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APLICACIONES DE LA MULTICONMUTACIÓN EN QUÍMICA ANALÍTICA

EVA RÓDENAS TORRALBA

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Facultat de Química
Departament de Química Analítica

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TESIS DOCTORAL

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Los directores de Tesis:

El Dr. Ángel Morales Rubio, Profesor Titular de Universidad, y el Dr. Miguel de la Guardia Cirugeda, Catedrático de Universidad, del Departamento de Química Analítica de la Universitat de València,

Certifican:

Que Dña. **Eva Ródenas Torralba**, Licenciada en Química por la Universitat de València, ha realizado bajo nuestra dirección y supervisión la presente Tesis Doctoral que lleva por título:

«Aplicaciones de la Multiconmutación en Química Analítica»

para poder optar al grado de Doctora en Química y autorizamos la presentación y defensa de la correspondiente memoria en la Facultad de Química de la Universitat de València.

Y para que así conste y a los efectos oportunos, expedimos y firmamos la presente autorización.

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ÍNDICE DE IMPACTO DE LAS PUBLICACIONES

Los índices de impacto (IF) de las revistas científicas de carácter internacional en las que están publicados o pendientes de aceptar los diez trabajos que se recogen en la presente Tesis se indican a continuación. Los datos han sido recogidos de la base de datos *ISI Web of Knowledge* (www.isiknowledge.com) y corresponden al 2004, último año publicado.

1. Boaventura F. Reis, Eva Ródenas-Torralba, José Sancenón-Buleo, Ángel Morales-Rubio, Miguel de la Guardia, *Improvement of the atomic fluorescence determination of mercury by using multicommutation*, **Journal of Analytical Atomic Spectrometry 17 (2002) 537-540. IF: 3.926.**

2. Boaventura F. Reis, Eva Ródenas-Torralba, José Sancenón-Buleo, Ángel Morales-Rubio, Miguel de la Guardia, *Multicommutation cold vapour atomic fluorescence determination of Hg in water*, **Talanta 60 (2003) 809-819. IF: 2.532.**

3. Eva Ródenas-Torralba, Patricia Cava-Montesinos, Ángel Morales-Rubio, M. Luisa Cervera, Miguel de la Guardia, *Multicommutation as an environmentally friendly analytical tool in the hydride generation atomic fluorescence determination of tellurium in milk*, **Analytical and Bioanalytical Chemistry 379 (2004) 83-89. IF: 2.098.**

4. Josep F. Ventura-Gayete, Eva Ródenas-Torralba, Ángel Morales-Rubio, Salvador Garrigues, Miguel de la Guardia, *A multicommutated flow system for determination of bismuth in milk shakes by hydride generation atomic fluorescence spectrometry incorporating on-line neutralization of waste effluent*, **Journal of AOAC International 87 (2004) 1252-1259. IF: 1.147.**

5. Eva Ródenas-Torralba, Ángel Morales-Rubio, Miguel de la Guardia, *Multicommutation hydride generation atomic fluorescence determination of inorganic tellurium species in milk*, **Food Chemistry 91 (2005) 181-189. IF: 1.535.**

6. Eva Ródenas-Torralba, Boaventura F. Reis, Ángel Morales-Rubio, Miguel de la Guardia, *An environmentally friendly multicommutated alternative to the reference method for anionic surfactant determination in water*, **Talanta 66 (2005) 591-599. IF: 2.532.**

7. Fábio R. P. Rocha, Eva Ródenas-Torralba, Boaventura F. Reis, Ángel Morales-Rubio, Miguel de la Guardia, *A portable and low cost equipment for flow injection chemiluminescence measurements*, **Talanta 67 (2005) 673-677. IF: 2.532.**

8. Fábio R. P. Rocha, Eva Ródenas-Torralba, Ángel Morales-Rubio, Miguel de la Guardia, *A clean method for flow-injection spectrophotometric determination of cyclamate in table sweeteners*, **Analytica Chimica Acta 547 (2005) 204-208. IF: 2.588.**

9. Eva Ródenas-Torralba, Ángel Morales-Rubio, Miguel de la Guardia, *Determination of phenols in waters using micro-pumped multicommutation and spectrophotometric detection. An automated alternative to the standard procedure*, **Analytical and Bioanalytical Chemistry 383 (2005) 138-144. IF: 2.098.**

10. Eva Ródenas-Torralba, Fábio R. P. Rocha, Boaventura F. Reis, Ángel Morales-Rubio, Miguel de la Guardia, *Evaluation of a multicommuted flow system for photometric environmental measurements*, **Journal of Automated Methods & Management in Chemistry (2005) en impresión. IF: 0.389.**

Agradecimientos

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ÍNDICE

La frase más excitante que se puede oír en ciencia, la que anuncia nuevos descubrimientos, no es

¡*Eureka*! (¡Lo encontré!)

sino

***Es extraño* …**

Isaac Asimov (1920 – 1996)

1. INTRODUCCIÓN

1. INTRODUCCIÓN

El perfeccionamiento de técnicas y métodos de análisis ha sido uno de los principales objetivos de las investigaciones en Química de las últimas décadas, con el fin de disminuir los errores resultantes de la intervención humana y utilizar los mínimos recursos en la menor cantidad de tiempo.

En la sociedad actual se ha producido un incremento en la demanda de información analítica: aparecen nuevos objetos de análisis, existe mayor necesidad de alcanzar concentraciones cada vez más bajas, con mayor eficiencia en la exactitud y precisión de las medidas, en tiempos cada vez más breves y a menor coste. Esta necesidad ha llevado a los investigadores a buscar alternativas y, a lo largo de los años, se han ido desarrollando nuevas áreas de investigación en el campo de la instrumentación y automatización de procedimientos analíticos que sean capaces de proveer información de forma continua, *in situ*, en tiempo real y, por supuesto, en solidaridad con el medio ambiente.

1.1. AUTOMATIZACIÓN DE SISTEMAS

De un modo general, desde las primeras contribuciones, los sistemas automatizados han experimentado una gran evolución y diversificación. La automatización ha destacado como una alternativa eficiente en diversas ramas de la ciencia y, principalmente, en Química Analítica.

La IUPAC (*International Union of Pure and Applied Chemistry*) define la *automatización* como «el uso de dispositivos instrumentales y mecánicos para sustituir, refinar, extender o complementar el esfuerzo humano en la ejecución de un proceso determinado, en el cual, al menos una operación principal debe

ser controlada sin la intervención humana a través de un proceso de realimentación» [IUPAC: 1994]. Inicialmente, el término automatización era utilizado en el sentido opuesto a los sistemas manuales, ya que en los sistemas en flujo no había manipulación de las disoluciones de muestras y reactivos. Actualmente, el término automatización de sistemas es mucho más exigente y se incluye, principalmente, en campos del área del análisis en flujo.

Luque de Castro y Valcárcel [Luque: 1995] definen como sistema automatizado aquél en el cual las disoluciones de muestras y las disoluciones de reactivos se introducen a través de muestreadores automáticos y/o válvulas controladas electrónicamente. Además, el funcionamiento automático de las bomba peristáltica debe presentar un control preciso, la automatización del sistema de reacción/transporte debe reducir la intervención humana y la adquisición de datos y el control del módulo de análisis deben ser realizados por ordenador, equipado con interfaces adecuadas, a través de programas informáticos. Sin embargo, no se ha llegado a un consenso sobre la idea de *grado de automatización*. Por ejemplo, algunos autores consideran que los sistemas por inyección en flujo son únicamente sistemas parcialmente automatizados, con relación a las operaciones preliminares del procedimiento analítico [Zhi: 1994]. Todos estos requisitos los cumple de manera inequívoca la multiconmutación, metodología objeto de esta tesis. En las sucesivas secciones se abordará y justificará su importancia y contribución a la evolución de los sistemas automatizados y mecanizados.

La expresión *sistema automatizado* hace referencia principalmente a sistemas capaces de tomar decisiones. Por tanto, en la presente Tesis sería más correcto utilizar el término *mecanización* en sustitución a *automatización*, ya que aunque la multiconmutación permite desarrollar sistemas totalmente automatizados, todos los estudios realizados se han llevado a cabo utilizando sistemas mecanizados.

1.1.1. IMPORTANCIA DE LA AUTOMATIZACIÓN / MECANIZACIÓN

La reducción de la participación humana en los procedimientos analíticos es, actualmente, uno de los objetivos primordiales de la Química Analítica [Valcárcel: 1988] [Valcárcel: 1990]. El proceso analítico se divide básicamente en tres estadios: (i) pretratamiento de las muestras, (ii) determinación de los analitos, (iii) procesamiento y análisis de los datos. En el primer estado, pretratamiento de las muestras, la minimización de la intervención del operador, mediante la automatización o la mecanización, desarrolla su principal objetivo en etapas críticas como la dilución de las muestras, la separación o la preconcentración de éstas, las reacciones químicas a las que son sometidas y su transporte hasta el detector. El interés de reducir al mínimo la intervención humana en estas etapas es evitar realizar actividades tediosas y repetitivas e intentar disminuir la exposición directa a muestras y reactivos tóxicos.

La automatización tiene también como objetivo la posibilidad de diseñar equipamientos robustos y autónomos para obtener información analítica sobre un determinado analito de forma continua y totalmente automatizada y con largos períodos de régimen operacional independiente.

1.1.2. DESARROLLO Y EVOLUCIÓN DE LOS SISTEMAS AUTOMATIZADOS / MECANIZADOS

A partir de los años 70, la creciente demanda de métodos rápidos y medioambientalmente sostenibles propició la investigación y el desarrollo de sistemas mecanizados. Los equipos presentaban como ventajas un aumento de la precisión y exactitud y, también, una disminución del coste operacional. Estos sistemas se dividían principalmente en dos grandes grupos: medidas en régimen estático (*batch*) y en flujo continuo. Los inconvenientes que presentaban las

medidas en *batch* eran la adición discreta de disoluciones de muestras y reactivos, la complejidad de sus componentes y su coste. Por su parte, los analizadores de flujo continuo presentaban como desventaja la contaminación entre disoluciones de muestras y el efecto memoria. No obstante, este inconveniente fue solventado por Skeggs, en 1957, con el diseño de un analizador en flujo segmentado con burbujas de aire (CFA) [Skeggs: 1957].

Un nuevo concepto de análisis en flujo, denominado Análisis por Inyección en Flujo (FIA) fue propuesto en 1975 [Ruzicka: 1975]. Su aparición representó un avance importante en la Química Analítica debido a su versatilidad y comodidad, ya que se suprimió la necesidad de manipular las muestras y los reactivos, disminuyendo así el riesgo de contaminación. El principio del FIA era muy simple. Se basaba en la inserción de una alícuota de disolución de la muestra en el curso analítico y su conducción hasta el detector por una disolución portadora, que podía ser el mismo reactivo.

En años posteriores se investigaron diversas variaciones de la propuesta original. Se estudió la adición de reactivos por confluencia [Bergamin: 1978 a] para mejorar la homogenización de las muestras, se intentó la economización de los reactivos a través de procesos de flujo intermitente [Bergamin: 1978 c] [Zagatto: 1980] y se realizaron estudios de dilución en línea de las disoluciones de muestra para que la concentración de la misma se situase en el rango de operación óptimo del instrumento [Reis: 1981].

En 1985, Pasquini y Oliveira [Pasquini: 1985] propusieron el análisis por flujo monosegmentado (MSFA) que englobaba las ventajas del FIA y CFA. En MSFA, muestras y reactivos se inyectan en el reactor entre dos burbujas de aire. Bajo estas condiciones pueden alcanzarse largos tiempos de residencia (10 – 15 min), con una mínima dispersión de la muestra y altos rendimientos de proceso.

Paralelamente al desarrollo y a la automatización de los sistemas FIA,

inicialmente asociados a la espectrofotometría, surgieron nuevos sistemas combinando el análisis en flujo y otras técnicas espectrométricas, intentando superar las deficiencias y aprovechando las ventajas de las técnicas respectivas. El primer acoplamiento FIA-FAAS (*Flame Atomic Absorption Spectrometry)* fue propuesto por Zagatto *et al*. en 1979 [Zagatto: 1979]. La asociación FIA-ICP-AES (*Inductively Coupled Plasma Atomic Emisión Spectrometry*) fue propuesta por Jacintho *et al*. [Jacintho: 1981] dos años después. Además de los métodos espectrométricos, el análisis por inyección en flujo también fue adaptado a los métodos electroquímicos y destacaron la potenciometría [Montenegro: 1993], la amperometría [Nóbrega: 1994] y la fluorimetría [Laassis: 1994], entre otros. Para mejorar en términos de sensibilidad se utilizaron métodos de preconcentración asociados al análisis en flujo, por extracción con disolvente [Bergamin: 1980] o intercambio iónico [Porta: 1992] [Ebdon: 1993].

Ruzicka y Marshall [Ruzicka: 1990], siguiendo la evolución de los procesos del análisis en flujo, introdujeron un nuevo concepto acuñado bajo la denominación SIA (Análisis por Inyección Secuencial). Las principales características que destacan los autores del SIA son robustez, versatilidad y simplicidad. Sin embargo, presenta una baja frecuencia de muestreo como principal inconveniente.

En 1994, Reis *et al*. [Reis: 1994] introdujeron el concepto de multiconmutación empleando el muestreo binario. En este trabajo el módulo de análisis consistía en un conjunto de válvulas solenoides de 3 vías, que actuaban como inyectores-conmutadores independientes. El concepto de muestreo binario se refería a la inserción de alícuotas de muestra en tándem con alícuotas de reactivo, en el tubo de reacción. Los sistemas de análisis en flujo con multiconmutación presentaban características análogas a los sistemas SIA, pero las frecuencias de muestreo eran comparables a las obtenidas por FIA o MSFA.

1.2. CLASIFICACIÓN DE LOS SISTEMAS EN FLUJO

De un modo general, los sistemas en flujo pueden clasificarse, según el proceso de muestreo, en continuos o discretos, y según las características del flujo, en segmentados, no-segmentados o monosegmentados. La clasificación recomendada por la IUPAC [Van der Linder: 1994] se resume en la Fig 1.

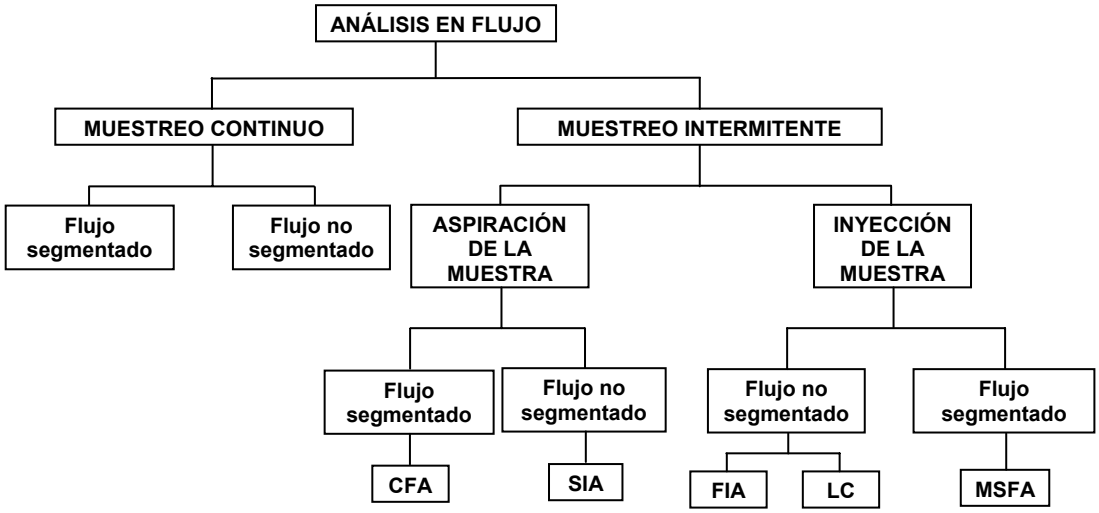


Fig. 1. Clasificación de los sistemas en flujo (IUPAC). CFA: flujo continuo multisegmentado. FIA: análisis por inyección en flujo. LC: cromatografía líquida. MSFA: análisis en flujo monosegmentado. SIA: análisis por inyección secuencial.

De los distintos términos indicados en la Fig. 1, para la realización de esta tesis se ha elegido la ***multiconmutación***. Este término se basa en un tipo de muestreo intermitente de flujo no segmentado, en el que las disoluciones de muestras y reactivos pueden ser tanto aspiradas como inyectadas. La multiconmutación puede entenderse como una forma elegante y mecanizada que engloba y amplia los sistemas FIA.

A continuación se describen brevemente los fundamentos de los distintos sistemas de flujo presentados en la Fig. 1. Es interesante el estudio de cada uno de ellos para poder posteriormente interrelacionarlos entre sí y complementarlos con la técnica objeto de la presente tesis, la multiconmutación.

1.2.1. ANÁLISIS EN FLUJO MULTISEGMENTADO (CFA)

En estos sistemas, las muestras se aspiran secuencialmente y son multisegmentadas por burbujas de aire [Skeggs: 1957] [Skeggs: 1966]. La segmentación tiene la función de favorecer el mezclado entre las disoluciones de muestras y reactivos en el curso analítico y aislarlas de la disolución portadora para evitar, de este modo, una dispersión excesiva, el efecto memoria y la intercontaminación entre las muestras (Fig. 2). Este sistema permite que la zona de muestreo pueda ser mantenida en el reactor por un largo intervalo de tiempo para garantizar que se alcance el estado de equilibrio de la reacción. Las burbujas de aire se eliminan en las proximidades del detector mediante un dispositivo adecuado diseñado para esta función [Skeggs: 1966].

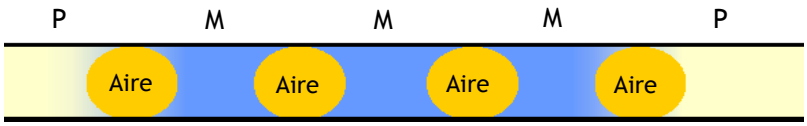


Fig. 2. Modelo de muestreo en el sistema CFA. P: portador; M: muestra.

En función de la tecnología empleada, los costes de adquisición y de mantenimiento son elevados y, por tanto, desfavorables para su implementación en laboratorios pequeños en comparación con los sistemas en flujo desarrollados en años posteriores. Sin embargo, estos sistemas tienen una importancia histórica y se consideran antecesores de los diferentes modelos de sistemas en flujo existentes en la actualidad.

1.2.2. ANÁLISIS POR INYECCIÓN EN FLUJO (FIA)

Como ya se ha comentado, este sistema alcanzó un gran impulso en la década de los 70 y fue desarrollado siguiendo los pasos de su antecesor, el CFA.

En los primeros años de nacimiento de esta técnica, el interés por ella creció exponencialmente, como demuestra el creciente número de publicaciones en revistas científicas que se representa en el gráfico acumulado de la Fig. 3.

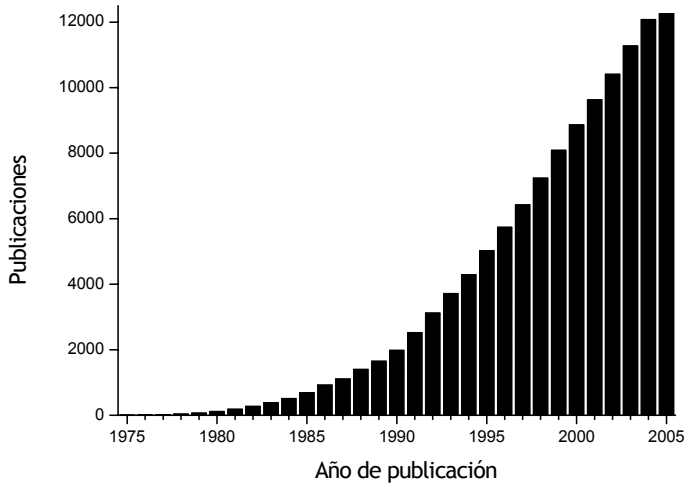


Fig. 3. Evolución cronológica de la literatura científica en FIA.

En esta metodología, las muestras se procesan individualmente (muestreo discreto), lo que posibilita efectuar medidas en estado no estacionario, evitando problemas relativos a la contaminación entre las disoluciones de muestra, una de las principales preocupaciones de los sistemas multisegmentados (Fig. 4). Los sistemas FIA presentaron un gran avance en relación a su precursor, ya que son más simples y económicos y utilizan equipamientos de uso común en los laboratorios de química. También posibilitan que la lectura de la señal se efectúe sin que la reacción química alcance las condiciones de equilibrio y la

homogenización. Así, la dispersión de la zona de muestra puede ser controlada en función de las características hidrodinámicas del módulo de análisis, que durante el transporte de las disoluciones genera un gradiente de concentración constante en cada punto del camino de reacción, es decir, el porcentaje de mezclado se mantiene constante entre determinaciones diferentes, propiciando una buena precisión de las medidas.

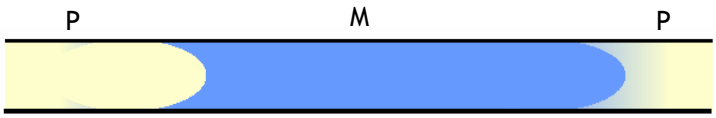


Fig. 4. Modelo de muestreo en el sistema FIA. P: portador; M: muestra.

CFA difiere conceptualmente de FIA, ya que en la primera técnica la segmentación proporcionada por el aire permite que las medidas puedan alcanzar el equilibrio químico, estado estacionario, con una mínima dispersión de la muestra. En FIA, al no emplearse segmentación con aire, las medidas pueden realizarse sin alcanzar el equilibrio químico, incrementándose la frecuencia de muestreo y reduciéndose el consumo de muestras y reactivos.

1.2.3. ANÁLISIS EN FLUJO MONOSEGMENTADO (MSFA)

Los sistemas de análisis en flujo monosegmentado fueron introducidos en 1985 por Pasquini y Oliveira [Pasquini: 1985], para la determinación de cromo y amonio. Estos sistemas combinan las ventajas ofrecidas por el sistema desarrollado por Skeggs [Skeggs: 1957] y por los sistemas FIA [Ruzicka: 1975].

El sistema de análisis MSFA tiene como principal característica la introducción de la muestra entre dos burbujas de aire, formando un monosegmento. La diferencia básica, en relación al sistema FIA, es la minimización de la dispersión y que permite trabajar con largos tiempos de residencia. Esto es particularmente importante en reacciones de cinética lenta

que requieren tiempos elevados para que sean cuantitativas o en reacciones que necesiten ser sometidas a tratamientos de temperatura. En este caso, tiempos largos de residencia no significa necesariamente baja frecuencia de muestreo, ya que las muestras pueden ser introducidas en el sistema secuencialmente, y esto es posible debido a la minimización de la contaminación. Además, en los sistemas de flujo monosegmentado no es necesario que las medidas se realicen en estado estacionario.

A pesar de las ventajas ofrecidas, como reproducibilidad, mayor frecuencia de muestreo y tiempos largos de residencia, el sistema originalmente propuesto no permitía el estudio de reacciones en las que era necesaria la adición de reactivos después de la inyección del monosegmento.

La Fig. 5 muestra el perfil de muestreo de los sistemas de análisis MSFA. La muestra y los reactivos se introducen en el sistema, el portador los arrastra a través del camino analítico y dos burbujas de aire encierran el bolo formado. La reacción tiene lugar en el reactor y, a continuación, los bolos pueden ser retenidos en una celda de permanencia (reacciones catalíticas [Andrade: 1991] y enzimáticas [Araújo: 1998 a] lentas) e incluso pueden ser agitados mecánicamente previamente a su detección. La posibilidad de inserciones consecutivas de muestra incrementa considerablemente la frecuencia de muestreo.

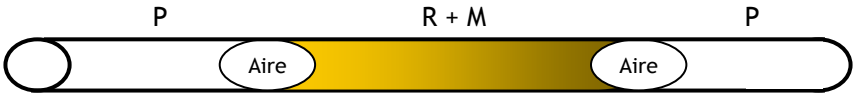


Fig. 5. Modelo de muestreo en el sistema en flujo monosegmentado. P: portador; R: reactivo; M: muestra.

El hecho de que la muestra se mantenga prácticamente aislada entre las dos burbujas de aire permite la inyección simultánea de varios reactivos y una eficiente homogenización, ya que las burbujas de aire favorecen movimientos de convección en el interior del monosegmento [Brito: 1998].

1.2.4. ANÁLISIS POR INYECCIÓN SECUENCIAL (SIA)

En 1990 fue propuesto el análisis por inyección secuencial [Ruzicka: 1990]. Este nombre se le dio en función de la forma de muestreo efectuada, ya que las disoluciones se insertaban en el camino analítico de forma secuencial (Fig. 6).

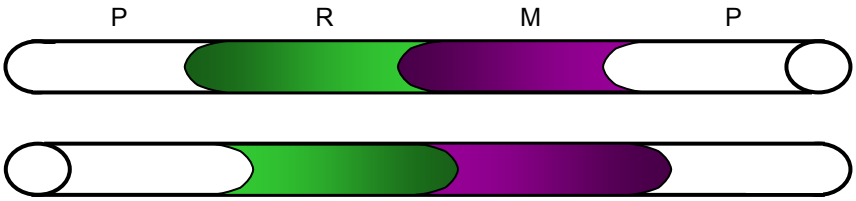


Fig. 6. Modelo de muestreo en un sistema SIA. P: portador; R: reactivo; M: muestra.

El procesamiento de las disoluciones se controla por un programa informático que permite modificar las variables operacionales sin la necesidad de modificar la configuración del módulo de análisis. El sistema requiere para su implementación el empleo de una válvula de selección o válvula multipuertos [Guzman: 1993]. Presenta como inconveniente la baja frecuencia de muestreo y la dificultad de implementar procedimientos que empleen varias disoluciones de reactivos.

El módulo de análisis posee una estructura y concepto distinto de los demás. La conmutación de los puertos de la válvula para la inserción de las alícuotas de muestras y reactivos es efectuada y controlada por un ordenador, en una secuencia previamente establecida. En primer lugar, las alícuotas de cada disolución se aspiran secuencialmente hacia una bobina receptora, conectada al puerto central de la válvula. A continuación, la válvula gira, permitiendo el paso de la zona de muestra hacia la bobina de reacción. Automáticamente, el sentido

de rotación de la bomba peristáltica se invierte y se desplaza la zona de muestra en dirección al detector. Inicialmente, la alícuota de la disolución de muestra tiene únicamente una interfaz de contacto con la alícuota de reactivo, y es en esta zona donde tiene lugar el mezclado. La inversión del sentido de bombeo contribuye a mejorar la condición de mezclado de las disoluciones, favoreciendo el desarrollo de la reacción. Además, se propicia el aumento de la dispersión de la zona de muestreo, factor que puede influir en la sensibilidad.

1.2.5. ANÁLISIS POR MULTICONMUTACIÓN

La multiconmutación es una técnica relativamente reciente [Reis: 1994] que utiliza como dispositivo fundamental válvulas solenoides de 3 vías. Los modelos que explotan la multiconmutación son implementados en módulos de análisis de una única línea. Destacan por su simplicidad y flexibilidad y ofrecen la posibilidad de determinar diferentes analitos en condiciones diversas, manteniendo la estructura del módulo constante.

La multiconmutación presenta la ventaja de poder integrar todos los modelos de flujo anteriormente expuestos, para obtener el perfil de inserción más adecuado a cada situación analítica. Permite la inserción de aire en el sistema [Ródenas-Torralba: 2005 c] [Comitre: 2005], el uso de reactivos múltiples [Rocha: 2000] [Reis: 1999] [Rocha: 2004], la posibilidad inmediata de variar el orden de inserción de muestras y reactivos, inversión del sentido de flujo [Ródenas-Torralba: 2005 c], etc.

La diferencia fundamental entre MSFA, SIA, FIA y multiconmutación reside en el modo en el que las muestras son introducidas en el reactor. La multiconmutación proporciona mezclado de muestras y reactivos diferentes a los obtenidos por MSFA, FIA y SIA, como consecuencia de la no segmentación producida por burbujas de aire y de la no inversión del flujo. Además, cuando es

necesario hacer reaccionar tres o más reactivos, resulta difícil obtener mezclados eficientes con los sistemas SIA [Ruzicka: 1990] [Cladera: 1995]. Con la multiconmutación se solventa este problema de una forma inmediata y sencilla.

1.2.5.1. Multiconmutación con muestreo en sándwich

El muestreo de las disoluciones se efectúa en el orden reactivo, muestra y reactivo, como se representa en la Fig. 7.

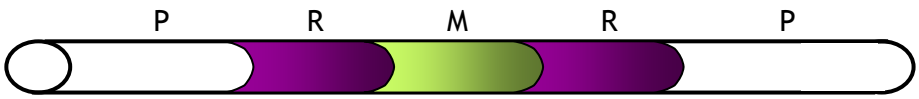


Fig. 7. Modelo de muestreo en sándwich. P: portador; R: reactivo; M: muestra.

El módulo de análisis utiliza válvulas solenoides controladas a través de programas informáticos. Esta estrategia de muestreo ha sido aplicada en la mecanización de diferentes métodos de análisis químicos, con detección tanto espectrofotométrica [Vieira: 1998 a] [Tumang: 1998] [Ferrer: 2004] como potenciométrica [Almeida: 2000 c] [Paim: 2002].

1.2.5.2. Multiconmutación con muestreo binario

El muestreo binario se asocia a la creación de una zona de muestra constituida por varias alícuotas de disolución de muestra, intercaladas con alícuotas de disolución de reactivos. El proceso de mezclado entre las disoluciones tiene lugar en función del contacto mutuo entre dos interfases de disoluciones diferentes y, por ello, en un principio, recibió la denominación de muestreo binario (Fig. 8).

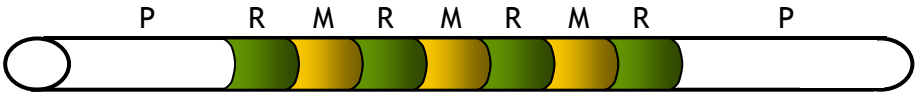


Fig. 8. Modelo de muestreo binario. P: portador; R: reactivo; M: muestra.

Normalmente, el módulo de análisis necesario para ejecutar el muestreo binario en multiconmutación está constituido por válvulas solenoides [Reis: 1994] [Reis: 2002] y el proceso de muestreo se controla por un programa informático. La inserción de las disoluciones en el tubo de reacción se realiza por accionamiento de las válvulas solenoides en una secuencia que posibilite la introducción de las disoluciones individualmente en un orden predefinido. Empleando esta estrategia, se introducen alícuotas de disolución de muestra separadas por alícuotas de disolución de reactivo. Durante el transporte hacia el detector, el mezclado entre las disoluciones se realiza rápidamente debido a las diferentes interfases de contacto de las alícuotas de las disoluciones de reactivos y se agiliza el desarrollo de la reacción química.

El concepto de muestreo binario ha propiciado el desarrollo de varios trabajos de análisis químico y se ha aplicado en la automatización y/o mecanización de diferentes metodologías, tales como valoraciones empleando detección fotométrica [Korn: 1995], espectrofotométrica [Oliveira: 1996] [Comitre: 2000] y potenciométrica [Almeida: 2000 a], entre otros. Es importante destacar que en éstas y otras aplicaciones se determinan diferentes analitos en diferentes matrices, lo que demuestra la gran flexibilidad del sistema.

1.2.5.3. Multiconmutación con el empleo de buretas multijeringas

El empleo de buretas en multiconmutación fue descrito por primera vez en 1999 [Cerdà: 1999] [Albertús: 1999] y constituye una potente herramienta para la inserción automática de microfluidos. Este término combina la robustez del SIA y

la flexibilidad de la multiconmutación y FIA, evitando los problemas asociados al uso de bombas peristálticas, al usar buretas multijeringa, y evitando, de este modo, los inconvenientes del envejecimiento de los tubos flexibles de Tygon. Además, la variabilidad de jeringas de distintos volúmenes disponibles en el mercado (0.5 – 25.0 mL) permite el desarrollo de diferentes volúmenes de inyección. En esta técnica, además, se han incorporado válvulas solenoides de las usualmente empleadas en multiconmutación para eliminar los aspectos críticos de las válvulas rotatorias.

Las Fig. 9 y 10 muestran el funcionamiento de las buretas multijeringa acopladas a válvulas solenoides para la inserción de las disoluciones.

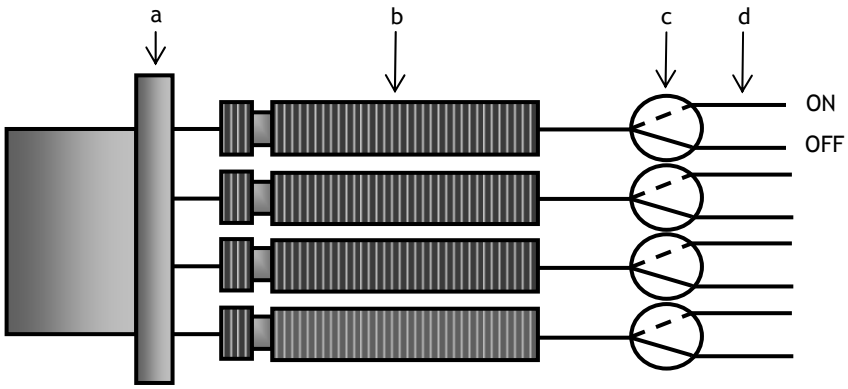


Fig. 9. Vista frontal de una bureta multijeringa. a: motor; b: jeringas; c: válvulas solenoides de tres vías; d: conectores y tubos de teflón o polivinilo. ON y OFF: válvula situada en la posición electrónica correspondiente al bit 1 y bit 0, respectivamente.

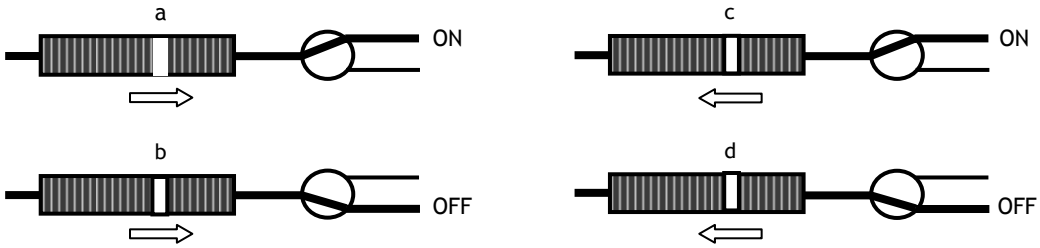


Fig. 10. Representación esquemática del mecanismo de distribución de una bureta multijeringa. a y b: movimiento del pistón en posición de propulsión de las disoluciones; c y d: movimiento del pistón hacia la posición de inicio. ON: conexión al camino de reacción; OFF: recirculación a los recipientes de disolución de partida o conexión al desecho.

1.2.5.4. Multiconmutación con flujo multipulsado

Esta estrategia propone el uso de minibombas [Lapa: 2002] [Lavorante: 2005] como sustitutas de las válvulas solenoides de 3 vías y de la bomba peristáltica. Las minibombas actúan como miniconmutadores propulsores de las disoluciones. Esta modalidad combina todas las ventajas de la multiconmutación con las válvulas solenoides y abarata costes al no hacer necesaria la bomba peristáltica.

1.3. LA MULTICONMUTACIÓN EN LA QUÍMICA ANALÍTICA

La multiconmutación es una metodología relativamente reciente [Reis: 1994] en el análisis en flujo, orientada al diseño de métodos analíticos con un alto grado de automatización y un mínimo impacto medioambiental.

Un parámetro que evalúa el interés de la multiconmutación es el creciente número de publicaciones desde 1994, cuando apareció el primer artículo sobre esta metodología llevado a cabo por el grupo de investigación

brasileño de Reis y Zagatto [Reis: 1994]. Actualmente diversos grupos de investigación de diferentes países se dedican al estudio de esta técnica, como muestra la Fig. 11.

Fig. 11. Distribución geográfica de los países en los que se investiga en multiconmutación.

La Fig. 12 muestra el número acumulado de artículos publicados desde 1994 hasta la actualidad (mayo 2005). En el gráfico acumulado puede observarse un crecimiento exponencial con un total de artículos publicados durante el período considerado superior a 120.

Respecto a las revistas analíticas en las que se han realizado las publicaciones, se indica en la Fig. 13 que la revista *Analytica Chimica Acta* recoge el mayor número de publicaciones (42.7 %) seguida por la revista *Talanta* (15.3 %). Otras revistas en las que se han publicado trabajos han sido *Journal of Brazilian Chemistry Society*, *Analytical and Bioanalytical Chemistry*, *Journal of Pharmaceutical Biomedical Analysis* o *Analytical Sciences*, entre otras. Durante los primeros años de publicación (desde 1994 hasta 1996), la revista *Analytica Chimica Acta* ha supuesto el 100% de los artículos publicados en dichos años y un porcentaje considerable en los siguientes (Fig 14).

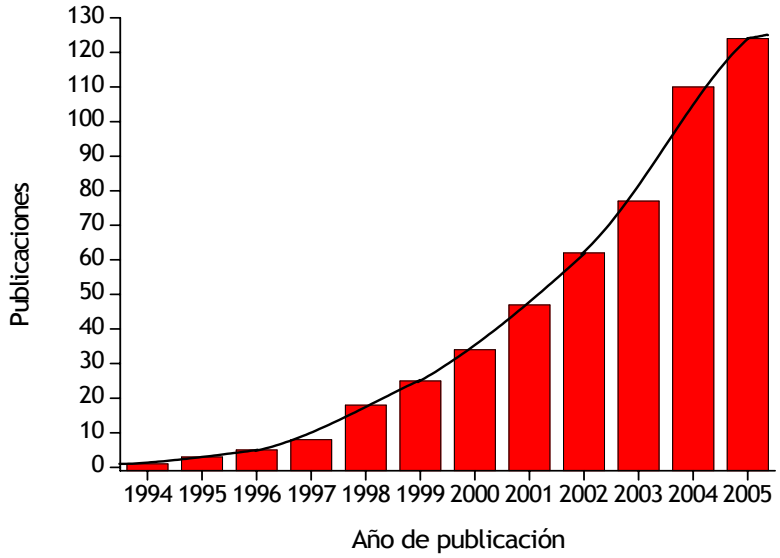


Fig. 12. Evolución cronológica de la literatura científica sobre multiconmutación.

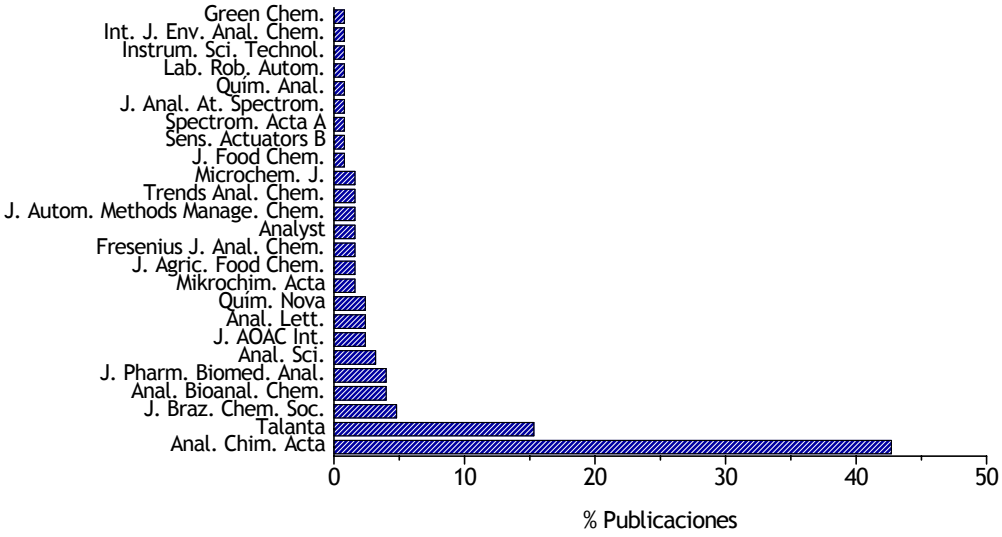


Fig. 13. Revistas analíticas que recogen artículos sobre multiconmutación.

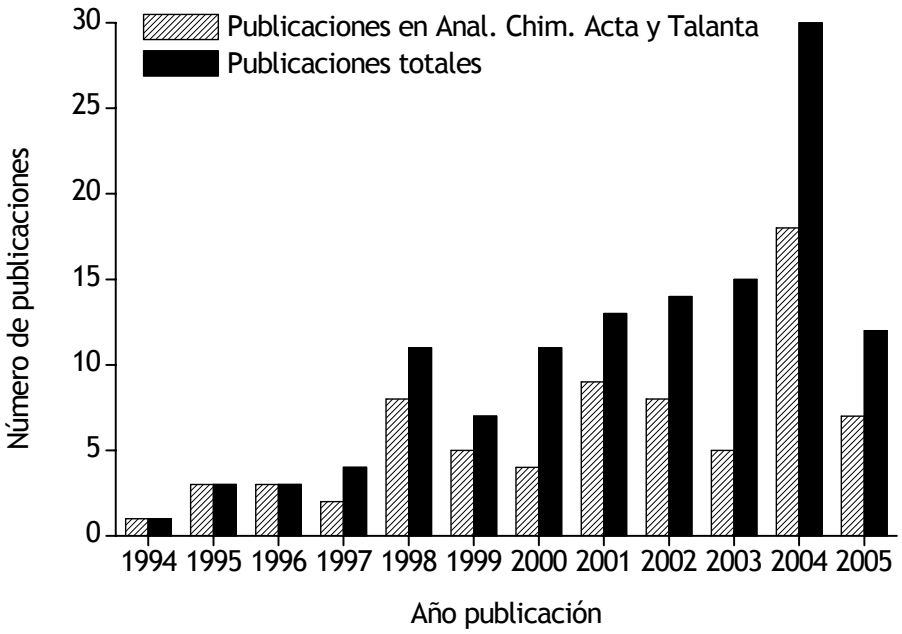


Fig. 14. Número de artículos publicados en Anal. Chim. Acta y Talanta, respecto al número total de artículos publicados por año sobre multiconmutación.

La multiconmutación se ha denominado usando diversos términos en la bibliografía. Fue referida originalmente como: «multicommutation flow system» o «multicommutation approach". El término «binary sampling» fue adoptado en el primer artículo de una serie de aplicaciones empleando la multiconmutación [Reis: 1994]. La expresión «multi-insertion principle» se propuso en la determinación de nitrato y nitrito en muestras de agua, fertilizantes y alimentos [Calatayud: 1998]. Sin embargo, los autores escogen la estrategia de «tandem flow» en el procedimiento de determinación de cloro por difusión de gas [Icardo: 2001]. Una técnica similar denominada «pulsed flow» [Wang: 1998 b] comprende la inserción de diferentes alícuotas a una frecuencia típica, para obtener turbulencias instantáneas que contribuyan a la mejora del mezclado y reduzcan la dispersión axial. Los autores acuñan el nombre «time-division multiplex

technique» para crear perfiles de concentraciones en la determinación potenciométrica de calcio y espectrofotométrica de fosfato [Wang: 1998 a]. Otros nombres que se encuentran en la literatura son «flow networks», «binary search» o «automated mono-segmented flow system».

1.3.1. LIMITACIONES DEL FIA Y DE LAS VÁLVULAS ROTATORIAS DE SEIS VÍAS

El diseño FIA presenta el inconveniente de ser menos flexible y versátil que el de otras alternativas, principalmente para el análisis simultáneo de varios analitos y, además, el volumen de la muestra queda definido por la longitud del tubo de reacción y por el volumen interno de la válvula de seis vías. Esta válvula rotatoria únicamente permite introducir el volumen de disolución correspondiente a la longitud del bucle. La introducción de volúmenes distintos exige interrumpir el flujo, reemplazar el tubo y consumir tiempo de análisis. Además, el FIA limita los volúmenes de la muestra a inyectar: volúmenes inferiores a pocos microlitros no son factibles, mientras que los volúmenes grandes presentan dispersiones de la muestra con el medio portador, en ambos extremos del bolo, impidiendo un mezclado eficiente de la zona central. Tras cada inyección, el bucle debe lavarse con un volumen adicional de muestra, para limpiar las paredes internas de la disolución portadora.

El resultado es un modelo rígido y difícil de mecanizar o automatizar, que exige un considerable consumo de muestras, patrones y reactivos y no es adecuado a las expectativas medioambientales.

Estas limitaciones pueden superarse de forma satisfactoria mediante el diseño de sistemas multiconmutados que operen con válvulas solenoides de tres vías o minibombas que suministren únicamente los volúmenes necesarios.

1.3.2. MULTICONMUTACIÓN

El diseño de sistemas multiconmutados presenta la ventaja de la capacidad individual de apertura y cierre de cada dispositivo solenoide [Reis: 1993] [Reis: 1994] [Reis: 2002] [Ródenas-Torralba: 2004 b]. La inserción de las alícuotas de muestras, patrones y reactivos puede realizarse en un único canal en el que se desarrollan las reacciones químicas. El movimiento del flujo por el interior de los tubos de reacción puede ser modificado sin cambiar la estructura física y es posible trabajar con diferentes disoluciones.

El dispositivo fundamental en multiconmutación es la válvula solenoide de tres vías o la minibomba solenoide. Su funcionamiento se realiza mediante un ordenador que controla el tiempo y, por tanto, el volumen de inserción de las distintas disoluciones, el número de ciclos de muestras y reactivos, y sincroniza el comienzo de cada ciclo analítico. Con el método de inserción de muestra basado en el control electrónico del tiempo durante el cual la válvula solenoide está activada por un pulso de corriente, y a un caudal constante y conocido, la incertidumbre del volumen de muestra depende principalmente de la precisión con la que se controla dicho tiempo.

Por tanto, la principal aportación de la multiconmutación al análisis en flujo es la sustitución de los *volúmenes* de inserción por *tiempos* de inserción, lo que permite desarrollar diseños basados en el tiempo, más precisos y reproducibles.

La introducción de disoluciones en los sistemas de multiconmutación se realiza, bien por la aspiración a través de canales de bombeo o bien aprovechando el avance por gravedad [Rocha: 2000] [Vieira: 2001] [Ródenas-Torralba: 2005 c]. Las válvulas o minibombas facilitan la inserción de pequeños pulsos de muestras y reactivos de forma alternada, consecuentemente, el tubo de reacción se carga con un flujo formado por pulsos de muestra en tándem con pulsos de

reactivos. En su transporte hacia el detector tiene lugar un rápido proceso de mezclado homogéneo debido a las dispersiones en las interfases líquido-líquido, lo que proporciona las condiciones para un desarrollo adecuado de las reacciones químicas.

Así, la multiconmutación aprovecha las ventajas que proporciona la combinación de la informática y la electrónica con la Química Analítica al:

- Incrementar el grado de automatización / mecanización.
- Miniaturizar los montajes de flujo.
- Controlar los procesos de inserción de microsegmentos de muestras y reactivos electrónicamente.
- Sincronizar el muestreo con distintas etapas como la de detección, etc.

y permite desarrollar sistemas en flujo continuo más versátiles, robustos y automatizables que los de la metodología FIA.

Con cada nuevo trabajo publicado se observa cómo se van descubriendo nuevas posibilidades y también nuevos problemas asociados que se resuelven de manera ingeniosa.

1.3.3. DISPOSITIVOS UTILIZADOS EN LA INSERCIÓN DE LAS DISOLUCIONES

Actualmente se encuentran en el mercado diferentes válvulas para la inserción de disoluciones, como por ejemplo las rotatorias, electromecánicas y solenoides [Ruzicka: 1977] [Reis: 1993 b] [Tumang: 1998] [Reis: 1999] [Ródenas-Torralba: 2005 d].

Entre los diferentes modelos de válvulas, las solenoides (principalmente válvulas de 3 vías y minibombas) han sido usadas para trabajos desarrollados en multiconmutación. Permiten disminuir el consumo de las disoluciones, pues posibilitan efectuar un muestreo discreto, insertando únicamente los volúmenes necesarios para llevar a cabo la reacción, ya que durante el intervalo de lectura puede fluir únicamente la disolución portadora. Por tanto, estos dispositivos son muy importantes en la economía de reactivos y muestras, en la minimización de los residuos y, como consecuencia, en la reducción de la contaminación y en el enfoque hacia una química medioambientalmente sostenible.

1.3.3.1. Válvulas solenoides de 3 vías

El dispositivo para la inserción de las disoluciones más empleado en multiconmutación es la válvula solenoide de 3 vías (1.8 cm x 1.8 cm x 3.0 cm, 32 g peso).

La Fig. 15 muestra el funcionamiento de una válvula solenoide de 3 vías. Cuando pasa la corriente por la bobina solenoide, el pistón es atraído hacia la cubierta de la válvula, comprimiendo el resorte y haciendo, de esta forma, que cualquier pieza unida al pistón siga esta trayectoria. Cuando cesa el voltaje, el resorte empuja el pistón lejos de la cubierta de la bobina y, en consecuencia, cualquier pieza unida al pistón también se mueve.

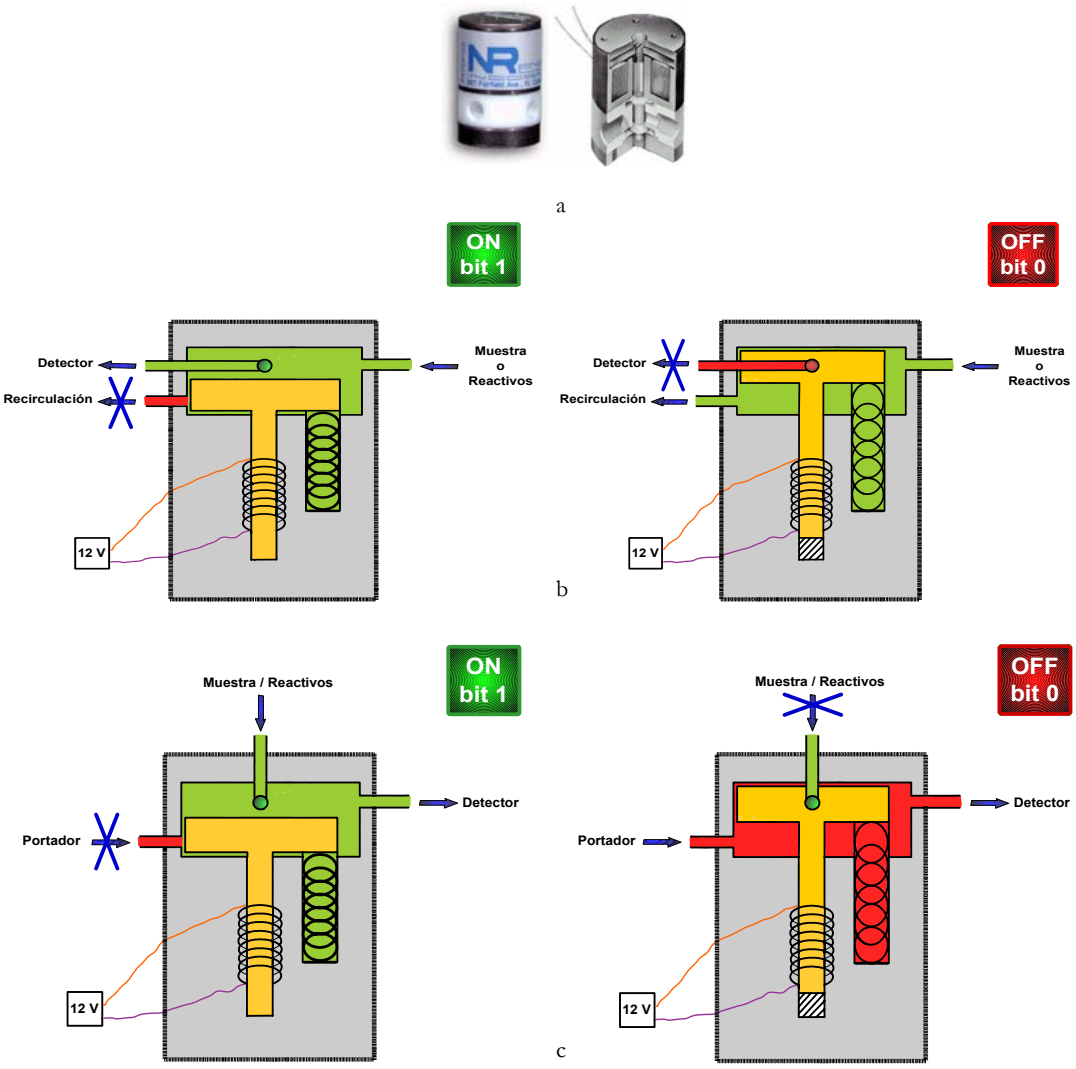


Figura 15. Funcionamiento de una válvula solenoide. *a*: aspecto externo e interno. *b*: Configuración IN, OUT_1 y OUT_2. *c*: IN_1, IN_2 y OUT.

Una válvula solenoide de 3 vías se comporta, de este modo, como un interruptor en dos estados posibles: ON y OFF. Dichos estados se programan utilizando un registro conectado a la válvula de forma que un 1, en un determinado bit del registro, abre la válvula en una posición y un 0 la abre en la

posición contraria, según se describe en la Fig. 16.

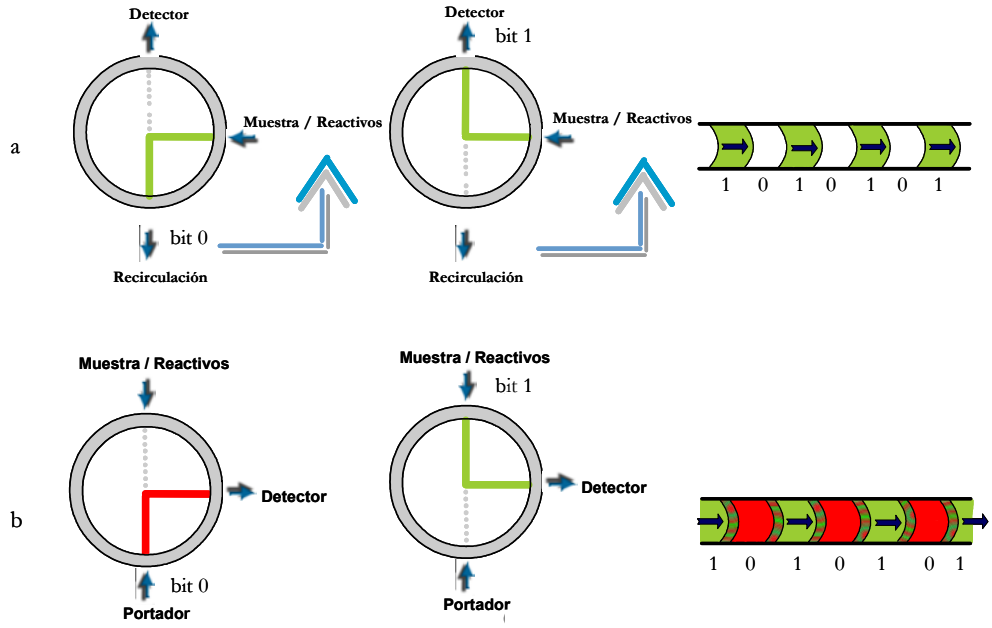


Figura 16. Estados de la válvula, proyectada desde su parte superior, y perfiles de inserción.

Dos de los tres puertos de la válvula están conectados permanentemente. Cuando la válvula está en OFF (bit 0) la disolución de muestra se recircula (a) o la disolución portadora fluye hacia el detector (b). Cuando cambia a la posición ON (bit 1), un pulso electrónico de una longitud programable permite la inserción de la muestra en el detector (a y b). El volumen de la muestra introducido es proporcional a la duración del pulso y puede ser alterado cambiando el perfil de la secuencia de inserción. El resultado es un sistema flexible que permite la inserción de volúmenes variables de muestra vía *software*.

El control electrónico de la duración de los pulsos asegura una inserción reproducible de los volúmenes de muestra. El uso de pequeños segmentos de muestra formando un sándwich entre los microsegmentos de portador facilita el mezclado, incluso con grandes volúmenes de inyección.

1.3.3.2. Minibombas

El uso de estos dispositivos es relativamente reciente en multiconmutación. Únicamente 9 trabajos [Lapa: 2002] [Carneiro: 2002] [Dias: 2003] [Lavorante: 2005] [Carneiro: 2005] [Rocha: 2005 a] [Rocha: 2005 b] [Ródenas-Torralba: 2005 a] [Ródenas-Torralba: 2005 d] de la literatura utilizan las minibombas como sistemas propulsores. No obstante, las minibombas sustituyen y complementan a las válvulas, ya que evitan el uso de las bombas peristálticas y reducen considerablemente el precio del sistema, facilitando su miniaturización.

Las bombas peristálticas presentan inconvenientes como su alto coste, gran tamaño y peso considerable, lo que reduce la portabilidad de los sistemas e incrementa el precio de los equipos. En este sentido, el reemplazo de las bombas peristálticas por estos propulsores de pequeño tamaño (1.8 cm x 1.8 cm x 5.0 cm, 58 g peso) constituye una alternativa para reducir drásticamente el coste y el tamaño de los sistemas, incrementando la versatilidad y portabilidad necesarias para efectuar estudios de campo.

Además, las minibombas solenoides pueden ser controladas individualmente a baja potencia: el consumo de potencia medio que requieren cuatro minibombas es aproximadamente 1/20 de la potencia que usa una bomba peristáltica [Weeks: 1996].

La Fig. 17 muestra el funcionamiento de una minibomba. Cuando pasa corriente a través de la bobina solenoide de la minibomba (ON), el diafragma altera su posición de reposo y retrocede aspirando una alícuota de disolución, cuyo volumen depende de la capacidad de la minibomba, hacia la cámara interior a través del canal de entrada. Cuando cesa el voltaje aplicado (OFF), el diafragma retorna a su posición de inicio bombeando la disolución hacia el exterior a través del canal de salida. Las minibombas bombean un volumen

constante por pulso, por lo tanto el control efectivo del volumen de muestras y reactivos se realiza seleccionando el número apropiado de pulsos ON/OFF. Los volúmenes bombeados normalmente son de 8 ± 2 µL en los trabajos que recoge la literatura [Lavorante: 2005] [Ródenas-Torralba: 2005 a]. Sin embargo, estos volúmenes pueden variar de 8 a 250 µL dependiendo de la capacidad interna de las minibombas disponibles comercialmente.

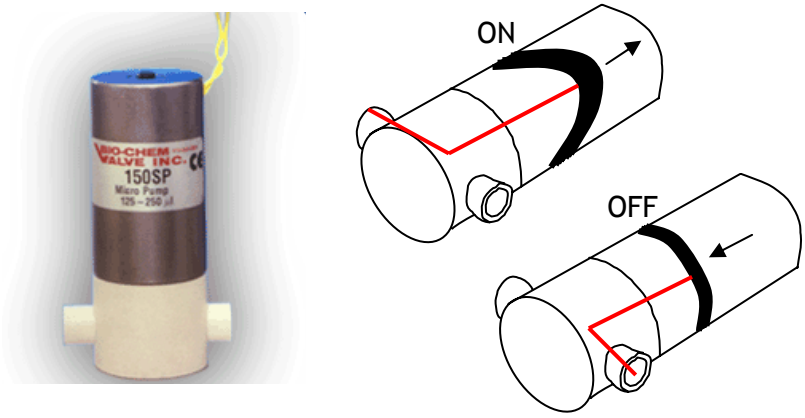


Fig. 17. Esquema del funcionamiento de una minibomba. En el dibujo puede apreciarse el movimiento que realiza el diafragma para propulsar la disolución a su paso por la minibomba.

1.3.4. POSIBLES CONFIGURACIONES MECÁNICAS EN MULTICONMUTACIÓN

La Fig. 18 muestra diferentes configuraciones mecánicas en el análisis de flujo explotando la multiconmutación. El mecanismo más simple de la conmutación se desarrolla redireccionando un único flujo (Fig. a y b), que se consigue fácilmente usando válvulas de 3 vías [Krug: 1986]. La estrategia ha sido explotada para insertar corrientes de flujo de forma intermitente e incrementar el número de inyecciones de muestra [Zagatto: 1980]. En el caso de la Fig. a, el proceso de recirculación permite la reducción del consumo de muestras y reactivos y, por tanto, la reducción de residuos [Ruzicka: 1989]. Por otra parte, la conmutación también permite alimentar la ruta analítica con segmentos (pulsos)

de disoluciones distintas (Fig. b). El dispositivo se diseña con dos entradas IN_1 e IN_2 y una salida OUT. Esta estrategia se usa normalmente en técnicas de sándwich [Alonso: 1987], inyecciones secuenciales [Ruzicka: 1990] y en tándem por conmutaciones sucesivas y rápidas (multiconmutación), originando una cadena binaria constituida por segmentos de las disoluciones involucradas. Otro tipo de conmutación mecánica implica el intercambio entre los componentes del montaje (Fig. c), de forma que se alcancen tiempos de residencia de la muestra distintos. Esta técnica es beneficiosa principalmente para implementar determinaciones simultáneas con discriminación cinética [Krug: 1983] [Arruda: 1987]. La configuración d ofrece la posibilidad de selección entre diversos caminos, siendo muy atractiva para realizar inyecciones con bucles [Krug: 1986], mini-columnas de intercambio iónico [Krug: 1986], detección múltiple [Zagatto: 1992], etc.

La configuración *e* permite la inserción de múltiples pulsos de reactivos diferentes y una única salida hacia el camino de reacción. Esta configuración es muy versátil y permite determinaciones no simultáneas de varios analitos sin modificar el esquema de flujo [Reis: 2004] [Ventura-Gayete: 2004 a].

Las minibombas ofrecen menores posibilidades, ya que únicamente presentan una entrada y una salida y siempre en una posición fija (*f*). Combinando diferentes minibombas puede conseguirse la inserción de varias disoluciones en un único canal de bombeo. Un ejemplo es el que se muestra en el diseño *g* con 3 minibombas [Carneiro: 2002]. Incrementando el número de minibombas se consiguen más posibilidades de inserción de reactivos.

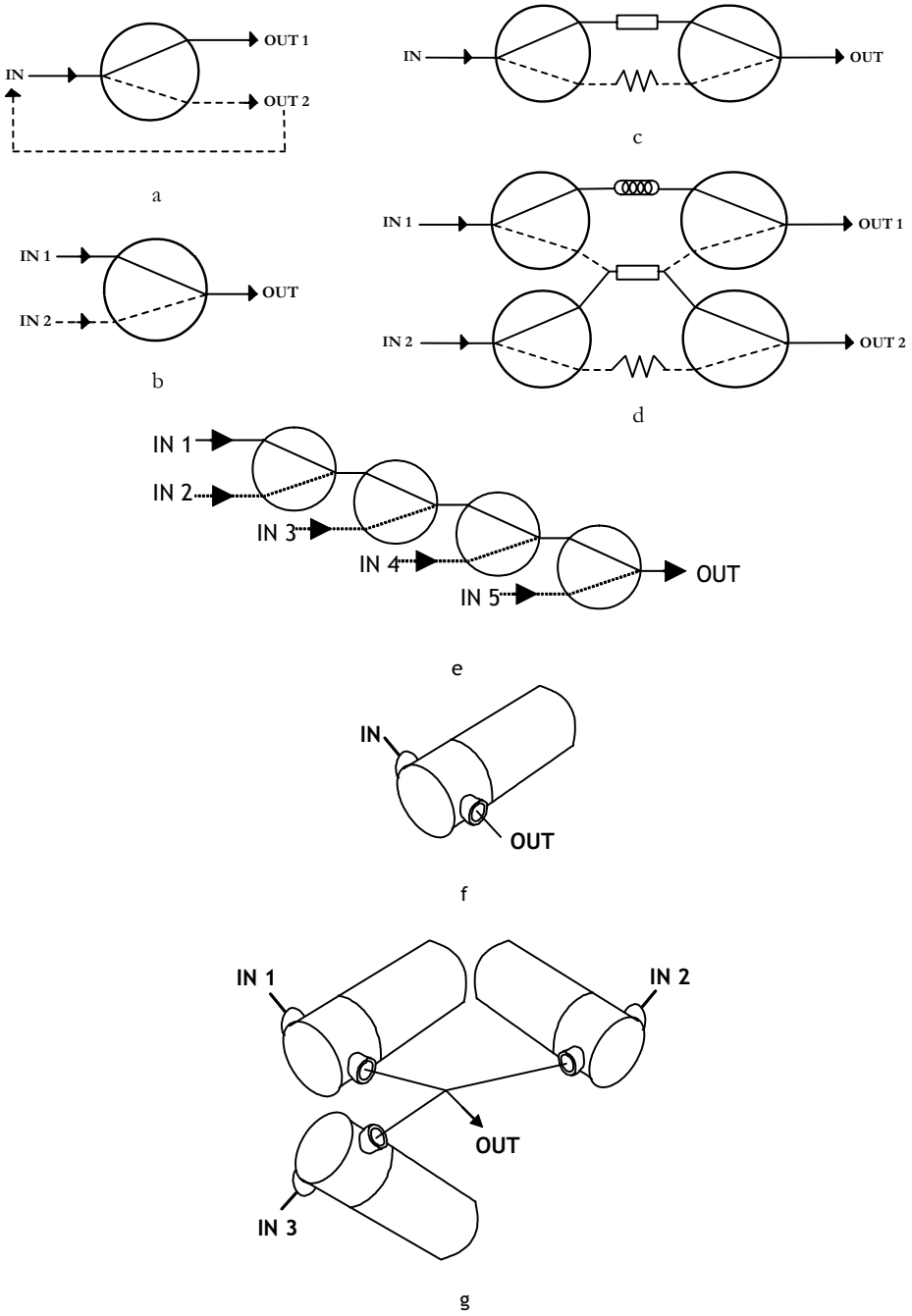


Fig. 18. Configuraciones mecánicas que permite la conmutación.

Tanto las minibombas como las válvulas solenoides pueden ser conmutadas ON/OFF al mismo tiempo o secuencialmente, una por una, dependiendo de las necesidades del procedimiento analítico.

Sistemas más elaborados comprenden usualmente más dispositivos activos. Sin embargo, la presencia de varias válvulas en el sistema se ha tomado erróneamente como un indicador de un sistema multiconmutado. Ésta no es una condición imprescindible, ya que los sistemas multiconmutados pueden operar con una única válvula [Almeida: 2000] y sistemas con varios conmutadores no son claramente representativos de la multiconmutación [Stewart: 1980] [Zagatto: 1987] [Araújo: 1998 b].

1.3.5. EL PERFIL DE INSERCIÓN EN MULTICONMUTACIÓN

Una de las principales características de la multiconmutación es el establecimiento de flujos en tándem. Un número de alícuotas de diferentes disoluciones miscibles pueden ser introducidas en el montaje por la conexión rápida y secuencial de los conmutadores. De esta forma, segmentos muy próximos entre sí se mezclan rápidamente mientras son transportados a través del tubo de reacción. El mezclado se mejora disminuyendo el volumen de las alícuotas e incrementando el número de pulsos. La inserción de n pares de pulsos de muestra/reactivo resulta en $2n$-1 interfases en las que el proceso de mezclado ocurre por dispersión axial. Al contrario de lo que ocurre en la mayoría de los sistemas en flujo, las interacciones muestra/reactivo comienzan en la etapa de muestreo, incrementando así el tiempo de residencia.

La Fig. 19 representa como a medida que se incrementa el número de pulsos (segmentos o alícuotas) de muestra y reactivo se consigue un mayor número de zonas de contacto entre disoluciones, con lo que se mejora el mezclado entre ellas. De esta forma, con un número elevado de pulsos se logra

un mezclado óptimo entre muestras y reactivos. El volumen de muestra insertado es proporcional a la duración del pulso y puede ser alterado modificando el perfil de la secuencia de inserción.

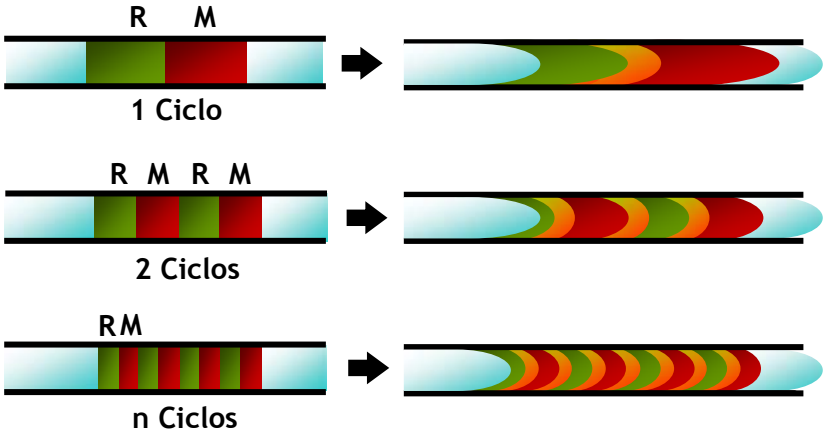


Fig. 19. Perfil de inserción que siguen muestras y reactivos en un modelo multiconmutado.

La Fig. 20 esquematiza el perfil de dispersión alcanzado por el bolo de muestra utilizando un sistema multiconmutado. Esta dispersión es, generalmente, un arma de doble filo, ya que por una parte mejora el mezclado entre el analito y los reactivos e incrementa la sensibilidad y, por otra, causa dilución, se ensanchan los picos y se reduce el número de determinaciones por hora en el laboratorio. Normalmente, el efecto de mejora de mezclado predomina, obteniéndose un incremento de la sensibilidad con el incremento de la dispersión. Es importante, pues, establecer un compromiso entre ambos efectos.

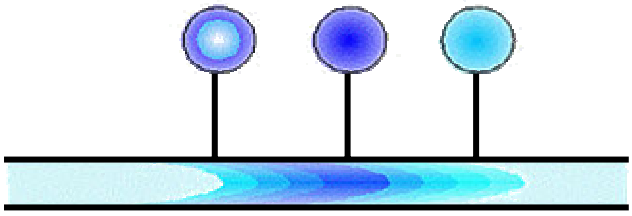


Fig. 20. Perfil de dispersión del bolo de muestra.

Los inconvenientes causados por diferencias en el índice de refracción en medidas espectrofotométricas pueden ser considerablemente minimizadas en sistemas en flujo con corriente en tándem, como se verifica en la determinación de la preparación farmacéutica pindolol [Lapa: 1998 a] y en la determinación de etanol en bebidas alcohólicas [Comitre: 2000]. Estos sistemas en tándem también se proponen para la mejora del proceso de mezclado en la determinación espectrofotométrica del ácido ascórbico [Paim: 1998] y de la clomipramina [Lima: 2002] en preparados farmacéuticos, y en la determinación de glicerol en zumos de fermentación alcohólica [Kronka: 2001]. Otros ejemplos ilustrativos se presentan en el apartado de aplicaciones.

1.3.6. MONTAJES EXPERIMENTALES BÁSICOS EN MULTICONMUTACIÓN

La multiconmutación ha permitido mejorar la simplicidad y la eficiencia en el control de muestras y reactivos y es interesante considerar algunas de sus configuraciones básicas.

La Fig. 21 muestra una de las configuraciones más simples que puede diseñarse. Este montaje está formado por tres válvulas solenoides de tres vías y se observa cómo se va introduciendo en cada pulso de válvula una alícuota de portador, muestra o reactivo, marcado por el programa informático realizado. El perfil de inserción formado es en forma de sándwich o tándem. Montajes de este tipo se han llevado a cabo para la detección fotométrica del punto final entre HCl y NaOH [Korn: 1995], para la determinación espectrofotométrica de Mn en soja [Smiderle: 1999] y en la determinación de Hg y Te por CV-AFS [Reis: 2002] y HG-AFS [Ródenas-Torralba: 2004 a], respectivamente.

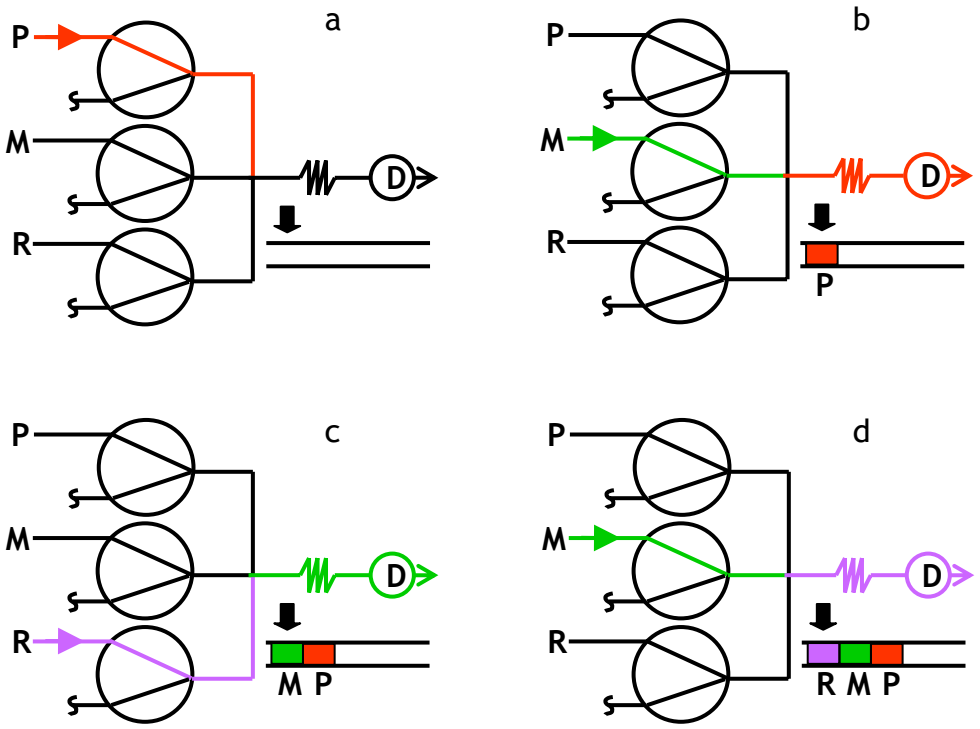


Fig. 21. Montaje experimental más simple en multiconmutación. (a): inserción del portador en el camino analítico. (b): inserción de una alícuota de muestra. (c): inserción de una alícuota de reactivo en tándem con la muestra. (d): inserción de una alícuota de muestra en tándem con el reactivo. P: portador. M: muestra. R: reactivo. D: detector.

El diseño de la Fig. 22, con cinco válvulas solenoides, es más complicado y permite la recirculación de las disoluciones que pasan a través de las válvulas V_1, V_2, V_4 y V_5 y, por tanto, la disminución en el consumo de muestras y reactivos y la reducción de los desechos generados. A su vez, la válvula V_3 permite el paso a través de dos caminos de reacción diferentes, lo que permite un control cinético de la reacción. El montaje realizado para la especiación de nitrógeno inorgánico en aguas [Rocha: 2000], para la determinación espectrofotométrica de aniones [Rocha: 2001 a] y para la determinación quimioluminiscente de ácido fólico en yogur [Martelli: 2001] permiten, además de dos posibilidades de reacción distintas, la recirculación de sus reactivos a los recipientes de inicio.

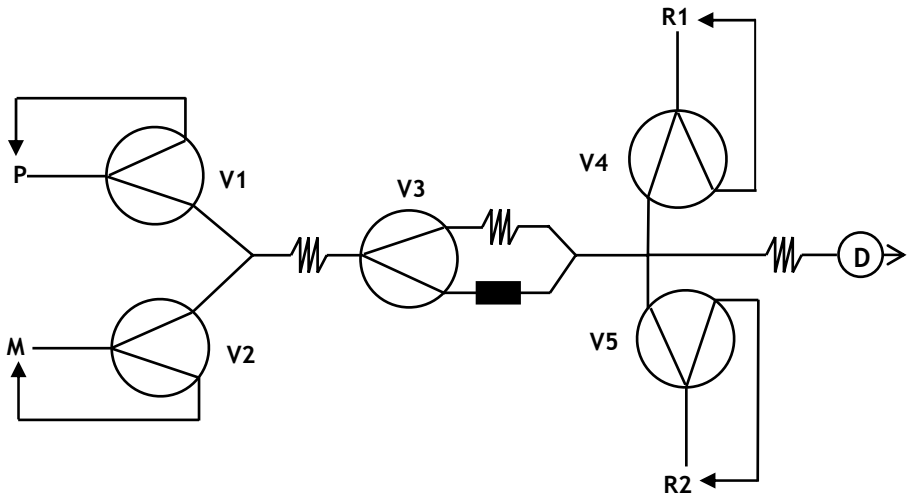


Fig. 22. Diagrama de flujo de un sistema multiconmutado mecanizado con recirculación y dos caminos de reacción.

La Fig. 23 representa un esquema de flujo que facilita la multidetección en dos caminos de análisis diferentes. El analito se trata con distintos reactivos según la línea analítica por la que es dirigido. Con pequeñas variaciones de este montaje básico se pueden determinar diversos analitos. Un esquema similar al presentado en la Fig. 23 se ha utilizado para la determinación espectrofotométrica simultánea de cationes y aniones [Rocha: 2004] y de amonio y orto-fosfato en aguas naturales [Fernandes: 2002].

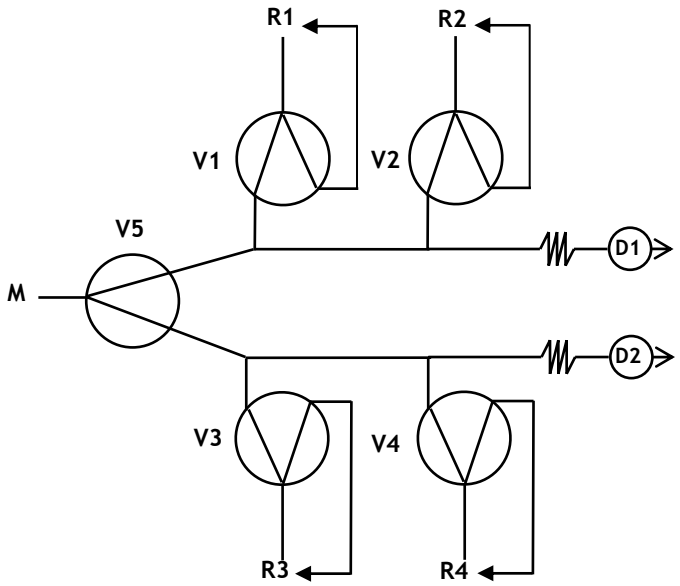


Fig. 23. Montaje para realizar multidetección en multiconmutación. V_i: válvulas solenoides. M: muestra. R_i: reactivos. D_i: detectores.

La Fig. 24 reproduce dos montajes experimentales diferentes y típicos en multiconmutación. En el primer sistema (*a*), las válvulas solenoides se colocan *en paralelo* y todas las disoluciones pasan a través de un mismo reactor, mientras que en el segundo (*b*) las válvulas se colocan en forma de cadena (montaje *en serie*) y las disoluciones pasan a través de distintos reactores. Montajes en paralelo han sido estudiados para las determinaciones espectrofotométricas de cloruros en aguas de río [Oliveira: 1997], de ácido ascórbico en fármacos [Paim: 1998] y de L(+)lactato en ensilaje (alimento que resulta de la fermentación anaeróbica de un material vegetal húmedo) [Tumang: 2001] y en la determinación turbidimétrica de sulfato en plantas [Vieira: 1998 a]. Los montajes en serie han sido menos explotados: en la literatura pueden encontrarse ejemplos de este tipo de diseños en las determinaciones de Cu, Cr, Fe y Pb en aceites lubricantes por AAS [Reis: 2004] y de sulfonato sódico en detergentes por ATR-FTIR [Ventura-Gayete: 2004 a].

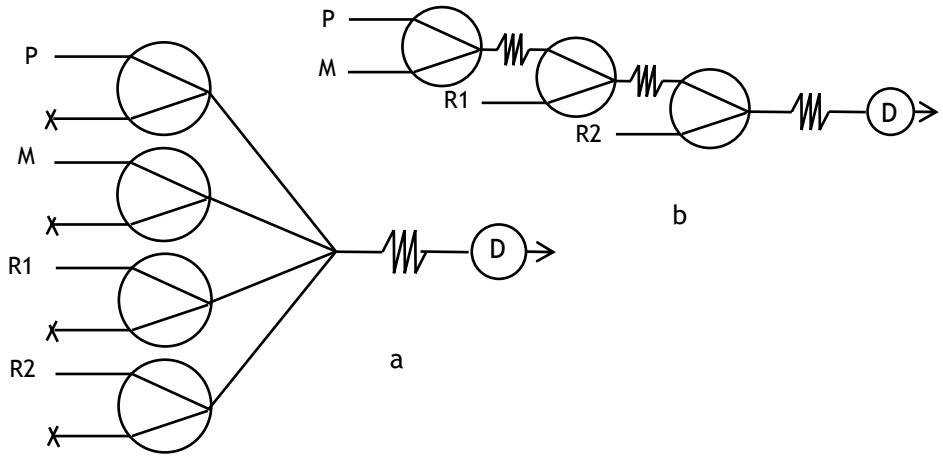


Fig. 24. Montaje en paralelo (a), montaje en serie (b).

1.3.7. VENTAJAS E INCONVENIENTES DE LA MULTICONMUTACIÓN

Las ventajas que se deducen de las configuraciones que hacen uso de la multiconmutación se enumeran a continuación:

1. *Amplio abanico de posibilidades*. El control electrónico y la flexibilidad de la multiconmutación hacen posible la implementación fiable de modos en gradiente (flujo parado, zonas de mezclado, zonas de muestreo), de métodos automatizados para determinaciones simultáneas de varios analitos [Rocha: 2004], sistemas multicanal complejos que pueden operar de forma simple y efectiva, preparación de calibrados y adiciones estándar en línea [Ródenas-Torralba: 2004 b], etc.

2. *Consumo muy reducido de muestras y reactivos*. El consumo de muestras y reactivos es mínimo, ya que pueden insertarse volúmenes de pocos microlitos correspondientes a tiempos de inserción de fracciones de segundo de una forma muy precisa.

3. *Economía y simplicidad*. Las válvulas solenoides y las minibombas son dispositivos económicos (el precio oscila en torno a 100 €) en comparación con otros equipos y aparatos utilizados en los laboratorios [Rocha: 2005 b] [Ródenas-Torralba: 2005 d]. Además, no es necesaria una fuente adicional de voltaje, ya que las válvulas solenoides y las minibombas pueden ser conectadas por un simple pulso eléctrico (12 V y 100 mA). La tarjeta insertada en la placa base del ordenador dirige y controla el sistema entero. Las operaciones pueden ser controladas mediante software flexible y sencillo desarrollado en lenguajes de programación cotidianos, como Quick BASIC o Visual BASIC. El carácter modular de los sistemas multiconmutados permiten su adaptación a las diferentes necesidades que hoy en día tiene la sociedad, análisis *in situ*, y cuya resolución compete a la Química Analítica.

4. *Flexibilidad y versatilidad*. La multiconmutación permite variar la longitud de los tubos de reacción e insertar volúmenes variables, sin necesidad de modificar físicamente el montaje y sin que el perfil de inserción se vea afectado. Los cambios pueden ser realizados simplemente reajustando la duración de los pulsos eléctricos alterando la secuencia de conmutación.

5. *Incremento de la reproducibilidad y automatización/mecanización*. Las válvulas y las minibombas solenoides requieren una intervención mínima por parte del operador. El proceso de inserción (ciclos ON/OFF) puede ser controlado vía software de forma reproducible y durante tiempos considerablemente prolongados.

6. *Incremento de la productividad en el laboratorio*. La multiconmutación reduce el tiempo de análisis y, por tanto, incrementa el número de determinaciones realizadas por hora, como queda demostrado por la gran mayoría de los artículos registrados en la bibliografía.

7. *Incremento del tiempo de residencia*. Al contrario de lo que ocurre en la mayoría de los sistemas en flujo, las interacciones muestra/reactivo comienzan en la etapa de muestreo, incrementándose el tiempo de residencia.

8. *Independencia y comodidad*. Todos los pasos desarrollados pueden ser implementados independientemente y, además, los sistemas de flujo pueden ser reconfigurados por control de *software*.

9. *Miniaturización del sistema de flujo*. El pequeño tamaño de las válvulas solenoides, minibombas y de las interfaces electrónicas, junto con su bajo peso, permite el desarrollo de sistemas compactos e integrados y equipos móviles para el análisis *in situ*.

10. *Rapidez*. La rapidez de la multiconmutación tiene su mayor incidencia en la frecuencia o velocidad de muestreo, siendo incomparablemente superior a las técnicas manuales. Factores como volumen de muestra, volumen interno del reactor y caudal son decisivos al establecer la velocidad de muestreo. Deben elegirse en cada caso las condiciones adecuadas a las necesidades.

11. *Sencillez*. La multiconmutación se caracteriza por una gran sencillez debido a montajes no sofisticados, de fácil ensamblaje y manejo. Su aplicación al análisis rutinario es muy útil y la manipulación de su diseño para adaptar el montaje a diferentes analitos resulta sencillo y rápido.

12. *Ventajas frente a FIA y SIA*. La multiconmutación, como alternativa al FIA, minimiza la generación de residuos [Reis: 1994] y con respecto al análisis de inyección secuencial consigue una disminución del tiempo de análisis [Ruzicka: 1990]. La determinación espectrofotométrica de carbaril con p-aminofenol (PAP) ilustra de forma significativa estas afirmaciones [Reis: 1999]: la multiconmutación consigue reducir el volumen total de residuos en un factor de 3.6 respecto al método FIA e incrementa 3.5 veces la frecuencia de muestreo con respecto al SIA.

Sin embargo, la multiconmutación también está sujeta a los siguientes inconvenientes:

1. *Disponibilidad comercial limitada*. La escasez de equipamiento disponible comercialmente (principalmente interfaces electrónicas y *software*) para el control de las válvulas solenoides y minibombas se refleja en el hecho de que todas las aplicaciones que existen en multiconmutación usan *hardware* y *software* de diseño propio. Por tanto, previamente al desarrollo de un sistema de multiconmutación es necesario aprender a programar y

controlar los dispositivos solenoides.

2. *Necesidad de una unidad de bombeo*. Debido a que las válvulas de 3 vías actúan como conmutadores, una de sus dos posiciones de entrada o de salida debe invariablemente estar en OFF y la circulación a través de este canal parada. Esto requiere una unidad de propulsión situada antes o después de las válvulas. Sin embargo, este problema en algunos casos puede solventare haciendo fluir las disoluciones por gravedad [Rocha: 2000] [Ródenas-Torralba: 2005 c] o con el uso de las minibombas [Lima: 2004] [Lavorante: 2005] [Rocha: 2005 b].

3. *Restricciones en los volúmenes de inserción*. Con segmentos de muestras y reactivos muy pequeños, la operación del sistema de propulsión debe estar sincronizada con las microinserciones. En caso contrario, los pulsos de bombeo introducen distorsiones irreproducibles en los perfiles de inserción.

1.3.8. APLICACIONES DE LA MULTICONMUTACIÓN

La simplicidad de la multiconmutación ha permitido desarrollar procedimientos analíticos diversos. Ejemplos son la determinación simultánea de dos o tres analitos por espectrofotometría [Kronka: 1998] [Rocha: 2000], procedimientos de titulación en espectrometría y potenciometría [Rocha: 1998] [Araújo: 1995] y el alcance de altos grados de dilución en línea [Martelli: 1999] [Paim: 2000]. Sin embargo, y hasta el momento, sólo nuestro grupo de trabajo ha desarrollado aplicaciones en técnicas como la espectroscopia de fluorescencia atómica por vapor frío (CV-AFS) o generación de hidruros (HG-AFS) o en técnicas de Infrarrojo por Transformada de Fourier (FT-IR) empleando la multiconmutación [Reis: 2002] [Reis: 2003] [Ródenas-Torralba: 2004 a] [Ródenas-Torralba: 2004 b] [Cava-Montesinos: 2004 b] [Ventura-Gayete: 2004 b].

Se han desarrollado sistemas de extracción líquido-líquido automatizados y/o mecanizados [Comitre: 2003] [Comitre: 2005] [Ródenas-Torralba: 2005 c] y sistemas de bajo coste y portátiles [Rocha: 2005 b] [Ródenas-Torralba: 2005 d]. Es de destacar que la multiconmutación también se ha aplicado a la especiación: de N por espectrofotometría [Calatayud: 1998] [Rocha: 2000] [David: 2001] [Rocha: 2001 a] y de Te por HG-AFS [Ródenas-Torralba: 2005 b].

La flexibilidad de la multiconmutación permite el uso de redes de flujo con un número elevado de válvulas solenoides [Rocha: 2000] [Miranda: 2002]. Más de la mitad de los trabajos utilizan sistemas de flujo básicos, aunque con ligeros cambios, siendo los fármacos y las aguas las muestras que han recibido la mayor atención, entre un 20 y 30 %, respectivamente, de los artículos publicados. La multiconmutación permite el uso de toda clase de detectores ópticos y electroquímicos. Más de la mitad de los trabajos involucran el uso de la espectroscopia UV-visible. También es interesante la proporción (alrededor del 10 %) de trabajos que emplean la detección por quimioluminiscencia.

En la Tabla 1 se muestran los trabajos publicados en multiconmutación desde su origen. Se incluyen los analitos determinados, las matrices, las técnicas empleadas, el número de válvulas o minibombas, así como las características analíticas más destacadas de los métodos desarrollados.

Tabla 1. Aplicaciones de la multiconmutación registradas en la literatura científica.

Año	*Nº de (v) /(b)*	*Técnica de detección*	*Analito*	*Matriz*	*Frecuencia de muestreo (h^{-1})*	*Intervalo lineal (µg/mL)*	*LD (ng/mL)*	*DER (%)*	*Ref.*
1994	4 v	S (480 nm)	Fe (III)	Plantas	220	0 - 10		< 3	[Reis: 1994]
1995	3 v	S	Creati-nina	Orina	24			3.0	[Araújo: 1995]
1995	3 v	S (540nm)	Fe		20				[Korn: 1995]
1995	4 v (Ni) 5 v (Fe/Cr)	S (Ni: 460 nm) (Fe/Cr: 526 nm)	Ni/Fe/Cr	Aceros	60 (Ni) 130 (Fe/Cr)	5 - 50		1.0	[Martelli: 1995]
1996	5 v (Ca) 4 v (K)	AAS/AES	Ca/K	Plantas	50 (Ca) 70 (K)				[Giné: 1996]
1996	6 v	S (660 nm)	NH_4^+/PO_4^{3-}	Plantas	80			2.0(NH_4^+) 1.5(PO_4^{3-})	[Kronka: 1996]
1996	1 v	S (620 nm)	Cu/Zn	Plantas	45	0 - 1.0 (Cu) 0 - 2.0 (Zn)	50 (Cu) 40 (Zn)	0.7 - 1.7	[Oliveira: 1996]
1997	v	S	PO_4^{3-}	Aguas de río y residuales	60			2.0	[Kronka: 1997]
1997	3 v	F	Ácido fólico	Preparados farmacéuticos	25	0.1 - 40.0		3.0	[Lapa: 1997]
1997	2 v	S	Ni/Fe	Aceros, aguas	80 (Ni) 60 (Fe)			1.0 (Ni) 1.5 (Fe)	[Martelli: 1997]
1997	5 v	S (460 nm)	Cl^-	Aguas de río	25	0.5 - 10.0		0.5	[Oliveira: 1997]
1998	1 v	FAAS	Ca/Mg	Aguas minerales	110	0.03 - 10.00 y 0.58 - 200.00 (Ca) 0.03 - 1.5 y 0.80 - 50.0 (Mg)	580 (Ca) 800 (Mg)	3	[Araújo: 1998 b]
1998	1 v	S (328 nm)	NO_3^-/NO_2^-	Aguas, suelos, fertilizantes, carne	15	0 - 12.3 (NO_3^-) 0 - 10.0 (NO_2^-)	28 (NO_3^-) 23 (NO_2^-)	0.4	[Calatayud: 1998]
1998	6 v	S (540 nm)	Fe/Al	Plantas	60			1.8 (Al) 1.2 (Fe)	[Kronka: 1998]
1998	5 v	S (635 nm)	Pindolol	Preparados farmacéuticos	30	5 - 120		1.1	[Lapa: 1998 a]
1998	4 v	S (480 nm)	Cl^-	Fármacos		0 - 10000		1.8	[Lapa: 1998 b]
1998	v	S	Ni/Fe	Aceros y aguas	80 (Ni) 60 (Fe)			1.0 (Ni) 1.5 (Fe)	[Martelli: 1998]
1998	4 v	S	Ác. Ascórbico	Fármacos	120	17.6 - 176.0	10600	1.5	[Paim: 1998]
1998	5 - 6 v	S (575 nm)	Ca	Agua, leche, material biológico, preparados farmacéuticos, fertilizantes	60	0.25 - 1000	7	0.8	[Rocha: 1998]
1998	v	S	B	Plantas	35	0.25 - 6.00		2.5	[Tumang: 1998]
1998	5 v	T (410 nm)	SO_4^{2-}	Plantas	100	10 - 150 y 100 - 500		2	[Vieira: 1998 a]
1998		S (510 nm)	Fe (II)	Aguas naturales				1.1	[Vieira: 1998 b]
1999	5 v	P	H_2SO_4	Licores	97			5	[Albertús: 1999]
1999			Review	Aguas					[Cerdà: 1999]
1999	4 v	S (510 nm)	Glucosa/ Sucrosa	Zumos y refrecos	30	0.05 - 0.20		0.12	[Kronka: 1999]
1999	5 v	P		Vinagre, cola, limón, tónica y zumos de naranjas naturales e industriales	120			1.0	[Martelli: 1999]
1999	5 v	S (596 nm)	Carbaril	Pesticidas	70		26	0.5	[Reis: 1999]
1999	4 v	S (548 nm)	Mn	Plantas	50	2.5 - 40.0	1200	0.27	[Smiderle: 1999]
1999			Review						[Zagatto: 1999]
2000	1 v	P	Cl^-	Aguas naturales embotelladas		2 - 110		3.2	[Almeida: 2000 a]
2000	v	P		Aguas residuales				3.5	[Almeida: 2000 b]

Año	Nº de (v) /(b)	Técnica de detección	Analito	Matriz	Frecuencia de muestreo (h^{-1})	Intervalo lineal (µg/mL)	LD (ng/mL)	DER (%)	Ref.
2000	1 v	P	HCl	Vinagre		0.15 - 1.2		2.5	[Almeida: 2000 c]
2000	v	P		Refrescos, bebidas isotónicas, cervezas sin alcohol, zumos de frutas	22			1.0	[Borges: 2000]
2000	4 v	S (600 nm)	Etanol	Bebidas alcohólicas	40	10.0 - 50.0 %		1.6	[Comitre: 2000]
2000	5 v	S (545 nm)	Hidro-cloruro de amilorida	Prep. farmacéuticos	30	0 - 120	1000	2.2	[Lapa: 2000 a]
2000	3 v	F	Isoniazid a		50	0 - 1.37	34.3	1.6	[Lapa: 2000 b]
2000	v	Q	Fenol	Aguas naturales	12 - 60		5.0	1.1	[Michalowski: 2000]
2000		AAS	Cu	Plantas, alimentos	48				[Miranda: 2000]
2000	v	S	Ác. Ascórbico	Zumos de frutas, refrescos	5 - 30			1.1	[Paim: 2000]
2000	8 v	S (540 y 660 - 690 nm)	NH_4^+ / NO_3^- /NO_2^-	Aguas de río	60	0.025 - 1.000 (NO_2^-) 0.10 - 5.00 (NO_3^-) 0.1 - 2.0 (NH_4^+)	5.0 (NO_2^-) 15 (NO_3^-) 25 (NH_4^+)	0.3 (NO_2^-) 0.4 (NO_3^-) 0.7 (NH_4^+)	[Rocha: 2000]
2001	1 v	P		Aguas residuales				2.4	[Almeida: 2001]
2001	1 v		NO_3^-/NO_2^-						[David: 2001]
2001	3 v	S (445 nm)	Cl^-	Formulaciones industriales, aguas	38	0.05 - 1.30	50	1.5	[Icardo: 2001]
2001	6 v	Q	Ácido láctico	Yogur	55	10 - 125	1200	1.9	[Martelli: 2001]
2001	3 v	ICP-MS	Cd		30	0.002 - 0.02			[Paker: 2001]
2001	9 v	S	NO_3^- / NO_2^- / Cl^- / PO_4^{3-}	Aguas de río	50	0.03 - 0.3 (NO_3^-) 0.0001 - 0.001 (NO_2^-) 0.001 - 0.01 (Cl^-) 0.00005 - 0.0025 (PO_4^{3-})	6 (NO_3^-) 40 (NO_2^-) 400 (Cl^-) 360 (PO_4^{3-})	1.6 (NO_3^-) 2.2 (NO_2^-) 2.3 (Cl^-) 1.5 (PO_4^{3-})	[Rocha: 2001 a]
2001	8 v	S	Zn(II), Fe(III), Cu(II), Ca(II), Mg(II)	Prep. Farmacéuticos	60		200 (Fe(III)) 200 (Zn(II)) 50 (Cu(II)) < 10 (Ca(II)) < 10 (Mg(II))	1.0 (Fe) 1.5 (Zn) 1.4 (Cu) 2.5 (Ca) 2.0 (Mg)	[Rocha: 2001 b]
2001			Review						[Rocha: 2001 c]
2001	6 v	S	Fe(III), Cu(II), Ni(II), Zn(II)	Prep. Farmacéuticos	80			2.0	[Rocha: 2001 d]
2001	4 v	S	Cafeína y amino-filina	Productos farmacéuticos	170	0.4 20.0			[Sales: 2001]
2001	6 v	S	L(+)lac-tato	Ensilaje	16	10 - 100	2000	2	[Tumang: 2001]
2001		T (420 nm)	K	Fertilizantes	> 240	6.0 - 60.0		1 - 3	[Vicente: 2001]
2001	2 v	T	SO_4^{2-}	Plantas, hígado de bovino y suero	40	40 - 200	30000	2	[Vieira: 2001]

Año	Nº de (v) /(b)	Técnica de detección	Analito	Matriz	Frecuencia de muestreo (h^{-1})	Intervalo lineal (µg/mL)	LD (ng/mL)	DER (%)	Ref.
2002	3 b	S (525 nm)	Ácido fítico	Plantas	150	5 - 100	1000	< 1	[Carneiro: 2002]
2002	5 v	S	Al	Bebidas	154	0.01 - 0.50	0.5	0.6	[de Armas: 2002]
2002	6 v	S	NH_4^+/ PO_4^{3-}	Aguas de río y lago	112	0.1 - 1.0 (NH_4^+) 0.25 - 3.00 (PO_4^{3-})	7.0 (NH_4^+) 17.0 (PO_4^{3-})	1.1 (NH_4^+) 0.7 (PO_4^{3-})	[Fernandes: 2002]
2002			Review						[Icardo: 2002]
2002	2 b	S	Cr (VI)	Aguas naturales	80			0.5	[Lapa: 2002]
2002	2 v	S (620 nm)	Clomipramina	Prep. farmacéuticos	15	0 - 50		< 2	[Lima: 2002]
2002	11 v	ICP-AES	Cd, Ni, Pb	Centeno, riñón de cerdo, hojas de tomate y harina de arroz	90		1.0 (Cd) 4.0 (Ni) 2.0 (Pb)	< 4	[Miranda: 2002]
2002			Review						[Miró: 2002]
2002	1 v	P	Ác. ascórbico	Prep. Farmacéuticos	15	1320 - 2640		1.0	[Paim: 2002]
2002	5 v	Q	Co (II)	Vitamina B12 (cianocobalamina)	180	0.000015 - 0.005	0.015	1.0	[Pizà: 2002]
2002	3 v	CV-AFS	Hg (II)	---	49.5	0 - 0.0015	1.3	< 0.1	[Reis: 2002]
2002			Review						[Rocha: 2002]
2002	v	V	Diacetil	Cervezas	12				[Rodrigues: 2002]
2002	v	S		Ensilaje	16			1.0	[Tumang: 2002]
2003	6 v	S (475nm)	Mo (VI)	Plantas	25	0.025 - 0.150	4.6	2.5	[Comitre: 2003]
2003	3 b	S (550 nm)	Bromhexina	Prep. farmacéuticos	45	0 - 400	2000	1.5	[Dias: 2003]
2003	4 v	S	Fe/Cr	Aceros	160			0.4 (Fe) 0.2 (Cr)	[Fernandes: 2003]
2003	4 v	S	Cu(II)	Agua	20		30	5.2	[Li: 2003]
2004	5 v	S (500 nm)	Fenoles	Aguas naturales y residuales	90	0.010 - 0.100	1	0.6	[Lupetti: 2004]
2003			Review	Aguas					[Miró: 2003]
2003	v	S (340nm)	3-hidroxi-butirato	Suero y plasma	60	10 - 150	2000	1.2 - 1.4	[Pires: 2003 a]
2003	v	Q	Glucosa	Suero y sangre de animal	60	50 - 600	120000	3.5	[Pires: 2003 b]
2003	v	Q	Colesterol	Sangre de animal	40	25 - 125	3700	2.3	[Pires: 2003 c]
2003	1 v	S (550 nm)	Isoniazida	Prep. farmacéuticos	20	0 - 18.0		1.5	[Prior: 2003 a]
2003	v	S	Trimipramine	Prep. Farmacéuticos	26	0.001 - 0.018		1.7	[Prior: 2003 b]
2003	3 v	CV-AFS	Hg (II)	Aguas	63	0.00005 - 0.0015	0.0015	0.1	[Reis: 2003]
2003	8 v	S 700 nm (ác. ascórbico) 367 nm (tiamina) 510 nm (riboflavina) 684 nm (piridoxina)	Vitaminas: ác. Ascórbico, tiamina, riboflavina, piridoxina	Prep. Farmacéuticos	60	0.5 - 10.0 (ác. ascórbico) 2.0 - 50.0 (tiamina) 5.0 - 50.0 (riboflavina) 0.5 - 8.0 (piridoxina)	80 (ác. ascórbico) 800 (tiamina) 200 (riboflavina) 100 (piridoxina)	1.0	[Rocha: 2003]
2003	v	S	Carbohidratos y azúcares	Forraje	32	0.2 - 0.8 %		< 2.0 %	[Tumang: 2003]
2003	1 v	P	Cl^-	Leche y vino				1.0	[Vieira: 2003]
2004	4 v	CV-AFS	Hg (II)	Leches	70	0 - 0.001	0.9	1.8	[Cava-Montesinos: 2004 b]
2004	3 v	Q	Asulam (pesticida)	Aguas naturales y residuales	30	0 - 5	40	5.8	[Chivulescu: 2004]

Año	Nº de (v) /(b)	Técnica de detección	Analito	Matriz	Frecuencia de muestreo (h^{-1})	Intervalo lineal (µg/mL)	LD (ng/mL)	DER (%)	Ref.
2004	3 v	Q	Hidroquinona	Prep. farmacéuticos, aguas residuales	103	0.1 - 15.0	30	2.9	[Corominas: 2004]
2004	7 v	S (666 nm)	S^{2-}	Aguas residuales	4	0.5 - 20	30	0.7	[de Armas: 2004]
2004	4 v	S (340 nm)	Glicerol	Vinos	33	2000 - 10000	6000	1.8	[Fernandes: 2004 a]
2004	v	Q	Etanol	Vinos	23	2.5 - 25 %	0.3 %	1.8	[Fernandes: 2004 b]
2004	5 v	S	S^{2-}	Aguas naturales y residuales	80	0.5 - 5.0	90 - 150	< 1.5	[Ferrer: 2004]
2004	4 v	S (500 nm)	Zn	Fármacos	16	0.005 - 0.025	2.0	1.2	[Jerónimo: 2004 a]
2004	3 v	S (500 nm)	Cu (II)	Orina	14	0.005 - 0.080	3.0	2.0	[Jerónimo: 2004 b]
2004	4 v	S (515 nm)	Bi	Fármacos	45	0.125 - 0.875	7.0	0.8	[Jerónimo: 2004 c]
2004			Review						[Lima: 2004]
2004	5 v	S (546 nm)	Albúmina y proteínas	Sangre de animal	45	0 - 0.015		0.8 - 1.5	[Luca: 2004]
2004	4 v	Q	Clomipramina	Prep. farmacéuticos	19 - 32	2.5 - 60.0	650 - 700	4.6	[Marques: 2004]
2004	4 v	S	Metronidazole	Drogas	60			2.3	[Medeiros: 2004]
2004			Review	Aguas					[Miró: 2004 a]
2004			Review	Aguas					[Miró: 2004 b]
2004	6 v	Q	o-Fosfato	Aguas medioambientales	11	0.005 - 0.050		3.0	[Morais: 2004]
2004	v	F	Al	Plantas y aguas naturales	60	0.1 - 1.0	40	1.7	[Paim: 2004]
2004	3 v	Q	Aldicarb	Formulaciones técnicas (Temik) y aguas minerales	17	0.0022 - 0.1000	0.069	3.7	[Palomeque: 2004]
2004	6 v	S	Fe (III)	Aguas, formulaciones farmacéuticas y productos agrícolas	22	0.025 - 0.500 y 2.0 - 40.0	8.4	2.5	[Pons: 2004]
2004	3 v	FAAS	Cu/Cr/ Fe/Pb	Aceites lubricantes	50	0 - 40 (Cu) 0 - 40 (Fe) 0 - 15 (Cr) 0 - 15 (Pb)		1.0 (Cu) 8.0 (Cr) 8.0 (Fe) 8.0 (Pb)	[Reis: 2004]
2004	4 v	S	PO_4^{3-} / NH_4^+	Aguas	40	0.005 - 0.3	1.0 (PO_4^{3-}) 1.0 (NH_4^+)	2.6 (PO_4^{3-}) 3.2 (NH_4^+)	[Rocha: 2004]
2004	3 v	HG-AFS	Te (IV)	Leche	85	0 - 0.0005	0.20	2.1	[Ródenas-Torralba: 2004 a]
2004	3 v	FT-IR	Benceno	Gasolinas	81	0 - 3.75 %	0.004 %	1.2	[Ródenas-Torralba: 2004 b]
2004	v	S	Mn (II)	Plantas	22	5.0 - 30.0	1200	1.3	[Smiderle: 2004]
2004	v	S	Drogas: sulfame-toxazoltri-metoprim, hidroxo-cloratiazida -captopril)	Medicamentos					[Tomsu: 2004]
2004	5 v	S (545 nm)	Al	Frutas		0.5 - 5.0, 5.0 - 25.0 y 10.0 - 100.0	100, 600 y 800	2.4	[Toth: 2004]
2004	4 v	ATR - FTIR	Sulfonato sódico α-olefínico	Detergentes	23	0 - 14.5 %	1.2 %	0.3	[Ventura-Gayete: 2004 a]
2004	5 v	HG-AFS	Bi (III)	Batidos	72	0 - 0.010	0.067	11.3	[Ventura-Gayete: 2004 b]

Año	Nº de (v) /(b)	Técnica de detección	Analito	Matriz	Frecuencia de muestreo (h^{-1})	Intervalo lineal (µg/mL)	LD (ng/mL)	DER (%)	Ref.
2005	9 b	S (410 nm)	Glucosa y fructosa	Jarabes	50			2.0	[Carneiro: 2005]
2005	5 v	S (520 nm)	Pb	Plantas	15	0.050 - 0.200	12	1.8	[Comitre: 2005]
2005	5 v	S	Tensio-activos aniónicos	Aguas de charca	2	0.03 - 1.00	10	3.7	[Hu: 2005]
2005	3 v	S	Dextrosa	Disoluciones de hemodiálisis y parenterales	90	0 - 1000		2.4	[Knochen: 2005]
2005	3 v	S (510 nm)	L(+)lac-tato	Caña de azúcar	36	5 - 100		2.0	[Kronka: 2005]
2005	4 b	S (464 nm)	Tensio-activos aniónicos	Aguas	60	0.5 - 50.0	0.034	0.8	[Lavorante: 2005]
2005	v	Q	Clorsulfu rón (herbicid a)	Aguas minerales	25	0.1 - 1.3	60	6.3	[Mervartova: 2005]
2005	3 b (3hidroxibut.) 4 b (glucosa) 4 b (colesterol)	S 340 nm (3-hidroxibut.) Q (glucosa y colesterol)	3-Hidroxi-butirato Glucosa Colesterol	Sangre de animal	55 (3-hidroxibut.) 40 (glucosa) 40 (colesterol)	10 - 150 (3-hidroxibut.) 50 - 600 (glucosa) 25 - 125 (colesterol)	1500 (3-hidro-xibut.) 14000 (glucosa) 4000 colesterol	1 (hidroxibut.) 2 (glucosa) 2 (colesterol)	[Pires: 2005]
2005	4 b	S (350 nm)	Ciclamato	Edulcorantes	60	0 - 600	6000	1.7	[Rocha: 2005 a]
2005	4 b	Q	H_2O_2 / NH_4^+	---	120	0.03 - 2.72 (H_2O_2) 0.03 - 3.21 (NH_4^+)	13.6 (H_2O_2) 3.21 (NH_4^+)	1.0 (H_2O_2) 1.8 (NH_4^+)	[Rocha: 2005 b]
2005	4 b	S (700 nm)	Fenol	Aguas	65	0.050 - 3.500	13	0.5	[Ródenas-Torralba: 2005 a]
2005	4 v	HG-AFS	Te(IV)/ Te(VI)	Leches	82	0 - 0.004	0.023	6.3	[Ródenas-Torralba: 2005 b]
2005	6 v	S (654 nm)	Tensio-activos aniónicos	Aguas	40	0.2 - 1.7	1.7	5.9	[Ródenas-Torralba: 2005 c]
2005	4 b	S 470 nm (Fe(III)) 546 nm (NO_2^-) 596 nm (carbaril) 700 nm (fenol)	Fe(III)/ NO_2^- / carbaril/ fenol	---	100 (Fe(III)) 110 (NO_2^-) 72 (carbaril) 65 (fenol)	1.0 - 10.0 (Fe(III)) 0.15 - 25.0 (NO_2^-) 0 - 50 (carbaril) 50 - 3500 (fenol)	22 (Fe(III)) 60 (NO_2^-) 60 (carbaril) 25 (fenol)	2.3 (Fe(III)) 1.0 (NO_2^-) 0.8 (carbaril) 1.8 (fenol)	[Ródenas-Torralba: 2005 d]

(v): válvulas solenoides; (b): minibombas solenoides; LD: Límite de detección; DER: Desviación estándar relativa; S: Espectrofotómetro; T: Turbidimetría; F: Fluorimetría; V: Voltamperometría; Q: Quimiluminiscencia; P: Potenciometría; CV-AFS: Espectroscopia de Fluorescencia Atómica por vapor frío; HG-AFS: Espectroscopia de Fluorescencia Atómica por generación de hidruros; FAAS: Espectroscopia de Absorción Atómica con llama; ATR: Reflectancia Total Atenuada; FTIR: Espectrometría Infrarroja por Transformada de Fourier.

La flexibilidad de la multiconmutación permite el uso de sistemas de flujo con un número relativamente alto de válvulas solenoides: usando un esquema experimental compuesto por 8 válvulas pueden determinarse espectrofotométricamente NO_3^-, NO_2^- y NH_4^+ [Rocha: 2000], mientras que la determinación de Cd, Ni y Pb por ICP-AES requiere 11 válvulas solenoides [Miranda: 2002]. También existe un número considerable de artículos que utilizan tan sólo 1 o 2 válvulas, como las determinaciones de ácido ascórbico [Paim: 2002] y de isozianida [Prior: 2003 a] en preparados farmacéuticos. La Fig. 25 muestra que el número más utilizado de válvulas/minibombas en los artículos publicados es de 4, aunque en gran cantidad de publicaciones también se utilizan 3, 5 y 6 dispositivos solenoides.

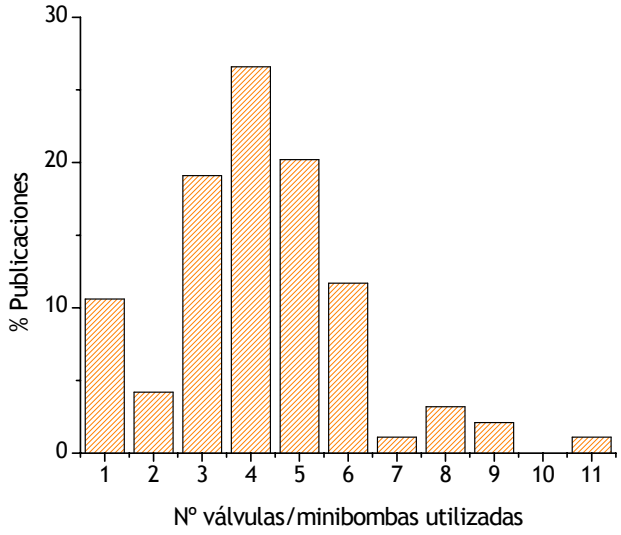


Fig. 25. Relación de las válvulas/minibombas solenoides empleadas en multiconmutación.

La multiconmutación se ha empleado principalmente para la determinación de metales (36 %) y compuestos orgánicos (38 %). También se han determinado analitos inorgánicos en un porcentaje elevado (18 %) y ácidos (8 %), según indica la Fig. 26.

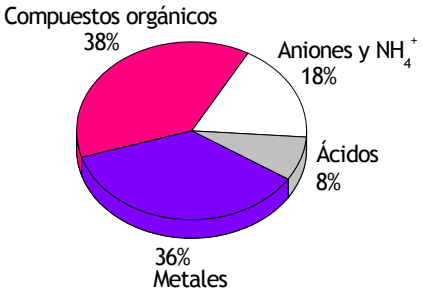


Fig. 26. Porcentaje de los distintos analitos determinados por multiconmutación.

Las matrices que han tenido una mayor atención han sido las aguas (28 %), los preparados farmacéuticos (19 %), las plantas (12 %), las bebidas (10 %) y los alimentos (8 %), como puede apreciarse en la Fig. 27. La mayoría de los trabajos que analizan aguas y plantas han sido investigados por el grupo de Piracicaba (Brasil).

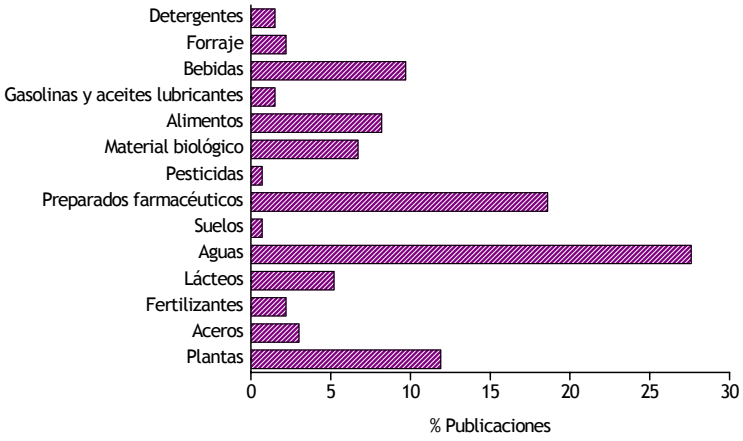


Fig. 27. Porcentaje de las distintas matrices analizadas.

En la Fig. 28 se representan en forma de sectores, los analitos determinados y las técnicas utilizadas en matrices acuosas. Se puede observar que por espectrofotometría (64 % de los artículos publicados) se determinan por igual compuestos orgánicos, inorgánicos y metales.

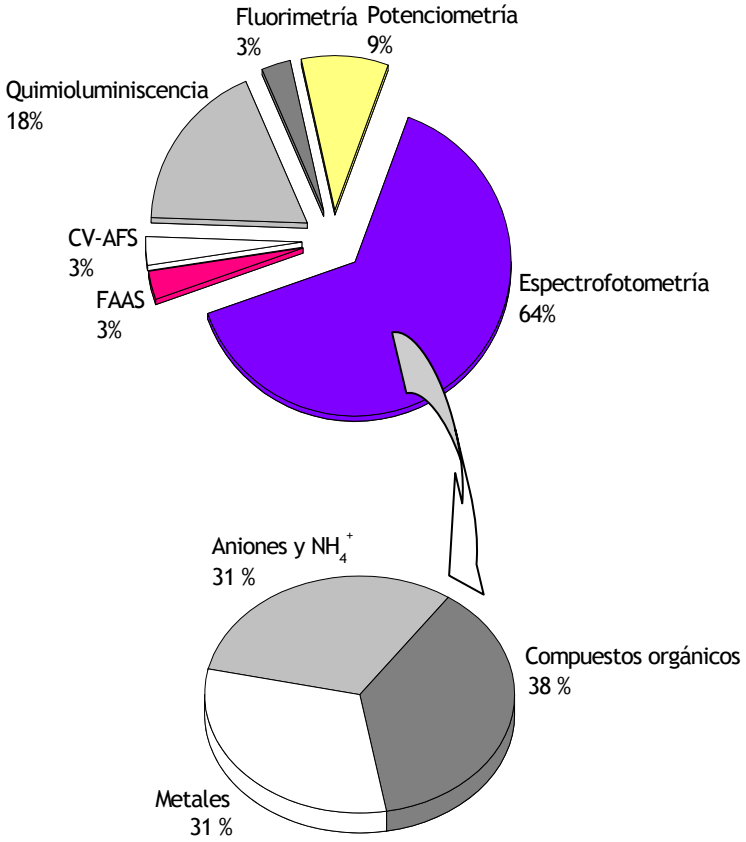


Fig. 28. Técnicas utilizadas y analitos determinados en el análisis de aguas.

La multiconmutación permite el uso de toda clase de detectores. En la Fig. 29 se indican los detectores ópticos y electroquímicos empleados. Más de la mitad de los trabajos (62 %) emplean la espectroscopia UV-vis. También es de destacar el porcentaje de trabajos que hacen uso de la quimioluminiscencia (11 %) y potenciometría (8 %), seguido de un 3 % de trabajos que emplean la espectroscopia de Fluorescencia Atómica (AFS), la turbidimetría, la espectroscopia de Absorción Atómica (AAS) o de Emisión Atómica (EAS) y la fluorimetría.

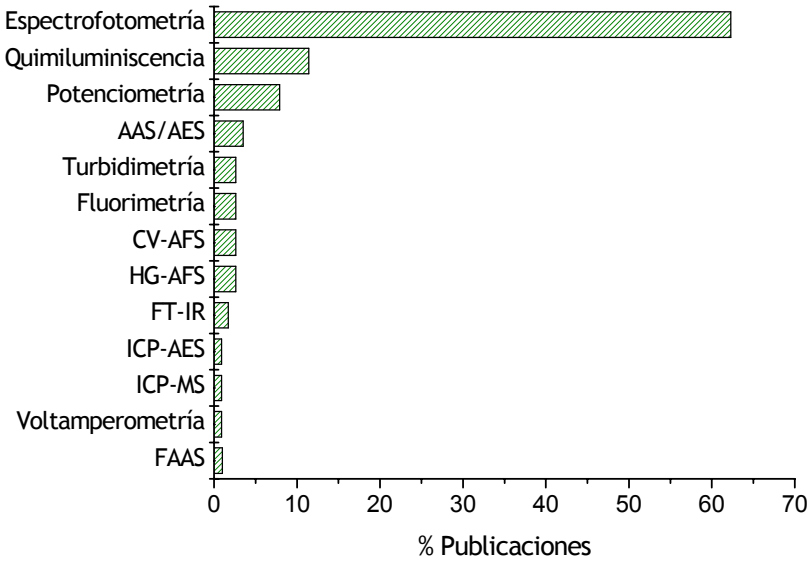


Fig. 29. Técnicas acopladas a la multiconmutación.

De los trabajos realizados mediante detección espectrofotométrica, un 41 % de éstos se dedican a la determinación de compuestos orgánicos y un 34 %, 19 % y 5 % a la de metales, compuestos inorgánicos y ácidos, respectivamente. En la Fig. 30 también puede apreciarse que las matrices más analizadas en estos artículos son las aguas (26 %), los fármacos (23 %) y las plantas (13 %).

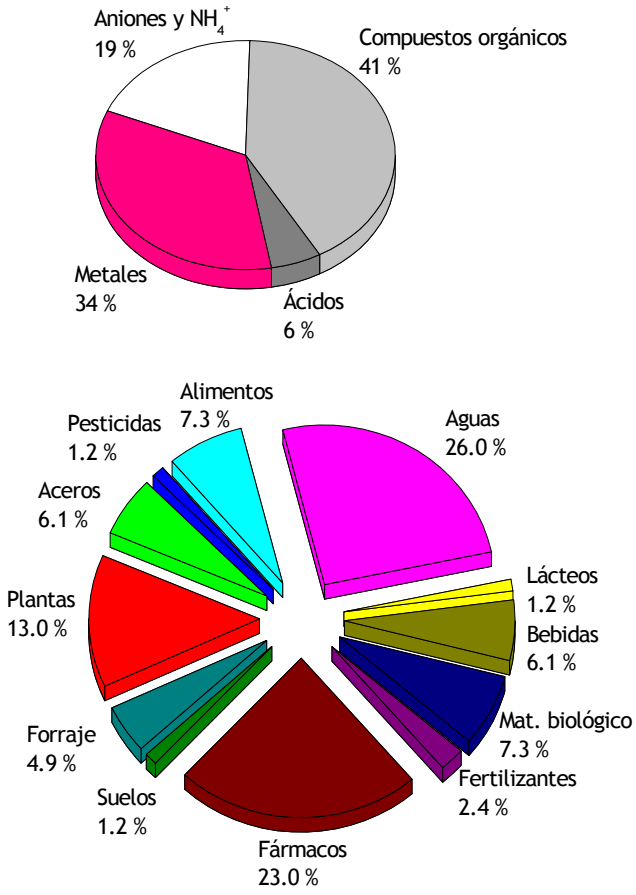


Fig. 30. Analitos determinados y matrices analizadas en detecciones espectrofotométricas.

Como se ha indicado, una cantidad destacable de los trabajos publicados en multiconmutación realiza valoraciones potenciométricas y espectrofotométricas. El esquema experimental no siempre es el mismo; de hecho, la inserción de muestra y reactivos se realiza en proporciones variables dependiendo del tiempo que las válvulas permanecen en ON. Este tipo de valoraciones puede considerarse como una estrategia de aproximaciones sucesivas por variación de la fracción volumétrica de la disolución valorante hasta localizar el punto final. Ejemplos de estas valoraciones son la titración de Ca con ácido etilenglicol-bis-N,N'-tetraacético (EGTA) [Wang: 1998 a], la determinación de la acidez en vinagres y zumos [Martelli: 1999] [Almeida: 2000 c] o en aguas residuales [Almeida: 2000 b] [Almeida: 2001].

El esquema básico de este tipo de valoraciones es el que se indica en la Figura 31:

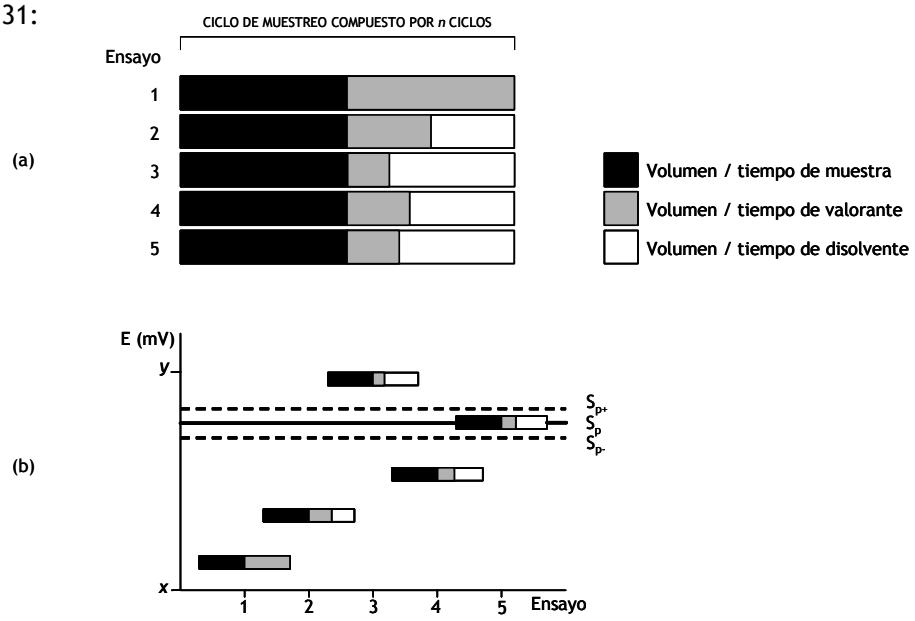


Fig. 31. Representación esquemática de una valoración por multiconmutación: (a) Perfil de muestreo, (b) Resultados hipotéticos. Sp: lectura promedio de la línea base; Sp+ = Sp + *q*s; Sp- = Sp - *q*s.

La señal generada por el electrodo cuando únicamente la disolución portadora fluye a través del camino de reacción se registra previamente a comenzar con los ciclos analíticos (Sp, medida de la línea base). Este valor se guarda como medida de referencia para decidir sobre el curso de la valoración tras cada ensayo y, también, para hallar el punto final. Si la señal generada por un ciclo analítico es superior al valor inicial de Sp existe un exceso de disolución de muestra, por lo que se incrementa una fracción volumétrica de disolvente en el siguiente ciclo. En caso contrario, si la señal es más baja que Sp se reduce la disolución de valorante, ya que existe un exceso del mismo en la disolución.

La Fig. 31 permite estudiar la estrategia seguida en el desarrollo de una valoración hipotética. Los volúmenes de muestra y valorante se introducen en el sistema en función del tiempo. Tras la introducción de pulsos de muestra y valorante, el ordenador lee la diferencia de potencial generada por el potenciómetro. Las señales recogidas se procesan para decidir si incrementar o reducir la alícuota de valorante. Como se indica en la Fig. 31, el volumen de disolución de valorante se varía según los límites que se establecen en la siguiente ecuación:

$$Ss = Sp \pm qs \qquad (1),$$

donde Ss es la señal leída tras cada ciclo, Sp es la señal de la línea base, q es una constante arbitraria y s es la desviación estándar de la línea base.

Para desarrollar el primer ciclo se insertan volúmenes iguales de muestra y valorante. Si no se alcanzan las condiciones estequiométricas, el volumen de valorante se incrementa o reduce con respecto a la última alícuota de valorante, según las siguientes ecuaciones:

$\Delta V_0 = V_1/2$ (2)

$V_2 = V_1 + \Delta V_0$ (3)

$V_2' = V_1 - \Delta V_0$ (4)

donde ΔV_0 es la variación del volumen de valorante, V_1 es el volumen de valorante del primer ciclo, V_2 y V_2' son los volúmenes de valorante de los siguientes ciclos.

Si el valor de la lectura es más alto que el rango definido en la ecuación (1), la concentración de la disolución de muestra es mayor que la de valorante, y la fracción volumétrica de valorante debe incrementarse como indica la ecuación (3). Si es menor, la disolución de valorante se diluye según se indica en la ecuación (4). De forma general, en los siguientes ciclos se procede siguiendo las relaciones matemáticas que se indican a continuación:

$\Delta V_i = V_{i-1}/2$ (5)

$V_i = V_{i-1} + \Delta V_i$ (6)

$V_i' = V_{i-1} - \Delta V_i$ (7)

Esta estrategia se continúa hasta que se alcanzan las condiciones establecidas en la ecuación (1).

Todos los caminos seguidos por la valoración hipotética llevada a cabo por multiconmutación se concluyen en el paso 5 de la Fig. 31, donde se alcanza el punto final.

2. OBJETIVOS

2. OBJETIVOS

Una línea prioritaria en la Química Analítica es el desarrollo de dispositivos que incrementen el grado de automatización y, por tanto, reduzcan la participación humana en los procedimientos de análisis. Sin embargo, no es suficiente con mecanizar los métodos, sino que es necesario que dichos métodos proporcionen resultados adecuados a las exigencias de la sociedad actual, en términos de sensibilidad, exactitud, precisión, robustez y rapidez. Además, las estrategias adoptadas deben estar encaminadas hacia una química "verde", evitando el uso de contaminantes, minimizando el consumo de reactivos y muestras y reduciendo los residuos generados, bien tratados químicamente o bien reciclados, al máximo.

Por otra parte, el ritmo acelerado de la sociedad, la aparición diaria de nuevos problemas y la mejora de la calidad de vida exigen, cada vez más, la determinación inmediata y a bajo coste de los analitos. Por ello, también es necesario dirigir las investigaciones hacia el desarrollo de sistemas portátiles y económicos que permitan realizar análisis in situ con la mínima intervención del operador y con recogida puntual de datos durante tiempos prolongados. E incluso es interesante el envío simultáneo de la información a centros de control para su tratamiento y posterior evaluación.

Toda esta demanda de información hace que los químicos analíticos nos planteemos el estudio de nuevas estrategias y de alternativas a los métodos de análisis oficiales y tradicionales para el desarrollo de metodologías sencillas, de bajo coste, rápidas e inocuas para el medio ambiente, que permitan la determinación de múltiples analitos y que estén al alcance de cualquier laboratorio de control o de investigación.

El principal objetivo de esta Tesis es generalizar el empleo de la

multiconmutación como la herramienta adecuada para la determinación de una amplia variedad de analitos, desde compuestos orgánicos a metales, en el análisis de distintas matrices y en el uso de técnicas de detección como la espectroscopia de fluorescencia atómica, la espectrofotometría molecular y la quimioluminiscencia, mediante el desarrollo de una serie de procedimientos analíticos directos, sencillos y limpios, que sean una alternativa en coste y rapidez a los métodos tradicionales.

Los diferentes métodos de análisis se desarrollarán en base a incrementar la frecuencia de muestreo, reducir consumos de reactivos, generar la mínima cantidad de residuos contaminantes y, todo ello, en términos de máxima versatilidad, sencillez y economía, con el fin de que puedan ser implantados tanto en laboratorios como en la industria. Se determinarán, en cada caso, las características analíticas y se destacarán las ventajas de cada uno de los trabajos.

Para alcanzar estos propósitos se plantean los siguientes objetivos:

1. Proponer la multiconmutación para el desarrollo de procedimientos de análisis en diversas áreas de la Química Analítica.
   Para generalizar el uso de la multiconmutación se propondrán procedimientos que permitan la determinación de analitos de distinta naturaleza (Hg(II), Bi(III), Te(IV), tensioactivos, fenol, ciclamato, H_2O_2, NH_4^+, Fe(III), NO_2^-, carbaril) en diferentes tipos de muestras (aguas, lácteos, edulcorantes), de forma que se cubran diversos campos de la Química Analítica.

2. Estudio y desarrollo de los respectivos programas informáticos en Quick BASIC 4.5 y Visual BASIC 6.0.

3. Búsqueda de las condiciones de medida más adecuadas para la

determinación cuantitativa de Hg por CV-AFS y Bi y Te por HG-AFS, tensioactivos aniónicos, fenol, ciclamato, Fe(III), NO_2^- y carbaril, por espectrofotometría molecular, H_2O_2 y NH_4^+ por quimioluminiscencia, en términos de máxima sensibilidad, precisión, exactitud, robustez y rapidez. Se establecerán y se intentarán mejorar las características analíticas de los métodos propuestos en la bibliografía.

3.1. Sensibilidad. Los métodos deben ser capaces de proporcionar límites de detección apropiados a los niveles de concentración del analito en la muestra.

Se comprobará que la multiconmutación acoplada a técnicas de detección como la fluorescencia atómica, la espectrofotometría molecular y la quimioluminiscencia, proporciona una sensibilidad adecuada y comparable a la de los métodos de referencia.

3.2. Exactitud. El procedimiento debe estar libre de errores sistemáticos y, al mismo tiempo, las variaciones debidas a la existencia de errores aleatorios deben permanecer dentro de unos intervalos definidos. Para comprobar la exactitud de los procedimientos se validarán los resultados empleando distintas estrategias que se fundamentarán principalmente en el empleo de métodos de referencia publicados. Cuando ello no sea suficiente o posible, se recurrirá al estudio de recuperaciones en el análisis de muestras enriquecidas o a la comparación con muestras certificadas o ejercicios interlaboratorio.

3.3. Precisión. Con el fin de obtener un valor óptimo de este parámetro, y considerando que a ello contribuyen tanto las características instrumentales del equipo utilizado como toda la manipulación realizada sobre las muestras, se realizará una selección adecuada de los parámetros instrumentales que proporcionen las mejores condiciones de medida y se intentará reducir o eliminar al máximo

las etapas de pretratamiento de las muestras.

3.4. Robustez. La capacidad de resolución del método no debe verse afectada por pequeñas variaciones de los parámetros del procedimiento y, por otra parte, los resultados deben ser reproducibles siempre que el procedimiento se aplique correctamente bajo idénticas condiciones. Para conseguir que los métodos de análisis propuestos por multiconmutación sean robustos, se intentará reducir, siempre que sea posible, el número de variables experimentales a controlar durante el proceso de análisis, de manera que mejore simultáneamente la repetibilidad y reproducibilidad de los resultados.

3.5. Rapidez. Se pretende también que los procedimientos desarrollados proporcionen una alta frecuencia de análisis. La multiconmutación permite realizar los análisis en línea disminuyendo el tiempo de análisis en comparación con los métodos de referencia. Se intentará desarrollar procedimientos que permitan la determinación secuencial de más de un analito, para contribuir a incrementar la productividad.

4. Minimizar el tiempo de análisis, el consumo de reactivos y muestras y la generación de residuos.
   Se desarrollarán métodos analíticos multiconmutados que permitan la minimización de la cantidad de los residuos generados y paralelamente se obtendrá un ahorro de reactivos, una mayor productividad, reducción de costes de gestión y tratamiento de residuos y mejoras en las condiciones de seguridad e higiene en el trabajo.

5. Desarrollo de un sistema mecanizado, rápido y menos contaminante, al reducir los consumos de reactivos y muestras y la generación de residuos, para la determinación de Hg en muestras de agua.

6. Diseño y explotación de un separador gas-líquido de tamaño reducido, para la determinación de Hg en aguas por CV-AFS, para incrementar la sensibilidad analítica sin sacrificar la frecuencia de muestreo y reducir consumos y desechos.

7. Desarrollo de metodologías con formación de suspensiones, menos agresivas que las digestiones asistidas por microondas, y mecanización de los procedimientos analíticos para la determinación de Te y Bi en productos lácteos por HG-AFS.

8. Estudio de un sistema en línea para el tratamiento de los residuos ácidos y metales pesados generados en la determinación de Bi, con el fin de conseguir una metodología medioambientalmente sostenible.

9. Desarrollo de una metodología no cromatográfica, sensible, rápida y sencilla para la especiación de Te (IV) y Te (VI) en muestras de leche utilizando HG-AFS.

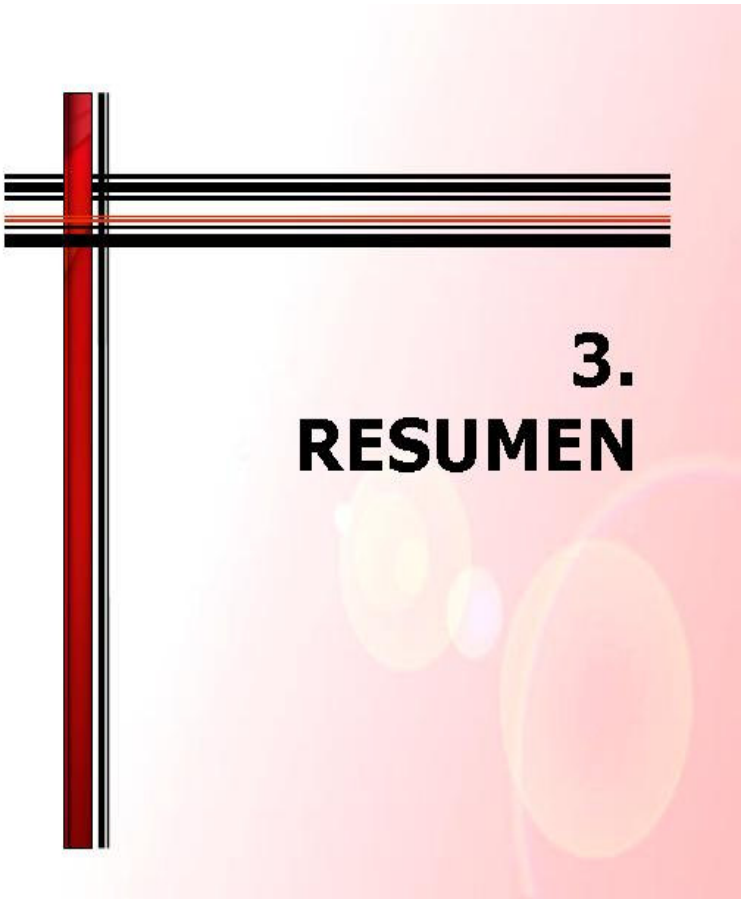
10. Puesta a punto de un procedimiento rápido, y con consumo mínimo de disolventes, para realizar extracciones líquido-líquido en línea para la determinación de tensioactivos aniónicos.

11. Puesta a punto de métodos rápidos, miniaturizados, poco contaminantes y económicos haciendo uso de minibombas solenoides y evitando el uso de la bomba peristáltica.

    11.1. Determinación de fenoles en muestras de agua por espectrofotometría molecular.
    11.2. Determinación de ciclamato en edulcorantes comerciales por espectrofotometría molecular.

12. Diseño de equipamientos de bajo coste y portátiles: un luminómetro para la determinación directa de H_2O_2 e indirecta de NH_4^+ y un fotómetro con LEDs de diferentes longitudes de onda, para la determinación de hierro, nitrito, carbaril y fenol sin modificar el esquema de flujo.

Por último, se expondrán las conclusiones más importantes y se analizarán las limitaciones y las ventajas que presenta el uso de la multiconmutación.



3. RESUMEN

3. RESUMEN

La presente Tesis Doctoral integra 10 trabajos que pueden estructurarse en tres áreas distintas: 1) Mecanización de las medidas de AFS; 2) Nuevos avances en la mecanización de las medidas obtenidas por espectrofotometría molecular; 3) Incorporación de la multiconmutación a instrumentos portátiles y de bajo coste.

1) En el primer bloque, la multiconmutación permite la mecanización para la determinación de metales por CV-AFS y HG-AFS. Consta de tres trabajos en los que se estudian las condiciones óptimas para la determinación del contenido en Hg, Bi y Te y un trabajo dedicado a la especiación en línea de Te. Éstos son los primeros trabajos recogidos en la bibliografía sobre el acoplamiento de la multiconmutación a la fluorescencia atómica. Según el metal y la estrategia desarrollada los trabajos se han dividido en: (i) Determinación de Hg inorgánico en aguas por CV-AFS. (ii) Determinación de Bi (III) en batidos por HG-AFS. (iii) Determinación de Te por HG-AFS en muestras de leche. (iv) Especiación de Te(IV) / Te(VI) en muestras de leche.

2) En el segundo bloque se ha desarrollado la multiconmutación como herramienta analítica para la mecanización de las aplicaciones espectrofotométricas. Los tres trabajos que incluyen este bloque se han dividido en dos líneas de trabajo: (i) Extracción líquido-líquido en línea para la determinación de tensioactivos aniónicos en aguas. Esta mecanización consigue una gran mejora en la frecuencia de muestreo y constituye uno de los primeros trabajos para la aplicación de la multiconmutación a la preconcentración en línea mediante la extracción líquido-líquido. (ii) Empleo de las minibombas como unidades propulsoras de fluidos y como sustitutas de las válvulas solenoides, con el objetivo de abaratar costes al no ser necesaria la bomba peristáltica. Esta estrategia se ha propuesto para las determinaciones de fenol en muestras de agua y ciclamato en edulcorantes.

3) El tercer apartado está dedicado a los beneficios de la multiconmutación en su aplicación a instrumentos portátiles. Se desarrollan dos equipos de bajo coste y peso: (i) Luminómetro, para la determinación directa de H_2O_2 e indirecta de NH_4^+. (ii) Fotómetro de LEDs, para la determinación de Fe (III), NO_2^-, fenol y carbaril.

Todos estos estudios presentan como rasgo común el uso de la multiconmutación para elaborar metodologías contrastadas, sencillas, rápidas, económicas, portátiles y medioambientalmente sostenibles, en distintos campos de la Química Analítica.

3.1. *HARDWARE* Y *SOFTWARE*

3.1.1. MONTAJE DE LOS MÓDULOS DE ANÁLISIS

Previamente a la aplicación de la multiconmutación a cualquier procedimiento analítico o a cualquier técnica de detección, es necesaria su conexión al ordenador y la programación de los dispositivos solenoides.

En los trabajos con válvulas de 3 vías, el equipo se ensambló para ser controlado por un ordenador PC-486 o superior compatible, con una tarjeta interfaz entre el ordenador y las válvulas PCL-711S (American Advantech CA) acoplada a su placa base. Esta interfaz suministra ocho líneas digitales de entrada y ocho de salida, un convertidor analógico-digital (ADC) y un convertidor digital-analógico (DAC), ambos con un rango de 12 bits de resolución.

Las válvulas solenoides necesitan una diferencia de potencial eléctrica de 12 V y una intensidad de corriente de aproximadamente 100 mA para ser activadas, sin embargo esta corriente no puede ser proporcionada por la interfaz

PCL-711S desde el ordenador. Para salvar este obstáculo, la interfaz se diseña según la Fig. 32 [Reis: 1994].

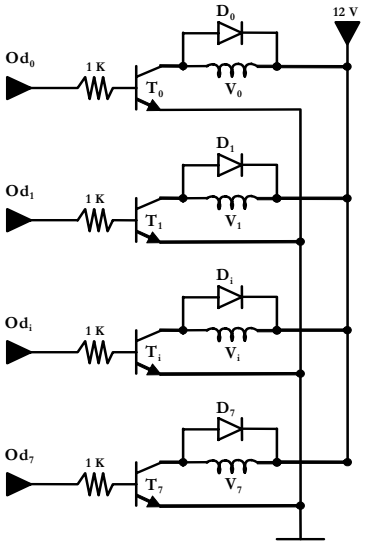


Fig. 32. Diagrama electrónico de la interfaz que conecta las válvulas solenoides. Vi = válvulas solenoides; Ti = transistores BC547; Di = diodos 1N4002; Odi = líneas de salida digital de la tarjeta interfaz PCL-711S. Todas las resistencias se expresan en Ohm.

En los trabajos desarrollados haciendo uso de las minibombas, el módulo de análisis se montó utilizando la misma estructura física aplicada para las válvulas solenoides, sin embargo la parte electrónica fue alterada de modo que el accionamiento de las minibombas se realizara por el puerto paralelo de la impresora, eliminándose la interfase electrónica modelo PCL-711S. Se confeccionó un sistema electrónico simple utilizando resistores y transistores, como muestra la Fig. 33 para la minibomba 1. Las conexiones con el resto de minibombas son semejantes a las representadas en la Fig. 33.

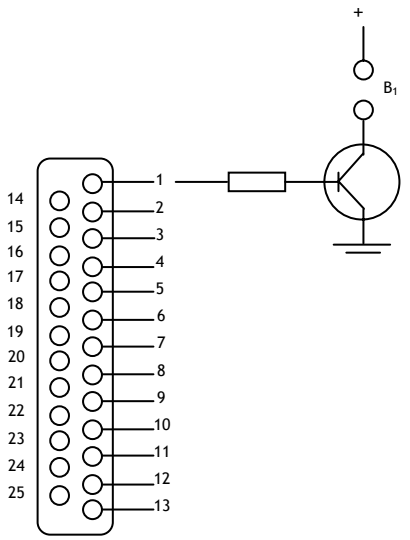


Fig. 33. Esquema electrónico para el accionamiento de las minibombas por el puerto paralelo de la impresora. B_1 – minibomba 1.

3.1.2. DESCRIPCIÓN DE LOS PROGRAMAS INFORMÁTICOS

Los programas computacionales de control y adquisición de datos para implementar los procesos de multiconmutación han sido escritos en Quick BASIC 4.5, Visual BASIC 3.0 y Visual BASIC 6.0. En estos programas, las opciones de entrada o de alteración de las variables de entrada se encuentran distribuidas en *menús* disponibles en la pantalla del monitor.

En la Fig. 34 se esquematiza el diagrama de flujo del funcionamiento del programa que controla las válvulas y minibombas. El programa está constituido por un conjunto de subrutinas que permiten escoger la opción deseada y en las que se introducen los parámetros de control que el ordenador necesita cuando el programa se inicia. Una vez proporcionadas estas informaciones, el ordenador efectúa todas las etapas del procedimiento analítico seleccionado, sin asistencia por parte del operador.

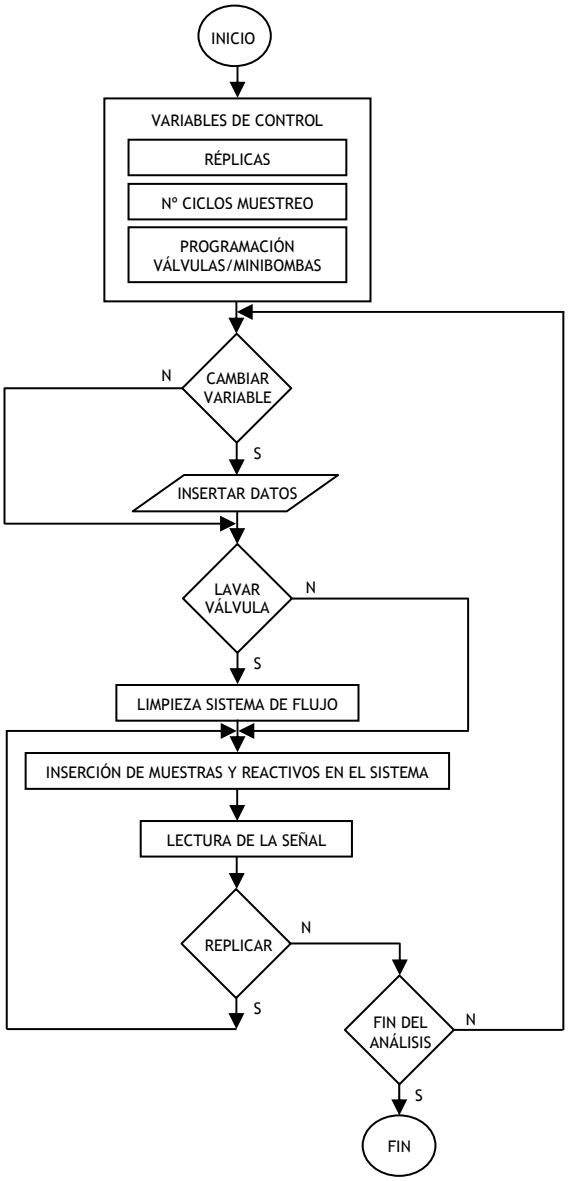


Fig. 34. Diagrama de flujo diseñado para multiconmutación por CV-AFS y HG-AFS.

En la Fig. 35 se muestra, a modo de ejemplo, la ventana principal del programa diseñado para la determinación de tensioactivos aniónicos en aguas por espectrofotometría molecular, utilizando válvulas solenoides. Se divide en diferentes líneas de comando con las entradas de las respectivas variables, vía teclado del ordenador, permitiendo a los usuarios interactuar con el programa seleccionando las variables de entrada de datos a través de cajas de texto. Todos los trabajos desarrollados en esta Tesis presentan modelos similares de programa informático.

Las opciones de trabajo del programa se describen a continuación:

- ***Muestrear:*** Se dan las opciones de comenzar con el muestreo o parar de forma inmediata el funcionamiento de las válvulas.
- ***Nº réplicas:*** Variable de entrada para el número de réplicas, es decir, para el número de determinaciones estipulado para cada muestra. El programa también realiza un cómputo del número de réplicas ejecutadas.
- ***Nº ciclos muestreo:*** Variable de entrada y cómputo del número de pulsos necesarios para efectuar una determinación.
- ***Programación válvulas:*** Variables de entrada y cómputo del tiempo en ON de las válvulas solenoides, es decir, del tiempo necesario para la inserción de reactivos y muestras, SDS, MB y $CHCl_3$, en el ejemplo escogido, y para el mezclado, extracción, separación de fases, desplazamiento de la fase orgánica y vaciado de la celda de flujo. Junto a cada una de las secuencias llevadas a cabo se indican las válvulas implicadas en el proceso.
- ***Guardar/Leer:*** Guarda y lee las variables de entrada en un archivo creado para tal fin.
- ***Longitud de onda:*** Indicación de la λ de trabajo.

- ***Línea base*:** Variable de entrada del tiempo necesario para insertar la disolución adecuada para establecer la línea base, previamente a comenzar las lecturas.
- ***Lava válvulas:*** Permite seleccionar las válvulas para ser lavadas con agua o con las disoluciones de muestra o reactivo.

Los programas desarrollados en cada determinación junto con los dispositivos solenoides permiten construir módulos de análisis híbridos y de estructuras compactas y versátiles, que posibilitan la implementación de los diferentes sistemas, únicamente variando determinadas secuencias del programa y las variables de entrada.

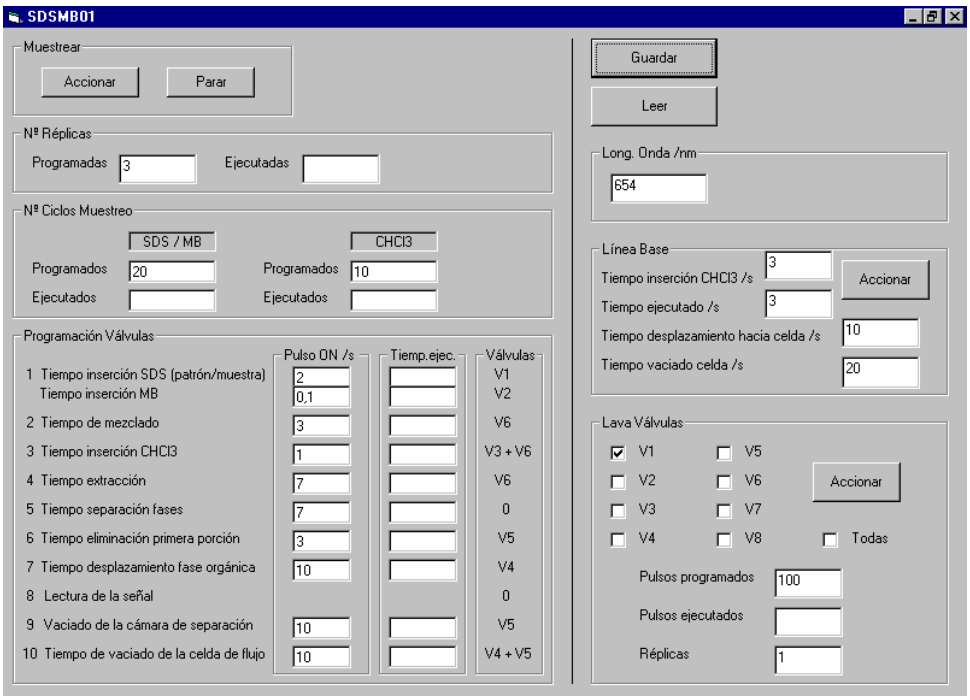


Fig. 35. Ventana de ejemplo de los programas informáticos diseñados para el control de las válvulas y minibombas, con sus correspondientes entradas para la inserción de variables y para la interacción con el usuario.

3.2. MECANIZACIÓN DE LAS MEDIDAS DE AFS

Las medidas por AFS ofrecen las siguientes ventajas: (i) separación del analito de la matriz, (ii) selectividad, (iii) posibilidad de especiar y (iv) fácil automatización. Sin embargo, la fluorescencia atómica es una técnica que se ve muy afectada por el estado de oxidación de los analitos y algunas condiciones experimentales, como la acidez de las muestras, la concentración del reductor y los caudales de los gases de transporte, por lo que es necesaria la optimización de estos parámetros para cada analito y un control estricto de las condiciones experimentales.

Para aprovechar al máximo las características de un equipo instrumental es necesario buscar aquellas condiciones experimentales en las que se obtenga una mayor señal y una mejor precisión de las medidas. Como etapa previa a esta búsqueda, se consideró conveniente determinar la sensibilidad que es posible alcanzar para los diferentes elementos en las condiciones propuestas por el fabricante y, de esta forma, tener un punto de referencia para, posteriormente, explotar los beneficios que ofrece la multiconmutación.

Hasta la publicación de los trabajos presentados en esta Tesis Doctoral no existían precedentes en la literatura del uso de la multiconmutación en el campo de la AFS. El uso de la multiconmutación acoplada a la fluorescencia atómica, en comparación con los métodos comerciales en continuo, repercute en un aumento de la productividad del laboratorio, incrementa el número de análisis por hora, minimiza al máximo el consumo de reactivos y muestras y el volumen de residuos y permite el tratamiento sencillo y en línea de los desechos generados.

3.2.1. VAPOR FRÍO Y GENERACIÓN DE HIDRUROS

Las técnicas de vapor frío y generación de hidruros reducen sustancialmente los límites de detección de la AFS. El análisis mediante estas técnicas incluye tres etapas: la generación del analito volátil, su transferencia al atomizador y su descomposición en átomos (esta etapa no es necesaria para el mercurio).

En la técnica del vapor frío, el mercurio se reduce a su estado elemental, Hg (0), seguido de su transporte y detección por fluorescencia. Esta técnica sólo es aplicable al mercurio, dado que es el único elemento metálico que es líquido a temperatura ambiente y posee una presión de vapor relativamente elevada (0.0016 mbar a 20 ºC).

La técnica de generación de hidruros se puede usar para generar los hidruros de diversos elementos: As, Bi, Ge, Pb, Sb, Se, Sn y Te. La reducción de los analitos (M) a sus hidruros se realiza habitualmente con tetrahidroborato sódico, de forma inmediata, según la reacción:

$$NaBH_4 + 3\ H_2O + HCl \rightarrow\ H_3BO_3 + NaCl + 8\ H^+ \xrightarrow{+\ M^{n+}}\ MH_n + H_2$$

La diferencia existente entre CV-AFS y HG-AFS es que en la detección por vapor frío, el reactivo $SnCl_2 \cdot 2H_2O$ reduce el Hg (II) a Hg metal a temperatura ambiente, mientras que en la técnica de generación de hidruros es necesario tratar las muestras y patrones con una disolución reductora de $NaBH_4$ para generar los hidruros volátiles de TeH_2 y BiH_3, elementos estudiados en la presente Tesis Doctoral, y, posteriormente, la existencia de una llama para atomizar el Te (IV) y el Bi (III). En el caso de CV-AFS es preferible utilizar el $SnCl_2$ como reductor, ya que el uso de $NaBH_4$ aumenta el riesgo de interferencias porque es un reductor más potente y se pueden cogenerar hidruros de otros elementos presentes en la muestra.

3.2.2. ANÁLISIS POR CV-AFS

Con la aplicación de la multiconmutación se pretende evaluar la influencia de una mecanización completa sobre las medidas de fluorescencia atómica por vapor frío. Con estos estudios se evalúan los parámetros analíticos del método en términos de exactitud, precisión, robustez, frecuencia de muestreo, consumo de reactivos y muestras y generación de residuos, en la determinación de Hg inorgánico en diferentes muestras de agua.

El uso de la multiconmutación repercute en una simplificación y completa mecanización del sistema, una drástica reducción del consumo de muestras, reactivos y Ar, en factores de 6.0, 8.4 y 6.0, respectivamente, una minimización de los residuos generados aproximadamente a la mitad, así como un incremento en la frecuencia de muestreo de 3.6 veces, en comparación con el método comercial de medidas en continuo. La multiconmutación ofrece, como consecuencia, una alternativa económica y medioambientalmente sostenible y una mejora en la técnica para la determinación de Hg inorgánico en muestras de agua.

El equipo comercial *PSA Merlín* está equipado con una cámara de separación de 17 mL de volumen interno. Para mejorar las medidas de fluorescencia del Hg, nuestro grupo ha diseñado un separador de volumen reducido de sólo 5 mL de volumen interno y se ha evaluado su comportamiento utilizando la multiconmutación. El separador diseñado puede incrementar la señal analítica para tiempos de muestreo y lectura fijos. Además, ofrece la posibilidad de usar un tiempo de muestreo menor, sin sacrificar la sensibilidad analítica, y de aumentar la productividad del laboratorio, incrementando el número de análisis por hora, ya que la cámara de separación de 5 mL, minimiza el tiempo de medida de 3.5 min a 1 min (considerando que el sistema de medida

podría alcanzar el estado estacionario en menos de 30 s). Adicionalmente, la reducción del volumen del separador gas-líquido proporciona una inserción más apropiada del flujo de Ar.

3.2.3. ANÁLISIS POR HG-AFS

La mayoría de los elementos capaces de formar hidruros (como el Bi, Te, Se, As, Sb) pueden existir en diferentes estados de oxidación. La forma del hidruro correspondiente depende del estado de oxidación, puesto que únicamente se forman de manera cuantitativa a partir del estado de oxidación IV (Te, Se) o III (As, Sb) y, por tanto, para determinar contenidos totales es necesario reducirlos previamente. El Bi no requiere etapa de prerreducción, ya que el estado V es metaestable, por lo que ya se encuentra como Bi (III).

Los análisis realizados por el método en continuo [Cava-Montesinos: 2003 a] [Cava-Montesinos: 2003 b] [Cava-Montesinos: 2004 a] han demostrado ser lentos, no económicos, ya que consumen elevadas cantidades de muestras y reactivos, y excesivamente contaminantes por el gran volumen de residuos generados, muy ácidos y con metales pesados.

En un intento por superar estos inconvenientes se ha aplicado la multiconmutación a la HG-AFS, y se han obtenido resultados muy positivos y beneficiosos: (i) mecanización del sistema, (ii) reducción de consumos, (iii) minimización de residuos, (iv) incremento de la productividad. Se ha determinado Bi y Te en batidos y leches, formando suspensiones [Cava-Montesinos: 2004 a] como método eficaz de digestión de las muestras, previamente a las medidas por HG-AFS. Los procedimientos desarrollados representan una alternativa sensible y precisa para el análisis de productos lácteos, así como la posibilidad de realizar sencillos pretratamientos de las muestras, basados en la sonicación a temperatura ambiente de las suspensiones formadas con agua regia.

La adecuada inserción del caudal de Ar, al igual que en el caso del Hg, minimiza el tiempo de análisis e incrementa la sensibilidad. El uso de un caudal adicional de hidrógeno ayuda al mantenimiento de la llama y a la formación eficiente de los hidruros.

Las determinaciones de Te y Bi por HG-AFS se realizan únicamente con el separador de 17 mL, ya que las características del sistema *PSA Excalibur* hacen inviable el uso de una cámara de tamaño más reducido.

3.2.3.1. Mecanización de la especiación

El diferente comportamiento en la generación de hidruros de la especie de Te puede aprovecharse para llevar a cabo una especiación no cromatográfica, seleccionando adecuadamente los parámetros experimentales.

Dado que la especie de Te (VI) no forma hidruros y no proporciona señal, se hace imprescindible una etapa previa de reducción. Se opera de la siguiente forma: se mide el contenido de Te (IV), se procede a la reducción cuantitativa de las especies presentes con KBr en menos de 2 min y se determina el Te total. El Te (VI) se obtiene por diferencia entre el Te total y el Te (IV). La reacción redox que tiene lugar es la siguiente:

$$TeO_4^{2-} + 4\,H^+ + 2\,Br^- \rightarrow H_2TeO_3 + H_2O + Br_2$$

La especiación de Te se aplicó a muestras de leche de vaca y cabra. El tratamiento de la leche se realizó por sonicación con agua regia, comprobándose previamente que este tratamiento no producía modificaciones en los estados de oxidación originales de la muestra. La combinación de la multiconmutación con la especiación permite la determinación de Te (IV) y Te (VI) en leche y contribuye a la mecanización de los métodos para especiar, mejorando

características previamente comentadas como la frecuencia de muestreo, consumos y minimización de residuos.

3.2.3.2. Tratamiento de los residuos generados

Uno de los problemas de la Química Analítica es la generación de residuos debido a los análisis químicos. En este sentido, la multiconmutación permite la mecanización del tratamiento de los residuos generados en AFS utilizando dos válvulas solenoides adicionales para el control de NaOH y $FeCl_3$. De esta forma se consiguen neutralizar los desechos ácidos (pH inferior a 1). A pH neutro, el hierro precipita como $Fe(OH)_3$ y los metales pesados en el residuo líquido precipitan o coprecipitan con el hierro, proporcionando un volumen de sólido reducido y una disolución medioambientalmente adecuada.

3.2.4. VENTAJAS DE LA MULTICONMUTACION EN LA INSERCIÓN DEL CAUDAL DE Ar

Uno de los parámetros que más influencia ejerce en la intensidad de fluorescencia es, sin lugar a dudas, el caudal del gas portador.

En el modo continuo (método seleccionado como referencia y recomendado por el fabricante), el argón se emplea para la separación y transporte del mercurio y los hidruros de teluro y bismuto en fase gas de la mezcla de reacción y, siguiendo el diseño del equipo, el Ar es burbujeado a través de la fase líquida al final del proceso. Por tanto, en esta modalidad el Ar se emplea únicamente para separar el Hg gaseoso y los hidruros volátiles de la mezcla de reacción, diluye el analito y lo transporta.

En multiconmutación el Ar se inserta en el camino analítico junto a la mezcla de los reactivos. En esta situación, el Ar actúa causando la multisegmentación de la zona de muestra, el transporte es más eficiente y las señales de fluorescencia alcanzan muy rápidamente el estado estacionario proporcionando la posibilidad de reducir el tiempo de medida.

3.3. EXTRACCIÓN LÍQUIDO-LÍQUIDO EN LÍNEA

Uno de los estudios más interesantes de la presente Tesis Doctoral consiste en la mecanización de un sistema de extracción líquido-líquido, para la determinación de tensioactivos aniónicos en muestras líquidas, empleando el método espectrofotométrico del azul de molibdeno (MB).

Los tensioactivos aniónicos, en general, disueltos en agua son débilmente solubles en cloroformo. Por otra parte, el MB se disuelve bien tanto en cloroformo como en agua y proporciona una disolución de color azul en ambos casos. El hecho de que el par iónico, formado entre los tensioactivos aniónicos y el MB, pueda ser extraído en cloroformo (esquema Fig. 36) es aprovechado, junto a la multiconmutación, para incrementar la frecuencia de muestreo y reducir consumos de muestras, de reactivos y de disolventes orgánicos.

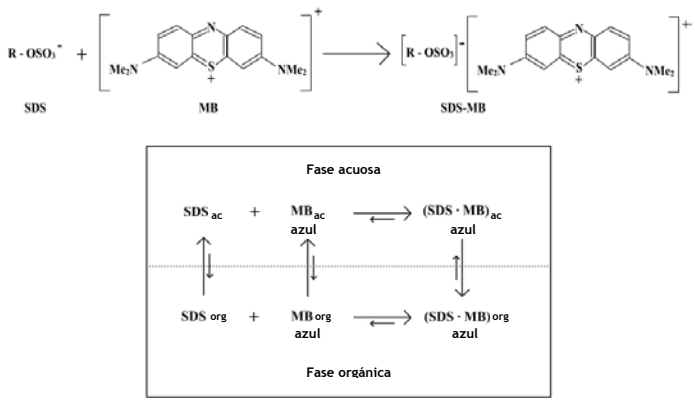


Fig. 36. Reacción entre el dodecilsulfato sódico y el azul de metileno en fase acuosa y orgánica.

Las extracciones líquido-líquido presentan como ventajas la separación de la matriz de la muestra y la preconcentración de los analitos. Adicionalmente, su automatización evita la manipulación directa de los disolventes orgánicos y, por tanto, los vapores generados peligrosos para la salud [Bergamin: 1978 b] [Kamburova: 1998].

En este sentido, el trabajo propuesto presenta diversas ventajas en comparación con el método de referencia [Council Directive EEC: 1982] y de otras alternativas previamente desarrolladas [Koga: 1999] [Chitikela: 1995] [Agudo: 1994] y ofrece una alternativa medioambientalmente sostenible a este tipo de determinaciones:

1. Elimina el uso de los embudos de decantación.
2. Reduce el volumen de muestra a sólo 3.6 mL, reducción considerable en comparación con 100 mL [Council Directive EEC: 1982], 50 mL [Koga: 1999] [Agudo: 1994] o 15 mL [Chitikela: 1995].
3. En términos de consumo de $CHCl_3$, únicamente se requieren 700 µL por determinación, volumen claramente inferior a los 45 mL del método de referencia [Council Directive EEC: 1982], a 30 mL [Chitikela: 1995] y a 5 mL [Koga: 1999], siendo únicamente superior a los 200 µL empleados en la extracción líquido-líquido en continuo [Agudo: 1994].
4. La multiconmutación ofrece una frecuencia de muestreo de 40 h^{-1}, doble que la obtenida por el procedimiento en FIA [Agudo: 1994].
5. Proporciona un límite de detección de 1.7 µg/L, por lo que el sistema es más sensible que trabajos anteriores (20 µg/L) [Koga: 1999] [Agudo: 1994].
6. La extracción y la detección se integran en el mismo esquema y todas las operaciones pueden ser controladas electrónicamente.

Otros procedimientos de análisis con extracción líquido-líquido que emplean la multiconmutación han sido propuestos en la literatura, concretamente en las determinaciones de molibdeno y plomo en plantas [Comitre: 2003] [Comitre: 2005]. Las ventajas del método desarrollado en nuestro grupo con respecto a éstos son: (i) un incremento en la frecuencia de muestreo, (ii) la inserción de las disoluciones en la cámara de extracción por gravedad, disminuyendo, de este modo, el número de canales de bombeo, (iii) no es necesario un bucle de reacción, ya que el mezclado y la extracción se realizan en la cámara líquido-líquido y (iv) la inserción de aire favorece el mezclado y mejora la propia extracción.

Por último, es de destacar que utilizando una cámara de extracción de dimensiones mayores (65 cm de altura y 0.7 cm de diámetro interno) se consiguen límites de detección inferiores, manteniendo la desviación estándar relativa del mismo orden.

3.4. UTILIZACIÓN DE MINIBOMBAS PARA LA INSERCIÓN DE DISOLUCIONES

El flujo pulsado se origina en un intento por mejorar los sistemas de introducción de disoluciones y el mezclado óptimo de pequeñas alícuotas de reactivos y muestras. Para intentar alcanzar estos objetivos se aplican las minibombas a la determinación de fenol en muestras de agua y a la determinación de ciclamato en edulcorantes, por espectrofotometría molecular.

El desplazamiento de las disoluciones empleando las minibombas se realiza mediante impulsos de un volumen fijo de fluido liberado por pulso de minibomba. Una determinada frecuencia de bombeo (pulsos s^{-1}) es la que fija el caudal de la disolución.

Se han llevado a cabo diversos estudios para determinar los mejores parámetros relativos al uso de la minibombas: duración del número de pulsos, relación de pulsos entre muestras y reactivos y número de ciclos de muestreo. En todos los experimentos realizados para las determinaciones de fenol y ciclamato utilizando el flujo multipulsado, las minibombas se colocan estratégicamente con el fin de direccionar de forma óptima las disoluciones y el orden de accionamiento se define en base al compromiso entre versatilidad, sensibilidad y repetibilidad. Los intervalos de tiempo, caudal de bombeo y estrategias de introducción de las disoluciones de muestras y reactivos son estudiados con el objetivo de encontrar las mejores condiciones de trabajo.

Con el fin de evaluar la repetibilidad de las minibombas, en cuanto a condiciones de dispersión, se varió el tiempo de accionamiento entre 0.1 y 1.0 s y se pesó la cantidad de agua bombeada en el intervalo de tiempo considerado. De estas medidas se deduce que el volumen correspondiente a cada pulso de la minibomba es de 8 ± 2 µL. Además, se observa que el volumen por pulso no varía en relación al caudal. Este hecho está de acuerdo con el fabricante, en el sentido en que el volumen inyectado es siempre de 8 µL por pulso y, por tanto, el caudal de bombeo está en función de la frecuencia de los pulsos. Las minibombas presentan buena estabilidad y repetibilidad, con desviaciones estándar relativas inferiores al 2 %.

La Fig. 37 muestra el efecto de la variación de tiempo (entre 0.1 y 0.3 s) entre aspirado/propulsión de las minibombas solenoides en la determinación de fenol por espectrofotometría molecular. Los valores de tiempo están referidos al tiempo durante el cual el diafragma de la minibomba aspira/impulsa los 8 ± 2 µL de disolución. No existen diferencias significativas entre el uso de 0.1 a 0.3 s. Sin embargo, los pulsos de 0.1/0.1 s demuestran ser los más adecuados para la obtención de mejor sensibilidad y picos más estrechos.

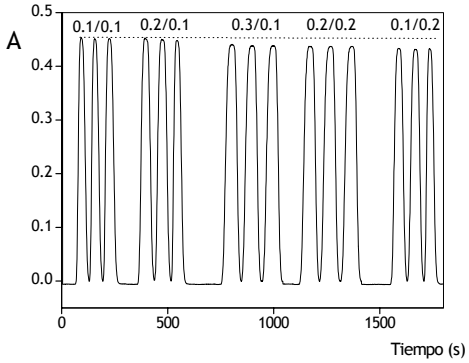


Fig. 37. Relación entre pulsos de llenado y vaciado.

Además, se estudia la variación de pulsos de muestreo hasta alcanzar condiciones adecuadas de mezclado. La Fig. 38 muestra, a modo de ejemplo, la variación del número de pulsos en la determinación de fenol. Se observa que 8 ciclos ya aseguran una reacción completa entre muestras y reactivos y que para un número de ciclos mayor a 11 se produce un ensanchamiento de los picos.

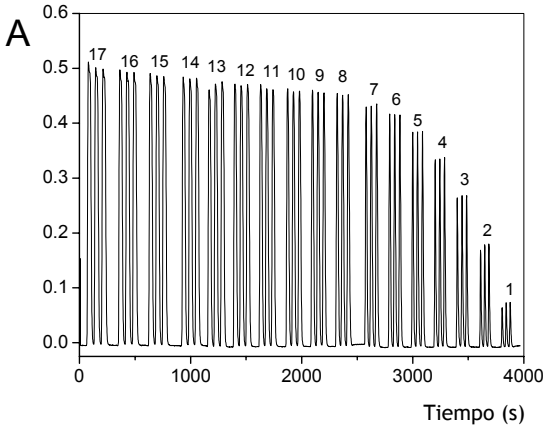


Fig. 38. Variación del número de pulsos.

Tras observar la estabilidad de la línea base, la repetibilidad de las señales transientes y la linealidad entre patrones, en los trabajos realizados para la determinación de fenol y ciclamato se demuestra la viabilidad de utilizar el flujo multipulsado como alternativa al análisis por inyección en flujo, sustituyendo las bombas peristálticas de elevado coste y tamaño por minibombas como unidades propulsoras de fluidos.

3.5. DESARROLLO DE DISPOSITIVOS DE BAJO COSTE PARA MEDIDAS POR QUIMIOLUMINISCENCIA Y FOTOMETRÍA

Las exigencias de la sociedad hacen necesario el diseño y desarrollo de sistemas económicos y portátiles que permitan realizar medidas *in situ*. Siguiendo este propósito, y aprovechando las ventajas que ha demostrado la multiconmutación, se han diseñado dos equipos simples, robustos, portátiles y de bajo coste: un luminómetro y un fotómetro que utiliza diferentes LEDs como fuente de radiación. El funcionamiento de los dispositivos se evalúa empleando como modelos la oxidación de luminol con peróxido de hidrógeno y la determinación indirecta de amonio, en el caso del luminómetro, y las determinaciones de hierro, nitrito, carbaril y fenol, en el caso del fotómetro.

El diseño electrónico de ambos dispositivos se muestra en la Fig. 39.

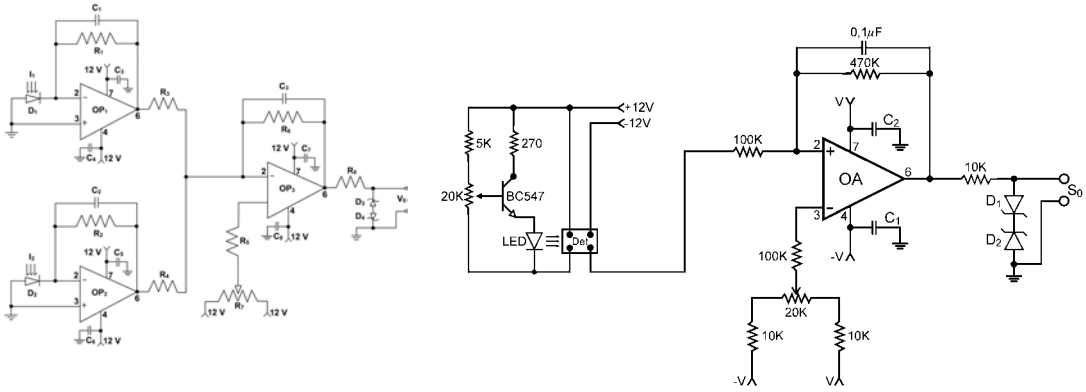


Fig. 39. Esquemas electrónicos del luminómetro (izquierda) y del fotómetro (derecha). *Izquierda*: OP1, OP2, OP3 = amplificadores operacionales OPOF; D1, D2 = fotodiodos; I1, I2 = intensidad de radiación electromagnética; R1, R2 = 20 MΩ resistores (1/4 W); C1, C2 = 0.01 μF y 250 V capacitores de poliéster; C3, C4, C5, C6, C7, C8 = 1 μF y 50 V capacitores de tántalo; R3, R4, R5 = 100 KΩ; R6, R8 = 470 KΩ y 10 KΩ resistores; R7 = 20 KΩ multivector; D3, D4 = 4.5 V diodo Zener, 0.4 W de potencia; V0 = salida de la señal en voltios. *Derecha*: Det = fotodiodo, RS 10530 DAL; OA = amplificador operacional, OP07; C1 y C2 = capacitores de tántalo, 1 μF; D1 y D2 = diodo Zener, 4.5 V; S0 = salida de la señal.

3.5.1. LUMINÓMETRO

El equipo para las medidas multiconmutadas por quimioluminiscencia puede ser fácilmente construido con un coste estimado de 600 € y 3 Kg de peso. El luminómetro se compone de una simple celda de polietileno enrollada alrededor de una superficie transparente y entre dos diodos de superficie igual a la longitud de la celda, D_1 y D_2 (Fig. 40).

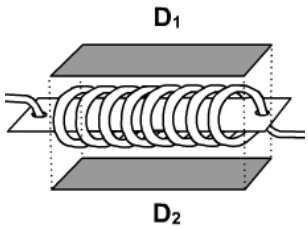


Fig. 40. Diseño de la celda de flujo empleada para las medidas por quimioluminiscencia.

Se obtienen características favorables en comparación con equipos comercialmente disponibles, como menor tamaño y bajo consumo de energía, alta frecuencia de muestreo (120 determinaciones por hora) y sensibilidad y precisión adecuadas.

La multiconmutación en combinación con el luminómetro y el uso de minibombas solenoides permite, además de minimizar el consumo de reactivos y la producción de residuos, el uso de sistemas miniaturizados compatibles con el empleo del equipo para medidas fuera del laboratorio.

3.5.2. FOTÓMETRO DE LEDs

El sistema está formado básicamente por tres componentes: (i) un juego de diodos emisores de luz (LEDs) de diversas longitudes de onda y de muy bajo coste, (ii) un detector de fotodiodo y (iii) un sistema multipulsado para la inserción de las disoluciones (Fig. 41). El sistema completo puede obtenerse por 650 €, pesa aproximadamente 3 Kg y no es necesario modificar su configuración física para llevar a cabo el estudio de diferentes reacciones químicas. Para evaluar el funcionamiento del sistema se llevan a cabo las siguientes determinaciones: (i) determinación de Fe^{3+} con SCN^-, (ii) determinación yodométrica de nitrito, (iii) determinación de fenol con nitroprusiato sódico y (iv) determinación de carbaril con p-aminofenol.

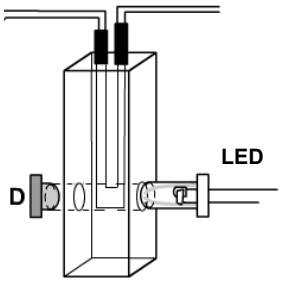


Fig. 41. Diseño del sistema empleado para las medidas fotométricas.

El aporte de la utilización de las minibombas al sistema de LEDs permite el diseño de un equipo compacto, con características destacables como las siguientes: portabilidad (pequeño tamaño y peso reducido), robustez, bajo consumo de reactivos (muestreo discreto de microvolúmenes) y minimización de energía y efluentes generados. Estas características hacen al equipo muy atractivo para su uso fuera del laboratorio, ya que los sistemas convencionales son difíciles de usar en trabajos de campo.

4. RESULTADOS

4. RESULTADOS

4.1. Mecanización de las medidas de AFS.

4.1.1. Determinación de Hg por CV-AFS.

4.1.1.1. *Mejoras en la determinación de Hg por fluorescencia atómica empleando la multiconmutación.*

4.1.1.2. *La multiconmutación en la determinación de Hg en aguas por CV-AFS.*

4.1.2. Determinación de Bi por HG-AFS.

4.1.2.1. *Un sistema en flujo multiconmutado para la determinación de Bi en batidos por HG-AFS, incorporando la neutralización en línea de los residuos.*

4.1.3. Determinación de Te por HG-AFS.

4.1.3.1. *Empleo de la multiconmutación como herramienta analítica medioambientalmente sostenible en la determinación de Te en leches por HG-AFS.*

4.1.3.2. *Determinación de las especies de Te inorgánico en leches por HG-AFS y multiconmutación.*

4.2. Nuevos avances en la mecanización de las medidas de espectrofotometría molecular.

4.2.1. Extracción líquido-líquido en línea.

4.2.1.1. *Una alternativa medioambientalmente sostenible al método de referencia para la determinación de tensioactivos aniónicos en aguas.*

4.2.2. Utilización de las minibombas para la inserción de disoluciones.

4.2.2.1. *Determinación de fenoles en aguas utilizando multiconmutación multipulsada y detección espectrofotométrica. Una alternativa automatizada al procedimiento estándar.*

4.2.2.2. *Un método limpio en flujo para la determinación espectrofotométrica de ciclamato en edulcorantes.*

4.3. Desarrollo de dispositivos de bajo coste para medidas por quimioluminiscencia y fotometría.

4.3.1. Luminómetro.

4.3.1.1. *Desarrollo de un equipo portátil y de bajo coste para mediciones en flujo por quimioluminiscencia.*

4.3.2. Fotómetro de LEDs.

4.3.2.1. *Desarrollo y evaluación de un sistema para análisis medioambientales, basado en medidas fotométricas en flujo multiconmutado.*

A continuación se presentan los artículos que componen la presente Tesis Doctoral. Nueve de ellos han sido publicados en revistas científicas de carácter internacional especializadas en el área de Química Analítica, mientras que el último está pendiente de impresión.

Los trabajos que se integran en esta Tesis Doctoral forman parte de los proyectos I+D siguientes: PHB2002-0054-PC (Ministerio de Educación, Cultura y Deporte), GV99-115-1-02 (Consellería de Cultura, Educación y Ciencia de la Generalitat Valenciana), PB98-0947-C02-0 (Dirección General de Enseñanza Superior e Investigación Científica), AGL2002-00729 (Ministerio de Ciencia y Tecnología) y CTESIN/2004/051 (Generalitat Valenciana), y constituyen una de las líneas de investigación dentro de la actividad que realiza el grupo SOLINQUIANA en el Departamento de Química Analítica de la Universitat de València.

4.1. MECANIZACIÓN DE LAS MEDIDAS DE AFS

«Improvement of the atomic fluorescence determination of mercury by using multicommutation»

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Improvement of the atomic fluorescence determination of mercury by using multicommutation

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Atomic fluorescence spectrometry (AFS) determination of Hg has been improved by exploiting the possibilities of the multicommutation approach in order to outline a fully mechanised system which supplies the same sensitivity as the use of continuous measurements, reducing drastically the reagents consumed and waste generation. The use of multicommutation with a simultaneous reduction of the liquid gas separator volume and the insertion point modification of argon transport gas provides a sensitivity of the AFS measurements of 300 mV ng ^{1} ml (using a full scale of 1000 mV), a limit of detection (3 *s*) of 1.3 ng l ^{1} and relative standard deviation values below 0.1% for 10 independent measurements of a 0.6 μg l ^{1} Hg solution. The Hg determination of one hundred samples could be carried out with 167 ml sample and 208 ml reagent mixture, as well as an argon consumption of 1450 ml and a waste generation of 375 ml. Thus, the sample and reagent consumption is reduced 6 times and the waste generation 2 times as compared with continuous measurements. Additionally, the sampling throughput increased from 13.5 h ^{1} to 49.5 h ^{1}.

Introduction

Nowadays, multicommutation is a well-established flow analysis technique, which has been proved to be highly useful in performing a complete mechanisation of analytical procedures involving UV Vis spectrophotometry,[1,2] potentiometry[3,4] and fluorimetry[5] as detection techniques. However, till now, there has been no application developed for the determination of Hg by multicommutation, nor for the mechanisation of any atomic fluorescence determination (AFS).

In a previous study on the spectrophotometric determination of carbaryl,[6] multicommutation was compared with classical flow injection (FIA) and modern sequential injection analysis (SIA) techniques, a strong reduction of waste generation at levels comparable to those found by SIA and six times lower than those of FIA being observed.

The main objective of the present study was to evaluate the influence of the complete mechanisation of cold vapour atomic fluorescence determination of Hg through the use of multicommutation. The analytical performance of the method and the practical parameters, such as laboratory productivity, reagents consumed and waste generation, have been evaluated.[7]

Experimental

Reagents and apparatus

All chemicals used (a) Hg^{2+}, (b) HCl, (c) $SnCl_2{\cdot}2H_2O$, (d) $KBrO_3$, (e) KBr and (f) hydroxylamine hydrochloride were of analytical grade and high purity water was obtained from a Milli-Q water purifier system.

The multicommutated flow system comprised three three-way solenoid valves (NResearch, 161T031), reaction coils and flow lines of 0.8 mm internal diameter PTFE tubing and two T-type junctions mechanized in acrylic. A 486 microcomputer furnished with a PCL-711S interface card (American Advantec Corp.) and running with a software written in Quick BASIC 4.5, was employed to control the proposed flow system.

A PSA 10.025 Millenium Merlin instrument (St. Mary Cray, Kent, England) was employed as a fluorescence mercury detector equipped with two peristaltic pumps, a liquid gas phase separation chamber, a Permapure dryer unit, a photomultiplier tube and a data acquisition system that was operated as indicated in the customer manual. The multicommutated flow system was coupled to the fluorescence detector equipment connecting its fluid outlet to the inlet of the liquid gas phase separation chamber.

The control signals to switch on/off the solenoid valves were generated by the microcomputer through the PCL711s interface card. The signal power was not enough to drive the solenoid valves, thus a home made electronic interface was employed to match the voltage and electric current requirements.

Flow diagram and procedure

The flow diagram depicted in Fig. 1 was outlined to handle solutions, either in the multicommutation mode or simulating the usual continuous procedure.

When all solenoid valves in Fig. 1 were switched off, the blank solution (B_k) was flowing towards liquid gas phase separation chamber. By switching on the valves V_1, V_2 and V_3, as indicated in the valves timing course diagram (a), sample solution (S) and reductant solution (R) were directed to the confluence point. At the same time blank solution was pumped towards its store vessel. After the sampling step was finished, (t_s), valves V_1 and V_2 were switched off. Under these valves working configuration, blank solution flowed again towards the gas phase separation chamber and sample solution recirculated (Re) to its store vessel. Mixing between sample and reductant solution take place during the transport through reaction coil (B) where reaction to reduce Hg^{2+} to elemental

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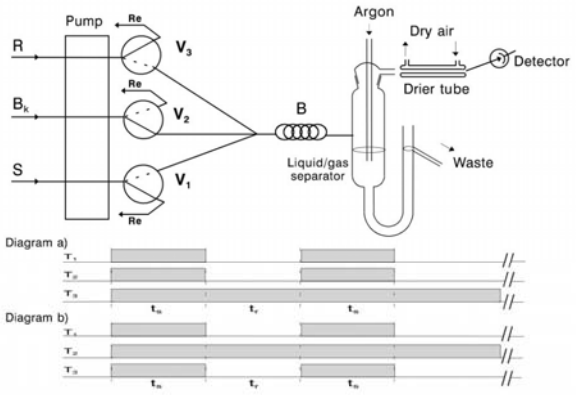

Fig. 1 Manifold scheme of the AFS system employed to operate in both the continuous and multicommutation modes. R = Reductant reagent; B_k = blank solution; S = standard solution; Re = return to the corresponding store vessel; V_1, V_2 and V_3 = three-way solenoid valves; B = reaction coil 120 cm length and 0.8 mm id. Solid and dotted lines in the valves symbol indicated the fluid pathway when switched off or on. T_1, T_2 and T_3 = Timing course for valves V_1, V_2 and V_3, respectively; t_s = sampling time; t_r = reading time. Dashed surface indicates the timing line during which the valve was switched on.

Hg occurred. The argon stream merged into the liquid–gas separation chamber to transport the Hg towards the detector.

The time intervals, t_s and t_r, could be settled long enough to allow the analytical signal to attain the steady state condition and return to the baseline. With the operational condition depicted in Fig. 1 these time intervals were selected at 120 and 90 s for t_s and t_r, respectively. To simulate the condition of the continuous mode, the reducer solution was maintained flowing towards the gas phase separator chamber while the analytical procedure was run.

As indicated in the valve timing course at diagram (b), the multicommutation operation mode could be implemented under software control without network reconfiguration. In this case solenoid valves V_1, V_2 and V_3 were switched on at the same time, thus sample and reductant solutions merge and flow through the reaction coils towards liquid–gas phase separation chamber, while blank solution was pumped towards its store vessel. When the sampling step was ended, valve V_1 was switched off and valve V_2 was maintained during a time interval of 20 s to transport the sample zone from reaction coil to the gas phase separation chamber. After finishing the signal reading step, t_r, another analytical run can be started. In this operation mode, the blank solution recirculates towards its store vessel throughput while samples are processed. After ending the analytical task, all valves were switched off and the blank solution was allowed to flow to wash the gas phase separation chamber. The pumping flow rates were maintained at 83 μl s^{-1} for all solutions, thus experiments to study the effect of sample volume were accomplished by varying the sampling time interval (t_s).

Results and discussion

Effect of argon on AFS determination of Hg

In the continuous AFS mode, argon is employed to do the gaseous Hg separation from the reaction mixture and thus, following the instrument design, the Ar is bubbled through the liquid phase at the end of the process (see Fig. 1).

The signal obtained for Hg increased slowly up to reach the steady state, 120 s being necessary to achieve the maximum signal.

A modification, introduced by us, consisted of inserting the Ar flow after mixing the reagents. In these conditions, Ar acts really as a carrier, Hg being evolved continuously from the liquid phase during the transport to the separation chamber, causing a multisegmentation of the sample zone.

In the aforementioned situation, the AFS signals reach the steady state very quickly (in only 60 s), thus providing the possibility of reducing the measurement time. However, in both cases the use of 1 or 2 min sample introduction time involves a minimum of 1.5 min to recover the base line signal.

Multicommutation AFS

Two three-way solenoid valves could be used to introduce simultaneously the sample or standard solution and the reaction mixture, thus reducing the number of channels of the system from three to two. Additionally, and taking into account the need for separation of the liquid and gaseous phases, the pumping of solutions is required. However, and according to results reported in the previous section, the insertion of an Ar flow after mixing the reagents provides appropriate transport of the analyte to the measurement zone.

Effect of Ar on the multicommutation AFS

Fig. 2 shows the effect of the sample injection volume (controlled by the sampling time) on the signals found for the fluorescence of 1 μg l^{-1} Hg, both using direct bubbling of a 0.24 l min^{-1} Ar flow, introduced from the top of the liquid–gas separator and, alternatively, Ar as a carrier introduced after mixing the reagents. From the data found, it could be concluded that the increase in the sample volume increases the fluorescence signal continuously until reaching the steady state for 2.92 ml (corresponding to 35 s sampling time) in the case of using the Ar segmented flow. On the other hand, the insertion of Ar in the liquid–gas separator provides substantially lower data than those found in the previous mode, and the steady state cannot be attained for a sampling time lower than 40 s.

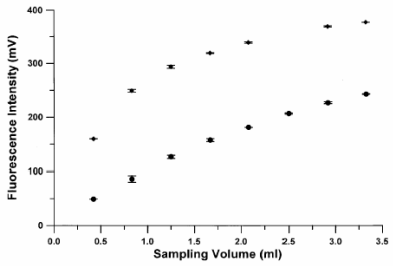

Fig. 2 Effect of sample volume on the multicommutation AFS determination of Hg as a function of the Ar insertion point using Ar as a carrier of reaction mixture in the segmented mode (◆), or bubbling the Ar flow through the top of the liquid–gas phase separator (●). Signals from 1 μg l^{-1} Hg using a 17 ml liquid–gas separator.

Effect of the liquid–gas phase separator volume

The commercially available PSA instrument is equipped with a 17 ml liquid gas phase separator. In order to improve the Hg AFS measurements a reduced volume separator with only 5 ml was designed in our laboratory.

For a fixed sampling time of 5 s, which corresponds to a sample volume of 0.42 ml, and using a reading time of 30 s in the multicommutation AFS mode, a calibration line was found for Hg fluorescence of $Y - 130\ C + 5$, with $r - 0.9990$, for the high volume separator, and a calibration line of $Y - 150\ C + 7$, with $r - 0.9993$ for the reduced volume phase separator, thus indicating that the designed separator can increase the analytical signal for fixed sampling and reading times. This also offers the possibility of the use of a reduced sampling time, without sacrificing the analytical sensitivity and thus affecting the productivity of the laboratory by increasing the sampling throughput based on the fact that the use of a reduced volume liquid gas phase separator reduces the time required to recover the base line signal after reading the maximum signal from 50 s to 30 s.

In short, as compared with data reported on the effect of Ar in the continuous mode, the use of multicommutation AFS for Hg determination, combined with the reduction of the size of the phase separator, moves the total measurement time from 3.5 min to probably 1 min (by considering that the system could reach the steady state in less than 30 s).

Effect of sample volume on multicommutation AFS

As can be seen in Fig. 2, the increase of the sampling volume from 0.42 to 3.36 ml provides an increase in the fluorescence signal, having practically reached the steady state for a sampling time of 20 s when a reduced volume phase separator was used. So, as compared with the use of the continuous mode or the commercially available phase separator, the multicommutation approach involves a strong reduction of sample volume, required to attain the maximum sensitivity, thus opening new possibilities of increasing the laboratory productivity through the sampling throughput and reducing the side effects of waste generation.

In the strategy developed the use of a sampling volume of 1.67 ml (corresponding to 20 s sampling time) could be recommended for maximum sensitivity or, alternatively, the use of a sampling time of 5 s when the Hg concentration is comparatively high.

Additional modifications

Additional experiments, carried out by pulsation of the argon stream during Hg generation, involve the reduction of argon volume at the sampling step, thus reducing by a factor of 2 the gaseous phase dilution. In the aforementioned conditions the Hg signal increases by 140% as compared with that obtained when, during the reading time, the argon was flowing continuously.

Table 1 shows the analytical features of AFS Hg determination by using the continuous and multicommutation modes. In the case of continuous mode, the original phase separator was employed, but for multicommutation a reduced volume separator was also used.

Conclusions

The displacement of the argon insertion point, during the AFS Hg determination, minimises the time required to attain the maximum sensitivity, thus improving the sample throughput.

The reduction of the volume of liquid gas phase separator increases the sensitivity of AFS by reducing the sample dispersion, thus affecting the measurements found before to reach the steady state. Additionally, the use of a small

Table 1 Analytical parameters found for the AFS determination of Hg using the continuous mode and the multicommutation mode, also indicating the effect of liquid–gas separation systems and different sampling times

	Continuous AFS	Multicommutation AFS	
	Large LGS[c] Reading time 90 s, sampling time 120 s	Large LGS Reading time 30 s, sampling time 5 s	Small LGS Reading time 40 s, sampling time 20 s
LOD[d]/pg ml^{-1}	0.3	2	1.3
SD[e]	3.0	0.8	0.16
RSD (%)[f]	1.5	0.8	0.09
Linear range/ng ml^{-1}	0–1.5	0–1.5	0–1.5
Calibration line	$y = 300x + 11$	$y = 130x + 5$	$y = 300x + 14$
Correlation coefficient (r)	0.9995	0.9990	0.9990
Sampling time/s	120	5	20
Sample/ml[a]	1000	42	167
Reagent/ml[a]	1750	83	208
Waste/ml[b]	471	100	193
Ar consumed/ml[b]	1730	70	290
Throughput/h^{-1}	13.5	63.0	49.5

[a]Sample and reagent consume correspond to a batch of 100 analysis. [b]Waste generated and Ar consume were established for 1 h working session. [c]LGS: liquid–gas separator. [d]LOD: limit of detection. [e]SD: standard deviation of 10 measurements obtained for a 0.6 μg l^{-1} Hg solution. [f]RSD (%): relative standard deviation.

liquid gas separator reduces the time required to recover the base line, thus reducing the time of analysis.

Multicommutation provides a simplification of the experimental set-up, a fully mechanised operation of the system and a drastic reduction of sample and reagents consumed and waste generated, thus offering a sustainable and environmentally friendly alternative.

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Multicommutation cold vapour atomic fluorescence determination of Hg in water

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Abstract

A multicommutation-based method has been developed for the on-line direct atomic fluorescence spectrometric (AFS) determination of Hg in waters without any previous sample treatment. The performance of the proposed procedure has been compared with that of a conventional AFS system based on continuous mode measurements. In short, the use of multicommutation, together with a reduction of the size of the liquid–gas phase separator, provides an increase of the laboratory productivity by improving the sample throughput by a factor of 3.6 and strongly reduces the sample consumed by a factor of 6 and reagent consumed by a factor of 8.4. The waste generation is reduced by a factor of 2.4 and the Ar consumed by a factor of 6, thus the developed method is an environmentally and economically sustainable alternative to the methodology based on continuous measurements, without any reduction of the analytical sensitivity and with an enhancement of the repeatability of measurements. Only the limit of detection was poorer for the methodology developed (1.3 ng l^{-1}) than that found by the classical continuous mode (0.3 ng l^{-1}). The aforementioned methodologies were applied to the determination of Hg in water samples having obtained comparable values by both procedures and with those found by an external laboratory.

Keywords: Multicommutation; Hg determination; Atomic fluorescence spectrometry; Cold vapour; Water analysis

1. Introduction

From the introduction of flow injection analysis (FIA) 28 years ago [1] different systems have been proposed to collect sample aliquots and reagents and to insert them into a carrier, as hypodermic syringes [1], proportional injectors [2], six and eight port valves [3,4] and three-way solenoid valves [5].

Three-way solenoid valves can be individually switched on and off in order that the flow inside of reaction coils and the amount of reagent could be modified without changing the manifold employed, thus increasing its ability to handle various

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solutions. Based on the systematic use of the three-way valves, multicommutation and binary sampling were developed as alternatives to traditional FIA [6] and modern sequential injection analysis (SIA) [7]. In short, multicommutation-based mechanised procedures use a quick-basic time programmed set of three-way valves which act on each one of involved reagent, analyte or carrier solutions in order to control the amount of reagents by controlling the switching on time of each valve and the order of addition by incorporating the different solutions in the valve sequence.

Multicommutation offers the possibility to drastically reduce: (i) the complexity of flow manifolds required for determinations involving the use of several reagents, (ii) the reagent consumption and (iii) the total amount of waste generated.

Multicommutation has been proved to be highly useful to perform a complete mechanisation of analytical procedures involving UV–Vis spectrophotometry [8,9], potentiometry [10,11] and fluorometry [12]. Furthermore, it has been used to automate true titration procedures [13] and also to detect and to circumvent sources of inaccuracy by using feedback [14].

However, till now, there are only two studies about the use of multicommutation in atomic spectrometry, one based on inductively coupled plasma optical emission spectrometry [15] and a preliminary study on the improvement of Hg determination by cold vapour atomic fluorescence spectrometry (CV-AFS) [16].

In a previous study on the comparison between classical FIA, SIA and multicommutation [17] it was confirmed that multicommutation can offer a strong reduction of waste generation at levels comparable to those found by SIA and six times lower than those of FIA. Furthermore, multicommutation did not supposed any reduction of the sensitivity attainable by FIA, and even, in some conditions, it could offer an increase of the analytical sensitivity.

CV-AFS is a powerful analytical technique for Hg determination at ultratrace levels [18,19], as it has been demonstrated in pioneering works on AFS which provided limits of detection at sub nanogram per liter levels [20–22] and because of that, it offers an important tool for mercury determination and speciation [23,24]. So, the Spanish [25,26] and the European legislation establishes for the control of human consumption a maximum admissible Hg concentration of 1 $\mu g\,l^{-1}$ and maximum tolerated Hg levels of 1 $\mu g\,l^{-1}$ in superficial waters, 0.5 $\mu g\,l^{-1}$ for estuarine waters and 0.3 $\mu g\,l^{-1}$ for marine water [27]. So, it is clear that CV-AFS is an interesting tool for Hg determination in natural waters and that, from the analytical point of view it could be interesting to exploit the advantages of the aforementioned technique in order to reduce reagent consumption and waste generation [28,29], without sacrifying the analytical performance of measurements carried out in the continuous mode.

In a previous short note [16] We noticed the advantages which could be derived from the automation of CV-AFS through multicommutation but a detailed comparison and in deep evaluation of parameters involved were out of the scope of the previous note.

The main objective of the present study has been to evaluate the influence of the complete mechanisation of CV-AFS determination of Hg through the use of multicommutation. The analytical performance of the method and the practical parameters, such as laboratory productivity, reagents consumption and waste generation, have been evaluated. A multicommutated flow analysis procedure by using AFS detection has been applied to the determination of mercury in water samples, comparing this approach with the conventional continuous mode CV-AFS.

2. Experimental

2.1. Apparatus

A CV-AFS system PSA 10.025 Millennium Merlin instrument (Kent, England) equipped with two peristaltic pumps, a 17 ml liquid gas phase separation chamber, a Permapure dryer unit, an ultraviolet detector and a data acquisition system was employed for fluorescence measurements in the continuous mode. Fig. 1 shows the

manifold used for CV-AFS also indicating the operation of the injection valve.

Fig. 2 shows the manifold for multicommutated flow system which includes three three-way solenoid valves (NResearch, 161T031), reaction coils and flow lines of 0.8 mm internal diameter PTFE tubing and two T-type junctions mechanised in acrylic. A 486 microcomputer furnished with a PCL-711S interface card (American Advantec Corp.) and running with a software wrote in QUICK BASIC 4.5, was employed to control the proposed system.

The control signals to switch on/off the solenoid valves were generated by the microcomputer through the PCL711s interface card. The signal power was not enough to drive the solenoid valves, thus a home made electronic interface was employed to match the voltage and electric current requirements. Fig. 2 indicates the timing course for valves using a continuous flow of Ar (diagram a)

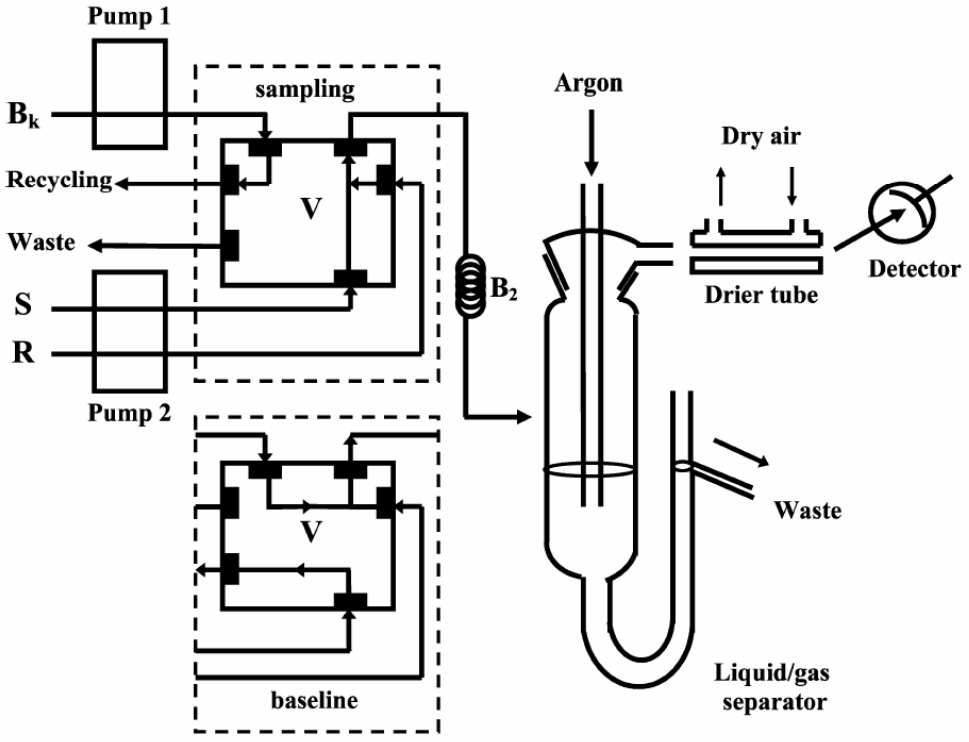

Fig. 1. Manifold employed to operate AFS in the continuous mode. R, reductant reagent; B_k, blank solution; S, standard solution; *V*, sampling valve; B_2, reaction coil. The scheme indicates the two positions of the mixing valve incorporated to control alternatively the sample or standard fluorescence and the baseline.

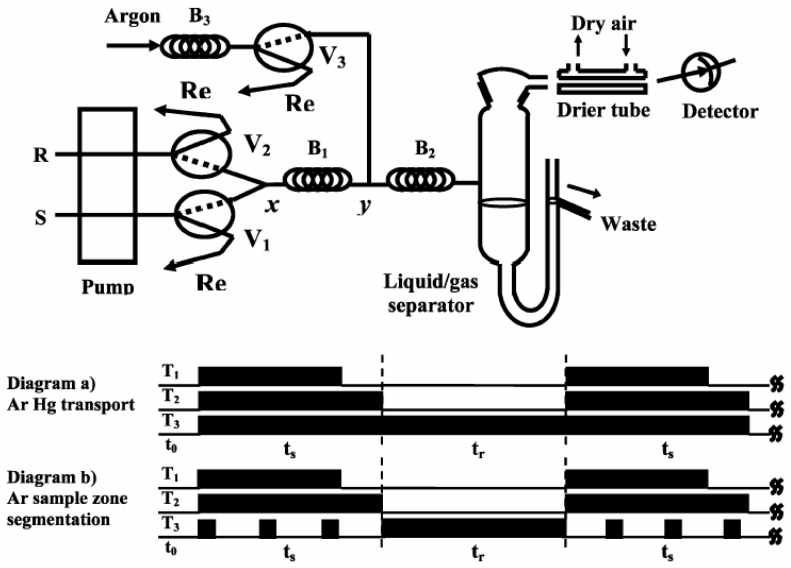

Fig. 2. Manifold scheme of the system employed for multicommutation AFS using argon stream for sample zone segmentation. R, reductant reagent; S, standard solution; Re, return to the corresponding store vessel; V_1, V_2 and V_3, three-way solenoid valves; B_1, reaction coil 20 cm length and 0.8 mm i.d.; B_2, sample segmentation coil, 80 cm length and 1.2 mm i.d.; x and y, confluence points. Solid and dotted lines in the valves symbol indicated the fluid pathway when switched off or on, respectively. T_1, T_2 and T_3, timing course for valves V_1, V_2 and V_3, respectively; t_s, sampling time; t_r, reading time. Diagram (a) indicates the continuous flow of argon, using V_3 in the open position and diagram (b) shows the sequence of valves for sample zone segmentation.

and a pulsed Ar flow (diagram b) which were employed during this study.

Table 1 resumes the operational conditions employed for the determination of Hg by both continuous and multicommutated procedures.

2.2. Reagents

All chemicals used were of analytical grade and high purity water was obtained from a Milli-Q water purifier system.

A 2% (w/v) $SnCl_2 \cdot 2H_2O$ solution in 10% HCl (v/v) was prepared from $SnCl_2$ Merck (Darmstadt, Germany) and HCl Scharlau (Barcelona, Spain). This solution was purged by bubbling nitrogen for 30 min.

A 0.033 mol l^{-1} $KBrO_3$ and a 0.200 mol l^{-1} KBr solutions were prepared daily from the corresponding Merck reagents and mixed in a 1:1 proportion.

Table 1
Operational conditions employed for the determination of mercury in water samples by CV-AFS in both, continuous and multicommutation, modes

Parameter	Continuous	Multicommutation
Wavelength (nm)	354.7	354.7
Range	100	100
Measurement mode	Peak height	Peak height
HCl% (v/v)	10	10
$SnCl_2$% (w/v)	2	2
Argon flow rate (ml min^{-1})	240	240
Blank flow rate (ml min^{-1})	5.0	
Sample flow rate (ml min^{-1})	5.0	5.0
$SnCl_2$ flow rate (ml min^{-1})	5.0	5.0
B_1 reaction coil length (cm)		20
B_2 reaction coil length (cm)	25	80
B_3 argon coil length (cm)		100

A 12% (w/v) hydroxylamine hydrochloride (NH_2OH HCl) was prepared each week from the Merck salt.

A blank solution, containing 0.002 mol l^{-1} KBr, 3.3×10^{-4} mol l^{-1} $KBrO_3$ and 9.6×10^{-2}% (w/v) of NH_2OH HCl in 5% (v/v) HCl, was employed to establish the Hg AFS baseline.

A 1000 mg l^{-1} Hg^{2+} certified standard stock solution was obtained from Merck. Standard solutions of 0.0, 0.05, 0.2, 0.4, 0.6, 1.0, 1.5 and 2.0 μg l^{-1} were prepared from appropriate dilution of the Hg stock and adding KBr, $KBrO_3$ and NH_2OH HCl at the same concentration level than that used for the blank.

2.3. Samples

Water samples were obtained from the 'INTER2000 Program' organized by the Generalitat de Catalunya (TR), and from the 'Programa de intercomparación de metales pesados' (IMP) organized by the Spanish Ministerio de Agricultura, Pesca y Alimentación. To 1 ml of sample it was added 2 ml KBr/$KBrO_3$ solution, 5 ml HCl and 2 ml NH_2OH HCl prior to dilute to 250 ml and diluted samples measured in front of external standards by CV-AFS using both, the continuous measurement mode and multicommutation. Data found by us were also compared with those found by an external laboratory, the Laboratorio Agroalimentario of the Generalitat Valenciana (Burjassot, Spain) and with the reference values established by the intercalibration programs.

2.4. General procedure

The manifold depicted in Fig. 1 corresponds to that provided by the instrument supplier. In the sampling operation, sample or standard solutions, were mixed with the reductant reagents inside the mixing valve. The mixture was transported into the liquid–gas separation chamber and the generated vapour of Hg was transported through a drier tube, to the detector, by an argon flow. Alternatively, in the baseline operation, a blank solution was merged with reagents in order to clean the system and to establish the baseline. In the continuous mode only the blank solution was recycled and samples and reagents were continuously pumped, being the consume of both a function of the time of operation and flow rate values employed.

Manifold scheme depicted in Fig. 2 was used for multicommutation measurements. In this case the argon stream was inserted into the analytical path to favour the gas phase transport. In the configuration showed all solenoid valves, V_1, V_2 and V_3 were switched off at the beginning, thus sample and reductant solutions flowed to their storage vessels by a recirculation system. Following the valves timing course strategy, summarised in diagram a, when the software was run the solenoid valves V_1 and V_2 were switched on at the same time, and sample and reductant solutions were merged together through the confluence point x and mixed into the reaction coil B_1, and V_3 valve was opened to assure the Ar transport. The mixing between these solutions causes Hg^{2+} reduction to Hg^0 during the displacement towards the point y. The insertion of the argon stream at point y causes a multisegmentation of the sample zone into coil B_2 and, as a consequence, the generation of a stream comprising several solution plugs separated by argon bubbles. The Hg atoms, resulting from the reduction reaction, should be extracted to the gas phase during the displacement towards the liquid/gas separation chamber. In order to verify the effectiveness of this proposal, a set of experiments were carried out in which the following system parameters were considered: reaction coil (B_1) length 10, 20 and 30 cm; segmentation coil (B_2) length 40, 80 and 120 cm; and variation of sampling time (t_s) from 5 to 40 s.

When running the system as indicated at the valves timing course diagram b, while valves V_1 and V_2 were switched on, to perform the sampling step (t_s), valve V_3 was switched on/off several times. Under these conditions, the insertion of the argon stream into the analytical path was interrupted several times, and thus the net flow rate of argon through coil B_2 and gas phase separation chamber was reduced. The pressure of the argon stream was usually adjusted at 35–40 psi, since the working pressure of the solenoid valves (NResearch, 161T031) was about 30 psi. So, the back-pressure reduction coil (B_3) was coupled to the

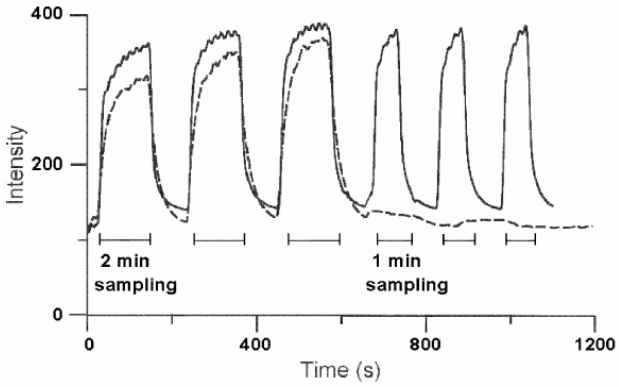

Fig. 3. Effect of the argon flow on the Hg AFS signal profile (-----), use of Ar for liquid/gas phase separation in continuous operation mode; ( ), use of Ar as gas phase carrier in multicommutation mode. Hg concentration employed was 1 $\mu g\,l^{-1}$ When using the continuous system, three signals and three blanks were presented. Using Ar as carrier in the multicommutation mode three signals were obtained for 2 and 1 min sampling times, respectively.

flow network in order to maintain its working performance. To study the effect that this operation mode could cause on the analytical signal, experiments were carried out maintaining the valve V_3 switched on during 0.25 s and switched off for 0.75 s. These experiments were done using a 1 $\mu g\,l^{-1}$ Hg standard solution.

Aiming to save analysis time the 17 ml liquid/gas phase separation chamber provided by the supplier was replaced by a new one made in our laboratory, with the same shape and with a volume of 5 ml. Experiments comprising the inserted sample volume, time required for the signal return to baseline, repeatability of the measurements and linear response range were carried out, also evaluating the reagent consumption and waste generation.

3. Results and discussion

3.1. Effect of argon on AFS determination of Hg

In the CV-AFS continuous operation mode Ar is bubbled directly through the liquid phase at the end of the reduction process, as it is shown in Fig. 1. So, argon is employed only to separate the gaseous Hg from the reaction mixture and thus it dilutes the analyte and is only used for analyte transport. As can be seen in Fig. 3, the signal obtained for 1 $\mu g\,l^{-1}$ Hg, in the aforementioned conditions, increases slowly up to reach the steady state, being necessary 120 s to achieve the maximum signal and, after that, it is necessary to wait 1.5 min till to recover the baseline signal.

In the multicommutation mode, and using the diagram b, the Ar flow was discontinuously introduced as carrier after mixing of the reagent. By using this modification in the manifold, the mercury vapour produced was evolved more efficiently than on the original system from the liquid phase during the transport through the reaction coil B_2. In this situation, the AFS signals reach very fastly the steady state (in only 60 s), thus providing the possibility of reducing the measurement time, from 2 to 1 min, as it can be seen in the signals shown in Fig. 3. However, in both cases, the use of 1 or 2 min sample introduction time involves between 1.0 and 1.5 min delay time to recover the base line signal.

3.2. Effect of reaction coil length on multicommutation AFS

The discrete insertion of small volumes of sample and reagents by using multicommutation

Table 2
Effect of reaction coil length on multicommutation AFS determination of Hg

B_1 (cm)	B_2 (cm)	Fluorescence signal ($x \pm s_{n-1}$)
10	120	559±1
20	120	555±1
30	120	554±1
20	40	550±10
20	80	548±4

2.0 $\mu g\,l^{-1}$ Hg concentration; 0.8 mm internal diameter coil; Ar being inserted at point y of the manifold depicted in Fig. 2; $x \pm s_{n-1}$ average±standard deviation of five independent measurements.

mode makes necessary to manage the reduction of Hg^{2+} to Hg^0 and gas phase evolution by controlling the time of contact between solutions.

Using the manifold depicted in Fig. 2, the influence of B_1 and B_2 reaction coil lengths was evaluated using a standard solution of 2 $\mu g\,l^{-1}$ Hg and internal diameter tubes of 0.8 mm. For a 120 cm fixed length of B_2, the effect of B_1 length was evaluated from 10 to 30 cm and, additionally, for a fixed 20 cm length of B_1, the effect of B_2 was evaluated from 40 to 120 cm. As it can be seen in Table 2 the size of reaction coils is not at all a critical parameter on the Hg fluorescence signal. However, in order to obtain a sensitivity as high as possible and reproducibility of AFS signals in as shorter as possible time, a length of 20 and 80 cm for B_1 and B_2 was selected, respectively.

3.3. Effect of sample volume on multicommutation AFS

Fig. 4 shows the influence of the sampling volume on the AFS measurements obtained in the multicommutation mode and using the manifold depicted in Fig. 2 with the small volume phase separator. For a fixed concentration of 0.6 $\mu g\,l^{-1}$ of Hg, the increase of the sampling volume, from 0.42 to 3.36 ml, provides an increase of the fluorescence signal, having reach practically the steady state for a sampling time of 20 s as can be seen from the shape and size of peaks in the inset of Fig. 4. So, as compared with the use of the commercially available phase separator, in multicommutation and continuous mode, the phase separator developed by us involves a strong reduction of sample volume required to attain the maximum sensitivity, thus to increase the laboratory productivity, through the enhancement of the sampling throughput and reduce the side effects of waste generation.

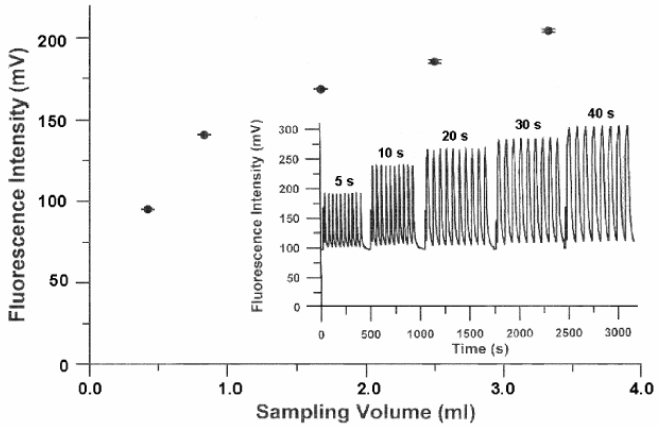

Fig. 4. Effect of sampling volume on the multicommutation AFS determination of Hg using a reduced volume liquid/gas phase separator. Hg concentration of 0.6 $\mu g\,l^{-1}$. *Inset:* signals obtained at different sampling times (from 5 to 40 s) using a reading time of 30 s, for 5 and 10 s sampling time, and a reading time of 40 s for high sampling time intervals.

Table 3
Analytical parameters found for the CV-AFS determination of Hg using the continuous mode and the multicommutation mode also indicating the effect of liquid/gas separation systems and different sampling times

	Continuous AFS	Multicommutation AFS					
	Big LGS	Big LGS	Small LGS				
	Reading time 90 s	Reading time 30 s	Reading time 30 s		Reading time 40 s		
	Sampling time 120 s	Sampling time 5 s	Sampling time 5 s	Sampling time 10 s	Sampling time 20 s	Sampling time 30 s	Sampling time 40 s
LOD ($ng\,l^{-1}$)	0.3	2	2	1.5	1.3	1.0	0.9
S.D.	3.0	0.8	0.12	0.14	0.16	1.2	1.1
%R.S.D.	1.5	0.8	0.13	0.10	0.09	0.6	0.5
Lineal range ($\mu g\,l^{-1}$)	0 1.5	0 1.5	0 1.5	0 1.5	0 1.5	0 1.5	0 1.5
Calibration line	$y=300x+11$	$y=130x+5$	$y=150x+7$	$y=200x+9$	$y=300x+14$	$y=300x+12$	$y=300x+17$
Correlation coefficient (r)	0.9995	0.9990	0.9993	0.9991	0.9990	0.9985	0.9988
Sampling time (s)	120	5	5	10	20	30	40
Cleaning time (s)	90	45	45	55	60	60	60
Sample (ml)[a]	1000	42	42	83	167	250	333
Reagent (ml)[a]	1750	83	83	125	208	292	375
Waste (ml)[b]	471	100	100	150	193	244	283
Ar consume (ml)[b]	1730	70	70	140	290	430	580
Throughput (h^{-1})	13.5	63.0	63.0	63.0	49.5	45.0	43.2

LGS, liquid/gas separator; LOD, limit of detection; S.D., standard deviation of 10 measurements obtained for a 0.6 $\mu g\,l^{-1}$ Hg solution; R.S.D.%, relative standard deviation.

[a] Sample and reagent consume correspond to a batch of 100 analysis.

[b] Waste generated and Ar consume were established for 1 h working session.

Table 4
Analysis of water samples for Hg determination by CV-AFS[a]

	Reference value	External laboratory	Continuous mode	Multicommutation mode
Sample TR-117[b]	94±22	100±3	90±2	96±6
Sample TR-23-00[b]	67±23	70±3	54.2±0.8	58±5
Sample IMP 804967[c]	19.9±2.7	21.4±0.6	20±1	21.6±1.0
Sample IMP 909307[c]	9.8±1.0	9.1±0.68	9.1±0.2	9±1
Sample IMP 804965[c]	10.2±1.8	10.9±0.5	8.1±0.4	10.0±0.1

[a] Mean values in microgram per litre and standard deviations based on three replicates.
[b] Obtained from 'Inter 2000 Program' organized by Generalitat de Catalunya.
[c] Obtained from the 'Programa de intercomparación de metales pesados' organized by the Spanish Ministerio de Agricultura, Pesca y Alimentación.

In the developed strategy the use of a sampling volume of 1.67 ml (corresponding to 20 s sampling time) could be recommended for improving the sensitivity or, alternatively, the use of a sampling time of 5 s when the Hg concentration is comparatively high, as can be seen in data shown in Table 3.

3.4. Analytical features of AFS determination of Hg

Table 3 shows the analytical features of CV-AFS Hg determination by using the continuous and multicommutation modes. In the case of continuous mode, the original phase separator was employed, but for multicommutation a reduced volume separator was also used.

From data reported in Table 3 it can be concluded that the sensitivity of multicommutation AFS increases on raising the sampling time from 5 to 20 s, as it has been commented before, having found a maximum sensitivity of 300 mV l μg^{-1} for sampling times higher than 20 s (1.67 ml), which is the same than that found in the continuous mode.

The theoretical limit of detection of the Hg AFS determination, established for a probability level of 99.6% ($k=3$), varies from 0.0003 to 0.002 $\mu g\,l^{-1}$, as a function of the analytical sensitivity and the standard deviation of blank measurements having not observed any enhancement of this parameter by using multicommutation. However, the precision of measurements (evaluated from the relative standard deviation of 10 independent measurements) is enhanced from a typical value of 1.5% found in the continuous mode to 0.1–0.6% in multicommutation at sampling times at which steady state was reached.

The main advantages of using multicommutation in Hg determination by CV-AFS concern both, the strong reduction of reagent consume per sample analysed and the reduction of waste generation. On the other hand an enhancement of the productivity of the laboratory was obtained which, without sacrifying the analytical sensitivity, provided a sampling frequency of 49 injection per hour, that compared with the 13.5 injections per hour obtained in the continuous mode. So the time of analysis was reduced by a factor of 3.6.

As Table 3 indicates, a sample volume of 1.67 ml ($t_s=20$ s), and a $SnCl_2$ (2% w/v) in HCl (10% v/v) volume of 2.09 ml is enough to obtain a sensitive fluorescence signal, being produced a waste of 193 ml h^{-1} for 49.5 injections in the multicommutation mode. These values are clearly lower than the 10 ml ($t_s=120$ s) sample consumption and 17.5 ml reagent required for the continuous mode measurement which also involves a waste generation of 471 ml h^{-1} for only 13.5 injections.

On the other hand, the reagent consumption and waste generation could be reduced by sacrifying the analytical sensitivity, by a factor of 2, for a sample volume of only 0.42 ml ($t_s=5$ s).

3.5. Effect of Ar pulsation on multicommutation AFS

As it has been commented in Fig. 2, the Ar inserted in point *y* of the manifold used for multicommutation, brings the Hg vapour to the

Table 5
Recovery studies on the determination of Hg by CV-AFS using both, continuous and multicommutation modes[a]

	Hg concentration ($\mu g l^{-1}$)	Continuous $R\%$	Multicommutation $R\%$
Sample TR 117[b]	0.4	102.3±0.2	109±2
	0.6	109.3±0.1	88±1
Sample TR-23-00[c]	0.6	102.5±0.1	102±4
	1.5	103.04±0.02	100±2
Sample IMP 804967[c]	0.6	105±1	98.74±0.05
	1.0	108.9±0.7	104.77±0.03
	1.5	103.0±0.5	100.97±0.02
Sample IMP 804965[c]	1.0	92.1±0.5	81.5±0.1
	1.5	95.6±0.3	87.1±0.1

[a] Mean recoveries based on three replicate analysis and expressed in percentage.
[b] Obtained from 'Inter 2000 Program' organized by Generalitat de Catalunya.
[c] Obtained from the 'Programa de intercomparación de metales pesados' organized by the Spanish Ministerio de Agricultura, Pesca y Alimentación.

measurement zone favouring its separation from the reaction mixture. However, it has been noticed that Ar flow dilutes the analyte in the measurement zone and, because of that, the introduction of a pulse in valve 3 operation (diagram b in Fig. 2) could offer a possibility to reduce this effect.

When valve 3 was maintained 0.25 s in the 'on' position and 0.75 s switched off, a 140% fluorescence signal increase was obtained as compared to the continuous introduction of Ar, thus offering an additional improvement of the multicommutation mode in CV-AFS determination of Hg.

3.6. Determination of Hg in waters

Hg was determined in 5 samples for which, previous results from an external laboratory were available.

Both, the continuous and the multicommutation, operation modes were used for CV-AFS determination of Hg. Table 4 summarizes the data obtained and, as can be seen, values of the same order were found in all the cases.

The regression between data obtained by the developed procedure and those obtained by continuous measurement provided an equation $y = (0.4 \pm 0.6) + (1.06 \pm 0.01)x$ with a regression coefficient $r^2 = 0.9998$ thus evidencing the good comparability of both approaches, when the same operator was working using the same instrument.

On the other hand, the regression between data found by us, using the developed procedure and those obtained by an external laboratory provided an equation $y = (0.5 \pm 1.8) + (0.94 \pm 0.03)x$ with $r^2 = 0.997$ which demonstrates a good comparability and confirms the validation of the methodology of multicommutation CV-AFS for Hg determination in water.

Table 5 shows the results obtained in additional experiments concerning recovery studies on samples spiked with known amounts of Hg. It is evident that Hg concentration, ranging from 0.4 to 1.5 $\mu g l^{-1}$, could be recovered quantitatively by using both assayed measurements modes.

4. Conclusions

The developed procedure for Hg determination, based on CV-AFS measurements in the multicommutation mode, offers a sensitive and accurate way for water analysis without requiring any sample pre-treatment.

On comparing the multicommutation mode with the continuous operation of the CV-AFS system, it can be noticed that the multicommutation approach increases the productivity of the laboratory strongly reducing the sample and reagent consumption. However, the use of multicommutation can reduce the sensitivity of the AFS

measurements when sampling times lower than 20 s were employed. On the other hand, the reduction of the liquid gas phase separator volume, the increase of the sampling time to reach the steady-state, and the appropriate insertion of the Ar transport flow can compensate the loss of sensitivity inherent to injection approaches as compared with continuous measurements.

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«A multicommutated flow system for determination of bismuth in milk shakes by hydride generation atomic fluorescence spectrometry incorporating on-line neutralization of waste effluent»

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RESIDUES AND TRACE ELEMENTS

A Multicommutated Flow System for Determination of Bismuth in Milk Shakes by Hydride Generation Atomic Fluorescence Spectrometry Incorporating On-Line Neutralization of Waste Effluent

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A highly sensitive method was developed for determination of bismuth in milk shakes by multicommutation hydride generation atomic fluorescence spectrometry (HG-AFS) based on off-line sonication for 10 min with aqua regia 8% (v/v) and on-line waste treatment. The instrumentation and chemistry variables were studied in order to provide the best performance. The limit of detection in the original samples, established for a probability level of 99.6% (k = 3), was 1.67 ng/g Bi. The method provides a fast alternative in control analysis with a sampling throughput of 72 h as compared with 31 h obtained by the classical continuous measurement. Additionally, multicommutation reduces waste generation by a factor of 2.6. The consumption of sample, reductant, and blank, as compared with continuous mode HG-AFS, was reduced 9.6, 4.5, and 13.3 times, respectively. To confirm the accuracy of the method, recovery studies were performed, and excellent agreement between multicommutation and continuous measurement-based values was obtained. Application of the developed methodology for bismuth determination in milk shake samples from the Spanish market provided concentrations ranging from 4.2 to 15.0 ng/mL, and good comparability with data obtained by continuous measurements after microwave-assisted total digestion of samples for a 95% probability level and 12 degrees of freedom was found.

Efforts must be carried out in order to reduce or eliminate the undesirable side effects of chemical activities, such as the consumption of dangerous products and the production of toxic wastes. Therefore, in the development of a new analytical procedure, the amount and toxicity of the wastes are important analytical features to be considered in the evaluation of a method. The replacement of old in batch experimental practices by mechanized procedures such as flow injection-based methodologies, including modern multicommutation, will result in a drastic reduction of wastes. In this regard, multicommutation has brought a new dimension to analytical chemistry, providing a high number of measurements with reduced consumption of reagents and amount of wastes.

The potential of the multicommutation approach to reduce the consumption of reagents was exemplified by the development of procedures for the determinations of carbaryl pesticide and anions in waters by molecular spectrophotometry (1, 2) and for the determination of Hg by cold vapor atomic fluorescence spectrometry (CV-AFS; 3, 4).

Bismuth compounds are poorly absorbed by the organism, and bismuth can be found in saliva and excreted in the urine only at ultra-trace levels (5). The bismuth content in milk and dairy samples is very low, normally only a few ng/mL. However, the possible introduction of bismuth in the food chain from industrial activities could be dangerous because it passes the placenta and is eliminated in the body fluid. No work has been reported in the literature for bismuth determination in milk shake samples, and there are only a few concentration data on bismuth in milk. A concentration of 0.05 μg/g in cow's milk determined by stripping voltammetry has been reported (6), as well as values ranging from 0.09 to 20 ng/mL in human milk (7) and from 11.8 to 28.8 ng/g in commercialized cow's milk (8). Therefore, sensitive methods are required for the determination of this element in dairy products.

Numerous analytical techniques are available for the determination of traces of bismuth, and the most common is hydride generation coupled with atomic spectrometric techniques (9). AFS offers advantages in terms of selectivity, linearity, and detection levels (10) compared to traditional atomic absorption and emission, providing limits of detection

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(LOD) similar to those obtained by inductively coupled plasma-mass spectrometry (ICP-MS).

In commercially available hydride generation-AFS (HG-AFS) instruments, the analytes are introduced into the atomization cell as their gaseous hydrides, which are generated in a continuous hydride generator using sodium borohydride as reductant and an argon–hydrogen diffusion miniflame as the atomizer. The hydrogen for feeding the flame is chemically generated as a by-product of the sodium tetrahydroborate oxidation. So, this instrumentation involves excessive consumption of reagents and a high volume of waste generation.

The present study describes the development of a multicommutation-based system for the determination of bismuth in milk shakes using HG-AFS, in order to provide an environmentally friendly methodology for the analytical control of milk and dairy products. Additionally, an off-line sample pretreatment procedure, based on room-temperature sonication of samples with aqua regia and direct feed in the HG-AFS system, was compared to classical procedures based on the total digestion of samples previous to hydride formation.

Experimental

Apparatus

(a) *Multicommunication devise.*—A PSA Millennium Merlin 10025 from PS Analytical (Orpington, UK) was used to generate and detect bismuth hydride. The apparatus was equipped with 2 independent peristaltic pumps (P_1 and P_2), a 17 mL gas–liquid separation chamber, a Perma Pure® drier unit, a photomultiplier tube, and a data acquisition system. A boosted-discharge hollow cathode lamp (Photron, Victoria, Australia) operating at 223.1 nm was used as the excitation source.

Figure 1 shows the multicommutation device: It comprises five 3-way solenoid valves (NResearch, West Caldwell, NJ; 161T031), junctions mechanized in acrylic, and a PTFE reaction coil with 0.8 mm id. A Gilson (Middleton, WI) minipuls peristaltic pump (P_2) was used to transport NaOH and Fe(III) solutions through valves V_4 and V_5 for the on-line treatment of residues.

(b) *Microcomputer.*—PCL-711S interface board (American Advantec Corp., (Sunnyvale, CA), operating with homemade software written in QuickBASIC 4.5, was used for data acquisition and for the ON/OFF switching of the solenoid valves. An external power supply interface was used to match the electric current intensity and voltage required by the 3-way solenoid valves.

(c) *Microwave.*—Microwave-assisted digestion of the milk shake samples was carried out with a domestic microwave oven LG Intellowave (Manchester, UK), with a 2450 MHz frequency and operating at 500 W. Homemade 115 mL hermetically sealed PTFE reactors were used for the pressurized digestion.

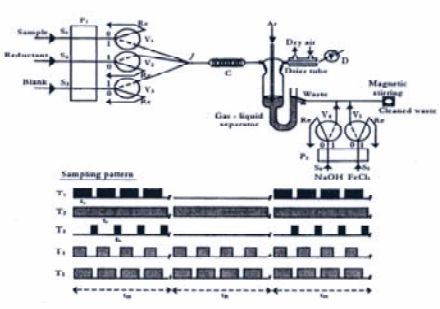

Figure 1. Manifold employed for bismuth determination by HG-AFS using multicommutation. The sampling pattern shows the valve sequence. S_1 = Milk shake sample or standard solution at flow rate of 4.6 mL/min; S_2 = reductant solution of $NaBH_4$ at flow rate of 5.0 mL/min; S_3 = blank solution of HCl at flow rate of 5.0 mL/min; S_4 = NaOH 6M solution at flow rate of 3.5 mL/min; S_5 = Fe(III) 50 μg/mL solution at flow rate of 3.5 mL/min; V_1, V_2, V_3, V_4, and V_5 = 3-way solenoid valves corresponding to sample or standard, reductant, blank, NaOH, and Fe(III) solutions, respectively; C = reaction and gas diffusing coil, 64 cm length, 0.8 mm id; J = 4-way type joint device machined in acrylic; P_i = peristaltic pumps; D = detector; Re = recirculation; T_1, T_2, T_3, T_4, and T_5 = times corresponding to V_1, V_2, V_3, V_4, and V_5, respectively; t_S = sampling time; t_r = reductant aspiration time; t_b = blank aspiration time; t_m = mixing time; t_R = reading time; (—) Bit 1 position (valve switched ON); (—) Bit 0 position (valve switched OFF). The shadow surface beneath the timing course lines indicates that valve was switched ON.

Reagents

The chemicals used were of analytical grade, and solutions were prepared in nanopure water, obtained from a Milli-Q water purifier system (Millipore, Bedford, MA), with a minimum resistivity of 18.2 MΩ cm.

(a) *Sodium borohydride utilized as the reductant.*—Prepared by dissolving the appropriate amount of the solid salt from Fluka (Seelze, Germany) in NaOH (Scharlau, Barcelona, Spain) in order to obtain a 1.0% (w/v) $NaBH_4$ in 0.4% (w/v) NaOH solution.

(b) *Blank solutions.*—Prepared by diluting hydrochloric acid (Scharlau) to 3.9 and 2.5M for the multicommutation and continuous modes, respectively.

(c) *Aqua regia (8%, v/v) for the sonication procedure.*—Prepared by mixing HCl (Scharlau) with HNO_3 (J.T. Baker, Deventer, Holland).

(d) *Nitric acid (J.T. Baker), H_2O_2 (Scharlau), and ascorbic acid (Merck, Darmstadt, Germany).*—Used in the microwave digestion procedure.

(e) *Working stock solution.*—A 100 ng/mL Bi(III) working stock solution was prepared daily by adequate dilution of a 1000 mg/L Bi(III) standard solution (Merck). Analytical solutions (0 to 10 ng/mL) in 3.9 and 2.5M HCl for the multicommutation and continuous procedure, respectively, were prepared by convenient dilution of the working stock solution.

(f) *Antifoam A.*—Sigma (Steinheim, Germany); was used to avoid foam formation during the hydride generation step.

(g) *Synthetic air and C-45 argon (Carburos Metálicos, Barcelona, Spain).*—Used for bismuth hydride drying and as the carrier gas, respectively.

Samples

Different milk shake samples obtained from the Spanish market were analyzed by the multicommutation and continuous modes: egg-and-sugar-flavored milk, yogurt shake, chocolate milk shake, mixed milk shake, strawberry milk shake, fruit milk shake, and cocoa milk shake. In the HG-AFS optimization studies, a milk shake sample spiked with 8.4 ng/mL bismuth was used.

Microwave-Assisted Digestion

Milk shake samples (2.0000 ± 0.0001 g) were weighed accurately inside a PTFE reactor. Concentrated HNO_3 (1 mL) was added, and the vessel was closed and irradiated for 4 cycles of 1 min at 500 W plus 10 min at 0 W. The reactor was allowed to cool, then 1 mL H_2O_2 (33%, w/v) and 0.5 mL concentrate HNO_3 were added and the irradiation cycles were repeated. A completely clear solution was obtained. It was allowed to cool, and 0.5 g ascorbic acid was added in order to eliminate excess HNO_3. Then, the digested sample was transferred quantitatively to a 50 mL volumetric flask.

To avoid damage of the magnetron as a result of nonabsorbed radiation, a beaker with 100 mL deionized water was placed inside the microwave oven in a fixed position, and its water load was changed after each heating cycle.

Table 1. Experimental conditions used for determination of bismuth in milk shakes by HG-AFS

Parameter	Continuous	Multicommutation
Wavelength, nm	223.1	223.1
Measurement mode	Peak height	Peak height
HCl, M/L	2.5	3.9
$NaBH_4$ %, w/v	1	1
Argon flow rate, mL/min	250	190
Sample flow rate, mL/min	5.2	4.6
$NaBH_4$ flow rate, mL/min	9.8	5
Blank flow rate, mL/min	5	5
Gas–liquid separator chamber, mL	17	17
Coil length, cm	150	64
t_s: ON/OFF sampling time, s	—	0.75/0.25
Sampling time cycles	—	18
t_r: ON/OFF reductant time, s	—	18/0
t_b: ON/OFF blank time, s	—	0.25/0.75
Blank time cycles	—	18
t_m: Mixing time, s	—	18

Sonication Treatment

Samples of milk shake (2.0000 ± 0.0001 g) were weighed accurately into a 50 mL volumetric flask, 2 mL antifoam A and 4 mL aqua regia were added, and the mixture was sonicated for 10 min in an ultrasonic water bath to leach bismuth quantitatively. Treated samples were acidified with HCl to final concentrations of 2.5 or 3.9M, depending on the detection mode.

HG-AFS Determination

In order to determine bismuth in milk shake, 2 measurement procedures were employed: a commercial PSA system in which the solutions or sample slurries were continuously flowing, and an alternative procedure based on multicommutation. Table 1 shows the experimental conditions used in both procedures for the determination of bismuth by HG-AFS.

In the continuous mode, sample or standard solutions were mixed with the $NaBH_4$ reductant reagent through a reaction coil, and the mixture was introduced into the 17 mL gas–liquid separation chamber. The bismuth hydride formed was transported by argon flow to the detector through a drier tube in which an air stream was flowing. Without delay, an HCl blank solution was merged with the $NaBH_4$ reductant reagent in order to clean the system, establish the baseline, and maintain the flame burn up. With this procedure, only the blank solution was recirculated and the consumption of sample and reagent depended on the flow rate values shown in Table 1.

For the introduction of previously leached samples in the HG-AFS system, the multicommutation setup indicated in Figure 1 was used. Valves V_1 and V_3 were switched ON/OFF alternatively (0.75/0.25 and 0.25/0.75 s, respectively), and V_2 was kept in Bit 1 position (ON) during all of the mixing time (t_m). Sample, blank, and reductant solutions merged together through the confluence point *J* into the reaction coil *C*. Figure 1 also indicates the valves' switching sequence, 0.75 s sample insertion time (t_s), 0.25 s blank insertion time (t_b), and permanent reductant insertion (t_r). The flow network was operated according to the valves' switching course shown in Table 2. After finishing the mixing time (t_m), valves V_1 and V_3 were switched OFF (in Bit 0 position), and valve V_2 was kept ON (in Bit 1 position) during the reading time (t_R). This was essential for supporting the flame burn up, because the HCl remaining in the gas–liquid separator was sufficient for $NaBH_4$ decomposition during t_R. When the solenoid valves were switched OFF (dotted line), sample solution (S_1), reductant solution (S_2), and blank solution (S_3) were pumped back to their reservoir vessels.

Table 2. Valve switching course for determination of bismuth in milk shakes by multicommutation HG-AFS

Step	V_1[a]	V_2[a]	V_3[a]	t, s	Cycles	Waste volume, μL[b]	Operation
1[c]	1	1	1	10	1	2433	Washing conditioning
2[d]	1	1	0	0.75	18	2160	Sample or standard + reductant insertion
	0	1	1	0.25		750	Blank + reductant insertion
3[e]	0	1	0	32	1	2667	Sample zone transport
							Hydride separation
							Signal reading

[a] Per measurement.
[b] Numbers 0 and 1 indicate that the valves (V) in Figure 1 are switched OFF or ON, respectively.
[c] Step was only necessary at the beginning of the measurement process.
[d] Mixing time sequence was repeated 18 times, corresponding to 18 s.
[e] Step corresponds to the period drawn in Figure 1, mentioned as a reading time.

Waste treatment was carried out on-line by introducing NaOH and $FeCl_3$ solutions through V_4 and V_5 valves, respectively. Alternatively switching valve V_4 ON/OFF for 0.5/0.5 s and V_5 for 0.8/0.2 s was enough to neutralize the acid waste solution to a pH around 7.5. At this pH, iron was precipitated as $Fe(OH)_3$ and heavy metals present in the liquid waste precipitated or coprecipitated with iron, thus providing a reduced volume of a passivated solid waste and a clear aqueous neutralized solution.

Results and Discussion

Conditions for Bismuth Determination by HG-AFS

Effect of the argon flow.—The effect of argon flow was evaluated in the 50 to 395 mL/min and 50 to 365 mL/min ranges for the continuous and multicommutation modes, respectively. As can be seen in Figure 2, the increase of the argon flow rate increases the atomic fluorescence signals up to maximum values at 250 and 190 mL/min for the continuous and multicommutation modes, respectively.

In the multicommutation mode, Ar introduction before the coil *C* was also studied. However, its direct insertion in the gas–liquid separation chamber was preferred once it provided a larger fluorescence signal in comparison to the other configuration.

Effect of flow rates.—The PSA instrument has only 3 speed positions for the 2 peristaltic pumps for the transport of $NaBH_4$, samples or standards, and HCl blank.

Upon combining the different speeds, the most appropriate were those indicated in Table 1. In the continuous mode, the blank, sample, and reductant flow rates selected were 5.0, 5.2, and 9.8 mL/min, respectively. These conditions ensured flame stability and avoided overpressure. However, the multicommutation mode required blank, sample, and reductant flow rates of 5.0, 4.6, and 5.0 mL/min, respectively, permitting less reagent consumption and waste generation.

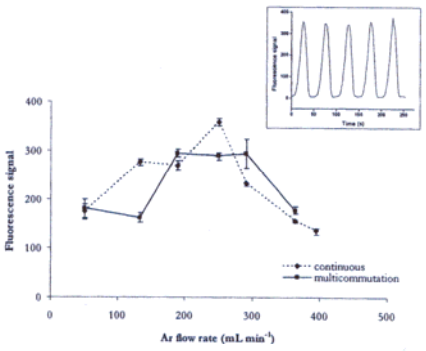

Figure 2. Effect of the Ar carrier flow AFS signal of slurries by continuous and multicommutation modes. Experiments corresponding to an original milk shake sample of 8.4 ng/mL bismuth concentration. Inset corresponds to the multicommutation recording of 3 ng/mL bismuth concentration.

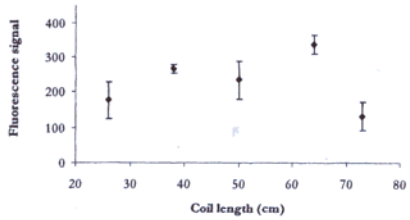

Figure 3. Effect of the reaction coil length on the fluorescence signal for a bismuth concentration of 8.4 ng/mL. Measurements made by multicommutation HG-AFS with a sample flow rate of 4.6 mL/min, $NaBH_4$ flow rate of 5.0 mL/min, and blank flow rate of 5.0 mL/min.

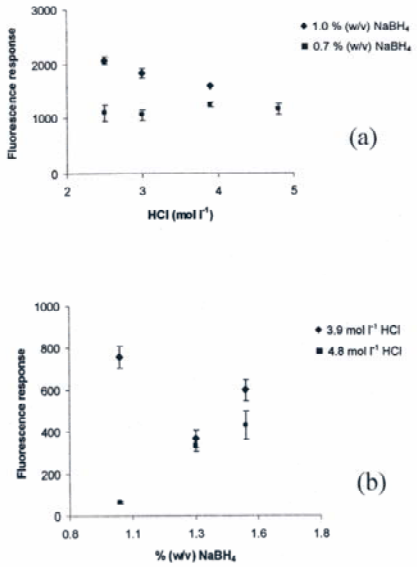

Figure 4. Effects of $NaBH_4$ and HCl concentrations on bismuth determination by HG-AFS using (a) continuous and (b) multicommutation modes.

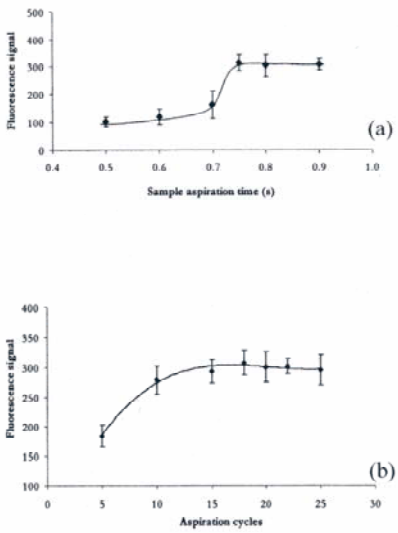

Figure 5. Effects of sampling time (a) and aspiration cycles number (b) on the fluorescence signals obtained by multicommutation HG-AFS. Data correspond to a milk shake sample with a bismuth concentration of 8.4 ng/mL. In each experiment, sample plus blank aspiration was complete in 1 s. The number of cycles was fixed at 20. In (b), V_1 was switched ON 0.75 s and OFF 0.25 s, while V_3 was switched ON/OFF 0.25/0.75 s, respectively. Therefore, 1 cycle corresponded to 1 s. In these experiments, the following conditions were maintained at constant values: 1.0% (w/v) $NaBH_4$ at 5.0 mL/min flow rate, 3.9M HCl at 5.0 mL/min, Ar at 190 mL/min, sample at 4.6 mL/min, and 64 cm coil length.

Effect of coil length.—The length of the reaction coil *C* (*see* Figure 1) controls the hydride formation time and, thus, must be large enough to provide a quantitative reaction between Bi(III) and $NaBH_4$ in the acidic medium. However, an increase in the coil length also increases the dispersion of the sample plug. Thus, this parameter must be controlled to ensure both total formation of hydrides and minimum dispersion.

A 150 cm PTFE coil with 0.8 mm id was used for mixing reagent solutions in the continuous mode, as it was shown to be best in a previous work (8). Figure 3 shows the coil length effect on the fluorescence signals obtained in the multicommutation mode by using the parameters shown in Tables 1 and 2. The coil length was varied from 26 up to 73 cm, and it was observed that 64 cm provided the maximum fluorescence signal for bismuth.

Influence of Reductant and HCl Concentrations

Figure 4 shows the effects of the HCl and $NaBH_4$ concentrations on the bismuth fluorescence. Figure 4a shows results obtained for the continuous mode. For the low $NaBH_4$ concentration, no influence of the HCl concentration was observed in the studied range. For the large reductant concentration, the largest signals were observed at 2.5M HCl. Furthermore, for reductant concentrations larger than 1.0% added, the overpressure was unpredictable and hydrogen formation became violent. On the other hand, the multicommutation mode (Figure 4b) required larger HCl concentrations than the continuous measurements to maintain the flame burn up. An HCl concentration of 3.9M and a reductant concentration of 1.0% were chosen to obtain the best sensitivity.

On the one hand, the use of HCl concentrations lower than 2.0 and 3.5M for the continuous and multicommutation modes, respectively, provided an extremely unstable flame that was easily extinguished for all of the $NaBH_4$ concentration ranges assayed. On the other hand, when reductant concentrations greater than 1.5% were used, the flame was in general excessively brilliant and intense, providing high background values and poor signal-to-noise ratios and increasing the standard deviation of measurements.

Multicommutation Parameters

For the multicommutation mode, variations in sample injection time and number of cycles were evaluated. Figure 5a

Table 3. Effect of microwave-assisted digestion conditions on the recovery of bismuth[a]

Conditions	Bismuth recovery, %
1 mL HNO_3 3 cycles 1 min 500 W + 1 mL H_2O_2 3 cycles 1 min 500 W	48 ± 1
1 mL HNO_3 3 cycles 1 min 500 W + 0.5 mL HNO_3 and 1 mL H_2O_2 3 cycles 1 min 500 W	53 ± 3
1 mL HNO_3 4 cycles 1 min 500 W + 1 mL H_2O_2 4 cycles 1 min 500 W	79 ± 8
1 mL HNO_3 4 cycles 1 min 700 W + 1 mL H_2O_2 4 cycles 1 min 700 W	93 ± 14
1 mL HNO_3 4 cycles 1 min 500 W + 0.5 mL HNO_3 and 1 mL H_2O_2 4 cycles 1 min 500 W	96 ± 9

[a] In all cases, 2.0000 ± 0.0001 g fruit milk shake was digested.

shows the effect of the sampling time on the fluorescence signal. The values for sample aspiration were the times during which V_1 was on ON (Bit 1), and was OFF (Bit 0) the rest of time to complete 1.0 s. A sample aspiration time of 0.75 s was the smallest adequate to obtain the best sensitivity. Using the aforementioned valve, the number of cycles necessary for complete mixing between sample and reagents was tested. As can be seen in Figure 5b, fluorescence response increased quickly with the number of cycles, reaching a constant value at approximately 18 cycles. In this way, 2160 µL sample plus reductant were introduced into the gas–liquid separator with 750 µL HCl blank plus reductant during the mixing time, as is indicated in Table 2 for each replicate analysis. The feeding of the flame during the reading time (32 s) required an additional reductant volume of 2667 µL.

Microwave-Assisted Digestion of Milk Shake Samples

Previous research (8) found that the microwave-assisted digestion of milk using small volumes of HNO_3 and H_2O_2 and a series of short irradiation steps of 4 min at a 500 W power provides clear solution. Therefore, milk shakes were analyzed in this way as can be seen in Table 3. Using the power irradiation program described in the *Experimental* section, quantitative recoveries were observed for both the continuous (90–106%) and multicommutation (90–106%) modes for all samples studied, showing that the conditions used in this work were adequate for quantitative recovery. As shown in Table 4, different spiked concentrations of bismuth in different samples gave recoveries close to 100%.

Table 5 shows the bismuth concentrations in all samples studied that were obtained after the microwave treatment.

Sonication Sample Treatment for Bismuth Determination in Milk Shake

A set of assays was performed with milk shake samples spiked with bismuth to assess the feasibility of sonication pretreatment using the experimental conditions previously reported (8). As can be seen in Table 4, recoveries from 95 to 104% were obtained for the continuous precedure, whereas in multicommutation mode the values varied from 96 to 106%. These data are similar to those obtained after microwave-assisted digestion, both by the continuous or multicommutation modes. Milk shake samples were analyzed after sonication using both

Table 4. Bismuth recoveries (%) found in 7 commercially available milk shake samples by HG-AFS after microwave-assisted digestion and sonication treatment[a]

		Microwave-assisted digestion		Sonication	
Sample	Bismuth added, ng/mL[b]	Continuous, %	Multicommutation, %	Continuous, %	Multicommutation, %
a	1	90 ± 3	101 ± 8	102 ± 6	106 ± 6
b	6	93 ± 2	106 ± 4	96 ± 4	98 ± 1
c	2	100 ± 2	100 ± 1	101 ± 7	104 ± 11
d	4	105 ± 2	92 ± 8	95 ± 3	104 ± 3
e	5	94 ± 1	97 ± 4	101 ± 4	96 ± 12
f	3	96 ± 9	110 ± 1	102 ± 3	99 ± 6
g	7	106 ± 7	103 ± 9	104 ± 4	97 ± 9
$\bar{x} \pm \sigma_{n-1}$[c]		98 ± 6	101 ± 6	100 ± 3	101 ± 4

[a] Measurements were carried out by continuous and multicommutation modes.
[b] Bismuth was spiked before sample treatment.
[c] n = 6 independent analyses.

Table 5. Total bismuth concentration (ng/mL ± σ_{n-1}[a]) found in milk shake samples after sonication and microwave-assisted digestion[b]

	Microwave-assisted digestion		Sonication	
Sample	Continuous, ng/mL	Multicommutation, ng/mL	Continuous, ng/mL	Multicommutation, ng/mL
a	14.2 ± 0.9	15.0 ± 1.2	15.0 ± 1.0	14.4 ± 0.8
b	5.4 ± 0.4	6.0 ± 0.8	4.6 ± 0.8	4.5 ± 0.6
c	10.7 ± 0.9	10.7 ± 0.3	10.3 ± 0.6	10.2 ± 0.5
d	7.8 ± 0.8	7.5 ± 0.6	8.2 ± 0.5	8.6 ± 0.8
e	8.5 ± 1.0	8.2 ± 1.0	8.5 ± 0.6	7.4 ± 0.9
f	4.6 ± 0.9	4.2 ± 1.0	4.8 ± 0.7	5.4 ± 0.7
g	9.2 ± 1.0	9.6 ± 0.4	10.1 ± 0.6	9.4 ± 0.6

[a] n = 3 independent analyses.
[b] Measurements were carried out by continuous and multicommutation modes.

multicommutation and continuous mode measurements, and the results are summarized in Table 5. As can be seen, the bismuth content varied between 4.2 and 15.0 ng/mL; the results were comparable for both modes and not significantly different from those obtained after microwave-assisted digestion.

Analytical Features of the HG-AFS Determination of Bismuth

The main features of the continuous and multicommutation modes for the determination of bismuth by HG-AFS are depicted in Table 6. As can be seen, the results obtained were comparable with those shown in the literature (8).

The LOD of the multicommutation mode, established for a probability level of 99.6% (k = 3), was 0.067 ng/mL, slightly higher than that found by the continuous mode (0.045 ng/mL); relative standard deviation (RSD) values for both modes were adequate and comparable. Analytical sensitivity for the multicommutation mode was lower than that obtained in the continuous mode.

However, a clear advantage of the multicommutation mode was the large reduction of reagent comsumption and waste generation. As can be seen in Table 6, sample consumption was reduced nearly 10 times, while the reductant consumption was reduced 4.5 and 13.3 times, respectively. The total waste generated was only 4015 µL for each determination made by multicommutation, whereas continuous determination produced 10 462 µL waste per replicate. Moreover, improvement of the sample throughput was remarkable: from 31 determinations/h for continuous measurements to 72 determinations/h, as can be seen in the Figure 2 inset, for multicommutation. In this way, a 2× enhancement of laboratory efficiency can be attained, without reducing the analytical advantages of continuous HG-AFS.

Comparison of Assay Methods

The comparison of data found for bismuth by multicommutation HG-AFS after sonication (y) and microwave-assisted digestion (x) provided an equation $y = (0.9 \pm 0.1)\,x + (0.7 \pm 1.0)$ with a regression coefficient of 0.96. Calculated t values for the slope (1.0) and intercept (0.7) were lower than the theoretical t value (1.78) for a 95% probability level and 12 degrees of freedom, thus showing good comparability of the results. It can be concluded that the fast sample pretreatment offered by sonication is sufficient for bismuth determination in these kinds of samples.

However, the comparison between continuous HG-AFS after microwave sample digestion (x) and sonication (y) provided a relation $y = (1.09 \pm 0.08)\,x - (0.6 \pm 0.7)$ with a regression coefficient of 0.99 and t calculated values for the slope (1.1) and for the intercept (0.8) lower than the theoretical one (1.78).

All of the aforementioned data evidenced a good comparability between the 2 measurement procedures, indicating that multicommutation approach provides results as valid as those found by operating the HG-AFS system in the continuous commercially available mode. Considering the excellent throughput together with the reduced reagents consumption and waste production, the determination of bismuth by multicommutation HG-AFS after milk shake sample sonciation with aqua regia is the recommended procedure for routine analysis.

Accuracy was estimated throughout this research by determining recovery from spiked samples because no certified reference materials for bismuth in dairy products were available.

On-Line Waste Treatment

Multicommutation, like other flow techniques, offers a unique possibility to minimize the problems related to the environmental contamination created by analysis wastes. During the hydride generation, bismuth standard solution with a concentration of only a few ng/mL passes into the air and does not represent a risk for human health. However, the liquid waste generated has a very acidic pH, lower than 1, and contains all the metal ions that do not form covalent hydrides.

Table 6. Analytical parameters of HG-AFS determination of bismuth by using continuous and multicommutation modes

	Continuous AFS	Multicommutation AFS
Calibration line	$I_F = (1161 \pm 61)\ C_{Bi} + (125 \pm 46)$	$I_F = (819 \pm 16)\ C_{Bi} + (118 \pm 97)$
Correlation coefficient, r	0.9995	0.996
RSD, %[a]	13.2	11.3
RSD, %[b]	6.1	9.3
LOD, ng/mL[c]	0.045	0.067
LOD, ng/g[d]	1.12	1.67
Sample, mL[e]	996.7[f]	103.5
Reductant, mL[e]	1878.3[f]	416.7[g]
Carrier blank, mL[e]	500.0[h]	37.5
Ar consumption, mL/min	250	190
Waste, mL[i]	1046.2	401.5
Throughput, h^{-1}	31	72

[a] RSD % = Relative standard deviation corresponding to 9 independent measurements of a solution containing 1.0 ng/mL Bi.
[b] RSD % = Relative standard deviation corresponding to 6 independent analyses of a sample containing 8.4 ng/mL Bi.
[c] LOD = Limit of detection for diluted samples.
[d] LOD = Limit of detection for the original sample.
[e] Sample and reagent consumption corresponding to 100 analyses.
[f] Values calculated for 115 s [analysis time (55 s) plus delay and memory time (60 s)].
[g] Value including 18 s of mixing time and 32 s of reading time.
[h] Value calculated for 60 s (delay and memory time) because blank solution was recycled during analysis time in continuous mode.
[i] Waste generated was established for 1 h working session.

The possibility of doing on-line waste treatment was evaluated using the multicommutation device, by mixing the effluents with Fe(III) and NaOH solutions in order to obtain a neutral waste, as shown in Figure 1. A few grams of a solid residue consisting of ferric hydroxide containing coprecipitated heavy metals was obtained instead of 401.5 mL of polluted aqueous waste for a 1 h working session. To do the on-line treatment, valve V_5 controlling the Fe(III) solution flow was switched at intervals of 0.8 s (ON) and 0.2 s (OFF). Valve V_4 controlling the introduction of NaOH solution was switched (ON/OFF) at intervals of 0.5/0.5 s.

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Multicommutation as an environmentally friendly analytical tool in the hydride generation atomic fluorescence determination of tellurium in milk

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Abstract The aim of this study is to show the advantages of the emerging multicommutation methodology based on the use of solenoid valves for Te determination in milk by hydride generation atomic fluorescence spectrometry (HG-AFS). The delivery of a series of alternating sequential insertions of small volumes of samples and reagents gives rise to new hydrodynamic processes and exciting analytical potentials by controlling the time of flow through the on/off-switched solenoid valves. This drastically reduces the reagent consumption by a factor of 4 and the generation of effluents (590 mL h^{-1} instead of 750 mL h^{-1} generated by the continuous-mode measurement) and also provides an improvement in the laboratory productivity by an increase of the sample throughput (85 h^{-1} compared to 20 h^{-1} found in the continuous mode). So, multicommutation is an environmentally and economically sustainable alternative to the methodology based on continuous measurements. The multicommutation-based method developed was applied to tellurium determination in commercially available milk samples; a calibration range of 0.0–0.5 ng mL^{-1} and a detection limit of 0.20 ng L^{-1} with average relative standard deviation of 2.1% were found. Comparable results were obtained for a series of samples using both continuous and multicommutation HG-AFS modes.

Keywords Multicommutation · Tellurium determination · Atomic fluorescence spectrometry · Hydride generation · Milk analysis

Introduction

Multicommutation was introduced nine years ago and it can be considered as a flow analysis technique [1]. The ability of the multicommutation approach to handle samples and reagents using simple manifolds has been exploited to develop several analytical procedures. Among these are the determination of two or three analytes at a time by spectrophotometry [2, 3], those for the true titration in spectrophotometry and potentiometry [4, 5] and the high dilution degree obtained on-line for samples in potentiometry [6, 7]. However, at this moment, there is no application involving the use of multicommutation in hydride generation atomic fluorescence spectrometry (HG-AFS); the only precedent for the use of this methodology is provided by our preliminary studies on Hg determination by cold vapour atomic fluorescence spectrometry (CV-AFS) [8, 9, 10].

In this work, we intend to exploit multicommutation's ability to handle solutions for the determination of tellurium in milk samples by HG-AFS, thus evaluating both the advantages reported by multicommutation to save reagents and reduce wastes, and the preservation of the best analytical performance of the procedure, in comparison with the continuous mode atomic fluorescence dry-ashing reference method [10].

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Experimental

Apparatus

The atomic fluorescence equipment set-up comprised a PSA Millennium Excalibur 1055 from PS Analytical (Kent, UK) with two peristaltic pumps, a Permapure® dried air unit, a boosted discharge lamp from Photron and filters, which permits the use of a specific wavelength, and a 17 mL gas–liquid separation chamber. Software provided by PSA (Avalon Windows Software) was used to control the fluorescence equipment and perform data acquisition.

A hydrogen generation system Claind HG-2000 (Lenno, Italy) was employed to improve the stability of the flame.

The multicommutation manifold (Fig. 1) comprised three three-way 161T031 NResearch solenoid valves, reaction coils and flow lines of PTFE with 0.8 mm and 1.0 mm internal diameter, and T-type joint devices machined in acrylic. A 486 microcomputer equipped with a PCL711S Advantec interface card was employed to control the flow system using software written in Quick BASIC 4.5. An external power supply interface was employed to match the electric current intensity and voltage required by the three-way solenoid valves.

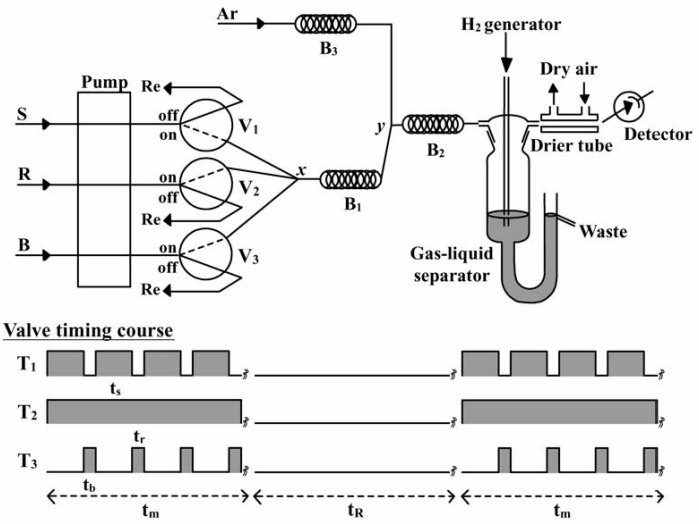

Fig. 1 Flow diagram of the multicommutation manifold for HG-AFS determination of tellurium. V_1, V_2 and V_3 three-way solenoid valves; B_1 reaction coil, 30-cm length, 0.8-mm i.d.; B_2 gas diffusing coil, 47-cm length, 0.8-mm i.d.; B_3 back-pressure-reducing coil, 60-cm length, 1.0-mm i.d.; *S* sample solution, flow rate at 8.6 mL min^{-1}; *R* tetrahydroborate reagent solution, flow rate at 4.9 mL min^{-1}; *B* blank solution, flow rate at 10.2 mL min^{-1}; *x* and *y* T-type joint devices machined in acrylic; *Re* solution circulate to it reservoir vessel; T_1, T_2 and T_3 switching timing course for valves V_1, V_2 and V_3, respectively; t_s sampling time; t_r reducing time; t_b blank time; t_R reading time; t_m mixing time. *Solid* and *dotted lines* in valve symbols indicate the solution pathway with valve switched off (bit 0 electric position) and on (bit 1 electric position), respectively. The *shaded area* beneath the timing course lines indicates that the valve was considered switched on

Table 1 Instrumental conditions for tellurium determination in milk samples by HG-AFS

Parameter	Continuous mode	Multicommutation mode
Wavelength (nm)	214.3	214.3
Measurement mode	Peak height	Peak height
HCl (mol L^{-1})	4	4
$NaBH_4$% (w/v)	0.5	1.0
Ar flow rate (mL min^{-1})	400	250
Hydrogen flow rate (mL min^{-1})	120	20
Blank flow rate (mL min^{-1})	10.2	10.2
Sample flow rate (mL min^{-1})	9.6	9.6
$NaBH_4$ flow rate (mL min^{-1})	4.9	4.9
Gas–liquid separator volume (mL)	17	17
B_1 coil length (cm)	150	30
B_2 coil length (cm)	–	47
B_3 coil length (cm)	–	60
t_s: on/off sampling time (s)	–	0.75/0.25
Sampling time cycles	–	30
t_r: on/off reducing time (s)	–	30/0
t_b: on/off blank time (s)	–	0.25/0.75
Blank time cycles	–	30
t_m: Mixing time (s)	–	30
t_R: Reading time (s)	–	12[a]

[a]All valves were switched off during reading time

Table 1 summarises the operational parameters employed in both continuous and multicommutation modes for tellurium determination.

A domestic microwave oven LG Intellowave (Manchester, UK) of 2.450-MHz frequency and operating at a maximum exit power of 700 W was employed for microwave-assisted digestion using an hermetically sealed homemade 120 mL internal volume PTFE reactor for the treatment.

Reagents and solutions

All chemicals used were of analytical grade and high-purity water with a resistivity higher than 18.2 MΩ cm was obtained from a Milli-Q water purifier system (Bedford, USA).

In multicommutation mode, a 1.0% (w/v) $NaBH_4$ solution and in continuous mode a 0.5% (w/v) $NaBH_4$ solution in 0.1 mol L^{-1} NaOH were prepared by dissolving the appropriate amount of $NaBH_4$ supplied by Fluka (Sigma–Aldrich, Germany) and NaOH from Scharlau (Barcelona, Spain). Those solutions were prepared daily and filtered through a 0.45 µm nylon membrane.

KBr from Merck (Darmstadt, Germany) was used to assure the correct form of tellurium in the samples. The role of KBr solution was to reduce Te(VI) to Te(IV) in samples, which provides the fluorescence signal.

Antifoam A from Sigma (Steinheim, Germany) was employed to avoid foam formation in sample analysis.

A blank carrier was prepared by taking 333 mL of conc. hydrochloric acid from Scharlau (Barcelona, Spain) and 10.0 g of KBr, and the volume was made up to 1,000 mL with water.

Standard solutions of 0.00, 0.05, 0.10, 0.25 and 0.50 ng mL^{-1} were prepared from appropriate dilutions of the 100 ng mL^{-1} Te(IV) stock solution. Concentrated hydrochloric acid solution (33.3 mL) and 1.0 g of KBr were added and diluted to 100 mL with water.

Samples

Cow milk samples were commercially available and were selected from different parts of Spain, with references: (1) full cream milk, (2) full cream with added Ca, (3) partially skimmed milk, (4) skim with added folic acid, (5) partially skim with added vitamins and minerals, (6) skimmed milk with added omega 3 acid, (7) skimmed milk, (8) vegetable base milk and (9) partially skimmed milk with added propolis.

Slurry sonication treatment

About 1 g (±0.0001) of milk was accurately weighed in a 25 mL volumetric flask and 2 mL of *aqua regia* and 1 mL of antifoam A were added. The mixture was sonicated for 10 min in an ultrasound water bath to achieve a complete extraction of Te into the aqueous solution from the sample slurries. After this treatment, 0.25 g KBr was added and the slurry was acidified with conc. HCl to a final concentration of 4 mol L^{-1} by diluting up to 25 mL with water. The sample was then transferred to a 50 mL beaker and heated for 15 min in a water bath at 60°C. After cooling to room temperature the sample slurries were introduced into the HG-AFS system [11].

Microwave-assisted digestion

About 1 g (±0.0001) of milk was accurately weighed into a PTFE reactor. A 1 mL aliquot of conc. HNO_3 was added and the vessel was closed, placed inside the microwave oven and irradiated for 4 cycles of 1 min at 500 W plus 10 min without irradiation. The reactor was cooled and 1 mL H_2O_2 30% (w/v) and 0.5 mL conc. HNO_3 were added; the irradiation was then repeated for 4 cycles of 1 min at 500 W plus 10 min without irradiation. At the end of this process, completely clear solutions were obtained. Ascorbic acid (0.5 g) was added to eliminate the excess of HNO_3 and the digested sample was transferred quantitatively to a 50 mL volumetric flask. For total Te determination 16.7 mL of conc. HCl and 0.5 g of KBr were added and then solutions were diluted to 50 mL and heated at 60°C for 30 min. The solution was allowed to cool to room temperature before feeding samples into the HG-AFS system. In order to prevent damage of the magnetron from reflected unabsorbed radiation, a beaker with 100 mL of deionized water was introduced inside the microwave oven in a cool position and the water changed after every step [12].

Reference method for sample dry-ashing

Milk (5.00±0.01 g) was weighed into a beaker and 0.25 mL of ashing aid (1% (w/v) MgO and 10% (w/v) $Mg(NO_3)_2$) was added and the mixture was evaporated to the dryness in a convective oven at 140°C for a night. The residue was treated with 2 mL 35% (w/v) HNO_3 and evaporated on a hot plate. When the residue was completely dried, it was placed into a muffle furnace and the following heating program was applied: 150°C for 1 h, 200°C for 2 h, 250°C for 1 h, 300°C for 3 h, 350°C for 30 min, 400°C for 30 min and finally 450°C for 14 h. At the end of the process 1 mL 35% (w/v) HNO_3 was added before repeating the preceding procedure in order to obtain white ashes. Ashes were dissolved with 25 mL of 4.5 mol L^{-1} HCl. Finally 10 mL of this solution was transferred to a 50-mL volumetric flask and 12.9 mL conc. HCl and 0.5 g KBr were added before dilution to 50 mL with water. The resulting solution was heated at 60°C for 30 min [10].

Flow manifolds

In continuous mode (manifold not shown), sample or standard solutions were mixed with the sodium tetrahydroborate reducing reagent inside a reaction coil. The mixture was introduced inside the gas–liquid separation chamber from which the tellurium hydride (H_2Te) was transported, by an argon flow, through the drier tube and finally to the detector. In this configuration only the blank solution is recycled and sample and reagent consumption depend on the time of operation and the flow rate. Alternatively, the blank solution was merged with reagents in order to clean the system, establish the baseline and maintain burn up the flame.

The flow diagram of the multicommuted system is depicted in Fig. 1. When the solenoid valves V_1, V_2 and V_3 are switched off (solid lines), sample solution (S), reagent solution (R) and blank solution (B) are pumped back to their reservoir vessels. Meanwhile, an argon stream flowed through coil B_2 towards the gas–liquid separation chamber and an additional hydrogen stream was introduced into this chamber. When the software was run the microcomputer sends a set of electric pulses through the PCL711S interface card following the pattern shown in the valve timing course. Under these conditions, the solenoid valve V_1 was switched on (discontinuous line) and off several times during the sampling time interval (t_s) inserting into the coil B_1 a sample volume v_s, where $v_s=n\varphi_s t_s$ (φ_s is the sample flow rate, n is the number of cycles and t_s is the sampling time). The solenoid valve V_2 was switched on during the reducing time (t_r) and inserted a reducing volume of v_r through the coil B_1, where $v_r=\varphi_r t_r$ (φ_r is the reducing flow rate and t_r is the reducing time). At the same time, the valve V_3 was switched on/off to insert into coil B_1 a volume of the blank solution. As can be seen, the reducing solution is flowing throughout the mixing time (t_m).

Sample and reagent solutions merged into the coil B_1, thus promoting the reaction to form H_2Te. The insertion of the argon stream in the joint point y caused a multisegmentation of the sample zone that was directed towards the gas–liquid separation chamber where it was mixed with the hydrogen stream coming from an external generator, also promoting the transfer of the hydride to the gaseous phase. This mixture of gas was directed towards the burner and tellurium fluorescence signal was measured as in the continuous mode procedure.

The argon and hydrogen streams, the sample, blank and reducing solutions flow rates were maintained at 250, 20, 9.6, 10.2 and 4.9 mL min^{-1} respectively, as indicated in Table 1.

Results and discussion

Experimental conditions for tellurium determination by AFS

In the continuous HG-AFS mode, Ar is bubbled through the liquid phase and inserted before the phase separation. Argon is therefore only employed to separate the gaseous Te hydride from the reaction mixture and thus, following the instrument design, it dilutes the analyte and is used for hydride transport.

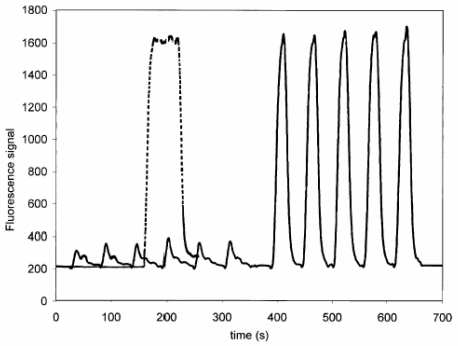

Fig. 2 Fluorescence signal of Te obtained by continuous (*dashed line*) and multicommutation (*dashed line*) modes. Te concentration=3 ng mL^{-1}. When using the continuous system, one signal was presented. Using multicommutation mode six blanks and five signals were obtained, measured in lower sensitivity range than that used for continuous measurements

A modification, introduced by us, consisted of inserting the Ar flow before the separation chamber, as can be seen in Fig. 1. Under these conditions, Ar really acts as a carrier of the hydride causing a multisegmentation of the sample zone; Te hydride is continuously evolved from the liquid phase during the transport to the separation chamber.

In the aforementioned situation, the AFS signals obtained by multicommutation very rapidly reach the steady state, thus providing the possibility of reducing the measurement time. In Fig. 2 an increase of the sample throughput can be observed when using the multicommutation mode (continuous line). Therefore, compared with the use of the continuous mode, the manifold developed here leads to a pronounced lessening of the analytical time required to attain the same sensitivity, thus increasing the laboratory productivity through the enhancement of the sampling throughput and reduces the side effects of waste generation.

Multicommutation compared to continuous mode

All preliminary experiments were carried out by processing sequentially a 3 ng mL^{-1} tellurium standard solution and a blank solution. The two parameters initially searched to define the best operational conditions were the amount of sample solution loaded into reaction coil B_1 and the volume ratio between aliquots of sample, blank and reducing solution. In the first assay, the microcomputer switched valves V_1, V_2 and V_3 on simultaneously and the time interval to maintain each valve open was varied from 5 to 40 s varied (mixing time). The reading time (t_R) was maintained at 12 s. Other assays were performed by varying the time interval to switch on valve V_1. While V_1 (sample valve) was maintained on, valve V_3 (blank valve) was switched off and valve V_2 (reducing valve) was switch on all the time.

The reaction to form the H_2Te occurred into the coil B_1; thus, to define the best conditions, experiments were carried out using coils of 19.5, 30 and 47 cm length and 0.8-mm i.d. Valve V_1 was switched on/off during 0.75/0.25 s; valve V_3 was switched on/off 0.25/0.75 s; and V_2 reducing valve was switched on all the time, to maintain the flame burn up and to displace the sample zone up to the joint *y* point, as indicated in the valve timing course in Fig. 1. The sampling time intervals 0.75/0.25 s could be set long enough to allow the analytical signal to attain steady state conditions and return to the baseline. In this sense, mixing time values of 5, 10, 20, 30, 40 s were evaluated to reach an appropriate signal. The steady state was attained with a 30 s mixing time and using V_1 in intervals of 0.75/0.25 s.

During the signal reading step (t_R), all valves were switched off and sample, blank and reagent solutions were pumped back to their respective reservoir vessels. As indicated in the valves timing course, after the reading time (t_R) was completed, another analytical run could be started (t_m).

Once the system's operational variables were studied, a set of experiments was carried out by using tellurium standard solutions ranging from 0.0 to 0.5 ng mL^{-1}. These standard solutions and nine milk samples were also processed using the procedure for tellurium determination suggested in the supplier's manual for the fluorescence spectrometer equipment and based on continuous measurements.

Effect of flow-rate values and sample and reagents volumes employed for multicommutation HG-AFS determination of tellurium

In the continuous mode HG-AFS, sample and reagents were continuously merged. In contrast, on using multicommutation, the discrete insertion of small volumes of sample and reagents makes it necessary to manage the reduction of Te and gas phase evolution by controlling the time of contact between solutions.

Argon and hydrogen streams, sample, blank and reagent solutions flow rates were maintained at 250, 20, 8.6, 10.2 and 4.9 mL min^{-1} respectively. It was observed that these flow rates were the best to obtain H_2Te species and to maintain the adequate flame providing the highest and most stable fluorescence signals.

The use of different mixing times from 5 to 40 s was evaluated for a fixed hydrochloric acid concentration of 4.0 mol L^{-1} with a flow rate of 10.2 mL min^{-1} and a sodium tetrahydroborate solution of 1.0% (w/v) or 0.5% (w/v) $NaBH_4$ (for multicommutation or continuous modes, respectively) with a flow rate of 4.9 mL min^{-1} and a sample flow rate of 9.6 mL min^{-1} using a fixed reading time of 40 s.

In a previous experiment, valves V_1, V_2 and V_3 were opened and closed at the same time. In these conditions, the steady state can be reached for a mixing time of 30 s, which corresponds to a sample volume of 4,300 µL (data not shown).

In order to reduce the sample consumption and to search for a better mixture between sample and reagents, the effect of valve V_3 pulsation during the time at which valve V_1 was switched off was evaluated. V_2 was opened all the time to maintain the flame burn up. It was observed that for a sampling pulse of 0.75 s and a blank pulse of 0.25 s, during 30 times (which corresponds to a mixing time of 30 s), the sensitivity of the signals does not change by introducing the V_3 pulsation as compared with the continuous sampling. However, sample consumption for the pulsation mode was reduced to 3,225 µL. Thus, we selected this better procedure for application of the method.

Additional experiments (results not shown) evidenced that the use of 0.5/0.25 s (on/off, sample/blank) pulses reduces the sensitivity by about 12%. On the other hand, the use of 0.75/0.1 s (on/off, sample/blank) pulses reduces the blank dilution effect and thus increases the sensitivity of Te reading, but increases the standard deviation of the signals. Additionally, this pulse (0.1 s) is at the limit of the stable valve response and thus it was chosen to use the 0.75/0.25 s (on/off) pulse as the best time step.

Effect of reaction coils

Coil B_3 was employed to protect the valves from an overpressure of Ar because these valves can support only 30 psi and the Ar pressure imposed by the HG-AFS equipment is of the order of 35–40 psi [8]. Experiments were carried out by using the coil B_3 with inner diameter of 1.0 mm and 60 cm length.

B_1 and B_2 coils were used to assure the complete generation of H_2Te from the reaction with $NaBH_4$. For a fixed internal diameter of 0.8 mm, 19.5 to 47 cm coils were assayed. To define the best conditions, experiments were carried out using coils B_1 and B_2 of 30 and 47 cm, respectively.

Analytical features of HG-AFS determination of Te

Table 2 shows the analytical features of HG-AFS Te determination by using the continuous and multicommutation modes. It can be concluded that the sensitivity of procedures compared is of the same order.

The limit of detection for Te HG-AFS determination, established for a probability level of 99.6% (k=3), varies from 0.13 to 0.20 ng L^{-1} as a function of the analytical sensitivity and the standard deviation of blank measurements having not observed any significant difference of this parameter by using multicommutation or continuous modes. The precision of measurements was also the same; typical values of 1.6–2.4% were found in the continuous mode compared to 2.1% in the multicommutation mode.

As Table 2 indicates, a sample volume of 3.2 mL and a reagent volume of 2.4 mL per analysis are enough to carry out the determination of tellurium with an appropriate sensitivity; a waste of 590 mL h^{-1} is produced for 85 determinations in the multicommutation mode. These consumptions are clearly lower than the 17.2 mL sample consumption and 9.8 mL reagent required for the continuous mode measurement, which also involves a waste generation of 750 mL h^{-1} for only 20 determinations. Therefore, the main advantages of using multicommutation for tellurium determination by HG-AFS concern the pronounced reduction of sample and reagent consumption and the reduction of waste generation. On the other hand, an enhancement of the productivity of the laboratory, from 20 to 85 h^{-1}, was obtained without sacrificing the analytical sensitivity.

Table 2 Analytical parameters found for the HG-AFS determination of Te using the continuous mode and the multicommutation mode

	Continuous AFS using H_2	Multicommutation AFS using H_2
LOD (ng L^{-1})	0.13	0.20
RSD%	1.6	2.1
Calibration range (ng mL^{-1})	0–0.5	0–0.5
Calibration line	y=(27.2±0.8)+(997±6)C	y=(60±10)+(810±40)C
Correlation coefficient (r)	0.9998	0.996
Mixing time (s)	60	30
Sample consumption (mL)*	1720[a]	322
Reducing consumption (mL)*	980[a]	245
Blank consumption (mL)*	1020[b]	127
Waste (mL)**	750	590
Ar consumption (L min^{-1})	0.4	0.25
H_2 consumption (L min^{-1})	0.12	0.02
Throughput (h^{-1})	20	85

*Sample and reagent consumption correspond to a batch of 100 analysis
**Waste generated was established for 1 h working session
[a]Values calculated for 120 s (mixing time plus delay and memory time)
[b]Values calculated for 60 s (delay and memory time), because blank solution was recycling during mixing time in continuous mode
LOD limit of detection
RSD% relative standard deviation, corresponding to three replicates

Sample pre-treatment

Complete sample digestion through dry-ashing on a microwave-assisted digestion was employed for the previous sample preparation in order to obtain data on Te content in milk by HG-AFS. Dry-ashing and continuous-mode HG-AFS was used as a reference procedure and for multicommutation AFS microwave-assisted digestion, together with a simple room-temperature sonication of sample slurries with *aqua regia* were employed.

Table 3 summarizes data found for commercially available milk samples obtained from the Spanish market. Analysis of this table reveals that all the procedures assayed provided comparable results.

Determination of Te by using slurry sonication

Aqua regia at room temperature and sonication for 10 min seems to be enough to extract tellurium from milk (see Table 3). Additionally, in order to test the accuracy of this simple procedure recovery studies were carried out on different samples and at different spiked levels before the sonication with *aqua regia* by using the criteria of adding the half amount of Te present in each sample. Results obtained are shown in Table 4 and provided recoveries of 94–103%.

On the other hand, Table 3 shows that results obtained for three milk samples analysed by using slurry sonication and by HG-AFS employing both continuous and multicommutation modes are statistically comparable, thus confirming the validity of the sonication strategy.

Recommended procedure for determination of Te in milk samples

Te was determined in nine different samples by using slurry treatment, microwave-assisted digestion and dry-

Table 3 Determination of Te in milk by HG-AFS using continuous and multicommutation modes[a]

	Continuous		Multicommutation	
	Dry ashing	Sonication	Microwave digestion	Sonication
1 Full cream milk	6.3±0.3	–	6.1±0.2	6.0±0.1
2 Full cream with Ca	8.1±0.7	–	8.5±0.3	8.3±0.4
3 Partially skimmed milk	6.6±0.3	–	6.2±0.9	6.3±0.8
4 Skim with folic acid	8.7±0.4	–	9.1±0.3	8.9±0.2
5 Partially skimmed with vitamins and minerals	9.0±0.3	–	9.3±0.5	9.4±0.5
6 Skim with omega 3	9.4±0.7	–	9.7±0.5	9.6±0.5
7 Skimmed milk	1.3±0.7	0.9±0.1	1.0±0.2	1.0±0.2
8 Vegetable base milk	2.4±0.5	2.9±0.2	2.8±0.9	2.7±0.3
9 Partially skimmed with propolis	9.3±0.5	9.0±0.2	9±1	8.6±0.2

[a]Mean values and uncertainties (ng mL^{-1}) based on three independent analysis of each sample

Table 4 Tellurium recoveries (%) found in five commercially available milk samples by slurry sampling. Standard deviation corresponding to three independent replicates

Milk	Te Spiked (ng mL^{-1})	Recoveries (%)
7 Skimmed milk	0.5	96±3
9 Partially skimmed with propolis	1	103±3
8 Vegetable base milk	2	99±4
1 Full cream milk	3	97±4
6 Skim with omega 3	4	94±5

Table 5 Regression lines between data found by dry-ashing, slurry sonication and microwave-assisted digestion HG-AFS determination of Te in milk samples using also continuous and multicommutation measurement modes

Comparison	Regression line[a]	R^2	t_{cal} slope[b]	t_{cal} intercept[b]
Method 1–4	y=(1.02±0.04)x–(0.1±0.3)	0.990	0.41	0.30
Method 2–4	y=(1.01±0.05)x–(0.1±0.3)	0.990	0.24	0.32
Method 1–2	y=(0.99±0.02)x–(0.0±0.1)	0.998	0.72	0.10
Method 3–2	y=(0.98± 0.02)x+(0.03±0.09)	0.998	1.04	0.33

Method 1 microwave and multicommutation; *method 2* slurry sonication and multicommutation; *method 3* slurry sonication and continuous measurement; *method 4* dry-ashing and continuous mode
[a] Regression lines were established between all the partial data found for the analysis of nine commercially available milk samples
[b] t theoretical values for slope and intercept were 2.120 for a probability level of 95% and 16 freedom degrees, which correspond to the comparison between 9 pairs of data for each element.

ashing. Data of tellurium determination in milk obtained by multicommutation were compared to those found by measurements in continuous mode after milk digestion.

Table 5 provides the corresponding regression lines determined by dry-ashing, slurry sonication and microwave-assisted digestion procedures, together with continuous or multicommutation mode HG-AFS determination of Te. As can be seen, the comparison between all the results obtained for the methods employed provided linear regressions with slope values statistically comparable to one and intercept values which are comparable to zero for a confidence level of 95%, thus indicating the excellent comparability of the methods employed.

By taking the simple and fast sample treatment involved in slurry sonication and the advantages of the multicommutation approach in terms of laboratory productivity, sample consumption and waste generation reduction, this procedure seems the best alternative for Te determination in milk.

Conclusions

The developed procedure for tellurium determination based on HG-AFS measurements in the multicommutation mode offers a sensitive and accurate method for milk analysis and features a simple sample pre-treatment based on room-temperature sonication of samples slurries with *aqua regia*.

The displacement of the argon insertion point during the HG-AFS Te determination from the gas–liquid phase separator to a previous point (y) minimises the time required to attain the maximum sensitivity, thus improving the sample throughput. Additionally, the use of a complementary hydrogen flow constitutes an economical appropriate alternative to maintain the flame burn up and to form tellurium hydrides efficiently.

On comparing the multicommutation mode with the continuous operation of the HG-AFS system it should be clear that the multicommutation approach provides a simplification of the experimental set-up, a fully mechanised operation of the system and a drastic reduction of sample and reagents consumption and waste generation, thus offering a sustainable and environmentally friendly alternative.

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Multicommutation hydride generation atomic fluorescence determination of inorganic tellurium species in milk

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Abstract

A multicommutated flow system has been developed for hydride generation, atomic fluorescence (HG-AFS) determination of tellurate (Te^{VI}) and tellurite (Te^{IV}) in milk samples. After a batch leaching of Te by sonication at room temperature for 10 min with aqua regia, sample slurries in acidic medium were merged with sodium borohydride and HCl to obtain data on Te^{IV}. Another portion of the acidic slurry was mixed with KBr and passed through a reaction coil introduced inside a microwave oven to reduce quantitatively Te^{VI} to Te^{IV} which was analyzed by HG-AFS. The detection limit was 0.57 ng g $^{-1}$ in the original samples. The linear range obtained was till 4 ng ml $^{-1}$ and the average recovery of different amounts of Te^{VI} and Te^{IV} added to real milk samples were 98 ± 4% and 98 ± 2%, respectively, indicating the absence of analyte losses or contaminations and original species modification. Average relative standard deviation of 6.3% was found for Te determination in a series of commercially available milk samples containing from 1.0 to 10.1 ng ml $^{-1}$ total Te. The proposed method provided a high sampling frequency of 24 h $^{-1}$ for the determination of both, free Te^{IV} and total Te, in a same sample with a two times reduced waste generation and a four times reduced reagent consumption as compared with the continuous hydride generation. Additionally, the method developed requires a minimum operator attention and sample manipulation.

Keywords: Multicommutation; Speciation; Tellurium; On-line microwave-assisted reduction; Hydride generation atomic fluorescence spectrometry; Milk analysis

1. Introduction

Tellurium is a non-essential toxic element widely spread in nature, usually at low concentration levels (Klaasen & Watkins, 1999). However, tellurium could be accumulated in milk samples in which it replaces the essential selenium. Thus, it is important to determine the content of Te in milk. Furthermore, the fact that the toxicity of Te depends on its oxidation state, tellurite is 10 times more toxic than tellurate, is an important reason to look for suitable methodologies in order to provide information about inorganic Te species in food, as well as to obtain the total Te content (Yu, Cai, Guo, Yang, & Khoo, 2003).

Hydride generation coupled with atomic fluorescence spectrometry HG-AFS is one of the most powerful analytical tools for ultra trace determination of heavy elements belonging to IVa–VIa groups, such as tellurium. HG has a marked selectivity for Te^{IV} determination, since only this oxidation state is able to generate volatile species in diluted hydrochloric media.

In comparison with extensive speciation studies with other elements, such as arsenic and selenium, only few works have dealt with the speciation of tellurium. The methods proposed for Te speciation are complex and involve several sequential steps, thus making their use very difficult in routine analysis.

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Narukawa (1999) described a procedure for the fractionation and determination of Te^{IV} and Te^{VI} using a tungsten furnace and this procedure was based on the use of cobalt(III) oxide powder as collector and 3-phenyl-5-mercapto-1,3,4-thiadiazole-2(3H)-thione potassium salt (bismuthiol II) as auxiliary agent for the discrimination between Te^{IV} and Te^{VI} always depending on the pH of the sample solution.

Korez, Eroglu, Volkan, and Atamon (2000) suggested a separation–preconcentration method using a mercapto-modified silica microcolumn for the determination of trace amounts of Te^{IV} in waters by HG-AAS technique.

Yu et al. (2003) depicted the speciation analysis of tellurium by using solid-phase extraction in the presence of ammonium pyrrolidine dithiocarbamate and the detection was carried out by inductively coupled plasma mass spectrometry.

Recently, our research group has developed a highly sensitive non-chromatographic procedure (Cava-Montesinos, de la Guardia, Teustch, Cervera, & de la Guardia, 2004) for the speciation of Se and Te in milk samples basing our research on HG-AFS determination before and after the pre-reduction with solid KBr at 80 °C during 30 min inside a water bath.

On-line microwave-assisted procedures have received an increased attention as they are very fast and can be integrated in the fully mechanization of the complete analytical process, from sample pre-treatment to the final determination of the analytes (Carbonell, de la Guardia, Salvador, Burguera, & Burguera, 1990; Carbonell et al., 1992; de la Guardia, Carbonell, Morales-Rubio, & Salvador, 1993).

Furthermore, the potential of the multicommutation approach as a way for mechanization of AFS determinations has been demonstrated recently in both, the reduction of reagents consumption and the increase of the sampling throughput for the determination of Hg by cold vapour – AFS in water (Reis, Ródenas-Torralba, Sancenón-Buleo, Morales-Rubio, & de la Guardia, 2003) and milk (Cava-Montesinos, Ródenas-Torralba, Morales-Rubio, Cervera, & de la Guardia, 2004) and in the determination of Bi by HG-AFS in milk shake samples (Ventura-Gayete, Ródenas-Torralba, Morales-Rubio, Garrigues, & de la Guardia, 2004).

The use of microwave treatments integrated with multicommutation and flow injection has several goals such as the following: (i) to develop novel procedures for sample digestion, (ii) to reduce the time involved in the aforementioned operations, (iii) to increase the automation level of these steps, (iv) to simplify the processes, (v) to reduce the consumption of reagents in general, and (vi) to avoid the use of dangerous chemicals, in particular. These last two objectives do agree with the current green chemistry trends of minimizing contamination of the environment. The scientific community requires a dramatic reduction of the toxicity and volumes of chemicals emitted to the environment. Moreover, multicommutation manages the chemical reagents and implements different reaction conditions in order to determine the involved species without changing the flow manifold.

In the proposed methodology, the speciation of inorganic tellurium by multicommutation HG-AFS has been developed. The multicommutation approach was employed in order to implement all the necessary conditions for the speciation of tellurium with the proposed manifold. Thus, the main aim of this report was to research a pre-reduction of Te^{VI} to Te^{IV} by KBr in a hydrochloric acidic medium with the help of microwave radiation. The method was tested with different milk samples and compared to complete digestion procedures (Cava-Montesinos, Cervera, Pastor, & de la Guardia, 2003; Cava-Montesinos, Cervera, Pastor, & de la Guardia, 2004).

2. Experimental

2.1. Apparatus and flow set-up

The multicommutation flow network was assembled with a set of four three-way solenoid valves NResearch 161T031 (West Caldwell, USA). A PC 486 microcomputer with electronic interfaces Advantech Corp. PCL 711S controlled the commutation devices and performed data acquisition and processing. The software was written in QUICK BASIC 4.5. An electronic interface was used to generate an electric potential of 12 V and a 100 mA current required to switch the solenoid valves.

A Gilson Minipuls 3 peristaltic pump Model M312 (Villiers-le Bel, France) was employed to propel the reagent solutions and sample slurries.

Microwave digestion was performed with a domestic MW oven LG Intellowave (Manchester, UK) operating at the maximum exit power of 700 W. MW was equipped with a magnetron of 2450 MHz. On-line reduction of Te^{VI} to Te^{IV} was achieved in a 3 m length (0.8 mm i.d.) PTFE coil located inside the microwave oven.

For tellurium measurements, a HG-AFS PSA Millennium Excalibur 10055 from PS Analytical (Orpington, UK) was equipped with two independent peristaltic pumps a boosted discharge hollow cathode lamp for Te, from Photron (Victoria, Australia), and a specific filter was employed. The gaseous hydride formed, after mixing the sample with $NaBH_4$, was separated by a gas–liquid separator, passed through a hygroscopic membrane Perma Pure® (Farmingdale, NJ, USA) and atomised using a hydrogen diffusion mini-flame fed by the excess of H_2 generated by the reaction between HCl and $NaBH_4$. Mixing coils and transmission lines

were made of polyethylene tubing (0.8 and 1.0 mm i.d.). Operating and chemical conditions employed by both, multicommutation and batch analysis, are given in Table 1.

The manifold employed for the mechanization of inorganic tellurium species determination is indicated in Fig. 1. It is integrated by two parts, the first one for on-line microwave-assisted pre-reduction of Te^{VI} to Te^{IV} and the second one for the hydride generation and AFS determination of free Te^{IV} directly from the sample slurries and total Te after their reduction.

Sample slurries, obtained by in batch sonication of milk with aqua regia, were sequentially aspirated on using valve V_2 for free Te^{IV} determination and by using valve V_1 for the on-line reduction of Te^{VI} to Te^{IV} with KBr and determination of total Te through aspiration using valve V_2.

Solenoid valves V_3 and V_4 were employed for the introduction of $NaBH_4$ and HCl prior to the HG-AFS determination of both, Te^{IV} and total Te. The pre-reduction manifold includes also a closed system for cool water recirculation, in order to avoid magnetron damage during sample treatment and an ice bath to cool the previously reduced sample slurry, being incorporated a well stirred open vessel to vent the samples before to be introduced in the HG system and to maintain stable the solid dispersion.

2.2. Reagents and samples

All reagents used were of the highest purity available and all solutions were prepared in ultrapure water, with a resistivity of 18.2 MΩ cm obtained from a *Milli*-Q system Millipore (Bedford, USA).

Stock solutions of 1000 mgl^{-1} Te^{IV} and 1000 mgl^{-1} Te^{VI} were prepared by dissolving the appropriate amounts of Na_2TeO_3 and H_6O_6Te, respectively, from Merck (Darmstadt, Germany) in ultrapure water. KBr, used as reductant agent, was obtained from Panreac (Barcelona, Spain). Sodium tetrahydroborate solutions were prepared from the solid product Fluka (Steinheim, Germany) dissolved in NaOH 0.1 $moll^{-1}$ Probus (Barcelona, Spain). The $NaBH_4$ solutions were filtered through a 0.45 μm nylon membrane Lida (Kenosha, USA). Calibration and $NaBH_4$ solutions were prepared daily.

Table 1
HG AFS operating parameters used for Te^{IV} and total inorganic Te determination in milk samples by both, multicommutation and batch modes

	Multicommutation		Batch
Atomic fluorescence spectrometer parameters			
Wavelength (nm)		214.3	
Measurement mode		Peak height	
Primary current (mA)		15	
Boost current (mA)		17.5	
Filter		45	
Multicommutation and continuous hydride generation parameters			
Gas–liquid separator (ml)		17	
Reaction coil length (cm)	–		150
B_1 coil length (cm)	300		–
B_2 coil length (cm)	200		–
B_3 coil length (cm)	47		–
ON/OFF sampling time (s) S_1 (ON)/S_1 (OFF)	0.25/0.25		–
ON/OFF reductant time (s)R_1 (ON)/R_1 (OFF)	0.25/0.25		–
ON/OFF sampling time (s) S_2 (ON)/S_2 (OFF)	0.75/0.25		–
Sampling time cycles	30		–
ON/OFF reductant time (s) R_2(ON)/R_2(OFF)	30/0		–
ON/OFF blank time (s) R_3(ON)/R_3(OFF)	0.25/0.75		–
Blank time cycles	30		–
Reagent concentrations			
KBr R_1 (%, w/v)		30	
$NaBH_4$ R_2 (%, w/v)		1.2	
HCl R_3 ($moll^{-1}$)		4.0	
Sample and reagent flow rates			
KBr R_1 ($mlmin^{-1}$)		4.6	
$NaBH_4$ R_2 ($mlmin^{-1}$)		6.5	
HCl R_3 ($mlmin^{-1}$)		9.3	
Sample S_1 ($mlmin^{-1}$)		4.6	
Sample S_2 ($mlmin^{-1}$)		8.5	
Ar ($mlmin^{-1}$)	200		330
Air ($mlmin^{-1}$)	250		250

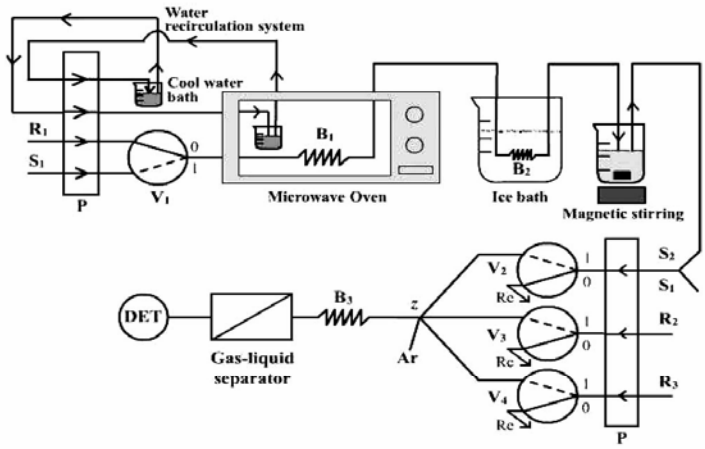

Fig. 1. Flow manifold for multicommutation HG-AFS speciation of inorganic tellurium. V_i: three-way solenoid valves (dashed lines represent the flow paths when the valves are in position 1); S_1: sample and standard solutions for total Te determination; S_2: sample and standard solutions for Te^{IV} determination; R_1: KBr reductant solution at flow rate of 4.6 ml min^{-1}; R_2: $NaBH_4$ reductant solution at flow rate of 6.5 ml min^{-1}; R_3: HCl blank solution at flow rate of 9.3 ml min^{-1}; B_1: reduction reaction coil, 3 m length; B_2: cooling coil, 2 m length; B_3: mixing coil, 47 cm length. Det: detector; Re: recycling to the corresponding vessels; P: peristaltic pumps; z: merging zone machined in acrylic.

Aqua regia was prepared by mixing HNO_3 and HCl 1:3 (v/v) from the concentrated suprapure solutions from Scharlau (Barcelona, Spain) and used for previous in batch leaching of Te from milk samples. Antifoam A, from Sigma (Steinheim, Germany), was used to stabilize the slurries and to avoid foam formation during hydride generation.

Synthetic air and high purity argon C-45 (99.995%), from *Carburos Metálicos* (Barcelona, Spain), were employed to dry volatile hydrides in the Perma Pure® membrane system and to transport them to the atomiser, respectively.

Ten commercial milk samples were analysed by using the developed procedure and data obtained were compared with those found by HG-AFS after complete digestion of samples by dry ashing (Cava-Montesinos et al., 2003) and by microwave assisted treatment in sealed reactors (Cava-Montesinos et al., 2004).

2.3. Multicommutation procedure

Two portions of 1.0000 ± 0.0001 g of milk were accurately weighed in 25 ml volumetric flasks, and 2 ml aqua regia and 1 ml antifoam A were added to each one before sonication in an ultrasound water bath for 10 min, following the treatment suggested in a previous study (Cava-Montesinos et al., 2004). After that, the obtained slurries were acidified with conc. HCl to a final concentration of 4.0 mol l^{-1} and diluted in both cases to 25 ml with water.

Using the manifold depicted in Fig. 1 the valves switching course shown in Table 2, the acidic sample slurries were aspirated alternately in the HG-AFS system using V_1 and V_2 or only V_2, depending of total Te or free Te^{IV} determination.

The sample slurry obtained after sonication was mixed with KBr for total Te determination. It was passed through the reaction coil located inside the MW oven by using valve V_1 (0.25/0.25 s) at a flow rate of 4.6 ml min^{-1}.

During Te^{VI} pre-reduction, in the B_1 coil, the microwave oven was operated at 700 W and valves V_2, V_3 and V_4 did not take part in the process. At the outside of the microwave oven, the treated slurry was cooled, in an ice bath, and vented and homogenized in an open beaker before feeding the pretreated samples into the HG-AFS system through valve V_2.

To determine free Te^{IV} and total Te the solenoid valve V_2 was switched to position 1 (0.75 s) and to position 0 (0.25 s) 30 cycles during the sampling time interval inserting sample solution S_1 directly, for free Te^{IV} determination, or pre-reduced slurry S_2 for total Te determination. During this time, a reductant solution (R_2) was also introduced through valve V_3 and both merged in the coil B_3, as can be seen in Fig. 1. At the same time, valve V_4 was switched to position 1 and 0, 0.25/0.75 s to insert into the coil B_3 a set of blank solution, as can be seen in step 2 shown in Table 2.

During the reading time, valves V_3 and V_4 were commutated. Valves inserting reductant solution (valve V_3)

Table 2
Valve switching course for the sequential determination of Te^{IV} and total Te by HG-AFS

Step	V_1	V_2	V_3	V_4	t (s)	Waste volume (μl)	Description of the task
1	1	–	–	–	0.25[a]	–	Microwave-assisted pre-reduction
	0	–	–	–	0.25		
2[b]	–	1	1	0	0.75	7600	Sample + reductant insertion
	–	0	1	1	0.25		Blank + reductant insertion
3[c]	–	0	0	1	1.0	3687	Transport of sample zone. Hydride separation step. Signal reading
	–	0	1	0	1.0		

0 and 1 represent that the solenoid valves are switched OFF and ON, respectively (see Fig. 1 for details). Determination of total Te: steps 1 to 3. Determination of Te^{IV}: steps 2 and 3. The waste volume was established from each sample or standard measurement.

[a] V_1 was switched in position 1 or 0 the necessary number of cycles to introduce KBr and sample solutions into B_1 reaction coil till to treat the original 25 ml sample slurry.

[b] This mixing time sequence was repeated 30 times, corresponding to 30 s.

[c] Step corresponding to the period defined as reading time, repeated seven times (14 s).

and HCl solution (valve V_4) were switched ON/OFF a set of times of 1.0/1.0 s during 14 s, as it is indicated in the step 3 of Table 2. The insertion of R_2 and R_3 solutions in the gas–liquid separation chamber is a necessary condition to maintain the mini-hydrogen diffusion flame burn up.

Data found from the fluorescence of samples were interpolated in a calibration line obtained from the insertion of Te^{IV} standards through valve V_2 and were used the same conditions than from samples.

3. Results and discussion

The determination of Te^{VI} and Te^{IV} in a conventional HG-AFS system is difficult because tellurate does not provide any fluorescence signal. Moreover, our preliminary studies based on the on-line reactions by using microwave-assisted treatments directly coupled to AFS measurements produced signal instability due to the overpressure generated during the on-line reduction of tellurate which extinguished the flame. These drawbacks were circumvented by using the set-up indicated in Fig. 1, exploiting the use of separate discrete commutation devices for a microwave-assisted reduction and fluorescence determination.

3.1. Determination of Te by using slurries

Previous studies made in our laboratory evidenced that the sonication at room temperature of 1 g milk with 2 ml aqua regia for 10 min was enough for the quantitative extraction of As, Sb, Se, Te and Bi (Cava-Montesinos et al., 2004). However, this treatment does not provide acidic slurries from which the corresponding hydride could be generated for total Te determination and a previous reduction of Te^{VI} to Te^{IV} was necessary.

3.2. Speciation analysis strategy

Te^{IV} can be directly determined by HG-AFS and speciation of inorganic forms, Te^{IV} and Te^{VI}, can be easily carried out by making measurements before and after the pre-reduction, in order to obtain Te^{IV} and total Te, respectively. Te^{VI} can be calculated by the difference between the two analysis performed.

KBr reductant solution has been extensively used to determine total Te, but the recommended concentrations of the reagents differ significantly (He, Moreda-Piñeiro, Cervera, & de la Guardia, 1998). Thus, the concentration of the reagent was varied in order to achieve the best sensitivity and precision.

The complete on-line reduction of Te^{VI} to Te^{IV} was assured by evaluating other parameters such as the reduction coil length and the carrier flow rates which control the residence time of the sample inside the microwave oven.

3.2.1. Reductant concentration effect on fluorescence signal

Concentrated HCl is usually employed for the reduction of tellurate to tellurite but KBr can be used to reduce the samples, thereby avoiding the deleterious effect of sample dilution involved in the reduction with HCl.

To ascertain the most appropriate KBr concentration, the fluorescence signals obtained for total Te in an actual full cream milk sample were evaluated by varying the reductant concentration from 20% to 50% (w/v) for different coil lengths of 2, 3 and 4 m, inside the oven operating at 700 W. As can be seen in Fig. 2, an increase in the KBr concentration increases also the reduction yield up to reach a maximum value, which depends on the reaction coil length. It seems clear that a KBr concentration of 30% (w/v) and a reaction coil of 3 m provide the most sensitive results. Hence, these conditions were chosen for further experiments.

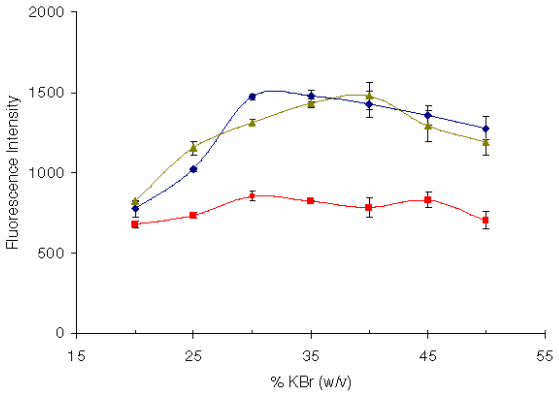

Fig. 2. Effect of KBr concentration on the fluorescence signal of total Te in a full cream milk with 6.1 ng ml^{-1} Te. In these experiments, the flow rate was fixed at 4.6 ml min^{-1}. Bars indicate the variability ($\pm s$) of three independent assays using different reduction coil lengths: (■) 2 m; (♦) 3 m; (▲) 4 m.

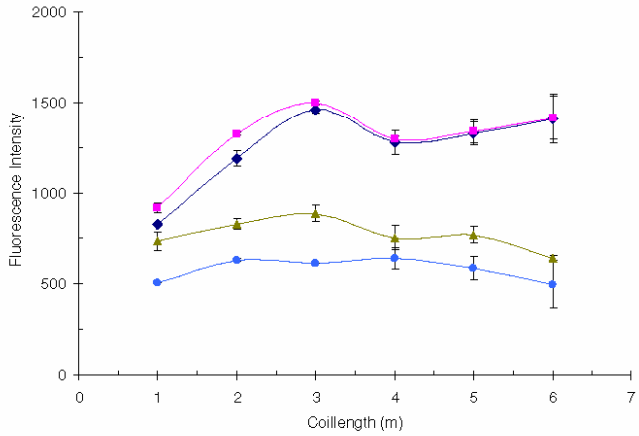

Fig. 3. Effect of the reduction coil length for different carrier flow rates on the fluorescence signals of a full cream cow milk with 6.1 ng ml^{-1} Te. In all experiences it was used a KBr concentration of 30% (w/v). Measurements were made using the multicommutation approach and error bars indicate the variability ($\pm s$) of three independent assays. (♦) 3.4 ml min^{-1}; (■) 4.6 ml min^{-1}; (▲) 5.7 ml min^{-1}; (●) 6.5 ml min^{-1}.

3.2.2. Reduction coil length and carrier flow rate effects on fluorescence intensity

Different coil length reactors were placed inside the microwave oven to reduce Te^{VI} to Te^{IV}. Fig. 3 shows the fluorescence signals obtained for a full cream sample treated with 30% (w/v) KBr and using a 700 W power radiation at different flow rates, from 3.4 to 6.5 ml min^{-1} and for different coil lengths, from 1 to 6 m.

As can be seen, the increase of the flow rate above 4.6 ml min^{-1} involves a drastic reduction of the fluorescence signals. It could be due to an incomplete reduction of Te^{VI} because of the small residence time of the sample inside the oven. For 3.4 and 4.6 ml min^{-1} flow rates, were found comparables results, increasing the fluorescence signals on increasing the reaction coil length, till to reach a maximum value for 3 m, from which the dispersion effects in the flow manifold tend to reduce the fluorescence signals and to increase the variability of the results. Thus, 4.6 ml min^{-1} and 3 m coil length seem to be the best compromise between the maximum reduction yield of Te^{VI} to Te^{IV} and the minimum dispersion, offering also a sampling throughput of 24 h^{-1}.

A 25 ml volume of a milk sample slurry could be treated for microwave-assisted reduction of Te^{VI} to Te^{IV} in approximately 326 s before aspirating a discrete volume of this reduce slurry into the multicommutation HG-AFS system to do the determination of total Te. It provides a dramatic decrease of the time required for the reduction of Te^{VI} as compared with the approximately 30 min involved in the batch reaction (Ródenas-Torralba, Cava-Montesinos, Morales-Rubio, Cervera, & de la Guardia, 2004).

3.3. Analysis of milk samples

The developed methodology was applied to the determination of Te species in cow and goat milk samples obtained from the Spanish market. These samples had different compositions, from full cream to skimmed milk, and contained also different additives, such as Omega 3, folic acid, Ca, minerals and vitamins, propolis or vegetable fat.

Results shown in Table 3 indicate the presence of free Te^{IV} concentrations from 0.6 to 6.5 $ng ml^{-1}$ and Te^{VI} levels from 0.4 to 4 $ng ml^{-1}$. In all samples, free Te^{IV} was the dominant specie in a nearly 3:2 ratio to the Te^{VI}.

Besides, total tellurium concentrations obtained by the proposed procedure for the samples analyzed are statistically comparable with those obtained after in batch dry-ashing microwave-assisted digestion or sonication following in all the cases by continuous HG-AFS determination.

The regression between data found by multicommutation and on-line reduction (y) and those obtained in batch after dry ashing (x_1) was $y = (1.02 \pm 0.04)x (0.1 \pm 0.3)$ with $R^2 = 0.99$ and the regression between y and in batch measurement after pressurized microwave-assisted digestion (x_2) was $y = (1.00 \pm 0.02) x + (0.2 \pm 0.1)$ with $R^2 = 0.998$, thus indicating the good comparability of the fully mechanized developed methodology with continuous hydride generation after complete off-line digestion.

3.4. Analytical figures of merit

Table 4 shows the analytical features of HG-AFS Te determination in milk by using the batch and the multicommutation modes. Typical regression lines between peak height fluorescence measurements and Te concentrations were $I_F = (20 \pm 15) + (850 \pm 40)\ C_{Te}$ ($ng ml^{-1}$) with a regression coefficient of $R = 0.999$ for batch mode and $I_F = (20 \pm 12) + (810 \pm 30)\ C_{Te}$ ($ng ml^{-1}$) with $R = 0.998$ for the multicommutation approach. In the best conditions, detection limits of 0.021 and 0.023 $ng ml^{-1}$ were obtained for in batch and multicommutation, respectively. Thus, LODs of 0.52 and 0.57 $ng g^{-1}$ were found in terms of Te concentration in the original milk sample. So, it can be concluded that the main ana-

Table 3
HG-AFS determination of Te species in milk samples using slurries and both, multicommutation and batch modes

Milk sample	Total Te			Te^{IV}		Te^{VI}[c]	
	Multicommutation	Batch		Multicommutation	Batch	Multicommutation	Batch
		Sonication	Dry-ashing (Cava-Montesinos et al., 2003)[a]	MW (Cava-Montesinos et al., 2004)[b]			
Cow full cream	6.1 ± 0.2	6.2 ± 0.3	6.3 ± 0.3	6.0 ± 0.1	3.9 ± 0.1	3.9 ± 0.2	2.2 ± 0.2	2.3 ± 0.3
Cow full cream with Ca	8.5 ± 0.4	8.3 ± 0.5	8.1 ± 0.7	8.2 ± 0.2	5.1 ± 0.5	4.9 ± 0.6	3.4 ± 0.4	3.4 ± 0.5
Cow partially skimmed	6.3 ± 0.8	6.4 ± 0.6	6.6 ± 0.3	6.2 ± 0.3	3.9 ± 0.5	3.9 ± 0.5	2.4 ± 0.5	2.5 ± 0.9
Cow skimmed with folic acid	9.0 ± 0.4	8.9 ± 0.4	8.7 ± 0.4	9.0 ± 0.1	5.7 ± 0.2	5.8 ± 0.4	3.3 ± 0.2	3.1 ± 0.3
Cow partially skimmed with vitamins and minerals	9.4 ± 0.5	9.1 ± 0.4	9.0 ± 0.3	9.3 ± 0.4	6.5 ± 0.4	6.3 ± 0.2	2.9 ± 0.5	2.8 ± 0.3
Cow skimmed with Omega 3	9.7 ± 0.6	9.5 ± 0.6	9.4 ± 0.7	9.2 ± 0.3	6.0 ± 0.6	5.8 ± 0.5	3.7 ± 0.5	3.7 ± 0.7
Cow skimmed	1.0 ± 0.2	1.2 ± 0.4	1.3 ± 0.7	0.8 ± 0.3	0.6 ± 0.3	0.8 ± 0.3	0.4 ± 0.3	0.4 ± 0.4
Cow vegetable base	2.8 ± 0.6	2.6 ± 0.7	2.4 ± 0.5	2.5 ± 0.3	1.9 ± 0.8	1.8 ± 0.6	0.9 ± 0.6	0.8 ± 0.5
Cow partially skimmed with propolis	8.9 ± 0.6	9.2 ± 0.7	9.3 ± 0.5	8.7 ± 0.1	5.3 ± 0.7	5.5 ± 0.7	3.6 ± 0.7	3.7 ± 0.7
Goat full cream	10.1 ± 0.2	10.4 ± 0.3	–	–	6.1 ± 0.1	6.3 ± 0.3	4.0 ± 0.2	4.1 ± 0.2

Te concentrations in milk are indicated in $ng ml^{-1}$, being also reported the standard deviation of three independent analysis of each sample.
[a] Data obtained after dry-ashing and batch HG-AFS (Cava-Montesinos et al., 2003).
[b] Data obtained after pressurized digestion by MW oven and batch HG-AFS (Cava-Montesinos et al., 2004).
[c] Te^{VI} concentrations were obtained from the difference between total tellurium and Te^{IV}.

Table 4
Analytical features of HG-AFS determination of Te by using batch and multicommutation modes

	Batch AFS	Multicommutation AFS
Calibration line[a]	$I_F = (20 \pm 15) + (850 \pm 40)\ C_{Te}$	$I_F = (20 \pm 12) + (810 \pm 30)\ C_{Te}$
Correlation coefficient (R)	0.999	0.998
RSD (%) [b]	11.2	10.5
RSD (%) [c]	5.4	6.3
LOD ($ng ml^{-1}$)[d]	0.021	0.023
LOD ($ng g^{-1}$)[e]	0.52	0.57
Sample consumption (ml)[f]	1700	318
Reagent consumption (ml)[f]	1300	324
Carrier blank consumption (ml)[f]	930	120
Ar consumption ($ml min^{-1}$)	330	200
Waste (ml)[g]	1179	625
Throughput (h^{-1})	30[h]	82[h]
		24[i]

[a] A minimum of four standards and a blank were used for each calibration line in the concentration range from 0 to 4 $ng ml^{-1}$. The average data were obtained from three calibration lines.
[b] RSD (%): The mean relative standard deviation was established from the average of the variation coefficient found for three replicate analysis of 10 commercially available samples.
[c] RSD (%): The relative standard deviation was established from five independent measurements of a solution containing 1.0 $ng ml^{-1}$ of Te^{IV}.
[d] LOD: limit of detection concerning diluted samples.
[e] LOD: limit of detection regarding the original sample.
[f] Sample and reagent consumption corresponding to 100 analysis.
[g] Waste generated was established for 1 h working session.
[h] Throughput corresponding to HG-AFS detection system.
[i] Throughput corresponding to the microwave-assisted digestion of samples.

lytical parameters of the off-line sonication of milk samples followed by in batch reduction with KBr and continuous HG-AFS determination are comparable to those obtained by the same procedure but making the reduction of Te^{VI} to Te^{IV} on-line and using the multicommutation strategy for sample and reagents insertion in the experimental set-up.

Moreover, as Table 4 indicates, a sample volume of 3.18 ml and a reagent volume of 3.24 ml per analysis are enough to carry out the determination of tellurium by the developed procedure, being produced a waste of 625 ml for 82 fluorescence measurements, made in one hour, on using the multicommutation HG-AFS system. These parameters are clearly better than the 17.00 ml sample consumption and 13.00 ml reagent required for the batch measurements which also involves a waste generation of 1179 $ml h^{-1}$ for only 30 measurements. So, it can be concluded that the methodology proposed in this study is a sustainable, fast and environmentally friendly alternative to previous traditional approaches.

Recovery experiments were made on several milk samples in order to evaluate the accuracy of the method and different concentrations of Te ^{IV} and Te^{VI} were added. As can be seen in Table 5, recovery values from $93 \pm 7\%$ to $104 \pm 8\%$ for Te^{IV} and from $94 \pm 7\%$ to $101 \pm 2\%$ for Te^{VI} were obtained, for ac-

Table 5
Recovery of Te^{IV} and Te^{VI} spiked concentrations added to different actual milk samples obtained by using both, the developed multicommutation approach an a traditional in batch reduction and continuous HG-AFS determination

Milk sample	Te^{IV} spiked	Te^{VI} spiked	Recovery % Te^{IV}		Recovery % Te^{VI}	
			Multicommutation	Batch	Multicommutation	Batch
Cow full cream	3	–	99 ± 6	101 ± 4	–	–
	–	3	–	–	101 ± 2	94 ± 7
	1.5	1.5	98 ± 4	95 ± 6	97 ± 5	96 ± 5
Cow partially skimmed	3	–	95 ± 8	96 ± 7	–	–
	–	3	–	–	98 ± 1	98 ± 2
	1.5	1.5	93 ± 7	94 ± 7	96 ± 4	96 ± 5
Cow skimmed with Omega 3	4	–	104 ± 8	102 ± 1	–	–
	–	4	–	–	97 ± 4	100 ± 4
	2	2	97 ± 2	99 ± 6	94 ± 7	95 ± 4
Cow partially skimmed with propolis	4	–	103 ± 4	100 ± 3	–	–
	–	4	–	–	98 ± 3	101 ± 5
	2	2	97 ± 2	97 ± 6	101 ± 2	99 ± 2

Tellurium recoveries (%) found in commercially available milk samples by using batch sonication and microwave-assisted pre-treatment. Measurements were carried out by batch and multicommutation modes. Standard deviation corresponding to three independent replicates. Te spikes in $ng ml^{-1}$ were added before sample treatment.

tual samples spiked with a single one or both of the Te species considered, thus evidencing the absence of analyte losses or contamination during the sonication, the microwave-assisted reduction of samples and the hydride generation, evidencing also the lack of modification of the spiked species during the different analysis steps.

The regression between the two data populations obtained for Te^{IV} and Te^{VI} determination using in batch (x) and multicommutation (y) approaches (see Table 3 data) provided equations: $y = (1.01 \pm 0.03)x \quad (0.1 \pm 0.2)$ [Te^{IV}] and $y = (0.98 \pm 0.03)x + (0.05 \pm 0.09)$ [Te^{VI}] with regression coefficients of 0.996 and 0.996, respectively. Slope values of the aforementioned equation are comparable to 1 ($t_{\text{calculated}} = 0.484$ (Te^{IV}) and 0.690 (Te^{VI}), for a $t_{\text{theoretical}} = 1.734$, for 95% probability level and 18 freedom degrees). Ordinate values are around 0 ($t_{\text{calculated}} = 0.437$ (Te^{IV}) and 0.640 (Te^{VI}), for a $t_{\text{theoretical}} = 1.734$, for 95% probability level and 18 freedom degrees). So, it can be concluded that the on-line microwave-assisted reduction of Te^{VI} and multicommutation HG-AFS determination of free Te^{IV} and total Te does not modifie the accuracy of the traditional in batch and continuous HG-AFS determinations.

4. Conclusions

A non-chromatographic and sensitive method with good linearity, reproducibility, high sampling frequency and low waste generation has been proposed for speciation of inorganic tellurium in milk samples.

The on-line microwave-assisted reduction system reduces sample handling and makes possible the complete automation of the inorganic tellurium species determination. The use of a KBr solution in an acidic medium permits the quantitative reduction of Te^{VI} to Te^{IV} in less than 2 min, approximately, by using a microwave oven operating at 700 W.

Moreover, multicommutation minimizes the reagent consumption by a factor of 4 and the generation of toxic residues by a factor of 2 and improves the speed of analysis over three times as compared with the classical approaches. Thus, it is an economically sustainable and environmentally friendly alternative to previously reported methods.

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4.2. NUEVOS AVANCES EN LA MECANIZACIÓN DE LAS MEDIDAS DE ESPECTROFOTOMETRÍA MOLECULAR

«An environmentally friendly multicommutated alternative to the reference method for anionic surfactant determination in water»

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An environmentally friendly multicommutated alternative to the reference method for anionic surfactant determination in water

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Abstract

It has been developed a fully mechanized procedure for the spectrophotometric determination of anionic surfactants in water expressed in terms of SDS concentration. The reference method, based on the reaction of SDS with methylene blue (MB) followed by extraction in chloroform, was mechanized in order to reduce the consumption of organic solvents. The system was based on the multicommutation approach and provided a 35 times reduction of the waste production without sacrificing the figures of merit of the method in terms of sensitivity and repeatability, for a dynamic linear range from 0.2 to 1.7 mg l^{-1}. Results obtained for washing water samples were comparable with those obtained using the reference method and no significant differences, at 95% confidence level, were observed. Other useful characteristics are a solvent consumption of 0.7 ml per determination, a sampling throughput of 40 determinations per hour, a relative standard deviation of 5.9% ($n = 10$) for a sample containing 2×10^{-6} mol l^{-1} (576 μg l^{-1}) surfactant and a limit of detection of 6.1×10^{-9} mol l^{-1} (1.7 μg l^{-1}).

Keywords: Multicommutation; Flow; Spectrophotometry; Surfactant; Washing water

1. Introduction

Anionic surfactants comprise a series of products widely used for washing proposals, both for personal hygiene and industrial cleaning applications. The extensive use of surfactants makes necessary their determination in natural waters as potential contaminants. In this sense, the authorities of the European Union established a maximum tolerated limit of 200 μg l^{-1} for anionic surfactants in water supplies for human consumption [1].

The official method for anionic surfactants determination in water is based on the reaction of these compounds with methylene blue (MB) followed by an extraction with chloroform prior to the spectrophotometric determination at 654 nm [2]. This analytical procedure, carried out manually, is very tedious. The use of large volumes of chloroform (45 ml per determination) and a lot of laboratory glassware, make these operations extremely expensive, time consuming and uncomfortable for the operator. So it seems necessary to search for alternatives of the aforementioned method in order to increase the laboratory productivity and operator safety and comfort and to reduce drastically the reagents consumption and waste production.

Koga et al. [3] proposed a reduction of the size of sample employed for anionic surfactant determination in water, being modified this method to use only 50 ml of water and 5 ml $CHCl_3$, having obtained a six times increase of the laboratory productivity.

On the other hand, the use of small tubes and Pasteur pipettes to do the solvent extraction and phase separation of the ion pair between anionic surfactants and MB provided a 20 times reduction of the sample size, a five times reduction

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of $CHCl_3$ consumption and avoids the use of expensive glassware [4].

Probably, the most innovation approach was that developed by Agudo et al. [5] based on the continuous solvent extraction inside the measurement cell by flow injection analysis, which provides a sampling throughput of 20 h^{-1} and a $CHCl_3$ consumption of 200 μl per determination.

In the last years, multicommutation has opened a new way for the mechanization of analytical procedures [6]. Multicommutation could be understood as a means to handle solutions in flow system by inserting sequentially small aliquots of sample and reagent solutions into the reacting device (coiled reactor or chamber). The figures of merit of the procedures based on multicommutation are usually similar to those observed employing ordinary flow process, nevertheless presenting a significant reduction on reagent consumption and waste production [7].

In this work, a multicommutation automated procedure for the determination of anionic surfactants in water employing the methylene blue spectrophotometric method has been developed.

2. Experimental

2.1. Apparatus and flow set-up

The equipment set-up comprised a Hewlett-Packard Model 8452A diode array spectrophotometer (Waldbronn, Germany) equipped with a 10 mm optical pathway flow cell, with 50 μl inner volume, a Gilson Minipuls P2 peristaltic pump (Villiers Lebel, France) furnished with Viton©(Iso-Versinic) pumping tubes, a PC 386 microcomputer furnished with a PCL711S Advantec electronic interface card and running software in QUICK BASIC 4.5, 6 three-way solenoid valves NResearch, 161T031 (West Caldwell, USA), three home-made Mariotte vessels of 1000 ml and home-made water/organic phase separation chambers. Reactor coils and conducts were of PTFE (0.8 mm i.d.). Coils employed for the connection between Mariotte vessels and solenoid valves were of PTFE (1.6 mm i.d.). The peristaltic pump was operated to provide a flow rate of 3.2 ml min^{-1} and the Mariotte vessels located 100 cm over the top of the separation chamber provided constant flows of 180 μl s^{-1} for sample and standards, 60 μl s^{-1} for the MB solution and 70 μl s^{-1} for $CHCl_3$.

The manifold employed for the mechanization of the anionic surfactant determination is indicated in Fig. 1. Samples and standards containing sodium dodecylsulphate (SDS), the MB solution and $CHCl_3$ were supplied through the switching ON valves V_1, V_2 and V_3, respectively, and using the Mariotte flask for reagent delivery.

PTFE fragments were used to fill the lower part of the separation chamber in order to provide a turbulent flow which can improve the reaction between MB and SDS and the mass transfer process during extraction. Additionally, an air flow of 106 μl s^{-1} was counter current introduced through the base of the separation chamber for shaking the mixture of phases, using V_6 valve.

Two additional solenoid valves, V_4 and V_5, were employed to select the organic phase to be measured in the detector.

Different designs of the separation chamber were home-made and assayed for this study. Fig. 2 indicates the five ones which provided the best results. Basically, the separation chamber was constructed in glass from cylindrical pieces with three reagent intakes located at the top for the introduction of SDS, MB and $CHCl_3$ and two outsides located at the bottom, one for air bubbling in counter current and the second one for aspiration of the separated phases. This basic design (A) was modified to let open the system to favour phases separation (B–E) and additionally, in the case of B, a single outside was employed for liquid aspiration, shaking the phases with a magnetic bar. The final design was improved by using different volumes of the thin part to increase the volume of the aqueous phase employed for extraction thus enhancing the preconcentration from a maximum volume of 35 ml (C and D) to a volume of 100 ml (E).

2.2. Reagent solutions

Sodium dodecylsulphate was employed as a representative anionic surfactant for calibration. It was obtained from Sigma (St. Louis, USA) (SDS [$CH_3(CH_2)_{11}OSO_3Na$], purity 99.0% or higher). Methylene blue (trihydrates) was used as a cationic dye and obtained from Merck (Steinheim, Germany) (MB [$C_{16}H_{18}N_3SCl{\cdot}3H_2O$], purity 98.5% or higher). Chloroform stabilized with ethanol (Scharlau, Barcelona, Spain) was used as extractant. For other products, analytical grade reagents were used.

2.3. Reference procedure

Hundred millilitre of sample was placed into a 250 ml separating funnel and 10 ml of a 1×10^{-3} mol l^{-1} methylene blue solution and 15 ml chloroform were added. After shaking the mixture vigorously for 1 min, the two phases were let to separate and chloroform layer taken for analysis. Each sample was extracted additionally three times using 10 ml portion of chloroform and absorbance measurements were made at 654 nm in front of an external calibration prepared from SDS. Solutions in the range between 0.1 and 0.5 mg l^{-1} were extracted in the same way than samples [2].

2.4. Recommended procedure

Using the manifold depicted in Fig. 1 and the experimental steps shown in Table 1, a total volume of sample of 3.60 ml, corresponding to 20 pulses of 1.0 s was placed in the separation chamber through valve V_1, at the same time than a total volume of 0.24 ml of a 1×10^{-3} mol l^{-1} MB solution,

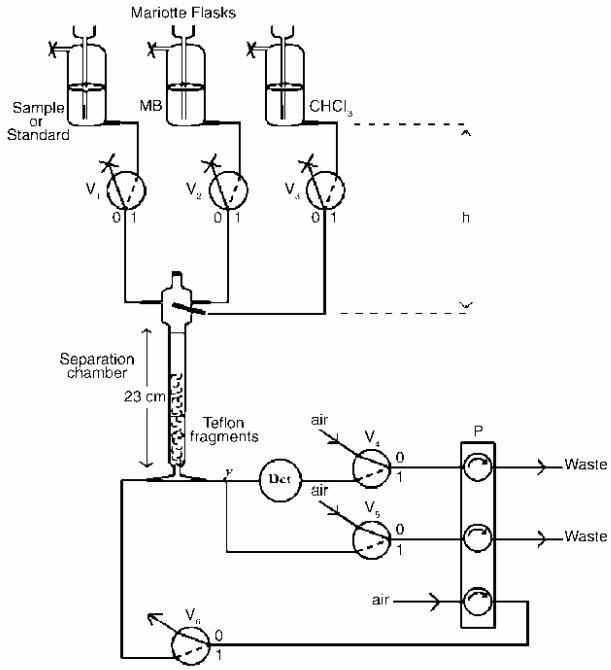

Fig. 1. Manifold employed for the multicommutation determination of anionic surfactants. Note—SDS: sodium dodecylsulfate; MB: methylene blue; $CHCl_3$: organic solvent extractant; V_1, V_2, V_3, V_4, V_5 and V_6: three-way solenoid valves; *h*: height necessary for the gravity flow (100 cm); *y*: Y type joint devices machined in acrylic; Det: detector; P: peristaltic pump; (- - - -) valve switched ON (bit 1 electronic position); (—) valve switched OFF (bit 0 electronic position). Separation column of 23 cm length.

corresponding to 20 pulses of 0.2 s was added through valve V_2. A time of 3.0 s was programmed as mixing step between the aforementioned solutions, inside the separation chamber. An air flow of 106 $\mu l\,s^{-1}$ (through the use of valve V_6) was bubbled in counter current to favour the reaction between anionic surfactant and MB to form an ionic pair. After the mixing step, valve V_3 was switched ON/OFF for 10 cycles of 1.0 s in order to introduce 0.70 ml of chloroform inside the mixing chamber. During this time, and additional 7.0 s, air was also bubbled through the chamber to improve the mass transfer of the ionic pair to the organic phase. To provide phase separation, all valves were switched OFF during 7.0 s.

Table 1
Multicommutated program employed to determine anionic surfactants

Event sequence	Parameter	Valves switched ON	Settled time (*s*)
1	Inserting sample solution (is)	V_1	1.0 (20 cycles)
	Inserting reagent solution (is)	V_2	0.2 (20 cycles)
2	Mixing step (ms)	V_6	3.0
3	Inserting organic solvent step (ios)	V_3+V_6	1.0 (10 cycles)
4	Extraction step (es)	V_6	7.0
5	Separation step (sp)	–	7.0
6	First portion delivering step (fd)	V_5	3.0
7	Displacing organic phase step (dos)	V_4	10.0
8	Aqueous phase delivering step (aq)	V_5	10.0
9	Flow cell empting step (fs)	V_4+V_5	10.0

Step 1 involves the sequential injection of small volumes of sample or standards and reagent solution through a series of 20 cycles of 1.0 and 0.2 s duration, respectively.

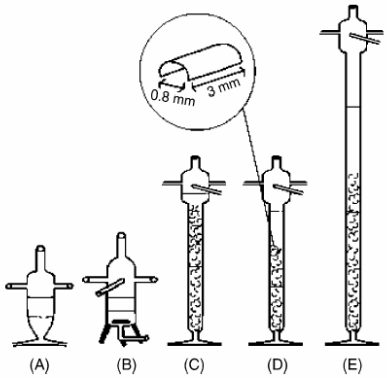

Fig. 2. Extraction cell designs. (A) Separation chamber of 4 cm length and 1.2 cm internal diameter. (B) Separation chamber of 3 cm length and 2 cm internal diameter, with a magnetic bar stirring inside. (C) Separation chamber of 35 ml upper part and 23 cm length, 0.7 cm internal diameter with 23 cm filled with fragments of Teflon separation area. (D) Separation chamber of 35 ml upper part and 23 cm length, 0.7 cm internal diameter with 16 cm filled with fragments of Teflon separation area. (E) Separation chamber of 100 ml upper part and 65 cm length, 0.7 cm internal diameter with 27 cm filled with fragments of Teflon separation area. Inset: PTFE packed pieces.

Afterwards, valve V_5 was switched ON for 3.0 s to eliminate the rests of aqueous phase which could remain at the bottom of the separation chamber. After switching OFF V_5, V_4 was switched ON to let the organic layer to reach the detector for 10.0 s. After that, V_4 was switched OFF and V_5 was switched ON for 10.0 s to displace the aqueous phase from the separation chamber to the waste making during this time the absorbance measurements in the stopped-flow mode. At the end of the process, V_4 and V_5 were switched ON for 10.0 s to clean the set-up.

Absorbance values at 654 nm, were corrected with the absorbance at 750 nm, to compensate base-line shift. These measurements were taken for both, samples and aqueous standards of SDS and used for anionic surfactant determination using $CHCl_3$ stabilized with ethanol as a blank.

3. Results and discussion

3.1. Reaction of SDS and MB

The equilibrium between SDS and MB and the distribution of SDS–MB ion pair in water and chloroform has been qualitatively reported in the literature [3]. The SDS, and other anionic detergents, dissolved in water are slightly soluble in $CHCl_3$. On the other hand, MB dissolves well in both, $CHCl_3$ and water, providing a blue colour solution in all the cases. When pure water is mixed with a $CHCl_3$ solution of MB, the blue colour is rapidly transferred to the water phase.

3.2. Experimental conditions for multicommutation analysis

In the experimental set-up indicated in Fig. 1, sample and reagent volumes were inserted sequentially into the separation chamber. The ionic pair extraction occurred by falling down chloroform and bubbling air in counter current. Good mixing conditions were attained by this way.

Therefore, to attain maximum sensitivity it is necessary to consider the separation chamber length and geometry, additionally than the sampling cycles and the use of a packing material to create a turbulent flow which can favour the reaction between MB and SDS and the mass transfer process.

3.2.1. Selection of the separation chamber

Two empty separation chambers and two packed ones (see Fig. 2) were assayed for making the on-line extraction of the SDS–MB ion pair into $CHCl_3$ using in all the cases, except for chamber B, the manifold depicted in Fig. 1 (for chamber B, V_6 and air line were unnecessary).

In these experiments, SDS and MB solutions were merged by using V_1 and V_2 pulses and let to stand before the introduction of $CHCl_3$.

When thin separation area chambers were employed (C–E), it was also evaluated the use of different portions of the chamber filled with PTFE fragments from the total height (23 cm for C) to 16 cm in the case of D and 23 cm over a total length of 65 cm for E.

Different sample volumes from 3.6 to 21.6 ml were assayed through the use of different inserting times and cycles for a fixed 1×10^{-3} mol l^{-1} MB solution and a $CHCl_3$ volume from 0.7 to 1.4 ml was used for extraction.

Table 2 shows the absorbance values found in each case for a solution of 576 μg l^{-1} SDS with the corresponding standard deviation and the typical calibration line obtained for each system in terms of absorbance units of the extracted chloroformic solution per mol^{-1} l SDS.

As can be seen in Table 2, separation chambers A and B provided a reduced sensitivity and a poor repeatability, especially in the case of A, due to the difficulties on making a quantitative extraction. Separation chambers with a thin separation area provided sensitive and reproducible data, based on the quantitative extraction of SDS and easy phase separation in case D and E and a high aqueous/organic phase ratio of 10.3 in case C. However, as it can be seen in the case of C and D both, the volume of sample and the relation between sample and $CHCl_3$ volumes together with the use of packing materials control the analytical sensitivity to be obtained. In fact aqueous/organic phase ratios from 2.6 to 5.1 could be found on using separation chamber D filled partially with PTFE fragments and a 26 times preconcentration was found on using a big separation chamber (E).

The best results in terms of sensitivity in the organic phase and precision were found on using chamber D and for 20 cycles of 1.0 s for samples and 20 cycles of 0.2 s for MB and using 0.7 ml of $CHCl_3$. However, for highly diluted samples,

Table 2
Effect of the extraction cell design on the analytical sensitivity and reproducibility

Cell	Loading plugs			Total volume (ml)	$A \pm s^a$	Calibration curve	R	LOD and RSD
A	SDS	45 s	1 cycle	8.10	0.17 ± 0.03	$y = (80000 \pm 2000)x + (0.01 \pm 0.02)$	0.997	LOD = 1.1×10^{-7} mol l^{-1} (31.7 ng ml^{-1}), RSD = 17.6%
	MB	2 s	1 cycle	0.12				
	$CHCl_3$	15 s	1 cycle	1.05				
B	SDS	40 s	1 cycle	7.20	0.123 ± 0.005	$y = (53700 \pm 500)x + (0.015 \pm 0.003)$	0.999	LOD = 2.8×10^{-7} mol l^{-1} (80.6 ng ml^{-1}), RSD = 4.1%
	MB	2 s	1 cycle	0.12				
	$CHCl_3$	15 s	1 cycle	1.05				
C	SDS	2 s	20 cycles	7.20	0.22 ± 0.01	$y = (101000 \pm 1000)x + (0.023 \pm 0.007)$	0.997	LOD = 3.0×10^{-7} mol l^{-1} (86.4 ng ml^{-1}), RSD = 4.5%
	MB	0.2 s	20 cycles	0.24				
	$CHCl_3$	1 s	10 cycles	0.70				
D	SDS	1 s	20 cycles	3.60	0.21 ± 0.01	$y = (141000 \pm 7000)x - (0.06 \pm 0.02)$	0.997	LOD = 6.1×10^{-9} mol l^{-1} (1.7 ng ml^{-1}), RSD = 4.8%
	MB	0.2 s	20 cycles	0.24				
	$CHCl_3$	1 s	10 cycles	0.70				
	SDS	2 s	10 cycles	3.60	0.116 ± 0.009	$y = (61800 \pm 700)x - (0.014 \pm 0.004)$	0.9998	LOD = 5.0×10^{-8} mol l^{-1} (14.2 ng ml^{-1}), RSD = 7.7%
	MB	0.2 s	10 cycles	0.12				
	$CHCl_3$	2 s	10 cycles	1.40				
E	SDS	2 s	60 cycles	21.60	0.22 ± 0.01	$y = (127000 \pm 4000)x - (0.02 \pm 0.01)$	0.998	LOD = 5.0×10^{-10} mol l^{-1} (0.14 ng ml^{-1}), RSD = 4.5%
	MB	0.2 s	60 cycles	0.72				
	$CHCl_3$	2 s	6 cycles	0.84				

SDS: anionic surfactant; MB: methylene blue; $CHCl_3$: solvent extractant. % RSD: relative standard deviation of the reference standard solution; LOD: limit of detection. Measurements for $k = 3$ in the original sample.
[a] Absorbance corresponding to six measurements of a solution containing 2×10^{-6} mol l^{-1} (576 μg l^{-1}) of SDS in the aqueous phase.

design E, which involves the use of a maximum sample volume of 21.6 ml for a $CHCl_3$ volume of 0.84 ml, provided a limit of detection of 0.14 μg l^{-1} anionic surfactant in the original sample, thus offering the best alternative.

3.2.2. Study of the effect of reagents and sample volumes

Sample or standard, reagents and air flow rates were fixed by using different positions of the Mariotte flasks and different rotation speeds and pump tubes.

Flow rates of 180, 60, 70 and 106 μl s^{-1} for sample, dye, solvent and air streams, respectively, were found to be convenient for introducing ml volumes on using short insertion times from 3 to 10 s.

On using the separation chamber C and D indicated in Fig. 2, it was evaluated the effect of the volumes of sample, MB and $CHCl_3$ on the extract absorbance measurements.

The total sample volume to be employed strongly depends on the volume of the separation chamber. Total volumes from 3.6 to 21.6 ml were assayed on varying the chamber design (see Table 2).

The volume of the organic plug introduced in the separation chamber is one of the most important variables in order to obtain a good aqueous/organic phase ratio for a fixed sample volume. It is clear that small $CHCl_3$ volumes are highly convenient in order to reduce the reagent consumption and side effects of toxic reagents. However, volumes smaller than about 0.5 ml $CHCl_3$ create practical difficulties to fill the detection cell and provided a poor precision. A volume of 0.7 ml per determination (10 cycles of 1.0 s) was finally selected as a compromise between technical difficulties and sensitivity.

The best MB amount in the separation chamber was obtained for a 1×10^{-3} mol l^{-1} solution and 0.24 ml insertion through the use of 20 cycles of 0.2 s. Higher MB amounts than that led to molecular aggregations of the reagent which can be extracted by chloroform, thus increasing the blank signal and adversely affecting the sensitivity. Moreover, a high concentration of MB involved long times for the washing step between samples.

3.2.3. Study of the sampling cycles in multicommutation

The analytical sensitivity of multicommutation methods increases on increasing the total sample volume introduced in the system [8].

For a fixed MB concentration and $CHCl_3$ volume, it was evaluated the effect of increasing volumes of SDS solutions as a function of the use of an increasing number of cycles of 1.0 s insertion time (see Fig. 3).

It can be seen that on using separation chamber D the absorbance signals increase linearly till to reach a plateau for 20 sampling cycles, which corresponds to 3.6 ml of sample, independently on the use of different SDS concentrations, thus indicating a limited extraction of SDS in the experimental conditions selected based on the shaking of both aqueous SDS and $CHCl_3$ MB solutions (see Fig. 3a).

On the other hand, on using the separation chamber E, the plateau was reached for 60 sampling cycles, corresponding to 21.6 ml (Fig. 3b). These data indicated that, in spite of the

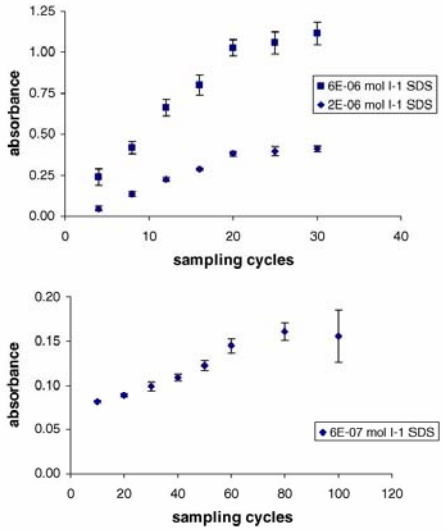

Fig. 3. Effect of the number of sampling cycles on the absorbance signal of SDS determined with MB. Note: each cycle corresponds to 1 s of loading time. Experiments were carried out for 2×10^{-6} and 6×10^{-6} mol l^{-1} SDS using the separation chamber D with 20 cycles of 0.2 s for 1×10^{-3} mol l^{-1} MB and 10 cycles of 1 s $CHCl_3$ (a) and for a concentration of 6×10^{-7} mol l^{-1} SDS in the separation chamber E using 60 cycles of 0.2 s 1×10^{-3} mol l^{-1} MB and 6 cycles of 2 s of $CHCl_3$ (b).

use of excess concentration of MB, the extraction efficiency, for a fixed mass of SDS strongly depends on the geometry and shaking characteristics of the system.

On comparing with in batch experiments, it was confirmed that the extraction efficiency of the ion pair between SDS and MB in $CHCl_3$ was 78 ± 3% and that this value is reproducible for both, sample and standard solutions.

3.3. Analytical figures of merit

In the best operational conditions determined previously, additional experiments were performed in order to establish the linear dynamic range and limit of detection. These assays were done running a set of anionic surfactant reference solutions and the blank solution.

Table 3 shows the main characteristics of anionic surfactants determination by using multicommutation compared with those found by the reference procedure. As can be seen, the slope of a typical calibration graph obtained by multicommutation is 1.8 times higher than that found in the reference method. The limits of detection obtained by both reference method and multicommutation were 1.8×10^{-8} and 6.1×10^{-9} mol l^{-1}, respectively, related to the original sample. The coefficient of variation of eight independent analysis of a solution containing 2×10^{-6} mol l^{-1} surfactant was of the same order for both the reference (6.2%) and the multicommutation approach (5.9%). So it can be concluded that multicommutation does not sacrifice any of the analytical basic properties of the method as compared with the reference procedure.

The sampling throughput was 40 injections per hour, providing a total waste volume of 4.5 ml per determination. So, it means a 40 times enhancement of the laboratory productivity and a 34 times reduction of laboratory wastes as compared with the reference procedures. Concerning sample and reagent consumptions, it can be seen in Table 3 that the reference method involves a total consumption of samples and reagents for 100 determinations of 101 of sample, 11 of dye solution and 4.51 of $CHCl_3$ being required 360, 24 and 70 ml, respectively, for the multicommutation approach,

Table 3
Analytical parameters of SDS–MB spectrophotometric determination by using the reference and the proposed multicommutated methods

	Reference method	Multicommutation[a]
Calibration line[b]	$y = (79000 \pm 3000)x + (0.062 \pm 0.003)$	$y = (141000 \pm 7000)x - (0.06 \pm 0.02)$
Correlation coefficient (r)	0.998	0.997
Linear range (mol l^{-1})	0.4×10^{-6} to 1.8×10^{-6}	0.7×10^{-6} to 6.1×10^{-6}
Linear range (mg l^{-1})	0.1–0.5	0.2–1.7
RSD (%)[c]	6.2	5.9
LOD (mol l^{-1})[b]	3.9×10^{-8}	3.1×10^{-8}
LOD (mol l^{-1})[d]	1.8×10^{-8}	6.1×10^{-9}
LOD (μg l^{-1})[b]	11.4	8.8
LOD (μg l^{-1})[d]	5.2	1.7
Sample consumption (ml)[e]	10000	360
Dye consumption (ml)[e]	1000	24
Solvent consumption (ml)[e]	4500	70
Waste (ml)[e]	15500	450
Throughput (h^{-1})	1	40

[a] Multicommutation measurements were made on using separation chamber D.
[b] Concentrations and LOD values are related to the organic phase.
[c] RSD (%): relative standard deviation corresponding to 10 independent measurements of a solution containing 2×10^{-6} mol l^{-1}.
[d] LOD values are related to the original sample.
[e] Sample and reagent consumptions and waste generation corresponding to 100 analysis.

Table 4
Analytical comparison of the proposed method with previous ones

	Proposed method	Previously reported methods						
		[3]	[4]	[5]	[9]	[10]	[11]	[12]
Correlation coefficient (r)	0.997	0.9993	0.997–0.999	0.990	0.999	–	0.9999	0.999
Linear range (mg l^{-1})	0.2–1.7	0.02–0.5	0–2	–	0–20	0.01–0.4	1.4–2.5	0–1.5
RSD %	5.9	7.2–7.5	6	6.7	0.5	2	0.4	1.5
LOD (μg l^{-1})	1.7	20–50	–	20	100	5	200	20
Sample consumption (ml)	3.6	50	15	50	0.2	50	0.18	0.15
Throughput (h^{-1})	40	–	–	20	50	–	90	30

thus reducing the consumptions by a factor of 28, 42 and 64 for sample, MB and $CHCl_3$, respectively. So, it can be concluded that the developed procedure is a sustainable and environmentally friendly alternative to the reference procedure.

On comparing the proposed method with previous spectrophotometric procedures available in the scientific literature (see Table 4), it can be concluded that all these methods work in the linear range till 0.4 to 2.5 mg l^{-1}, except a method which covers till 20 mg l^{-1} [9]. RSD values range comprises from 0.4 to 7.5% but not so many data are available about the criterion and concentration levels employed to establish the repeatability. Concerning the LOD, it can be concluded that the method developed is one of the most sensitive and it must be emphasized that a sample consumption of 3.6 ml of the proposed method is clearly lower than 50 ml [3,5,10] or 15 ml [4] required for previous procedures and that methods involving less than 1 ml of sample [9,11,12] only provided LOD values between 20 and 200 μg l^{-1}. So, it can be concluded that the proposed method clearly improves the reference ones and provides comparable or better performance than that offered by precedent studies.

3.4. Study of interferences

It was evaluated the effect of Ca^{2+}, Mg^{2+}, Cd^{2+}, Cl^-, NO_3^-, $C_2O_4^{2-}$ and Triton X-100 on the determination of anionic surfactants by the developed procedure. For a fixed concentration of 2×10^{-6} mol l^{-1} (576 μg l^{-1}) SDS, the maximum tolerance levels which do not modify the absorbance values of SDS, beyond the limits of $\bar{A} \pm 3$ s of those obtained in the absence of interferences, were established and results reported in Table 5 were found. In this table, there are also indicated the tolerance levels described in the literature for foreign ions [9–14] on using different procedures for anionic surfactants determination. As can be seen, only Cd^{2+} and Mg^{2+} interfere at concentration levels under the 1000 mg l^{-1}. It can be seen that the method developed clearly enhances the selectivity of this determination as compared with some of the reported methods [9,12,13]. On the other hand, the method proposed inhibits the Triton X-100 interference reported before [11,13] and improves the selectivity of NO_3^- [9,10,12,13] and Cl^- [9,12–14].

3.5. Evaluation of the accuracy

The evaluation of the developed procedure was made by two ways: (i) making recovery studies on washing water samples spiked with known concentrations of SDS from 5 to 30 mg l^{-1} and on natural irrigation channels waters spiked at a concentration level of 0.2 mg l^{-1} and (ii) by the comparison of results found by both, the reference and the developed procedure, on the analysis of a series of different actual samples.

Recovery studies on washing samples were made by using both methods, the reference and the proposed one, in order to evidence the absence of losses or contaminations on the determination of anionic surfactants. Data found for different

Table 5
Tolerance levels of foreign ions in mg l^{-1} for a SDS concentration of 2×10^{-6} mol l^{-1}

Ion	Developed method	Previously reported methods					
		[9]	[10]	[11]	[12]	[13]	[14]
Ca^{2+}	>1000	200	400	1000	–	300	4000
Mg^{2+}	>500	100	1500	1000	–	300	2430
Cd^{2+}	>500	–	–	–	–	30	–
Cl^-	>1800	100	24900	>16000	1000	300	1750
NO_3^-	>1000	400	6.2	>16000	100	30	–
$C_2O_4^{2-}$	>1000	–	–	8000	–	–	–
Triton X-100	>10000	–	–	400	–	0.3	–

Note: Reported methods are based on: [9] FIA reaction in water with different dyes; [10] in batch extraction in toluene of the reaction product with a Co(III) complex; [11] FIA displacement of the ion pair between methyl orange and cetyl pyridine in waters; [12] FIA extraction of MB and SDS in $CHCl_3$; [13] in batch extraction in benzene or toluene of the ion pair with ethyl violet; [14] in batch extraction in different solvents of the reaction product between a Co(III) complex and SDS.

Table 6
(a) Total anionic surfactant recoveries (%) found in washing samples[a]; (b) recovery of SDS added to natural irrigation channel waters at a spiked level of 0.2 mg l^{-1} on using the multicommutation developed procedure

Water	SDS added (mg l^{-1})	Reference method (%)	Multicommutation (%)
Tap	5	99 ± 9	91 ± 5
Clothes washing	10	105 ± 9	97 ± 7
Washing machine	20	102 ± 9	98 ± 2
Crockery washing	30	101 ± 5	95 ± 4

Irrigation channel	Concentration (mg l^{-1})	Recovery (%)
Port Saplaya	0.31 ± 0.01	94 ± 3
Pobla de Farnals	0.73 ± 0.03	103 ± 6
Puig	0.24 ± 0.02	95 ± 3

[a] Measurements were carried out by the reference method and by the multicommutation developed procedure. Standard deviation values correspond to three independent analysis.

Table 7
Total anionic surfactant concentrations found in washing waste water samples

Water sample	Reference method (mg l^{-1})	RSD (%)	Multicommutation (mg l^{-1})	RSD (%)
Crockery washing	860 ± 50	5.8	870 ± 10	1.1
Clothes washing	42 ± 4	9.5	43 ± 2	4.7
Clothes washing	15 ± 2	13.3	17 ± 1	5.9
Washing machine	15 ± 2	13.3	14.7 ± 0.8	5.4
Hand washing	1110 ± 50	4.5	1140 ± 30	2.6
Glassware washing	20 ± 2	10.0	21.0 ± 0.5	2.4
Soil washing	33 ± 2	6.1	33 ± 1	3.0
Car washing	12 ± 2	16.7	13.5 ± 0.3	2.2

Results indicated are the average of three independent determinations (six measurements) ± the corresponding standard deviation.

kind of samples spiked with SDS concentrations ranging from 5 to 30 mg l^{-1} are presented in Table 6a.

Average recoveries of 95% for multicommutation and 102% for the reference procedure were found.

Water samples collected from three irrigation channels near the city of Valencia containing from 0.24 to 0.73 mg l^{-1} of anionic surfactants, were spiked with a 0.2 mg l^{-1} concentration of SDS and recovery values reported in Table 6b evidenced that SDS was recovered between the 94 and 103%.

Anionic surfactants were determined by the developed procedure in eight different washing waste water samples, and the results obtained are shown in Table 7 also indicating data found by the reference standard method.

Data reported evidenced the presence of concentrations of anionic surfactants from 12 (car washing waste water) to 1140 mg l^{-1} (hand washing waste water).

Precision (RSD values) found for real sample analysis varied from 1.1 to 5.9% in the case of multicommutation measurements and from 4.5 to 16.7% for the reference method being average RSD values 3.4 and 9.9%, respectively. These data indicate the good repeatability obtained by the developed procedure.

The comparison of data found by multicommutation with those found by the reference method provided an equation $y = (1.02 \pm 0.08)x + (0 \pm 4)$ with a regression coefficient of 0.999 and Student's t calculated values for the slope (0.322) and for the intercept (0.107) which were in both cases lower than the theoretical t value (1.682) for a 95% probability level and 46 freedom degrees, thus showing a good comparability of the results, which evidences that both procedures provide statistically comparable results.

4. Conclusions

The proposed method, for the determination of anionic surfactants in waters, offers several advantages over the reference procedure [2] and previously developed alternatives [3–5]. It has been reduced the sample size from 100 ml [2], 50 ml [3,5] or 15 ml [4] to only 3.6 ml. In terms of $CHCl_3$ consumption, the 700 μl required per determination are clearly lower than the 45 ml of the reference method [2] and the 30 ml [4] and 5 ml [3] required by the simplified methods, being only higher than that employed in continuous liquid–liquid extraction, which was 200 μl [5].

Separatory funnels and expensive glassware were avoided as in the case for some of the previous alternatives [4,5].

On the other hand, the multicommutation approach offers a sampling throughput of 40 h^{-1}, two times better than the FIA procedure [5] and provides the best limit of detection reported, 1.7 μg l^{-1} as compared with the 20 μg l^{-1} indicated in previous papers [3,5].

The proposed method is characterized by its simplicity and reliability. Extraction and detection are integrated in the same manifold and all the operations can be controlled from the computer. Additionally, as it has been indicated, multicommutation offers an environmentally friendly alternative to do this kind of determinations.

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«Determination of phenols in waters using micro-pumped multicommutation and spectrophotometric detection. An automated alternative to the standard procedure»

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Determination of phenols in waters using micro-pumped multicommutation and spectrophotometric detection. An automated alternative to the standard procedure

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Abstract

An automated and greener spectrophotometric procedure has been developed for the determination of phenol in waters at 700 nm. The method uses the reaction between phenol, sodium nitroprusside and hydroxylamine hydrochloride in a buffered medium at pH 12.3. The flow manifold comprises 4 solenoid micro-pumps employed for samples and reagents introduction to the reaction coil and to transport of colored product formed to the detector. The linear dynamic range was 50 – 3500 ng mL^{-1} (R = 0.99997; n = 6) and the method provided limit of detection (3σ) of 13 ng mL^{-1}. The sampling throughput was estimated as 65 measurements per hour and the coefficient of variation was 0.5 % (n = 10) for a 1.0 µg mL^{-1} phenol concentration. Recoveries from 92 % to 105 % were obtained for phenol determination in spiked water samples at concentration levels from 50 to 5000 ng mL^{-1}. The use of multicommutation reduces 25 times the reagents consumption, 225 times the sample consumption and 30 times the waste generation as compared with the batch procedure. The proposed method is an environmentally friendly alternative to the 4-aminoantipyrine official method since it avoids the use of chloroform.

Keywords: *phenol; solenoid micro-pumps; multicommutation; spectrophotometry; waters.*

Introduction

Nowadays, there is an increasing need of inexpensive and greener analytical methods that can provide reliable results for monitoring water chemical parameters as phenol concentration.

The environmental concern of phenol pollution results from the high toxicity of many substituted phenolic compounds to mammals, fish and other aquatic life. As a result, the maximum allowed levels of total phenolics in a wide range of industrial wastes and natural waters are regulated by the legislation in each country. Spanish norms tolerate phenol concentrations of even 100 ng mL^{-1} in non chlorinated streams and 10 ng mL^{-1} in chlorinated waters [1].

The official ASTM standard method is based on the oxidative coupling of phenols with 4-aminoantipyrine (4-AAP) in alkaline medium, extracting the colored derivative compound in chloroform [2]. However, the main disadvantages of this procedure are: (i) the use of big amounts of $CHCl_3$, a depleting ozone layer product [3], (ii) the use of hazardous solutions, as H_2SO_4 and (iii) tedious operations. Additionally, some phenolic compounds can not be extracted by chloroform. Automated methods based on the aforementioned colored derivative, but avoiding the extraction step, have been also proposed [4, 5].

Other spectrophotometric methods have been developed in the last years by using different chromogenic reactions with 3-methyl-2-benzothiazolinone hydrazone (MBTH) [6], iodine

monobromide [7], the iron (II) 1,10-phenantroline complex [8] or 2,4,6-trimethylaniline [9], and recent alternatives are based on the use of solid phase UV spectrophotometric measurements [10] or electrochemical detection [11].

Interesting procedures have been proposed based on the Berthelot reaction, that develops a blue-colored product by reaction between phenol and sodium nitroprusside in presence of reagents that contain amino groups in a buffered medium [12-14]. However, these procedures consume big amounts of reagents, involve a high dilution of samples and were not enough sensitive (limit of detection around 100 ng mL^{-1}) to be suitable for the determination of phenol at the levels tolerated by the law.

The Berthelot reaction applied to the determination of phenols has been improved by Kang *et al*. [15] enhancing its sensitivity and reducing the limit of detection till 50 ng mL^{-1}. However, this procedure even involves a high reagents consumption and waste generation and a reduced sampling throughput.

The use of the multicommutation approach, employing a set of micro-pump devices, offers exciting answers to the disadvantages of available methodologies for phenol determination by increasing the sensitive of analytical procedures, reducing the consumption of reagents and samples and the waste generation and increasing the productivity of the laboratory [16]. Taken in consideration this new approach, the present study describes an automated and green spectrophotometric method based on the use of multicommutation for the fast determination of phenols in waters.

Experimental

Apparatus

The flow system comprised four solenoid micro-pumps Bio-Chem. 090SP (Boonton, USA), flow lines of 0.8 mm i.d. PTFE tubing, a reaction polyethylene coil of 0.8 mm i.d. and a home-made 5-channels confluence connector. A Hewlett-Packard Model 8452A diode array spectrophotometer (Waldbronn, Germany), furnished with a 50 µL flow cell and 10 mm path length, was used as detection system. A Pentium 133 MHz microcomputer, equipped with an electronic interface Advantech PCL-711S, was employed for system controlling and data acquisition by means a software written in Microsoft Visual Basic 6.0. A home-made electronic interface was used to generate the electric potential and current required to switch the solenoid pumps (12 V, ca. 100 mA). The micro-pump delivers 7 µL of solution per stroke, therefore the volume of the sample and reagent solutions inserted into the analytical path should be controlled by varying the number of the switching cycles, being the switching frequency settled at 5 Hz.

Reagents and Solutions

All solutions were prepared with nanopure water (18.2 MΩ cm) and analytical grade chemicals.

Phenol, from Merck (Darmstadt, Germany), standard solutions were daily prepared by appropriate dilutions of the stock one. The stock solution was stable at least 60 days maintained in a refrigerator at + 4 °C. Sodium nitroprusside, from Merck (Darmstadt, Germany), and hydroxylamine hydrochloride, from Panreac (Barcelona, Spain), were prepared weekly and sodium nitroprusside was maintained in the dark. A pH 12.3 buffer solution was prepared with NaH_2PO_4 from Panreac (Barcelona, Spain) and NaOH from Scharlau (Barcelona, Spain) and was used as a carrier.

A 4-AAP solution buffered at pH 10.0 from Acros Organics (New Jersey, USA) and a $K_3[Fe(CN)_6]$ solution from Panreac (Barcelona, Spain) were employed to develop the procedure used as reference.

Solutions of $NaNO_3$ from Panreac (Barcelona, Spain), NH_4Cl from Merck (Darmstadt, Germany), KNO_3, $Ba(NO_3)_2$, $CaSO_4$ from Panreac (Barcelona, Spain) and $MgSO_4$ from F.E.R.O.S.A. (Barcelona, Spain) were prepared to evaluate the interference agents.

Solutions containing phenol, o-cresol, m-cresol, m-xylenol, 2,6-dichlorophenol-indophenol, 2-(2-thiazolylazo)-p-cresol, 5-dimethylamino-2-(thiazolylazo)phenol from Merck (Darmstadt, Germany), p-aminophenol, resorcinol from Fluka (Buchs, Switzerland) and naphtol from F.E.R.O.S.A. (Barcelona, Spain) were employed to evaluate the relative response of the system for different phenols.

Natural waters were collected in polyethylene bottles from the Valencia city area and filtered with 0.45 µm nylon filters to remove the suspended solids, which could impair the diaphragm of the solenoid micro-pumps.

Flow set-up and proposed procedure

Fig. 1 shows the manifold employed in the present study. Four solenoid micro-pumps (P_1 to P_4) with pulses of 0.1/0.1 s

(aspiration of 7 µL solution into the micro-pump / ejection of 7 µL from the micro-pump) were used to handle the samples or standards, sodium nitroprusside, hydroxylamine hydrochloride and buffer, respectively. These solutions were mixed at the confluence point *x* and the coiled polyethylene tube *B* was used as reactor and mixing coil, in which the reaction scheme depicted at the bottom of the figure takes place

When the control software was run, the microcomputer requested the values of the time intervals to switch the micro-pumps P_1, P_2, P_3 and P_4, in the states 1/0 or 0, being 1/0 a sequence to open and close the pump and 0 just to remain without voltage. Afterwards, the flow system was operated following the switching course summarized in Table 1.

In the first step, 4 pulses of sample or standard (S), 1 pulse of sodium nitroprusside solution (R_1), 1 pulse of hydroxylamine hydrochloride (R_2) and 2 pulses of buffer (C) provided the aspiration of 28 µL of sample or standard, 7 µL of each one of the two reagents and 14 µL of buffer through the sequential operation of P_1, P_2, P_3 and P_4 micro-pumps. These solutions were mixed at the confluence point *x* towards reactor *B* (Fig. 1). This sequence was repeated 8 times. Next, in the step 2, 1155 µL of the buffer solution were flowed into the system by using 165 pulses of P_4 to remove the sampling zone to the detector and to clean the reactor coil *B*.

Reference batch method

45.0 mL of sample, 1.0 mL of 1.0 x 10^{-2} mol L^{-1} sodium nitroprusside solution, 1.0 mL of 4.0 x 10^{-2} mol L^{-1} hydroxylamine hydrochloride solution and 3.0 mL of buffer were introduced inside a 50 mL volumetric flask. After mixing well, it was allowed to stand for 15 min and the absorbance at 700 nm was measured against a reagent blank [15]. Sample absorbance values were interpolated in an external calibration line, prepared in the same way, for a concentration range between 50 and 5000 ng mL^{-1} phenol.

4-Aminoantipyrine method

A 0.10 % (m/v) 4-AAP solution buffered at pH 10.0 and a 0.2 % (m/v) $K_3[Fe(CN)_6]$ solution were employed as reagents. Pure water was used as a carrier. Sample and reagent solutions were sequentially introduced using the manifold indicated in Fig. 1 by switching the pumps P_1 – P_4 during 10 cycles, in the relation 6:1:1:5 (S:R_1:R_2:C) towards a 80 cm coil. For sample replacing, the pump of the sample was switched ON/OFF during 200 cycles and the system was washed with the sample. Measurements were carried out at 500 nm using a modification of the multicommuted method developed by Lupetti *et al*. [5] through the use of micro-pumps.

Results and Discussion

Developed multi-pumped system

The micro-pumps employed for this study are constituted by solenoid diaphragm devices, which by means of an inner spring mechanism are maintained open or closed, depending if the voltage is applied or not [17]. This opening action permits to flow the solutions into the pump chamber and next, these solutions are dispensed from the pumps by dropping the applied voltages. The de-energizing of the solenoid coils forces the diaphragms back to the closed position. The employed devices were characterized by a fixed stroke volume (7 µL per pulse), so that, a precise and effective control of the volume of sample and reagents, at a given flow rate, is accomplished by appropriate dimensioning of the frequency (5 Hz) and number of pulses.

In this study, four micro-pumps were employed to move the samples or standards (P_1), the two reagents used in the selected procedure (P_2 and P_3) and the buffer carrier flow (P_4).

Chemical aspects of the phenol determination

The developed procedure is based on the reaction between phenol, sodium nitroprusside and hydroxylamine hydrochloride in a basic buffered medium, according to the mechanism studied by Kang *et al*. [15] described in the bottom of Fig. 1. In alkaline medium, the phenol group is deprotonated and makes the benzene ring very active. The nitroso group of the nitroprusside attacks the benzene ring and the reaction takes place providing a blue product with a maximum absorbance at 700 nm.

It was evaluated the best conditions to improve the aforementioned reaction, to reduce reagents consumption and waste generation and to enhance the sensitivity of the phenol determination.

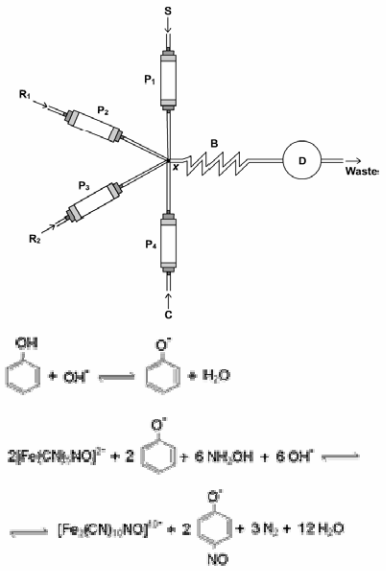

Fig. 1 Multicommutated flow manifold diagram. S – sample or standard solution; R_1 – 3.0 x 10^{-2} mol L^{-1} sodium nitroprusside solution; R_2 – 3.0 x 10^{-2} mol L^{-1} hydroxylamine hydrochloride solution; C – carrier buffer solution, pH 12.3; P_i – solenoid micro-pumps; x – confluence point; B – 100 cm mixing tube 0.8 mm i.d.; D – detector.
Bottom: Mechanism of the reaction between phenol, sodium nitroprusside and hydroxylamine hydrochloride in a strong basic medium.

Effect of experimental conditions

Fig. 2 shows the effect of different concentrations of hydroxylamine hydrochloride for different concentrations of sodium nitroprusside. It was observed that concentrations of sodium nitroprusside lower or higher than 3.0 x 10^{-2} mol L^{-1} provided a reduction of the analytical sensitivity and hydroxylamine hydrochloride solution between 3.0 x 10^{-2} mol L^{-1} and 5.0 x 10^{-2} mol L^{-1} provided the best analytical results. So, 3.0 x 10^{-2} mol L^{-1} for hydroxylamine hydrochloride and 3.0 x 10^{-2} mol L^{-1} for sodium nitroprusside were chosen for this study.

Two parameters related to the time in which the coloured reaction occurs were studied. The reaction coil length controls the blue-green product formation time that must be long enough to provide a quantitative reaction between phenol and reagents in the buffer medium, according to the equation drawn in Fig. 1. However, the increase of the coil length also increases the dispersion of the sample plug. Thus, this parameter must be controlled to assure both, total formation of the reaction product and minimum dispersion, being 200 cm chosen as the best coil length.

However, it was possible to use small reaction coils, by fitting a delay time for measurement, to complete the reaction in the stopped flow mode. It was observed that, for a coil length of 100 cm, stopping the flow for 15 s was appropriate to obtain the same sensitivity than using a 200 cm coil length with an excellent repeatability.

Several studies were carried out to determine the best multicommutation parameters with regard to sampling conditions: the duration of the micro-pumps pulses, the pulses relation between samples and reagents and the number of sampling cycles. The effect of the filling/propulsion time variation of the solenoid micro-pumps, was evaluated between 0.1 and 0.3 s. The values of times are refereed to the time during which the diaphragm of the micro-pump aspirates/propels the 7 µL solution. It was found not significant differences in the use of 0.1 or 0.3 s. However, pulses of 0.1/0.1 s were found as the most adequate to obtain the best sensitivity and the narrowest peaks. So, combinations of 0.1/0.1 s to propel the solutions were chosen for following studies.

It was evaluated the effect of different relations between sample and reagent pulses. The following pulse series were researched, according to the sequence $S:R_1:R_2:C$ (see Fig. 1 and Table 1 for details) — 1:1:1:1, 2:1:1:1, 3:1:1:1, 4:1:1:1, 5:1:1:1, 4:2:1:1, 4:3:1:1, 4:1:2:1, 4:1:3:1, 4:1:1:2, 4:1:1:3, 4:1:1:4. The solutions insertion was carried out sequentially (pulse by pulse) or simultaneously (all pulses at the same time) for all switching courses evaluated. The best conditions, in terms of sensitivity and precision, were obtained for sequential injection in a 4:1:1:2 relation.

The analytical sensitivity of multicommutation methods increases on increasing the total sample volume introduced in the system [18]. For fixed $Na_2[Fe(CN)_5NO]\cdot 2H_2O$ and $NH_2OH\cdot HCl$ concentrations, it was evaluated the effect of increasing the number of sampling cycles of pulses of 0.1/0.1 s, according to the selected sequence 4:1:1:2 from 1 to 17. Results indicated that the absorbance signals increase till to reach a plateau for 8 sampling cycles, thus indicating a complete reaction between samples and reagents.

Table 1 Solenoid micro-pumps switching course for spectrophotometric phenol determination [a]

Step	Description	P_1	P_2	P_3	P_4	Pulses	
1	Sampling and mixing of S, R_1, R_2 and C	1/0	0	0	0	4	
		0	1/0	0	0	1	8 [b]
		0	0	1/0	0	1	
		0	0	0	1/0	2	
2	Sample zone removal, reading and cleaning	0	0	0	1/0	165	

[a] 1/0 indicates the activation of pulses of the solenoid micro-pump to be opened and closed; 0 indicates that solenoid micro-pumps remain inactive

[b] Sequence of pulses 4:1:1:2 was repeated 8 times per sampling cycle

Additionally, for a number of sampling cycles higher than 11, it can be observed a widening of peaks. So, 8 cycles was selected as the best choice.

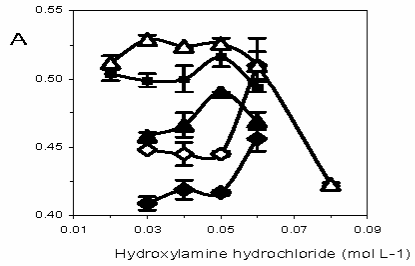

Fig. 2 Effect of sodium nitroprusside and hydroxylamine hydrochloride concentrations on the absorbance of a 2.5 µg mL^{-1} phenol solution. Sodium nitroprusside concentrations: ◆ 0.005 mol L^{-1}, ◇ 0.01 mol L^{-1}, ▲ 0.02 mol L^{-1}, △ 0.03 mol L^{-1}, ■ 0.04 mol L^{-1}. The number of cycles were 8 for sampling and mixing and 165 for removing of sampling zone and cleaning (see table 1 for details).

Study of interferences

The interference of different inorganic ions that may be encountered in natural waters was investigated. It was observed that until 150 µg mL^{-1} of Na^+, K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Cl^- did not interfere the signal of a phenol solution of 1.0 µg mL^{-1}.

Relative responses for different phenols

The relative responses of selected phenolic compounds at 2.0 µg mL^{-1} concentration level were compared using the above established parameters and results found are summarized in Table 2. The analytical response varied with the degree of substitution, type and position of substituents. As can be seen, phenol, p-aminophenol and o-cresol present a high sensitivity by this procedure, followed by other phenolic compounds as m-cresol, resorcinol or napthol.

Table 2 Reaction features for several phenolic compounds using the proposed method*

Compound	λmax (nm)	$A_{\lambda max}/A_{700\ phenol}$
Phenol	700	1.00
p-Aminophenol	696	1.00
o-Cresol	690	0.94
m-Cresol	690	0.85
Resorcinol	628	0.76
Naphtol	680	0.55
m-Xylenol	690	0.30
2,6-dichlorophenol-indophenol	670	0.14
2-(2-thiazolylazo)-p-cresol	696	0.11
5-dimethylamino-2-(thiazolylazo)phenol	690	0.11

*2.0 µg mL^{-1} solutions were employed for measuring of all compounds and conditions indicated in Fig. 1 were used in all cases.

Analytical features of the developed procedure

In the best operational conditions previously determined, additional experiments were performed in order to establish the analytical features of the procedure. These assays were done running a set of phenol standard solutions and a blank solution.

Table 3 Analytical features of different spectrophotometric procedures for phenol determination

		Reagents employed	*λ (nm)*	*Path length (cm)*	*Calibration equation*	*R*	*Linear range (ng mL^{-1})*	*LOD* (ng mL^{-1})	*CV (%)* [a]	*Sampling rate (h^{-1})*	*Waste volume (mL)* [b]	*Reagents consumption (mL)* [b]		
												Sample	*Reagents*	*$CHCl_3$*
Micro-pumping multicommuted developed method		$Na_2[Fe(CN)_5NO]\cdot 2H_2O$ $NH_2OH\cdot HCl$	700	1	A=(0.028±0.002)+ +(0.217±0.001)·C	0.9997	50 - 3500	13	0.5	65	1.6	0.2	0.2	---
Batch methods	[15]	$Na_2[Fe(CN)_5NO]\cdot 2H_2O$ $NH_2OH\cdot HCl$	700	1	A=(-0.018±0.009) + +(0.212±0.003)·C	0.9998	50 - 5000	50	1.3	4	50	45	5	---
	[12]	$Na_2[Fe(CN)_5NO]\cdot 2H_2O$ chloramine	680	NA	NA	NA	50 - 1000	3.8	NA	NA	NA	1	9	---
	[7]	Iodine monobromide Cyclohexane	ultraviolet	NA	NA	NA	8 – 160	11	NA	NA	NA	NA	NA	---
	[8]	Iron (III) 1,10-phenantroline	510	NA	A=0.047+ +0.0047·C	NA	420 – 6380	44	NA	NA	NA	25	15	---
SIA method	[4]	4-AAP $K_3[Fe(CN)_6]$	540	1	A=0.145+ +0.098·C	0.9998	500 - 25000	100	2.2	12	10	0.2	NA	---
Solenoid valves multicommuted method	[5]	4-AAP $K_3[Fe(CN)_6]$	500	100	NA	0.999	10 - 100	1	0.6	90	4.0	0.7	0.2	---
FIA methods	[19]	4-AAP $K_3[Fe(CN)_6]$	500	1	NA	NA	50 - 15000	30	5.0	40 – 60	4.8	0.4	3.2	---
	[9]	2,4,6-trimethylaniline	550	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	---
	[10]	Methanol KCl NaOH	237	1	A=0.0072+ +0.0104·C	0.9997	1000 – 30000 (40 µL) 100 – 10000 (600 µL)	105 (40 µL) 1 (600 µL)	1.5 - 3.2 (40 µL) 1.1 – 2.2 (600 µL)	39	NA	0.05	NA	---
Segmented flow	[6]	MBTH [c] $CHCl_3$	NA	1	NA	NA	0.5 - 10	0.2	3.6	10	60	NA	NA	3.0

[a] Estimated for 10 measurements of a 1.0 µg mL^{-1} concentration of phenol.
[b] Estimates per determination
[c] MBTH = 3-methyl-2-benzothiazolinone hydrazone.
NA: not available

Table 3 shows the main characteristics of phenol determination by using multicommutation with solenoid micro-pumps compared to those found by using other previously procedures reported in the literature employing different reagents. As can be seen, the slope of typical calibration graphs obtained by using both, micro-pump devices and the batch procedure are of the same order for the reaction with sodium nitroprusside.

Micro-pumps-based multicommutation method achieves a limit of detection of 13 ng mL^{-1}, that is higher than those obtained by using the 4-AAP reaction with solenoid valves multicommutation [5], iodine monobromide [7] and the MBTH reaction in a segmented flow method [6], but 3, 3.4 and 10 times lower than those obtained by FIA [19], batch [8] and SIA [4] measurements with 4-AAP and 5 times lower than that found with sodium nitroprusside in batch [15]. It must be also noticed that methods providing the lowest limit of detection values involve an extraction with $CHCl_3$ [6] or the use of an optical path length of 100 cm [5].

The repeatability obtained by using micro-pumps-based multicommutation is comparable to that obtained by using solenoid valves multicommutation and better than values found for the rest of methods.

For the micro-pumping developed method, the sampling frequency was 65 h^{-1} as compared with 4 h^{-1} for the in batch method [15], thus evidencing a high productivity of the multicommutation approach. Only the method that employs solenoid valves [5] exceeds in speed the procedure reported in this paper.

Considering waste generation, Table 3 shows that the use of micro-pumps-based multicommutation generates a waste of 1.6 mL per determination and provides a 31 times reduction of waste volume as compared to the in batch procedure, and 6, 2.5, 3 and 37.5 times reduction of waste as compared with the flow alternative procedures summarized in Table 3. The sample and reagent consumptions per determination were also reduced in the developed system in comparison to the other methods. So, the developed procedure is a suitable and environmentally friendly alternative as compared to methods available in the literature.

Evaluation of the accuracy

Recovery studies on water samples were made in order to evidence the absence of losses or contaminations on the determination of phenol. Data found for different kind of water samples, spiked with known concentrations of phenol from 50 to 5000 ng mL^{-1}, showed quantitative recoveries from 92 % to 105 %.

Additionally, phenols were determined by the developed procedure in six different water samples and results compared with those obtained by reference procedures. Table 4 shows the data found by micro-pumping multicommutation for the reaction with sodium nitroprusside and those found by the batch reference method and the flow injection 4-AAP procedure. On comparing the micro-pumping multicommutation data with those found by the batch reference and flow injection methods, the following equations were found $y = (-1 \pm 1) + (1.000 \pm 0.002)\,x$ and $y = (-5 \pm 5) + (0.987 \pm 0.009)\,x$, respectively, with regression coefficients of 0.99999 and 0.9998. Student's t calculated values for the slope (0.0 and 1.4) and for the intercept (1.0 and 1.0) were in all cases lower than the theoretical t value (1.812) for a 95 % probability level and 10 freedom degrees. So, it can conclude that the accuracy of the developed procedure is comparable to that found in batch on using the same reaction and in flow analysis using an alternative reagent.

Table 4 Determination of phenol in water samples

Sample	Phenols (mg $m L^{-1}$)		
	Proposed method	Batch method [15]	4-AAP method [5]
Wastewater 1	307 ± 9	311 ± 8	324 ± 4
Wastewater 2	280 ± 7	284 ± 3	301 ± 9
Wastewater 3	1320 ± 20	1320 ± 30	1340 ± 15
Spiked tap water	56.8 ± 0.9	57 ± 2	55.9 ± 0.7
Spiked spring water	92 ± 2	92 ± 1	87 ± 3
Spiked rain water	110 ± 4	110 ± 10	121 ± 5

Standard deviation values correspond to three independent analysis

Conclusion

The proposed method offers advantageous analytical features in comparison to previously reported procedures for phenols determination, in terms of precision and sampling frequency. Moreover, this flow system avoids the use of organic solvents and reduces sample and reagents consumption and waste generation in comparison to the in batch method and other flow alternatives.

The use of solenoid micro-pumps facilitates the miniaturization of the system and its total automatization. The application of the developed system shows that it is a low cost and fast technique, yielding results in good agreement with those provided by reference methodologies.

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A clean method for flow injection spectrophotometric determination of cyclamate in table sweeteners

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Abstract

A flow system based on the multicommutation is proposed for fast and clean determination of cyclamate. The procedure exploits the reaction of cyclamate with nitrite in acidic medium and the spectrophotometric determination of the excess of nitrite by iodometry. The flow system was designed with a set of solenoid micro-pumps to minimize reagent consumption and waste generation. The detection limit was estimated as 30 μmol L^{-1} (99.7% confidence level) with linear response ranging up to 3.0 mmol L^{-1}. The coefficient of variation was estimated as 1.7% for a solution containing 2.0 mmol L^{-1} cyclamate ($n = 20$). About 60 samples can be analyzed per hour, consuming only 3 mg KI and 1.3 μg $NaNO_2$, and generating 2.0 mL of effluent per determination, thus providing an environmentally friendly alternative to previously proposed procedures. Common artificial and natural sweeteners did not interfere when present in concentrations 10-times higher than cyclamate. The procedure was successfully applied for determination of cyclamate in artificial table sweeteners with results in agreement with the reference method at the 95% confidence level.

Keywords: Flow injection; Multicommutation; Solenoid pumps; Spectrophotometry; Cyclamate; Artificial sweeteners

1. Introduction

Efforts to develop environmentally friendly procedures have continuously increased in order to minimize the impact of chemical activities. This field of investigation, named Green Chemistry, encompasses a number of strategies to minimize or eliminate the use of toxic substances and generation of wastes [1]. The main focus has been the development of new synthetic routes to minimize the amount of side products and replace toxic solvents [2]. The development of greener analytical procedures is a clear need as well, because several current analytical methods generate considerable amounts of toxic wastes [3].

The use of flow-based procedures has contributed to achieve greener analytical methods, by means of automation and miniaturization [4]. In-line reagent recycling [5] and treatment of wastes [6] are also interesting alternatives. The multicommutation approach [7], formerly proposed to increase system versatility, presents also the advantage of minimizing both, reagent consumption and waste generation [4], mainly when the binary sampling strategy is exploited [7]. In this approach, micro-volumes of sample and reagent solutions are sequentially inserted in the reaction coil of a single line manifold, providing system simplicity, suitable mixing conditions, facilities to optimize the sample/reagent ratio and avoiding waste of reagents. Multicommuted flow systems can be also designed with solenoid micro-pumps that can reproductively dispense micro-volumes of solutions [8]. These devices can replace both the injection and propulsion units, yielding compact manifolds with low power requirements.

Cyclamate is an artificial sweetener about 30-times as sweet as refined cane sugar [9]. This edulcorant is widely used in diet and medical products even being prohibited in some

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countries [10] in view of the possible carcinogenic effect of the cyclamate and its metabolite.

Several methods for cyclamate determination involve the use of toxic substances like lead [10], *N*-naphthylethylenediamine [11] and organic solvents [12,13]. Flow-based procedures have also been proposed, involving detection by chemiluminescence [14], spectrophotometry [11,15], flame atomic absorption spectrometry [10] or amperometry [16]. All procedures involve continuous reagent addition, generating considerable amounts of wastes, and some require a previous sample treatment [14,15].

In this work it is described a clean flow-based method for cyclamate determination in artificial table sweeteners, based on the reaction of the analyte with sodium nitrite in slightly acid medium and measurement of the excess of the reagent by iodometry, thus replacing toxic reagents previously used [10,11,14–16]. Solenoid micro-pumps were employed for minimizing reagent consumptions and waste generation. Analytical performance is not deteriorated and many characteristics are better than those achieved in previous works.

2. Experimental

2.1. Apparatus

The flow system comprised four solenoid micro-pumps Bio-chem 090SP with a nominal volume of 8 μL per pulse (Boonton, USA), flow lines of 0.8 mm i.d. PTFE tubing and two confluence connectors. A Pentium 133 MHz microcomputer was employed for system controlling by a parallel interface through a software written in Microsoft Visual Basic. A lab-made electronic interface, analogous to the previously described [17], was used to generate the electric potential and current required to switch the solenoid micro-pumps (12 V, ca. 100 mA). Measurements were carried out with a Hewlett-Packard Model 8452A diode array spectrophotometer equipped with a 10 mm optical path and 50 μl inner volume flow cell (Waldbronn, Germany).

2.2. Reagents and solutions

All solutions were prepared with deionized water (18.2 MΩ cm) and analytical grade chemicals. A 0.25 mol L^{-1} cyclamate stock solution was prepared from the sodium salt and working solutions (0.50–5.0 mmol L^{-1}) were prepared from dilution of the stock one in water. Reagents solutions were 0.20 mmol L^{-1} $NaNO_2$ (R_1) and 0.20 mol L^{-1} KI (R_2). A 0.10 mol L^{-1} H_3PO_4 was employed as carrier.

The effect of concomitants was evaluated using solutions containing 1.0 mmol L^{-1} cyclamate and up to 100-times excess of each one of the following substances: saccharin, aspartame, acesulfame-K, sucrose, glucose, fructose, maltose, galactose, lactose, sorbitol, caffeine, phenylalanine, ascorbic, benzoic and citric acids.

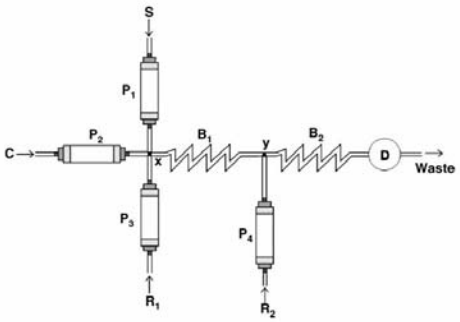


Fig. 1. Multicommuted flow-system for cyclamate determination. P_1, solenoid micro-pumps; D, spectrophotometric detector (350 nm); B_1, B_2, coiled reactors (100 and 50 cm); x, y, confluence points; S, sample; R_1, 0.20 mmol L^{-1} $NaNO_2$, R_2, 0.20 mol L^{-1} KI; C, carrier (0.1 mol L^{-1} H_3PO_4).

Samples of table sweeteners were purchased at the local market. Samples analyzed contained also sodium saccharin, sodium bicarbonate, sodium citrate and silicon dioxide (sample 1); dextrose and sodium sulfimidobenzoate (samples 2 and 5); sodium saccharin, dextrose and aspartame (sample 3); sodium saccharin and dextrose (sample 4). Samples were analyzed after simply dissolving 50–100 mg of the solids in 10 mL water.

2.3. Flow diagram and procedure

The solenoid micro-pumps were arranged as shown in Fig. 1, employing one device for each solution handled. During actuation, the devices were operated at 2 Hz, thus providing a flow of 840 μL min^{-1}.

System was operated as described in Table 1, exploiting the binary sampling approach [7] to mix the solutions. Each step was repeated until to complete the number of cycles. Sample (S), sodium nitrite (R_1) and carrier (C) solutions were sequentially inserted in coil B_1 (step 1) in which the main reaction took place. Sample zone was transported by the carrier towards the confluence point y (step 2). An optional stopped flow period could be then implemented (step 3). Then, aliquots of R_2 reagent were inserted between the sample zone (step 4) to determine the excess of nitrite by reaction with iodide. Transient signal measurement and sample removal by the carrier were carried out in step 5.

The effect of the stop period was evaluated by processing different aliquots of a 1.0 mmol L^{-1} cyclamate solution in different stopping times (0, 5, 10, 15, 20, 30 and 60 s). Interferences were evaluated by comparing the analytical signal obtained for cyclamate with or without the interfering species in different concentrations. All measurements were carried out in triplicate.

Table 1
Solenoid micro-pumps switching course for cyclamate determination

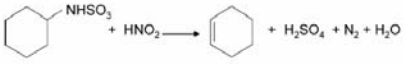
Step	Description	P_1	P_2	P_3	P_4	Pulses	Cycles
1	Mixing of S and R_1	1/0	0	0	0	2	8
		0	0	1/0	0	2	
		0	1/0	0	0	1	
2	Transport of the sample zone	0	1/0	0	0	30	1
3	Stopped flow (optional)	0	0	0	0	–	–
4	Addition of R_2	0	1/0	0	0	1	15
		0	0	0	1/0	1	
5	Transport of the sample zone and reading	0	1/0	0	0	200	1

Numbers 1/0 indicate a pulse of the solenoid micro-pump. Number 0 indicates that the applied voltage was turned off.

3. Results and discussion

3.1. General characteristics

Reaction between nitrite and cyclamate has been exploited in some analytical methods for determination of this artificial sweetener [11,13]. However, the procedures are tedious and time consuming [13] or use toxic reagents to determine the excess of nitrite [11]. In this study a green procedure based on this reaction has been developed by replacing toxic reagents and minimizing reagent amounts by using a flow system with solenoid micro-pumps. The method is based on the reaction of cyclamate with nitrite in acid medium (Eq. (1)) followed by reaction of the excess of nitrite with iodide to form the triiodide anion (Eq. (2)), with absorption maximum at 350 nm. This last reaction was previously applied for nitrite determination in a flow injection system with continuous reagent addition [18]:



(1)

$$2NO_2^- + 3I^- + 4H^+ \rightarrow 2NO + I_3^- + 2H_2O \quad (2)$$

The binary sampling approach [7] was selected for solution handling, by introducing small aliquots of each solution in tandem. With the proposed configuration, nitrite, phosphoric acid and cyclamate are formerly mixed in the reaction coil B_1, in which the main reaction (Eq. (1)) occurs. The iodide solution (R_2) is then added to the sample zone in the confluence point y to form the measured species. Thus, the height of the transient signals diminishes by increasing the cyclamate concentration in view of the minor amount of the remaining nitrite. The analytical signal was then defined as the difference between the reference signal, obtained by replacing the cyclamate by the blank solution (water), and the transient signals for each sample and reference solution.

The binary sampling strategy made feasible the optimization of sample/reagent ratios by varying the number of pulses of each solution (0–4 for solutions S and C; 10–25 for solution R_2). A nitrite concentration of 0.20 mmol L^{-1} and two pulses were selected for attaining a reference signal close to 0.9. For the other solutions, the effect of the number of pulses is shown in Fig. 2. In all cases a maximum is observed and the analytical signal diminishes either due to lack of reagent or excessive dilution of the sample zone. By considering the main reaction (Eq. (1)) the best sensitivity was achieved for the proportion sample:nitrite:phosphoric acid (carrier) of 2:2:1. The number of sampling cycles (number of repetitions of the reagents injection sequence) was also evaluated and sensitivity increased up to 8 cycles that correspond to a 240 μL sample zone for the 500 μL reactor used.

Two parameters related to the iodometric determination of the excess of nitrite (Eq. (2)) were important: the instant of addition and the number of pulses of iodide. It was verified in experiments with dyes that 30 pulses in step 2 (Table 1) were enough to place the center of the sample zone in the confluence point y. The addition of 15 pulses of the iodide solution was then suitable to attain best sensitivity (see Fig. 2).

As the reaction between cyclamate and nitrite (Eq. (1)) is relatively slow, high sample residence times in reactor B_1 can be exploited to increase sensitivity. This can be implemented in the proposed system by stopping the flow after mixing the reagents in the confluence point x (Table 1, step 3). The effect of the stop period on the analytical signal (S) was evaluated for stopping times from 5 to 20, 30 and 60 s, and can be

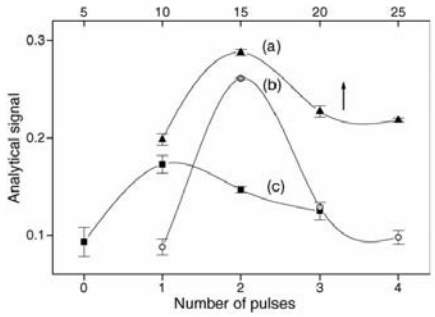


Fig. 2. Effect of the number of pulses of: (a) iodide (upper scale), (b) cyclamate and (c) phosphoric acid (carrier).

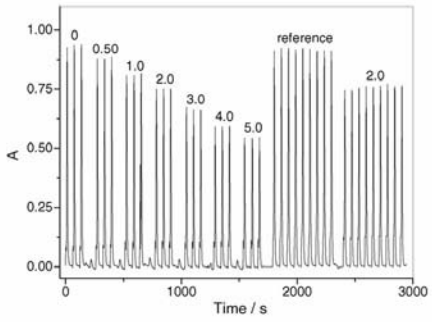


Fig. 3. Transient signals obtained for different solutions of sodium cyclamate. Numbers indicate concentrations in mmol L^{-1}.

described by the Eq. (3), which indicates a two-times increase in sensitivity for a 60 s stop period. On the other hand, the sampling rate would be reduced in 50%. As the sensitivity was not a critical aspect for the samples analyzed, subsequent measurements were carried out without stopping the sample zone in reactor B_1:

$$S = 0.394 + 0.0108t - 6.38 \times 10^{-5}t^2,$$
$$r = 0.987, \quad 0 < t < 60\,\text{s} \qquad (3)$$

3.2. Analytical features

System stability and signal repeatability can be visualized in Fig. 3, which shows a set of transient signals obtained for several cyclamate solutions. The detection limit, defined as the low cyclamate concentration that produces a signal significantly different of the reference signal at the 99.7% confidence level, was estimated as 30 μmol L^{-1} without stopping the flow. Linear response was observed up to 3.0 mmol L^{-1} and the coefficient of variation was estimated as 1.7% for 20 measurements of a solution containing 2.0 mmol L^{-1} cyclamate. About 60 samples can be analyzed per hour, consuming 3 mg KI, 1.3 μg $NaNO_2$ and 125 μmol H_3PO_4 per determination and generating a waste volume of 2.0 mL. As the most toxic chemical ($NaNO_2$) is completely decomposed in acid media, the waste generated is a solution containing triiodide and iodide ions.

A comparison of the analytical features achieved by the proposed and other flow-based procedures for cyclamate determination is presented in Table 2. The proposed procedure presents similar linear response range and detection limit and higher sampling rate as compared with the procedure proposed by Gouveia et al. [11], which uses continuous addition of toxic reagent to determine the excess of nitrite. Detection limit is 80 times better and sampling rate is 30% lower than that achieved in the procedure with amperometric detection that also employs nitrite as reagent [16]. The procedures proposed by Psarellis et al. [14] and Cabero et al. [15] also use toxic reagents and lack selectivity [14] or requires previous sample treatment in batch (hydrolysis for 2 h at 100 °C) [15]. The indirect procedure based on atomic absorption measurements [10] presents the lowest detection limit but generates ca. 50 mg of each toxic chemical ($Pb(NO_3)_2$ and $NaNO_2$) to implement 100 measurements.

3.3. Effect of concomitants and application

The effect on the cyclamate determination of several natural sugars like sucrose, glucose, fructose, maltose, galactose and lactose and artificial sweeteners as saccharin, aspartame, acesulfame-K as well as other common additives, like sorbitol, caffeine, phenylalanine, ascorbic, benzoic and citric acids was evaluated. The most severe interference was observed for ascorbic acid. Indeed, this species interfered severally even when present in concentration equal to the analyte, increasing the analytical signal in ca. 80%. The positive interference is caused by the reduction of both nitrite and triiodide anions by ascorbic acid. This interference was also observed in the procedures proposed by Gouveia et al. [11] (that exploits the reaction within nitrite and cyclamate) and by Miura and Kusakari [18] that proposed an iodometric procedure for nitrite determination. However, ascorbic acid is not commonly present in table sweeteners. For other kind of samples, such as soft drinks, the interference can

Table 2
Comparison of the characteristics of flow injection procedures proposed for cyclamate determination

	Proposed method	Psarellis et al. [14]	Gouveia et al. [11]	Yebra and Bermejo [10]	Cabero et al. [15]	Fatibello-Filho et al. [16]
Reagents	KI, $NaNO_2$, H_3PO_4	Sulfite, Ce(IV), H_2SO_4	$NaNO_2$, H_3PO_4, sulphanilamide, NED[a]	$Pb(NO_3)_2$, $NaNO_2$ acetic acid, ethanol	H_2O_2, HCl, NQS[b]	$NaNO_2$, H_3PO_4
Detection	Spectrophotometry	Chemiluminescence	Spectrophotometry	Atomic absorption spectrometry	Spectrophotometry	Amperometry
Linear range (μmol l^{-1})	100–3000	5–250	60–2600	5–450	<1000	5000–40000
LOD (μmol l^{-1})	30	–	30	1.2	7.7	2500
R.S.D. (%)	1.7	–	0.5	3.1	3.5	1.7
Sampling rate (h^{-1})	60	100	24	35	–	90

[a] *N*-1-naphthylethylenediamine.
[b] 1,2-naphthoquinone-4-sulfonate.

Table 3
Mean values and uncertainties ($n = 3$) for determination of cyclamate in commercial table sweeteners

Sample[a]	Sodium cyclamate (mg/g)	
	Proposed method	Reference method [11]
1	703 ± 8	714 ± 7
2	107 ± 2	106 ± 4
3	45 ± 1	47 ± 2
4	60.6 ± 0.9	60 ± 2
5	148 ± 4	144 ± 4

[a] Samples analyzed also contained, sample 1: sodium saccharin, sodium bicarbonate, sodium citrate and silicon dioxide. Sample 2: dextrose and sodium sulfimidobenzoate. Sample 3: sodium saccharin, dextrose and aspartame. Sample 4: sodium saccharin and dextrose. Sample 5: dextrose and sodium sulfimidobenzoate.

be avoided by previous oxidation of the ascorbic acid by bubbling oxygen or air through the samples [11]. Benzoic acid and maltosa interfere when in concentration 25 times higher than cyclamate; aspartame interferes when in a 50 times excess in relation to the analyte. Other species did not present any interference even in the maximum concentration level evaluated, 100-times excess in relation to cyclamate.

The proposed procedure was applied for the determination of cyclamate in five table sweeteners (see Table 3) which were also analyzed by the reference method [11]. A good correlation within the results attained by both procedures was obtained, as described by Eq. (4), indicating that systematic errors are absent:

$$[\text{Cyclamate}]_{\text{proposed}} = (0.32 \pm 1.7) + (0.993 \pm 0.019)[\text{Cyclamate}]_{\text{reference}}, \quad r = 0.999 \qquad (4)$$

4. Conclusions

The proposed procedure is a green alternative for cyclamate determination, avoiding the use of toxic reagents and minimizing the amounts of chemicals consumed and wastes generated. In spite of this, analytical features are comparable with those attained by other flow injection procedures for cyclamate determination.

On the other hand, this work presents a global performance better than other previous procedures for cyclamate determination (including the flow-based ones). In our opinion, this advantages over previous.

Application to other kind of samples is also possible because common additives did not interfere and ascorbic acid interference can be previously eliminated.

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4.3. DESARROLLO DE DISPOSITIVOS DE BAJO COSTE PARA MEDIDAS POR QUIMIOLUMINISCENCIA Y FOTOMETRÍA

«A portable and low cost equipment for flow injection chemiluminescence measurements»

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Short communication

A portable and low cost equipment for flow injection chemiluminescence measurements

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Abstract

A compact, reliable and low cost flow injection chemiluminescence system is described. The flow system consists of a set of solenoid micropumps that can dispense reproductive micro-volumes of solutions. The luminometer was based on a coiled cell constructed from polyethylene tubing that was sandwiched between two large area photodiodes. The whole equipment costs about US$ 750 and weights ca. 3 kg. Equipment performance was evaluated by measuring low concentrations of hydrogen peroxide by oxidation of luminol and for the determination of ammonium, based on its inhibition of the luminescence provided by the reaction of luminol and sodium hypochlorite. Linear responses were achieved within 1.0–80 μmol L^{-1} H_2O_2 and 0.6–60 μmol L^{-1} NH_4^+ with detection limits estimated as 400 nmol L^{-1} H_2O_2 and 60 nmol L^{-1} NH_4^+ at the 99.7% confidence level. Coefficients of variation were 1.0 and 1.8%, estimated for 20 μmol L^{-1} H_2O_2 and 15 μmol L^{-1} NH_4^+ ($n = 20$), respectively. Reagent consumption of 55 μg luminol, effluent volume of 950 μL per determination and sampling rate of 120 samples per hour were also achieved.

Keywords: Chemiluminescence; Flow injection; Multicommutation; Solenoid pumps; Luminol

1. Introduction

Analytical procedures based on chemiluminescence (CL) usually employ a spiral quartz flow cell placed as close as possible to the detector, which is in general a photomultiplier tube [1]. As the involved reactions are typically fast, precision and sensitivity are highly dependent on the ability of mixing the solutions and measuring the emitted radiation. Thus, CL procedures are often carried out in continuous flow systems, with solutions flowing at relatively high flow rates [1,2]. This design provides attractive characteristics such as high sensitivity, low detection limits and high sampling rates, but also some drawbacks like limited robustness, costs relatively high (instrumentation and reagent consumptions) and high generation of wastes. The usual flow cell geometry also limits the amount of radiation detected to lower than 50%. Some alternatives have been proposed in order to overcome these hindrances, such as the employment of photodiodes [3–6], specially designed cells [2,7,8] and immobilization of the luminogenic reagents [9].

Multicommutation is an alternative to increase versatility of flow systems, by employing discrete commuting devices for solution handling [10,11]. This process has also the advantage of minimizing both reagent consumption and production of wastes [12,13]. One recent proposal, also related to the employment of discrete devices, is the use of solenoid micropumps that can reproductively dispense micro-volumes of solutions [14,15]. In contrast to conventional flow injection systems, these devices can replace the injection and

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propulsion units, yielding compact manifolds that provide low reagent consumption and minimize the production of wastes. An additional advantage is the lower power requirement of the solenoid micro-pumps as compared with the conventional flow injection devices.

In this work, we describe a compact and low cost flow injection chemiluminescence system that involves solenoid micro-pumps for solution handling and a lab-made luminometer based on a simple coiled polyethylene cell sandwiched between two large area photodiodes. The analytical performance has been evaluated by means of measurements of hydrogen peroxide and ammonium employing luminol as luminogenic reagent.

2. Experimental

2.1. Apparatus

The flow system comprised four solenoid micro-pumps Bio-Chem. 090SP with a nominal volume of 8 μL per pulse (Boonton, USA), flow lines of 0.8 mm i.d. PTFE tubing and two confluence connectors. A Pentium 133 MHz microcomputer equipped with an electronic interface Advantech, PCL-711S was employed for system controlling and data acquisition by means of a software written in Microsoft Visual Basic. A lab-made electronic interface, analogous to the previously described [10], was used to generate the electric potential and current required to switch the solenoid pumps (12 V, ca. 100 mA).

The flow cell was made from polyethylene tubing (20 cm length, 0.8 mm i.d.) coiled around a transparent and rectangular acrylic piece (10 mm wide and 2.0 mm thickness). This configuration provided a mechanical support and two large observation surfaces with a cell with 100 μL internal volume. The cell was inserted between two 100 mm^2 photodiodes Hamamatsu, 12337-1010BR and directly connected to the flow system. The whole detection system was placed into a dark box to protect it from ambient light.

2.2. Reagents and solutions

All solutions were prepared with deionized water (18.2 $M\Omega\,cm^{-1}$) and analytical grade chemicals. Stock sodium hypochlorite solution was previously standardized by iodometric titration and hydrogen peroxide stock solution was valorated with potassium permanganate. Ammonium stock solution (0.1 mol L^{-1}) was prepared from ammonium chloride previously dried at 110 °C for 2 h. Ammonium and hydrogen peroxide work solutions were daily prepared by dilution of the stocks.

Luminol solution (4.5 mmol L^{-1}) was prepared by dissolving 5-amino-2,3-dihydro-1,4-phthalazinedione in 0.2 mol L^{-1} sodium carbonate with pH adjusted to 9.8 with hydrochloric acid. This solution was employed preferably after 48 h, according to literature recommendations [5,16].

For ammonium determination, a 1.0×10^{-2} mol L^{-1} NaClO solution was daily prepared. A 5.0×10^{-2} mol L^{-1} $K_3[Fe(CN)_6]$ solution was employed as catalyst in the luminol-hydrogen peroxide reaction.

2.3. Flow injection chemiluminescence system

The chemiluminescence detector comprised two photodiodes coupled to operational amplifiers. The output voltage of the two operational amplifiers showed a direct relation to the light intensity from the chemiluminescence cell. A third operational amplifier was assembled as a voltage summing, thus the output signal was directly proportional to the sum of the signals from both devices. A variable resistor was assembled to set up the reference voltage, thus permitting baseline adjustment that was carried out with the flow cell filled with carrier and photodiodes in the dark.

The solenoid micro-pumps were arranged as shown in Fig. 1, employing one device for each solution handled. The devices were operated at 2 Hz (840 μL min^{-1}). The polyethylene cell acted as both, reactor coil and detection cell. The confluence point *y* was placed as close as possible (ca. 5 mm) of the flow cell.

For hydrogen peroxide determination, the system was operated as described in Table 1. In the first step, hydrogen peroxide (S) and luminol solution (R_1) were simultaneously mixed, merged at confluence point *x* and inserted in reactor B. Then, the catalyst (R_2, hexacyanoferrate (III)) was added in the sample zone in the confluence point *y* and data acquisition was started (step 2). Finally, removal of the sample zone and washing was performed by using the carrier solution (step 3).

For ammonium determination, the system was analogously operated (see Table 1). Ammonium (S) and sodium hypochlorite (R_1) were simultaneously inserted in reactor B, merging at confluence point *x* (step 1) and, in the next step, luminol solution (R_2) was added to the sample zone in

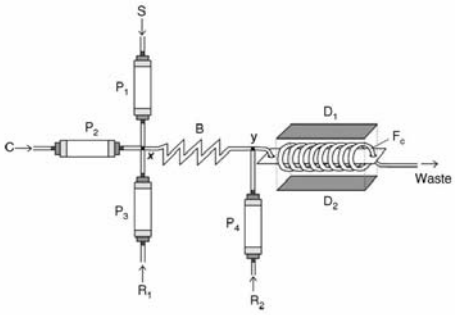


Fig. 1. Flow injection chemiluminescence system. P_i, solenoid micro-pumps; D_i, 100 mm^2 active area photodiodes; FC, 20 cm-coiled polyethylene flow cell; B, 25 cm mixing tube; *x*, *y*, confluence points; S, sample; R_i, reagents; C, carrier (water).

Table 1
Solenoid micro-pumps switching course for CL peroxide and ammonium determination[a]

Step	Description	P_1	P_2	P_3	P_4	Pulses H_2O_2[b]	Pulses NH_4^+[c]
1	Mixing of S and R_1	1/0	0	1/0	0	10	25
2	R_2 addition and reading	0	1/0	0	1/0	10	10
		0	1/0	0	0	–	
3	Sample zone removal and reading	0	1/0	0	0	150	150

[a] Numbers 1/0 indicate a pulse of the solenoid micro-pump.
[b] Hydride peroxide determination.
[c] Ammonium determination.

the confluence point *y* and data acquisition was immediately started. The binary sampling approach [10] was exploited to add luminol aliquots, in order to save the reagent and improving its distribution into the sample zone. As above described, transport of the sample zone and washing was performed by the carrier (step 3).

3. Results and discussion

3.1. General characteristics

Commercial solenoid micro-pumps can dispense volumes within 3 and 50 μL and can be operated in frequency of up to 5 Hz [15]. The employed devices have nominal volume of 8 ± 2 μL per pulse. It was verified that the mean volume was 7 μL for pumps P_1–P_3 and 6 μL for pump P_4 (see Fig. 1). The proposed flow system operates at the pulsed-flow mode that shows advantages of improving sample-reagent mixing [15,17]. This is an important characteristic, mainly for chemiluminogenic reactions usually characterized by fast and short-lived emissions [18].

Polyethylene is reasonably transparent to electromagnetic radiation emitted by luminol chemiluminescence (λ_{max} 420–440 nm) and can thus be employed to construct a robust and low cost cell for CL measurements. The flow cell geometry and the large area of the photodiodes increase the amount of radiation detected. In view of the low cost of this kind of detector, additionally more than one device can be employed for maximizing sensitivity. With the electronic circuit employed, baseline oscillation was estimated as 20 mV (0.4% of the full scale). Baseline drift was lower than 35 mV h^{-1}.

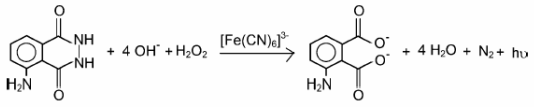
Excluding the microcomputer, the whole equipment weighs about 3 kg and costs about US$ 750 (US$ 650 for the flow system and US$ 100 for the lab made luminometer). The whole system can be conditioned in a box with 30 cm × 10 cm × 25 cm and a notebook can be employed for system controlling and data acquisition. A 12 V car battery can be employed as power supply to drive the micro-pumps. As the OP07 operational amplifier can work with voltages ranging from ±3 to ±18 V, the luminometer can be energized using two 9 V alkaline batteries configured to supply ±9 V.

To enhance the performance of the system, it was evaluated the effect of the flow cell coil length from 5 to 100 cm and that of the coil B from 5 to 100 cm, being selected coils of 20 and 25 cm, respectively, as the most appropriate to obtain a high sensitivity.

On the other hand, it was evaluated the best position of detection in front of the coiled polyethylene flow cell.

3.2. Hydrogen peroxide determination

The classical oxidation of luminol by hydrogen peroxide catalyzed by hexacyanoferrate(III) was initially exploited to evaluate analytical features of the proposed equipment. Reaction occurs slowly in the absence of the catalyst but become very fast after its addition (see reaction scheme (1)). The flow system was then operated for adding the catalyst at the confluence point *y* (see Fig. 1), placed as close as possible of the chemiluminescence cell.



(1)

To establish the best conditions for hydrogen peroxide determination, it was evaluated the effect of the luminol concentration from 0.75 to 9 mmol L^{-1}, being selected a 4.5 mmol L^{-1} value. It were studied the pH range (from 9.4 to 10.6), being chosen a pH of 9.8, and the number of pulses of the luminol solution (from 5 to 20), being selected 10 pulses. The number of pulses of the H_2O_2 solution was varied from 2 to 20, being selected 10 pulses.

Analytical features attained by the proposed equipment and those reported in previously published procedures for hydrogen peroxide determination by CL with photodiodes as detector are presented in Table 2. The detection limit was better than those previously reported and sampling rate is comparable or higher than those found in the literature. Low reagent consumption (55 μg per determination) and waste generation (900 μL per determination) were also observed. Linear response range was comparable to that found in previous

Table 2
Analytical features of flow procedures for hydrogen peroxide determination by luminol chemiluminescence and using photodiode as detector

Analytical characteristics	Proposed system	Preuschoff et al. [4]	Borges et al. [6]	Leite et al. [8]
Linear range (μmol L^{-1})	1.0–80	10–1000	2.5–500	2.5–315
Detection limit (nmol L^{-1})	400	1000	800	1000
Sampling rate (determination h^{-1})	120	65	150	70
CV (%), 20 μmol L^{-1} H_2O_2 (n=20)	1.0	2.5	0.9	1.2
Waste volume (mL/determination)	0.9	0.9	3.0	3.9
Luminol (μg/determination)	55	41	478	500

procedures, being described by the equation:

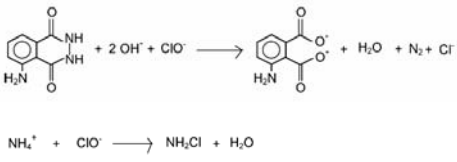
$$\text{CL intensity (mV)} = 155 + 35.5C_{H_2O_2}\ (\mu g\ L^{-1}),\quad r = 0.999 \qquad (2)$$

Analytical signals obtained for different hydrogen peroxide solutions are shown in Fig. 2a. In view of the transient characteristics of the flow system and the kinetics of the luminogenic reaction, good precision is only attained with highly reproductive solution volumes, mixing and timing. Coefficient of variation of 1.0% is then a clear indication that these conditions were attained.

Some analytical features compare unfavorably with those attained by Hayashi et al. [5]—detection limit of 3 nmol L^{-1}, linear response up to concentrations 3000-fold higher than LD and sampling rate of 180 determinations per hour. However, these authors employed a more expensive catalyst (peroxidase from *Artheromyces ramosus*) that according to their own descriptions intensify the emission of radiation in comparison with the previously reported ones.

3.3. Ammonium determination

The performance of the equipment was also evaluated with an indirect procedure based on the inhibition of luminol chemiluminescence. Hypochlorite reacts very fast with luminol even in the absence of catalysts. CL intensity reaches a maximum in about 800 ms [19]. Ammonia reacts with hypochlorite in alkaline media to form chloramines that do not react with luminol. Thus, ammonium can be indirectly determined by the consumption of hypochlorite [2,9,20] (see reaction scheme (3)). However, for obtaining reliable data, the reference signal related to the initial concentration of the oxidant need to be highly reproducible.



(3)

For ammonium ion determination, the conditions fixed for H_2O_2 determination were retained and, additionally, it was evaluated the effect of the ionic strength for 0.1–1.0 mol L^{-1} K_2CO_3 and the number of pulses of ammonium solution (from 10 to 40), being selected a K_2CO_3 concentration of 0.2 mol L^{-1} and 25 pulses.

As it can be seen in Table 3, analytical features for ammonium obtained with the developed device compare favorably with those attained by literature procedures, all those employing photomultiplier tubes as detector. The lowest detection limit and highest sampling rate was attained, with good precision and minimized generation of wastes. The reagent consumption was lower than that reported by Kraus and Crouch [20], but ca. two and four times higher than that attained by Li and Dasgupta [2] and Quin et al. [9], respectively. In these works, low reagent consumption was attained due to the low flow rate (100 μL min^{-1}) [2] or to the immobilization of the luminogenic reagent [9].

Sensitivity found for ammonium determination was very dependent on the hypochlorite concentration, and best results

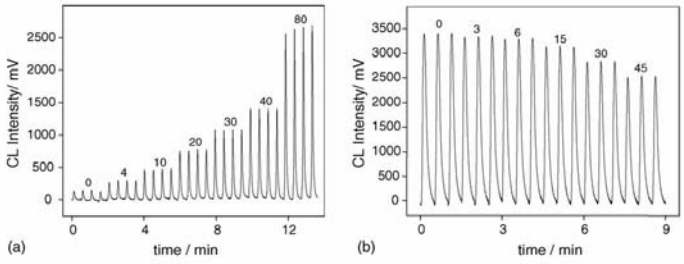


Fig. 2. Transient signals obtained for (a) hydrogen peroxide and (b) ammonium. Numbers indicate concentrations in μmol L^{-1}.

Table 3
Apparatus and analytical features of flow procedures for ammonium determination by chemiluminescence

Analytical characteristics	Proposed system	Li and Dasgupta [2]	Qin et al. [9]	Kraus and Crouch [20]
Detector	Photodiode	Photomultiplier	Photomultiplier	Photomultiplier
Flow cell	Coiled poliethylene tubing	Teflon AF-2400 liquid-core waveguide	Spiral quartz tubing	Coiled
Linear range (μmol L^{-1})	0.6–60.0	<120	1.0–100	250–1900
Detection limit (nmol L^{-1})	60	120	400	100000
Sampling rate (determination h^{-1})	120	42	60	30
CV (%), 15 μmol L^{-1} NH_4^+ (n=20)	1.9	–	<6.0	–
Waste volume (mL/determination)	0.95	0.9	17.5	11.4
Luminol (μg/determination)	55	25	12	2800

were observed for 1.0×10^{-2} mol L^{-1} NaClO. This concentration is considerably higher than the employed in previous works, because reagent dilution is implemented in situ in the proposed flow system. In view of the instability of very diluted hypochlorite solutions [16], previous works exploited the electrochemical generation of the reagent [2,9]. However, in the present work, satisfactory performance was observed by preparing the oxidant freshly for each working day.

Linear response was also comparable to the previous procedures that employ a photomultiplier tube as detector, showing that the limitation should be imposed by the hypochlorite concentration, magnitude and stability of the reference signal. By considering the analytical signal as the difference between the reference signal and that obtained in the presence of ammonium, the calibration curve can be described by the equation:

$$\text{CL intensity (mV)} = 91.9 + 28.3C_{NH_4^+}\ (\mu\text{g L}^{-1}),$$
$$r = 0.999 \quad (4)$$

Analytical signals obtained for ammonium solutions of different concentrations are shown in Fig. 2b, which also evidences the high repeatability and sampling throughput of the developed system.

4. Conclusions

The association of a flow system constructed with solenoid micro-pumps and a lab-made photodiode luminometer yields a compact equipment with profitable characteristics: portability (small size and weight), robustness (high precision in volumes of reagent dispensed), low consumption of reagent (discrete sampling of micro-volumes) and energy as well as minimized generation of effluents. These features make the equipment attractive for measurements out of laboratory, in which conventional flow systems and luminometers are difficult to use. The low cost of the photodiode make feasible the use of more than one detector to improve the amount of radiation detected. In addition, the proposed flow cell is simple, robust, easy to construct and as cheaper as possible.

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«Evaluation of a multicommuted flow system for photometric environmental measurements»

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Evaluation of a multicommuted flow system for photometric environmental measurements

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Abstract

A multipurpose, low cost, reliable and portable flow analysis instrument is described for in situ photometric measurements. This system is based on a set of inexpensive light emitting diodes (LEDs) and a photodiode detector, coupled to a versatile multi-pumping flow system. The whole equipment presents dimensions of 25 cm x 22 cm x 10 cm, weights ca. 3 kg and costs 650 €. System performance was evaluated for different applications and involved different chemistries without changing hardware configuration: (i) Fe^{3+} determination with SCN⁻, (ii) iodometric nitrite determination, (iii) determination of phenol with sodium nitroprusside and (iv) carbaryl determination with p-aminophenol. The calibration equations obtained for all applications were linear with regression coefficients of 0.999 or better. The detection limits were estimated as 22, 60, 25 and 60 ng mL^{-1} for iron, nitrite, phenol and carbaryl, at the 99.7 % confidence level with coefficients of variation of 2.3, 1.0, 1.8 and 0.8 %, respectively. Reagent consumptions and waste volumes were lower than those obtained by conventional spectrophotometric measurements and flow systems with continuous reagent addition. Sampling rates of 100, 110, 65 and 72 determinations per hour were achieved for iron, nitrite, phenol and carbaryl determinations, respectively. The proposed system is simple, compact and versatile. The combination of such profitable features with low reagent and sample consumption and the minimized production of undesirable wastes make feasible its application for in situ environmental studies.

Keywords: *flow analysis; led-based photometer; solenoid micro-pumps; multicommutation.*

1. Introduction

Nowadays, portable instruments are becoming very important in environmental analytical chemistry due to the increasingly interest for easy field deployment. In this sense, studies with LED-based photometers have been carried out because they provide the most suitable approach to achieve portable methodologies.

Light-emitting diodes (LEDs) developed since the 1960s, constitute a stable and sufficiently narrow monochromatic light source, with high intensity and available in a large variety of wavelengths in the visible and near UV spectrum with a long lifetime, providing simple and inexpensive devices for photometric measurements. In the last decades, LEDs [1] have been used mainly for absorbance [2-9] and fluorescence [10, 11] measurements.

LEDs have been also employed in polarimetry [12], liquid-chromatography and capillary electrophoresis [13].

In the analytical field, the majority of LED applications involve absorbance measurements in flow cells. Dasgupta group has been working in LED-based detectors as a good commercial alternative and had been observed that a homebuilt LED-based detector provides the same performance as a commercial adjustable wavelength detector [14]. LED-based detectors have been used in several illustrative experiments combining long path length absorption (LPLA), chemiluminescence (CL) and fluorescence detectors [15] using peristaltic pumps as a fluid propelling device. However, peristaltic pumps present characteristics as high cost, big size and high weight, which decrease the portability of the systems and raise the price of the equipments. In this sense, the replacement of the peristaltic pump units by smaller propulsion devices as solenoid micro-pumps (1.8 cm x 1.8 cm x 5.0 cm, 58 g weight) constitutes an alternative to reduce drastically the cost and the size of the systems, to increase versatility and portability for in *situ* studies.

Multi-pumping flow systems, based on the utilization of solenoid micro-pumps, allow the miniaturization of continuous flow methodologies. This attractive strategy presents also the ability to perform rapid and reproducible analyses using simple and robust instrumentation for minimizing both, reagent consumption and waste generation [16]. Moreover, solenoid micro-pumps can be individually controlled with low power requirements: the average power consumption used for four solenoid pumps is about 1/20 the power used by a peristaltic pump [17].

In the present work we describe a compact and low-cost equipment involving a LED-based photometer and a flow system based on a set of solenoid micro-pumps for samples and reagents handling. The system is applicable to a large variety of analytes permitting in-field measurements and real-time monitoring of the analyte by suitable selection of LEDs and involved chemistries. The analytical performance of the proposed system has been evaluated by monitoring iron, nitrite, phenol and carbaryl in water.

2. Experimental

2.1. Apparatus

The flow system comprised four solenoid micro-pumps Bio-Chem. 090SP (Boonton, USA), nominal volume of 8 ± 2 μL per pulse, flow lines of 0.8 mm i.d. PTFE tubing and one 5-channels confluence connector. A Pentium 133 MHz microcomputer equipped with an electronic interface card Advantech, PCL-711S was employed for system controlling and data acquisition by means a software written in Microsoft Visual Basic. A lab-made electronic interface based on the integrated circuit LM2803 was used to drive the solenoid pumps. This device was coupled to the digital output of the PCL711S interface card to allow the control of the solenoid pumps by the microcomputer. The pumps were coupled to the output lines (D_0, D_1, D_2, D_3) of the LM2803 device as indicated in Fig.1. The voltage to feed the solenoid pumps (12 V) was obtained from the microcomputer. The signal measurement was performed using a homemade photometer described in the next section.

A Hewlett-Packard Model 8452A diode array spectrophotometer (Waldbronn, Germany) furnished with a 50 μL flow cell with a 10 mm path length was used to compare the performance of the proposed equipment.

2.2. Reagents and Solutions

Stocks solutions were prepared using analytical grade chemicals and nanopure water (18.2 MΩ cm^{-1}). Working solutions were daily prepared.

Fe^{3+} and SCN^- solutions (100 μg mL^{-1}) were prepared by dissolving $FeCl_3$ and KSCN, from Scharlau (Barcelona, Spain), directly in water. Working solutions were prepared by dilution from 1.0 to 10.0 μg mL^{-1}.

Standard stock solution 100 μmol L^{-1} nitrite was prepared by dissolving sodium nitrite from Probus (Barcelona, Spain). No measurable concentration change was found in this solution when it was stored at room temperature (20 °C) protected against light. Working nitrite solutions with concentration ranging from 0.15 to 25.0 μg mL^{-1} nitrite were daily prepared by suitable dilution of the stock

solution with water. A 6 mmol L^{-1} iodide solution in a 0.1 mol L^{-1} perchloric acid medium was prepared by dissolving the appropriate amount of KI salt, both reagents obtained from Panreac (Barcelona, Spain).

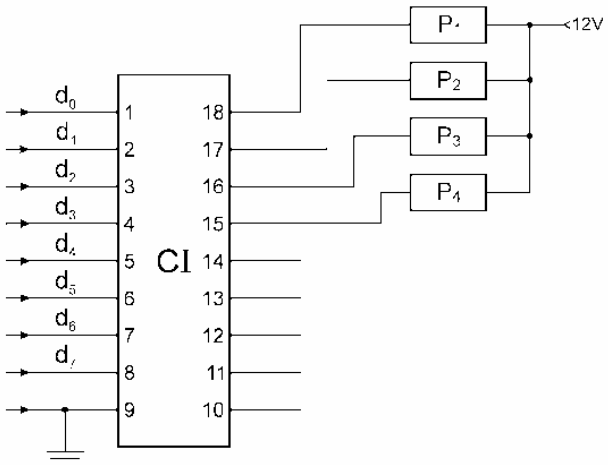

Figure 1. Electronic diagram of the interface to control the micro-pumps. CI = integrated circuit LM2803; d_0, d_1, …d_7 = input lines, P_1, P_2 , P_3 and P_4 = micro-pumps.

Phenol was obtained from Merck (Darmstadt, Germany). A stock 1000 μg mL^{-1} phenol solution was prepared by dissolving the reagent in water. The solution was stable for at least 30 days maintaining in refrigerator at + 4 °C. The working standard solutions ranging from 50 to 3500 μg mL^{-1} phenol were prepared by appropriate dilution of the stock with water before use. The sodium nitroprusside solution ($3.0x10^{-2}$ mol L^{-1}) from Merck (Darmstadt, Germany) and the hydroxylamine hydrochloride solution ($3.0x10^{-2}$ mol L^{-1}) from Panreac (Barcelona, Spain) were prepared by dissolving the reagents in water. A buffer solution 0.1 mol L^{-1} NaH_2PO_4 (pH =12) was prepared by adjusting the pH with a 6.0 mol L^{-1} NaOH solution, reagents provided by Panreac and Scharlau (Barcelona, Spain), respectively.

Carbaryl (purity 99.5 %) was obtained from Union Carbide. A 15 μg mL^{-1} carbaryl stock solution was prepared by dissolving the pesticide in water. This solution was very stable to light, heat and hydrolysis under laboratory conditions, thus no protection was required. A 50 μg mL^{-1} p-aminophenol working solution was freshly prepared by dissolving 0.025 g of PAP, purchased from Fluka (Buchs, Switzerland) in 500 mL of boiled and cooled water. This solution is stable for more than 8 hours. A 0.001 mol L^{-1} KIO_4 solution was prepared by dissolving the corresponding salt in water. A 1.0 mol L^{-1} NaOH solution was prepared by dissolving the appropriate mass of solid. Both reagents were obtained from Probus (Barcelona, Spain).

2.3.The photometer

The photodiode was the core of the photometer designed to use LEDs as radiation source and its electronic diagram is depicted in Fig.2. The photodiode and LED were coupled together to the flow cell in order to improve light measurements. Aiming the use of the equipment to monitor different analytes, each LED was fitted in a PVC block, which was machined to permit easy replacement.

Because LED emission intensity can vary from one component family to other, the electronic network (Fig.2), comprising the transistor (BC547) and the potentiometer (20 KΩ), was designed to permit the adjustment of the LED emission intensity. The photometer was built up by associating a set of LEDs (blue – 466 nm; green -566 nm; orange -590 nm; red - 660 nm) with a photodetector (RS 10530DAL). This latter consists of a silicon photodiode combined with a high-gain low-noise operational amplifier. The LED can be coupled before use according to the chemical specie to be determined. The photodetector (Det in Fig. 2) response is a function of the light intensity and the output signal (volt) could be read directly by microcomputer by coupling the device output to the analog input of the PCL711 interface card. The electronic network comprising the OP07 operational amplifier and other electronic components was assembled to permit signal conditioning and baseline adjustment, which was done through the variable resistor (20 kΩ), avoiding no inverting input of the operational amplifier.

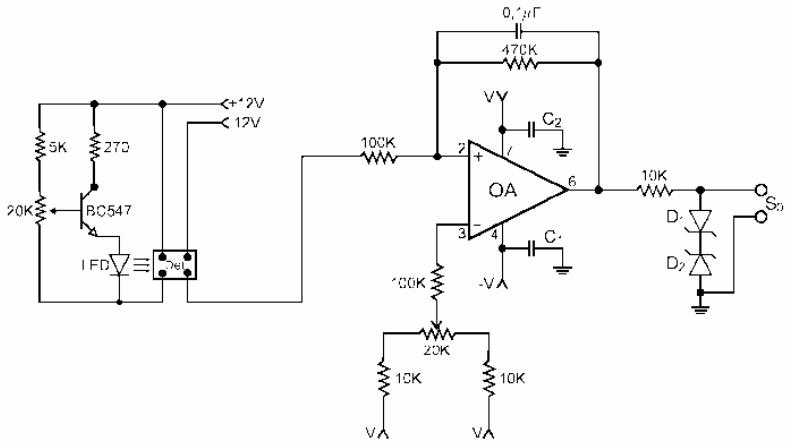

Figure 2. Diagram of the photometer. Det = Photodiode, RS 10530 DAL; OA = Operational amplifier, OP07; C_1 and C_2 = Tantalum capacitor, 1 µF; D_1 and D_2 = Zener diode, 4.5 V; and S_0 = output signal.

2.4. Flow system

The flow system manifold was designed employing four solenoid micro-pumps, which were assembled to allow the handling of four different solutions, as it can be shown in Fig. 3. The micro-pumps were switched ON/OFF by programming the microcomputer to send through the digital output of the PCL711 interface card a sequence of electric pulses. When the solenoid coil of the micro-pump was energized (ON) a sucking action was carried out, thus permitting the solution insertion into the micro-pump chamber through the input channel. When the applied voltage was turned OFF, the inner diaphragm go back to rest position and the fluid was dispensed through the micro-pump output channel. The micro-pumps employed in the proposed system delivered a constant volume of 7 µL per pulse, so that, up to a given flow rate, the effective control of the volume of sample and reagents solutions can be accomplished by settling the appropriate frequency to switch ON/OFF each micro-pump.

In this work, the switching frequency was settled at 2 Hz, thus the flow rate per channel could attaint 840 µL min^{-1}. The device data sheet pointed out that solution volume delivered per struck could be 8 ± 2 µL, nevertheless laboratory tests showed that the correct value was 7 µL.

The micro-pumps could be switched ON/OFF at the same time or sequentially one by one, or combining two or three at a time in order to perform the requirement of the analytical procedure. These operation modes could be

made by software, thus permitting to implement several applications without any reconfiguration of the manifold. The solutions merged into the reaction coil *B* through the joint device *x*, thus permitting that mixing and chemical reaction occurred while sample zone was displaced towards the detector. The analytical signal was read by the microcomputer through the analog input of the PCL711 interface card and stored as an CSCII file to permit further treatment. While measurements were performed a plot of the signal was displayed as a time function on the microcomputer screen to allow its visualization in real time.

The solenoid pumps switching courses settled for the four analytical procedures are summarized in Table 1. For iron (III) determination in the sampling step (step 1) micro-pumps P_1 and P_2 were switched ON at the same time, thus aliquots (7 μL) of sample (R_1) and reagent R_2 (KSCN) merged into the reaction coil (*B*). This sequence of event was named a sampling cycle and in this case it was repeated 10 times to insert into the coil (*B*) 140 μL of sample and reagent solutions. Afterwards, the data acquisition was carried out (step 2) while micro-pump P_3 was switched ON/OFF several times (150) to propel the carrier solution (R_3 = water) in order to displace the sample zone to the flow cell towards waste, carrying out the signal reading and the cleaning of the manifold.

As indicate in Table 1 for nitrite determination the sampling cycle comprised one pumping pulse of reagent solution R_1 (KI), two pumping pulses of sample (R_2) and one pumping pulse of $HClO_4$ solution (R_3) during step 1. This sampling cycle was repeated 20 times. In the step 2, data acquisition was carried out while sample zone was removed towards the detector. In this case, the $HClO_4$ solution (R_3) was used as carrier fluid. To carry out this step, the micro-pump P_3 was switched ON/OFF sequentially 150 times.

The procedures for both, phenol and carbaryl determination, required the use of four micro-pumps. In the step 1 (see Table 1) for phenol determination, the sampling cycle comprised four pumping pulses for sample (R_1), one for sodium nitroprusside (R_2), one for hydroxylamine hydrochloride (R_3) and two for buffered solution (R_4). The sampling cycle was repeated 8 times. Afterwards the sample zone was displaced towards the detector by switching the micro-pump P_4 ON/OFF 165 times.

For carbaryl determination the sampling cycle included three pumping pulses for sample (R_1), one for PAP solution (R_2), two for sample (R_1) and one for KIO_4 solution (R_3). The sampling cycle was repeated 8 times. In this case, a NaOH solution (R_4) was used as carrier and the micro-pump P_4 was switched ON/OFF 150 times to remove the sample zone towards the detector.

In all the cases, the data acquisition was performed while running the step 2 indicated in Table 1.

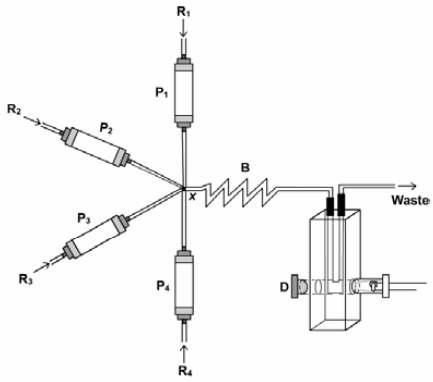

Figure 3. Flow diagram of the system. P_1, P_2, P_3 and P_4 = solenoid micro-pumps; R_1, R_2, R_3 and R_4 = sample and reagent solutions, (for details see text); *x* = joint device; B = reactor coil, 120 cm length and 0.8 mm i.d.; D = mortise to fit the photodiode.

3. Results and discussion

In the present work, the attention was focused to develop a portable and low cost equipment. Aiming to demonstrate its feasibility, procedures for determination of four chemical species of interest for water quality were selected as models. The selected methods presented light absorption bands at different wavelengths and these features were considered also as an opportunity to prove the setup functionality and performance, which are commented below.

3.1. Hardware features of the proposed setup

In the usual flow system the manifold should be designed to present small dimensions mainly those based on multicommutation approach, nevertheless the use of peristaltic pumps to propel solutions could be considered an impediment for the equipment downsizing. Nowadays, the availability of the solenoid micro-pumps could avoid this difficulty. The pumping devices were assembled to replace peristaltic pump and solenoid valves in order to obtain a portable and low cost equipment. In fact, the whole equipment employed through this study weights about 3 kg and it cost was about 650 €, including four solenoid micro-pumps (600 €) and electronic components (50 €).

The whole system was conditioned inside a metallic box (25 cm x 22 cm x 10 cm), thus a desirable portability feature was accomplished. For field measurements, a 12V car battery could be employed as power supply to drive the micro-pumps. The operational amplifier can work with voltage ranging from ± 3 V to ± 18 V, therefore the photometer can be energized using two low cost 9 V alkaline batteries configured to supply ± 9 V. The running of the system module can be controlled through the parallel port of the microcomputer, which is normally used to drive the printer. In this sense, for work outside laboratory a portable microcomputer could be used to control the flow system and to perform data acquisition. A digital voltmeter furnished with facility for serial communication, presenting resolution of 0.1mV and conversion rate of three measurements per second could be used for data acquisition. In this case, the cost is lower than that dispend to buy a PACL711 interface card.

3.2. Detection system

The compounds formed in the studied reactions show absorption maxima at 470, 546, 596 and 700 nm, respectively and to accomplish this requirement four LEDs (blue – 466 nm; green -566 nm; orange -590 nm and red - 660 nm) were employed as light source. The absorption spectra of the monitored compounds are shown in Fig.4. As it can be seen, a suitable matching between product absorption and LED emission spectra was achieved, thus indicating that it was possible to perform the measurements employing the corresponding radiation sources. In addition, the spectra bandwidths of the LEDs blue, green, orange and red were 30, 26, 33 and 29 nm, respectively, therefore they were suitable for the photometric measurements of the selected analytes.

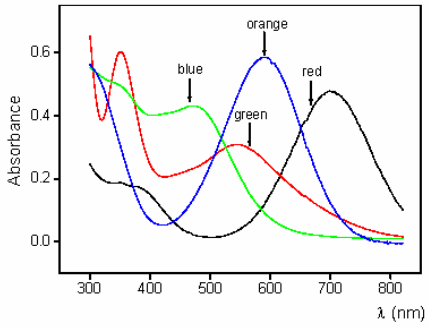

Fig. 4. Absorption spectra of the chemical products measured with indication of the LEDs emission for Fe^{3+} (blue LED) nitrite (green LED), carbaryl (orange LED) and phenol (red LED) determinations, with 470, 546, 596 and 700 nm of maximum absorbance, respectively. The arrows show the maximum emission point of the employed LEDS (466, 566, 590 and 660 nm, respectively).

In preliminary experiments, it was verified that the emission intensity of the LEDs affected both, sensitivity and dynamic range of the photometer. This drawback was overcome employing the electronic network (see Fig.1) comprised by the transistor and the variable resistor. Under these conditions, the LED emission intensity was easily adjusted controlling the electric current that was drained through the variable resistor and the base of the transistor. Prior to carry out this adjustment, the flow cell was filled with the carrier solution. The photometer must be switched ON at least 20 min before. Working continuously for four hours no significant baseline variation was observed. This feature was always observed, thus indicating that the long-term stability of the

photometer was very good. In this sense, we can affirm that the performance of the proposed photometer was suitable to carry out measurements for chemical determination.

3.3. Iron and nitrite determination

The procedure for iron determination was developed based on the classical reaction of Fe^{3+} with SCN^- to form a red complex that was detected using a blue LED with emission band around 470 nm. The procedure for nitrite employed the reaction with iodide in the presence of $HClO_4$ [18], generating a compound that was detected using a green LED (λ = 546). The results obtained for both analytes are showed in Table 2, where we can see that the analytical features were similar to those observed using a commercial spectrophotometer employing the same analytical procedure. Additionally, it was indicated the reagents consumption and waste generation additionally than the sample throughput obtained on using the micro-pump multicommuted system.

3.4. Phenol determination

The performance of the equipment was evaluated implementing a procedure for phenol determination. Aiming to assure a good evaluation the analyte was also determined employing measurements with diode-array spectrophotometer [19, 20] yielding the results shown in Table 3. As it can be seen, the sensitivity was a little but smaller than that obtained using diode array spectrophotometer and the limit of detection 2 times higher than that found with a conventional spectrophotometer. However, a sampling rate of 65 determinations per hour, low reagent consumption (56 µL sodium nitroprusside and 56 µL hydroxylamine hydrochloride per determination) and reduced waste generation (1.6 mL per determination) were obtained on using the micro-pumps. So, the overall analytical features of the proposed system attained the requisites expected for a portable analytical equipment.

3.5. Carbaryl determination

The procedure for carbaryl determination with PAP was evaluated based on a set of experiments designed to provide a complete comparative study with previous reported flow procedures based on conventional flow injection analysis (FIA), sequential injection analysis (SIA) and multicommutation with three-way solenoid valves [21] (see Table 4). As it can be seen, the slope of the calibration graph obtained with the proposed system was 1.9 or 1.5 times (for spectrophotometer or LED photometer, respectively) higher than that found using the FIA procedure. When it was used the SIA strategy the sensitivity was 3.2 times lower than that achieved using the proposed system. Using a flow manifold based on three-way solenoid valves, sensitivity was similar to that obtained using the proposed flow module. In this case, the structure of the flow system manifold was similar to that based on multicommutation, therefore this result prove that micro-pumps can be effectively used to replace peristaltic pumps and solenoid valves to implement reliable automatic flow procedure.

The limit of detection obtained by using solenoid micro-pumps was higher than those obtained by the others procedures. Nevertheless, the difference was not significant (2.3 times in the worst case), therefore indicating that multicommutation (using micro-pumps or three-way solenoid valves) is a convenient tool to implement automatic analytical procedures for carbaryl determination.

The sampling rates were 20 h^{-1} for SIA, 70 h^{-1} for multicommutation with three-way solenoid valves 72 h^{-1} for the flow system with micro-pumps and 90 h^{-1} for FIA, thus indicating that micro-pump can be considered as a reliable alternative to replace peristaltic pumps.The waste generation from the data in Table 4 considering the sampling rates are 10.7, 1.4, 1.7 and 1.4 mL per determination for FIA, SIA, multicommutation using solenoid valves and multicommutation using solenoid micro-pumps, respectively. So, the procedure implemented using the proposed equipment reduced the waste generation at the same order than that obtained with SIA, nevertheless it provided better analytical performance. The low waste generation could be a

parameter to define the usefulness of the analytical procedure and it was favourable to the proposed system. Analysing the data concerning to reagents consumption it can been observed that this parameter compares favourably to the procedure based on multicommutation.

The results obtained for the four analytes prove that the solenoid micro-pumps are an effective alternative for solution propelling in flow analysis system. Furthermore, each micro-pump can be driven as an independent commutation device, thus replacing the three-way solenoid valves used in flow system based on multicommutation [21]. This double function was efficiently exploited in this work to obtain downsized equipment, which associated with the LED-based photometer, offers a cheapest alternative for the development of portable automated devices.

4. Conclusions

The characteristics of solenoid micro-pumps associated to a lab-made LED-based photometer afford a very attractive strategy for the implementation of simple and efficient automated analytical procedures.

The equipment designed combine robustness, small size and weight and low energy consumption, which comprise the set of features desirable for portable equipment. Additionally, it is simple, fast, precise, provides low reagent consumption, low waste generation and minor operator intervention, without reducing the analytical performance of methods based on the use of conventional spectrophotometers. These characteristics suggest that this portable setup could be advantageously used for fieldwork.

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Table 1.
Micro-pumps switching course for iron(III), nitrite, phenol and carbaryl determination

Step	*Description*	P_1	P_2	P_3	P_4	*Pulses* [a]	*Sampling cycles*
1	Insertion of sample and reagent solutions for **iron** (III) determination	ON/OFF	ON/OFF	OFF	OFF	10	1
2	Sample zone displacing towards waste (reading and cleaning)	OFF	OFF	ON/OFF	OFF	150	1
1	Insertion of sample and reagent solutions for **nitrite** determination	ON/OFF	OFF	OFF	OFF	1	20[b]
		OFF	ON/OFF	OFF	OFF	2	
		OFF	OFF	ON/OFF	OFF	1	
2	Sample zone displacing towards waste (reading and cleaning)	OFF	OFF	ON/OFF	OFF	150	1
1	Insertion of sample and reagent solutions for **phenol** determination	ON/OFF	OFF	OFF	OFF	4	8[c]
		OFF	ON/OFF	OFF	OFF	1	
		OFF	OFF	ON/OFF	OFF	1	
		OFF	OFF	OFF	ON/OFF	2	
2	Sample zone displacing towards waste (reading and cleaning)	OFF	OFF	OFF	ON/OFF	165	1
1	Insertion of sample and reagent solutions for **carbaryl** determination	ON/OFF	OFF	OFF	OFF	3	8[d]
		OFF	ON/OFF	OFF	OFF	1	
		ON/OFF	OFF	OFF	OFF	2	
		OFF	OFF	ON/OFF	OFF	1	
2	Sample zone displacing towards waste (reading and cleaning)	OFF	OFF	OFF	ON/OFF	150	1

[a] Pulses: the digits indicate the number of times that the corresponding micro-pump was switched ON/OFF to perform each sampling cycle.
[b] Sequence 1:2:1 is repeated 20 cycles
[c] Sequence 4:1:1:2 is repeated 8 cycles
[d] Sequence 3:1:2:1 is repeated 8 cycles

Table 2.

Analytical performance of the systems employed for the determination of iron (III) and nitrite in water

Analyte	*Detection*	*Calibration equation* [a]	*r (n)* [b]	*LOD* [c] *(ng mL^{-1})*	*CV* [d] *(%)*	*Total waste (mL)* [e]	*Sample throughput (h^{-1})*	*Reagent consumption (mL/determination)* [f]	
								R1	R2
Iron	Spectrophotometer	A = (-0.047 ± 0.007)+ + (0.105 ± 0.001) C	0.9998 (6)	25	1.7				
	LED photometer	A = (-0.00 ± 0.01) + + (0.126 ± 0.003) C	0.999 (6)	22	2.3	1.2	100	0.07	0.07
Nitrite	Spectrophotometer	A = (-0.06 ± 0.02) + + (0.058 ± 0.003) C	0.997 (5)	60	0.9				
	LED photometer	A = (-0.04 ± 0.03) + + (0.06 ± 0.01) C	0.9990 (5)	60	1.0	1.7	110	0.14	0.28

[a] A = absorbance, C = concentration in μg mL^{-1}, values are mean ± SD for 3 measurements at each point.

[b] Regression coefficient, number of standards in parenthesis.

[c] Limit of detection (3σ).

[d] Coefficient of variation for 10 independent analysis of a sample containing 5.0 μg mL^{-1}.

[e] Total waste (mL/determination).

[f] Reagent consumption (mL/determination)

R_1 = KI; R_2 = $NaNO_2$ (nitrite determination).

R_1 = $FeCl_3$; R_2 = NH_4SCN (iron determination).

Table 3.

Analytical performance of the system employed for the determination of phenol in water

Strategy	*Calibration equation* [a]	*r (n)* [b]	*LOD* [c] *(ng mL^{-1})*	*CV (%)* [d] *(n; C)*	*Total waste (ml)* [e]	*Sample throughput (h^{-1})*	*Reagent consumption (mL/determination)* [f]	
							R1	*R2*
Spectrophotometer [19]	A = (0.028 ± 0.002) + + (0.217 ± 0.001) C	0.99997 (5)	13	0.5 (10; 2.5)				
LED Photometer	A = (0.020 ± 0.004) + + (0.199 ± 0.003) C	0.9998 (8)	25	1.8 (10; 3.5)	1.6	65	0.056	0.056

[a] A = absorbance, C = concentration in μg mL^{-1}, values are mean ± SD for 3 measurements at each point.

[b] Regression coefficient, number of standards indicated in parenthesis.

[c] Limit of detection (3σ).

[d] Coefficient of variation for *n* independent analysis of a sample containing C μg mL^{-1}.

[e] Total waste (mL/determination).

[f] R_1 = sodium nitroprusside; R_2 =hydroxylamine hydrochloride.

Table 4.

Analytical performance of different automated strategies for carbaryl determination with PAP using a spectrophotometer and a LED-based photometer

Strategy	*Detector*	*Calibration equation* [a]	*r* [b]	*LOD* [c] *(ng mL^{-1})*	*CV (%)* [d] *(n; C)*	*Sampling (h^{-1})*	*Total waste (mL h^{-1})*	*Reagent consumption (g/1000 determinations)*		
								NaOH	*KIO_4*	*PAP*
FIA [21]	Spectroph.	A=(0.0000 ± 0.0002)+ + (0.03015 ± 0.00004) C	0.9999 (6)	26	0.14 (4;4.8)	90	960	216	2.48	0.135
SIA [21]	Spectroph.	A=(0.019 ± 0.003)+ + (0.014 ± 0.003) C	0.9990 (5)	40	1 - 3 (3;10.0)	20	27	1.7	0.193	0.011
Solenoid valves multicommutation [21]	Spectroph.	A=(0.021 ± 0.003)+ + (0.047 ± 0.002) C	0.99998 (6)	26	0.5 (8;5.8)	70	120	2	0.092	0.005
Micro-pumps multicommutation	Spectroph.	A=(0.054 ± 0.003)+ + (0.0586 ± 0.0008) C	0.996 (7)	51	0.76 (10;6.0)	72	104	4.5	0.013	0.0028
Micro-pumps multicommutation Developed system	LED-based photometer	A=(0.021 ± 0.003)+ + (0.0445 ± 0.0008) C	0.9993 (7)	60	0.8 (10;6.0)	72	104	4.5	0.013	0.0028

[a] A = absorbance, C = concentration in μg mL^{-1}, values are mean ± SD for 3 measurements at each point.

[b] Regression coefficient, number of values in parenthesis.

[c] Limit of detection (3σ).

[d] Coefficient of variation for *n* independent analysis of a sample containing C μg mL^{-1} carbaryl.

5. CONCLUSIONES

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1. Se ha puesto a punto una nueva estrategia totalmente mecanizada para la determinación de Hg inorgánico, empleando la multiconmutación en CV-AFS, técnica para la que no había antecedentes en la literatura. El sistema desarrollado permite una sencilla mecanización en la etapa de introducción de muestra y de medida, la simplificación del equipo instrumental, la recirculación de muestras y patrones que permite la reducción drástica de su consumo en un factor de 6 y de la generación de residuos en un factor de 2 y ofrece, a su vez, una vía sensible y exacta para la determinación de este elemento.

   La reducción del volumen del separador gas-líquido incrementa la sensibilidad y reduce el tiempo de análisis.

2. Se ha aplicado el procedimiento desarrollado para la determinación de Hg por CV-AFS en aguas sin ser necesario un tratamiento previo de la muestra.

   El procedimiento propuesto se ha comparado con las medidas llevadas a cabo convencionalmente por AFS en modo continuo y se ha comprobado que ambos procedimientos son estadísticamente comparables entre sí y con los datos procedentes de un laboratorio externo.

3. Se ha acoplado la multiconmutación a HG-AFS y se ha desarrollado un método altamente sensible para la determinación de Bi y Te en productos lácteos, basado en la sonicación, durante 10 minutos y a temperatura ambiente, con agua regia y antiespumante, y se ha confirmado la validez de las características analíticas de este procedimiento para el análisis de productos lácteos, por comparación con tratamientos de digestión asistida por microondas.

   Nuevamente, la multiconmutación proporciona una vía rápida para el análisis de control, incrementando la frecuencia de muestreo de 31 h^{-1} y 20

h^{-1} (método en continuo) a 72 h^{-1} y 85 h^{-1}, para la determinación de Bi y Te, respectivamente. Adicionalmente, reduce la generación de residuos en un factor de 2.6 (Bi) y 4.0 (Te) y los consumos de muestra, reductor y portador los disminuye 9.6, 4.5 y 13.3 veces, en el caso del Bi y 5.3, 4.0 y 8.0 en la determinación del Te.

4. La multiconmutación ofrece la posibilidad de minimizar los problemas relativos a la contaminación medioambiental derivada de los residuos ácidos e iones metálicos generados tras los análisis por HG-AFS y CV-AFS, incorporando a los métodos desarrollados para la determinación de metales un tratamiento de neutralización en línea.

5. Se ha desarrollado un procedimiento mecanizado, rápido y altamente sensible para llevar a cabo la especiación de Te (IV) y Te (VI) en leche por HG-AFS, basado en la extracción asistida por ultrasonidos y el análisis de las suspensiones directamente y tras la reducción con KBr. De esta forma, se determina primero el Te(IV) libre y, a continuación, el Te total, en menos de 2 min, obteniéndose el Te (VI) por diferencia.
   La ventaja que ofrece la multiconmutación en esta metodología, paralelamente a una elevada productividad y a la reducción de 2 y 4 veces los residuos generados y el consumo de reactivos en comparación con el método manual, es una mínima atención por parte del operador y un mínimo tratamiento de la muestra.

6. Se ha desarrollado un procedimiento totalmente mecanizado por multiconmutación para la determinación espectrofotométrica de tensioactivos aniónicos en aguas, por extracción con cloroformo en línea.
   El método de referencia, llevado a cabo manualmente, es tedioso, utiliza gran cantidad de material de laboratorio y consume elevados volúmenes de cloroformo (45 mL por determinación), lo que lo hace caro, lento e incómodo para el operador. La alternativa basada en la multiconmutación

permite el incremento de la productividad, el aumento de la seguridad y comodidad del analista y la reducción de la producción de residuos en un factor de 35, sin sacrificar las características analíticas. Además, reduce el volumen de muestra a sólo 3.6 mL por determinación.

7. Se ha desarrollado una metodología analítica basada en la multiconmutación para la determinación de fenoles en muestras de agua mediante espectrofotometría molecular, utilizando como dispositivo propulsor de las disoluciones minibombas solenoides.
   El método propuesto ofrece características favorables en términos de precisión, frecuencia de muestreo, consumos de reactivos y generación de residuos, en comparación con el método manual y otros trabajos propuestos en la literatura. Además, este sistema de flujo evita el uso de disolventes orgánicos, altamente tóxicos y contaminantes.
   El uso de las minibombas facilita la miniaturización del sistema, reduce su coste y posibilita los estudios de campo.

8. Se ha desarrollado un nuevo método limpio, utilizando la multiconmutación, para la determinación de ciclamato en muestras de edulcorantes comerciales y se han reemplazado los reactivos tóxicos usados en la bibliografía. Se emplean minibombas solenoides para minimizar el consumo de reactivos y la generación de residuos y dotar al sistema de economía y portabilidad.

9. Se ha diseñado y validado un sistema de bajo coste por quimioluminiscencia en inyección en flujo, consistente en un conjunto de minibombas solenoides para la inserción de los reactivos y en un luminómetro construido en el laboratorio, formado por un simple tubo de polietileno enrollado entre dos fotodiodos. Las características analíticas del sistema se han evaluado en las determinaciones de H_2O_2 por oxidación del

luminol y de NH_4^+, basada en la inhibición de la luminiscencia proporcionada por la reacción entre el luminol y el hipoclorito sódico.

10. Se ha diseñado y desarrollado un equipo de análisis en flujo de bajo coste para medidas fotométricas *in situ*, basado en la combinación de un conjunto de económicos LEDs, un detector de fotodiodo y un sistema de flujo multipulsado. El sistema se ha evaluado para diferentes aplicaciones: (i) determinación de Fe (III) con tiocianato; (ii) determinación yodométrica de nitrito; (iii) determinación de fenol con nitroprusiato sódico y (iv) determinación de carbaril con p-aminofenol. Se han obtenido características analíticas comparables a las de los métodos presentados en la bibliografía y la reducción de consumos y residuos generados.

De forma general, de todos estos trabajos desarrollados en multiconmutación, se puede afirmar que:

- La multiconmutación proporciona un incremento considerable en la productividad del laboratorio, disminuye el tiempo empleado en los análisis y abarata costes.

- La multiconmutación utiliza racionalmente reactivos y muestras, lo que conduce a una reducción tanto de los residuos generados como de las muestras y reactivos empleados, ya que únicamente se insertan los volúmenes requeridos para el análisis y no es necesario que las disoluciones fluyan continuamente como en el caso FIA. Esto se traduce en mejores condiciones de seguridad e higiene así como en una reducción de los costes, tanto directos como de gestión y tratamiento de los residuos. Adicionalmente, se reduce el impacto negativo generado en el medioambiente gracias a esta reducción en la generación de residuos y a la sustitución de disolventes y reactivos tóxicos por otros de menor impacto en los casos en que esto sea posible. Además, se reducen al

máximo los riesgos para el operador que puedan derivarse de la manipulación de reactivos tóxicos.

- La multiconmutación proporciona versatilidad, flexibilidad, economía, robustez y miniaturización a los sistemas. Además es fácilmente automatizable en cada una de las etapas del análisis, característica que se aprovecha para diseñar equipos portátiles para análisis *in situ*, como por ejemplo en hospitales, monitoreo de aguas, análisis medioambientales, control de procesos industriales, etc.

- La multiconmutación genera sistemas inteligentes y polivalentes: tratamiento de la muestra en línea, tratamiento de los residuos generados, extracción con disolventes, incremento de la sensibilidad mediante flujo parado, etc.

- La validación llevada a cabo para cada una de las metodologías propuestas ha puesto de manifiesto su adecuada exactitud, de modo que en todos los casos los resultados obtenidos empleando la multiconmutación han sido estadísticamente comparables a los encontrados por los métodos de referencia utilizados.

- La precisión alcanzada por la multiconmutación para la determinación de los analitos propuestos en los trabajos estudiados es adecuada y comparable con la consultada en los métodos bibliográficos.

En conclusión, y tal como se había planteado al definir los objetivos iniciales, esta Tesis Doctoral ha aportado soluciones sencillas a diversas aplicaciones de la multiconmutación en la Química Analítica. Y puede afirmarse que la multiconmutación constituye una metodología medioambientalmente sostenible para el futuro, complementando y mejorando al clásico FIA. En

palabras del grupo de Piracicaba (Brasil), padres de la técnica: "*A tendency towards improvement in versatility, simplicity and ruggedness has been verified during the development of flow analysis, and multicommutation is a key feature in this context (…) matching the present tendency towards Green Chemistry*" [Rocha: 2002].

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ANEXO

ANEXO

Ejemplo de programa Basic (implementado utilizando el compilador Visual Basic 6.0 de Microsoft) para la determinación de tensioactivos aniónicos en aguas por espectrofotometría molecular

```
Declare Sub vbOut Lib "WIN95IO.DLL" (ByVal nPort As Integer, ByVal ndata As Integer)
Declare Sub Sleep Lib "kernel32" (ByVal dwMilliseconds As Long)
Global parar As Integer

Private Sub accionar_muestreo_Click()
parar = False
For i = 1 To rep_prog.Text
   If parar = True Then  'Sale de accionar muestreo...
      Exit Sub
   End If
   DoEvents
   rep_ejec.Text = i
   DoEvents

   For j = 1 To sds_prog.Text
      DoEvents
      If parar = True Then
         Exit Sub
      End If
      sds_ejec.Text = j
      DoEvents
      'permite a windows realizar otras actividades como refrescar pantalla
      vbOut &H378, 1 'v1 en decimal
      Sleep tiempo1_1.Text * 1000  'ya que sleep funciona con milisegundos...
      tej1_1.Text = (sds_ejec.Text) * (tiempo1_1.Text)
      vbOut &H378, 2 'v2 en decimal
      Sleep tiempo1_2.Text * 1000  'ya que sleep funciona con milisegundos...
      tej1_2.Text = (sds_ejec.Text) * (tiempo1_2.Text)
    Next j
```

```
 For ta = 1 To tiempo_2.Text
    If parar = True Then
          Exit Sub
    End If
    tej_2.Text = ta
    DoEvents
    vbOut &H378, 32 'v6 en decimal
    Sleep 1000  'ya que sleep funciona con milisegundos...
 Next ta


For tb = 1 To clor_prog.Text
       If parar = True Then
          Exit Sub
       End If
       clor_ejec.Text = tb
       DoEvents  'permite a windows realizar otras actividades como refrescar
        pantalla
       vbOut &H378, 4 + 32 'v3 + v6 en decimal
       Sleep tiempo_3.Text * 1000  'ya que sleep funciona con milisegundos...
       tej_3.Text = (clor_ejec.Text) * (tiempo_3.Text)
Next tb


For tc = 1 To tiempo_4.Text
       DoEvents
       If parar = True Then
          Exit Sub
       End If
       tej_4.Text = tc
       vbOut &H378, 32 'v6 en decimal
       Sleep 1000  'milisegundos
Next tc
```

```
For td = 1 To tiempo_5.Text
      DoEvents
      If parar = True Then
         Exit Sub
      End If
      tej_5.Text = td
      vbOut &H378, 0 'todas las válvulas en OFF para la separación de fases
      Sleep 1000  'milisegundos
Next td


For te = 1 To tiempo_6.Text
      DoEvents
      If parar = True Then
         Exit Sub
      End If
      tej_6.Text = te
      vbOut &H378, 16 'v5
      Sleep 1000  'milisegundos
Next te


For tf = 1 To tiempo_7.Text
      DoEvents
      If parar = True Then
         Exit Sub
      End If
      tej_7.Text = tf
      vbOut &H378, 8 'v4
      Sleep 1000  'milisegundos
Next tf


vbOut &H378, 0  'todas las válvulas en OFF
Beep
Sleep 1000
Beep
MsgBox "Presione aceptar una vez completada la lectura de la señal"
Beep
```

```
   For tg = 1 To tiempo_9.Text
         DoEvents
         If parar = True Then
            Exit Sub
         End If
         tej_9.Text = tg
         vbOut &H378, 16 'v5
         Sleep 1000  'milisegundos
   Next tg


   Beep
   Sleep 1000
   Beep
   MsgBox "Presione aceptar tras el total vaciado de la cámara de separación"
   Beep


   For th = 1 To tiempo_10.Text
         DoEvents
         If parar = True Then
            Exit Sub
         End If
         tej_10.Text = th
         vbOut &H378, 8 + 16 'v4 + v5
         Sleep 1000  'milisegundos
   Next th


Beep
Next i
End Sub
```

```
Private Sub Guardar_Click()
Open "c:/variables_sdsmb.txt" For Output As #1
Write #1, rep_prog.Text
Write #1, sds_prog.Text
Write #1, clor_prog.Text
Write #1, tiempo1_1.Text
Write #1, tiempo1_2.Text
Write #1, tiempo_2.Text
Write #1, tiempo_3.Text
Write #1, tiempo_4.Text
Write #1, tiempo_5.Text
Write #1, tiempo_6.Text
Write #1, tiempo_7.Text
Write #1, tiempo_9.Text
Write #1, tiempo_10.Text
Write #1, long_onda.Text
Write #1, lb_prog.Text
Write #1, lava_prog.Text
Write #1, lava_rep.Text
Close #1
End Sub


Private Sub lava_accionar_Click()
For j = 1 To lava_rep.Text
   For i = 1 To lava_prog.Text
      lava_ejec.Text = i
      DoEvents
      If lava_v1.Value = 1 Then
         vbOut &H378, 1 'v1
      End If
      If lava_v2.Value = 1 Then
         vbOut &H378, 2 'v2
      End If
      If lava_v3.Value = 1 Then
         vbOut &H378, 4 'b3
      End If
      If lava_v4.Value = 1 Then
         vbOut &H378, 8 'v4
      End If
      If lava_v5.Value = 1 Then
```

```
        vbOut &H378, 16 'v5
      End If
      If lava_v6.Value = 1 Then
        vbOut &H378, 32 'v6
      End If
      If lava_v7.Value = 1 Then
        vbOut &H378, 64 'v7
      End If
      If lava_v8.Value = 1 Then
        vbOut &H378, 128 'v8
      End If
      If lava_todas.Value = 1 Then
        vbOut &H378, 1 + 2 + 4 + 8 + 16 + 32 + 64 + 128
      End If
      Sleep 100
      vbOut &H378, 0
      Sleep 100
      Next i
Beep
Next j
End Sub


Private Sub lb_accionar_Click()
For ta = 1 To lb_prog.Text
  lb_ejec.Text = ta
  DoEvents
  vbOut &H378, 4 'Válvula V3
  Sleep 1000  'en milisegundos (1 seg)
Next
For tb = 1 To 10
  lb_desplaz.Text = tb
  DoEvents
  vbOut &H378, 8 'Válvula V4
  Sleep 1000  'en milisegundos (1 seg)
Next
vbOut &H378, 0
Beep
Sleep 1000
```

```
Beep
MsgBox "Presione aceptar cuando haya realizado la lectura de la línea base"
Beep
For tc = 1 To 20
   lb_vaciocelda.Text = tc
   DoEvents
   vbOut &H378, 8 'Válvula V4
   Sleep 1000  'en milisegundos (1 seg)
Next
vbOut &H378, 0
End Sub


Private Sub Leer_Click()
Dim aux As String
Open "c:/variables_sdsmb.txt" For Input As #1
Input #1, aux
rep_prog.Text = aux
Input #1, aux
sds_prog.Text = aux
Input #1, aux
clor_prog.Text = aux
Input #1, aux
tiempo1_1.Text = aux
Input #1, aux
tiempo1_2.Text = aux
Input #1, aux
tiempo_2.Text = aux
Input #1, aux
tiempo_3.Text = aux
Input #1, aux
tiempo_4.Text = aux
Input #1, aux
tiempo_5.Text = aux
Input #1, aux
tiempo_6.Text = aux
Input #1, aux
tiempo_7.Text = aux
Input #1, aux
tiempo_9.Text = aux
Input #1, aux
```

```
tiempo_10.Text = aux
Input #1, aux
long_onda.Text = aux
Input #1, aux
lb_prog.Text = aux
Input #1, aux
lava_prog.Text = aux
Input #1, aux
lava_rep.Text = aux
Close #1
End Sub


Private Sub parar_muestreo_Click()
parar = True
End Sub
```

Ventana de interacción con el usuario