown in Table A19 indicate that recovery rates using different matrices (river, well, rain, and salty water) were significantly affected. In this case, recoveries were calculated considering the concentration factor obtained previously, this is, considering the concentration factor as a starting point to obtain a 100% of recovery and evaluating the matrix effect from the increase or decrease of this value as a reference.

**Table A19. Matrix influence in PAHs recovery using the bonded β-CD-silica xerogel.**

| Compound | Recovery (%) | | | |
|---|---|---|---|---|
| | River water | Well water | Rain water | Salty water |
| Benzo(a)anthracene | 130 ± 20 | 156 ± 9 | 160 ± 9 | 115 ± 4 |
| Benzo(b)fluoranthene | 50 ± 11 | 54 ± 5 | 110 ± 10 | 96 ± 6 |
| Benzo(k)fluoranthene | 95 ± 11 | 121 ± 4 | 127 ± 11 | 111.4 ± 1.0 |
| Benzo(a)pyrene | 94 ± 12 | 129 ± 7 | 109 ± 12 | 91 ± 6 |
| Dibenzo(a,h)anthracene | 120 ± 20 | 146 ± 6 | 141 ± 16 | 104 ± 6 |
| Benzo(g,h,i)perylene | 128 ± 7 | 150 ± 5 | 145 ± 17 | 100 ± 2 |
| Indeno(1,2,3)pyrene | 83 ± 4 | 110 ± 5 | 80 ± 4 | 84 ± 2 |

* Recovery values of 100% correspond to the concentration factor obtained for each analyte to the method developed. Results in this table show the recovery increase or decrease when working with different matrices.

As can be seen, recoveries were generally affected upwards when using the different matrices studied with some exceptions. For example, benzo(b)fluoranthene in the river and well water cases, as well as some analytes in the salty water cases such as benzo(a)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene, decreased their recoveries. Moreover, it seems important to highlight that results obtained for the river water are in general lower when compared to the other water matrices, although differences were not significant at all. Oppositely, some recoveries increased notably such as those of benzo(a)anthracene in the well and the rain water cases, for example. In conclusion, the results of the study of the matrix effect indicated that the use of a standard addition calibration would be advisable due to the existing matrix influence for future applications of the method designed.

Finally, the proposed procedure was applied to the determination of the target PAHs in four water samples. As can be observed in Table A20, the analytes whose sample presence was richer were benzo(b)fluoranthene, benzo(k)fluoranthene, and benzo(a)pyrene, with the exception of the rain water sample. Most compounds detected in the analyzed samples were below the LOQs established. Some of the obtained results must be highlighted, as in the case of the rain sample. PAHs are compounds usually found in the atmosphere as pollution products and, for this reason, it was expected that the rain water collected them. As can be seen, this was not the case, probably due to their low solubility in water (Garcia Londoño et al., 2013). Besides, it seems surprising that the river water showed PAHs presence since it was collected in a natural and protected zone, rather isolated from pollution sources.

**Table A20. Analysis of PAHs in real samples using the bonded β-CD xerogel (μg $L^{-1}$, $\bar{x} \pm s$).**

| Compound | Túria river | Well water | Malvarrosa beach | Rain water |
|---|---|---|---|---|
| Benzo(a)anthracene | < LOD | < LOD | < LOQ (0.07) | < LOD |
| Benzo(b)fluoranthene | < LOQ (0.2) | < LOQ (0.3) | < LOD | < LOD |
| Benzo(k)fluoranthene | < LOQ (0.03) | < LOQ (0.06) | < LOQ (0.06) | < LOD |
| Benzo(a)pyrene | < LOQ (0.09) | < LOQ (0.09) | 0.15 | < LOQ (0.08) |
| Dibenzo(a,h)anthracene | < LOD | < LOD | < LOD | < LOD |
| Benzo(g,h,i)perylene | < LOD | < LOD | < LOQ (0.2) | < LOD |
| Indeno(1,2,3)pyrene | < LOD | < LOD | < LOD | < LOD |

Also, the concentration of benzo(a)pyrene was below 0.27 μg $L^{-1}$, the maximum permissible concentration in inland surface water, in all samples where it could be quantified (2013/39/EU, 2013). For the rest of PAHs regulated, results indicate that the total content of benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, and indeno(1,2,3)pyrene was below 0.1 μg $L^{-1}$ only in the rain sample case, in accordance with the minimum requirements for human drinking water established (98/83/EC, 1998). Anyway, any of the analyzed water samples are supposed to be consumed.

At this point, a comparison between the included CD-silica xerogel studied previously and the here assessed bonded CD-silica xerogel was carried out. Results can be observed in Figure A16. They confirm that, on the one hand, the bonded CD-silica material improves results for PAH retention in the included CD-silica xerogel and, on the other hand, no CD losses occur this time when analyzing aqueous samples treated with the present solid phase.

As it is shown in Figure A16, recoveries are in all cases higher for the bonded CD-silica material than those obtained for the included CD-silica material excepting the case of benzo(b)fluoranthene. Recovery improvements can be highlighted in the case of benzo(a)anthracene, benzo(k)fluoranthene, and especially in the case of indeno(1,2,3)pyrene, which has a lower sensitivity. In addition, it is important to highlight the higher precision obtained with the bonded CD-silica solid phase in comparison with the included one, obtaining lower deviations in the recovery results.

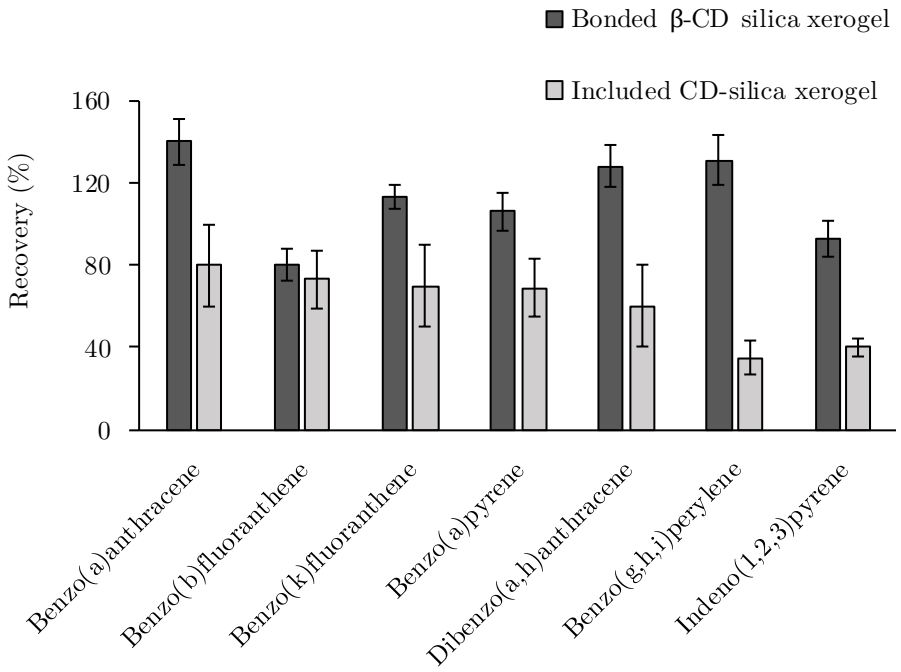



**Figure A16. Comparison of the included β-HPCD-silica xerogel and the bonded β-CD-silica xerogel for PAHs extraction from water samples.**

Moreover, UV spectra of the passed-through-the-cartridge water were measured and they supported the idea of the bonded material not presenting CD leaching during the extraction procedure, whereas the included material did present these losses, which can affect the signal observed during the quantification procedure.

In general, the bonded β-CD-silica material improves the previous results obtained for the included CD-silica xerogels. On the one hand, the PAHs retention and precision have been improved and, on the other hand, the material application in the SPE using aqueous samples was successfully conducted with no CD leaching occurring during the process in comparison with included materials. Furthermore, it was found that the bonded materials evaluated are reusable at least for three consecutive extractions, in comparison with the experiments carried out for the included CD-silica solid phases. In this way, the bonded β-CD-silica presents a great advantage.

From this study, it can be concluded that β-CD-silica xerogels are a good alternative to other sorbents used for the concentration of PAHs from water samples through SPE. The solid phase preparation and the construction of the cartridge are easy and relatively inexpensive. Recoveries obtained are comparable

to those found using other solid phase types in extraction procedures (Bruzzoniti et al., 2010; Kueseng et al., 2010; Payanan et al., 2013) being the analytical conditions also similar or even better in this case. Additionally, LODs and LOQs can be also compared with those obtained using different extraction procedures such as LLE, being important to highlight the lower use of organic solvents in this case. Also, the proposed procedure can be used to determine PAHs in different water types, including the control of natural and drinking waters in accordance with the regulated limits (2013/39/EU, 2013; 98/83/EC, 1998), as the LODs established are below the required values.

To conclude with this study, it seems important to highlight that the possibility of quantifying these analytes using method variations that permit increasing the sensitivity or selectivity such as varying the solid phase properties must be considered. For this reason, bonded γ-CD-silica xerogels were assessed.

### 3.3.3. Use of bonded γ-CD-xerogels

As mentioned, the extraction conditions for the retention and subsequent desorption of PAHs in the bonded γ-CD-silica material were re-optimized based on those already studied for the bonded β-CD-silica material.

First, the recoveries were evaluated using 150, 200, 250, and 300 mg of solid phase. In this case, the best results were obtained when using 200 mg of material (81, 91, 90, 86, 77, and 79% of recovery for benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, and dibenzo(a,h)anthracene, respectively). A very significant decrease in the extraction performance was observed when 250 mg and 300 mg of solid phase were used, which was understood as proper retention of the analytes followed by an incomplete elution, where a greater volume of organic solvent may be necessary to drag PAHs down. Therefore, 200 mg of solid phase were selected to carry out the SPE procedure.

Regarding the pH of the water sample, it did not influence the PAHs recovery, as expected, since these analytes do not possess acid-base properties.

Concerning the sample volume used, the extraction performance when using 10 mL was in all cases higher than the obtained by using 50 mL (the recovery decreased to 68, 59, 60, 56, 54, and 54% for benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, and dibenzo(a,h)anthracene, respectively). However, since it is advisable to use a greater volume when working with real samples due to the increase in the sensitivity that can be obtained, even if there are analyte losses, it was decided

to use 50 mL of sample with the only precaution of preparing the standards in the same conditions and treating them in parallel to the sample.

The loading capacity study showed that the concentration did not significantly influence the results obtained either using 4.5 or 20 µg $L^{-1}$, with the only differences observed in the recovery obtained being attributable to the typical variations during the experimental procedure. To our purpose, this is a great advantage since it allows performing a past-through-the-cartridge standard calibration, as previously recommended.

To improve the extraction of the target analytes, it was decided to review the nature and volume of the eluent used with respect to those working better in the case of the bonded β-CD-silica material. The study was carried out by independently eluting with 4 mL of ACN, 4 mL of MeOH, and 4 mL of hexane due to its apolar nature, subsequently evaporating at 60°C in the vacuum concentrator and redissolving the residue in 500 µL of methanol. Results showed that the best recoveries were obtained when acetonitrile and hexane were used as solvents, with values of around 60%, while the values for the use of methanol were somewhat lower, of around a 50%. Thus, it was decided to change in this case the extraction eluent to 4 mL of ACN followed by 4 mL of hexane. The decision of using 4 mL of each solvent was taken in based on their elution profile, which indicated that most analytes were eluted in the first 4 mL of both.

Results for the analysis of the matrix effect carried out with salty water showed a difference between the recoveries obtained using ultrapure water spiked with 4.5 µg $L^{-1}$ of PAHs (83, 71, 70, 68, 66, and 66% for benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, and dibenzo(a,h)anthracene, respectively) in comparison with the use of a salty sea water matrix (60, 51, 53, 48, 46, and 56% for the previously named analytes). Therefore, it was concluded that the matrix did influence the extraction performance, so, parallel to the previous cases, it is advisable the use of a standard addition method.

Besides, a reuse study was carried out. From it, it was concluded that there were no significant differences between the extraction carried out with a newly synthesized material and the extraction using a reused material. Indeed, results clearly indicated that cartridges are reusable at least twice, as expected.

Other parameters such as the ionic strength were not studied in this case because the mechanism affecting the improvement or the decrease in the recoveries obtained should be the same using either β-CD or γ-CD in the solid phase, so the conditions were maintained from the previous study.

Once the working conditions were optimized, the comparison study of the bonded β-CD-silica xerogel and the γ-CD-silica xerogel for PAHs concentration from water samples was conducted. Results are shown in Table A21. As can be seen, based on these results it can be concluded that the bonded γ-CD-silica material improved the extraction performance obtained when using the bonded β-CD-silica xerogel in recoveries between 10 and 40%.

This improvement can be attributed to the size of the PAHs molecules and the size of the hydrophobic cavity of both CDs. The average diameter of the target analytes is established between 0.8 nm and 0.9 nm (Table A11), while the β-CD cavity measures in average 0.70 nm and the γ-CD cavity measures in average 0.85 nm (Table 2). Therefore, it is logical to think that, in the case of the material containing β-CD, the PAH-cyclodextrin interaction occurring may be lower since, in most cases, the molecules do not completely fit inside the cavity, so only a part of them is positioned and interacts within. Oppositely, in the case of γ-CD, whose cavity is greater, all PAH molecules should, in principle, fit into it. Thus, when the analyte enters completely inside it, the interaction occurring must be greater, obtaining in this way greater retentions and also a higher extraction performance.

**Table A21. Comparison of the bonded β-CD-silica and the bonded γ-CD-silica xerogels for PAHs extraction from water samples.**

| Compound | Recovery (%) | |
|---|---|---|
| | Bonded β-CD-silica xerogel | Bonded γ-CD-silica xerogel |
| Benzo(a)anthracene | 47 ± 3 | 81 ± 9 |
| Chrysene | 51 ± 4 | 80 ± 10 |
| Benzo(b)fluoranthene | 49 ± 2 | 82 ± 7 |
| Benzo(k)fluoranthene | 57 ± 2 | 79 ± 6 |
| Benzo(a)pyrene | 54 ± 3 | 73 ± 7 |
| Dibenzo(a,h)anthracene | 63 ± 32 | 76 ± 7 |

This conclusion is supported by the previous water adsorption-desorption shown (Figure A10), which showed the fine working of the γ-CD material with water samples, as well as by other similar studies available in the literature (Tijunelyte et al., 2017). In them, it was concluded that the size between analytes and the CD cavities strongly correlates with their ability to engage in complexation. Thus, the selection of the CD type based on the size of the target analytes plays an important role in the inclusion complex formation as well as in the strength of the interaction between the molecules involved.

**Table A22. Analytical figures of merit established for the PAHs extraction method using the bonded γ-CD-silica xerogel.**

| Compound | CV (%) | | LOD[a] (ng $L^{-1}$) | LOQ[a] (ng $L^{-1}$) | Linearity[b] (µg $L^{-1}$) |
|---|---|---|---|---|---|
| | Intra-day | Inter-day | | | |
| Benzo(a)anthracene | 4 | 11 | 0.3 | 0.9 | 0.3 – 100 |
| Chrysene | 8 | 20 | 3 | 9 | 3 – 100 |
| Benzo(b)fluoranthene | 6 | 9 | 1.4 | 4 | 1.4 – 100 |
| Benzo(k)fluoranthene | 6 | 8 | 0.2 | 0.6 | 0.2 – 100 |
| Benzo(a)pyrene | 5 | 9 | 0.2 | 0.7 | 0.2 – 100 |
| Dibenzo(a,h)anthracene | 9 | 9 | 0.8 | 2 | 0.8 – 100 |

[a] LOD and LOQ are referred to the water sample.
[b] Linearity is referred to the measuring solution.

The analytical figures of merit for the use of the bonded γ-CD-silica solid phase are shown in Table A22, including the coefficient of variation, the limits of detection and quantification, and the linearity range. As it is observed, the repeatability of the method designed is acceptable, with CVs not exceeding 9% in the intra-day variations. However, the repeatability between different days worsened significantly, with coefficients of variation that, in the case of chrysene, reached 20%, probably due to its lower sensitivity. In all cases, the obtained LOQs were lower than 9 ng $L^{-1}$, which allows us to quantify very trace levels of PAHs in water samples. These quantities also allow evaluating the compliance with the legislation about the presence of PAHs in water (2013/39/EU, 2013; 98/83/EC, 1998). Besides, the chromatographic response of analytes is linear in all cases up to concentrations equal or greater than 100 µg $L^{-1}$ in the measuring solution.

**Table A23. Analysis of PAHs using the developed extraction method with the bonded γ-CD xerogel (ng $L^{-1}$, $\bar{x} \pm s$) and its validation.**

| Compound | Malvarrosa beach | | Valencia boat harbor | |
|---|---|---|---|---|
| | $C_{18}$ | Bonded γ-CD silica xerogel | $C_{18}$ | Bonded γ-CD silica xerogel |
| Benzo(a)anthracene | 88 ± 9 | 81 ± 4 | 203 ± 13 | 219 ± 14 |
| Chrysene | < LOD | < LOD | < LOD | < LOD |
| Benzo(b)fluoranthene | < LOD | < LOD | 247 ± 12 | 220 ± 20 |
| Benzo(k)fluoranthene | 102 ± 5 | 104 ± 2 | 198 ± 2 | 203 ± 7 |
| Benzo(a)pyrene | 103 ± 3 | 97 ± 3 | 181 ± 12 | 185 ± 3 |
| Dibenzo(a,h)anthracene | < LOD | < LOD | 185 ± 8 | 201 ± 2 |

Finally, the proposed extraction procedure was applied to determine PAHs from real water samples, and results were validated with those obtained using a reference method (Bruzzoniti et al., 2010). The values quantified can be observed in Table A23. As it is shown, the concentration of benzo(a)pyrene was lower than 270 ng $L^{-1}$ in both samples, which is the maximum allowable concentration of this compound in inland surface waters (2013/39/EU, 2013). However, it should be noted that a small increase in the concentration of this compound in the water of the Valencia boat harbor could lead to exceeding this maximum. Additionally, the total content of benzo(b)fluoranthene and benzo(k)fluoranthene does exceed the maximum of 100 ng $L^{-1}$ established for human drinking water (98/83/EC, 1998), meaning that, logically, the analyzed waters are not recommended for human consumption. Regarding the comparison of both methods, results obtained in both cases were comparable through the Fisher-Snedecor's test for the comparison of variances and the Student's test for the comparison of means at a 95% of confidence level. In short, the developed procedure supposes a lower investment of time and, in addition, a lower necessity of passing such high volumes of sample to achieve an adequate detection of the analytes in comparison with the reference method chosen.

**Table A24. Comparison of the method for PAHs extraction with the bonded γ-CD-silica xerogel with other methods in the literature.**

| Sample treatment | Sorbent | Recovery (%) | LOQ (ng $L^{-1}$) | Reference |
|---|---|---|---|---|
| 100 mL | Sulphur microparticles | 78 – 108 | 21 – 144 | (Khalili-Fard, Ghanemi, Nikpour, & Fallah-Mehrjardi, 2012) |
| 500 mL<br>Evaporation and reconstitution | $C_{18}$ | 20 – 92 | 27 – 520 | (Bruzzoniti et al., 2010) |
| 500 mL | Multi-walled carbon nanotubes | 66 – 122 | 6 – 25 | (Ma et al., 2010) |
| 50 mL<br>Evaporation and reconstitution | Bonded γ-CD-silica xerogel | 73 – 82 | 6 – 90 | Proposed method |

To end, Table A24 compares the characteristic features of the SPE procedure using bonded γ-CD-silica xerogels as a solid phase with other methods reported for PAHs concentration from water samples. In this context, our recovery values

and our LOQs were also comparable or even better than those found in the literature. Besides, the sample volume used in our case is smaller than other treatments proposed, permitting this a time saving. Nevertheless, the extraction solvent used (not only the nature but also the volume) as well as the larger process of evaporation and reconstitution step could be still improved and should, therefore, be acknowledged.

## 3.4. Conclusion

An improvement of the results obtained for PAHs concentration from aqueous samples using included CD-silica xerogels is here described through the assessment of new applications of bonded CD-silica xerogels. These solid phases give the possibility of adapting to the type of analyte under study by taking into account the type and size of cyclodextrin as well as to the medium in which analytes are contained, air or water, as proven through the adsorption-desorption studies performed described in Chapter 1. Thus, the bonded γ-CD-silica xerogel has also given the chance to improve the extraction performance of PAHs from water samples in comparison with the bonded β-CD-silica xerogel despite the low porosity that it seems to have for its use in air sampling (Figure A8 and Figure A9). This improvement can be explained by the larger size of the γ-CD cavity and the hydration of the solid phase when using aqueous samples, whose bases have been previously supported.

In short, a double improvement has been achieved in this chapter for the concentration of PAHs from water samples: first, from included β-CD-based to bonded β-CD-based silica xerogels, avoiding in this way CD leaching and permitting thus lower amounts of solid phase being used, and then, from bonded β-CD-based to bonded γ-CD-based xerogels, allowing in this sense a better adaption of the solid phase used to the target analytes chosen.

# 4

# Extraction of polychlorinated biphenyls from water

## 4.1. Introduction

Persistent organic pollutants (POPs) are organic compounds that are found virtually everywhere all around the world because of their resistance to environmental degradation and their facility to be transported by wind and water. They have been classified as high-priority compounds by the WHO (World Health Organization, 2020) and are of global concern due to their bioaccumulation and biomagnification through the food chain, with potential adverse impacts on health and the environment (Omwoma et al., 2019). Human exposure to POPs can lead, among others, to increased cancer risk, reproductive disorders, alteration of the immune system, neurobehavioral impairment, endocrine disruption, or increased birth defects.

Among the main POPs, polychlorinated biphenyls (PCBs) are widely known. Chemically, they are thermodynamically stable chlorine-substituted biphenyl rings and comprise up to 209 individual congeners, which can contain one to eight chlorine atoms (Helou, Harmouche-Karaki, Karake, & Narbonne, 2019; Wahlang, Hardesty, Jin, Falkner, & Cave, 2019). These compounds have been used since the 1930s as insulating fluids in electric equipment and as additives in sealants. Thus, their environmental presence comes from industries, improper disposal, leakage from landfills, incineration, volatilization, and harbor activities. Nowadays, PCBs can be therefore found in insulating oils, surface water, and human milk or serum (Helou et al., 2019; Raffetti et al., 2020) at trace level, being the most important sources for human contamination meat, fish, poultry, and other common products (Bandow, Conrad, Kolossa-Gehring, Murawski, & Sawal,

2020). Due to their toxicity and all-around occurrence, there is a need to control environmental samples to develop concrete policies in their management (Bandow et al., 2020) currently.

While low-chlorinated PCBs are often metabolized and eliminated by humans, highly chlorinated ones tend to bioaccumulation (Helou et al., 2019) and lead to health disorders. Thus, some PCBs are particularly known for their persistence. These are recognized as indicator PCBs and are assumed suitable as representative of the group, as they are the predominant congeners in biotic and abiotic matrices at the same time they represent different chlorination degrees. Thus, the seven indicator PCBs are PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, and PCB180 (Afful, Awudza, Twumasi, & Osae, 2013).

Most of the analytical techniques reported for the determination of pollutants in environmental samples are based on liquid chromatography and gas chromatography (Arenas, Martín, Santos, Aparicio, & Alonso, 2021), in most cases coupled to mass spectrometry (HPLC-MS and GC-MS) due to the high selectivity and sensitivity provided by this detection type. Specifically, GC is widely applied to determine volatile and thermally-stable pollutants such as pesticides or PCBs, being the electron-capture detector (GC-ECD) commonly used due to the enhanced selectivity it shows towards halogens (Atmaca, Das, Yavuz, & Aksoy, 2019). However, the assessment of these pollutants represents nowadays a challenge since they are usually difficult to determine due to their usual low concentrations. Therefore, developing analytical procedures that combine pre-concentration and low detection and quantification limits for environmental samples is nowadays a necessity.

A wide variety of methodologies have been developed and applied in the last years for the determination of PCBs in different types of samples as a sign of the environmental problem represented by them. For example, focused ultrasound extraction has been used to extract PCBs from toad liver tissue (Flores-Ramírez et al., 2017). Oppositely, SPE and DSPE were the selected techniques to concentrate them from the soil by using an aptamer-functionalized solid phase (S. Lin, Gan, Cao, Chen, & Jiang, 2016), from human serum by using commercial $C_{18}$ cartridges (Čonka, Drobná, Kočan, & Petrík, 2005), and from the air by using a commercial polyurethane foam (Birgül et al., 2017). It can be highlighted that SPME is one of the most popular methodologies to extract PCBs from fish, soil (F. Lv et al., 2017), and water (Y. Y. Wu, Yang, & Yan, 2014).

The use of materials based on CDs may be a feasible alternative to other sorbents based on the studies reported to this end concerning PCBs. Specifically, γ-CD showed to be capable of adsorbing and recovering a wide variety of PCBs

congeners from insulating oils through cooperative binding by plural cyclodextrin cavities (Kawano et al., 2014). Moreover, β-CD was also described as a proper choice to adsorb PCBs at room temperature through experimental evidence and density functional theory calculations (Manlin Wang et al., 2015). In this last research, results lead to think that these molecules prefer to enter the CD cavity from the wide rim. Besides, PCB28 seems to be the one with a better ability to completely enter the CD hydrophobic cavity. The rest of the PCBs under study accommodated in the cavity only with one phenyl ring, while the other part of the molecules is exposed outside owing to the steric hindrance of the chlorine atoms on 5, 5' sites.

Accordingly, the potential use of bonded CD-silica xerogels as SPE sorbents in a new environmental proposal for the extraction and subsequent determination of six indicator PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180) from water samples is assessed in this chapter. After an initial comparison with other solid sorbents containing not only different types of CDs (β- or γ-CD) but also different CD-silica ratios (Table A1) to evaluate the performance, the relevant features of the method have been established. Finally, real samples are analyzed and the results obtained are compared with those calculated with a reference method using commercial $C_{18}$ as sorbent.

## 4.2. Experimental

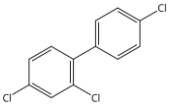
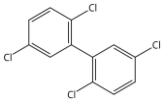
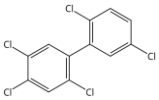
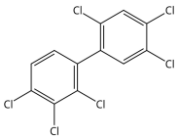
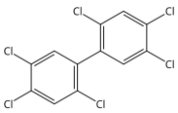
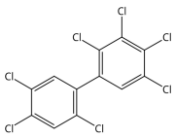
### 4.2.1. Reagents, materials, and instrumentation

A standard 10 mg $L^{-1}$ calibration mix of the six target PCBs in acetonitrile and quintozene, which was used as internal standard, were purchased from LGC Standards (Teddington, United Kingdom). The working solutions were prepared by dilution and stored at -18 °C. Other reagents such as NaCl (s) ≥ 99.5%, HCl (aq.) 37%, and NaOH (s) ≥ 98.5% were from Panreac AppliChem (Barcelona, Spain). Besides, all organic solvents used (HPLC quality, ≥ 99%) were acquired in Merck Millipore (Massachusetts, United States). To carry out the comparison with a reference method (Environmental Protection Agency, 1995), commercial 200 mg $C_{18}$ Varian Bond Elut cartridges from Agilent Technologies (California, United States) were used. To end, ultrapure water from an Adrona purification system (Riga, Latvia) was employed during the whole experimental procedure, and all samples were previously filtered with Nylon 0.45 µm filters from Sartorius Stedim Biotech (Göttingen, Germany).

A Vac Elut 20 connected to a vacuum pump model CKNF from Agilent Technologies (California, United States) and 3 mL polypropylene cartridges from Análisis Vínicos (Ciudad Real, Spain) were used during the SPE procedure.

Moreover, a miVac sample concentrator from SP Scientific (Warminster, United States) was used for solvent evaporation.

**Table A25. Physicochemical properties of the studied PCBs (Scifinder Scholar Database, 2021).**

| Compound | Structure (CAS) | Boiling point (°C) | Vapor pressure[a] (kPa) | logP[a] | Molecular size (nm) |
|---|---|---|---|---|---|
| PCB28 |  (7012-37-5) | 330 | $4.29 \cdot 10^{-5}$ | 5.71 | 0.845 |
| PCB52 |  (35693-99-3) | 345 | $1.70 \cdot 10^{-5}$ | 5.83 | 0.863 |
| PCB101 |  (37680-73-2) | 371 | $3.01 \cdot 10^{-6}$ | 6.44 | 0.879 |
| PCB138 |  (35065-28-2) | 400 | $4.04 \cdot 10^{-7}$ | 6.98 | 0.896 |
| PCB153 |  (35065-27-1) | 396 | $5.29 \cdot 10^{-7}$ | 7.04 | 0.896 |
| PCB180 |  (35065-29-3) | 424 | $6.86 \cdot 10^{-8}$ | 7.50 | 0.911 |

[a] Vapor pressure and logP measured at 25 °C.

PCBs were quantified during the optimization study by using a Thermo Scientific Trace GC Ultra gas chromatograph (Massachusetts, United States) equipped with an electron capture detector system (GC-ECD). The analytical column was an Agilent HP-5 (30 m x 0.32 mm x 0.25 µm film thickness) and the separation was carried out using nitrogen as carrier gas at a 1 mL $min^{-1}$ flow rate. The sample injection (1 µL) was done in splitless mode using the following temperature program: 60°C for 2 minutes followed by a ramp at 26°C $min^{-1}$ up to

190°C, and then a ramp at 5°C $min^{-1}$ to 270°C (hold 3 minutes). Under these conditions, a good resolution was achieved for all PCBs under study. Also, an Agilent 5977A gas chromatograph (California, United States) with mass spectrometry detection (GC-MS) was used to carry out the qualitative and quantitative analysis of the real water samples. In this case, helium was the carrier gas at a 0.7 mL $min^{-1}$ flow with the same conditions. The range used in SCAN mode was m/z 50 – 400, whereas ions m/z 237, 186, 292, 326, 360, 145 and 394 were used, respectively, for quintozene, PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 in SIM mode.

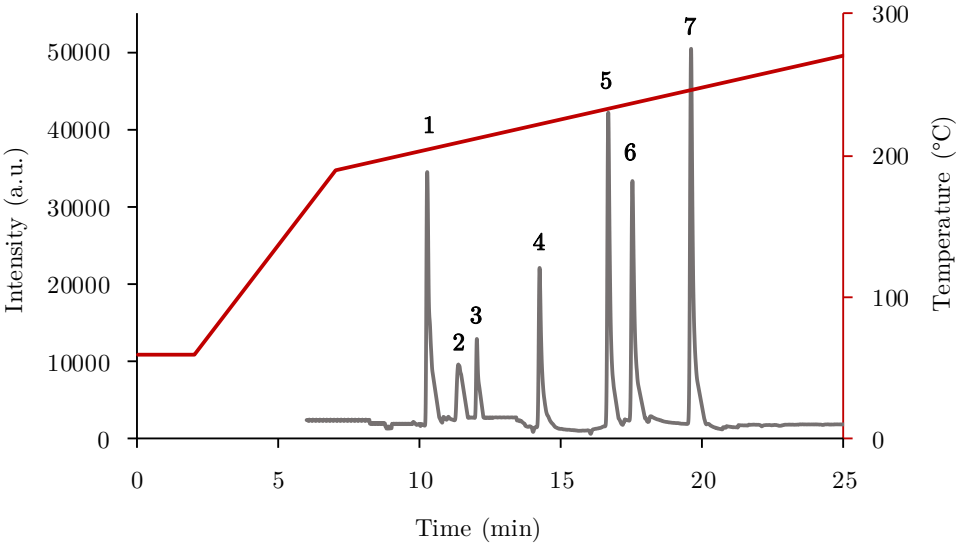



**Figure A17. GC-FID chromatographic profile of a multianalyte solution of the target PCBs containing the internal standard in acetone: (1) quintozene (2) PCB28 (3) PCB52 (4) PCB101 (5) PCB138 (6) PCB153 (7) PCB180.**

### 4.2.2. Optimization and validation of the SPE procedure

Several parameters can influence the concentration factor obtained by the application of a SPE procedure. For this reason, factors affecting the retention and elution of the six selected indicator PCBs were evaluated and optimized. The optimization study was carried out based on the recovery obtained from spiked ultrapure water samples at the µg $L^{-1}$ level. The recovery was calculated from the ratio between the obtained concentrations and the theoretical concentration in triplicates (n=3). In this sense, the procedure was optimized by varying one parameter at a time, while keeping the others constant. The studied parameters were those affecting the retention (type of solid phase, pH, ionic strength, washing

step, quantity of solid phase, sample volume, and influence of concentration) and the elution process (type of solvent, volume of solvent, and possibility of carrying out a post-concentration). In addition, the option of reusing the material was also evaluated. Finally, the matrix effect was assessed by spiking diverse real water matrices.

Once the SPE method was optimized, the analytical figures of merit of the optimized method were established with respect to linearity, sensitivity, and precision. This time, the limit of detection (LOD), the limit of quantification (LOQ), and the linearity were estimated following the latest IUPAC recommendations (Olivieri et al., 2006) with a confidence level of 95% and considering the LOQ as the lower limit of linearity. In this way, different calibration solutions were injected into the GC-ECD system to evaluate the linear range and the sensitivity of the method. In addition, the precision was assessed by studying the intra-day and inter-day repeatability. The intra-day precision was determined by analyzing five replicates within the same day, whereas the inter-day precision was established by analyzing three series of three independent extractions carried out on three different days.

Therefore, the extraction procedure designed was as follows. First, conditioning of the extraction cartridges is carried out with methanol (3 mL) followed by an equilibration step with water (5 mL). Then, a volume of 100 mL of water sample is filtered to remove any particulate matter and then suctioned at a 1 mL $min^{-1}$ flow rate through an extraction cartridge containing 400 mg of the B2 β-CD silica material (Table A1). Next, the cartridge is washed with 2 mL of water and dried at a high vacuum during 5 minutes. Analytes are eluted with 6 mL of acetone. The solvent is evaporated in a sample concentrator at 40°C and the residue is then re-dissolved in 1 mL of acetone. All solutions are filtered before their chromatographic use.

Finally, the recommended protocol was used to quantify the target PCBs in six real water samples (M1 to M6) coming from the effluent and influent of different wastewater treatment plants of the Valencian Community (Spain), and results were validated with those obtained using a reference method (Environmental Protection Agency, 1995). Importantly, a standard addition method combined with the addition of an internal standard in the quantification step must be used to avoid both the matrix effect and the instrumental error derived from the analysis of complex environmental samples.

## 4.3. Results and discussion

The optimization of the SPE procedure gave rise to the following results.

First, the study of the nature of the sorbent was evaluated by testing B1, B2, B3, G1, G2, G3 (Table A1), and the bare silica xerogel as a blank. To this end, cartridges containing 100 mg of each one were tested with 10 mL of water samples spiked at 10 µg $L^{-1}$ of PCBs. After the elution step, the best recovery efficiencies were reached for solid phases B2 and B3 (95, 90, 73, 71, 74, and 81% for PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180, respectively, compared to 86, 84, 74, 64, 65, and 68% for the same analytes using xerogel B1). These results showed that the presence of CDs as active complexing points in the material improved the performance of the solid phases, as expected. For this reason, material B2 was selected to continue with the study to reach a compromise between obtaining enough good recoveries and the quantity of CD used in the synthesis process.

Regarding the study of the pH, solutions with pH 4.5 (acetic/acetate 0.01 M buffer), uncontrolled pH, and pH 9 (ammonium/ammonia 0.01 M buffer) were studied with 10 mL of spiked water samples at 10 µg $L^{-1}$. Results indicated that the pH did not directly affect the retention of the target analytes, as expected, probably due to the lack of acid-base properties of PCB molecules. In short, results suggested that it was not necessary to control the pH of the sample.

The effect of the ionic strength was also studied. As it is well known, the salting-out effect can decrease the solubility of apolar analytes in the aqueous sample while enhancing their distribution coefficient into the sorbent. In this sense, NaCl concentrations of 0, 1, 2, 3, and 3 M were tested. Results indicated that, as long as the salt concentration increased, recoveries for all PCBs decreased. Specifically, recoveries of 90, 91, 78, 76, 79, and 70% were obtained for PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180, respectively, at a 0 M NaCl concentration, while recoveries of 35, 29, 17, 14, 17, and 18 were calculated for the same analytes at a 3 M NaCl concentration. Thus, the adjustment of the ionic strength was discarded. These results showed that the conductivity must be measured and evaluated when working with real samples since the natural ionic strength of the sample matrices may directly decrease the recovery of PCBs.

The use of an in-between washing step in order to clean undesired compounds coming from the water matrix was also studied. The washing step was carried out with 2 mL of ultrapure water in comparison with a non-washed cartridge. The washing did not influence recoveries, probably due to the successful formation of the inclusion complexes, which avoided water to affect PCBs retention. For this reason, the washing step should be carried out to eliminate matrix interferences.

The flow rate of the sample through the cartridges can influence the retention of PCBs. For this reason, the effect of using 1, 2, and 3 mL $min^{-1}$ flow rates in

the recovery was evaluated. The flow rate did not affect the recovery of PCBs. However, a flow rate of 1 mL $min^{-1}$ was chosen to ensure and ease the interaction PCB-sorbent.

Results for the loading capacity of the material are shown in Table A26. They were tested by passing through the cartridges 10 mL of water samples with rinsing concentrations of PCBs (10, 100, and 1000 µg $L^{-1}$). As can be seen, results obtained showed that the concentration did not influence the recovery of the analytes, obtaining values that were comparable between them (Harris, 2007) at a 95% of confidence level. These results suggested that the studied material can be properly used for the extraction of PCBs up to the higher limit.

**Table A26. Influence of the concentration of PCBs in the water sample in the recoveries obtained for their SPE using the bonded B2 β-CD-silica xerogel.**

| Compound | Recovery (%) | | |
|---|---|---|---|
| | 10 µg $L^{-1}$ | 100 µg $L^{-1}$ | 1000 µg $L^{-1}$ |
| PCB28 | 86 ± 5 | 90 ± 5 | 91 ± 2 |
| PCB52 | 88 ± 6 | 85 ± 4 | 86 ± 4 |
| PCB101 | 87 ± 4 | 81 ± 4 | 88 ± 3 |
| PCB138 | 71.6 ± 1.2 | 76 ± 3 | 76 ± 5 |
| PCB153 | 82 ± 6 | 81 ± 2 | 79 ± 4 |
| PCB180 | 73 ± 6 | 71 ± 2 | 76 ± 2 |

At this point, the nature of the eluent was tested. Ethyl acetate, acetone, methanol, acetonitrile, and hexane were assessed as eluents due to their mainly apolar nature and so their ability to displace the molecules of PCBs from the CD cavity. The best recoveries were obtained using acetone in all cases (96, 91, 74, 71, 73, and 73% for PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180, respectively) followed by the use of ethyl acetate (78, 77, 70, 55, 70, and 62% for the same mentioned PCBs. Thus, acetone was selected as the eluting solvent since it presents, in addition, lower toxicity than other organic solvents.

To determine the breakthrough volume, 10, 25, 50, and 100 mL of sample were tested by fixing the total amount of PCBs in the respective solutions. Results showed that recoveries maintained from 10 to 25 mL, but a decrease was observed with larger volumes. This fact makes sense since the sample can displace the retained analytes little by little. To improve the enrichment factor, 100 mL was chosen as sample volume and an increase in the solid phase quantity, as well as in the eluent volume, were subsequently tested.

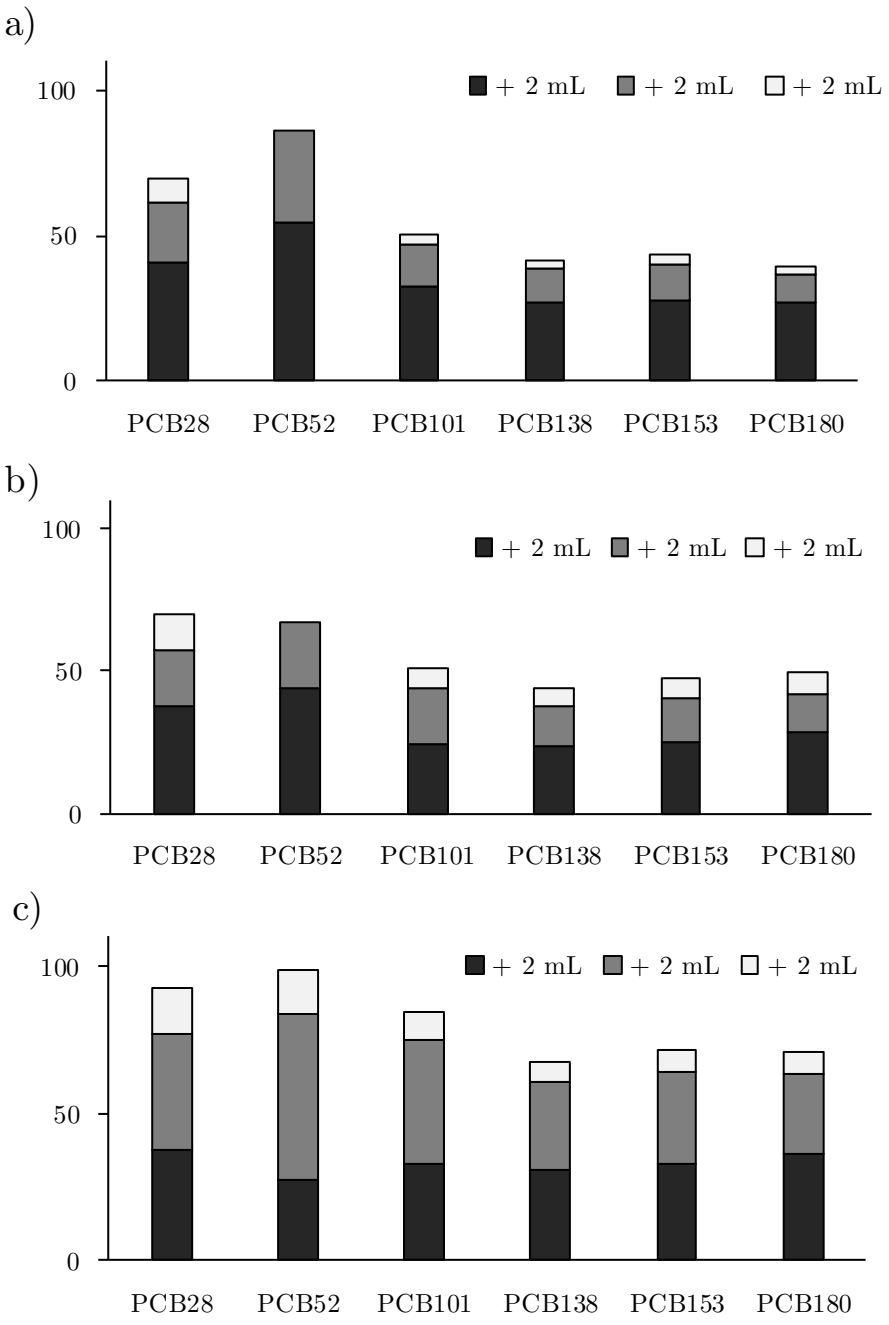



**Figure A18. Study of the amount of solid phase and volume of elution solvent on the recovery of PCBs during the optimization study: (a) 100 mg (b) 250 mg, and (c) 400 mg.**

The quantity of solid phase and the volume of eluent were studied at the same time since they can influence each other. The results obtained can be observed in Figure A18. Quantities of 100, 250, and 400 mg were tested whereas portions of 2 mL of eluent were used to assess the elution profile. From the results, it was concluded that 100 mL of water sample needed 400 mg of solid phase and 6 mL of elution solvent. In the following 2 mL portions, results obtained were in most cases under the LODs established.

Besides, the possibility of increasing the sensitivity of the method by evaporating the eluent and redissolving the residue in 1 mL of acetone was assessed. Acetone was evaporated at 40°C for 15 minutes in a sample concentrator. No significant differences in recoveries were obtained after evaporation. This indicated that there exists the possibility of carrying out this pre-concentration step. In fact, it was recommended due to the volatility of acetone, which can evaporate easily during the extraction and affect the repeatability of the results calculated.

To conclude, the reuse of the B2 material was also assessed. Recoveries remained constant for five different and consecutive uses.

In Chapter 1, an increase in the CD size induced a decrease in the porosity of the solid phases (Figure A8, Figure A9, and Figure A10), leading to lower surface areas and pore volumes determined from $N_2$ adsorption-desorption isotherms (Table A3) was observed. The porosity results also reinforced the idea that, when CD molecules are covalently bonded to the silica, a loss of porosity is observed when compared to silica materials containing included CDs. These previous observations suggested that higher sizes and rigidity of CDs cooperatively contributed to an apparent micropore closing towards $N_2$ at 77 K. Thus, these results invited to study if an increase in the CD content from B1 to the here used B2 material (ca. doubled content) also led to this behavior.

First, results for the adsorption-desorption of $N_2$ can be observed in Table A27. From them, it can be mentioned that the expected deportment of the B2 material was proven, showing it a significantly lower porosity to this gas than silica xerogels containing lower concentrations of bonded CDs (Table A3).

Conversely, the porosity of material B2 was appreciated by the adsorption of $CO_2$ at 273 K, whose size is slightly lower than that of $N_2$. This change seemed to be enough to open the sub-nanometric pores of the silica xerogels under study. The $CO_2$ adsorption-desorption isotherms are shown in Figure A19 in comparison with the adsorption of $N_2$. The material showed a typical isotherm of microporous materials. By doubling the amount of anchored CD, the adsorption of $CO_2$ was reduced from 39.2 to 14.5 $cm^3\ g^{-1}$ (Table A27) and the hysteresis cycle became

more pronounced. These facts suggested a more tortuous hindered $CO_2$ diffusion, probably ascribed to a higher density of anchored β-CD, which acts as an obstacle.

**Table A27. Comparison of the physical and textural parameters of bonded β-CD-silica xerogels containing different amounts of CDs.**

| Solid phase | Si/CD molar ratio | BET surface[a] ($m^2\ g^{-1}$) | Pore volume[b] ($cm^3\ g^{-1}$) | $CO_2$ adsorption[c] ($cm^3\ g^{-1}$ STP) | Surface[c] ($m^2\ g^{-1}$) | Pore volume[c] ($cm^3\ g^{-1}$) |
|---|---|---|---|---|---|---|
| Bare silica xerogel | - | 530.5 | 0.26 | 72.3 | 656.4 | 0.26 |
| B1 | 57 | 347.2 | 0.16 | 39.2 | 353.6 | 0.14 |
| B2 | 28 | < 1 | - | 14.5 | 128.5 | 0.05 |

[a] Determined through the BET model from the $N_2$ adsorption isotherm branches.
[b] Determined from the N2 adsorption isotherm.
[c] Determined from the $CO_2$ adsorption isotherms using the Dubinin-Radushkevich model.

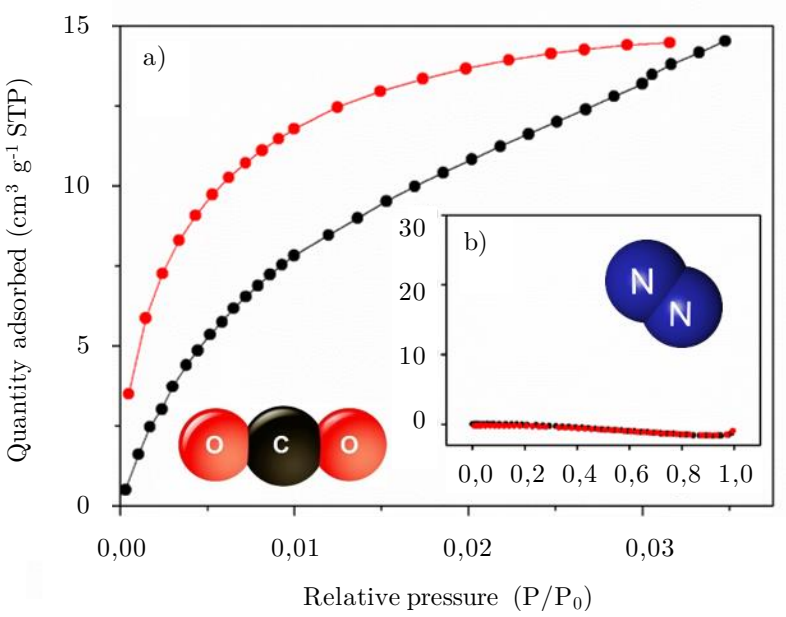



**Figure A19. Adsorption-desorption (a) $CO_2$ and (b) $N_2$ isotherms for the B2 material. Curves in black and red correspond to the adsorption and desorption isotherm branches, respectively. $N_2$ isotherms were acquired at 77 K, whereas $CO_2$ isotherms were measured at 273 K. Samples were previously degasified at 50°C during 15 hours at a $10^{-6}$ Torr pressure.**

Finally, the adsorption of water molecules progressed in a regular way versus the humidity degree (Figure A20). Although the covalent anchoring of higher amounts of β-CD strongly diminished the $N_2$ and $CO_2$ adsorption, its porosity seemed to be accessible for water (and, consequently, for the analytes in aqueous solution, as in this case). This behavior is similar to the one observed for bonded

γ-CD-silica xerogels described previously, whose successful application when working with aqueous samples was also proven in Chapter 3.

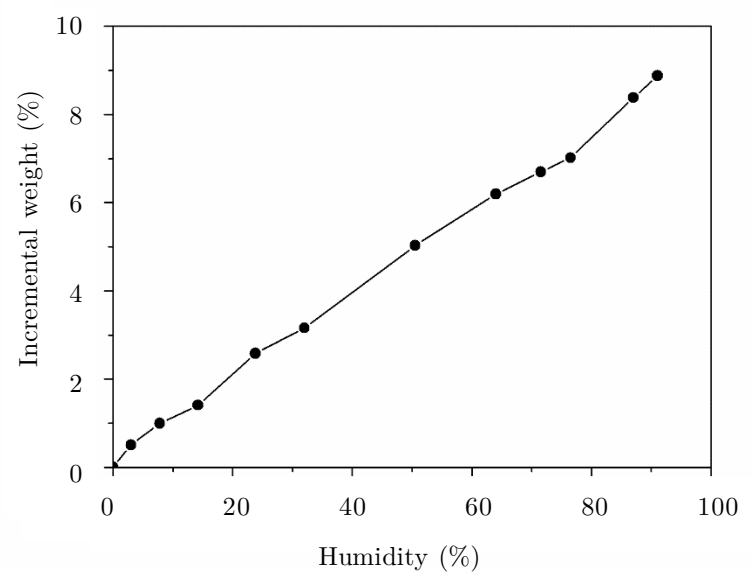



**Figure A20. Water adsorption curve for the B2 material. Isotherms were acquired at 37°C and the sample was previously dried to constant weight under 0% relative humidity.**

Regarding the analytical performance of the method for determining PCBs in environmental water, the analytical figures of merit are shown in Table A28. As can be seen, results indicated good precision, with coefficients of variation below 11% for intra-day repeatability and below 8% for the repeatability on different days. Concerning the sensitivity, LOQs were lower than 5 ng $L^{-1}$ for all PCBs by using the proposed extraction method combined with GC-ECD instrumentation. This allowed quantifying PCBs in the concentration ranges established by legislation, not only in the water policy field (2013/39/EU, 2013) but also for the food control (1881/2006/EC, 2006). Besides, good extraction efficiencies were achieved (between 79 and 91%), and enrichment factors for all the analytes were calculated by considering 100 mL of sample volume and 1 mL of final extract. They were also in the range from 78 to 91. Finally, the calibration curve obtained remained linear in concentrations > 2000 µg $L^{-1}$ in the measuring solution, which is quite acceptable since it covers the concentration range of interest to work.

Also, Table A29 shows a comparison of the characteristic features of the here developed method with other methods reported in the literature for the determination of PCBs in different samples. As can be observed, SPE is widely used to extract and determine PCBs (Čonka et al., 2005; X. Song et al., 2012). With respect to the analytical performance of the proposed method, recoveries

obtained were comparable to others in the literature since the recovery values reported covered our range of values. In this case, our LODs and LOQs were likewise comparable with the limits reported in the selected works, specifically with those using commercial solid phases (Čonka et al., 2005; X. Song et al., 2012). The main advantage of the developed material in comparison with commercial materials is that the presence of CDs can prevent the competition of apolar analytes with humidity and improve the adsorption step. In short, the proposed method constitutes a fast and simple extraction procedure for the subsequent determination of PCBs in aqueous samples. The sample treatment step was easier than other treatments proposed in the literature that use a temperature stage (X. Song et al., 2012) or less green solvents such as hexane or dichloromethane (Čonka et al., 2005). In this case, the solvent proposed for its use was acetone, which has a wider occupational limit exposure value in comparison with other proposed solvents that are more toxic. In addition, the evaporation and reconstitution step proposed can help to improve the sensitivity of the method (Birgül et al., 2017; Flores-Ramírez et al., 2017) by using more convenient water volumes. Finally, it can be highlighted that the easy and relatively cheap synthesis of the solid phase, which has proven to be reusable, together with the extraction procedure of PCBs from water, constituted a method supporting the main principles of green chemistry.

Finally, the matrix effect was studied by spiking water samples at 10 µg $L^{-1}$ and then calculating the recovery after the extraction. The conductivity was measured in all cases and it proved to be significantly higher than that of ultrapure water. Recoveries obtained were 84, 64, 59, 34, 31, and 37% for PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180, respectively. The presence of a matrix effect was also assessed by comparing the slope with a standard addition calibration. From the results, it was concluded that an environmental water matrix significantly affected the recovery obtained, probably due to their conductivity, which increases the ionic strength of the solutions under treatment among other parameters. For this reason, the determination of PCBs in these types of water must be carried out by applying a standard addition method.

The method was applied to the determination of PCBs in six real water samples to assess its applicability. Results are shown in Table A30. Among the target analytes, PCB138 and PCB153 were not detected in any of the samples. Besides, PCB101 was the one whose presence was most remarkable, while PCB28, PCB52, and PCB180 were only present in a few samples. In any case, all the PCBs were detected and quantified at trace level, as expected due to the environmental nature of the samples under study (M. Yu et al., 2019).

Table A28. Analytical figures of merit established for the PCBs extraction method using the B2 bonded β-CD-silica xerogel.

| Compound | CV (%) | | Recovery (%) | LOD[a] (ng $L^{-1}$) | LOQ[a] (ng $L^{-1}$) | Linearity[b] (µg $L^{-1}$) |
|---|---|---|---|---|---|---|
| | Intra-day | Inter-day | | | | |
| PCB28 | 6.1 | 5.2 | 91 ± 4 | 0.5 | 1.5 | 0.15 – 2000 |
| PCB52 | 7.2 | 1.6 | 79 ± 5 | 1.7 | 5 | 0.5 – 2000 |
| PCB101 | 11.3 | 5.6 | 78 ± 3 | 1.0 | 3 | 0.3 – 2000 |
| PCB138 | 11.0 | 5.1 | 82 ± 2 | 0.5 | 1.4 | 0.14 – 2000 |
| PCB153 | 5.5 | 5.4 | 83 ± 4 | 0.3 | 1.0 | 0.10 – 2000 |
| PCB180 | 8.0 | 8.1 | 81 ± 3 | 0.2 | 0.6 | 0.06 – 2000 |

[a] LOD and LOQ are referred to the water sample.
[b] Linearity is referred to the measuring solution.

**Table A29. Comparison of the developed method for PCBs extraction using the B2 bonded β-CD-silica xerogel with other methods in the literature.**

| Analytes | Sample | Extraction | LOD (ng $L^{-1}$) | Recovery (%) | Reference |
|---|---|---|---|---|---|
| PCB28, PCB52, PCB101, PCB105, PCB114, PCB118, PCB123, PCB138, PCB153, PCB156, PCB157, PCB167, PCB170, PCB180, PCB189 | Human serum | SPE $C_{18}$ | 1.2 – 5.1 | 99 – 120 | (Čonka et al., 2005) |
| PCB1, PCB5, PCB29, PCB47, PCB98, PCB154, PCB171, PCB201 | Water | SPME Commercial fibres (PDMS and CW-DVB) | 0.3 – 7.5 | 35 – 99 | (X. Song et al., 2012) |
| PCB28, PCB52, PCB72, PCB101, PCB106 | Soil | DSPE Aptamer-based adsorbent ($Fe_3O_4$@PDA@UiO-66-Apt) | 10 – 15 | > 80 | (S. Lin et al., 2016) |
| PCB18, PCB22, PCB28, PCB31, PCB41, PCB44, PCB49, PCB52, PCB54, PCB56, PCB60, PCB70, PCB74, PCB87, PCB90, PCB99, PCB101, PCB104, PCB105, PCB110, PCB114, PCB118, PCB123, PCB132, PCB138, PCB141, PCB151, PCB157, PCB158, PCB167, PCB170, PCB180, PCB183, PCB187, PCB188, PCB189, PCB194, PCB199, PCB203 | Air | Passive sampling PUF | 300 – 3000 | 88 – 105 | (Birgül et al., 2017) |
| PCB28, PCB52, PCB99, PCB101, PCB105, PCB118, PCB128, PCB138, PCB153, PCB180, PCB183, PCB187 | Liver toad tissue | Focused ultrasound-assisted extraction | 1500 – 3300 | 79 – 116 | (Flores-Ramírez et al., 2017) |
| PCB28, PCB52, PCB101, PCB136, PCB153, PCB180 | Water | SPE Bonded β-CD-silica xerogel | 0.2 – 1.7 | 78 – 91 | Proposed method |

Table A30. Analysis of PCBs using the extraction method developed with the bonded B2 β-CD-silica xerogel (μg $L^{-1}$, $\bar{x} \pm s$) and its validation.

| Compound | M1 | | M2 | | M3 | | M4 | | M5 | | M6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bonded β-CD-silica xerogel | $C_{18}$ | Bonded β-CD-silica xerogel | $C_{18}$ | Bonded β-CD-silica xerogel | $C_{18}$ | Bonded β-CD-silica xerogel | $C_{18}$ | Bonded β-CD-silica xerogel | $C_{18}$ | Bonded β-CD-silica xerogel | $C_{18}$ |
| PCB28 | < LOD | < LOD | 0.11 ± 0.02 | 0.31 ± 0.09 | < LOD | < LOD | < LOD | < LOD | 0.9 ± 0.2 | 1.0 ± 0.3 | < LOD | < LOD |
| PCB52 | < LOD | < LOD | < LOD | < LOD | 0.32 ± 0.07 | 0.7 ± 0.3 | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| PCB101 | 2.4 ± 0.4 | 1.7 ± 0.4 | 2.3 ± 0.3 | 1.8 ± 0.3 | 1.4 ± 0.3 | 1.2 ± 0.2 | 1.57 ± 0.12 | 1.3 ± 0.3 | < LOD | < LOD | 0.88 ± 0.11 | 0.76 ± 0.07 |
| PCB138 | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| PCB153 | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| PCB180 | 0.8 ± 0.3 | 0.52 ± 0.06 | < LOD | < LOD | 2.3 ± 0.3 | 2.3 ± 0.3 | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |

Regarding the validation of the developed method in comparison with an alternative one chosen as a reference (Environmental Protection Agency, 1995), it should be noted that the overall recovery of analytes in spiked water samples by using this reference procedure was in the range from 35 to 70% due to the also existing matrix effect, as expected. Besides, its precision was calculated on the same day and on different days, with coefficients of variation between 5 and 20%. As can be seen, the repeatability of the reference method was worse in comparison with the here proposed methodology. Finally, it was also noteworthy that results for the analysis of wastewater samples with both methods were comparable among them by using the paired t-test for comparing individual differences (Harris, 2007) with a 95% of confidence level (Table A30).

## 4.4. Conclusion

The determination of PCBs in environmental water is of great importance, not only to bring new knowledge to the state of the art regarding extraction and concentration methodologies but also to support the necessity of monitoring and eliminating them in the framework of the management plan against PCBs by 2028 agreed at the Stockholm Convention.

The novelty and advantages of this study can be highlighted in terms of the already described synthesis of the bonded CD-silica solid phase used, which is easy, cheap, and environmentally sustainable due to the ability to be reused, and of the improvement of the experimental procedure in comparison with other existing methods with the same end. Specifically, the amount and toxicity of organic solvents and the time consumed are some of the features improved. In addition, the sensitivity of the method permits quantifying PCBs at very trace levels, reaching concentration values analogous to those established by legislation both in the water policy (2013/39/EU, 2013) and in the food control (1881/2006/EC, 2006) fields. Regarding the disadvantages observed, the selectivity of the method is quite acceptable in comparison with the use of other commercial solid phases, but it lies basically in the chromatographic separation step since GC-ECD and GC-MS have been used. As it is well known, organic and inorganic analytes that have an acceptable geometry and polarity can act as guest molecules with CDs. In this sense, the bonded CD-silica xerogel studied is likewise able to this approach regarding PCBs, which improves the selectivity of the SPE procedure. However, it is not entirely selective for the PCBs' family or a selected group of them (S. Lin et al., 2016).

Due to the proven capability of bonded CD-silica xerogels to be applied to the analytical determination of high-concern substances from environmental matrices,

also reaching enough sensitivity to be applied in food control for the established thresholds of several pollutants, as demonstrated here, the possibility of expanding their application field through the sorption of substances of interest from biological matrices that may be nowadays of notice was established as the last objective to verify the applicability of the CD-silica xerogels designed.

# 5

# Determination of synthetic cannabinoids in oral fluids

## 5.1. Introduction

New psychoactive substances (NPS) are mainly non-regulated chemical compounds whose effects on consumers are similar to those described in the 1961 Single Convention on Narcotic Drugs or the 1971 Psychotropic Substances Convention (United Nations, 2020; UNODC, 2021). Also referred to as *bath salts* or *legal highs* (Graziano, Anzillotti, Mannocchi, Pichini, & Busardò, 2019), NPS are found every year. A study carried out in the 2019-2020 New Year's Eve found up to 16 NPS in wastewater in eight different countries (Bade et al., 2021), showing that their ingesting among the population is a reality. The unawareness of their long-term effect on human health due to their only recent usage (Sumnall et al., 2011) together with their growing variety are nowadays a major concern.

Different classifications have been made on NPS. The EUROPOL divided them into 12 categories (EMCDDA-Europol, 2018) based on their composition, while the United Nations made an 8-group distribution based on their psychoactive effect (UNODC, 2020). The subgroup including a higher variety of structures is *synthetic cannabinoids*, where different generations arise as different chemical changes are applied to them. Advice on the third generation of synthetic cannabinoids was published in December 2014 by the Advisory Council on the Misuse of Drugs (Advisory Council on the Misuse of Drugs, 2014).

Third-generation synthetic cannabinoids are able to act as cannabinoid receptor agonists such as $\Delta^9$-THC, binding to CB1 and CB2 receptors of the brain (Cohen & Weinstein, 2018). However, while the main active compound found in

cannabis does not completely bind to the abovementioned receptor, some of these cannabinoids do (Cohen & Weinstein, 2018), creating a more unpredictable effect resulting from their consumption. For this reason, there is a need of developing approaches that allow for their isolation in complex matrices so that proper identification and quantification can be made (J. P. Smith, Sutcliffe, & Banks, 2015).

The most widely used separation techniques for NPS quantification (Favretto, Pascali, & Tagliaro, 2013; Uchiyama, Kikura-Hanajiri, Ogata, & Goda, 2010; Wohlfarth & Weinmann, 2010) are liquid and gas chromatography usually coupled to mass spectrometry detection (HPLC-MS and GC-MS). Some piperazines have also been analyzed in rat plasma using fluorescence detection (Wada et al., 2012) taking advantage of the fluorescent properties of these ring-structured substances. Another very common approach for their analysis is ion mobility spectrometry (IMS), which permits fast quantifications at low concentration levels (Armenta et al., 2015; Sorribes-Soriano, de la Guardia, Esteve-Turrillas, & Armenta, 2018) using high-resolution mass spectrometry for screening methods (Yanini, Esteve-Turrillas, de la Guardia, & Armenta, 2018). However, a previous removal of interfering substances may be important to carry out an accurate quantification in this case, being a multianalyte determination difficult.

Besides, different biological matrices such as oral fluids, blood, urine, or hair can be studied to establish NPS concentrations in them (Favretto et al., 2013; Ong et al., 2020; Seywright et al., 2016; Sorribes-Soriano, Verdeguer, Pastor, Armenta, & Esteve-Turrillas, 2021; Staeheli et al., 2019). However, synthetic cannabinoids are rapidly metabolized and the parent compounds are rarely detected in urine. Moreover, misidentification of synthetic cannabinoids with similar molecular structures may occur because they provide similar metabolites. Oppositely, a more common occurrence of parent compounds has been observed in the direct analysis of synthetic cannabinoids in serum, blood, and oral fluids (Sorribes-Soriano et al., 2021). Since the typical intake of these compounds is in the low mg range, very low concentrations are expected, requiring them the use of highly sensitive procedures.

In this sense, sorption techniques (Sorribes-Soriano et al., 2021) such as SPE are a commendable way for separating and pre-concentrating these target analytes. Indeed, when working with oral fluids, SPE has been used to isolate NPS using molecularly imprinted materials (Sorribes-Soriano, Esteve-Turrillas, Armenta, de la Guardia, & Herrero-Martínez, 2017; Sorribes-Soriano, Esteve-Turrillas, Armenta, Amorós, & Herrero-Martínez, 2019) or non-polar commercial

cartridges (Y. Lu, O'Donnell, & Harrington, 2009; Sundström et al., 2013) as sorbents. Thus, designing new materials for their application in SPE procedures is a challenge that must be overcome.

In this context, CDs emerge as a potential tool since they have been already applied in separation chemistry to encapsulate a variety of analytes from complex matrices, including biological fluids (Moon, Kim, et al., 2008; Zhou & Zeng, 2006). Cyclodextrins have also proven to form selective inclusion complexes with NPS. Some examples are those host-guest interactions formed with chiral amines, allowing for their separation with capillary electrophoresis (Hägele, Hubner, & Schmid, 2020; Řezanková, Kohoutová, Kuchař, Král, & Řezanka, 2018), and with natural cannabinoids (P. Lv et al., 2019). Therefore, CDs may constitute a feasible alternative to those already existing for the sample clean-up and extraction techniques of NPS from oral fluids.

This study aimed to assess the potential use of bonded CD-silica xerogels as sorbents for the solid-phase extraction of third-generation synthetic cannabinoids (ADB-CHMICA, MMB-CHMICA, and MDMB-CHMZCA) in human oral fluids. The incorporation of CD units to the silica structure results appropriate to enhance the selectivity of the sample treatment procedure and as a clean-up step. Although these materials have been used to pre-concentrate trace pollutants from different environmental samples, their application with clean-up purposes in biological fluids to encapsulate NPS has not been reported to date. To this end, an extraction procedure has been developed and its main analytical features have been established to evaluate the performance of the method. Then, field synthetically-spiked oral fluid samples have been analyzed following the experimental procedure developed.

## 5.2. Experimental

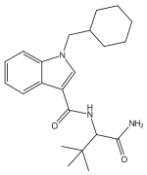
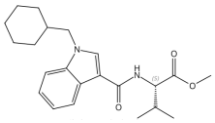
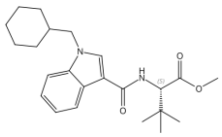
### 5.2.1. Reagents, materials, and instrumentation

ADB-CHMICA, MMB-CHMICA, and MDMB-CHMZCA standards were provided by the Unidad de Inspección de Farmacia y Control de Drogas (Valencia, Spain). Then, standard working solutions were prepared in methanol and stored at -18°C in amber glass vials.

Ultrapure water from an Adrona (Riga, Latvia) purification system was employed. Solvents used in extraction and analysis procedures were of HPLC grade quality in every case. Thus, methanol ≥ 99.8%, ACN ≥99.9%, and 2-propanol ≥99.8% were bought from Panreac AppliChem (Barcelona, Spain). Other reagents such as NaCl (s) ≥99.5%, HCl (aq.) 37%, and NaOH (s) ≥ 98.5%

were also acquired from this supplier. Buffer constituents were obtained from Scharlab (Barcelona, Spain). They were prepared from sodium acetate (pH 4.5), dipotassium hydrogen phosphate (pH 7.0), and ammonium chloride (pH 9.5) salts.

**Table A31. Physicochemical properties of the studied third-generation synthetic cannabinoids (Scifinder Scholar Database, 2021).**

| Compound | Structure (CAS) | Boiling point (°C) | Vapor pressure[a] (kPa) | logP[a] | Molecular size (nm) |
|---|---|---|---|---|---|
| ADB-CHMICA |  (2221100-70-3) | 630 | $1.11 \cdot 10^{-16}$ | 4.27 | 1.38 |
| MMB-CHMICA |  (1971007-94-9) | 573 | $5.29 \cdot 10^{-14}$ | 4.50 | 1.40 |
| MDMB-CHMZCA |  (1971007-95-0) | 577 | $3.58 \cdot 10^{-14}$ | 4.91 | 1.49 |

A Vac Elut 20 connected to a vacuum pump CKNF model from Agilent Technologies (California, United States) and 3 mL polypropylene cartridges from Análisis Vínicos (Ciudad Real, Spain) were used during the SPE procedure. In addition, a miVac sample concentrator from SP Scientific (Warminster, United States) was utilized for solvent evaporation. All samples were filtered with 0.45 mm Sartorius Stedim Biotech nylon filters (Göttingen, Germany).

Field oral fluid samples were obtained by expectoration into 15 mL Falcon conical tubes from volunteers who provided their consent after appropriate information about the study following the ethical guidelines established by the University of Valencia on drug analysis in biofluids (H1454687358321). Nine oral fluid samples from different volunteers were collected. Three of them were used as real matrices for recovery studies in the evaluation of the extraction conditions and six of them as field spiked oral fluid for the sample analysis part.

The optimum excitation and emission wavelengths of the analytes were established using a Jasco V-650 spectrophotometer and a Jasco FP-750 spectrofluorometer (Madrid, Spain). The best conditions (Figure A21) for all three analytes were similar between them.

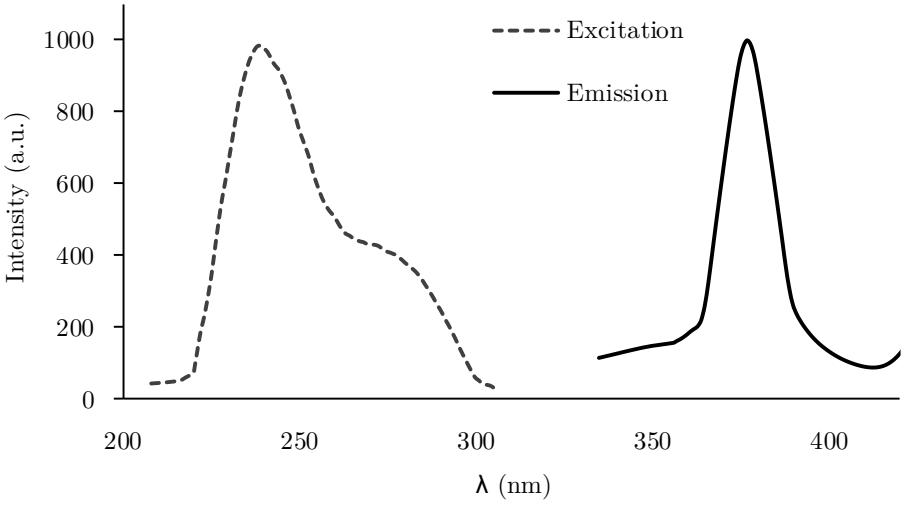



**Figure A21. Maximum excitation and emission wavelengths measured for the target synthetic cannabinoids under study.**

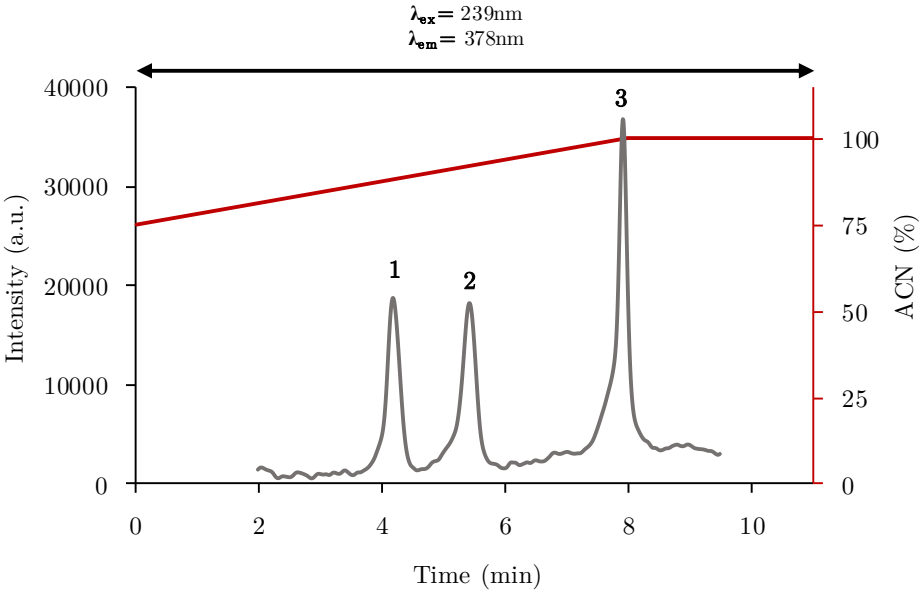



**Figure A22. HPLC-FLD chromatographic profile of a multianalyte solution of third-generation synthetic cannabinoids in methanol: (1) ADB-CHMICA (2) MMB-CHMICA (3) MDMB-CHMZCA.**

The analytes separation and quantification were done with an LC-2000 Plus liquid chromatograph equipped with an FP-2020 Plus Intelligent Fluorescent Detector, a PU-2089 Plus Quaternary Gradient Pump with integrated degasser, and an I/FLC/NetII/ADC interface from Jasco (Madrid, Spain). The injection volume was 20 µL in a six-way injection valve from Agilent Technologies (California, United States). The stationary phase used was a Kromasil $C_{18}$ column (150 x 4.6 mm, 5 µm particle size) from Análisis Vínicos (Ciudad Real, Spain). The separation was carried out at 1 mL $min^{-1}$ using a gradient-mode ACN:$H_2O$ mobile phase (from 75% to 100% ACN in 8 minutes). The fluorometric detection was carried out at a $\lambda_{ex}$ = 239 nm and a $\lambda_{em}$ = 378 nm.

### 5.2.2. Optimization and validation of the SPE procedure

The extraction procedure was assessed by studying those parameters that may influence the recovery obtained through a SPE procedure. They were evaluated by varying one parameter at a time while keeping the rest constant. Therefore, the study was carried out based on the recovery obtained from ultrapure water spiked with 10 µg $L^{-1}$ and it was calculated from the ratio between the obtained concentrations and the theoretical concentration in individual triplicates (n=3). The studied parameters were those affecting the retention (type of clean-up and extraction procedure, type of solid phase, pH, ionic strength, intermediate cleaning, and influence of the concentration) and the elution step (type of solvent, volume of solvent, and post-evaporation step).

Besides, the existing matrix effect was evaluated by spiking oral fluid samples in the same way as water to establish the recovery obtained.

The analytical performance of the final protocol was evaluated concerning the global recovery, precision, limits of detection (LODs) and quantification (LOQs), and linearity. The lower limit of the linearity range was defined for each analyte from the LOQ. Therefore, the analytical figures of merit were estimated according to the recommendations of the IUPAC (Olivieri et al., 2006).

Finally, the extraction of synthetic cannabinoids from oral fluid samples was carried out as follows. First, 150 mg of the bonded β-CD-silica xerogel are placed inside a 3 mL SPE cartridge with both top and bottom frits, being then conditioned with 2 mL of methanol and 5 mL of ultrapure water. Samples are initially centrifuged at 3500 rpm for 15 minutes to remove solids that may provide clogging. Then, 1 mL of sample is taken and weighed, and the final volume is completed to 10 mL by adding ultrapure water buffered at pH 7.0 with 1 mL of a 1 M $HPO_4^{2-}/H_2PO_4^-$ mixture. The final solution is loaded into the cartridges at 1 mL $min^{-1}$, washed with 3 mL of water, dried for 5 minutes, and then eluted with

2 mL of 2-propanol. Extracts are filtered. After concentration by evaporation at 60°C for 15 minutes and the subsequent recomposition with 500 µL of 2-propanol, HPLC-FLD determination is carried out.

### 5.3. Results and discussion

The first step of the optimization was to assess the influence of the type of sorbent in the retention of the target analytes during the extraction procedure. Based on the results obtained in Chapter 4, the bonded B2 β-CD-silica xerogel and the G2 γ-CD-silica xerogel, as well as the bare silica sorbent, were tested (Table A1) in this case. To this, SPE cartridges containing 150 mg of each solid phase were constructed in 3 mL extraction cartridges, and 10 mL of ultrapure water were spiked in each case with 10 µg $L^{-1}$ coming from a multianalyte solution. After the loading, washing, and drying steps, the elution was carried out with 3 mL of methanol. Besides, the water was collected after passing through the cartridge in order to check the retention together with the extraction performance achieved following this procedure. Results showed an improvement in the recovery from the bare silica sorbent to the xerogels containing cyclodextrin (Figure A23). Specifically, recoveries for the first one were around 60%, while recoveries for both the bonded β-CD-silica and the γ-CD-silica xerogels were 90% for the three analytes. The missing fraction of analytes was detected and quantified in the water. The recoveries obtained supported the idea of the CD influencing the efficiency and selectivity of the extraction through the interaction with the analytes under study (Hägele et al., 2020; Řezanková et al., 2018). However, contrary to what was observed in previous chapters, the size of the CD did not appear to influence the results obtained.

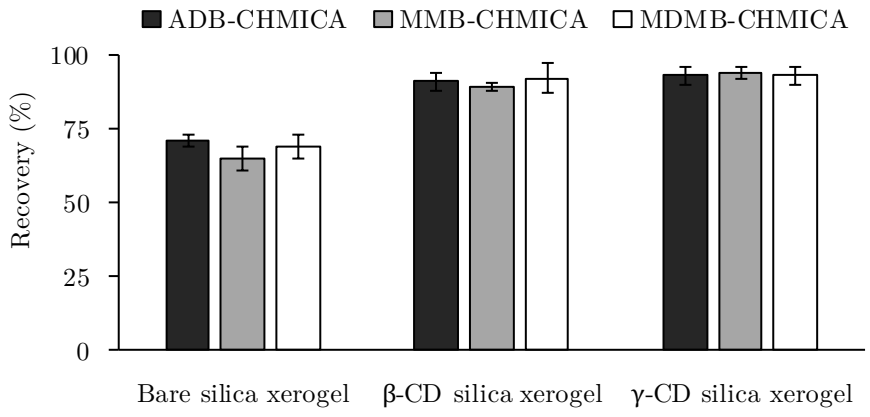



**Figure A23. Influence of the type of bonded CD-silica xerogel used on the recovery of the target synthetic cannabinoids.**

The most probable explanation for this phenomenon may be the higher size of the analytes under study (Table A31) with respect to the β-CD and the γ-CD cavities (Table 2). This would provoke that the interactions analyte-cyclodextrin are not mainly governed in this case by the host-guest interactions of the complete molecules, but they are based on different parts of the analytes' molecules interacting with CDs. For example, other interactions analyte-cyclodextrin such as an engagement with CD's external hydroxyl groups may also take place, as demonstrated previously (L. X. Song, Wang, Yang, & Xu, 2007). Therefore, the equal facility of formation of inclusion complexes of this hypothetic part of the cannabinoids molecules with β- and γ-CD may be a possible explanation. In any case, these results support the hypothesis of the presence of accessible cyclodextrin units onto the silica structure influencing the extraction performance observed. Due to the more affordable price and higher versatility of β-CD, the material containing the first one was selected to continue with the study.

The type of extraction was the next parameter to assess. To it, SPE and DSPE were evaluated. The conditions used to assess SPE were the same ones as the abovementioned, using 150 mg of the selected sorbent. To carry out the dispersive solid-phase extraction, 10 mL of ultrapure water were spiked with a multianalyte 10 μg $L^{-1}$ concentration and placed into a beaker. Then, 150 mg of the bonded β-CD-silica solid phase were added to it. The mixture was stirred for 30 minutes and loaded into 3 mL of extraction cartridges to separate the aqueous part from the sorbent used. After washing and drying, the elution was carried out with 3 mL of methanol. Results worsened for the use of the DSPE in comparison with the use of SPE. Specifically, recoveries in the first case were between 40 and 50%, whereas recoveries in the second case maintained at around 90%. This result indicates that the contact between the sample and the solid phase needs to be close, a characteristic that classic SPE provides to a higher extent compared to DSPE. For this reason, SPE was chosen as the extraction technique.

The loading pH may not be a critical parameter because physiological pH is not expected to provoke any protonation or deprotonation of the evaluated synthetic cannabinoids (Sorribes-Soriano et al., 2021). Nevertheless, 10 mL of spiked water was buffered at pH 4.5, 7.0, and 9.5 and treated following the same extraction procedure. Results obtained for pH 4.5 and 7.0 were comparable between them, being the recoveries calculated of 95, 98, and 96% for analytes ADB-CHMICA, MMB-CHMICA, and MDMB-CHMZCA, respectively. However, results for pH 9.5 were slightly lower, with recoveries in the 75 – 85% range. This behavior may be explained by taking into account the sensitivity of the silica

structure of the sorbent to basic pHs. In view of the recoveries observed, pH 7.0 was selected for being the closest to the expected physiological pH.

The increase of the ionic strength through salt addition can decrease the solubility of analytes in water and enhance the distribution coefficient of solutes to the cyclodextrins in the studied material. The influence of ionic strength was studied by adding sodium chloride at 0 M, 1 M, 2 M, and 3 M to the 10 mL of the spiked sample under study. All the recoveries obtained for the three target analytes were between 85 and 100%, existing no significant differences between them. Thus, the ionic strength did not show an important influence in the retention of NPS in this case.

Regarding the intermediate cleaning, a more aggressive washing may be beneficial for the removal of interfering compounds. For this reason, after charging the cartridges with 1 mL of the oral fluid sample completed to 10 mL with water and spiked at 10 µg $L^{-1}$, washings consisting of 3 mL of solutions containing 100% $H_2O$, 90:10 $H_2O$:2-propanol, 80:20% $H_2O$:2-propanol, and 70:30% $H_2O$:2-propanol were tested. Unfortunately, a decrease in the recovery obtained was observed while the proportion of 2-propanol increased (from around 80% when using 100% of water to around 40% when using 70:30% $H_2O$:2-propanol). However, taking into account the high selectivity already observed together with the high-selective detection technique being used, it was decided that the intermediate washing with 100% water was enough to clean the matrix.

Methanol and 2-propanol were evaluated as elution solvents. Comparable recoveries were obtained for both of them. However, 2-propanol was finally chosen as elution solvent due to its lower toxicity than methanol. Besides, the elution profile of 2-propanol was assessed to use the minimum but enough amount of solvent in the experimental procedure. Different extractions were carried out by using volumes of 0.5, 1, 2, 3, and 4 mL of 2-propanol for the elution process. Results showed that the elution of our target analytes was complete with only 2 mL of 2-propanol. Concretely, the first 0.5 mL used of 2-propanol extracted 80, 75, and 45% of ADB-CHMICA, MMB-CHMICA, and MDMB-CHMZCA, while the second 0.5 mL around 15% of ADB-CHMICA, MMB-CHMICA, and 35% of MDMB-CHMZCA, respectively. The other milliliter was also added in order to extract the remaining traces of MDMB-CHMZCA retained in the sorbent (between 5 and 12%). No analytes were extracted in the next two milliliters.

Also, the possibility of concentrating the 2 mL extract by evaporation and subsequent reconstitution was studied. The treatment was carried out under vacuum at 50°C and 60°C for 15 minutes followed by redissolution with 500 µL of 2-propanol. Results obtained can be observed in Figure A24. As can be seen,

no significant variations were observed in the final recovery at both temperatures, probably due to the high stability of these analytes to temperature (Table A31). Thus, it is recommended to carry out a post-concentration step at 60 °C for 15 minutes to increase the sensitivity of the method.

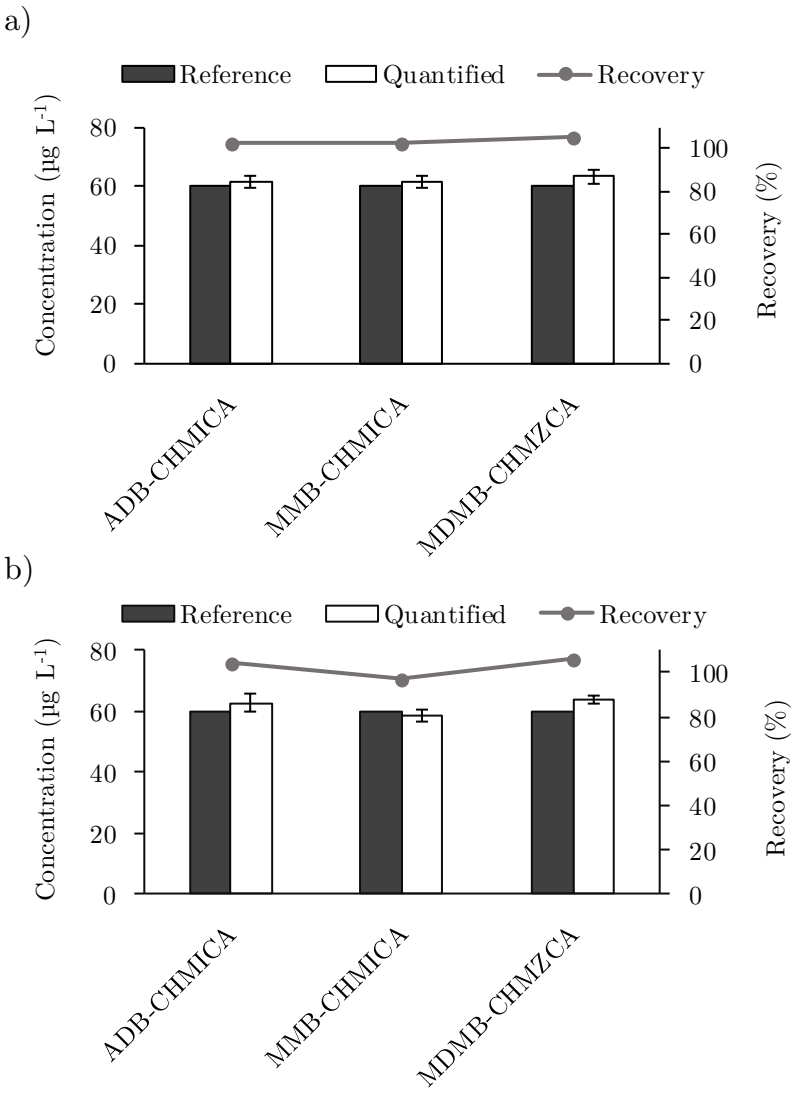



**Figure A24. Influence of the temperature in the evaporation and recomposition step on the recovery of third-generation synthetic cannabinoids at (a) 50°C for 15 minutes, and (b) 60°C for 15 minutes.**

To end, the loading capacity was evaluated with samples spiked at 10 µg $L^{-1}$, 50 µg $L^{-1}$, 200 µg $L^{-1}$, and 500 µg $L^{-1}$ in 150 mg of solid phase. Every recovery quantified for the three target analytes when using the different concentrations

mentioned was between 80 and 90%. The only little variations observed in the recoveries in the range studied can be simply due to the dispersion of the results and not to the influence of the concentration. Although no higher concentrations were assessed due to the expected low content of the target analytes under study in oral fluids, it can be stated that the loading capacity of the bonded β-CD-silica xerogel used is, at least, 33 μg $g^{-1}$ for each of the synthetic cannabinoids.

Regarding the analytical performance, the analytical parameters calculated are informed in Table A32. Among the studied parameters, the coefficients of variation were selected as dispersion measurements. Inter-day experiments were performed for three series of three extraction experiments carried out on three independent days, whereas intra-day experiments were established by analyzing three replicates within a day. As can be seen, CVs are below 6% in every case, and they are quite similar for the three selected compounds. Hence, good precision and repeatability of the method were proven. When it comes to sensitivity, both LODs and LOQs were calculated considering the concentration factor provided by the described procedure. The most sensitive compound is MDMB-CHMZCA, while similar results are observed for ADB-CHMICA and MMB-CHMICA. LODs and LOQs were at the trace level, showing the high sensitivity obtained. Thus, the experimental procedure developed together with the detection method selected provide a satisfactory sensitivity. To end, an acceptable linearity observed in the external calibration prepared supported the use of the developed methodology in an adequate range of concentrations of the measuring solution.

**Table A32. Analytical figures of merit established for the synthetic cannabinoids clean-up and extraction method using the bonded β-CD-silica xerogel.**

| Compound | CV (%) | | LOD[a] (μg $kg^{-1}$) | LOQ[a] (μg $kg^{-1}$) | Linearity[b] (μg $L^{-1}$) |
|---|---|---|---|---|---|
| | Intra-day | Inter-day | | | |
| ADB-CHMICA | 3.8 | 4.1 | 0.7 | 2.2 | 3 – 500 |
| MMB-CHMICA | 4.3 | 2.7 | 0.6 | 1.9 | 3 – 500 |
| MDMB-CHMZCA | 5.6 | 4.1 | 0.1 | 0.3 | 0.6 – 150 |

[a] LOD and LOQ are referred to the sample.
[b] Linearity is referred to the measuring solution.

These results are in good accordance with those published before, which focused on the analysis of synthetic cannabinoids, in terms of sensitivity and precision. A comparison of the method's performance can be seen in Table A33.

The study of the matrix effect showed that overall recoveries were greater than 90% were achieved for the application of the developed method to the extraction of spiked water solutions, being the concentration factors nearly 2 (Table A34). The great sensitivity shown by the procedure made it not necessary to increase this concentration factor, constituting the most important contribution of the developed method the clean-up step of the matrix. In short, these results showed a good performance of the overall procedure. The same experiments were carried out with spiked water and spiked oral fluid samples to study the matrix effect. In this case, real oral fluid matrices containing other possible interfering compounds such as caffeine and nicotine were used to carry out the study. Results shown in Table A34 also indicate the recovery obtained for the study of the matrix effect.

Thus, when real oral fluid matrices are analyzed, the recoveries display a decrease in the performance for ADB-CHMICA and MMB-CHMICA cases, falling to $> 75\%$. Oppositely, MDMB-CHMZCA shows a comparable result to the one obtained using the water matrix. In any case, these results can be considered as quantitative recoveries. Besides, it should be noticed that a lower dispersion of the results has been achieved when working with oral fluid matrices.

These results indicate that the oral fluid matrix interferes with the analytes when interacting with the CD units for ADB-CHMICA and MMB-CHMICA, while MDMB-CHMZCA seems to be equally retained regardless of the matrix type. This difference may be explained by the different chemical structures of the analytes (Table A31). On the one hand, ADB-CHMICA and MMB-CHMICA have a very similar aromatic backbone with an indazole and an indole base, respectively. On the other hand, MDMB-CHMZCA has a much bulkier aromatic structure, with an accessible benzene ring belonging to the carbazole structure. The driving forces originating the formation of inclusion complexes between CDs and analytes have been extensively described in the literature, being the most remarkable ones ejection of water from the CD cavity, hydrophobic interactions, hydrogen bonding, and induction forces (Duchêne, 2011). Consequently, it has been proven that CDs tend to form inclusion complexes with aromatic compounds very easily (Lucas-Abellán, Fortea, López-Nicolás, & Núñez-Delicado, 2007) due to the abovementioned non-polar interactions between the cavity and the compound. Overall, the difference in the aromatic structure of the synthetic cannabinoids may be the differential factor causing the better recoveries obtained for the MDMB-CHMZCA.

The lower recoveries for ADB-CHMICA and MMB-CHMICA in the oral fluid matrix can be explained taking into consideration the oral fluid composition (Humphrey & Williamson, 2001).

**Table A33. Comparison of the developed method for synthetic cannabinoids extraction using the bonded β-CD-silica xerogel with other methods in the literature.**

| Analytes | LOD (μg $L^{-1}$) | LOQ (μg $L^{-1}$) | CV (%) | Analytical technique | Reference |
|---|---|---|---|---|---|
| Up to 29 synthetic cannabinoids | 0.1 – 2.5 | No data | 3 – 29 | HPLC-MS | (Ong et al., 2020) |
| 5F-ADB, MMB-CHMICA, THJ-2201, CUMYL-4CN-BINACA, MDMB-CHMZCA | 10 – 20 | 30 – 60 | No data | GC-MS | (Sorribes-Soriano et al., 2021) |
| MDMB-CHMICA | 0.5 | No data | No data | HPLC-MS | (Seywright et al., 2016) |
| Up to 75 synthetic cannabinoids | 0.2 – 60 | No data | < 5 | HPLC-MS | (Sundström et al., 2013) |
| 8 synthetic cannabinoids | 0.05 – 2.5 | No data | No data | HPLC-MS | (Staeheli et al., 2019) |
| ADB-CHMICA, MMB-CHMICA, MDMB-CHMZCA | 0.1 – 0.7 | 0.2 – 2.2 | 3.8 – 5.6 (intra-day)<br>2.7 – 4.1 (inter-day) | HPLC-FLD | Proposed method |

**Table A34. Matrix influence in synthetic cannabinoids recovery using the bonded β-CD-silica xerogel.**

| Compound | Recovery (%) | |
|---|---|---|
| | Spiked water | Spiked oral fluid |
| ADB-CHMICA | 96 ± 4 | 79 ± 2 |
| MMB-CHMICA | 93 ± 6 | 76 ± 4 |
| MDMB-CHMZCA | 95 ± 7 | 96 ± 3 |

Table A35. Analysis of synthetic cannabinoids following the extraction method using the bonded β-CD-silica xerogel (μg $kg^{-1}$, $\bar{x} \pm s$).

| Sample | Compound | Spiked concentration | Quantified concentration | Trueness (%) |
|---|---|---|---|---|
| M1 | ADB-CHMICA | 35.6 | 33.4 ± 0.6 | 94 |
| | MMB-CHMICA | 35.6 | 38 ± 4 | 105 |
| | MDMB-CHMZCA | 10.7 | 11.3 ± 0.3 | 106 |
| M2 | ADB-CHMICA | 22.3 | 22.7 ± 1.5 | 102 |
| | MMB-CHMICA | 22.3 | 21.2 ± 1.7 | 95 |
| | MDMB-CHMZCA | 6.7 | 6.5 ± 0.4 | 97 |
| M3 | ADB-CHMICA | 24.7 | 22.6 ± 2.0 | 92 |
| | MMB-CHMICA | 24.7 | 24.4 ± 1.0 | 98 |
| | MDMB-CHMZCA | 7.4 | 7.0 ± 0.7 | 94 |
| M4 | ADB-CHMICA | 25.0 | 25.7 ± 2.1 | 103 |
| | MMB-CHMICA | 25.0 | 24.6 ± 0.7 | 99 |
| | MDMB-CHMZCA | 7.5 | 7.6 ± 0.6 | 101 |
| M5 | ADB-CHMICA | 14.0 | 14.8 ± 0.6 | 106 |
| | MMB-CHMICA | 14.0 | 14.0 ± 0.3 | 100 |
| | MDMB-CHMZCA | 4.2 | 3.8 ± 0.5 | 90 |
| M6 | ADB-CHMICA | 35.6 | 34 ± 3 | 96 |
| | MMB-CHMICA | 35.6 | 30.1 ± 1.2 | 84 |
| | MDMB-CHMZCA | 10.7 | 10.2 ± 0.7 | 95 |

Oral fluids, being 99% water, are also composed of inorganic compounds ($Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $HCO_3^-$, $HPO_4^{2-}$), complex organic compounds (proteins like immunoglobulins and enzymes), and simpler nitrogen-based compounds (ammonia or urea, for example). Additionally, it has a small amount of solid particles in suspension due to oral tissues. Although the common interfering compounds evaluated in this case proved not to influence the determination step (HPLC-FLD is a sufficiently selective technique), the complex oral fluid mixture may cause a more difficult interaction between CD and the analyte, probably occluding the pores in the solid phase (lowering the amount of available CD) or interfering by interacting with the CD itself. In any case, the extraction methodology developed constitutes an easy and efficient way of cleaning up the complex matrix being studied with a slight impact on the recovery of the analytes under study.

At this point, the target analytes were determined in six field oral fluid samples (Table A35). Since none of them was expected to be present in the samples selected due to their origin, the oral fluids under analysis were spiked and quantified. Previous reports on the determination of synthetic cannabinoids in oral fluids established their usual concentrations at the µg $L^{-1}$ level (Sorribes-Soriano et al., 2021). For this reason, the trueness of the developed procedure was assessed by the analysis of oral fluids spiked at trace level. As can be observed, all the obtained concentrations were statistically comparable to the theoretical ones using a 95% confidence level interval, ranging the accuracy calculated from 94 to 106%.

## 5.4. Conclusion

In this study, bonded β-CD-silica xerogels have been applied for the determination of three novel synthetic cannabinoids in oral fluids based on SPE and HPLC-FLD analysis. The extracting features have been validated from field spiked oral fluid samples. Good sensitivity and selectivity have been achieved, while the repeatability of the developed method can be classified as satisfactory. As an advantage, cyclodextrins in the sorbent certainly contribute to the matrix clean-up and the selectivity-enhance extraction of the target compounds. However, the possible adsorption of other undesired compounds may be an issue that can be evaded using fluorescence detection as a selective input in the quantification step.

The novelty of this study resides in the few research reported to date concerning the simultaneous isolation and analysis of the new psychoactive substances studied excepting the already reported direct screening methods. The

development of new methodologies for the analysis of oral fluids allows the identification of the parent compounds consumed instead of their respective metabolites, which significantly reduces potential misidentifications in routine control of other biological matrices such as, for example, urine.

# 6

# Overview on cyclodextrin-silica xerogels

New materials for adsorbing a variety of pollutants and other high-concern substances based on CD-silica microporous composites have been described and tested in a variety of conditions, including different types of analytes, different types of matrices, or different purposes of the sorption procedure.

In general, the solid phase preparation and the construction of the cartridges or samplers are easy and relatively inexpensive. As exposed, the success of these materials is found in an adequate design of the solid phase. Included and bonded CD-silica hybrid microporous solids combine a hydrophilic silica surface with well-dispersed hydrophobic CD sites. Thus, the solid design expands from the nanoscale (providing an open and accessible micropore system for easy diffusion of analytes) to the molecular level (preserving the CD conformation inside high hydrophilic silica cage-like pores). Moreover, it has been proven that their architecture can be modulated not only during the synthesis process but also when choosing the CD used in order to achieve selective materials for certain analytes, thus providing discrimination based on the molecular size or the chemical nature of the substances under study.

When talking about included CD-silica xerogels, they provide high advantages when used as air samplers if compared with other similar devices described. Not only are they able to work properly in a wide range of efficient operative conditions, but also the conditions for sorption and desorption processes are easier and greener than other described methods that use, for example, organic solvents such as dichloromethane or carbon disulfide.

Regarding bonded CD-silica xerogels, they have demonstrated to improve the results obtained previously with the included solid phases in different applications such as the phenolic compounds sampling or the solid-phase extraction of PAHs from water. Due to the chemical bonding of CDs to the silica structure, they are also capable of being reused for several times because cyclodextrin losses are avoided in this case, which adds enhanced value to them. In this case, the medium in which analytes are contained, air-type of water-type, can be freely changed while maintaining a proper analytical performance, as has been demonstrated through the adsorption-desorption studies carried out too. For this reason, they have been applied to the determination of several analytes in different matrices, including not only environmental ones but also biological fluids, without losing reliability in the determination carried out.

In general, the possibility of improving different analytical features such as the sensitivity, selectivity, or the clean-up potential of the method designed when using CD-silica-based materials represents a success, as demonstrated. Importantly, the possibility of adapting the material to the type of analyte under study by taking into account the type and size of the CDs contained in the silica network denotes thus a remarkable benefit.

# SECTION B

# Application of mesoporous cyclodextrin-silica solid phases

# 1

# Synthesis and characterization of mesoporous cyclodextrin-silica materials

## 1.1 Introduction

Technical advances in several fields such as analytical chemistry, where molecular recognition is needed, may sometimes require the design and development of ordered porous materials with controllable structures and systematic tailoring pore architecture (El Haskouri et al., 2008; Wan & Zhao, 2007). Indeed, several analytical applications (e.g., adsorption, separation, drug delivery, or sensors, among others) need solid phases with high porosity.

An increase in the porosity and thus in the specific surface area of materials often enhances reactivity and improves recognition properties, which helps to ensure high sensitivity. Furthermore, wide-open structures are likely to offer more rapid mass transport, which frequently constitutes a rate-determining step of the sorption processes (Walcarius & Collinson, 2009). Such advantages can be presented by silica and organically modified silica-based materials, specifically by mesostructured silica materials that are characterized for usually possessing high specific surface areas and pore volumes.

Mesoporous materials were developed in the early 1990s, when the synthesis of mesostructured silicas was first reported by Mobil scientists (Beck et al., 1992; C.T. Kresge et al., 1992) based on the use of surfactants as templates. This time, cationic surfactants as cetyl trimethylammonium bromide (CTAB) were used to prepare highly ordered M41S mesoporous silicate molecular sieves under hydrothermal and basic conditions. This kind of attractive material extended the uniform pore sizes from the micropore to the mesopore range (Wan & Zhao, 2007).

Thus, the discovery of the M41S family constituted a breakthrough finding by opening a new way towards ordered uniform mesoporous systems.

Although the surfactant self-assembly is particularly essential for the formation of highly ordered mesostructures (Walcarius & Collinson, 2009), mesoporous materials can be rationally designed and the synthesis can be controlled (Wan & Zhao, 2007) in different ways. Thus, their key feature is, besides their ultimate composition and homogeneity, the set of structural parameters characterizing their framework and texture (El Haskouri et al., 2008) which can help to enhance the accessibility of the active sites of these materials. Among them, pore dimensions and arrays, wall thickness, crystalline order, and particle size can be mentioned. To date, a huge collection of rationalized recipes in preparative chemistry has aimed at controlling some of these specific parameters. They include the managing of procedural variables such as the type and concentration of precursors and surfactants, solvents, pH, temperature, or thermal treatments, among others.

A combination and precise control of some of the previously mentioned structural parameters led to UVM-7 (*University of Valencia Material*) solid phases some years ago (Huerta, Amorós, Beltrán-Porter, & Corberán, 2006). Type UVM-7 materials, which are considered as a nanometric version of the MCM-41 derivatives (Pellicer-Castell et al., 2021) due to their particular architecture, are nanoparticulated materials with hierarchical porosity. They combine short pore length with a bimodal pore system consisting of intra-particle mesopores (porogen effect of the surfactant) and inter-particle macropores (formed by the aggregation of particles). Therefore, the diffusion of different compounds through the mesopores is eased by the macropore structure (El Haskouri et al., 2002; Pérez-Cabero et al., 2012).

Going forward to the analytical chemistry field, the remarkable features offered by mesoporous silica as structuring material of a variety of solid phases include high stability, large surface areas, and the possibility of adjusting the pore size (El Haskouri et al., 2008; Pellicer-Castell et al., 2019) to the application needed. Some applications of UVM-7 materials as extraction sorbents have been recently reported (Martínez Pérez-Cejuela et al., 2018), including decoration with metallic nanoparticles (Pellicer-Castell et al., 2018, 2021). However, their combination with the benefits offered by cyclodextrins as selectivity-enhancing units has been barely studied to date.

In this chapter, the development and characterization of silica-based type UVM-7 mesoporous materials containing accessible cyclodextrin units are described and discussed. Two types of CDs are used as functionalizing agents in

this case to assess the advantages offered by the solids synthesized: β- and γ-CD. The analytical benefits offered by the materials described are exposed, and their possibilities for their use in extraction techniques within the analytical chemistry field are finally commented on.

## 1.2. Experimental

### 1.2.1. Reagents, materials, and instrumentation

The synthesis of the type UVM-7 solid phases included a first functionalization step for the cyclodextrins used. To it, β- and γ-cyclodextrin were purchased from CycloLab (Budapest, Hungary), and 3-(triethoxysilyl)propyl isocyanate and dry pyridine ≥ 99.5% were acquired from Sigma-Aldrich (St. Louis, United States). An Azbil Telstar LyoAlfa 10/15 freeze drier (Barcelona, Spain) was used to dry CDs previously, and a Büchi rotary evaporator (Flawil, Switzerland) was used to remove the solvent after the reaction. Once CDs were modified, the obtention of the mesoporous silica materials was carried out using cetyl trimethylammonium bromide (CTAB) reagent grade from Sigma-Aldrich (St. Louis, United States), ethanol from VWR ProLabo Chemicals (Radnor, United States), as well as tetraethyl orthosilicate ≥ 98% (TEOS) and triethanolamine ≥ 99% (TEA) from Fluka (Fisher Scientific, Buchs, Switzerland).

The characterization of the solid phases was done using several techniques. Transmission electron microscopy (TEM) images of the materials were obtained using a Jeol microscope (Tokyo, Japan) model JEM-1010 operating at 200 kV. In addition, $^{13}C$ and $^{29}Si$ MAS NMR spectra were acquired at 128.3 MHz and 79.5 MHz, respectively, with a Varian Unity 300 spectrometer from Agilent Technologies (California, United States). X-ray diffractograms (XRD) were obtained using Cu $K_\alpha$ radiation in a Seifert 3000TT θ-θ from Spectro Analytical Instruments (Pomona, South Africa). Elemental CNH analysis was performed with an elemental analyzer 1100 of CE Instruments (Hindley Green, United Kingdom). Also, the porosity data were determined by using $N_2$ adsorption-desorption isotherms recorded at 77 K with a Micromeritics ASAP-2020 automated sorption analyzer (Norcross, United States). To end, thermogravimetric analysis (TGA) was operated with a Setaram Setsys 16-18 (Caluire-et-Cuire, France).

### 1.2.2. Synthesis of mesoporous cyclodextrin-silica materials

The first step of the synthesis corresponds to the CD silanization to make the covalent attachment to the silica structure possible. The functionalization

procedure has been already described in Section A. In short, 9.4 mmol of previously dried CD (10.7 g for β-CD and 12.2 g for γ-CD, respectively) are mixed with 9.3 mL of 3-(triethoxysilyl)propyl isocyanate in 130 mL of dry pyridine. The solution is stirred at 70 °C for 48 h. Then, the solvent is removed under pressure and the solid obtained is washed thoroughly with ethanol.

Then, the synthesis of the final UVM-7-type solid phases was adapted from the one used for obtaining bare UVM-7 materials elsewhere (Pellicer-Castell et al., 2018) through an experimental procedure in the presence of surfactant. The basic ingredients were mixed in the molar ratio 2TEOS:7TEA:0.5CTAB:180$H_2O$. In this case, the incorporation of CDs was carried out at a 2% with respect to the TEOS content. Thus, it should be 1.98TEOS:0.02CD:7TEA:0.5CTAB:180$H_2O$. The use of the atrane method implies that all reagents (both the silica source and other heteroelements or modified silanes) are forming atrane or pseudoatrane complexes before starting the cohydrolysis and cocondensation processes. Thus, all reagents are supposed to be first dissolved in TEA. However, the impossibility of dissolving the functionalized γ-CD in the TEA medium made it necessary to modify the general method in this case. The dissolution of both CDs (β- and γ-) was carried out in an aqueous medium, and they were incorporated into the synthesis in the last stage. Although functionalized β-CD appeared to be soluble in TEA medium, both CDs were dissolved in aqueous media in order to have homogeneity in the preparation of both materials here tested.

The synthesis process was as follows. Briefly, 50 mL of TEA are added to 23.7 mL of TEOS. The mixture is heated to 140°C. After short cooling, 9.8 g of CTAB are added to the mixture at 120°C and 1.8 mmol of functionalized cyclodextrin (2.3 g of β-CD and 2.6 g of γ-CD, respectively), previously dissolved in 180 mL of water, are slowly mixed with them under vigorous stirring. The incorporation of CDs to the rest of the reagents is carried out in short times and under stirring to avoid self-condensation processes. The mixture is aged at room temperature overnight and the resulting powder is afterwards filtered and washed. Then, the material is dried with vacuum. To end, acid extraction is used to remove the surfactant. To this end, 100 mL of ethanol and 8.5 mL of HCl 37% are added for each gram of the obtained material and mixed under stirring at 60°C.

## 1.3. Results and discussion

In the first place, TEM images were obtained for the CD-UVM-7 materials synthesized. In both cases, the incorporation of cyclodextrin units in the UVM-7 structure did not modify the typical architecture of the pure mesoporous silica

studied. Representative images are shown in Figure B1. In them, the characteristic UVM-7 structure includes a pore system combining large inter-particle macropores with intra-particle mesopores showing a hexagonal disordered array. In the micrographs shown, the continuous silica network constructed from aggregated small mesoporous nanoparticles was appreciated. It was observed that, in the case of the β-CD-UVM-7, there has been a greater aggregation among primary nanoparticles, whose clusters showed, in consequence, a slightly larger size when compared to γ-CD-UVM-7. In any case, it can be concluded that the modification of the synthesis method did not prevent the obtaining of materials with UVM-7 type architecture according to the obtained TEM images. However, it should be mentioned that a certain loss of organization and homogeneity was observed compared to similar materials containing β-CD obtained from the classical method, that is, dissolving β-CD in TEA. Despite this, TEM images showed that a highly accessible silica network was preserved, which is the key feature for the hypothetical analytical applications that may be conducted with them.

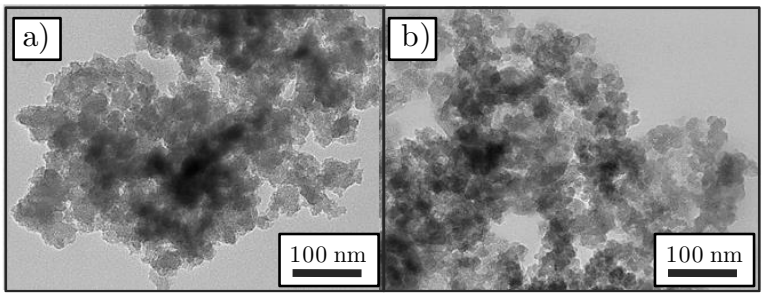



**Figure B1. Representative TEM images of (a) β-CD-UVM-7 and (b) γ-CD-UVM-7.**

The XRD patterns measured evidenced a broad signal at low angles (2° (2θ)), which is in concordance with the expected intrananoparticle organization based on a mesopore array with hexagonal pseudo-order. In both materials, this XRD signal (Figure B2) was wider and of lower intensity than that observed in materials prepared by the standard procedure using TEA as solvent. This feature suggested that the co-condensation process starting from the silica-based reagents in water did not allow such an ordered and effective self-assembly with the surfactant micelles when compared to the standard synthesis starting from TEA. This trend was even more pronounced in the case of the sample containing γ-CD, probably due to its larger size, which can generate greater steric hindrances during the mesostructure formation. In fact, in the case of the γ-CD-UVM-7, the XRD signal was observed as a shoulder. Then, the materials obtained, although clearly

of UVM-7 type according to TEM images, presented a slightly lower order in the intra-particle mesopore system. However, it is important to note that the order degree does not suppose an added value for certain applications, as exposed in Section A. In this case, the important characteristic is to achieve a good dispersion of active groups (β- and γ-CD) accessible for the different analytes to be retained. This objective seemed to be achieved according to the data presented.

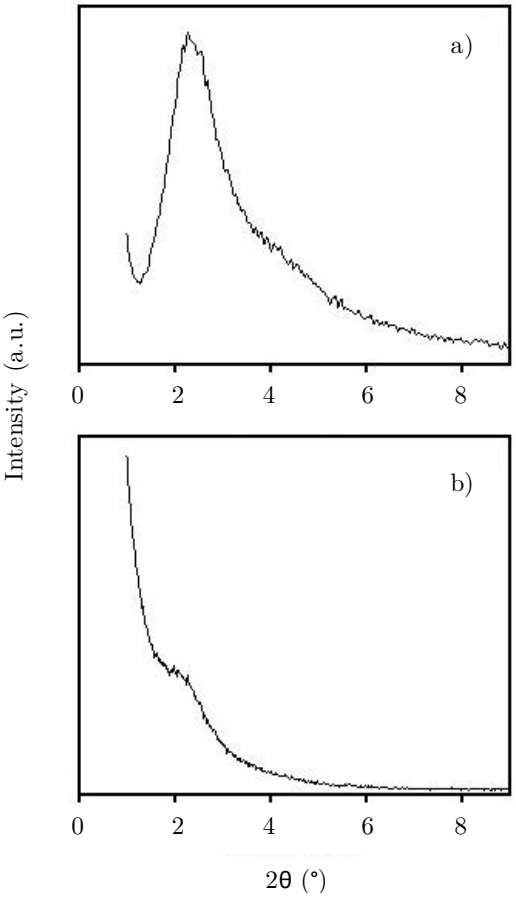



**Figure B2. Low-angle XRD patterns measured for (a) β-CD-UVM-7 and (b) γ-CD-UVM-7.**

The surface area and pore distribution of the prepared materials were determined based on the $N_2$ adsorption-desorption isotherms. In all cases, isotherms showed two adsorption steps (Figure B3), thus confirming their bimodal framework and high accessibility. Data in Table B1 is besides consistent with TEM and XRD observations. While in the case of the β-CD material the intra-particle porosity was significant (0.34 $cm^3\ g^{-1}$), it decreased for the material with γ-CD (0.14 $cm^3\ g^{-1}$). The inter-particle textural porosity was, oppositely,

higher for the γ-CD-UVM-7 (0.71 $cm^3$ $g^{-1}$) when compared to the β-CD-UVM-7 (0.25 $cm^3$ $g^{-1}$). The lower particle aggregation in the latter material also correlates with its larger textural pore size. On the other hand, the greater size of the intra-particle mesopore observed for the γ-CD-UVM-7 material is probably related to the greater size of the cyclodextrin and the steric problems it may induce in the self-assembly process among silica and the micelles. In any case, the preserved porosity is useful enough for the analytes trapping in the CD active centers of the solid phases within optimized extraction conditions and can contribute to higher selectivity of the analytical methodology applied.

**Table B1. Physical and textural parameters of CD-UVM-7 materials.**

| Solid phase | BET surface area ($m^2$ $g^{-1}$) | Mesopores | | Large pores | |
|---|---|---|---|---|---|
| | | Pore size (nm) | Pore volume ($cm^3$ $g^{-1}$) | Pore size (nm) | Pore volume ($cm^3$ $g^{-1}$) |
| **β-CD-UVM-7** | 685.5 | 2.38 | 0.34 | 30.1 | 0.25 |
| **γ-CD-UVM-7** | 352.7 | 2.80 | 0.14 | 41.4 | 0.71 |

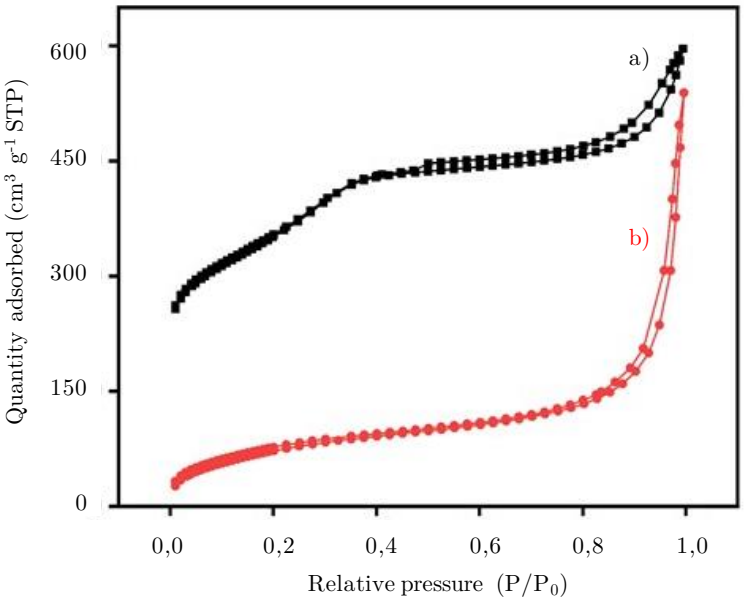



**Figure B3. $N_2$ adsorption-desorption isotherms of (a) β-CD-UVM-7 and (b) γ-CD-UVM-7.**

In addition, the amount of CD incorporated onto the silica network described was estimated from the TGA curves obtained (Figure B4). In both cases, a first weight loss at T < 90°C can be attributed to hydration water molecules of the phases. At higher temperatures (200 – 500°C), the relatively abrupt weight loss observed in both cases can be associated with the degradation and oxidation of cyclodextrins. Later, a less pronounced weight loss due to the silanol condensation occurs leading to the $SiO_2$ final residue.

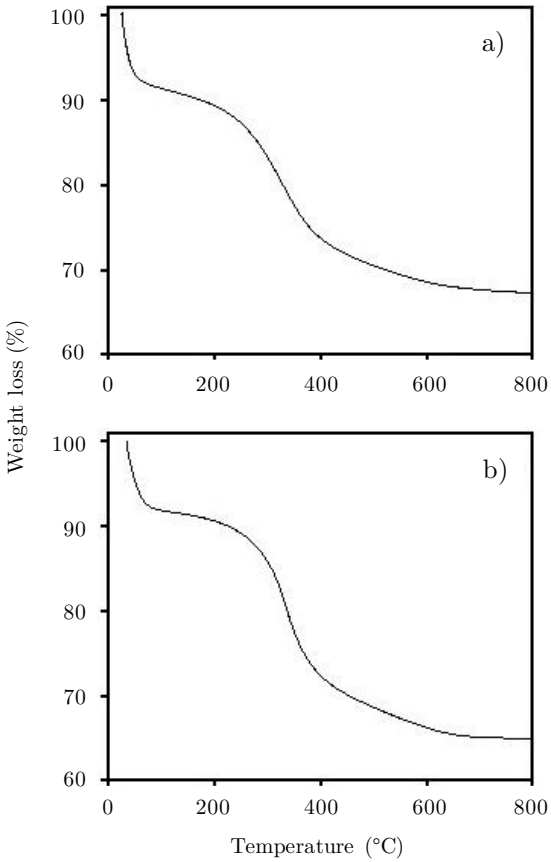



**Figure B4. Thermogravimetric analysis of (a) β-CD-UVM-7 and (b) γ-CD-UVM-7. Measurements were carried out in an $O_2$ environment and at a heating speed of 10 °C $min^{-1}$.**

It was calculated that the weight loss associated with cyclodextrins decomposition is 21 and 23% for β-CD-UVM-7 and γ-CD-UVM-7, respectively. Considering that the number of silanol arms attached to both cyclodextrins is very similar, ca. 4 (3.8 and 3.9 for the β-CD and the γ-CD, respectively, as described in Section A), it was possible to estimate the functionalization degree assuming a simplified $CD_x$-$SiO_2$ formulation. An identical value of x=0.01 was

obtained for both materials, which corresponds to a degree of CD incorporation of 0.13 mmol/g (matching to C wt% values of 9.7 and 10.4 for β-CD-UVM-7 and γ-CD-UVM-7, respectively). These values were in excellent agreement with the elemental analysis data; 9.9 C wt% (β-CD-UVM-7) and 10.6 C wt% (γ-CD-UVM-7). Due to the low nitrogen content of the modified CD structures, the C content was used as the most reliable data to determine the concentration of CD in our materials. Important is to mention that the results of the TGA experiments also permitted intuiting fine stability of the solid phase up to temperatures of at least 200°C, which is in accordance with previous studies on type UVM-7 materials (El Haskouri et al., 2002).

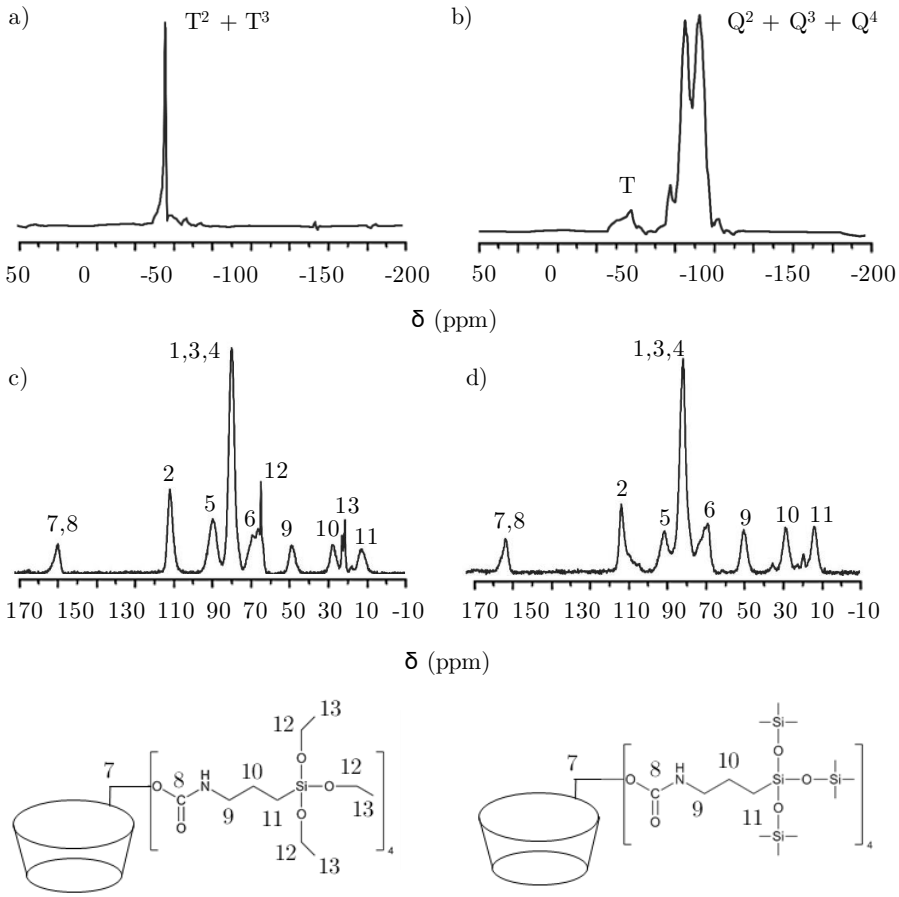



**Figure B5. (a) $^{29}$Si NMR spectra of the modified CDs, (b) $^{29}$Si NMR spectra of the CD-UVM-7 materials, (c) $^{13}$C NMR spectra of the modified CDs, and (d) $^{13}$C NMR spectra of the final CD-UVM-7 materials.**

Regarding the NMR experiments, the $^{29}Si$ and $^{13}C$ NMR spectra of the solid phases were recorded. Results are shown in Figure B5 and they were comparable for both materials. As can be seen, the $^{29}Si$ NMR of the modified cyclodextrins (either β- or γ-CD) showed a unique signal, which is compatible with the presence of silicon $T^2$ and $T^3$ sites presence corresponding to their structure. Oppositely, the final materials evidenced the existence of a minority of T silicon sites in comparison with the new presence of $Q^2$, $Q^3$, and $Q^4$ silicon sites, also coinciding with the expected molecular structure of the final material. The $^{13}C$ NMR spectra supported this information, also in accordance with CD-silica xerogels of Section A. In this case, both spectra were very similar. However, the one corresponding to the modified CDs showed two extra peaks in comparison with the spectra of the final material. This is consistent with the disappearance of the ethyl groups in the synthesis of the final solid phase due to the covalent attachment of the modified cyclodextrin to the silica structure through its silanol groups.

## 1.4. Conclusion

The analytical applications of the CD-based silica mesoporous materials characterized described hereafter are focused on testing their ability to be used for the sample treatment of different samples. Based on the results described in Section A, the analytes were chosen according to the analytical concern represented by them together with their appropriate physical characteristics to evaluate the influence of the sizes of the respective CD cavities to adsorb them. Therefore, the types of samples were chosen based on the usual environments where the selected analytes can be found all around.

# 2

# Determination of endocrine-disrupting chemicals in juice

## 2.1. Introduction

Synthetic resins are widely used to bestow some plastic properties (Bang et al., 2012) such as flame resistance, color, and softness. Polyethylene (PE), polyvinyl chloride (PVC), polyethylene terephthalate (PET), or polycarbonate (PC) can be named among the major ingredients for the manufacture of an extensive variety of goods (Bang et al., 2012; Khan, Alammari, Aqel, & Azam, 2020). However, they are usually a source of concern due to the presence of endocrine-disrupting substances in their structure, which may pose some risk to the environment and human health (Bang et al., 2012). Since they are not chemically bonded therein, unintentional contamination of endocrine-disrupting chemicals in food coming from plastic containers may happen easily, especially under specific conditions such as high temperature, acidic pH, and sunlight (Arfaeinia et al., 2020; Muncke, 2009).

Endocrine disruption is known as the disruption of the hormonal and physiological balance of the body as a result of natural or synthetic chemicals interfering with endocrine pathways (Kaya, Cetinkaya, Bakirhan, & Ozkan, 2020). Potential health concerns include diabetes, thyroid dysfunction, reproductive disorders, developmental defects, and cancer. Thus, several chemical substances have been identified as endocrine-disrupting chemicals (EDCs). Some common examples are alkylphenols and bisphenols, phthalates, parabens, pesticides, hormones, polychlorinated biphenyls, or heavy metals (Chormey, Zaman, Kasa, & Bakırdere, 2020), among others. As a matter of fact, most of them are included in the 2021 European Union's REACH (1907/2006/EC, 2006)

Substances of Very High Concern list and legislative regulations about their maximum concentrations allowed by the European Commission (2018/213/EU, 2018). The EPA has also established reference doses for them. Among them, bisphenols attract special attention as ubiquitous substances used to make food and beverage containers and as additives (Khan et al., 2020). Concretely, bisphenol A (BPA) triggers high concern and has been determined in beverages coming from cans and plastic bottles, for example (Bang et al., 2012; Khan et al., 2020; Wagner & Oehlmann, 2009). Besides, parabens are popular synthetic preservatives used not only in food products but also in pharmaceutical and cosmetic preparations. Our daily exposure to them has been estimated as significant, with 25 mg coming from pharmaceuticals, 50 mg from cosmetics, and 2.5 mg from food products (Matwiejczuk, Galicka, & Brzóska, 2020).

Due to the existing variety of EDCs, our lifestyle means that we are in constant contact with them. The strict regulations that require trace detection due to the lack of certain threshold values for adverse effects and their low concentrations in products make it necessary to develop sensitive monitoring methods (Kaya et al., 2020). A variety of procedures has been reported. They consist mostly of extraction methodologies including SPE (Azzouz, Rascón, & Ballesteros, 2016b; Brede, Fjeldal, Skjevrak, & Herikstad, 2003), LLE (Asimakopoulos, Wang, Thomaidis, & Kannan, 2014), pressurized liquid extraction (Ferrer et al., 2011), SPME (Mousa, Basheer, & Rahman Al-Arfaj, 2013), SBSE (M. S. Chang, Shen, Yang, & Wu, 2012), and LLME (Vela-Soria et al., 2013) mostly coupled to chromatographic detection (Kaya et al., 2020). Among them, SPE is the preferred choice for cleaning and preconcentrating these substances due to its suitability to the analysis of compounds of different polarity and properties. For this reason, some authors have used it to preconcentrate not only EDCs but also other substances in complex matrices (Azzouz, Rascón, & Ballesteros, 2016a). However, using commercial solid phases presents some limitations such as the lack of specificity. Besides, the simultaneous presence of polar groups may complicate the quantitation step of EDCs in certain determination routes (Azzouz et al., 2018) to require additional derivatization. Due to the need to use these extraction strategies, there is a special interest in developing new sorbent materials for the selective retention of analytes.

The use of self-designed nanostructured materials such as metal-organic frameworks or molecularly imprinted polymers in sorptive techniques has thus been recognized as a potential sample treatment step to ease the isolation of the analytes of interest (Azzouz et al., 2016a). Particularly interesting are host-guest complexing paths, which are already being exploited for the reversible adsorption

of substances and can provide an improvement in some features such as selectivity. Cyclodextrins are in this sense a promising tool due to their capability of encapsulating suitable analytes based on their polarity and physical properties.

This study aimed to assess the utility of type UVM-7 mesoporous silica modified with β-CD as SPE sorbent for the extraction of EDCs in widely consumed fruit juices. Since apple juices are characterized for showing a high acidic pH and are usually packaged in PET plastic containers while stored for a long time, the presence of EDCs in these liquids may be expected (Arfaeinia et al., 2020). To this, the relevant analytical features of the method were established. The results were compared with those obtained using a reference extraction method reported in the literature (Khan et al., 2020).

## 2.2. Experimental

### 2.2.1. Reagents, materials, and instrumentation

On the one hand, the individual bisphenol A (BPA), bisphenol AP (BPAP), and bisphenol C (BPC) analytical standards were purchased from Sigma-Aldrich (St. Louis, United States). On the other hand, methylparaben (MP), ethylparaben (EP), and propylparaben (PP) ≥ 99% were acquired from Fluka (Fisher Scientific, Buchs, Switzerland). The concentrated individual standard solutions were prepared in methanol and the working standard multianalyte solutions were obtained by their dilution and stored at -18°C. HPLC grade methanol and acetonitrile were acquired in VWR ProLabo Chemicals (Radnor, United States). In addition, ultrapure water from an Adrona (Riga, Latvia) purification system was employed during the experimental procedure. Other reagents such as NaCl (s) ≥ 99.5%, NaOH (s) ≥ 98.5%, and $H_2SO_4$ (aq.) 98% were bought from Panreac AppliChem (Barcelona, Spain).

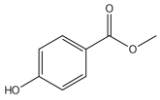
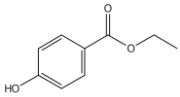
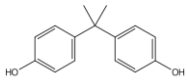
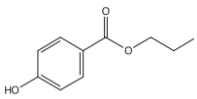
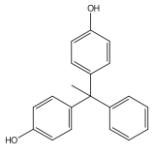
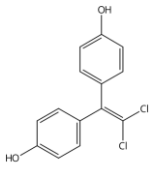
The reference extraction procedure (Khan, Alammari, Aqel, & Azam, 2020) was reproduced using 500 mg $C_{18}$ Varian Bond Elut cartridges from Agilent Technologies (California, United States).

For the SPE, a Vac Elut 20 from Agilent Technologies (California, United States) using 6 mL cartridges from Análisis Vínicos (Ciudad Real, Spain) was employed. All samples were previously filtered with Nylon 0.45 mm Sartorious Stedim Biotech filters (Göttingen, Germany). A miVac sample concentrator from SP Scientific (Warmister, United States) was utilized for solvent evaporation.

For the analytes separation and quantitation (Figure B6), an LC-2000 Plus liquid chromatograph equipped with an FP-2020 Plus Intelligent Fluorescent Detector, a PU-2089 Plus Quaternary Gradient Pump with integrated degasser,

and an I/FLC/NetII/ADC interface from Jasco (Madrid, Spain) were used. The injection volume was 20 µL in a Supelco Analytical (Sigma Aldrich, St. Louis, United States) six-way injection valve from Agilent Technologies, and the stationary phase used was a Kromasil $C_{18}$ column (150 x 4.6 mm, 5 µm particle size) from Análisis Vínicos (Ciudad Real, Spain). The separation was carried out at 1 mL $min^{-1}$ using a 55:45 ACN:$H_2O$ isocratic-mode mobile phase. To end, the fluorimetric detection was conducted at a $\lambda_{ex}$ of 254 nm and a $\lambda_{em}$ of 310 nm.

**Table B2. Physicochemical properties of the studied endocrine-disrupting chemicals (Scifinder Scholar Database, 2021).**

| Compound | CAS | Structure | Boiling point (°C) | logP[a] | Vapour pressure[a] (Pa) | Molecular size (nm) |
|---|---|---|---|---|---|---|
| Methylparaben | 99-76-3 |  | 265 | 1.88 | $7.4\ 10^{-1}$ | 0.736 |
| Ethylparaben | 120-47-8 |  | 298 | 2.39 | $1.0\ 10^{-1}$ | 0.767 |
| Bisphenol A | 80-05-7 |  | 400 | 3.64 | $7.1\ 10^{-5}$ | 0.859 |
| Propylparaben | 94-13-3 |  | 2.94 | 2.90 | $1.2\ 10^{-1}$ | 0.796 |
| Bisphenol AP | 1571-75-1 |  | 474 | 4.33 | $1.7\ 10^{-7}$ | 0.921 |
| Bisphenol C | 14868-03-2 |  | 405 | 4.98 | $5.2\ 10^{-5}$ | 0.859 |

[a] Vapor pressure and logP measured at 25 °C.

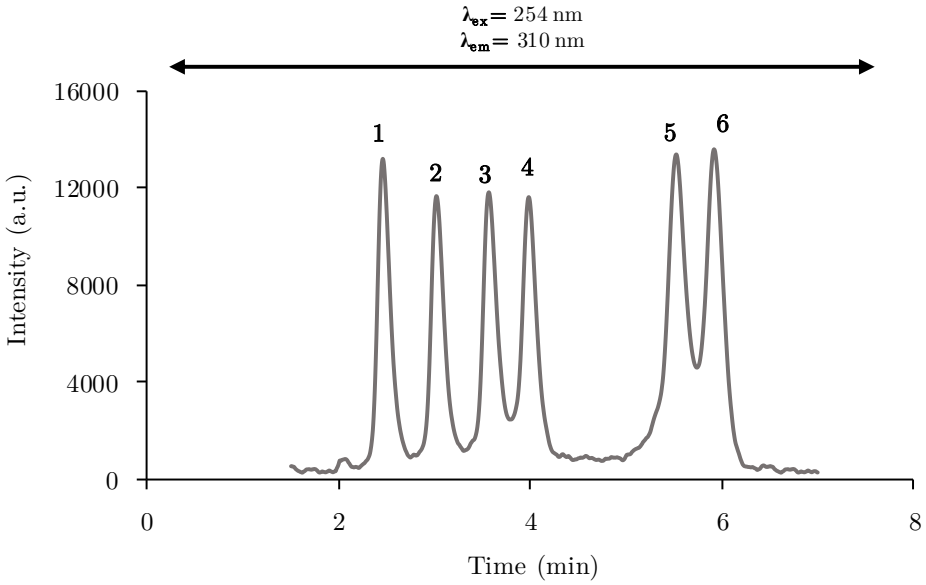



**Figure B6. HPLC-FLD chromatographic profile of a multianalyte solution of the endocrine-disrupting chemicals under study in methanol: (1) methylparaben (2) ethylparaben (3) bisphenol A (4) propylparaben (5) bisphenol AP (6) bisphenol C.**

### 2.2.2. Optimization and validation of the SPE procedure

The experimental procedure was optimized by studying the parameters that can influence the concentration factor obtained through SPE. Thus, they were evaluated and optimized independently, that is, by varying one parameter at a time while keeping the others constant. The optimization study was carried out based on the recovery obtained from spiked samples at the μg $L^{-1}$ level and the recovery was calculated from the ratio between the obtained concentrations and the theoretical concentration (n=3). The studied parameters were the type of adsorbent, ionic strength, pH, influence of the washing step, amount of solid phase, sample volume, loading capacity, the volume of extracting solvent, and the possibility of concentrating the elution by evaporation and later reconstitution. Besides, the chance of reusing the materials was tested. To end, the existing matrix effect was assessed by spiking juice samples to establish the variation of the recovery and the coefficient of variance of standard line slopes coming from standard addition calibration solutions. A study of CDs interfering in the determination when present in juices as food additives was also carried out.

Extraction cartridges were prepared by packing the β-CD-UVM-7 material between two polyethylene frits into 6 mL empty cartridges. The recommended SPE procedure consists of using 200 mg of β-CD-UVM-7 solid phase and

conditioning it with 3 mL of MeOH followed by 5 mL of ultrapure water. Then, 25 mL of sample, whose ionic strength and pH are previously adjusted to 4 M and pH 2.5, respectively, are passed through the cartridge at reduced pressure. The cartridges are subsequently washed with 5 mL of ultrapure water at the same pH and ionic strength as the samples and dried for 10 min. In the end, analytes are eluted with 2 mL of MeOH. After their concentration by evaporation and subsequent recomposition with 500 µL of MeOH, the extracts are analyzed by HPLC-FLD.

Regarding the analytical figures of merit, linearity, sensitivity, and precision of the final protocol were established. The limit of the detection (LOD) and the limit of quantification (LOQ) were estimated following the IUPAC recommendations (Olivieri et al., 2006). The LOQ was considered as the lower limit of linearity. Besides, the precision was evaluated by studying the intra-day and inter-day repeatability through the calculation of the coefficients of variation (CV). The intra-day precision was determined by analyzing five replicates within the same day, whereas the inter-day precision was established by analyzing three series of three independent extractions carried out at three different times.

The studied EDCs were determined in five commercial apple juices sold in PET containers (M1 and M2), Tetra Pack packages (M3 and M4), and glass bottles (M5) in Spanish supermarkets. As mentioned, the results obtained were validated with those obtained using a reference method in the literature (Khan et al., 2020). All samples were previously homogenized, filtered, and pre-treated following the recommended procedure in each case. After assessing the existing matrix effect, results were calculated by using a standard addition calibration.

## 2.3. Results and discussion

The first step of the optimization study consisted of assessing the influence of the sorbent nature on the extraction performance. This study was carried out by testing either bare type UVM-7 silica as well as β-CD-UVM-7 and γ-CD-UVM-7. To this, SPE cartridges containing 100 mg of solid phase were constructed and 10 mL of ultrapure water spiked with 50 µg $L^{-1}$ of each of the target analytes were loaded into the cartridges. The elution was accomplished with 3 mL of MeOH. Besides, water was collected after passing through the cartridge to be able to check the retention together with the extraction performance achieved. The best results (Figure B7) were obtained when using the β-CD-UVM-7 solid phase in all cases. In the case of parabens, recoveries were of 72, 100, and 108% for MP, EP, and PP, respectively. In the case of bisphenols, all recoveries were around 100% for BPA, BPAP, and BPC. Thus, results obtained strongly supported the idea of

the size of CDs influencing the formation of the inclusion complexes with the analytes exposed. For parabens, better recoveries were observed as the analytes' molecular size increased (Table B2). As a shift from methyl to propyl-substituents occurs, recoveries improve significantly due to a better fit between parabens and β-CD (Table 2). The same behavior was observed in the case of bare UVM-7, probably due to a slight contribution of the silica mesopores in the retention, as well as in the case of γ-CD-UVM-7, whose internal cavity is possibly too large for them (Table 2). In this case, analytes may be able to enter inside γ-CD, but the interactions taking place are not strong enough to ensure proper retention. The case of bisphenols is not entirely comparable. As shown, β-CD-UVM-7 worked better with them in all cases for the same reason as in the previous case. However, no differences were observed for the three analytes, probably due to their similarities at a structural level, which lead to thinking that the structural differences between them should not be as significant as in the case of parabens (Table B2). In any case, these results also support the influence of CD on the retention mechanism taking place, with higher recoveries being obtained in all cases with the presence of accessible cyclodextrin units onto the silica structure.

Then, the elution step was studied. Methanol was chosen as extracting solvent due to the quantitative recoveries with respect to the amount of analytes retained in the solid phase already observed. Moreover, its toxicity is lower than that of other solvents that may be tested such as acetonitrile. The elution profile of methanol was thus assessed to use the minimum but enough amount of solvent in the optimized procedure. Different extractions were carried out by using volumes of 1, 2, 3, and 4 mL of methanol for the elution process. Results obtained showed that the elution was complete with only 2 mL of methanol. Concretely, 1 mL of methanol extracted from 60 to 90% of the studied compounds, depending on the analyte observed, while the second milliliter eluted the rest (from 10% to 35% in addition to the previous amount). No analytes were extracted in the next third milliliter.

Also, the possibility of concentrating the extract by evaporation and subsequent reconstitution was studied. The treatment was carried out under vacuum at 60 °C for 15 minutes followed by redissolution with 500 µL of methanol. Variations lower than 4% were obtained in the final recovery, probably due to the high stability these analytes show to temperature (Table B2). Thus, the concentration step is recommended to increase the sensitivity of the method.

Regarding the loading conditions, the first parameter studied was the pH. As it is known, fruit juices usually present an acidic pH. Due to the acid-base properties that the compounds under study may present (Table B2), together

with the influence of pH on the encapsulation properties of cyclodextrin and the common acidic pHs found in fruit juices, it was decided to test the use of loading pH 2.5 in comparison to the neutral pH used so far. The mentioned value was adjusted by dropping concentrated $H_2SO_4$ into the spiked sample. For MP and EP, the recovery improved at a rate in the range of 5 – 24% from neutral pH to the acidic pH tested. The rest of the analytes maintained the already shown quantitative recoveries. These results reinforce the idea of obtaining good extraction results in the analysis of real juice samples.

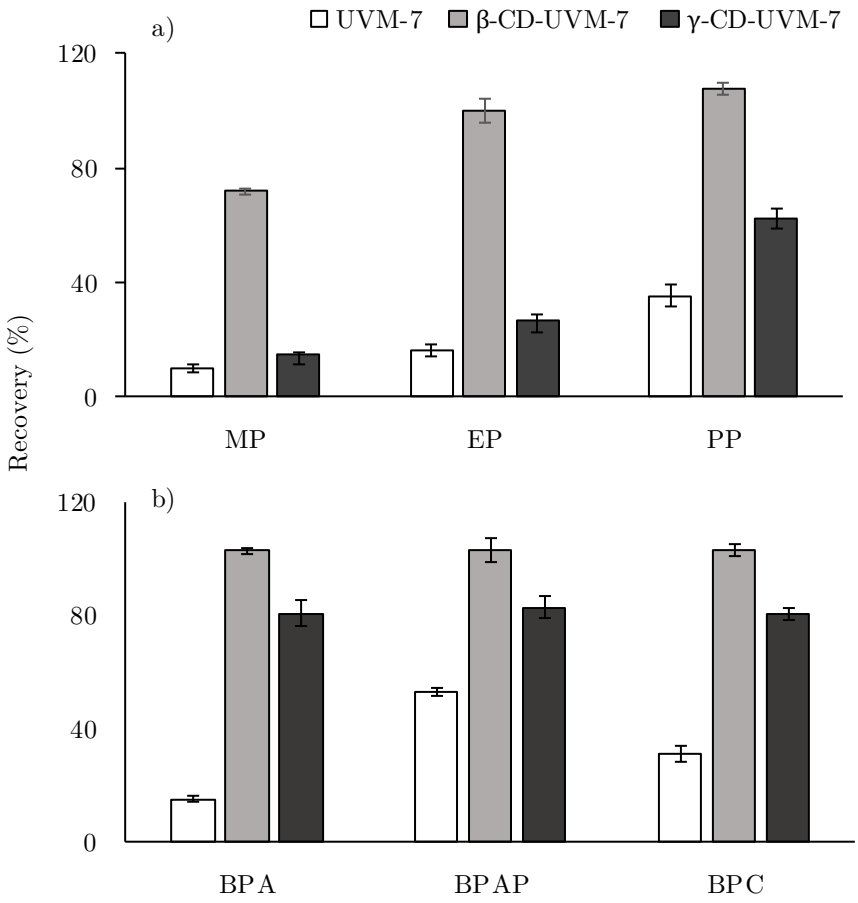



**Figure B7. Influence of the nature of the sorbent used on the recovery of EDCs of (a) parabens and (b) bisphenols.**

Besides, the ionic strength was evaluated through the modification of the NaCl content (from 0 M to 4 M). Recoveries improved considerably as the ionic strength increased to the best results with the use of 4 M. The recoveries, in this case, were 87%, 102%, 100%, 98%, 93%, and 92% for MP, EP, BPA, PP, BPAP, and BPC, respectively.

Other analytical parameters such as the breakthrough volume were also assessed. To this, several spiked samples were treated following the optimized SPE procedure, varying both the sample volume and the amount of solid phase each time. Results showed that good recoveries were obtained when using 100 mg of solid phase to extract 10 mL of sample. To improve the sensitivity, 25 and 50 mL of sample were then tested. However, significant losses were quantified in this case. For this reason, 200 mg of solid phase were also tested to extract 25 mL and 50 mL of sample. Results indicate that either 25 or 50 mL of sample can be treated by using 200 mg of the developed material, being 25 mL volume acceptable for the treatment of juice while achieving a good sensitivity.

The loading capacity was tested with spiked samples from the ng $L^{-1}$ level to the µg $L^{-1}$ level. No higher concentrations were assessed due to the expected low content of the studied compounds in the selected samples. The only little variations observed in the recoveries obtained for the different analytes in the range studied can be simply due to the dispersion of the results and not to the influence of the concentration.

To conclude, the reusability of the material was studied. Results showed that the solid phase is reusable for at least four extractions by maintaining the recovery. For this reason, the possibility of reusing the material can be included among its functionalities and constitutes therefore a great advantage. These results were expected due to those obtained in previous studies when using polymeric materials based on cyclodextrins.

At this point, the analytical performance of the SPE protocol was evaluated concerning global recovery, precision, limits of detection (LODs) and limits of quantification (LOQs), and linearity. Parameters informed in Table B3 were estimated according to the recommendations of the IUPAC (Olivieri et al., 2006). Concretely, the lower limit of the linearity range was established from the LOQs.

As mentioned, the precision of the method was evaluated by measuring the intra-day and inter-day repeatability from different extractions of spiked samples and it was expressed in terms of the obtained CVs. The intra-day precision was estimated by analyzing three replicates within a day, whereas the inter-day precision was calculated by analyzing three series of three experiments carried out on three days. As can be seen, the CVs ranged from 0.5% in the lower limit to 6.8% as the maximum variability. In any case, the method showed good precision with deviation values below 7%. Besides, both LOD and LOQ were calculated referred to the initial sample by considering the concentration factor provided by the described extraction procedure. LODs were calculated to be between 16 and 28 ng $L^{-1}$ and LOQs were below 86 ng $L^{-1}$, showing the high sensitivity obtained

when using the proposed method. The experimental procedure developed together with the detection method selected provide a satisfactory sensitivity. This would be expected since fluorescence detection is usually characterized for showing high selectivity and sensitivity. Also, overall recoveries greater than 90% were achieved for all analytes under study, which made the concentration factors being established in the range from 46.5 to 48.5. To end, an acceptable linearity range permits the use of the developed extraction methodology in a range of concentrations of the measuring solution.

**Table B3. Analytical figures of merit established for the endocrine-disrupting chemicals extraction method using β-CD-UVM-7.**

| Compound | CV (%) | | Recovery (%) | LOD[a] (ng $L^{-1}$) | LOQ[a] (ng $L^{-1}$) | Linearity[b] (µg $L^{-1}$) |
|---|---|---|---|---|---|---|
| | Intra-day | Inter-day | | | | |
| MP | 3.9 | 0.5 | 93 ± 4 | 26 | 80 | 2.5 – 500 |
| EP | 2.2 | 0.4 | 96 ± 3 | 28 | 86 | 2.7 – 500 |
| BPA | 4.6 | 3.3 | 95 ± 4 | 26 | 78 | 2.4 – 500 |
| PP | 4.7 | 1.8 | 97 ± 3 | 24 | 73 | 2.3 – 500 |
| BPAP | 6.8 | 6.4 | 94 ± 5 | 16 | 50 | 1.5 – 500 |
| BPC | 2.2 | 2.9 | 97 ± 3 | 17 | 52 | 1.6 – 500 |

[a] LOD and LOQ are referred to the water sample.
[b] Linearity is referred to the measuring solution.

To end, Table B4 compares the characteristic analytical features of the developed method with other methodologies reported in the literature for the determination of EDCs in different types of samples. Thus, it can be stated that the here-presented analytical parameters obtained were at least comparable or even better than those commonly found in the literature (Asimakopoulos, Wang, Thomaidis, & Kannan, 2014; M. S. Chang, Shen, Yang, & Wu, 2012; Chormey, Bodur, Baskın, Fırat, & Bakırdere, 2018; Mousa, Basheer, & Rahman Al-Arfaj, 2013; Vela-Soria et al., 2013). As can be seen, many of the reported methods use extraction systems that do not involve adsorbing solid phases. However, and as mentioned before, its use can help to considerably improve certain characteristics of the extraction, such as the selectivity achieved. This behavior can be clearly seen, given that the lowest detection limits and variation of the recoveries obtained are accomplished when the mentioned adsorbents are used. Furthermore, the usage of reusable materials results in greener and more sustainable analytical methodologies.

**Table B4. Comparison of the developed method for endocrine-disrupting chemicals using β-CD-UVM-7 with other methods in the literature.**

| Reference | Extraction | Sample | Material | Analytes | LOD ($ng\ L^{-1}$) | Recovery (%) |
|---|---|---|---|---|---|---|
| (Asimakopoulos et al., 2014) | LLE | Urine | - | Bisphenol A diglycidyl ethers, p-hydroxybenzoic acid esters, benzophenone-type ultraviolet filters, triclosan, triclocarban | 70 – 700 | 25 – 135 |
| (Mousa et al., 2013) | Electro-enhanced SPME | Blood and seawater | Commercial polydimethylsiloxane fiber | Diethylphthalate, butylbenzyl phthalate, di-n-butyl phthalate, bisphenol A | 4 – 150 | 74 – 95 |
| (M. S. Chang et al., 2012) | SBSE | Bottled water, personal care products, soap, lotions, and urine | Polydimethylsiloxane sorptive stir bars | 4-n-nonylphenol, triclosan, di-n-butyl phthalate | 1400 – 11000 | 47 – 92 |
| (Vela-Soria et al., 2013) | Dispersive liquid-liquid microextraction | Human serum | - | Methylparaben, ethylparaben, propylparaben, butylparaben | 100 – 200 | 96 – 103 |
| (Chormey et al., 2018) | LPME | Water | - | 4-n-octylphenol, diazinon, 4-nonylphenol, heptachlor, Aldrin, endosulfan α, cis-chlordane, bisphenol A, dieldrin, endosulfan β, estrone, 17-β-estradiol | 300 – 1300 | 91 – 111 |
| Proposed method | SPE | Apple juice | β-CD-UVM-7 mesoporous silica | Methylparaben, ethylparaben, propylparaben, bisphenol A, bisphenol AP, and bisphenol C | 16 – 28 | 93 – 97 |

Table B5. Influence of the matrix in the recovery of EDCs using β-CD-UVM7.

| Compound | Recovery (%) | |
|---|---|---|
| | β-CD-UVM-7 | $C_{18}$ |
| **MP** | 63 ± 3 | 48.5 ± 1.1 |
| **EP** | 69 ± 3 | 23 ± 5 |
| **BPA** | 80.0 ± 1.8 | 21 ± 4 |
| **PP** | 59 ± 3 | 11 ± 2 |
| **BPAP** | 64 ± 7 | 58 ± 2 |
| **BPC** | 64 ± 5 | 59 ± 2 |

Table B6. Analysis of endocrine-disrupting chemicals using the extraction method developed with β-CD-UVM-7 (μg $L^{-1}$, $\bar{x} \pm s$) and its validation.

| Compound | M1 PET container | | M2 PET container | | M3 Tetra Pack package | | M4 Tetra Pack package | | M5 Glass bottle | |
|---|---|---|---|---|---|---|---|---|---|---|
| | β-CD-UVM-7 | $C_{18}$ | β-CD-UVM-7 | $C_{18}$ | β-CD-UVM-7 | $C_{18}$ | β-CD-UVM-7 | $C_{18}$ | β-CD-UVM-7 | $C_{18}$ |
| **MP** | 1.92 ± 0.15 | 1.8 ± 0.1 | 16.2 ± 0.7 | 14 ± 3 | 56.0 ± 0.1 | 44 ± 3 | 2.5 ± 0.3 | < LOD | < LOD | < LOD |
| **EP** | < LOD | 1.2 ± 0.1 | < LOD | < LOD | 74 ± 2 | 68 ± 7 | 10 ± 2 | 11.0 ± 0.3 | 8.1 ± 0.4 | 8.9 ± 0.4 |
| **BPA** | 7.8 ± 0.4 | < LOD | 12.8 ± 0.8 | < LOD | 5 ± 1.3 | < LOD | < LOD | < LOD | 1.5 ± 0.3 | < LOD |
| **PP** | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| **BPAP** | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| **BPC** | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |

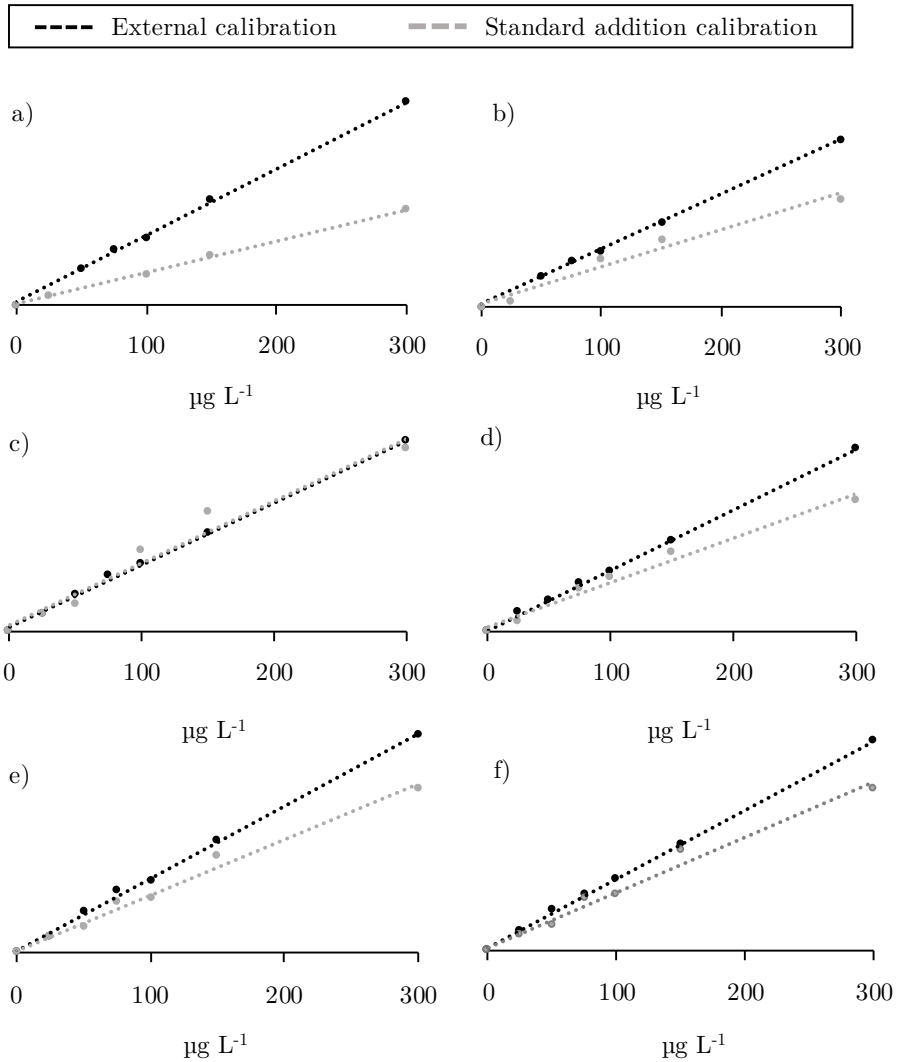



**Figure B8. Comparison of the external calibration curves and the standard addition calibration curves for (a) methylparaben (b) ethylparaben (c) bisphenol A (d) propylparaben (e) bisphenol AP, and (f) bisphenol C to complement the evaluation of the existing matrix effect. Concentration range 0-300 µg $L^{-1}$.**

The matrix effect was studied in a two-way process. First, the extraction efficiency of real juice samples spiked with 50 µg $L^{-1}$ of each of the EDCs under study, following both the proposed method and the chosen reference method (Khan et al., 2020), was assessed. Results are shown in Table B5. As expected, the recovery obtained was lower than that achieved during the optimization study with spiked ultrapure water due to the enhanced complexity of the matrices. It is important to highlight the significantly lower recoveries obtained in the case of

the reference method. Concretely, EP, BPA, and PP were mostly lost following the recommended extraction procedure. In the case of the proposed extraction methodology, it is important to highlight the case of BPA, whose losses were minor in comparison with those observed for the rest of the target compounds. Based on the results obtained, the determination of EDCs in juice samples must be carried out by applying a standard addition method.

For this reason, a standard addition calibration was measured in comparison with the external calibration used so far to confirm the matrix effect and analyze real samples. As can be seen in Figure B8, significant variations in the slopes of the calibration curves were obtained in all cases excepting the BPA, which would coincide with the behavior observed in the last experiment. Moreover, it can be stated that the matrix effect was higher for MP and EP (-53 and -33% of slope variation), followed by PP, BPAP, and BPC (-26, -24, and -20%, respectively).

Also, it must be highlighted that free cyclodextrins are commonly added in solution to natural fruit juices as additives to guarantee long-term preservation and to keep their good properties (Matencio, Navarro-Orcajada, García-Carmona, & López-Nicolás, 2020; Pereira et al., 2021). Since their presence may constitute potential interfering substances in the determination of EDCs developed through maintaining them in solution and avoiding their retention in the solid phase, a study of interferences was carried out to evaluate this possibility. To it, the extraction efficiency of real juice samples spiked with 50 µg $L^{-1}$ of each of the EDCs under study containing a 15 mM concentration of either free α-, β-, or γ-CD in solution (Andreu-Sevilla, López-Nicolás, Carbonell-Barrachina, & García-Carmona, 2011) was evaluated. Results showed that free CDs represent indeed potential interfering ingredients. Specifically, α-CD potentially interfered with a decrease in the recoveries for MP, EP, and PP (recoveries decreased to 10, 12, and 7%, respectively). In the same way, β-CD interfered for MP, EP, PP, BPC, and slightly for BPA and BPAP (recoveries quantified were of 12, 13, 60, 6, 11, and 55% for MP, EP, BPA, PP, BPAP, and BPC, respectively). Finally, γ-CD showed to disturb in the recovery for BPA, BPAP, BPC, and slightly for PP (recoveries decreased to 21, 25, 18, and 40%, respectively), while the rest of the analytes got their initial values back. The explanation for these phenomena may be found in the sizes of the CD cavities together with the sizes of the analytes molecules (Table B2), as commented previously. This is, analytes that fit better with each CD decreased the recovery obtained in the solid phase since they maintained in solution, while the rest were able to be quantified in the same conditions.

At this point, the target EDCs were determined in five commercial juice samples (Table B6) free of CDs in solution. Given them, it can be concluded that EDCs are present in juice at trace level, as expected. The compounds that are more commonly found in the packages studied are MP, PP, and BPA. Moreover, PET containers show an increased content of the variety of EDCs in comparison with, for example, Tetra Pack containers. The presence of PP, BPAP, and BPC is less remarkable.

Regarding the comparison of the results with those obtained using the reference extraction methodology, it can be stated that both methods are comparable among them (Harris, 2007). The differences observed in the results may be due to (i) the interferences in the reference method, since the solid phase used does not present a high selectivity and this fact may influence the detection step, and (ii) the higher detection limits of the reference method coming from its lower extraction efficiencies, which could convert a β-CD-UVM-7-detectable compound into non-detectable. However, it can be observed that the results obtained in both cases are at the same concentration level, that is, at the trace level, so the method developed can be considered validated.

## 2.4. Conclusion

In this study, the extracting features of the β-CD-UVM-7 developed sorbent have been validated through the SPE of endocrine-disrupting chemicals from acidic fruit juices for their subsequent quantification.

After the optimization of the extraction parameters, good sensitivity has been achieved and the repeatability of the method can be classified as satisfactory. The reported method represents a simple procedure as a promising tool for systematically analyzing migrating EDCs residues in bottled acidic fruit juices. As a future insight, the use of the developed sorbent to clean fruit juices from high-concern substances as EDCs prior to consumption may also be a topic of interest to study.

# 3

# Determination of fluoroquinolones in milk

## 3.1. Introduction

Antibiotics are widely used in the prophylaxis and treatment of a variety of food-producing animal diseases such as pulmonary, urinary, and digestive infections. They are also common feed additives employed for increasing the growth rate of livestock (Adachi, Yamamoto, Takakura, & Kawahara, 2013; Marazuela & Moreno-Bondi, 2004). However, their misuse and overuse in intensive cattle increase the possibility of finding excess residues in animal foods such as the milk coming from dairy cows. This overage may give rise to public health, environmental, and industrial problems. Allergic reactions, increasing antibiotic resistance, or production of dairy derivatives can be mentioned among the most distressed areas (Marazuela & Moreno-Bondi, 2004).

A class of non-steroidal synthetic antibiotics widely applied to poultry and livestock are fluoroquinolones (FQs) (K. Yu, Yue, Xu, & Jiang, 2020). They are characterized for their low price, low cross-resistance, and broad-spectrum antimicrobial activity due to the piperazinyl group in their structure (Lee, Peart, & Svoboda, 2007) while contributing with high drug tolerance and low toxicity (Blanka & Tőkés, 2011). However, excessive fluoroquinolone residues can cause serious safety and health problems because of their potential carcinogenicity and antibiotic resistance (M. Zhang, Chen, Zhao, & Zeng, 2020).

With the aim of ensuring food safety, the European Union has established strict regulations on the presence and control of residues of veterinary drugs in foods (2017/625/EU, 2017; 470/2009/EC, 2009). Therefore, developing sensitive

and reliable methods for the analysis of trace fluoroquinolones in foodstuff is advisable. Despite the progress made in recent years, there is still a strong demand for new strategies to improve the performance of these analytical methods (Aymard et al., 2022).

A variety of analytical methodologies for the detection or quantification of fluoroquinolones in food and tissues has been reported in the literature (El-Aziz, Fathy, El-Enany, Aly, & Tolba, 2022). On the one hand, screening methods allow the detection of fluoroquinolones through only qualitative or semi-quantitative tests such as direct enzyme-linked immunosorbent assays (S. Hu et al., 2019), or fluorescent and colorimetric sensors (Dolati et al., 2018; Rezende et al., 2019). On the other hand, confirmatory methods include different quantification techniques such as capillary electrophoresis (X. Xu, Liu, Jia, & Shu, 2015), liquid chromatography coupled to mass spectrometry, fluorescence, or UV detection (Bailac, Barrón, & Barbosa, 2006; Rocha, Santos, da Silva, Augusti, & Faria, 2015; Stoilova, Surleva, & Stoev, 2013), spectrophotometry (Attimarad & Nair, 2011), and spectrofluorimetry (Shah, Jan, Ullah, & Shah, 2013). Among them, fluorescence seems to offer the highest performance- and simplicity-to-cost ratio (Peris-Vicente, Tayeb-Cherif, Carda-Broch, & Esteve-Romero, 2017). However, these techniques require previous sophisticated extraction and clean-up processes (Aymard et al., 2022). Specifically, solid-to-liquid extraction is required to isolate fluoroquinolones from the original matrix (Peris-Vicente et al., 2017).

Sorption techniques such as solid-phase extraction (Zhiwen Zhang et al., 2016), liquid-liquid extraction (Bailac et al., 2006), QuEchERS (Castilla-Fernández et al., 2021), or immunoaffinity columns (Niu et al., 2019) are the most usual sample preparation procedures used for separating fluoroquinolones, being SPE the most used so far due to its advantages. Solid-phase extraction permits separating and pre-concentrating analytes in a fast and simple way. In this sense, different sorbents have been studied for preparing fluoroquinolone-containing samples through SPE as alternatives. Some examples are commercial solid phases (Prat, Benito, Compañó, Hernández-Arteseros, & Granados, 2004), molecularly imprinted polymers (Benito-Peña, Urraca, Sellergren, & Moreno-Bondi, 2008), or metal-organic framework-based materials (Lian et al., 2018). Due to its great influence on the overall performance of the analytical method applied, designing new sorbents for their use in SPE procedures is nowadays a challenge that must be overcome.

In this chapter, the γ-CD-UVM-7 material based on mesoporous silica is used as SPE sorbent for the isolation of fluoroquinolones (ofloxacin, norfloxacin, and ciprofloxacin) from milk samples. The use of a type UVM-7 material containing

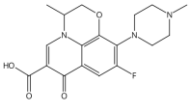
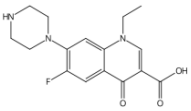
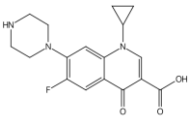
γ-CD was chosen based on the physicochemical properties of the analytes under study. To this end, the optimum experimental procedure and the analytical features of the method developed are established. Then, real spiked milk samples are analyzed following the experimental procedure proposed and the results are compared with those obtained using a reference extraction method reported in the literature.

## 3.2. Experimental

### 3.2.1. Reagents, materials, and instrumentation

The individual fluoroquinolone analytical standards were acquired from Alfa Aesar (Massachusetts, United States). Then, separate standard solutions were prepared in water at pH < 3, while the working standard multianalyte solutions were obtained from these by dilution in methanol and stored at -18°C in amber glass vials.

**Table B7. Physicochemical properties of the studied fluoroquinolones (Scifinder Scholar Database, 2021).**

| Compound | CAS | Structure | logP[a] | Molecular size (nm) | Boiling point (°C) | Vapor pressure[a] (Pa) | $pK_a$[a] |
|---|---|---|---|---|---|---|---|
| Ofloxacin | 82419-36-1 |  | 1.9 | 0.892 | 571 | $8.93 \cdot 10^{-12}$ | $pK_{a1}$: 5.19<br>$pK_{a2}$: 7.37 |
| Norfloxacin | 70458-96-7 |  | 1.7 | 0.799 | 556 | $4.60 \cdot 10^{-11}$ | $pK_{a1}$: 0.16<br>$pK_{a2}$: 8.68 |
| Ciprofloxacin | 85721-33-1 |  | 1.6 | 0.814 | 582 | $2.99 \cdot 10^{-12}$ | $pK_{a1}$: 6.43<br>$pK_{a2}$: 8.68 |

[a] Vapor pressure, logP, and $pK_a$ measured at 25 °C.

HPLC grade methanol (MeOH) ≥ 99.8%, acetonitrile (ACN) ≥ 99.8%, and acetone ≥ 99.5% were purchased in VWR ProLabo Chemicals (Radnor, United States). Ultrapure water from an Adrona (Riga, Latvia) purification system was

used during the whole experimental procedure. Other reagents such as NaCl (s) ≥ 99.5%, NaOH (s) ≥ 98.5%, formic acid, and glacial acetic acid were bought in Scharlab (Barcelona, Spain).

The procedure chosen as a reference method (Huang, Tran, & Young, 2015) was carried out using 150 mg Oasis extraction cartridges from Waters Corporation (Massachusetts, United States).

For the SPE procedure, a Vac Elut 20 from Agilent Technologies (California, United States) using 3 mL cartridges from Análisis Vínicos (Ciudad Real, Spain) was utilized. In addition, a miVac sample concentrator from SP Scientific (Warmister, United States) was operated for solvent evaporation after extraction.

The milk samples used in the study were acquired in a Spanish supermarket with three different fat rates: skimmed, semi-skimmed, and whole milk. The validation of the methodology with real matrices was conducted using the semi-skimmed milk as representative of the family, although its correct performance was also verified afterward with the other two types. Subsequently, milk samples were obtained by spiking these different fat-rate milk samples at two usual concentration levels of FQs. They acted as real samples since the analytes under study were not initially detected in them.

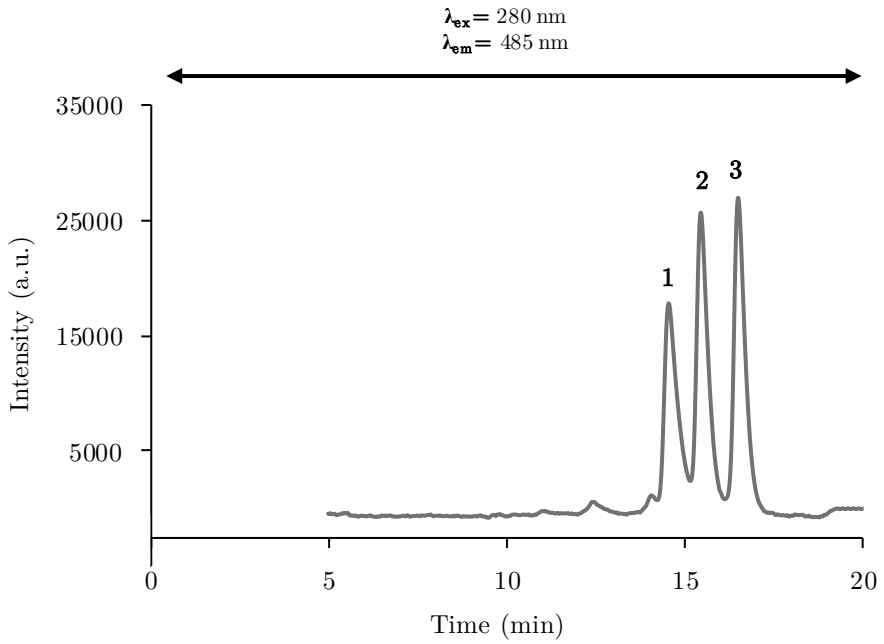



**Figure B9. HPLC-FLD chromatographic profile of a multianalyte solution of the fluoroquinolones under study in methanol: (1) ofloxacin (2) norfloxacin (3) ciprofloxacin.**

The separation of the analytes (Figure B9) was carried out using HPLC-FLD. To it, an LC-2000 Plus liquid chromatograph equipped with an FP-2020 Plus Intelligent Fluorescent Detector, a PU-2089 Plus Quaternary Gradient Pump with integrated degasser, and an I/FLC/NetII/ADC interface from Jasco (Madrid, Spain) were used. A six-way injection valve with 20 µL of injection volume from Agilent Technologies (California, United States) was utilized to inject extracts. The stationary phase used was a Kromasil $C_{18}$ column (150 x 4.6 mm, 5 µm particle size) from Análisis Vínicos (Ciudad Real, Spain). The separation was done at a 1 mL $min^{-1}$ flow using a gradient-mode $H_2O$:ACN:MeOH 3% $H_3PO_4$ (73:12:15 to 85:0:15 in 25 minutes). The fluorimetric detection was carried out at $\lambda_{ex}$=280 nm and $\lambda_{em}$=485 nm.

### 3.2.2. Optimization and validation of the SPE procedure

The optimal conditions for the adsorption of fluoroquinolones in the materials developed were evaluated by studying the parameters influencing the recovery obtained through SPE. They were evaluated based on the recovery obtained from ultrapure water spiked with 25 µg $L^{-1}$. The recovery was calculated from the ratio between the obtained concentrations and the theoretical concentration in individual triplicates (n=3).

The studied parameters were those affecting the retention (type of solid phase, ionic strength, pH, intermediate cleaning, volume of sample, and loading capability) and those affecting the elution step (type of solvent, volume of solvent, and post-evaporation step). In addition, the existing matrix effect was evaluated by spiking semi-skimmed milk samples to establish the recovery obtained in this case, and the possibility of reusing the material with milk matrices during successive extractions was also studied.

The optimized SPE procedure was established as follows. Samples are initially treated through a modification of the procedure proposed elsewhere (Pellicer-Castell, Belenguer-Sapiña, Amorós, Herrero-Martínez, & Mauri-Aucejo, 2020). In short, 2 g of NaCl are dissolved in 10 mL of milk. Then, 7.5 mL of ACN and 200 µL of acetic acid are added to the solution, and the mixture is shacked for 2 minutes. After centrifugation at 6000 rpm for 10 minutes, the upper phase, which contains FQs, is transferred into a glass tube and evaporated under vacuum at 60 °C for 20 minutes. The residue is dissolved with 10 mL of a NaCl 2 M aqueous solution at pH 7.0 for the SPE step.

The extraction of antibiotics from milk samples is carried out using 150 mg of the γ-CD-UVM-7 mesoporous silica sorbent, which are previously conditioned with 2 mL of methanol and 5 mL of ultrapure water in SPE cartridges. Then, the

10 mL solution described above is then loaded into the cartridges and subsequently washed with 2 mL of NaCl 2 M aqueous solution, dried for 10 minutes, and eluted with 2 mL of ACN. Extracts are filtered and, after their concentration by evaporation at 60°C during 20 minutes and subsequent recomposition with 500 µL of ACN, the HPLC-FLD determination is done.

### 3.3. Results and discussion

The milk pre-treatment procedure carried out before the SPE step of the fluoroquinolones of interest was first tested (Pellicer-Castell et al., 2020). Due to the acid-base activity of the target analytes (Table B7), it was evaluated whether the precipitation of the fat and proteins contained in milk using acetic acid may influence the extraction efficiency obtained. To this, before carrying out the centrifugation step and after the addition of the acidic solution, the pH of different replicates was adjusted to acid (this is, not doing anything else), neutral, and basic. The results permitted concluding that the pre-treatment procedure was efficient as described and that the higher the acidity of the sample before centrifugation, the better extraction of fluoroquinolones in the organic phase. For this reason, it was decided to treat the milk by adding 200 µL of acetic acid before centrifugation.

Then, the first step of the optimization process was to assess the influence of the type of sorbent in the retention of FQs during SPE. To this, β-CD-UVM-7 and γ-CD-UVM-7, as well as the bare UVM-7 mesoporous sorbent, were evaluated. First, 150 mg of each solid phase were packed in 3 mL extraction cartridges, and 10 mL of NaCl 2 M ultrapure water were spiked in each case with 25 µg $L^{-1}$ of a multianalyte solution containing the three target fluoroquinolones. After loading, cleaning, and drying, the elution step was carried out with 3 mL of MeOH. The passed-through-the-cartridge water was also collected in order to check the retention of the analytes in the respective sorbents together with the extraction performance reached. As can be seen in Figure B10, the best results were obtained when using the γ-CD-UVM-7 material, as expected, followed by the pure UVM-7 and, last, by the β-CD-UVM-7 sorbent. The missing fraction of analytes was detected and quantified in the water collected. These results support once more the idea of the CD size influencing the efficiency of the extraction through interaction with appropriate-sized analytes. The behavior observed may be attributed to the size of the analyte' molecules (Table B7) together with the size of the internal cavity of the cyclodextrins (Table 2), thus being host-guest interactions easier with γ-CD. Anyway, other interactions analyte-cyclodextrins that may also take place (for example, with the external hydroxyl groups in the

CDs structure) should not be neglected. Therefore, the γ-CD-UVM-7 material was selected to continue with the optimization process. The results observed corroborate the presence of cyclodextrin units into the mesoporous silica structure, which are more accessible in the case of γ-CD, thus influencing the extraction performance achieved. Probably, the trouble found in the retention of analytes into β-CD-UVM-7 can actually be due to β-CD molecules interfering with the porous structure of the sorbent when they do not directly form inclusion complexes with analytes. Therefore, even the interaction between mesoporous silica and FQs is more difficult in the case of β-CD-UVM-7 than in the case of the bare UVM-7 material.

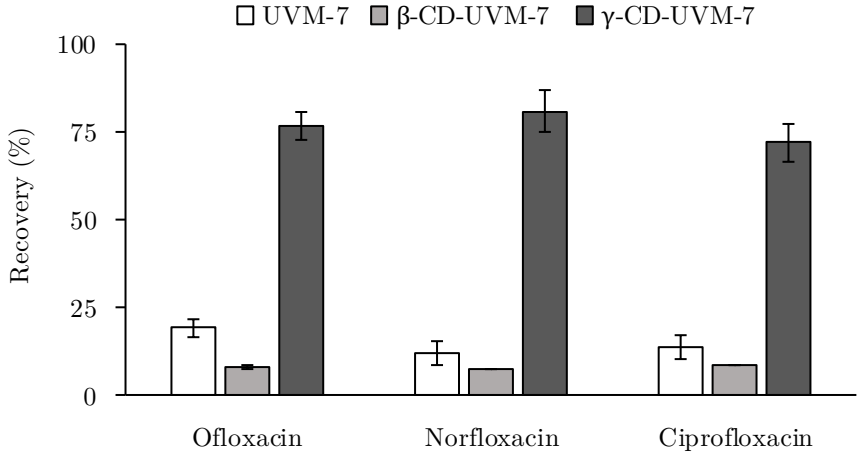



**Figure B10. Influence of the type of sorbent used on the recovery obtained for FQs.**

The distribution coefficient of analytes to the CDs in the γ-CD-UVM-7 studied may be enhanced through the salting-out effect, thus adding salt to decrease their solubility in water. The influence of ionic strength was studied by adding sodium chloride at 0 M, 1 M, 2 M, and 3 M to 10 mL of spiked water. The extraction was carried out with 3 mL ACN. As expected, the 0 M concentration gave to significant losses of analytes (recoveries of 10, 12, and 12% for ofloxacin, norfloxacin, and ciprofloxacin, respectively), whereas results improved as salt concentration increased to a maximum reached at 2 M (recoveries of 83, 88, and 92% for the same analytes as above). For this reason, a 2 M salt concentration was chosen to carry out the extraction.

Then, the loading pH was assessed due to the possibility of the acid-base activity of the target analytes influencing their interaction with CDs. To this, 10 mL of spiked water were buffered at pH 4.5, 7.0, and 9.5 and treated following the same extraction procedure. The only slight influence was observed with

ofloxacin, which improved the recovery obtained from pH 4.5 to pH 7.0 (the recovery increased from 73 to 84%). Norfloxacin and ciprofloxacin maintained the recoveries obtained at every different pH. Taking into account these results, pH 7.0 was selected to continue with the optimization since basic pH could spoil the structure of mesoporous silica although maintaining the CD units in it.

A relatively aggressive intermediate washing may help to remove other interfering compounds in the sample matrix. Therefore, different washings consisting of 3 mL of solutions containing 100% $H_2O$ 2 M, 10:90% MeOH:$H_2O$ 2 M, and 20:80% MeOH:$H_2O$ 2 M were assessed after charging the cartridges with 10 mL of spiked water. Unfortunately, the recovery achieved decreased while the content in MeOH increased. However, taking into account the sample treatment to remove fats, proteins, and other interfering compounds of milk, it was decided that the intermediate washing with 100% $H_2O$ 2 M was enough during the SPE procedure.

The volume of sample loaded into the cartridges was also evaluated for the use of 150 mg of solid phase. The recoveries obtained maintained from 10 mL to 25 mL at around 90, 85, and 85% for ofloxacin, norfloxacin, and ciprofloxacin, respectively, whereas a decrease in the recovery for norfloxacin and ciprofloxacin was already calculated when loading 50 mL (48 and 52%, respectively). Since the volume of sample used is actually defined by the initial volume of milk used in the pre-treatment step, it was decided to carry out the reconstitution of the sample after this first step with 10 mL of 2 M water to avoid loss of analytes due to carryover.

Besides, the loading capacity of the solid phase was tested with spiked samples at 5 µg $L^{-1}$, 50 µg $L^{-1}$, 100 µg $L^{-1}$, 500 µg $L^{-1}$, and 1 mg $L^{-1}$. No higher concentrations were assessed due to the expected trace concentrations of FQs found in milk. All the recoveries were maintained from 5 µg $L^{-1}$ to 500 µg $L^{-1}$, while 1 mg $L^{-1}$ conducted to significant losses, especially in the case of ciprofloxacin. This is, the working range for the method should not exceed 500 µg $L^{-1}$ of the target analytes in milk samples.

Regarding the elution step, MeOH, acetone, and ACN were assessed as solvents. While MeOH led to lower recoveries (66, 67, and 75% for ofloxacin, norfloxacin, and ciprofloxacin, respectively), acetone practically exceeded 100% of recovery in all cases, probably due to the ease of evaporation of this solvent. Since ACN led to good results and did not present the same problem as acetone, it was chosen as the best alternative. In addition, the elution profile of this solvent was evaluated in order to use just the necessary amount during the experimental procedure. Three subsequent extractions were carried out for the same loading by

using volumes of 1 mL to a final volume of 3 mL of ACN. The elution of FQs was complete with only 2 mL of ACN, while the last 1 mL used led to so low recoveries that they were below the limit of detection. Due to the results obtained, the elution process was established to be performed by using 2 mL of ACN.

Due to the optimal thermal and physicochemical properties of the fluoroquinolones treated (Table B7), the possibility of carrying out a final concentration step by evaporation of the extraction portions and subsequent reconstitution was studied. The treatment was done under vacuum at 60 °C for 20 minutes followed by redissolution with 500 µL of ACN. The results obtained indicated that no analyte losses were observed during an evaporation step, and so it is recommended to do it to improve the sensitivity of the extraction method used.

At this point, the analytical performance of the final protocol was evaluated with respect to the global recovery, precision, limits of detection (LODs) and quantification (LOQs), and linearity. The lower limit of the linearity range was established from the LOQs. Therefore, parameters informed in Table B8 were estimated according to the recommendations of the IUPAC (Olivieri et al., 2006). To this, the whole pre-treatment and extraction procedure was tested using semi-skimmed milk spiked with 25 µg $L^{-1}$ of a multianalyte solution of the target antibiotics.

**Table B8. Analytical figures of merit established for the fluoroquinolones extraction method developed using γ-CD-UVM-7.**

| Compound | CV (%) | | LOD[a] (ng $L^{-1}$) | LOQ[a] (ng $L^{-1}$) | Recovery (%) | Linearity[b] (µg $L^{-1}$) |
|---|---|---|---|---|---|---|
| | Intra-day | Inter-day | | | | |
| Ofloxacin | 4 | 8 | 39 | 120 | 69 ± 4 | 2.4 – 250 |
| Norfloxacin | 4 | 10 | 30 | 92 | 65 ± 3 | 1.8 – 250 |
| Ciprofloxacin | 8 | 5 | 33 | 100 | 60 ± 4 | 2.0 – 250 |

[a] LOD and LOQ are referred to the water sample.
[b] Linearity is referred to the measuring solution.

As can be seen, the final recovery of the extraction method decreased slightly (60 – 70%) due to the possible losses that may accumulate during the complete treatment of the sample. However, the recovery obtained has good precision and an adequate magnitude to be able to determine the analytes under study in milk samples with a high degree of confidence. There were precisely the coefficients of variation that helped to define the precision of the method. Inter-day experiments, which led to a variation lower than 8%, were performed for three series of three extraction experiments carried out on three independent days, whereas intra-day

experiments, which led to variations up to 10%, were established by analyzing three replicates within a day. Hence, good precision and repeatability of the method were demonstrated. Also, an acceptable linearity supported the use of the developed methodology in an adequate range of concentrations of the measuring solution. Regarding the LODs and LOQs, they were calculated for each analyte considering the concentration factor provided by the described procedure. Similar results were obtained for the three compounds under study, showing a high sensitivity since it was possible to quantify very trace concentrations. Thus, the experimental procedure developed together with the detection method selected provide a satisfactory sensitivity.

To end, the possibility of reusing the solid phases with real matrices coming from the pre-treatment step was assessed. Results showed that it is possible to reuse the material synthesized at least for six consecutive extractions while maintaining the recoveries initially observed for the analytes of interest.

Once the SPE procedure was optimized, a comparison of the here described method with others reported in the literature for the determination of fluoroquinolones in milk and food samples is shown in Table B9.

From this data, it can be stated that a previous pre-treatment or clean-up procedure of antibiotics from the sample under study is generally needed and that direct analysis methods for complex food samples are barely described. The most commonly used extraction technique is SPE, sometimes with slight modifications (e.g., magnetic SPE). Thus, a great variety of solid phases has been reported for the reversible adsorption of FQs. Among them, MOF and COF derivatives are currently widely used. However, it is necessary to mention the use of techniques based on immunoaffinity, including modifications of existing immunosorbents or immunotechniques that may represent an alternative for the selective analysis of these analytes. Their high degree of specificity is a great advantage but, at the same time, a problem if we think in terms of the price of each of the analyses carried out. On the other hand, MOFs and COFs with magnetic properties are novel types of sorbents widely used for the extraction of different families of pollutants and other compounds of interest recently. Besides, the magnetic properties described may ease the extraction step. However, their synthesis is usually laborious, and the use of a high variety of reagents could increase the price to obtain them. Furthermore, although some improved properties characterize the new generation of sorbents they represent, they are not recognized for having a particularly high selectivity. For this reason, new ways to increase the selectivity of the analysis must be found. Among them, CDs appear as a worthy opportunity.

**Table B9. Comparison of the developed method for fluoroquinolones using γ-CD-UVM-7 with other methods in the literature.**

| Analyte | Matrix | LOD ($\mu g\ L^{-1}$) | CV (%) | Methodology | Reference |
|---|---|---|---|---|---|
| Ciprofloxacin, enrofloxacin, lomefloxacin, gatifloxacin, levofloxacin, and pefloxacin | Milk, pork, and human plasma | 0.25 – 0.5 | 2 – 6 | Magnetic SPE with $Fe_3O_4$@COFs HPLC-DAD | (Min Wang et al., 2019) |
| Flumequine, levofloxacin, ofloxacin, enoxacin | Beef and shrimp | 1.09 | 4 – 7 | Biomimetic ELISA based on a molecularly imprinted polymer as selectivity affinity agent | (Weihua Liu, Wang, Yu, & Wang, 2020) |
| Ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, lomefloxacin, marbofloxacin, norfloxacin, ofloxacin, orbifloxacin, sarafloxacin | Milk | 2 – 20 | Non-specified High variations to be solved | Magnetic nanoparticle-based lateral flow immunochromatography assay | (Chang Liu et al., 2021) |
| Ofloxacin, ciprofloxacin, norfloxacin | Milk | 0.009 – 0.016 | 3 – 7 | Magnetic SPE, zinc-based 2D-MOF HPLC-MS/MS | (Bagheri, Al Lawati, Al Sharji, & Hassanzadeh, 2021) |
| Ofloxacin, norfloxacin, ciprofloxacin | Milk | 0.030 – 0.039 | 4 – 13 | Sample pre-treatment and SPE HPLC-FLD | Proposed method |

Regarding the analytical performance observed, all the methods described showed good and acceptable precision, with CVs that are comparable between them. Only one of the selected studies reported excessively high variations that should be corrected in the near future, probably due to a higher complexity or novelty of the extraction and quantification techniques used. Regarding the LODs achieved in this study, they are at least comparable or even better than those calculated from the use of other methodologies. This fact allows the detection and analysis of concentrations up to ng $L^{-1}$ with good confidence, which can ease broad compliance with international regulations on pharmaceuticals in food as well as greater protection of public health. To end, the usage of reusable materials as in this case may result in greener and more sustainable analytical methodologies to be developed.

The method proposed was then applied to the determination of ofloxacin, norfloxacin, and ciprofloxacin in three different fat-rate milks to assess its feasibility. As mentioned, the results obtained in this case were compared to those obtained using a reference method (Huang et al., 2015).

**Table B10. Analytical figures of merit established for the reference extraction method of fluoroquinolones in milk.**

| Compound | CV (%) | | LOD[a] (µg $L^{-1}$) | LOQ[a] (µg $L^{-1}$) | Recovery (%) |
|---|---|---|---|---|---|
| | Intra-day | Inter-day | | | |
| Ofloxacin | 6 | 11 | 0.14 | 0.43 | 19 ± 2 |
| Norfloxacin | 7 | 6 | 0.07 | 0.20 | 30 ± 4 |
| Ciprofloxacin | 4 | 13 | 0.09 | 0.26 | 23.4 ± 1.1 |

[a] LOD and LOQ are referred to the water sample.

First, the basic analytical features of the chosen reference method were established by reproducing the recommended experimental procedure with synthetically-spiked semi-skimmed milk at 25 µg $L^{-1}$ of the described multianalyte solution, parallel to how it was done previously with the here proposed method. In this case, inter-day repeatability experiments were also performed for three series of three extractions in three independent days, whereas intra-day repeatability was calculated from the analysis of three replicates within a day. Results can be consulted in Table B10. As observed, good precision was obtained following the extraction procedure proposed by the authors. However, worse detection and quantification limits, as well as worse recoveries in comparison with the UVM-7-based extraction method, must be acknowledged. One possible cause is that the reference method is designed for a multi-residue analysis, where the

performance depends essentially on the chosen instrumental technique. Oppositely, the performance achieved in this study must depend largely on the extraction method used, although the contribution of the separation and quantification technique used should not be forgotten either.

**Table B11. Analysis of fluoroquinolones using the extraction method developed with γ-CD-UVM-7 ($\bar{x} \pm s$) and its validation.**

| Sample | Analyte | Spiked (μg $L^{-1}$) | Reference method (Huang et al., 2015) | | Proposed method | |
|---|---|---|---|---|---|---|
| | | | Quantified (μg $L^{-1}$) | Trueness (%) | Quantified (μg $L^{-1}$) | Trueness (%) |
| **M1 Skimmed** | Ofloxacin | 50 | 59.3 ± 0.3 | 119 | 48 ± 4 | 96 |
| | Norfloxacin | | 68.3 ± 0.9 | 137 | 64.6 ± 1.1 | 129 |
| | Ciprofloxacin | | 71.3 ± 1.1 | 143 | 63.3 ± 2.0 | 127 |
| **M2 Skimmed** | Ofloxacin | 200 | 148 ± 4 | 74 | 141 ± 3 | 71 |
| | Norfloxacin | | 278 ± 2 | 139 | 234 ± 4 | 117 |
| | Ciprofloxacin | | 204 ± 6 | 102 | 224 ± 5 | 112 |
| **M3 Semi-skimmed** | Ofloxacin | 50 | 62.3 ± 1.1 | 125 | 55 ± 4 | 110 |
| | Norfloxacin | | 71.9 ± 1.0 | 144 | 61.5 ± 1.3 | 123 |
| | Ciprofloxacin | | 73.0 ± 1.4 | 146 | 66.7 ± 1.6 | 133 |
| **M4 Semi-skimmed** | Ofloxacin | 200 | 153 ± 14 | 77 | 138 ± 4 | 69 |
| | Norfloxacin | | 294 ± 6 | 147 | 239 ± 6 | 119 |
| | Ciprofloxacin | | 203 ± 4 | 102 | 192 ± 6 | 96 |
| **M5 Whole** | Ofloxacin | 50 | 68 ± 3 | 136 | 56 ± 2 | 112 |
| | Norfloxacin | | 70.1 ± 0.9 | 140 | 62.6 ± 1.1 | 125 |
| | Ciprofloxacin | | 72.8 ± 1.6 | 146 | 65.0 ± 1.5 | 130 |
| **M6 Whole** | Ofloxacin | 200 | 167 ± 5 | 83 | 189 ± 5 | 94 |
| | Norfloxacin | | 272 ± 5 | 136 | 272 ± 4 | 136 |
| | Ciprofloxacin | | 217 ± 3 | 108 | 220 ± 2 | 110 |

Then, the real content of the target antibiotics in the selected milk was evaluated following the recommended procedure in this study. The analysis carried out showed that every fluoroquinolone was under the limit of detection, this is, any of them was detected in the samples. For this reason, the milk samples were synthetically spiked at two concentration levels at the µg $L^{-1}$ level based on the maximum permitted residue limits (470/2009/EC, 2009). This made a total of six samples to be analyzed by both methods that permitted assessing the trueness of the proposed analysis. Results are shown in Table B11.

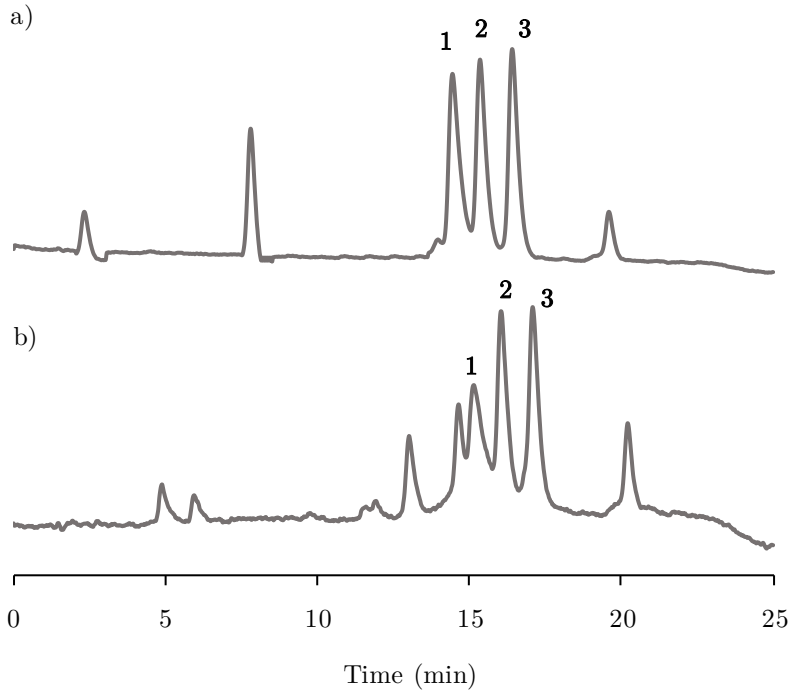



**Figure B11. HPLC-FLD chromatograms of milk spiked with 50 µg $L^{-1}$ of the target fluoroquinolones using (a) the developed method, and (b) the reference method chosen: (1) ofloxacin, (2) norfloxacin, (3) ciprofloxacin.**

The results obtained suggest that both methods, regardless of their specific analytical features, are valid for the determination of fluoroquinolones in milk. Furthermore, and contrary to what may be expected, a positive aspect found is that the amount of fat present in the sample did not influence the results obtained, with good results being reported in the three different fat-rate samples. Most of the compounds in the different samples were quantified at the expected level, some being quantified at the expected level, some being quantified below expectations, and others, somewhat, higher. Especially noteworthy is the case of ofloxacin, whose quantification tends to underestimate its content only when

working with higher concentrations, regardless of the fat content of the milk. This behavior was not observed during the optimization process in the evaluation of the influence of concentration on the recovery. Furthermore, the same behavior was also observed in the case of the reference method being applied. In any case, results were comparable between them when using the t-test of comparison of means at a 95% confidence level (Harris, 2007).

The greatest advantage of the method developed in this work results in the cleanliness of the extracted portion to analysis, which can help in a correct determination of the analytes of interest. While the extract obtained from the reference method returned us a chromatogram where some interferences could be observed, the extract obtained through the here proposed method returned a much cleaner chromatogram (Figure B11). In it, the interferences present did not overlap in any case with the peaks of interest. This fact may allow a much more accurate quantification in other applications.

## 3.4. Conclusion

In this chapter, the γ-CD-UVM-7 material has been applied to the extraction of substances of high concern from food samples. Specifically, the adsorption and subsequent analysis of fluoroquinolones that may be present in milk coming from dairy cows has been carried out through a complete pre-treatment, SPE, and quantification sequence. To this, the extracting conditions of the SPE procedure and analytical features of the whole method have been validated for the determination of ofloxacin, norfloxacin, and ciprofloxacin. After the evaluation of the extraction parameters, good sensitivity and selectivity have been achieved and the repeatability of the method can be classified as satisfactory.

The comparison of the results obtained when determining fluoroquinolones in real milk samples with another reference method using commercial solid phases shows good agreement with the results calculated using the here reported method. In this sense, the material proposed together with the extraction methodology developed represent an alternative for routine determination of antibiotics in milk samples as part of food safety controls.

# 4

# Overview on mesoporous cyclodextrin-silica materials

A new variation of the already existing type UVM-7 mesoporous silica materials for adsorbing several high-concern substances based on the existence of accessible cyclodextrins has been described. Different analytical applications have been described and tested for them in diverse conditions, including different types of analytes as well as different types of sample matrices.

The described solid phases are characterized for possessing high surface areas with interconnected porosity at different sizes for the adsorption of a variety of substances. Also, the possibility of modifying this surface with CD units helps to improve their sorption properties with enhanced sensitivity and selectivity. In this case, they have been applied for the analysis of organic pollutants in food samples, although the possibility of using them in different fields such as the environmental one or the biological analysis remains still open.

Parallel to the case of CD-silica xerogels exposed in Section A, they are also capable of being reused for several times because cyclodextrin losses are avoided in this case due to the chemical bonding of CDs to the silica structure, which adds enhanced value to them.

The positive influence of cyclodextrins in the structure of the material on the adsorption mechanism has been verified. Moreover, the idea of adapting the synthesis of the material to the analytes taking into account the influence of the cyclodextrin size on the retention of different families of compounds according to their physicochemical properties is supported here. Cyclodextrins certainly contribute to the matrix clean-up and the selectivity-enhanced extraction of the

target compounds prior to their analysis. In the case of juice analysis, other ingredients of the sample, such as free CDs present as additives, may result in interfering substances for the determination described and may be taken into account, which represents a disadvantage. In the case of the milk analysis, the high complexity of the matrix used may be an issue and makes the operator need a pre-treatment step to avoid undesired components that may hinder SPE.

# SECTION C
# Application of polymeric cyclodextrin-based materials

# 1

# Synthesis and characterization of cyclodextrin-based polymers

## 1.1. Introduction

Porous monolithic polymers are a category of materials that have attracted much interest in the last decades (Svec & Lv, 2015). Due to their advantageous properties such as their simple and *in situ* preparation, their high permeability and stability along a wide pH range, and the versatile chemistries of their surface (Vergara-Barberán, Lerma-García, Simó-Alfonso, & Herrero-Martínez, 2016), they have been converted with increasing force into a competitive alternative to conventional packed chromatographic columns (N. W. Smith & Jiang, 2008) and other common solid phases used in analytical chemistry. Their porous structure can aid to increase the loading capacity offered by them while helping to provide very low resistance to flow due to the rapid mass transport carried out within (Svec & Lv, 2015), although their effective surface area is not as high as would be desired. However, this drawback can be overcome by changing their retention efficiency with the introduction of different functional groups onto their surface, which may produce polymeric structures with enhanced selectivity (Carrasco-Correa, Ramis-Ramos, & Herrero-Martínez, 2015) in addition to the other advantages mentioned.

Although inorganic-natured supports have been the most widely used to date, polymer-based materials may present some advantages over silica-based ones, such as a simpler and faster preparation, greater choices of surface functionalities, lower pH-change sensitivity, and better biocompatibility. Therefore, several monolithic polymers based on polymethacrylate, polyacrylate, polyacrylamide,

and polystyrene have been described (Carrasco-Correa, Ramis-Ramos, Herrero-Martínez, & Lämmerhofer, 2014). As a result, monolithic stationary phases have been used not only in separation techniques (K. Liu, Aggarwal, Lawson, Tolley, & Lee, 2013; Ou et al., 2015) but also as solid sorbents in the field of sample treatment (Mompó-Roselló, Vergara-Barberán, Lerma-García, Simó-Alfonso, & Herrero-Martínez, 2021; Vergara-Barberán, Lerma-García, Simó-Alfonso, & Herrero-Martínez, 2017), constituting the possibility of tailoring their pore structure or functionalizing their surface two main benefits.

Two different strategies of surface modification can be used. In contrast to the single-step copolymerization using functional monomers (Carrasco-Correa, Ramis-Ramos, & Herrero-Martínez, 2013), post-modifications of the monolith allow the independent tuning of mechanical and flow-through porous properties and the surface chemistry of the parent polymeric support (Carrasco-Correa et al., 2014). This multi-step post-polymerization approach provides an excellent way of harmonizing incompatible reagents, including hydrophobic and hydrophilic substances, low solubility ion-pairing agents, or oxidizing and reducing agents.

Glycidyl methacrylate (GMA) is a well-known monomer that has been used to obtain functionalizable monoliths with several properties (Preinerstorfer, Lindner, & Lämmerhofer, 2005). Using ethylene dimethacrylate (EDMA) as a crosslinker, poly(GMA-*co*-EDMA) monoliths with a highly reactive surface are obtained. Different alternatives have been studied to open and modify the epoxy ring of the GMA structure, including bonding amines (Wieder, Bisjak, Huck, Bakry, & Bonn, 2006), amino acids (Dong, Dong, Ou, Zhu, & Zou, 2006), sodium sulfite (Ueki, Umemura, Li, Odake, & Tsunoda, 2005), or chiral agents (Preinerstorfer et al., 2005). Among them, click-chemistry reactions between surface thiol groups of the polymeric support and different types of substrates under soft conditions (Preinerstorfer, Bicker, Lindner, & Lämmerhofer, 2004) can be mentioned. Thiols are prone to react either via radical or catalyzed processes in mild environments with a multitude of substrates, which makes them suitable as reactive moieties on solid supports for surface functionalization (Carrasco-Correa et al., 2014).

The described family of monolithic polymeric materials has been mainly used as filling of traditional separation columns to date. However, they have also demonstrated many advantages when used in sample treatment as polymeric supports, where few works have been reported (Alwael et al., 2011). Also, CDs have been shortly studied as nanomodifiers of polymeric materials for separation columns (J. Guo et al., 2016; Q. Zhang, Guo, Wang, Crommen, & Jiang, 2014) and for their application as sorbents to SPE (Ruohua & Wenting, 2008).

In this chapter, the synthesis and characterization of porous GMA-*co*-EDMA polymeric solid phases containing surface cyclodextrin units are described and discussed. Based on the results for the analytical applications of CD-silica materials described before and the most common sizes of analyte molecules found all around, two types of CDs are studied as functionalizing agents: β- and γ-CD. Thus, the synthetic method of the poly(GMA-*co*-EDMA) nanohybrid modified with β-CD is later adapted to the use of γ-CD. In both cases, the polymeric materials are obtained in a multi-step process, where cyclodextrins are first modified with a reactive allyl group and then used to interact with the modified polymeric support employing a thiol-ene click reaction. The main features of the materials obtained are commented on, and their possibilities within the field of analytical chemistry are finally mentioned.

## 1.2. Experimental

### 1.2.1. Reagents, materials, and instrumentation

Glycidyl methacrylate ≥ 97%, ethylene dimethacrylate ≥ 98%, cystamine dihydrochloride ≥ 96%, p-toluenesulfonyl chloride ≥ 99%, 1-dodecanol ≥ 98%, and cyclohexanol 99% were acquired from Sigma-Aldrich (St. Louis, United States) for the synthesis of the polymeric materials. Cyclodextrins used in the synthesis processes described were β-CD and γ-CD, both of reagent grade and acquired from CycloLab (Budapest, Hungary). In addition, azo-bisisobutyronitrile 98% (AIBN) was from Honeywell Fluka (Fischer Scientific, Buchs, Switzerland), and allylamine ≥ 98%, as well as dimethylformamide (DMF) ≥ 99.8%, were purchased from Alfa Aesar (Massachusetts, United States). Tris(2-carboxyethyl)phosphine hydrochloride 98% (TCEP), ACN ≥ 99.5%, methanol ≥ 99.8% and ethanol ≥ 99.5% were bought in VWR ProLabo Chemicals (Radnor, United States). Finally, ultrapure water from an Adrona purification system (Riga, Latvia) was employed during the whole experimental procedure.

Different characterization techniques were used throughout the synthesis process. First, the β-CD modification process was studied with $^{1}H$ – $^{13}C$ nuclear magnetic resonance (NMR) using a Bruker DRX500 spectrometer (Massachusetts, United States) and through optical rotation, with a Perkin Elmer polarimeter (sodium light, D line 589 nm, 1 dm cell) (Massachusetts, United States). The modification of γ-CD was studied with $^{1}H$ NMR using a Bruker DRX500 spectrometer (Massachusetts, Unites States) and matrix-assisted laser desorption/ionization (MALDI) experiments with an Advance II 400 and an Autoflex Speed MALDI-TOF (Massachusets, United States).

To study the physical and chemical properties of the developed solid phases, other characterization techniques were used: scanning electron microscopy (SEM), with a Hitachi S-4800 (Chiyoda, Japan); $N_2$ adsorption-desorption, with a Micromeritics ASAP-2020 (Norcross, United States); thermogravimetric analysis (TGA), with a Setaram Setsys 16-18 (Caluire-et-Cuire, France); infrared spectroscopy with total attenuated reflectance (IR-ATR) with an Agilent Cary 630 (California, United States); Raman spectroscopy, with a Horiba-MTB Xplora (Kioto, Japan); elemental CNHS analysis, with an elemental analyzer CHNS1100 of CE Instruments (Hindley Green, United Kingdom); fluorimetry, with a Jasco FP-750 spectrofluorometer (Madrid, Spain), and confocal microscopy with an Olympus FV1000 (Tokyo, Japan). To it, difluoro{2-[1-(3,5-dimethyl-2H-pyrrol-2-ylidene-N)ethyl]3,5-dimethyl-1H-pyrrolato-N} boron 99% (Bodipy-Me) was purchased from Sigma-Aldrich (St. Louis, United States).

### 1.2.2. GMA-*co*-EDMA polymer synthesis and functionalization

The GMA-*co*-EDMA polymeric support (Figure C1) was prepared as it was already described (Vergara-Barberán et al., 2016). In short, a polymerization mixture containing 7.82 g of GMA (20 wt%), 1.95 g of EDMA (5 wt%), cyclohexanol (27.36 g, 70 wt%), 1-dodecanol (1.95 g, 5 wt%), and AIBN (98 mg, 1 wt% with respect to the monomers) is prepared by weighing in a 40 mL large glass vial the mentioned products. This mixture is sonicated for 5 minutes and then purged with nitrogen to remove oxygen for ten minutes. The polymerization is carried out in an oven at 60°C for 24 hours. Next, the material is extracted from the glass and washed thoroughly with 200 mL of MeOH to remove the porogenic solvents and any possible unreacted monomers. Then, it is grounded with a mortar and sieved to sizes between 100 µm and 250 µm with a steel sieve.

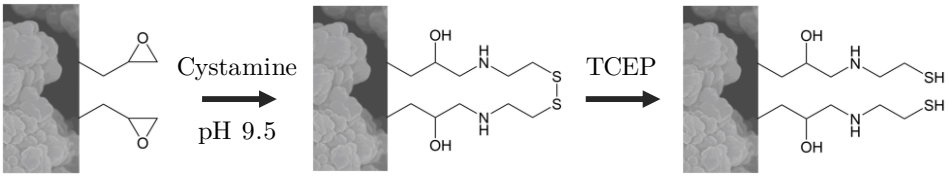



**Figure C1. Schematic representation of the GMA-*co*-EDMA synthesis and functionalization.**

Then, the synthesized powder porous material is chemically modified through a stoichiometric reaction with a cystamine aqueous solution (pH 9.5) for 5 hours at room temperature, whose product is washed with water until neutral pH.

Finally, TCEP is used to reduce the disulfide bridge in the cystamine-reacted polymeric support to obtain thiol groups that are able to react with allyl-CDs. To this, the modified powder porous material is allowed to react with a TCEP

aqueous solution for 5 hours at room temperature. Then, the GMA-SH polymeric support is repeatedly washed with water to clean it of unreacted products.

### 1.2.3. Modification of CDs

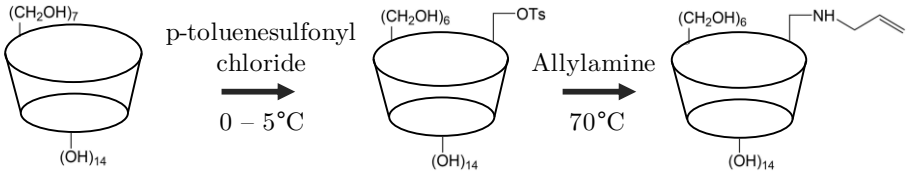



**Figure C2. Schematic representation of the synthesis of the allyl-β-CD.**

The synthesis of (6A-N-allylamino-6A-deoxy)-β-cyclodextrin (allyl-β-CD) was carried out in two steps (Figure C2). First, 6A-O-p-toluenesulfonyl-β-cyclodextrin (Ts-β-CD) is obtained in a one-pot and straightforward reaction from β-CD (Brady, Lynam, Sullivan, & Ahern, 2000). Due to the enhanced reactivity of the –OTs group, it is then used as starting product to synthesize the allyl- derivative with minor modifications on the procedure already described (Zhenbin Zhang et al., 2011). Specifically, a solution of 1.97 g (1.53 mmol) of Ts-β-CD in 30 mL (306 mmol) of allylamine is stirred at reflux (70°C) for 24 hours. The yellow solution obtained is cooled at room temperature and is then diluted with 30 mL of methanol. When adding 200 mL of ACN, the final colorless product is precipitated. To end, allyl-β-CD is filtered and dried at a high vacuum.

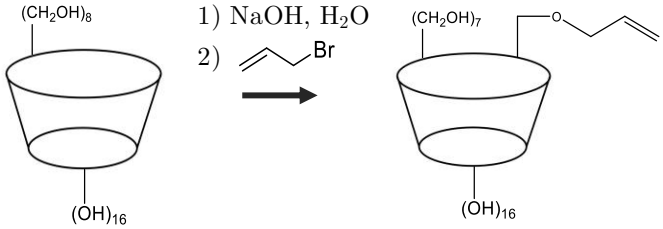



**Figure C3. Schematic representation of the synthesis of the allyl-γ-CD.**

The allylation of γ-CD to obtain 6I-O-allyl-γ-cyclodextrin (allyl-γ-CD) was carried out following an experimental procedure (Figure C3) based on the one already described (Řezanka, Eignerová, Jindřich, & Kotora, 2010). The impossibility of modifying γ-CD via tosylation (Brady et al., 2000) followed by reaction with allylamine (Zhenbin Zhang et al., 2011) led to this alternative one-pot and rapid way of functionalizing the cyclodextrin of interest. The synthetic procedure applied is easier, faster, and greener than the previous with β-CD. In short, 6.5 g of γ-CD (5 mmol) are dissolved in 50 mL of a 2 M NaOH aqueous solution. Then, 0.52 mL of allyl bromide (6 mmol) are subsequently added to the solution. After 5 hours at 50 °C, the pH is acidified to quench the reaction and

the product is precipitated with cold acetone. The solid product is therefore separated from the solution by centrifugation.

### 1.2.4. Synthesis of the final CD-based polymeric solid phase

The final GMA-S-CD materials development is done by reacting the respective allyl-CD with the GMA-SH polymeric support (Figure C4). To it, 500 mg of the GMA-SH support react with 2.5 mmol (1 eq.) of the modified cyclodextrin in each case and 33 mg of AIBN during 48 hours at 60°C in 100 mL of DMF. The amount of functionalized CD is established taking into account the modification yield of 2,3-epoxypropyl groups in the parent GMA-*co*-EDMA polymer studied previously (Carrasco-Correa et al., 2015) and the purity of the substituted CDs inferred from their characterization process. The final product is washed thoroughly with ethanol to remove DMF traces.

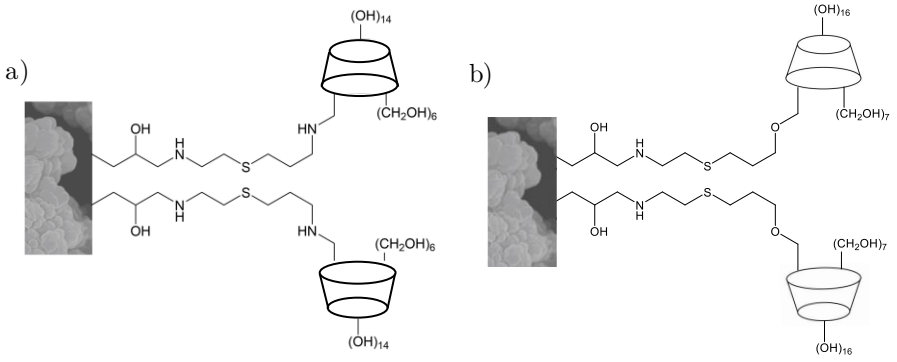



**Figure C4. Schematic representation of the final CD-based polymeric materials: (a) GMA-S-β-CD solid phase and (b) GMA-S-γ-CD solid phase.**

Also, different ratios allyl-CD:GMA-SH were synthesized to study the influence of the quantity of CD in the material analytical performance. Specifically, different synthetic procedures using 0.5 eq. and 2 eq. of allyl-CDs with respect to the GMA-SH support were carried out.

## 1.3. Results and discussion

### 1.3.1. Characterization of β-CD polymeric materials

The products obtained during the synthesis process were semi-quantitatively characterized using elemental CNHS analysis. From the analysis, and taking into account the difficulties due to the polymeric nature of the support, some general assumptions were made from the acquired data. First, it was observed that the molar relation of sulfur remained nearly constant from the cystamine-reacted polymeric support to the GMA-S-β-CD material with respect to the other

elements. In addition, the nitrogen molar relation increased its value from the GMA-SH support to the final GMA-S-β-CD material, as expected due to the binding of the allyl-β-CD carried out (Figure C4).

The description of $^{1}H$ and $^{13}C$ NMR experiments for Ts-β-CD and allyl-β-CD showed the presence of a quite low amount of the ditosylate product as an impurity for the Ts-β-CD synthesis in the aromatic region, as expected (Brady et al., 2000). Also, the characterization of these products led to a $[\alpha]_D^{20}$ of +118.4 (c=0.49 g/100 mL in dimethyl sulfoxide) for the Ts-β-CD and to a $[\alpha]_D^{20}$ of +133.4 (c=0.61 g/100 mL in dimethyl sulfoxide) for the allyl-β-CD.

Raman spectroscopy (Figure C5) was employed as the technique to verify the binding of allyl-β-CD onto the surface of the GMA-SH material. Thus, the characteristic stretching mode of thiol groups (between 2500 and 2700 $cm^{-1}$) was evidenced for the GMA-SH support, according to the CNHS analysis described. Before the thiol modification (parent GMA-*co*-EDMA polymeric support), and after the attachment of the allyl-β-CD onto the surface of the modified polymer (GMA-S-β-CD material), the mentioned characteristic stretching mode of thiol disappeared, this indicating the assembling between β-CD and the polymeric support through the disappearance of the thiol group.

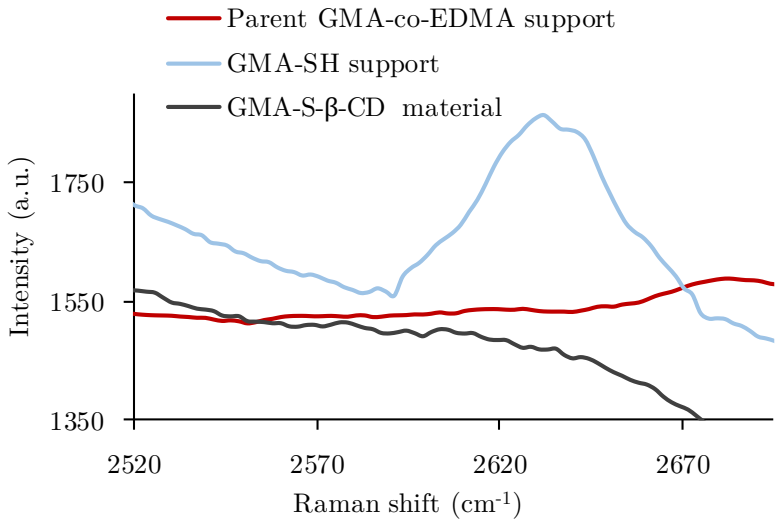



**Figure C5. Raman spectra of the bare polymeric supports in comparison with the modified GMA-S-β-CD material. Measurements were carried out with a visible green laser at λ=532 nm in the 500 – 3500 $cm^{-1}$ range.**

Besides, the amount of β-CD incorporated onto the polymeric support was roughly estimated from the thermogravimetric analysis curves (Figure C6). The comparison of the curve obtained for the GMA-*co*-EDMA polymeric support and

the final GMA-S-β-CD material showed some subtle differences. In both cases, an abrupt and major weight loss was detected between 250 and 320°C, but based on the percentage of weight loss this step seemed to be more important for the parent polymer. In fact, this stage may correspond to the polymer degradation that completes its thermal evolution in a second and less pronounced step occurring between ca. 300 and 450°C. In the case of the GMA-S-β-CD material, the shape of this second stage clearly differed from that of the original polymeric support. At this point, it must be remarked that, according to the literature (L. X. Song et al., 2008), the thermal decomposition of cyclodextrins expands up to higher temperatures (from 450 to 500°C) than those observed for the GMA-*co*-EDMA polymers. From the differences detected, a rough estimation about the maximum CD content of the synthesized material of ca. 3.7 wt% was intuited.

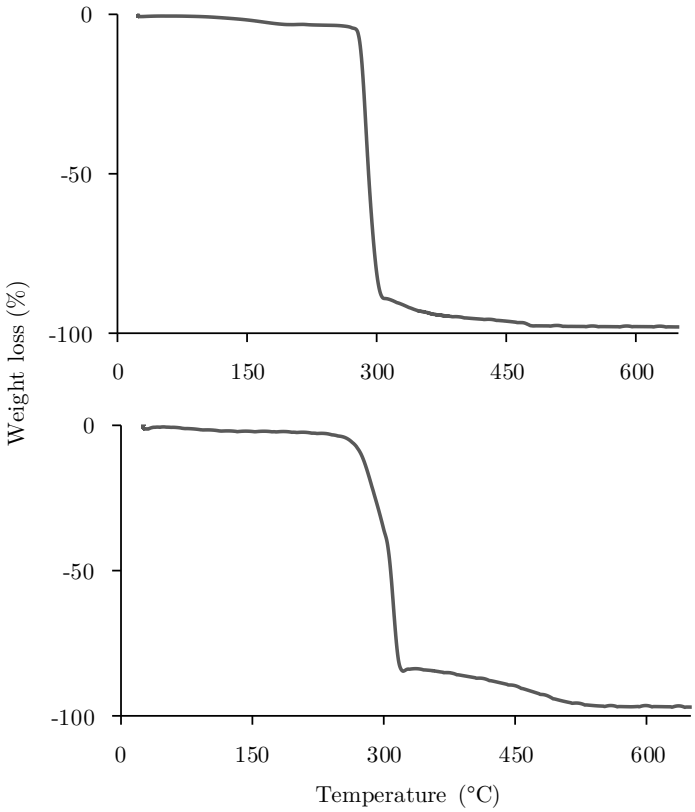



**Figure C6. Thermogravimetric analysis of (a) the parent GMA-*co*-EDMA support and (b) the GMA-S-β-CD material. Measurements were carried out in an $O_2$ environment and at a heating speed of 10°C $min^{-1}$.**

Moreover, the CD incorporation slightly altered the typical infrared spectrum of the parent polymer (Figure C7). The spectrum of the GMA-S-β-CD material

showed new signals appearing. The broad band centered at ca. 3500 $cm^{-1}$ can be assigned to the stretching O-H (21 units per CD molecule) and N-H (in a lower proportion) bonds, and the band intensity of the $\upsilon_s$ and $\upsilon_{as}$ $CH_2$ stretching modes (2900 – 3000 $cm^{-1}$) increased after the CD inclusion due to the effect of the $CH_2$ moieties. Furthermore, additional bands at the 1300 – 1500 $cm^{-1}$ and 900 – 1100 $cm^{-1}$ ranges appeared (partially overlapped with the polymer signals), which can be respectively assigned to δ(C-O-H) and υ (C-OH) vibration modes of the CD functional groups.

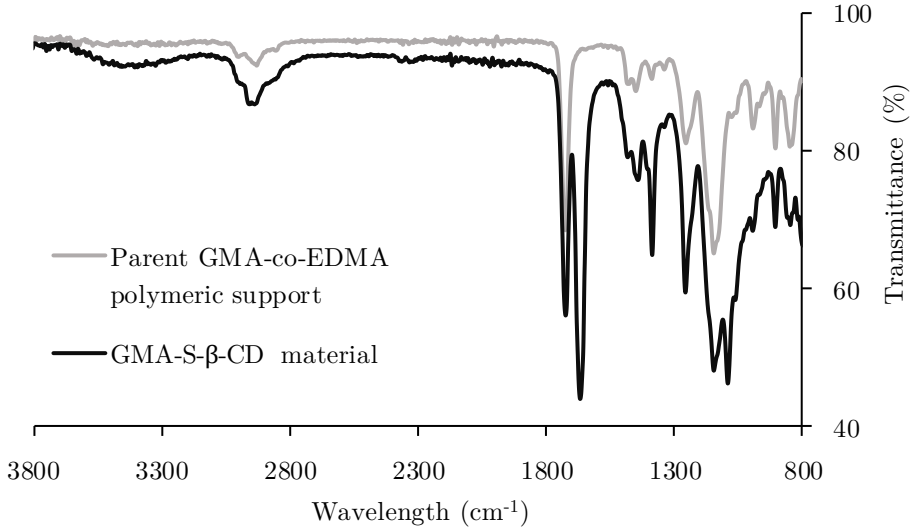



**Figure C7. FT-IR spectra of (a) the parent GMA-*co*-EDMA support and (b) the GMA-S-β-CD material.**

The morphology of the GMA-S-β-CD material was studied using SEM. As it is shown in Figure C8, the hybrid polymeric material containing cyclodextrin did not show significant changes in comparison with a normal GMA-*co*-EDMA porous polymer skeleton (Vergara-Barberán et al., 2016). In both cases, similar microglobular aggregates were detected with sizes between 1 µm and 3 µm. This observation is in agreement with the infrared spectroscopy and the thermogravimetric analysis data, which indicated a low amount of cyclodextrin in comparison with the polymer skeleton. This fact, together with the relatively small size of the cyclodextrin molecules, makes expecting no changes in the image observed. The $N_2$ adsorption-desorption isotherms also confirmed the preservation of the morphology and texture of the polymer. Although the typical macroporosity of the GMA-*co*-EDMA supports is out of the detection window of the experiment, it was possible to determine the specific surface areas through

the application of the BET model. As expected, statistically equivalent values of 3.3 ± 0.1 $m^2$ $g^{-1}$ and 3.2 ± 0.2 $m^2$ $g^{-1}$ were estimated for the GMA-*co*-EDMA polymeric support and the GMA-S-β-CD material, respectively. These values were in good agreement with the relatively low surface areas of these types of polymers, as expected. However, they were enough to apply the material to the purpose of this work.

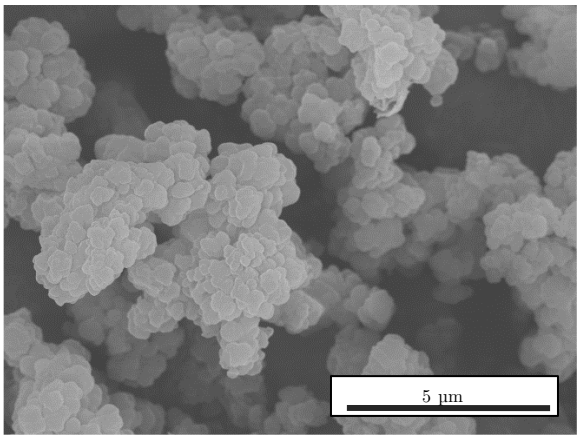



**Figure C8. Scanning electron microscopy micrograph of the GMA-S-β-CD material.**

Finally, in order to establish the degree of homogeneity of the CD molecules along the polymer surface, the confocal microscopy technique was used. To this end, 10 mL of a DMF Bodipy-Me solution (7.5 mmol $L^{-1}$) were forced to pass through 25 mg of the GMA-S-β-CD material following a typical SPE experimental procedure. Subsequently, the material was thoroughly washed with DMF until the washing solution discoloration was complete to preserve only the Bodipy-Me molecules captured by the cyclodextrin units (Ruiz-Esparza et al., 2014). This Bodipy-Me marked material was analyzed through confocal microscopy (Figure C9). The solid sample was irradiated with a 488 nm laser, and it emitted an intense green fluorescent signal. As can be seen, all material particles showed the characteristic green fluorescence due to the Bodipy-Me-CD inclusion complexes formed, and no polymeric particles were observed without emission. This data supported the theory of the CD functionalization occurring in a homogeneous and well-distributed way along the polymer surface, which is crucial to achieving an optimum analytical performance of the solid phase. Also, an indirect quantitative estimation of the CD content was carried out by analyzing the fluorescence ($\lambda_{ex}$=488 nm and $\lambda_{em}$=530 nm) of the solutions passed through the GMA-*co*-EDMA polymeric support and the GMA-S-β-CD material. Results showed that around a 4% of the Bodipy-Me was retained in the parent support, whereas the GMA-S-β-CD material trapped approximately a 60%. Therefore, although slight

participation of the bare polymeric support in the retention of the Bodipy-Me cannot be ruled out, the primary retention mechanism of the material may be the presence of cyclodextrin on its surface, which would be of interest for future analytical applications of the solid phase.

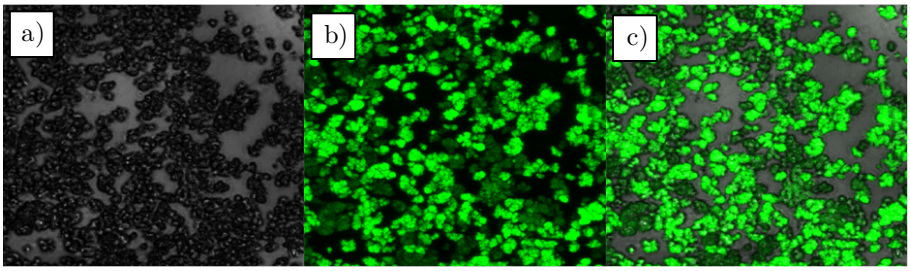



**Figure C9. Confocal microscopy images obtained from the fluorescence emission provided by the Bodipy-Me inclusion complexes with β-CD in the material (60x magnification lens, water media, thickness of particles approx. 6 µm): (a) bright-field image, (b) green fluorescence images due to the Bodipy-Me-CD complexes, and (c) merged a) and b) images.**

### 1.3.2. Characterization of γ-CD polymeric materials

First, allyl-γ-CD was characterized through a $^{1}$H NMR experiment. The spectrum was comparable to the one described previously (Řezanka et al., 2010), where the peaks were already identified according to the structure of the molecule. In this case, the presence of the protons corresponding to the functionalizing group was thus verified. Also, the MALDI analysis (Figure C10) showed a mass distributed mainly between the non-substituted and the mono-substituted γ-CD. A slight contribution of the di-substituted γ-CD was observed too. However, the most important factor in this case is not the purity of the product since it was not so important if cyclodextrins react by one or two functional groups because this should not affect the accessibility of γ-CD to analytes in the polymer.

Parallel to the previous case, the verification of the binding of allyl-γ-CD onto the GMA-*co*-EDMA surface was carried out by using Raman spectroscopy (Figure C11). The characteristic stretching mode of thiol groups (usually at 2500 $cm^{-1}$), was evidenced for the thiolized support at around 2450 $cm^{-1}$. However, it was not observable neither before the thiol modification, that is, for the parent polymeric support, nor after the reaction with allyl-γ-CD, that is, for the GMA-S-γ-CD material, this indicating the assembling between γ-CD and the polymer through the disappearance of the thiol group. Important is to mention the appearance of a small peak in the GMA-S-γ-CD case. It should be assigned to a small proportion of thiol groups not reacting with the modified CD, as initially expected.

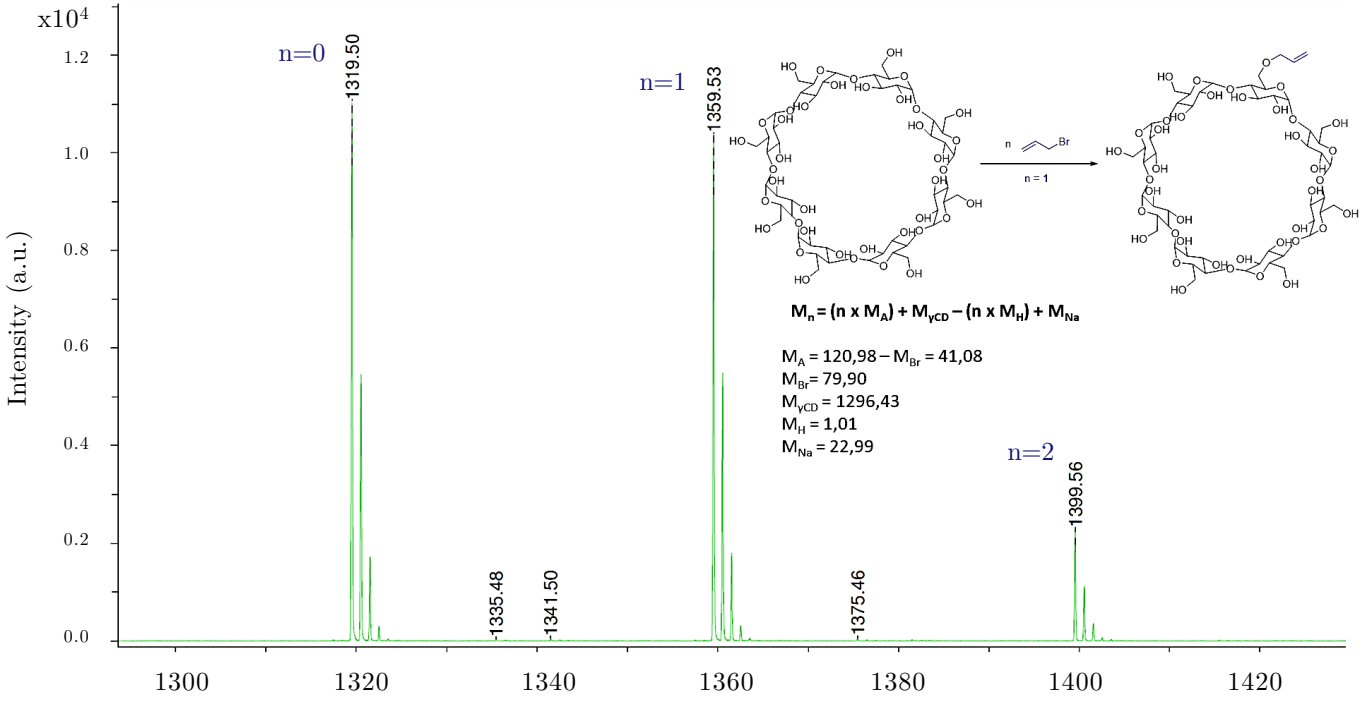



Figure C10. MALDI spectrum for allyl-γ-CD, where $M_n$=mass of the product, n=number of functionalizing units, $M_A$=mass of the functionalizing group, $M_{Br}$=mass of Br, $M_H$=mass of H, $M_{Na}$=mass of $Na^+$.

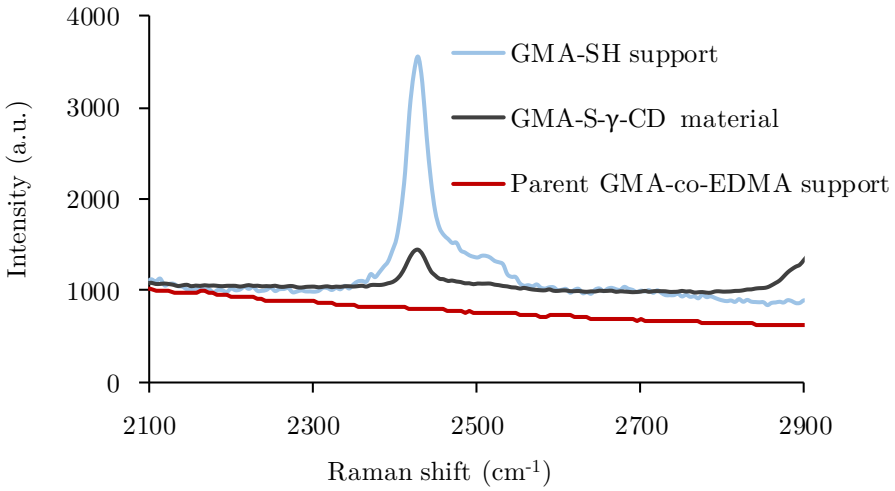



**Figure C11. Raman spectra of the bare polymeric supports in comparison with the modified GMA-S-γ-CD material. Measurements were carried out with a visible green laser at λ=532 nm in the 500 – 3500 $cm^{-1}$ range.**

Also, the amount of γ-CD incorporated onto the polymeric support was roughly estimated from the TGA curves obtained (Figure C12). Only few and slight differences were observed between the TGA curves corresponding to the parent GMA-*co*-EDMA material and the final GMA-S-γ-CD material. Two partially overlapped stems seemed to be involved in the GMA-*co*-EDMA support degradation. The first one was associated with the most abrupt weight loss occurring in the 250 – 320°C range. The second and less pronounced step occurred in the 300 – 500°C temperature range and completed the thermal polymer degradation. In the case of the GMA-S-γ-CD solid phase, the shape of the second stage clearly differed from that of the parent polymeric support. It is therefore evident that the anchored γ-CD units were in the origin of the observed changes. The thermal decomposition of the γ-CD starts at ca. 450°C and can be expanded to temperature values higher than those of the GMA-*co*-EDMA polymer degradation. From these small differences in the TGA curves, it was possible to roughly estimate the γ-CD content in the final solid phase at around a 2.9 wt%.

The relatively small size of the cyclodextrin molecules together with the observations in the infrared spectroscopy and the TGA curves made expecting no changes in the structure of the material. In fact, the observed morphology of the GMA-S-γ-CD material coincided with the normal GMA-*co*-EDMA porous polymer skeleton showed by SEM (Figure C13). Similar microglobular aggregates were detected with sizes between 200 and 700 nm (Figure C14), as expected. Besides, the preservation of the morphology and texture of the GMA-*co*-EDMA

polymer was also assessed through $N_2$ adsorption-desorption experiments. Specifically, the surface area was determined through the application of the BET model for the GMA-S-γ-CD material in comparison with both the GMA-S-β-CD material and the parent polymeric support. The values obtained can be observed in Table C1. As expected, equivalent values were obtained in all cases. The usually obtained surface areas of these types of polymers make the values obtained be expectable. Although relatively low, the quantified areas are indeed enough to apply the material obtained to the analytical purpose established.

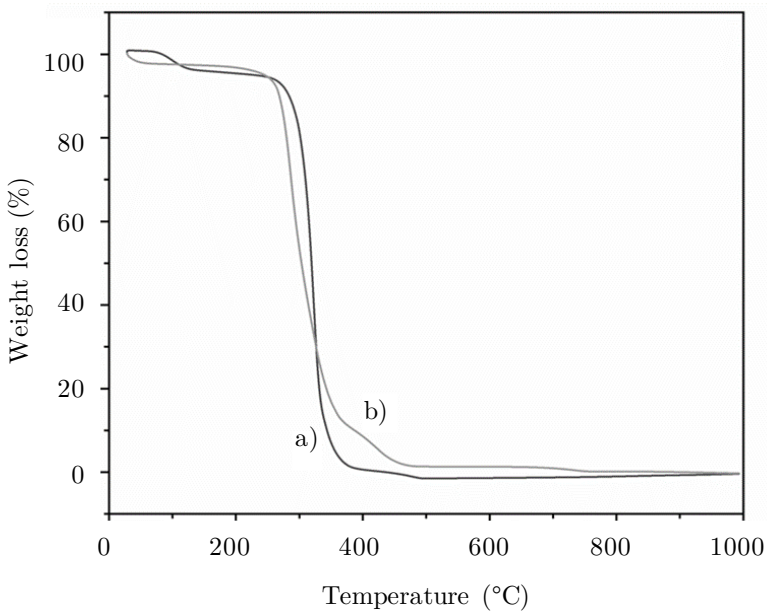



**Figure C12. Thermogravimetric analysis of (a) the parent GMA-*co*-EDMA support and (b) the GMA-S-γ-CD material. Measurements were carried out in an $O_2$ environment and at a heating speed of 10°C $min^{-1}$.**

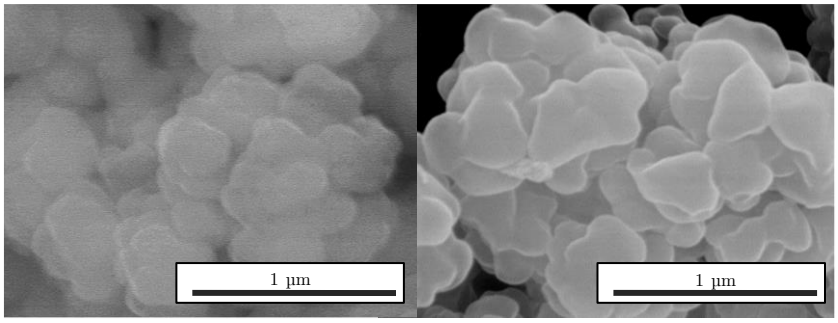



**Figure C13. Scanning electron microscopy micrographs of the GMA-S-γ-CD material.**

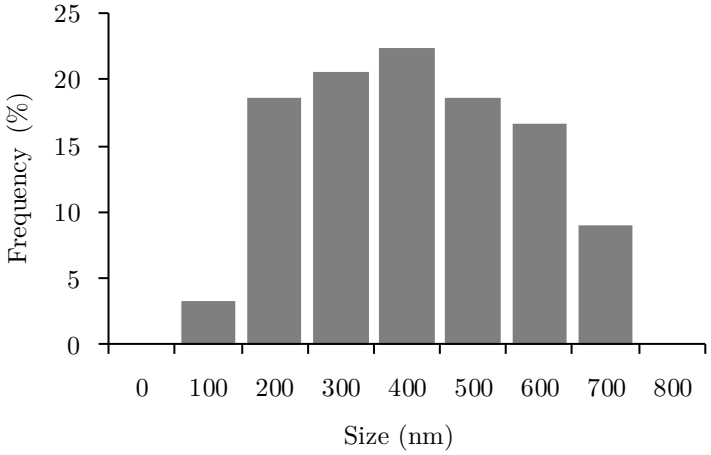



**Figure C14. Size distribution of the microglobular aggregates of the GMA-S-γ-CD material.**

**Table C1. Physical and textural parameters of GMA-S-CD polymeric materials.**

| Solid phase | CD content[a] (wt%) | CD content[a] (mmol $g^{-1}$) | Surface[b] ($m^2$ $g^{-1}$) |
|---|---|---|---|
| Parent polymer | - | - | 4.7 |
| GMA-S-β-CD | 3.7 | 0.326 | 3.2 |
| GMA-S-γ-CD | 2.9 | 0.226 | 5.2 |

[a] CD contents estimated from the TGA data.

[b] Total surface area determined through the BET model.

To end, the experiment with the confocal microscopy used previously was repeated in order to establish the homogeneity of the CD molecules along the polymeric support surface. To this, a stoichiometric quantity of Bodipy-Me in DMF solution was forced to pass through 25 mg of GMAS-γ-CD material. The material was subsequently washed thoroughly with DMF until the washing solution discoloration was complete to preserve only the Bodipy-Me molecules captured by the cyclodextrin units as far as possible (Discenza, Lynch, Feder, & Levine, 2018). Then, it was irradiated with a laser of 488 nm, emitting the Bodipy-Me-CD complexes an intense green fluorescence signal that was captured (Figure C15). As can be seen, all particles showed the characteristic green fluorescence due to the inclusion complexes formed, that is, to the presence of CD in the surface, in comparison with the parent polymeric support, which clearly kept a lower quantity of Bodipy-Me molecules after washing. The images obtained supported that the CD functionalization occurred in a well-distributed way along the polymer surface, which is important to achieving an optimum adsorptive response of the material. Imitating the previous procedure, an indirect

quantitative estimation of the CD content was carried out by analyzing the fluorescence ($\lambda_{ex}$ = 495 nm and $\lambda_{em}$ = 520 nm) of the solutions after passing through the material. Results showed that only around a 10% of the Bodipy-Me was retained in the parent polymeric support, whereas approximately a 50% was trapped in the GMA-S-γ-CD solid phase. With the images and the fluorescence results, it was concluded that the primary retention mechanism of the material was the presence of cyclodextrin on its surface, although slight participation of the polymeric support in the retention may not be completely ruled out.

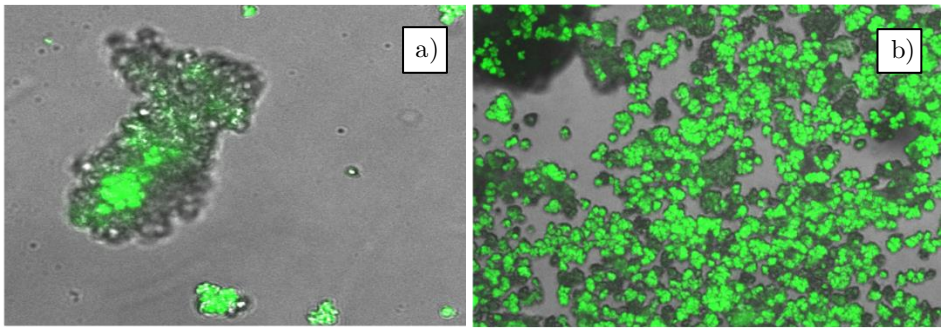



**Figure C15. Confocal microscopy images obtained from the fluorescence emission provided by the Bodipy-Me inclusion complexes with γ-CD in the material (60x magnification lens, water media): (a) the bare GMA-*co*-EDMA polymeric support and (b) the GMA-S-γ-CD material.**

## 1.4. Conclusion

The obtained characterization results of both solid phases justified the experiments described hereafter, where two different assessments to measure the ability of the polymeric materials to be used for sample treatment were conducted. The analytes families were chosen based on the sizes of the respective CD cavities in order to find the best performance in each case, following the directing theme of the Doctoral Thesis being presented. The matrices where the experimental procedures were applied were chosen based on the usual environments where the analytes under study can be found.

*A part of the experiments described in this chapter was conducted during the research stay carried out in the Synthesis of Nanoscale System research group led by Professor Doctor Bart Jan Ravoo at the University of Münster (Germany) during the period September – December 2019, which must be acknowledged.*

# 2

# Determination of phenolic compounds in tea samples

## 2.1. Introduction

The concept of food is changing these days from the need for survival to an emphasis on its properties to promote better health (Otemuyiwa, Williams, & Adewusi, 2017). Tea, a resource used by millions of people, is nowadays the most widely consumed beverage except water, probably due to the stimulating effect and taste it offers (Elmar, 1966; Otemuyiwa et al., 2017). Indeed, the annual output of tea seeds has been reported to be more than 2 million tons, which accounts for around 66% worldwide.

The literature describes a wide variety of positive biological activities of tea including obesity prevention, antioxidative activities, anti-inflammation, anticancer, and antithrombotic. Besides, it has good oxidative stability due to its richness in natural antioxidants (Guoyan Liu et al., 2022). Specifically, the high content of polyphenols such as catechins in tea may be behind the advantages mentioned (Güclü-Üstündag et al., 2016). In this way, there are claims that the consumption of tea infusions could help ameliorate free radical-induced diseases. For example, black tea, which is considered a rich source of polyphenols and other antioxidants, is one of the most popular teas due to its healing activity.

However, there have been found traces of other phenolic derivatives in tea that could not have such positive effects on health. The term *phenolic compounds* encompasses approximately 8000 occurring compounds, all possessing one common structural feature, an aromatic ring bearing at least one hydroxyl substituent called phenol (Leopoldini, Russo, & Toscano, 2011). Further

classification divides them into polyphenols and simple phenols, depending on the number of phenol subunits in their structure. Thus, compared to those phenolic compounds with a beneficial antioxidant activity, the finding of phenol and all isomers of cresol in black tea (Elmar, 1966), which are classified as VOCs and present, in fact, certain toxicity (Conde et al., 2006), has been reported too.

Generally, organic solvents are used to isolate phenolic compounds from tea, either with the purpose of recovering them or for their later determination. However, the volatilization of organic solvents may contribute to environmental pollution (L. Cui et al., 2017). Although several environmentally-friendly extraction techniques such as ultra-high pressure, pulsed electric field, ultrasound, and supercritical fluid extraction have been proposed, these methods present some technical limits due to the requirement of advanced and costly equipment used.

The special hollow cylinder in the CDs structure allows the inclusion of some bioactive compounds such as volatile oils and polyphenols in them. Indeed, it has been recognized as safe by the Food and Drug Administration in 1998 and is now extensively used as a flavor carried and protectant in the food industry (L. Cui et al., 2017). Recently, several researchers have reported β-CD as an alternative to extract phenolic compounds from grape and apple pomace or vine shoot cultivars, among others (López-Miranda et al., 2016; Rajha et al., 2015; Ratnasooriya & Rupasinghe, 2012). Compared to organic solvents, β-CD-assisted extraction is more economic, safer, and greener.

The interaction of guaiacol and eugenol with cyclodextrins has been already described. Specifically, these molecules exhibit identical orientations with the phenyl ring within the CD cavity and the hydroxyl and methoxyl groups projected outside (Divakar & Maheswaran, 1997; L. X. Song et al., 2007). Therefore, cyclodextrins stabilize VOCs and decrease their reactivity; thus their stability is increased. For these reasons, they have been widely used in analytical chemistry for the entrapment of VOCs, not only for remediation but also for their determination in different types of samples.

In this study, an SPE proposal for the extraction and determination of phenolic compounds by using a porous glycidyl methacrylate and ethylene dimethacrylate polymeric material with β-CD as a surface ligand (GMA-S-β-CD solid phase) is proposed. To this, a solid-phase extraction procedure is developed and its main analytical features are studied. Different volatile alkylphenols have been selected to carry out this study: phenol, guaiacol, cresol, 4-vinylphenol, ethylphenol, ethylguaiacol, 2-methoxy-4-vinylphenol, and eugenol. Finally, tea has been chosen as a real aqueous matrix where these analytes can be determined in order to evaluate the performance of the method developed.

## 2.2. Experimental

### 2.2.1. Reagents, materials, and instrumentation

On the one hand, phenolic compounds ≥ 98% were bought in Sigma-Aldrich (St. Louis, United States), and all standard stock solutions were prepared in acetonitrile and kept at -18°C. The physicochemical properties of the phenolic compounds studied can be observed in the previous Table A4. All solvents used during the experimental procedure were of HPLC grade and were purchased from VWR ProLabo Chemicals (Radnor, United States). Other reagents such as NaCl (s), NaOH (s), and HCl (aq.) 37% were from Panreac AppliChem (Barcelona, Spain). The comparison of the developed method (Bieniek, 1996) was carried out by using Varian $C_{18}$ Bond Elut extraction cartridges from Agilent Technologies (California, United States). To end, ultrapure water from an Adrona purification system (Riga, Latvia) was employed during the experimental procedure.

On the other hand, the sample treatment was carried out by using a Vac Elut 20 for SPE connected to a vacuum pump CKNF from Agilent Technologies (California, United States), using 3 mL polypropylene cartridges from Análisis Vínicos (Ciudad Real, Spain) and a 1 mL $min^{-1}$ constant flow. All samples were previously filtered with Nylon 0.45 mm Sartorius Stedim Biotech filters (Göttingen, Germany).

During the optimization study, analytes were separated with an LC-2000 Plus liquid chromatograph equipped with a FP-2020 Plus Intelligent Fluorescent Detector, a PU-2089 Plus Quaternary Gradient Pump with integrated degasser, and an I/FLC/NetII/ADC interface (Figure C16) from Jasco (Madrid, Spain). The injection volume was 20 µL in a Supelco (Bellefonte, United States) six-way injection valve, and the stationary phase was a $C_{18}$ ZORBAX column from Agilent Technologies (Santa Clara, USA) with 10 cm length, 4.6 mm internal diameter, and 3.5 µm thickness. The separation was carried out with a 1.7 mL $min^{-1}$ flow. The mobile phase was a gradient from a 95% acetic acid 0.1 M solution and 5% ACN to a 60% acetic acid 0.1 M and 40% ACN in 25 min, and the fluorimetric detection was carried out at $\lambda_{ex}$=280 nm/$\lambda_{em}$=310 nm.

For the determination of phenolic compounds in real samples, they were separated in this case through an Agilent 5977A gas chromatograph (California, United States) with mass spectrometry detection, where qualitative and quantitative analysis was carried out. A stationary phase 5% phenyl methylpolysiloxane 30 m/0.25 mm/0.25 µm was used to carry out the separation. Helium was the carrier gas at a 0.7 mL $min^{-1}$ flow. The temperature was initially

set at 40°C (2 min) and then heated to 280°C at 5 °C $min^{-1}$. The mass selective detector was operated in electron impact mode with an ionization potential of 70 eV and a source temperature of 250 °C. The range used in SCAN mode was m/z 40 – 340. In SIM mode, the ions used were selected at m/z 94, 108, 124, 107, 120, 152, 150, and 164 for phenol, cresol, guaiacol, 4-ethylphenol, 4-vinylphenol, 4-ethylguaiacol, 2-methoxy-4-vinylphenol, and eugenol, respectively (Figure A12). The interface temperature and the injector temperature were also set at 250°C, and 1 µL of the sample was injected in splitless mode.

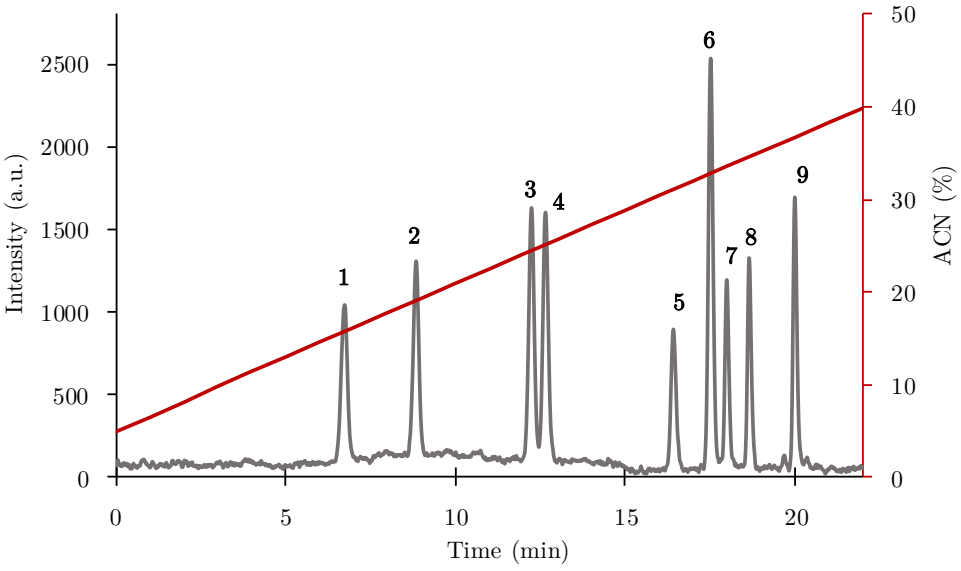



**Figure C16. HPLC-FLD chromatographic profile of a multianalyte solution of the phenolic compounds under study in methanol: (1) phenol (2) o-cresol (3) m+p-cresol (4) guaiacol (5) 4-ethylphenol (6) 4-vinylphenol (7) 4-ethylguaiacol (8) 2-methoxy-4-vinylphenol (9) eugenol.**

### 2.2.2. Optimization and validation of the SPE procedure

Several parameters can influence the concentration factor obtained by the application of a SPE procedure. Therefore, factors affecting the retention and elution of the target phenolic compounds were assessed. The optimization study was carried out based on the recovery obtained from spiked ultrapure water samples at the µg $L^{-1}$ level. The recovery was calculated from the ratio between the obtained concentrations and the theoretical concentration in individual triplicates (n=3). In this sense, the procedure was optimized by varying one parameter at a time, while keeping the others constant. The studied parameters were those affecting the sorption (pH, ionic strength, cartridges conditioning,

amount of solid phase, sample volume, and influence of concentration) and the extraction process (type and volume of solvent). In addition, the possibility of reusing the material was also evaluated. Finally, the matrix effect was assessed by spiking diverse real water matrices.

Once the SPE method was optimized, the analytical figures of merit of the optimized method were established. To this end, global recovery, repeatability, sensitivity, and linearity were studied. The limits of detection (LOD), limits of quantification (LOQ), and the linearity range were calculated following the recommendations of the IUPAC (Olivieri et al., 2006).

Thus, the extraction procedure designed was as follows. First, activation of the sorbent is carried out with MeOH (2 mL), and then it is equilibrated with water (3 mL). Then, 50 mL of sample are buffered at pH 4.5 (acetic acid/acetate 0.01 M). Also, NaCl is added to provide a 3 M salt concentration. The sample aliquot is placed in the extraction cartridge containing 100 mg of the solid phase, and it is suctioned at a 1 mL $min^{-1}$ constant flow. Next, it is washed with 2 mL of water and dried at vacuum for 2 minutes. Finally, the analytes are eluted with 3 mL of an ACN:MeOH (1:1) mixture. Previously to the injection in the chromatographic system, elution fractions must be filtered. In addition, the matrix effect of the samples has to be evaluated and a standard addition calibration must be used if necessary.

To end, the studied phenolic compounds were determined in four tea samples purchased in the form of tea bags (chai black tea, Marrakesh style tea, citrus tea, and cold lime tea) by applying the developed extraction method at the same time that the matrix effect was studied. The sample pre-treatment was carried out by immersing each bag in 250 mL of water at 80°C for 20 minutes. The consumable liquid portion was then let cool, filtered, homogenized, and extracted. Results were compared with those obtained with a reference method (Bieniek, 1996) with the same end. The overall recovery and the repeatability of the reference method were also studied in order to carry out the quantification of the target phenolic compounds reliably.

## 2.3. Results and discussion

As phenolic compounds possess acid-base properties, the retention of the analytes may be influenced by the pH of the sample solution. Thus, an acetic/acetate pH 4.5 buffer 0.01 M, an ammonium/ammonia pH 9.5 buffer 0.01 M, and water at neutral pH were proven as sample media. Results showed no differences between the studied pH. For this reason, pH 4.5 with the acetic/acetate buffer was selected to continue with the optimization study to

evade, in any case, the deprotonation of phenolic compounds. Also, the buffer concentration was studied. Concentrations of 0.01 M and 0.1 M were proven. Results showed that neither in this case did the buffer concentration influence the retention of analytes, and a 0.01 M concentration was selected to continue with the study to avoid its influence on the ionic strength as long as possible.

The salting-out effect can decrease the solubility of non-polar analytes in water and enhance the distribution coefficient of solutes to the studied material through the salt added to aqueous solutions. Thus, ionic strengths of NaCl 0 M, 1 M, 2 M, 3 M, and 4 M were studied. Based on the results, it can be concluded that an ionic strength of 3 M should be used to continue with the study, as the recovery increased from 0 M (34, 21, 55, 56, 82, 84, 79, 79, and 81% for phenol, guaiacol, m+p-cresol, o-cresol, 4-vinylphenol, 2-methoxy-4-vinylphenol, 4-ethylphenol, 4-ethylguaiacol, and eugenol, respectively) to 3 M (58, 60, 97, 94, 95, 89, 96, 100, and 92% for the same analytes). For a 4 M concentration, recoveries remained with the same values as the 3 M concentration.

Initial conditioning of the cartridge could also influence the retention of our phenolic compounds. The activation of the 100 mg of solid phase was studied with 2 mL of MeOH, 2 mL of ACN, and 2 mL of a MeOH:ACN (1:1) mixture. As results did not vary, it was concluded that the previous conditioning did not influence the recovery of the analytes. For this reason, it was decided to use MeOH since its toxicity is lower than that of ACN.

The breakthrough volume was also studied. To this end, 10 mL, 25 mL, and 50 mL of sample were studied with 50 mg of solid phase while fixing the amount of analyte. Results showed that 10 mL of the sample worked fine with 50 mg of solid phase, while 50 mL of sample diminished recoveries considerably. The study of the amount of solid phase for SPE was then carried out with 50 mg and 100 mg of material when using 50 mL of sample. Recoveries calculated were always higher when using 100 mg of solid phase (41, 50, 95, 96, 100, 82, 97, 94, and 100% for phenol, guaiacol, m+p-cresol, o-cresol, 4-vinylphenol, 2-methoxy-4-vinylphenol, 4-ethylphenol, 4-ethylguaiacol, and eugenol, respectively.), while recoveries using 50 mg maintained low since the breakthrough volume was reached in this case.

Moreover, the loading capacity of 100 mg of sorbent was studied by passing through the cartridge water samples with rising concentrations of phenolic compounds. Results showed that the concentration did not influence the recovery results from 45 µg $L^{-1}$ to 5 mg $L^{-1}$ of each phenolic compound in the loading solution, obtaining values that were comparable in all cases based on the comparison of means by Student's test, 95% of confidence level (Harris, 2007).

Higher concentrations were not studied since it is not expected to apply the method with samples containing those quantities.

At this point, the nature and amount of eluent were studied. An ACN:MeOH (1:1) mixture, an ACN:acetic acid 0.1 M (1:1) mixture, a MeOH:acetic acid 0.1 M (1:1) mixture, ACN, and MeOH were proposed as eluents since they were previously studied to extract phenolic compounds from cyclodextrin. Results obtained were comparable for ACN, MeOH, and the ACN:MeOH mixture, reaching values similar to those obtained previously. Recoveries using the ACN:acetic acid mixture and the MeOH:acetic acid mixture were around a 10% lower. With the aim of reaching a compromise, the mixture ACN:MeOH was selected because of the better chromatographic resolution it allowed. In addition, the elution profile of this mixture was also studied. Results indicated that most analytes were eluted in the first 3 mL of elution, obtaining results in the following portions that were in some cases lower than the calculated LOD.

As can be seen, recoveries for phenol and guaiacol were in all cases significantly lower than for the rest of analytes. Taking into account that phenolic compounds losses may arise when passing the sample through the cartridges due to incomplete retention, the previously passed-through-the-cartridge water was collected in order to verify the proper retention of analytes. It was concluded that the non-retained phenols fraction in the extraction cartridge was not so significant for the analytes. Only the concentration of phenol and guaiacol was higher than the rest, as expected, due to their non-complete retention (around 50% of the introduced analyte was in the extract in both cases). As proved, this lower retention could not be improved during the optimization process. The most probable explanation for this poor retention of phenol and guaiacol in our material is their polarity (Table A4). As it is well known, the lipophilic cavity of cyclodextrin provides an environment into which mainly non-polar molecules can enter to form inclusion complexes (Divakar & Maheswaran, 1997). The driving force for complex formation is the release of enthalpy-rich water molecules from the cavity, as water molecules are displaced by more hydrophobic guest molecules present in the solution to attain an apolar-apolar association and decrease of cyclodextrin ring strain, resulting in a more stable lower energy state (Del Valle, 2004). The logP is an extended form to measure the lipophilicity of a substance, which is related to its hydrophobicity and therefore to its polarity. In this way, this behavior can be attributed to the higher polarity of phenol and guaiacol in comparison with the rest of phenolic compounds studied ($\log P \geq 1.8$), which may arise in a higher difficulty to compete with water and displace it from the apolar cavity of cyclodextrin, making their retention difficult.

The analytical figures of merit of the proposed method are shown in Table C2. Results indicated good repeatability, with CVs below 7% in the same day and under 6% between days. It seems important to highlight that some inter-day coefficients were lower than those obtained for the intra-day analysis, contrary to what would be expected. In addition, detection and quantification limits are between 1.2 μg $L^{-1}$ and 400 μg $L^{-1}$, being the HPLC-FLD use more sensitive than that of the GC-MS. In both cases, phenol and 4-vinylphenol are those that present a lower sensitivity and, for this reason, standards were prepared in higher concentrations for these analytes. To end, linearity was quite acceptable as it covers the concentration range of interest to work, and the overall recovery was in all cases over 95% excepting phenol, guaiacol, and 2-methoxy-4-vinylphenol, as expected due to the optimization study carried out.

A proof of the enhanced functionalities of the polymer when modified with CDs was carried out. To this, two extractions were conducted in the same conditions, one using the bare GMA-*co*-EDMA support as sorbent and the other with the GMA-S-β-CD. As expected, the GMA-S-β-CD material worked better due to the host-guest chemistries taking place thanks to the cyclodextrin content. Besides, not only the parent polymer worked worse but also the analysis of the collected water passed through the cartridge showed, in general, the non-retention of phenolic compounds in the solid phase. Specifically, recoveries calculated for the GMA-S-β-CD material were 65, 54, 115, 110, 100, 90, 97, 96, and 98% for phenol, guaiacol, m+p-cresol, o-cresol, 4-vinylphenol, 2-methoxy-4-vinylphenol, ethylphenol, ethylguaiacol, and eugenol, respectively, whereas they were of 34, 32, 47, 45, 60, 44, 52, 41 and 43% for the parent GMA-*co*-EDMA support.

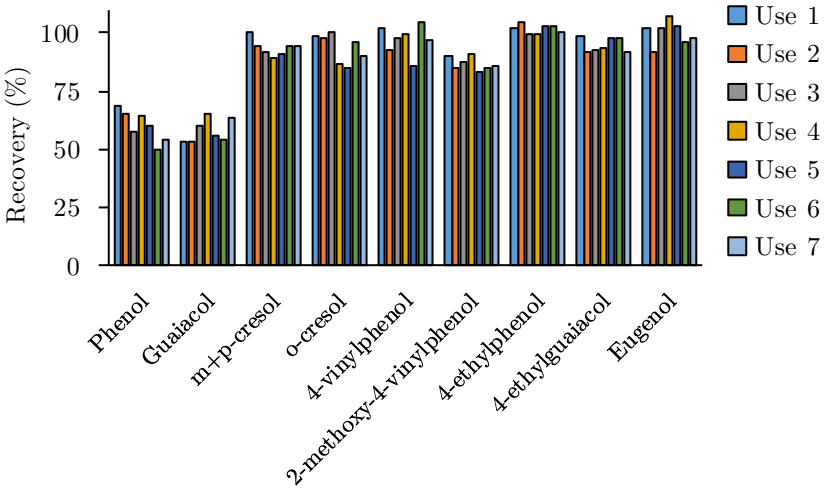



**Figure C17. Study of the reusability of the GMA-S-β-CD material in terms of the recovery.**

Table C2. Analytical figures of merit established for the extraction method of phenolic compounds using the GMA-S-β-CD polymeric support.

| Compound | CV (%) | | Recovery (%) | LOD[a] (μg $L^{-1}$) | | LOQ[a] (μg $L^{-1}$) | | Linearity[b] (μg $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| | Intra-day | Inter-day | | HPLC-FLD | GC-MS | HPLC-FLD | GC-MS | |
| Phenol | 1.9 | 2.9 | 58.9 ± 1.7 | 0.7 | 4.8 | 2.1 | 15 | LOQ – 120000 |
| Guaiacol | 0.6 | 6.3 | 57 ± 4 | 0.6 | 2.7 | 1.9 | 8.8 | LOQ – 30000 |
| m+p-cresol | 7.2 | 5.2 | 97 ± 5 | 0.4 | 1.5 | 1.2 | 4.6 | LOQ – 30000 |
| o-cresol | 3.1 | 3.0 | 97 ± 3 | 0.6 | 2.6 | 1.8 | 8.5 | LOQ – 30000 |
| 4-vinylphenol | 1.8 | 1.7 | 96.1± 1.7 | 17 | 140 | 50 | 400 | LOQ – 1200000 |
| 2-methoxy-4-vinylphenol | 0.7 | 0.8 | 85.7 ± 0.7 | 0.4 | 3.1 | 1.2 | 9.3 | LOQ – 30000 |
| 4-ethylphenol | 2.8 | 4.5 | 101 ± 5 | 1.0 | 2.4 | 3.1 | 7.4 | LOQ – 30000 |
| 4-ethylguaiacol | 4.0 | 2.9 | 95 ± 3 | 0.8 | 1.7 | 2.5 | 5.1 | LOQ – 30000 |
| Eugenol | 7.1 | 5.3 | 98 ± 5 | 0.7 | 6.7 | 2.0 | 22 | LOQ – 70000 |

[a] LOD and LOQ are referred to the water sample.

[b] Linearity is referred to the measuring solution.

Moreover, the reusability of the developed material was studied, and results showed that the solid phase is reusable for at least seven consecutive extractions by maintaining the recovery obtained in the first, as can be seen in Figure C17. For this reason, it can be concluded that the use of a polymeric support constitutes a great advantage for the reusability of the material.

In addition, the repeatability of the GMA-S-β-CD synthesis was evaluated through the repeatability of the recovery obtained using solid phases in different synthesis processes. Results can be observed in Table C3, and they indicate that, as expected, the synthesis is sufficiently repeatable to maintain the results obtained over time.

**Table C3. Evaluation of the repeatability of the GMA-S-β-CD synthesis through absolute recovery results.**

| Compound | Recovery (%) | | |
|---|---|---|---|
| | Synthesis 1 | Synthesis 2 | Synthesis 3 |
| Phenol | 68 ± 2 | 63 ± 4 | 61 ± 7 |
| Guaiacol | 55 ± 4 | 50 ± 6 | 60 ± 4 |
| m+p-cresol | 99 ± 5 | 100 ± 5 | 92.8 ± 1.5 |
| o-cresol | 111 ± 4 | 94 ± 7 | 93 ± 3 |
| 4-vinylphenol | 114 ± 4 | 106 ± 4 | 105 ± 11 |
| 2-methoxy-4-vinylphenol | 95.02 ± 0.05 | 108 ± 4 | 92 ± 8 |
| 4-ethylphenol | 100 ± 4 | 106 ± 2 | 96 ± 7 |
| 4-ethylguaiacol | 104 ± 7 | 96 ± 4 | 94 ± 5 |
| Eugenol | 97 ± 8 | 100 ± 2 | 101 ± 4 |

Table C4 compares the characteristic features of our method with others reported for the extraction of phenolic compounds by using different solid phases. Thus, recoveries are quite acceptable, since our range of values covers those reached in other cases. Regarding the analytical performance of the method, our LODs and LOQs are comparable to those in other works, except the method reported by Ni et al., whose sensitivity is remarkable (Ni et al., 2019). The oldest methods mostly choose the use of typically commercial phases, both silica-based and polymeric ones. In our case, the most significant advantage concerning commercial materials is that the competition of apolar analytes with humidity during the adsorption step is avoided thanks to the presence of CD, although a synthetic process must be carried out. Furthermore, silica can lead on some occasions to the irreversible adsorption of compounds. The method proposed here constitutes a rapid and straightforward extraction procedure for the subsequent determination of phenolic compounds in aqueous samples.

**Table C4. Comparison of the developed method for phenolic compounds using the GMA-S-β-CD material with other methods in the literature.**

| Analytes | Sample | Extraction | LOD (μg $L^{-1}$) | LOQ (μg $L^{-1}$) | Recovery (%) | Reference |
|---|---|---|---|---|---|---|
| Phenol, cresol isomers, 2,6-xylenol, 2,5-xylenol, 2,3-xylenol, 3,5-xylenol, 3,4-xylenol,1-naphthol, 2-naphthol | Urine | $C_{18}$ SPE | - | - | 74 – 100 | (Bieniek, 1996) |
| 4-ethylguaiacol, 4-ethylphenol, 4-vinylphenol, 4-vinylguaiacol | Wine | Polymeric Lichrolut EN (Merck) SPE | 500 – 2200 | 1600 – 7500 | 81 – 88 | (Domínguez, Guillén, & Barroso, 2002) |
| Eugenol | Fish | $C_{18}$ SPE | 2.5 | 5.0 | 95 – 104 | (J. Li, Zhang, & Liu, 2015) |
| Bisphenol F, bisphenol A, bisphenol AF | Water Orange juice | Microporous β-CD polymer with decafluorobiphenyl as cross-linker SPE | 0.15 | 0.45 | 93 – 107 | (Yarong Li, Lu, Cheng, Zhu, et al., 2018) |
| 2-chlorophenol, 4-methylphenol, 2,6-dimethylphenol, o-nitrophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol | Water | Zirconium and nitrogen co-doped ordered mesoporous carbon SPME | 0.0002 – 0.0017 | - | 84 – 108 | (Ni et al., 2019) |
| Phenol, cresol isomers, 2-methoxy-4-vinylphenol, 4-ethylphenol, 4-vinylphenol, 4-ethylguaiacol, guaiacol, eugenol | Tea | GMA-S-β-CD SPE | 1.5 – 140 | 4.6 – 400 | 57 – 101 | Proposed method |

Table C5. Analysis of phenolic compounds using the extraction method with the GMA-S-β-CD material (μg $L^{-1}$, $\bar{x} \pm s$) and its validation.

| Compound | Chai black tea | | Marrakesh style tea | | Citrus tea | | Lime cold tea | |
|---|---|---|---|---|---|---|---|---|
| | $C_{18}$ | GMA-S-β-CD | $C_{18}$ | GMA-S-β-CD | $C_{18}$ | GMA-S-β-CD | $C_{18}$ | GMA-S-β-CD |
| Phenol | 109 ± 7 | 97.4 ± 1.8 | 103 ± 3 | 96 ± 6 | 86.3 ± 0.2 | 93.84 ± 0.15 | 106.4 ± 1.0 | 109 ± 8 |
| Guaiacol | 22.4 ± 1.0 | 31.1 ± 1.8 | 22.6 ± 0.4 | 32.7 ± 1.5 | 24.4 ± 0.7 | 29.7 ± 0.3 | 29 ± 3 | 30.4 ± 1.4 |
| m+p-cresol | < LOD | < LOD | 15.0 ± 0.8 | 18.28 ± 0.02 | < LOD | < LOD | 16.7 ± 0.8 | 19.5 ± 0.2 |
| o-cresol | 10.25 ± 0.12 | 12.9 ± 0.3 | 10.6 ± 0.6 | 12.27 ± 0.10 | 10.5 ± 0.8 | 12.24 ± 0.13 | 10.65 ± 0.12 | 12.33 ± 0.13 |
| 4-vinylphenol | 670 ± 4 | 630 ± 30 | 720 ± 20 | 700 ± 20 | 670 ± 15 | 573 ± 7 | < LOD | < LOD |
| 2-methoxy-4-vinylphenol | 43.2 ± 0.5 | 44.6 ± 1.7 | 43.8 ± 0.3 | 42.03 ± 0.14 | < LOD | < LOD | < LOD | < LOD |
| 4-ethylphenol | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | 8.95 ± 0.14 | 8.6 ± 0.9 |
| 4-ethylguaiacol | 10.3 ± 0.6 | 12.65 ± 0.05 | 9.9 ± 0.7 | 12.5 ± 0.2 | 9.7 ± 0.5 | 12.34 ± 0.12 | 10.9 ± 0.4 | 12.47 ± 0.15 |
| Eugenol | 4200 ± 400 | 3700 ± 400 | 34.7 ± 0.3 | 55 ± 2 | 39.9 ± 0.3 | 44.5 ± 0.7 | 24.8 ± 0.9 | 38.0 ± 0.4 |

Regarding the selectivity of the sorbent, CDs are able to trap organic and inorganic analytes that have an acceptable geometry and a polarity lower than that of water due to the displacement of the water from the apolar cavity during the entrapment process (Del Valle, 2004; Szente & Szemán, 2013). Therefore, the material described is likewise able to this approach, so that it is not entirely selective for phenolic compounds although of course CD increases this property. In fact, the selectivity of the method lies in the chromatographic separation stage, where GC-MS was used when working with real matrix samples. For this reason, the method developed permits to catch, identify and quantify phenolic compounds in tea although other analytes can alike be retained and extracted during the SPE process.

The matrix effect was studied by spiking real tea samples (50 μg $L^{-1}$ of each analyte, 500 μg $L^{-1}$ of phenol and 4-vinylphenol) and then calculating the recovery. From the results, it can be concluded that the tea matrix significantly affected recoveries. Recoveries in the case of the ultrapure water were of 59, 57, 97, 97, 96, 86, 101, 95, and 98% for phenol, guaiacol, m+p-cresol, o-cresol, 4-vinylphenol, 2-methoxy-4-vinylphenol, 4-ethylphenol, 4-ethylguaiacol, and eugenol, respectively, whereas recoveries using a real tea sample were of 25, 31, 58, 61, 57, 60, 64, 63, and 57% for the same analytes mentioned. For this reason, the determination of phenolic compounds in tea must be carried out by applying a standard addition method.

Finally, the target phenolic compounds were determined in four tea samples by applying the extraction procedure. Results can be observed in Table C5. Based on them, it can be concluded that phenolic compounds are present in tea at trace level, as expected (Elmar, 1966), and those phenols that are present in all samples analyzed are phenol, guaiacol, o-cresol, and eugenol. On the contrary, the presence of 4-ethylphenol and 2-methoxy-4-vinylphenol was less remarkable. Moreover, concentrations for each analyte in the four samples were in general quite similar between them, excepting the eugenol, whose presence was notable in the chai black tea case, the higher aromatic one. It should also be noted that, although some analytes were not detected in all samples, results were in all cases above the established limits of quantification.

With respect to the comparison of the current method with a reference method (Bieniek, 1996) to the analysis of tea, the overall recovery of the analytes in spiked tea samples by using this reference procedure was in the range of 20 – 80%. In addition, the calculated intra-day precision with CV values was between 5% and 20%, whereas the inter-day precision gave CVs in the range of 5 – 40%. Finally, it can be stated that results for the analysis of tea samples with both methods

were comparable among them by using the paired t-test for comparing individual differences (Harris, 2007) for a 95% of confidence level.

## 2.4. Conclusion

In this study, the GMA-S-β-CD composite has been successfully applied as an effective sorbent for the extraction of trace phenolic compounds in tea before their quantification. The novelty of this achievement may be highlighted by the lack of existence of other methods that permit the determination of these phenolic compounds in aqueous matrices by using a polymeric material based on host-guest interactions. As a disadvantage, the selectivity of the extraction is an issue that can result in the extraction of some undesired compounds from the sample matrix. However, this can be evaded by using a selective analytical technique in the quantification step such as GC-MS. In short, the method is a promising tool for systematically analyzing trace phenolic compounds in tea samples.

At this point, the good features offered by the GMA-S-β-CD material justified the evaluation of the previously described GMA-S-γ-CD solid phase with other analytes according to the physicochemical properties offered by this type of cyclodextrin.

# 3

# Extraction of fluoroquinolones from water

## 3.1. Introduction

Incomplete or non-adequate treatment of water can have a variety of consequences. Among them, the presence of pharmaceuticals and personal care products at trace level in the environment is one of the most significant (W. Lu et al., 2019). From years ago, antibiotics are widely used in the prophylaxis and treatment of a variety of human and animal diseases, as well as to increase the growth rate of animals (Adachi et al., 2013). Since most of them are excreted in their initial form after their consumption, they have been widely identified in aquatic environments (Adachi et al., 2013), including drinking water. Antibiotics used against human diseases attract special attention (X. Cui et al., 2015) since they can poison plants and aquatic organisms and promote the proliferation of antimicrobial-resistant bacteria all around us (W. Lu et al., 2019). Overall, pharmaceutics pollution is recognized as an issue of concern (Andreu, Blasco, & Picó, 2007) and there exists a growing interest in increasing the knowledge of the consequences that the antibiotics present in the environment may have on human health. Therefore, the development of new ways for their removal and determination in water has become highly desired and very significant (W. Lu et al., 2019).

Fluoroquinolones (FQs) are a highly consumed group of relatively new and entirely human-made, non-steroidal amphoteric antibiotics (Lee et al., 2007). Chemically, they are 6-fluorinated piperazinyl derivatives of nalidixic acid (Lian et al., 2018). The piperazinyl group induces the long half-life shown by them and

their structure makes them efficient not only against Gram (-) but also against Gram (+) bacteria. They also contribute with low protein binding, high drug tolerance, and low toxicity (Blanka & Tőkés, 2011). Nowadays, fluoroquinolones are used for the treatment of infections in livestock farming and human medicine (Yang Wang, Tong, Xu, & Zhang, 2018), as well as to enhance production. Due to their widespread usage, FQs are considered an emergent class of environmental pollutants (Barahona, Albero, Tadeo, & Martín-Esteban, 2019) commonly found in water.

A worthy opportunity for monitoring emerging pollutants in the environment is represented by sorption techniques. Indeed, a separation step that eliminates matrix-origin interferences of the sample and selectively pre-concentrates the trace analytes (Ligang Chen et al., 2011) under study is mandatory to improve the sensitivity of the applied analytical methodologies. For this purpose, a number of different solid adsorbents are commercially available (Jung et al., 2013; T. Li et al., 2020; Payanan et al., 2013). However, the use of commercial solid phases usually presents some drawbacks such as the lack of selectivity, being possible the retention and extraction of other substances, in addition to the competition of analytes with water when working with aqueous matrices. Thus, the development of new materials with enhanced properties for their application in sorption processes is a challenge that must be overcome. In fact, improving selectivity through structural variations in the adsorbents is being developed with increasing force. In this sense, an area of ongoing research is the use of the advantages offered by host-guest chemistry for the reversible sorption of analytes (Szente & Szemán, 2013; Teyssandier et al., 2016). Here, cyclodextrins have led to an increasing interest in implementing the principles of supramolecular host-guest chemistry to molecular-surface interactions (Teyssandier et al., 2016).

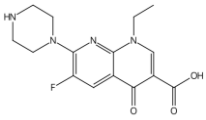
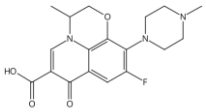
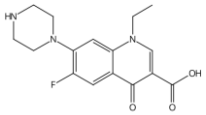
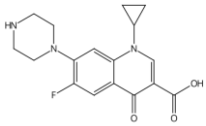
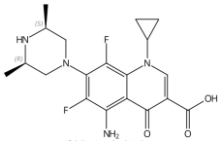
This chapter aims to assess the use of the porous glycidyl methacrylate and ethylene dimethacrylate polymeric materials described previously with γ-CD as a surface modifier (GMA-S-γ-CD) to extract and subsequently determine fluoroquinolones in groundwater. The diameter of the molecules makes the use of γ-CD instead of β-CD advisable to ensure a proper cyclodextrin-analyte interaction. Thus, a SPE proposal is developed. The target antibiotics selected to carry out the study are enoxacin, ofloxacin, norfloxacin, ciprofloxacin, and sparfloxacin. Prior to the application of the developed methodology for the determination of antibiotics in real samples, its main analytical features have been established. Then, real groundwater samples are analyzed and the results obtained are compared with those quantified using a reference extraction method reported in the literature.

## 3.2. Experimental

### 3.2.1. Reagents, materials, and instrumentation

The individual fluoroquinolone analytical standards were acquired from Alfa Aesar (Massachusetts, United States). From them, separate stock standard solutions were first prepared in water at pH > 3. Then, the working standard multianalyte solutions were obtained from these by dilution in methanol and stored at -18 °C in amber glass vials. The main physicochemical properties of the target analytes are informed in Table C6.

**Table C6. Physicochemical properties of the studied fluoroquinolones (Scifinder Scholar Database, 2021).**

| Compound | CAS | Structure | logP[a] | Molecular size (nm) | $pK_a$[a] |
|---|---|---|---|---|---|
| Enoxacin | 74011-58-8 |  | 1.6 | 0.786 | $pK_{a1}$: 6.04<br>$pK_{a2}$: 8.19 |
| Ofloxacin | 82419-36-1 |  | 1.9 | 0.892 | $pK_{a1}$: 5.19<br>$pK_{a2}$: 7.37 |
| Norfloxacin | 70458-96-7 |  | 1.7 | 0.799 | $pK_{a1}$: 0.16<br>$pK_{a2}$: 8.68 |
| Ciprofloxacin | 85721-33-1 |  | 1.6 | 0.814 | $pK_{a1}$: 6.43<br>$pK_{a2}$: 8.68 |
| Sparfloxacin | 110871-86-8 |  | 2.6 | 0.787 | $pK_{a1}$: 6.42<br>$pK_{a2}$: 8.59 |

[a] logP and $pK_a$ measured at 25 °C.

HPLC grade methanol ≥ 99.8%, ethanol ≥ 99.5%, acetonitrile ≥ 99.8%, and acetone ≥ 99.5% were purchased in VWR ProLabo Chemicals (Radnor, United States). Ultrapure water from an Adrona (Riga, Latvia) purification system was employed during the whole procedure. Other reagents such as NaCl (s) ≥ 99.5%, NaOH (s) ≥ 98.5%, formic acid, and glacial acetic acid were bought in Scharlab (Barcelona, Spain).

The procedure for the reference method was carried out using 150 mg Oasis WCX extraction cartridges from Waters Corporation (Massachusetts, United States) (Adachi et al., 2013). The extraction procedure was done with a Vac Elut 20 for solid-phase extraction from Agilent Technologies (California, United States) using 3 mL polypropylene cartridges from Análisis Vínicos (Ciudad Real, Spain). All samples were previously filtered with Nylon 0.45 mm Sartorius Stedim Biotech filters (Göttingen, Germany).

The analytical instrumentation used included a Merck Hitachi Lachrom liquid chromatograph equipped with a UV-Vis detector and an L-7100 quaternary pump for the optimization stage. The stationary phase used was a $C_{18}$ Kromasil column from Análisis Vínicos, 15 cm x 4.6 mm, 5 µm particle size (Ciudad Real, Spain) and the separation was carried out at a 1 mL $min^{-1}$ flow using a $H_2O$:ACN:MeOH 3% $H_3PO_4$ gradient mobile phase (80:10:10 to 60:30:10 in 30 min). The detection was carried out at 285 nm in this case (Figure C18). In addition, fluoroquinolones were separated and quantified in the real samples through an Acquity UPLC Waters liquid chromatograph (Massachusetts, United States) coupled to a triple quadrupole mass spectrometry detector (UPLC-MS/MS), where qualitative and quantitative analyses were carried out simultaneously. The stationary phase was a $C_{18}$ BEH Waters column (50 cm x 2.1 mm, 1.7 µm particle size). The mass selective detector identified ions m/z 321, 362, 320, 332, and 393 for enoxacin, ofloxacin, norfloxacin, ciprofloxacin, and sparfloxacin, respectively.

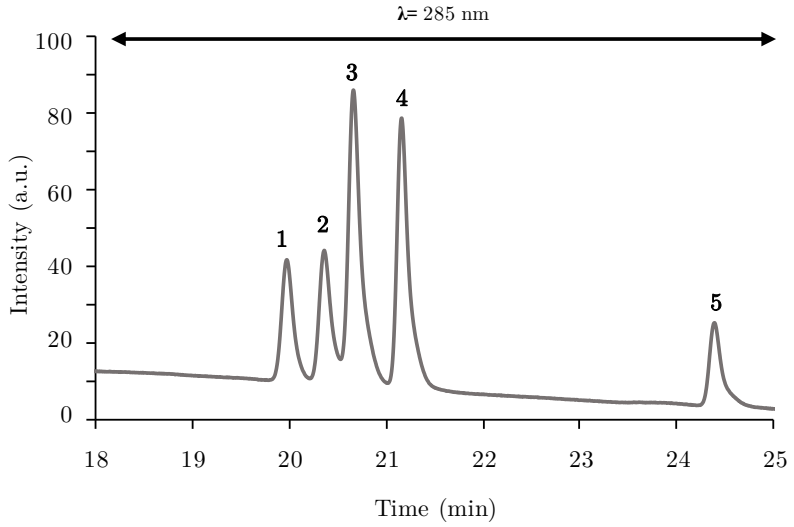



**Figure C18. HPLC-FLD chromatographic profile of a multianalyte solution of the fluoroquinolones under study: (1) enoxacin (2) ofloxacin (3) norfloxacin (4) ciprofloxacin (5) sparfloxacin.**

### 3.2.2. Optimization and validation of the SPE procedure

Different parameters affecting the concentration factor obtained by the SPE procedure developed were studied and optimized through the recovery obtained for synthetically-spiked water samples at the µg $L^{-1}$ level, calculated from the ratio between the obtained and the theoretical concentration (n=3). The procedure was optimized by varying one parameter at a time while keeping the others constant. The studied parameters were the type of solid phase, pH, ionic strength, intermediate washing, flow rate, quantity of solid phase, sample volume, loading capacity (retention conditions), type and volume of solvent (elution conditions), and post-evaporation. The reusability of the solid phase, as well as the matrix effect observed when working with real aqueous matrices, were also assessed.

Then, the analytical performance of the developed method was assessed through the establishment of global recovery, repeatability, sensitivity, and linearity. The limits of detection (LOD) and quantification (LOQ), as well as the linearity range, were calculated following the recommendations of the IUPAC (Olivieri et al., 2006).

The final optimized extraction procedure was as follows. First, SPE cartridges are prepared by packing 500 mg of the GMA-S-γ-CD material between two polyethylene frits into 6 mL empty polypropylene cartridges. The conditioning of the cartridges is carried out with 5 mL of MeOH followed by equilibration with 5 mL of ultrapure water. Then, the ionic strength of 100 mL of sample is adjusted to NaCl 3 M, where HCl 37% is added to obtain pH 1. The sample aliquot is placed in the extraction cartridge and suctioned at a 1 mL $min^{-1}$ flow. Next, it is washed with 3 mL of water and dried at vacuum for 5 min. The elution is accomplished with 6 mL of MeOH. Finally, the organic solvent is evaporated and recomposed with 1 mL MeOH. Then, UPLC-MS/MS is used for the quantitative analysis after filtration of the portions being measured.

To end, six real samples were chosen to apply the developed extraction method. Specifically, they consisted of two fecal waters with an expected strong matrix effect (M1 and M2, coming from the sewage system) and four groundwater samples (M3-M6) coming from different water wells geographically distributed all around the Valencian Community (Spain). The samples were first homogenized and filtered. As mentioned, the results for the analysis of the fluoroquinolones under study have been compared with those obtained with a reference method (Adachi et al., 2013). The overall recovery of the reference method was also studied to carry out the quantification of the fluoroquinolones reliably taking into account the corresponding effect of each sample matrix.

### 3.3. Results and discussion

The comparison between the developed GMA-S-γ-CD solid phase, the parent GMA-*co*-EDMA polymeric support, and the previously described GMA-S-β-CD polymeric material was first carried out to achieve proper retention of the target fluoroquinolones in the SPE cartridges constructed. As can be seen in Figure C19, a significant improvement in the recovery of the analytes was observed for the use of GMA-S-γ-CD in comparison with GMA-S-β-CD and the bare polymeric support. This behavior can be assigned to the size of the molecules of the analytes (ca. 0.80 – 0.90 nm) and that of the internal cavity of CDs (ca. 0.78 nm for β-CD and ca. 0.98 nm for γ-CD, as observed in Table 2), thus being host-guest interactions facilitated with γ-CD while β-CD probably offers not enough space. Moreover, the influence of the amount of CD in the retention was assessed using 0.5 eq., 1 eq., and 2 eq. of CD during the synthesis process with respect to the expected accessible epoxy groups corresponding to the GMA-*co*-EDMA support (Carrasco-Correa et al., 2015). Results, shown in Figure C20, showed better results when using 2 eq. of CD in the synthesis process. Therefore, this molar rate was selected to continue with the development of the method.

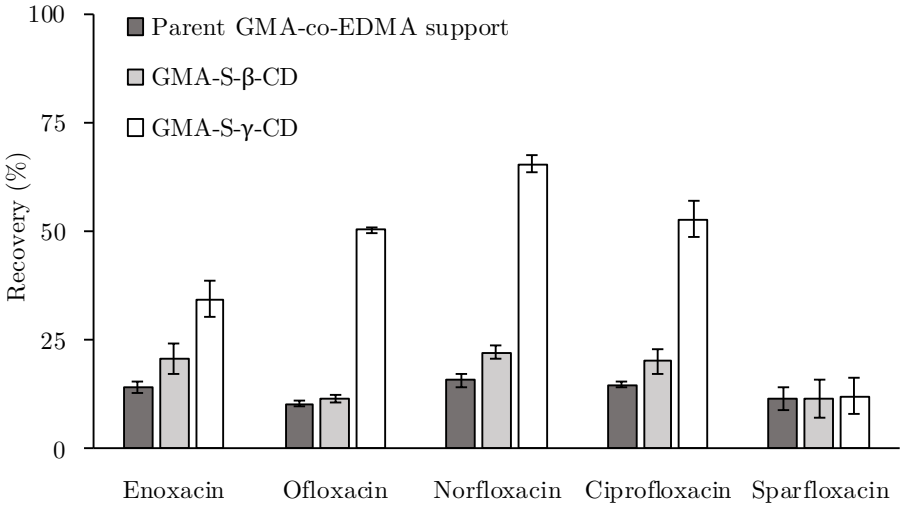



**Figure C19. Influence of the nature of the solid phase in the recoveries obtained for the SPE of fluoroquinolones.**

Then, other loading conditions such as the pH and the ionic strength were studied. First, spiked aqueous solutions at pH 1, 2, 3 (adjusted with HCl 37%), and 4 (acetic/acetate buffer) were studied. Results indicated that the recoveries improved significantly when working at pH 1. This behavior may be explained through the protonation of certain parts of the molecules of fluoroquinolones

(Table C6), which would make them able to interact more strongly not only through apolar interactions with the cavity of the cyclodextrin but also with the polar external hydroxyl groups. Results were of 80, 71, 74, 67, and 76% for enoxacin, ofloxacin, norfloxacin, ciprofloxacin, and sparfloxacin, respectively, when using pH 1, while they decreased to 62, 58, 61, 49, and 49% for the same analytes at pH 4. Thus, results made it advisable to use pH 1 for the proper retention procedure carried out. Besides, the influence of the ionic strength was evaluated through different concentrations of NaCl (from 0 M to 3 M) in the spiked testing solution. Results suggested that the higher the ionic strength, the higher the recovery calculated, this indicating higher retention of the target compounds in the solid phase. The salting-out effect supports these data. With a higher ionic strength, the solubility of the analytes in the aqueous phase decreases while increasing their retention into the sorbent. Consequently, the use of a 3 M concentration of NaCl during the extraction procedure is advisable.

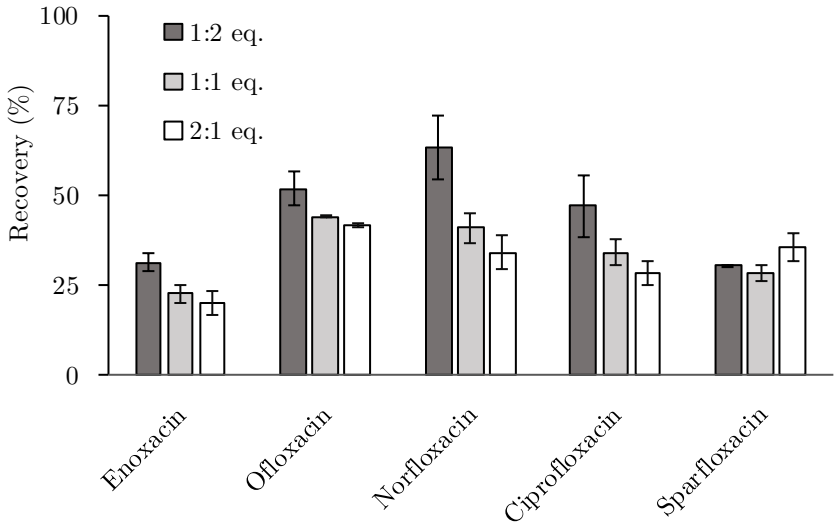



**Figure C20. Influence of the amount of γ-CD used during the synthesis process.**

At this point, the influence of an intermediate washing with water and the influence of the flow rate were studied. Regarding the intermediate washing, a comparison between the results obtained in absence of intermediate washing and with an in-between charge of 3 mL of water to clean the cartridge of interferences was conducted. Recoveries in both cases are comparable between them. Therefore, it can be concluded that the washing step is advisable since it does not worsen retention and is helpful to remove interferences from the sample matrix. Furthermore, the flow rate of the sample through the cartridges can influence the

retention of fluoroquinolones in them. Thus, the effect of 1, 2, and 3 mL $min^{-1}$ flow rates in the retention was assessed. Results worsened from the use of 1 mL $min^{-1}$ (75, 78, 71, 74, and 81% of recovery for enoxacin, ofloxacin, norfloxacin, ciprofloxacin, and sparfloxacin, respectively) to the use of 3 mL $min^{-1}$ (69, 70, 69, 68, and 71% for the same compounds). For this reason, flow 1 mL $min^{-1}$ was chosen as the most appropriate to continue with the optimization study to ensure and ease the interaction antibiotic-sorbent.

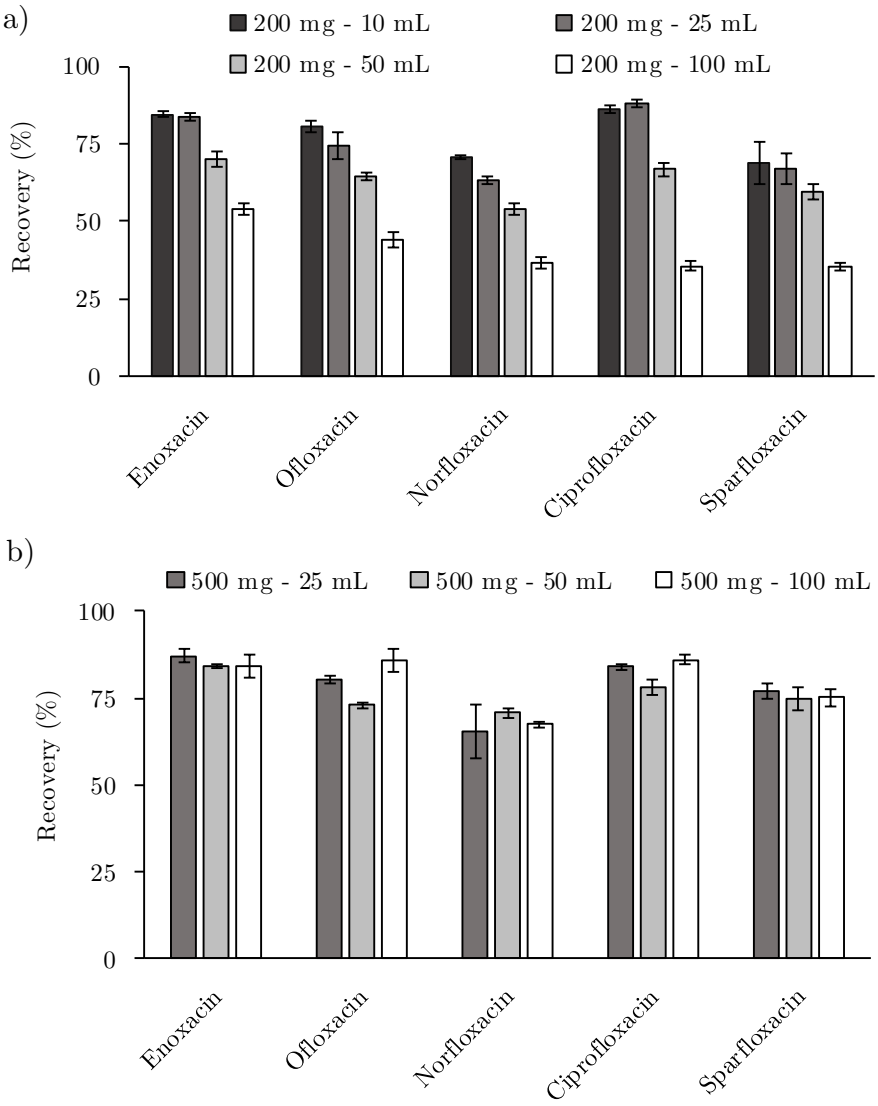



**Figure C21. Influence of the amount of material and the sample volume in the retention of fluoroquinolones. Recovery obtained for the use of (a) 20 mg and (b) 500 mg of solid phase.**

The amount of solid phase with respect to the sample volume was also studied. As it is known, the higher the sample volume, the better sensitivity can be achieved when working with analytes at trace level. Figure C21 displays the results obtained. From them, it can be concluded that the use of 200 mg of solid phase does not permit the use volumes higher than 25 mL since a higher amount of water may cause losses of analytes. Oppositely, the use of 500 mg of solid phase allowed using up to 100 mL of sample, which is preferable for this purpose.

Regarding the nature and volume of the eluent, MeOH, ACN, ethanol, and acetone were tested thinking of the mainly apolar nature of the retention and the best way of eluting our analytes. The recoveries obtained for the majority of analytes were comparable for MeOH, ACN, and acetone. However, acetone showed a significant decline in the recovery of ciprofloxacin and sparfloxacin. Between ACN and MeOH, MeOH was chosen in terms of a greener methodology being proposed. In addition, the elution profile showed that most of the analytes are eluted in the first 4 mL of eluent. However, a non-negligible percentage could be quantified in the next 2 mL. Thus, with the aim of increasing the sensitivity, the possibility of evaporating and redissolving the residue was tested. No significant differences were obtained after evaporation, indicating the absence of losses during this procedure. Therefore, there exists the possibility of carrying out this concentration step without affecting the results obtained. In this way, not only the sensitivity but also the repeatability can be enhanced due to the possibility of accidental evaporation of the eluent during the elution step. For all this, 6 mL of solvent were chosen to carry out the elution step. Then, their evaporation at 40°C for 20 minutes in a concentrator and the subsequent reconstitution completed the procedure.

The loading capacity of the material was tested by passing 10 mL of water samples spiked with concentrations of fluoroquinolones ranging from 50 µg $L^{-1}$ to 5 mg $L^{-1}$ through the cartridges. Results indicated that the concentration did not influence the recovery up to 2 mg $L^{-1}$ (recoveries of 80, 83, 65, 84, and 75% for enoxacin, ofloxacin, norfloxacin, ciprofloxacin, and sparfloxacin, respectively) For 5 mg $L^{-1}$, the upper limit of capacity may have been reached and, for this reason, the recovery was lower in that case (values of 64, 58, 40, 50, and 56% for the same analytes). Since fluoroquinolones are usually found in the environment at trace levels, the validated concentration range is enough for the intended purpose of the synthesized GMA-S-γ-CD.

To conclude, the reusability of the material was assessed. Recoveries remained constant for five different and consecutive uses tested, as expected.

Concerning the analytical figures of merit of the extraction method developed, the precision of the method was evaluated by measuring the repeatability from extractions of spiked samples and was expressed in terms of the coefficient of variation. As observed in Table C7, the coefficients of variation ranged from 2.5% in the lower limit to 5.1% in the upper limit, the lower values obtained in general for extractions within the same day, as would be expected. Besides, both LOD and LOQ were calculated referred to the water sample considering the described experimental procedure. LODs were established between 0.2 and 0.5 μg $L^{-1}$, and LOQs are below 1.6 μg $L^{-1}$, being the limits low enough to achieve the detection and quantification of fluoroquinolones at trace level in the environment as emerging pollutants. The experimental procedure together with the separation and quantification using the UPLC-MS/MS equipment provided a satisfactory sensitivity. The overall recovery was 78, 83, 76, 89, and 81% for enoxacin, ofloxacin, norfloxacin, ciprofloxacin, and sparfloxacin, respectively. A reasonable explanation for the observed results could be that the recoveries obtained coincide with a better or worse fit of the molecules in the central cavity of the γ-CD, as mentioned. To end, an acceptable linearity range permits the use of the developed extraction methodology in a range of concentrations.

**Table C7. Analytical figures of merit established for the extraction method of fluoroquinolones using the GMA-S-γ-CD polymeric support.**

| Compound | CV (%) | | Recovery (%) | LOD[a] (μg $L^{-1}$) | LOQ[a] (μg $L^{-1}$) | Linearity[b] (mg $L^{-1}$) |
|---|---|---|---|---|---|---|
| | Intra-day | Inter-day | | | | |
| Enoxacin | 2.9 | 4.2 | 78 ± 3 | 0.7 | 2.0 | 0.20 – 500 |
| Ofloxacin | 2.5 | 3.5 | 83 ± 4 | 0.8 | 2.5 | 0.25 – 500 |
| Norfloxacin | 5.1 | 3.6 | 76 ± 4 | 0.7 | 2.1 | 0.20 – 500 |
| Ciprofloxacin | 4.2 | 3.7 | 89 ± 2 | 0.7 | 2.0 | 0.20 – 500 |
| Sparfloxacin | 3.8 | 3.8 | 81 ± 3 | 0.1 | 0.4 | 0.20 – 500 |

[a] LOD and LOQ are referred to the water sample.
[b] Linearity is referred to the measuring solution.

The matrix effect was studied by measuring the extraction efficiency of real samples spiked with 2 μg $L^{-1}$ of each of the fluoroquinolones under study, following both the proposed method and the chosen reference method (Adachi et al., 2013).

Two different matrices were evaluated as a reference to study this effect: one for fecal water (M1) and another for groundwater (M3), corresponding with the type of matrices selected for the analysis of real water samples. The recovery was calculated in each case from the ratio between the obtained and the theoretical concentration by previous subtraction of the real fluoroquinolone content of each sample without spiking it. Results are shown in Table C8. As expected, the recovery obtained was significantly lower than that calculated during the optimization study with spiked ultrapure water due to the enhanced complexity of the matrices, especially in the case of fecal water. Significant is to mention the improvement of the results obtained for enoxacin and sparfloxacin when using the GMA-S-γ-CD material proposed in comparison with the WCX cartridges suggested in the reference. These results can be translated into advantageous properties of the solid phase developed in terms of the matrix effect that can occur when analyzing complex samples, which can be attributed to the enhanced selectivity provided by the use of cyclodextrins. In addition, it should be mentioned that in the case of the simplest type of matrix, that is, groundwater, the results obtained were comparable for all analytes, which allows validating the functionality of the proposed material. In any case, the use of a standard addition calibration method to carry out the analysis of real water samples is advisable.

**Table C8. Influence of the matrix in the recovery of FQs using the GMA-S-γ-CD.**

| Compound | Recovery (%) Fecal water | | Recovery (%) Groundwater | |
|---|---|---|---|---|
| | GMA-S-γ-CD | WCX | GMA-S-γ-CD | WCX |
| Enoxacin | 78 ± 4 | 51 ± 7 | 76 ± 4 | 73 ± 5 |
| Ofloxacin | 77 ± 7 | 74 ± 3 | 82 ± 4 | 73 ± 4 |
| Norfloxacin | 57 ± 7 | 62 ± 6 | 76 ± 7 | 70 ± 9 |
| Ciprofloxacin | 44 ± 8 | 42 ± 3 | 81 ± 3 | 85 ± 2 |
| Sparfloxacin | 73 ± 5 | 61 ± 4 | 75 ± 8 | 71 ± 4 |

The feasibility of the described method was assessed by applying it to the determination of antibiotics in real samples taking into account the matrix effect already described. Results are shown in Table C9.

Table C9. Analysis of fluoroquinolones using the extraction method developed with the GMA-S-γ-CD material (μg $L^{-1}$, $\bar{x} \pm s$) and its validation.

| Compound | M1 Fecal water | | M2 Fecal water | | M3 Groundwater | | M4 Groundwater | | M5 Groundwater | | M6 Groundwater | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GMA-S-γ-CD | WCX | GMA-S-γ-CD | WCX | GMA-S-γ-CD | WCX | GMA-S-γ-CD | WCX | GMA-S-γ-CD | WCX | GMA-S-γ-CD | WCX |
| Enoxacin | 2.2 ± 0.4 | 1.92 ± 0.15 | 2.5 ± 0.5 | 1.9 ± 0.2 | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| Ofloxacin | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| Norfloxacin | < LOQ | < LOQ | < LOQ | < LOQ | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| Ciprofloxacin | 4.7 ± 0.2 | 4.2 ± 0.3 | 4.6 ± 0.7 | 5.0 ± 0.6 | < LOQ | < LOQ | < LOQ | < LOQ | < LOD | < LOD | < LOD | < LOD |
| Sparfloxacin | < LOD | < LOD | 0.22 ± 0.02 | 0.22 ± 0.04 | < LOQ | < LOQ | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |

As can be seen, every target compound except ofloxacin was detected in the samples under study. Logically, M1 and M2 were the ones with a higher detection and quantitation rate due to the habitual consumption of antibiotics by the population. Specifically, ciprofloxacin and norfloxacin were detected in both of them, coinciding with the declared most commonly used medicines in this class (European Medicines Agency, 2018). These fluoroquinolones cover a wide range of infections since ciprofloxacin is commonly used to treat general infections such as those of the digestive system or pneumonia, while norfloxacin is recommended in cases of urinary infection. In this case, its consumption may be lower since it was detected, but the values were below the limit of quantification of the method. Other antibiotics detected were enoxacin and sparfloxacin. Enoxacin is also used in the case of urinary infections, which could explain the lower values of norfloxacin obtained due to higher consumption of one over the other, while sparfloxacin is used for very specific types of bacteria, which would explain its presence in only one of the two samples. Concerning the M3 to M6 samples, all analytes detected were below the limit of quantification, which constitutes good results. It may indicate that environmental water cleaning processes may be effective and that these pollutants in the groundwater do not arrive in many cases to human consumption.

Regarding the comparison with the reference method (Adachi et al., 2013), the results obtained for the analysis of real water samples with both methods are comparable between them by using the paired t-test for comparing individual differences (Harris, 2007) with a 95% confidence level.

## 3.4. Conclusion

In this work, the GMA-S-γ-CD solid phase has been applied to the isolation of fluoroquinolones from groundwater. Between the advantages presented, an enhancement in the extraction selectivity thanks to the host-guest interactions offered by the presence of cyclodextrins can be mentioned. The comparison of the results with other commercial solid phases and proposed methodologies shows a lower matrix effect when using the proposed extraction methodology with complex environmental samples. Thus, the reported SPE sorbent together with the developed methodology represent a promising alternative for the systematic determination of antibiotics in water samples.

*The groundwater samples used in this chapter were nicely provided by the City Council of Picanya (Valencia) within the project agreed upon as a result of the Concepción Aleixandre award to Woman in Science (2020) granted to the author, which is appreciated.*

# 4

# Overview on polymeric cyclodextrin-based materials

A new variation of already existing GMA-*co*-EDMA polymeric materials through their surface modification with cyclodextrin units and their use as sorbents for high-concern substances has been described and tested in different conditions, including different types of analytes, different types of matrices, or different types of purposes. For example, the analysis of phenolic compounds using the GMA-S-β-CD solid phase as sorbent may represent the application in food safety, whereas the isolation of fluoroquinolones from groundwater using the GMA-S-γ-CD material may represent the application to environmental monitoring and control.

Not only the solid phase preparation but also the SPE procedures developed are easy and relatively economical. In this sense, the possibility of reusing the materials synthesized represents a great advantage. However, the several steps constituting the synthesis process may represent a disadvantage.

Contrary to the silica-based materials described previously, this kind of polymeric supports are usually characterized for possessing low surface areas for the adsorption of different substances. However, the possibility of decorating their area through a variety of efficient selective chemistries such as click-chemistry reactions helps to overcome this inconvenience. Moreover, and parallel to the previous cases, it has been proven that their features can be modulated when choosing the CD used in order to achieve materials with enhanced selectivity for certain substances.

The described GMA-S-β-CD-polymeric material constitutes a feasible alternative to other sorbents containing cyclodextrin used in solid-phase extraction. Its availability as a satisfactory sorbent has been confirmed by the analytical features achieved during the extraction procedure of phenolic compounds from tea. This is in accordance with the covalent attachment of the β-CD to the polymeric network, which avoids losses when treating aqueous samples to the relative solubility of CD in water.

Also, the key role of γ-CD on the features of the GMA-S-γ-CD material synthesized has been proven. Hence, the idea of adapting the synthesis of a polymeric material to the type of analytes in use taking into account the influence of the cyclodextrin size on the retention of different families of compounds according to their physicochemical properties is supported here too. Thus, fluoroquinolones, which possess a higher size, fit better into the γ-CD size thus obtaining better retention results that influence the whole analytical procedure.

# SUMMARY AND CONCLUSIONS

As a conclusion of the research carried out during the development of this Doctoral Thesis, it can be extracted that the use of cyclodextrins as a part of sorbent materials for their operation in analytical applications has proven to be a feasible option. Broadly, the materials designed and characterized have shown to have good capacities to improve the features of some of the already existing analytical methods, being important to highlight an increase in the selectivity and sensitivity of the tested analytes.

As is well known, chromatographic techniques, specifically liquid and gas chromatography, turn out to be promising alternatives for the separation and quantification of different types of analytes due to their high efficiency and relative speed. In fact, its use is widespread for screening-type analysis when combined with detectors that are capable of undoubtedly identifying analytes, such as mass spectrometry detectors. However, these first-approach analyses do not always allow the detection of the substances of interest at trace level, which is the most usual situation, nor do they allow adequate reliability of the reported results. For this reason, the combination of chromatographic techniques with previous sample treatments using sorbent materials turns out to be a good solution to eliminate potential interferents while achieving fine pre-concentration of target analytes, which permits, in turn, the analysis of much lower concentrations of pollutants in a variety of complex samples such as those found in the environmental, food, and clinical fields. In the case of environmental samples, the abovementioned solid phases are applicable not only to the extraction of the substances of interest from aqueous samples but also to their sampling in air through the use of environmental samplers containing some of them.

The first section of the work (Section A) was devoted to the synthesis of porous cyclodextrin-silica xerogels for the retention of pollutants in several types of samples. As expected, cyclodextrins trapped the analytes of interest through the formation of selective host-guest complexes, and the accessibility to these cyclodextrin molecules was eased due to an adequate porosity of the solid phases under study. The main conclusions extracted from the research carried out with this type of materials is outlined as follows:

- Cyclodextrin-silica xerogels have been described and tested in a variety of conditions, including diverse analytes, types of matrices, or different purposes of the sorption procedure such as sampling or concentrating.
- These materials have been synthesized in two different ways. Not only the simple inclusion of cyclodextrins into the silica network was studied, but

also their covalent attachment to the silica support to observe the variation on the functionalities of the solid phases obtained.

- The materials preparation and the construction of cartridges or samplers were easy and relatively inexpensive.
- The adequate design of the solid phases, which combined hydrophilic silica with well-dispersed hydrophobic cyclodextrin sites, permits flexibility in the analytical application tested.
- Discrimination to adsorb certain analytes based on their molecular size together with their chemical nature was achieved thanks to the use of cyclodextrins in the sorption process.
- Cyclodextrin-silica xerogels have demonstrated fine capacities not only with environmental samples but also when working with biological fluids.
- The possibility of improving different analytical features such as the sensitivity, selectivity, or clean-up potential of an analytical method when using cyclodextrin-silica xerogels represents a success.

The next section (Section B) focused on the synthesis of mesoporous type UVM-7 silica materials with the same end. In this case, it was proven that the bimodal porosity of the solid phases influenced the sorption mechanism and the accessibility of cyclodextrins to analytes. In any case, the solids obtained also demonstrated good applicability in analytical chemistry with pre-concentration and clean-up purposes through the formation of host-guest complexes analyte-cyclodextrins that permitted enhanced selectivity parallel to cyclodextrin-silica xerogels. A summary of the main conclusions extracted is exposed hereunder:

- Type UVM-7 materials containing cyclodextrins have been designed and characterized. Later, they were applied to the analysis of different substances in food matrices.
- The low solubility of modified γ-cyclodextrin hampered the use of the common synthesis scheme established for UVM-7 materials. However, slight modifications of the obtaining process also led to the solid phases expected.
- The characteristic high surface areas with interconnected porosity of UVM-7 solid phases permitted fine adsorption of a variety of substances, making cyclodextrins accessible to target analytes too. However, and as expected, the mesoporous structure did not provide major benefits for certain analytical applications in comparison with xerogels.

- The possibility of adapting the material to the type of analyte under study by taking into account the type and size of the cyclodextrins contained in the silica network denotes a remarkable benefit.
- Type UVM-7 silica materials containing cyclodextrins have demonstrated fine capacities when working with food samples.
- The possibility of improving some of the analytical features of an analytical method such as the sensitivity, selectivity, or the clean-up potential when using mesoporous cyclodextrin-silica materials also represents an advantage in this case.

To end, the last section of this work (Section C) represented a change of approach in the research conducted. This time, the type of supporting material to cyclodextrins was changed to polymer-based solid phases. Therefore, the main objective this time was to synthesize polymeric cyclodextrin-based materials for the retention of pollutants. The main results of the research are summarized as follows:

- The surface of already described GMA-*co*-EDMA polymeric materials has been modified with cyclodextrin units and their use as sorbents for substances of high concern has been described and tested in different conditions, including food and environmental analysis.
- Contrary to silica-based materials, these polymeric solid phases are characterized for possessing low surface areas for the adsorption of substances. However, the possibility of decorating their area through the use of click-chemistry reactions helped to overcome this inconvenience.
- The synthesis of the solid phases is easy and economical. However, the several steps of the process may represent a disadvantage in comparison with the mainly one-pot reactions described for silica materials.
- Following the preceding steps, the idea of adapting the synthesis of the materials to the analytes taking into account the influence of the cyclodextrin size on the retention of the compounds of interest according to their physicochemical properties was supported here too. Cyclodextrins certainly contribute to the matrix clean-up and the improvement of the selectivity towards the target compounds prior to their analysis.
- The contribution of the presence of cyclodextrins together with the support design strengthened, parallel to previous cases, the possibility of improving the analytical figures of merit measured of the analytical methods developed in comparison with other existing methods.

- Polymeric materials containing accessible cyclodextrin molecules showed good features not only with environmental samples but also when treating food samples.

Based on the research exposed and the conclusions mentioned above, the future perspectives of the work started during this Doctoral Thesis should focus on increasing the field of application of the materials containing cyclodextrins obtained. Although they have shown good functionalities, their analytical applications have been limited to a few families of pollutants in very specific types of samples due to time obligations and resources economy. However, in view of the results, this good performance should be extrapolated to many other analytical situations, which would imply the confirmation of the high potential shown by the materials described. In this sense, their use has been mainly limited to solid-phase extraction. Nevertheless, as described in the Introduction, the number of extraction techniques and methodologies for sample preparation is today so wide that the use of the designed solid phases could be adapted to a variety of them. In addition, the study of their use as environmental air samplers, whose field is less studied and would require much more specific working conditions to obtain conclusive and reliable results, still remains an open door for further future investigations.

To end, it must be stated that this work constitutes one more example that the nanoscience applications are currently expanding to diverse areas. Sorbent materials and nanomaterials occupy an important place in current research in analytical chemistry. Indeed, their application as sorbents has achieved a sharp increase in recent years since they can play an outstanding role in sampling and pre-concentration processes. Their usefulness as sample preparation sorbents is a result of their varied morphologies, high surface area, surface-to-volume ratio, and porosity, as well as their ability to interact in a variety of ways that gives them low resistance to diffusion, large sorptive capacity, and fast adsorption kinetics in many cases. Thus, they have allowed the development of innovative analytical methodologies with a relevant contribution in concentration and interference removal, aspects of vital importance in the trace analysis of complex samples. As shown, the materials that scientists are able to produce have some uncertainties and irregularities in their physical and chemical characteristics. However, this fact does not usually limit their application as sorbents because it has been shown that a certain degree of control over them can ultimately be achieved. Therefore, it is clear that sorbent materials and nanomaterials have come to stay in our daily life and, in particular, in the analytical chemistry laboratory.

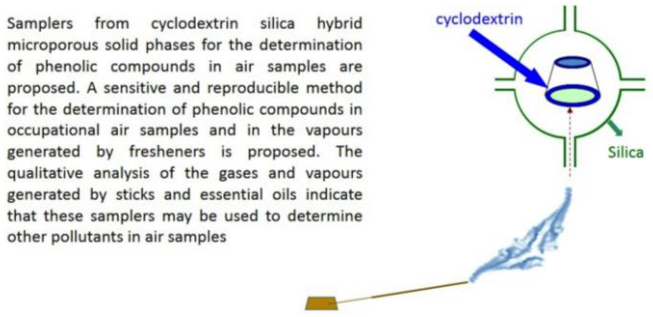
# CONTRIBUTION TO PUBLISHED CONTENT

The work carried out during the pre-doctoral period conducted to several publications that must be acknowledged.

First, the content in this Ph.D. work corresponds to the following scientific articles, listed according to the publication date:

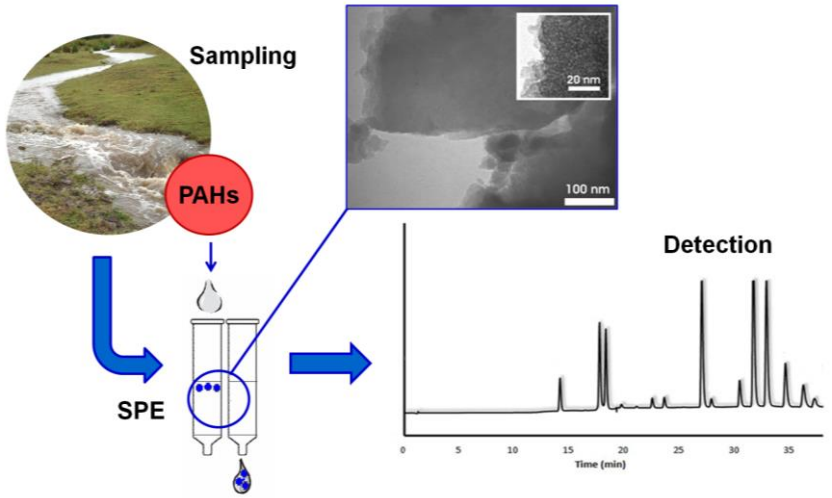
1. Mauri-Aucejo, A.R., Ponce-Catalá, P., Belenguer-Sapiña, C., Amorós, P. (**2015**) Determination of phenolic compound in air by using cyclodextrin-silica hybrid microporous composite samplers. *Talanta*, 134, 560-567. https://doi.org/10.1016/j.talanta.2014.11.057

   *An analytical method for the determination of phenolic compounds in air samples based on the use of cyclodextrin-silica hybrid microporous composite samplers is proposed. The method allows the determination of phenol, guaiacol, cresol isomers, eugenol, 4-ethylphenol and 4-ethylguaiacol in workplaces according to the Norm UNE EN 1076:2009 for active sampling. Therefore, the proposed method offers an alternative for the assessment of the occupational exposure to phenol and cresol isomers. The detection limits of the proposed method are lower than those for the NIOSH Method 2546. Storage time of samples almost reaches 44 days. Recovery values for phenol, guaiacol, o-cresol, m-cresol, p-cresol, 4-ethylguaiacol, eugenol and 4-ethylphenol are 109%, 99%, 102%, 94%, 94%, 91%, 95% and 102%, respectively with a coefficient of variation below 6%. The method has been applied to the assessment of exposure in different areas of a farm and regarding the quantification of these compounds in the vapors generated by burning incense sticks and an essential oil marketed as air fresheners. The acquired results are comparable with those provided from a reference method for a 95% of confidence level. The possible use of these samplers for the sampling of other toxic compounds such as phthalates is evaluated by qualitative analysis of extracts from incense sticks and essential oil samples.*



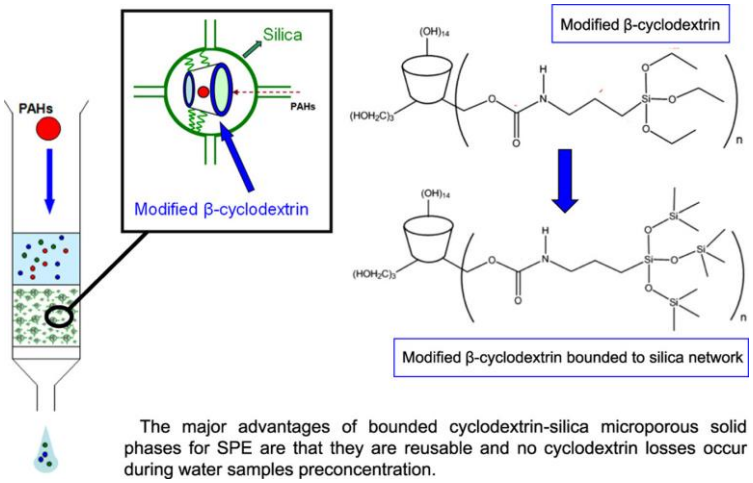
**2.** Soler-Seguí, S., Belenguer-Sapiña, C., Amorós, P., Mauri-Aucejo, A. (**2016**) Evaluation of a cyclodextrin-silica hybrid microporous composite for the solid-phase extraction of polycyclic aromatic hydrocarbons. *Analytical Sciences*, 32(6), 659-665.
https://doi.org/10.2116/analsci.32.659

*Solid-phase extraction (SPE) coupled with high-performance liquid chromatography (HPLC) with fluorescence detection were employed to determine trace polycyclic aromatic hydrocarbons in water samples. In this way, the use of cartridges containing cyclodextrin-silica hybrid microporous solid phases was proposed. The experimental results indicated that the method provided relative standard deviations of below 15% and detection limits recorded were 12, 1.2, 12, 38, 4, 6 and 4 ng $L^{-1}$ for benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, indeno[1,2,3]pyrene, benzo[a]pyrene, dibenzo[a,h]anthracene and benzo[a]anthracene, respectively. Moreover, the method was successfully applied for the determination of these organic compounds in water samples, where they were found to be in the 7 to 580 ng $L^{-1}$ range. It can be concluded that the major advantages of cyclodextrin-silica hybrid microporous solid phases are that they reduce the consumption and the toxicity of the solvent and the time consumption of the sample treatment step.*



**3.** Mauri-Aucejo, A.R., Amorós, P., Moragues, A., Guillem, C., Belenguer-Sapiña, C. (**2016**) Comparison of the solid-phase extraction efficiency of a bounded and an included cyclodextrin-silica microporous composite for polycyclic aromatic hydrocarbons determination in water samples. *Talanta*, 156-157, 95-103.
https://doi.org/10.1016/j.talanta.2016.05.011

*Solid-phase extraction is one of the most important techniques for sample purification and concentration. A wide variety of solid phases have been used for sample preparation over time. In this work, the efficiency of a new kind of solid-phase extraction adsorbent, which is a microporous material made from modified cyclodextrin bounded to a silica network, is evaluated through an analytical method which combines solid-phase extraction with high-performance liquid chromatography to determine polycyclic aromatic hydrocarbons in water samples. Several parameters that affected the analytes recovery, such as the amount of solid phase, the nature and volume of the eluent or the sample volume and concentration influence have been evaluated. The experimental results indicate that the material possesses adsorption ability to the tested polycyclic aromatic hydrocarbons. Under the optimum conditions, the quantification limits of the method were in the range of 0.09–2.4 μg $L^{-1}$ and fine linear correlations between peak height and concentration were found around 1.3–70 μg $L^{-1}$. The method has good repeatability and reproducibility, with coefficients of variation under 8%. Due to the concentration results, this material may represent an alternative for trace analysis of polycyclic aromatic hydrocarbons in water trough solid-phase extraction.*

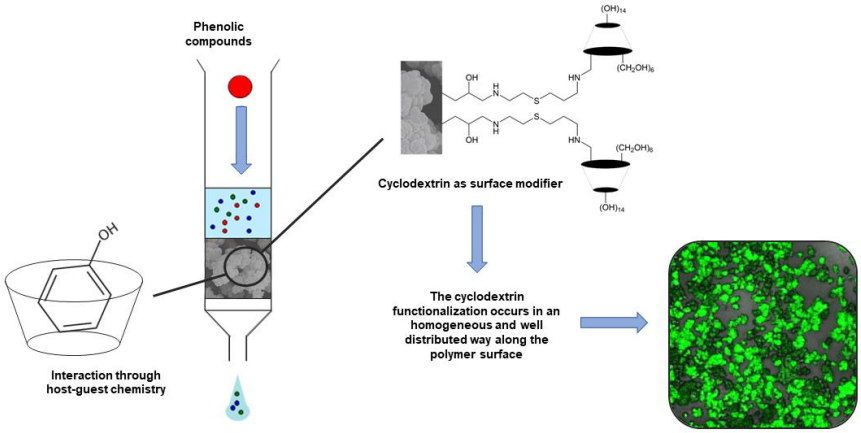


4. Belenguer-Sapiña, C., Pellicer-Castell, E., El Haskouri, J., Guillem, C., Simó-Alfonso, E.F., Amorós, P., Mauri-Aucejo, A.R. (**2018**) Design, characterization and comparison of materials based on β and γ cyclodextrin covalently connected to microporous silica for environmental analysis. *Journal of Chromatography A*, 1563, 10-19.
https://doi.org/10.1016/j.chroma.2018.05.070

*Determination of organic pollutants in environmental samples presents great difficulties due to the lack of sensitivity and selectivity in many of the existing analytical methods. In this work, the efficiency of materials based on silica structures containing bounded γ-cyclodextrin has been evaluated to determinate phenolic compounds and polycyclic aromatic hydrocarbons in air and water samples, respectively, in comparison with materials made of β-cyclodextrin. According to the results obtained for the material characterization, the new γ-cyclodextrin solid phase does not apparently present any porosity when used in air samples, but it has been shown to work efficiently for the preconcentration of polycyclic aromatic hydrocarbons in water, with recoveries around 80%. In addition, the use of the β-cyclodextrin material for phenolic compounds sampling can be highlighted with recoveries between 83% and 95%, and recoveries for 4-vinylphenol and 2-methoxy-4-vinylphenol have been especially improved in comparison with the use of materials containing trapped β-cyclodextrin in our previous researches. The observed phenomena can be explained on the basis of the analyte molecules size and the diameter of the cyclodextrin cavities, the influence of the cyclodextrin type in the material structure as well as on the interactions taking place with the pollutants and the influence of the matrix type in the retention and desorption mechanisms.*

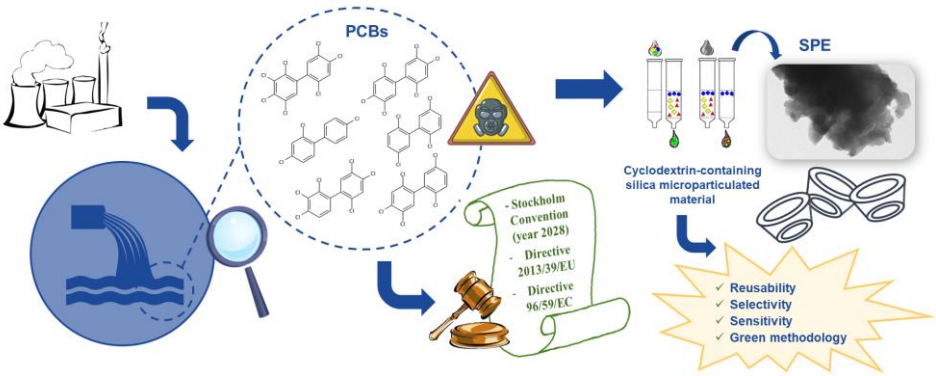
**5.** Belenguer-Sapiña, C., Pellicer-Castell, E., Vila, C., Simó-Alfonso, E.F., Amorós, P., Mauri-Aucejo, A.R. (**2019**) A poly(glycidyl-co-ethylene dimethacrylate) nanohybrid modified with β-cyclodextrin as a sorbent for solid-phase extraction of phenolic compounds. *Microchimica Acta*, 186(9), 615. https://doi.org/10.1007/s00604-019-3739-4

*A hybrid material made of β-cyclodextrin anchored to a polymeric network is described and evaluated as a sorbent for solid-phase extraction of phenolic compounds (phenol, cresol isomers, 2-methoxy-4-vinylphenol, 4-ethylphenol, 4-vinylphenol, 4-ethylguaiacol, guaiacol, and eugenol). The polymeric backbone of the sorbent consists of a poly(glycidyl-co-ethylene dimethacrylate) network, whose surface has been modified with β-cyclodextrin by a click-chemistry based procedure. The resulting material has been characterized by different techniques, and it has shown to be viable as a sorbent for its use in extraction cartridges. In this way, a method for the determination of the above analytes in tea has been validated. Under optimum conditions, the method has good repeatability, with coefficients of variation between 0.6 and 7.2%. In addition, recoveries from spiked samples at the level of 50 μg $L^{-1}$ are between 57 and 101%. The method has been then applied to the determination of phenolic compounds in the drinkable portion of infusions made from tea bags. The quantification has been carried out by using gas chromatography coupled to a mass spectrometry detector. Following their elution from the sorbent with a mixture of acetonitrile and methanol, the limits of quantification reached are between 4.6 and 400 μg $L^{-1}$. Results have been compared with those obtained with a reference method by using the paired t-test for comparing individual differences. The solid phase is reusable, and no cyclodextrin is lost during extraction due to its covalent anchoring to the polymeric support.*



6. Belenguer-Sapiña, C., Pellicer-Castell, E., Amorós, P., Simó-Alfonso, E.F., Mauri-Aucejo, A.R. (**2020**) A new proposal for the determination of polychlorinated biphenyls in environmental water by using host-guest adsorption. *Science of the Total Environment*, 724, 138266. https://doi.org/10.1016/j.scitotenv.2020.138266

*Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants whose wide industrial use has been banned over the years in most countries due to their persistence and bioaccumulation. In fact, the International Agency for Research on Cancer defined them in 2016 as carcinogenic to humans based on sufficient evidence of an increased risk of cancer, being children and pregnant or lactating women the most vulnerable population subgroups. In this work, a new alternative for the determination of polychlorinated biphenyls (PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180) in water samples has been developed by using a cyclodextrin-containing silica microparticulated material as an adsorbent in solid-phase extraction. Gas chromatography coupled to an electron capture detector has been used in the quantification step. The methodology allows quantifying polychlorinated biphenyls at very trace levels, with limits of detection between 0.2 and 1.7 ng $L^{-1}$. Other parameters such as the repeatability, with coefficients of variation lower than 11%, were likewise established. To end, real water samples were analyzed, and the results were comparable with those obtained with a reference method. The proposed methodology can be utilized for assessing the presence of these compounds in the environment and can come in handy for evaluation and remediation purposes.*

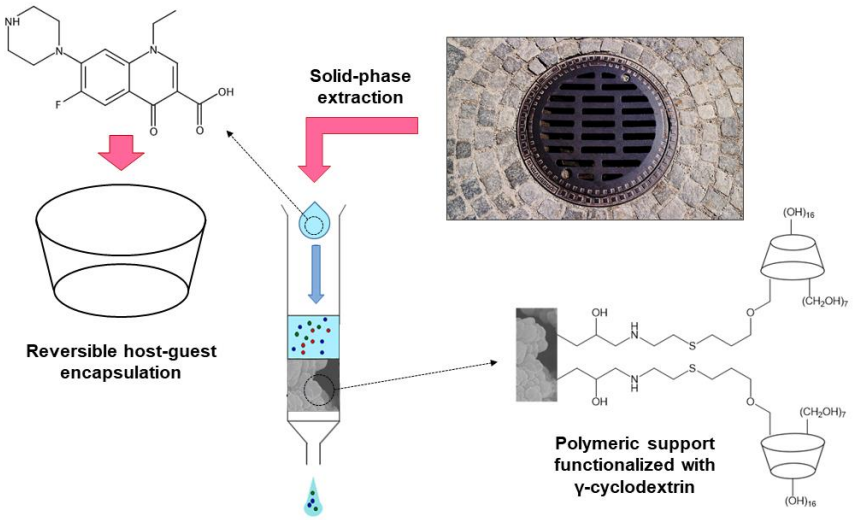


7. Belenguer-Sapiña, C., Pellicer-Castell, E., Mauri-Aucejo, A.R., Simó-Alfonso, E.F., Amorós, P. (**2021**) Cyclodextrins as a key piece in nanostructured materials: quantitation and remediation of pollutants. *Nanomaterials*, 11(1), 1-28.
https://doi.org/10.3390/nano11010007

*Separation and pre-concentration of trace pollutants from their matrix by reversible formation of inclusion complexes has turned into a widely studied field, especially for the benefits provided to different areas. Cyclodextrins are non-toxic oligosaccharides that are well known for their host–guest chemistry, low prices, and negligible environmental impact. Therefore, they have been widely used as chiral selectors and delivery systems in the pharmaceutical and food industry over time. However, their use for extraction purposes is hampered by their high solubility in water. This difficulty is being overcome with a variety of investigations in materials science. The setting-up of novel solid sorbents with improved properties thanks to the presence of cyclodextrins at their structure is still an open research area. Some properties they can offer, such as an increased selectivity or a good distribution along the surface of a solid support, which provides better accessibility for guest molecules, are characteristics of great interest. This systematic review reports the most significant uses of cyclodextrins for the adsorption of pollutants in different-origin samples based on the works reported in the literature in the last years. The study has been carried out indistinctly for quantitation and remediation purposes.*

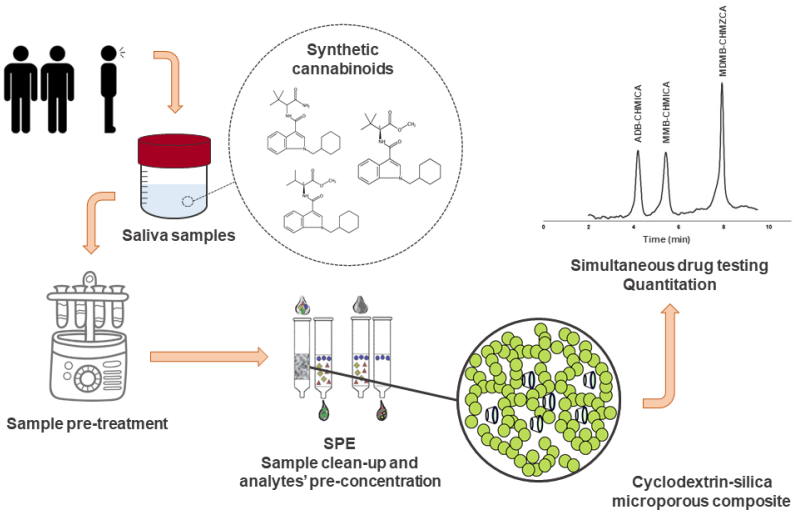
8. Belenguer-Sapiña, C., Pellicer-Castell, E., Pottanam Chali, S., Ravoo, B.J., Amorós, P., Simó-Alfonso, E.F., Mauri-Aucejo, A.R. (**2021**) Host-guest interactions for extracting antibiotics with a γ-cyclodextrin poly(glycidyl-co-ethylene dimethacrylate) hybrid sorbent. *Talanta*, 232, 122478. https://doi.org/10.1016/j.talanta.2021.122478

*A procedure for the solid-phase extraction of antibiotics (enoxacin, ofloxacin, norfloxacin, ciprofloxacin, and sparfloxacin) in water has been developed. The sorbent used is based on a poly(glycidyl-co-ethylene dimethacrylate) network, whose previously modified surface has been functionalized with γ-cyclodextrin through a click-chemistry reaction. The architecture of the material has been characterized by thermogravimetric analysis, $N_2$ adsorption-desorption, Raman spectroscopy, confocal microscopy, and scanning electron microscopy, showing good capability to be used as a filler for extraction cartridges. The optimization of the extraction methodology shows good intra-day and inter-day repeatability of the extraction procedure, with coefficients of variation between 2.5 and 5.1% and the possibility of reusing the material at least five times. The detection limits of the method have been established at the μg $L^{-1}$ level, confirming the possibility of quantifying trace levels. To end, real groundwater samples have been analyzed and the results are comparable with those obtained with a reference method. The proposed material can be used for assessing the presence of antibiotics in aqueous environments through an extraction procedure taking advantage of the presence of γ-cyclodextrin on its structure.*



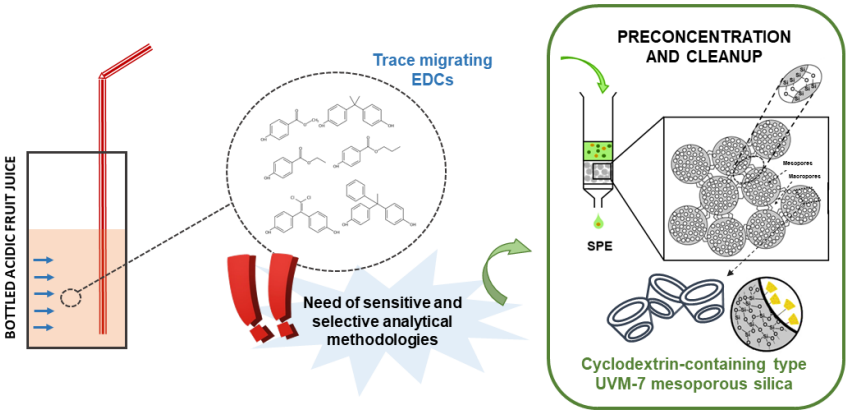
**9.** Belenguer-Sapiña, C., Sáez-Hernández, R., Pellicer-Castell, E., Armenta, S., Mauri-Aucejo, A.R. (**2022**) Simultaneous determination of third-generation synthetic cannabinoids in oral fluids using cyclodextrin-silica porous sorbents. *Microchemical Journal*, 172, 106915.
https://doi.org/10.1016/j.microc.2021.106915

*A microporous material made of cyclodextrin units covalently immobilized into a silica network has found an innovative bioanalytical application in the sample clean-up and simultaneous isolation of three synthetic cannabinoids (ADB-CHMICA, MMB-CHMICA, and MDMB-CHMCZCA) from human oral fluid through solid-phase extraction. The subsequent quantitation is carried out using liquid chromatography coupled to fluorescence detection. The optimized experimental procedure gives recoveries ranging from 76% to 96% in oral fluid samples spiked with the studied cannabinoids at the µg $L^{-1}$ level. High selectivity is obtained through the extraction procedure due to the presence of cyclodextrin in the silica network of the solid phase and thanks to the use of fluorescence detection in the separation technique. It is precisely the type of detection chosen that also gives the developed method a great sensitivity. The limits of detection of the proposed analytical method range from 0.11 to 0.73 µg $kg^{-1}$ and high repeatability is observed, with variations lower than 6%. The obtained results demonstrate the versatility and high potential of cyclodextrin-silica porous materials for the sorption of the studied substances, contributing in a novel way to the proper determination of third-generation synthetic cannabinoids in biological samples.*



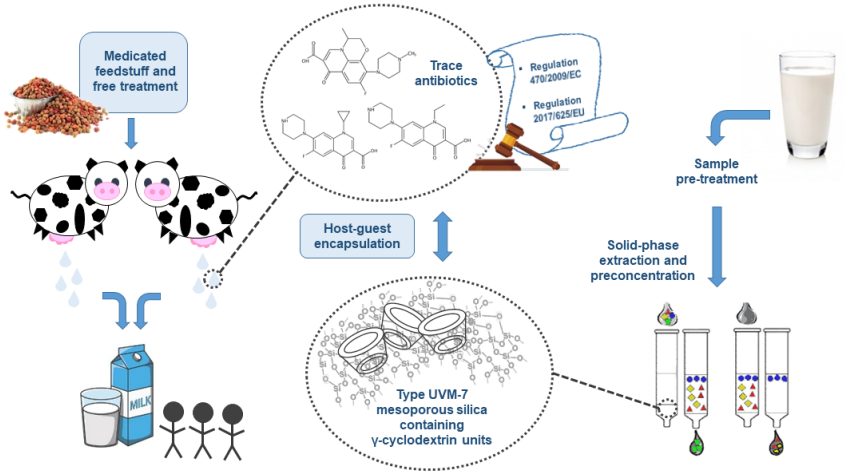
10. Belenguer-Sapiña, C., Pellicer-Castell, E., El Haskouri, J., Simó-Alfonso, E.F., Amorós, P., Mauri-Aucejo, A.R. (**2022**) Assessment of migrating endocrine-disrupting chemicals in bottled acidic juice using type UVM-7 mesoporous silica modified with cyclodextrin. *Food Chemistry*, 380, 132207. https://doi.org/10.1016/j.foodchem.2022.132207

*An innovative material based in type UVM-7 mesoporous silica containing analyte-accessible cyclodextrin units is described and assessed as a selectivity-enhancing sorbent for extracting endocrine-disrupting chemicals from bottled apple juice to subsequently quantify them. The synthesis procedure has been carried out using both β- and γ-cyclodextrin for their later comparison. Then, a complete analytical method for the isolation and determination of the above analytes has been validated. Following the optimized procedure, recoveries between 94% and 100% have been achieved and good repeatability is obtained with deviations under 6.8% for intra-day and inter-day experiments. The detection limits of the method have been established in the ng $L^{-1}$ level, which demonstrates the ability to quantify the trace concentrations established by sanitary restrictions. A low matrix effect is found when working with real samples. To end, a comparison with an alternative extraction method using $C_{18}$ extraction cartridges has been carried out and the results obtained with both procedures are comparable.*



**11.** Belenguer-Sapiña, C., Pellicer-Castell, E., El Haskouri, J., Simó-Alfonso, E.F., Amorós, P., Mauri-Aucejo, A.R. (**2022**) A type UVM-7 mesoporous silica with γ-cyclodextrin for the isolation of three veterinary antibiotics (ofloxacin, norfloxacin, and ciprofloxacin) from different fat-rate milk samples. *Journal of Food Composition and Analysis*, 109, 104463. https://doi.org/10.1016/j.jfca.2022.104463

*An analytical method based on the solid-phase extraction of three veterinary fluoroquinolones (ofloxacin, norfloxacin, and ciprofloxacin) from milk samples followed by their quantification with liquid chromatography coupled to fluorescence detection is described here. The sorbent used is a novel type UVM-7 mesoporous silica modified with analyte-accessible γ-cyclodextrin, which is useful to isolate fluoroquinolones through the selective host-guest interactions carried out between them. The whole extraction procedure has been validated and the recoveries obtained range from 83% to 92% in water and from 60% to 70% in real milk samples spiked with the studied compounds at µg $L^{-1}$. The complexity of the milk matrix is overcome through a high selectivity obtained due to the presence of γ-cyclodextrin in the silica structure of the material and thanks to the type of quantification technique used. Indeed, no interfering effect is observed during the chromatographic separation. Besides, the good precision of the method is shown through variations lower than 10% for intra-day and inter-day repeatability, and the detection limits have been established in the ng $L^{-1}$ level. To end, a comparison with an alternative extraction method of veterinary drugs from milk has been carried out and the results obtained are comparable with those achieved using the developed method. The method described here can play an outstanding effect in the target fluoroquinolones separation and purification, which are nowadays substances of high concern in food safety.*



Also, the following publications, whose content is not included in this work, have been generated during this time:

1. Weller, A., Carrasco-Correa, E.J., Belenguer-Sapiña C., Mauri-Aucejo, A.R., Amorós, P., Herrero-Martínez, J.M. (**2017**) Organo-silica hybrid capillary monolithic column with mesoporous silica particles for separation of small aromatic molecules. M*icrochimica Acta*, 184(10), 3799-3808.

2. Pellicer-Castell, E., Belenguer-Sapiña, C., Amorós, P., El Haskouri, J., Herrero-Martínez, J.M., Mauri-Aucejo, A.R. (**2018**) Study of silica-structured materials as sorbents for organophosphorus pesticides determination in environmental water samples. *Talanta*, 189, 560-567.

3. Pellicer-Castell, E., Belenguer-Sapiña, C., Borràs, V.J., Amorós, P., El Haskouri, J., Herrero-Martínez, J.M., Mauri-Aucejo, A.R. (**2019**) Extraction of aflatoxins by using mesoporous silica (type UVM-7), and their quantitation by HPLC-MS. *Microchimica Acta*, 186(12), 792.

4. Pellicer-Castell, E., Belenguer-Sapiña, C., Amorós, P., El Haskouri, J., Herrero-Martínez, J.M., Mauri-Aucejo, A.R. (**2020**) Comparison of silica-based materials for organophosphorus pesticides sampling and occupational risk assessment. *Analytica Chimica Acta*, 1110, 26-34.

5. Pellicer-Castell, E., Belenguer-Sapiña, C., Amorós, P., Herrero-Martínez, J.M., Mauri-Aucejo, A.R. (**2020**) Bimodal porous silica nanomaterials as sorbents for an efficient and inexpensive determination of aflatoxin M1 in milk and dairy products. *Food Chemistry*, 333, 127421.

6. Pellicer-Castell, E., Belenguer-Sapiña, C., Amorós, P., El Haskouri, J., Herrero-Martínez, J.M., Mauri-Aucejo, A.R. (**2021**) Enhancing extraction performance of organophosphorus flame retardants in water samples using titanium hierarchical porous silica materials as sorbents. *Journal of Chromatography A*, 1639, 461938.

7. Briz-Redón, A., Belenguer-Sapiña, C., Serrano-Aroca, Á. (**2021**) Changes in air pollution during COVID-19 lockdown in Spain: a multi-city study. *Journal of Environmental Sciences*, 101, 16-26.

8. Belenguer-Sapiña, C., Briz-Redón, Á., Domínguez-Sales, M.C. (**2021**) Do social chemophobic attitudes influence the opinions of secondary school students? *Journal of Chemical Education*, 98(7), 2176-2187.

9. Pellicer-Castell, E., Belenguer-Sapiña, C., Amorós, P., El Haskouri, J., Herrero-Martínez, J.M., Mauri-Aucejo, A.R. (**2022**) Mesoporous silica sorbent with gold nanoparticles for solid-phase extraction of organochlorine pesticides in water samples. *Journal of Chromatography A*, 1662, 462729.

10. Briz-Redón, Á., Belenguer-Sapiña, C., Serrano-Aroca, Á. (**2022**) A city-level analysis of $PM_{2.5}$ pollution, climate and COVID-19 early spread in Spain. *Journal of Environmental Health Science and Engineering*, Accepted.

Finally, the developed research has also been presented in different national and international scientific conferences:

- XVI Reunión Científica de la Sociedad de Cromatografía y Técnicas Afines (*SECyTA* **2016**). Three poster communications.
- XXI Reunión de la Sociedad Española de Química Analítica (*SEQA* **2017**). One poster communication.
- 15th Instrumental Analysis Conference (*JAI* **2017**) and XVII Reunión Científica de la Sociedad de Cromatografía y Técnicas Afines (*SECyTA* **2017**). Three poster communications.
- 2nd Workshop *Advances in Separation Techniques* (**2017**). Two oral communications.
- XVIII Reunion Científica de la Sociedad de Cromatografía y Técnicas Afines (*SECyTA* **2018**). Three poster communications.
- Congreso Internacional *De la Antigüedad al Renacimiento. Secretos de salud y belleza de las mujeres* (**2018**). One invited oral communication.
- 3rd Workshop *Advances in Separation Techniques* (**2018**). Two oral communications.
- 1st Workshop *Young Researchers in Chemistry* (**2019**). Two poster communications and two oral communications.
- 3rd Scholarship of Teaching and Learning Conference (*EuroSoTL* **2019**). One poster communication.
- 48th International Symposium of High-Performance Liquid Phase Separations and Related Techniques (*HPLC* **2019**). Two poster communications.

- VI *Jornades d'Innovació Educativa de la Universitat de València* (**2020**). One poster communication.
- I *Jornada de valorización de un nuevo procedimiento de síntesis asistida por microondas de sílice porosa* (**2020**). One invited oral communication.
- XXIII International Symposium on Advaces in Extraction Technologies (*Extech* **2021**). Six poster communications.
- XI Congreso Internacional de Docencia Universitaria e Innovación (*CIDUI* **2021**). One poster communication.
- VII *Jornades d'Innovació Educativa de la Universitat de València* (**2021**). One poster communication and one oral communication.

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