Smart bio-metal-organic frameworks for selective molecular recognition of hydrophilic vitamins

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Abstract

In this work, a bio metal-organic framework (bio-MOF) derived from the amino acid L-serine has been prepared in bulk form and the resulting material was evaluated as selective sorbent for the molecular recognition and extraction of B-vitamins. The functional pores of bio-MOF, with formula $\{Cu^{II}_6Ca^{II}$ [(S,S)-serimox]$_3(OH)_2(H_2O)\}39H_2O$ (Cu-Serimox-Ca), exhibit high amounts of hydroxyl groups jointly other directing supramolecular host-guest interactions allowed a selective recognition of B-vitamins by bio-MOF. Single-crystal X-ray diffraction studies reveal the specific B-vitamin binding sites and the existence of multiple hydrogen bonds between these target molecules and the framework. It offered unique snapshots to accomplish a highly efficient capture of these solutes in complex aqueous matrices. Indeed, this material demonstrated a high potential as solid-phase extraction sorbent with remarkable reproducibility and low detection limits (between 0.4 to 1.4 ng mL^{-1}). The application of bio-MOF was successfully evaluated by preconcentrating these hydrophilic vitamins in fruit juices and energy drinks.

Keywords: bio-MOF, host-guest interaction, solid-phase extraction, B-vitamins

1. Introduction

Metal-organic frameworks (MOFs) are crystalline materials, which are made up of coordination bonds between multidentate organic linkers and transition-metal cations into periodic structures [1, 2]. These tailored porous materials have developed quickly in the last twenty years and have shown promising features (*e.g.* easy preparation, high porosity and surface areas, large mechanical and good thermal stability, etc.) for several applications, such as gas storage, separation and catalysis [3, 4]. Also, these materials have been applied in chromatographic area and sample preparation [5, 6]. Despite the remarkable advances in MOFs as stationary phases; however, most of them have addressed to solid-phase microextraction (SPME) purposes [7]. Besides, in most cases, the selectivity of separation has been attributed to the hydrophobic and π-π interactions of solutes with the functional (commonly aromatic) ligands instead of taking advantages of their tunable pore size and molecular recognition capabilities.

In the last decade, a new subclass of MOFs combining supramolecular chemistry and bioscience has arisen, the so-called biological metal-organic frameworks (bio-MOFs) [8]. These materials have attracted wide attention due to of their particular structures, rich supramolecular chemistry and unique biomimetic properties. These porous materials constructed from biomolecules (amino acids, nucleobases, etc.) show the advantages of environmentally friendly designs, low toxicity, biocompatibility and intrinsic capabilities to drive molecular recognition processes [9]. These features can be tailored due to the presence of several accessible metal binding sites and the ability to self-organize into highly ordered structures through different interactions (hydrogen bonding, π-stacking or coordination-driven self-assembly) [10]. Besides, the size and shape of the bio-MOF pore can be match with the guest, thus resulting its immobilization within the confined space of the host framework. Indeed, these bio-materials have valuable applications in

biological motors [11], drug delivery [12], electrochemical sensors [13], biomimetic catalysis [14], and environmental protection materials [15]. However, bio-MOFs have been scarcely explored in analytical field as chromatographic supports. Thus, Zhang and co-workers [16] synthesized several homochiral MOFs from different amino acids ligands (L-tyrosine, L-histidine, L-tryptophan and L-glutamic acid) and metal ions (Zn^{2+} or Co^{2+}) and applied these materials to the HPLC separation of several types of enantiomers such as amines, organic acids, alcohols, etc. In another work, Navarro-Sánchez *et al.*[17] reported a chiral Cu(II) MOF based on the tripeptide Gly-L-His-Gly for enantioseparation of ephedrine and metamphetamine as solid-phase extraction (SPE) sorbent. The sorbent was able of separating >50% of (+)-ephedrine enantiomer as from a racemic mixture in only 4 min. Recently, Mon and co-workers [10, 18] have developed bio-MOFs based on enantiopure disubstituted oxamidato- and oxamato-ligands derived from natural aminoacids, specifically L-methionine (showing the material functional channels with thio-alkyl chains), as promising materials for a selective capture of metallic traces (Au and Hg) in electronic wastes. The high stability demonstrated by these bio-MOFs under environmental conditions, jointly with its easy preparation and tailored pore sizes and channels make these materials attractive SPE sorbents with enhanced molecular recognition capabilities.

It is well-known that vitamins are a group of indispensable compounds for the development and normal growth of the human body and, therefore, they are regularly added to foods and beverages. In particular, water-soluble vitamins belong to the B-group play specific and vital functions in metabolism, and their lack or excess can cause deficit disorder and diseases (anemia, headache, diarrhea, etc.) [19]. In order to overcome insufficiency of these vitamins in the diet and potential deficit disorders, some of these are included in energy and sport drinks to enhance their vital biological role as co-

enzymes and to increase mental alertness, concentration, and even mood. In particular, vitB1 (thiamine), vitB3 (niacin) and vitB6 (pyridoxine) are commonly added into energy drinks and juices formulations [20] and their determination in these products for quality control purposes is of interest. However, the challenge in B-vitamin analysis in these complex mixtures arises from their low abundance with respect to other additives and with a large amount of sugars. In this sense, one or more pretreatment steps (using SPE as extraction technique) are usually addressed to increase sensitivity and selectivity of the available instruments [21].

In this work, a bio-MOF based on the disubstituted oxamidato ligand derived from the natural L-serine was synthesized and applied as SPE sorbent to the selective isolation of B-vitamins in juices and energy drinks. The developed material was characterized and its resulting formula was $\{Ca^{II}Cu^{II}_6[(S,S)\text{-serimox}]_3(OH)_2(H_2O)\}\cdot 39H_2O$ (-Ca) (where serimox = bis[(S)-serine]oxalyl diamide). The high degree of crystallinity and robustness of this bio-MOF allowed the resolution of the crystal structures of several host-guest adsorbates, which offers a unique visualization of the interactions between the MOF and the guest molecules, thus governing the capture properties. On the basis of these advantageous features, this material was used as SPE sorbent. To our knowledge, this is the first application of this type of novel materials with sample pretreatment purposes. Thus, several extraction parameters influencing on the analytical performance were investigated (*e.g.* elution solvent, breakthrough volume and loading capacity). The effectiveness of the bio-MOF material as SPE sorbent was also compared with a commercial C18 cartridge usually used for isolation of these analytes. The-recommended protocol was successfully applied to the extraction and determination of four B-complex vitamins, such as B1, B3(acid), B3(amide) and B6 in juices and energy drinks samples.

2. Experimental section

The reagents and samples used, the synthesis of the bio-MOF and preparation of host-guest aggregates, the instruments and experimental conditions employed for the characterization of the materials, and the SPE protocol and vitamin extraction are described in detail in the Supporting Information

3. Results and discussion

3.1. Characterization of the bio-MOF

Elemental analysis and FT-IR data of this material were performed to confirm the formation of bio-MOF (see Supporting Information). Besides, the crystal structure of this material, determined by single-crystal X-ray diffraction (SCXRD), was consistent with the results previously obtained [22]. Indeed, its structure consists of a homochiral–3D calcium(II)-copper(II) network featuring hydrophilic hexagonal channels where the flexible hydroxyl (–OH) groups of the serine amino acid remain confined. The functionality and the virtual diameter of approx. 0.9 nm provided a good environment, in terms of polarity and size to host polar molecules.

Additionally, experimental powder X-ray diffraction (PXRD) patterns of polycrystalline samples of bio-MOF, vitB1@bio-MOF, vitB3(acid)@bio-MOF and vitB3(amide)@bio-MOF were carried out (Fig. S1). They were consistent with the theoretical pattern of bio-MOF, which confirmed the purity and homogeneity of the bulk samples. The solvent contents for all materials were also determined by thermogravimetric analysis (Fig. S2), and these data were in agreement with the established crystallographic structure.

The N_2 adsorption isotherm of bio-MOF was also done and it exhibited a type-I sorption behavior, which is typical of microporororous materials (Fig. S3), and with a Brunauer-Emmett-Teller (BET) surface area of 828 $m^2\ g^{-1}$. Moreover, the adsorption isotherms of

target compounds with this material (vitB1@bio-MOF, vitB3(acid)@bio-MOF and vitB3(amide)@bio-MOF) were obtained, where a severe reduction of the BET surface areas was produced (478, 341 and 407 $m^2 g^{-1}$ for vitB1@bio-MOF, vitB3(acid)@bio-MOF and vitB3(amide)@bio-MOF, respectively). This behaviour was attributed to a decrease in the accessible void space as a consequence of the presence of the inserted guest molecules that are filling the pores of the synthesized material.

3.2. Recognition ability of B-vitamins by bio-MOF

To investigate the selective recognition ability of bio-MOF sorbent over tested analytes, a preliminary study was conducted. Thus, the B-vitamins are a group of compounds with hydrophilic character, which suggests that they could be retained on retention mechanisms based on dipole-dipole and hydrogen-bonding interactions or ion-exchange interactions between ionizable solutes and the bio-MOF stationary phase. In a previous study [22], a systematic crystallographic study based on SCXRD analysis of bio-MOF loaded with several target compounds (like vitB6) was performed in order to define better the mechanisms/interactions responsible of the origin of the effective retention and organization of the molecules within the pores. A similar study was done here with the rest of B-vitamins (Fig. 1, Figs. S4 and S5). These figures show the structures of vitB3 (as representative examples) with bio-MOF determined by SCXRD. Thus, in vitB3(acid)@bio-MOF crystal structure unveils a direct H-bond interactions between serine hydroxyl moieties and carboxylic groups of this vitamin ([O⋯O distance of 2.83 Å], see Fig. S4), which was similar to that observed for vitB6 [22]. Besides, a further interaction occurs among nitrogen atoms of the niacin guests and copper (II) sites of the 3D net ([Cu⋯N distance of 2.51 Å], see Fig. S4). For the crystal structure of vitB3(amide)@bio-MOF, nicotinamide guest molecules orient in dissimilar way, where

nitrogen atoms are involved in weak H-bonds with serine arms ([N···O distance of 3.31 Å], see Fig. S5). In any case, all these results show insights on the target molecules with serine moiety interaction as well as on the molecular recognition process involved in the vitamins capture process. In fact, the real structures of the adsorbates, experimentally determined, allow not only to confirm these interactions but also to rigorously visualize the skillful molecules capture and the extraordinary structural flexibility of the system.

3.3. Optimization of extraction protocol

Once studied the recognition ability of bio-MOF, several parameters that can influence on the extraction efficiency of SPE protocol were examined. Thus, the optimization of this protocol SPE was performed using 25 μg mL^{-1} of vitB6 solution. All presented results were obtained from almost the mean value of three replicates. The composition of the sample loading solvent was first considered since it can improve the retention of analytes onto the SPE cartridge. Thus, Mon and co-workers [22] have proposed the use of aprotic solvents like CH_3CN in sample matrices in order to enhance the interactions between bio-MOF and hydrophilic solutes (like the analytes under study). Thus, loading solvents composed of different CH_3CN:H_2O percentages (from 95:5 to 0:100, v/v) were tried. The amount of retained vitB6 onto the sorbent was calculated by comparing the signal of analyte found in the percolated solution (measured by HPLC) with respect to the initial concentration loaded control. Maximum retention (98%) of vitB6 was found for CH_3CN:H_2O (95:5, v/v), and it was used for further studies. These results suggest that the retention of vitamin was mainly performed by hydrogen bond interaction with the residues of serine amino acid in the multiple channels of bio-MOF, as we mentioned above.

After choice of loading sample solution, an appropriate washing step was performed. For this purpose, CH_3CN was selected since it can help to remove low-polarity compounds presents in the matrix sample. Using this solvent, small losses (<1%) of vitB6 were evidenced in the washing step.

Then, the selection of an appropriate solvent to elute the retained vitB6 on the sorbent was accomplished. Since B-vitamins are hydrophilic compounds, several authors [23,24] have suggested that the use of mixtures of MeOH:H_2O in different proportions as efficient elution solvents. Thus, in order to achieve the highest eluting efficiency, different percentages of MeOH:aqueous solutions (from 0 to 85%. v/v), were tested (Figure 2A). As it can be seen, a progressive increase in MeOH content gave worse recoveries than those obtained using only deionized water. Consequently, water was selected for further studies because its simplicity and environmentally friendly behaviour.

In order to improve the recoveries of vitB6 in the eluates, a study of pH was performed. Thus, the pH of eluting solutions was studied in the range 5-10.5. Lower pHs than 5.0 were not tried to preserve the integrity of the bio-MOF sorbent. As shown in Fig. 2B, recovery of vitB6 increased with pH increasing from 5.0 to 7.0, reaching the maximum value (*ca*. 100%) when pH was 7.0, and then decreased with pH increasing up 10.5. This decrease could be explained by the progressive deprotonation of free hydroxyl groups present in the vitB6, whereas amino acid group of serine could remained protonated. Therefore, the presence of attractive interactions between the vitB6 and the bio-MOF sorbent could produce, thus hindering the desorption process.

The ionic strength effect in a SPE protocol may affect the partitioning of the analytes between sample solution and sorbent, which could enhance or decrease its extraction efficiency. Thus, the effect of addition of different contents of sodium chloride (up to 1000 mM) in the eluting solvent (aqueous buffer at pH 7.0) was studied. However, the

salt content did not have a significant effect on the extraction efficiency of vitB6, and consequently, the addition of NaCl was discarded.

Then, the best SPE conditions found for isolation of vitB6 were applied to the rest of B-vitamins investigated. Thus, recovery values around 100% were found for the vitB1, vitB3(acid) and vitB3(amide). These results confirmed that bio-MOF exhibited high selective binding affinity of vitB6 and some structure-related vitamins, and that all these analytes can be retained mostly through electrostatic and hydrogen bonding interactions between the hydroxyl groups of these solutes and the serine moieties presents in bio-MOF structure, as we commented above.

Once established the optimum SPE protocol, several parameters of bio-MOF as SPE sorbent were established. Thus, the adsorption capacity of bio-MOF (25 mg) was evaluated by increasing the amount of vitB6 without changing the loading solvent volume, giving a maximum value of 50 µg per mg sorbent. This value is about two times higher (25.6 µg per mg) and faster than the reported by other bio-material for isolation of B-vitamins [25].

The breakthrough volume of bio-MOF sorbent was also estimated. For this purpose, different sample volumes (1-5 mL) of the standard vitB6 solutions were passed through to the SPE material by keeping constant the total amount of this vitamin (20 µg per mg sorbent). Recoveries ranged from 96 to 75 % were obtained in the range tested, which indicated that no significant analyte loss happened. Also, the reusability of bio-MOF was carried out using the recommended SPE protocol (see supplementary information). The sorbent can be reused for 5 times without significant efficiency losses (recoveries values > 90%).

The extraction efficiency of the bio-MOF sorbent was compared to a commercial packed C18 cartridge. Using their SPE protocol [26]. The traditional SPE cartridge gave

238 percentages of losses values around 85 % for tested B-vitamins, which were significantly
239 higher than those obtained using the bio-MOF sorbent. This fact occurred when the big
240 amount of sorbent in the commercial cartridge (150 mg) was down-scaled to 25 mg to be
241 compared with our bio-MOF cartridges. In this sense, that low amount of sorbent used
242 implies the possibility of manufacturing several SPE cartridge from a unique synthesis of
243 bio-material, which certainly makes this SPE protocol economically very interesting.
244

245 **3.4. Extraction and analysis of B-vitamins from real samples**

246 The optimized SPE procedure using the synthesized bio-MOF sorbent was validated with
247 respect to linearity, limits of detection (LOD) and quantification (LOQ) and precision.
248 External calibrations curves were prepared at different concentrations (0.005 to 500 µg
249 mL^{-1}) and each calibration level was injected twice. For all the analytes, a good linearity
250 ($r > 0.9999$) was observed. These calibration curves were used since their slopes and those
251 obtained with spiked sample solutions tested did not differ significantly. Further, the LOD
252 and LOQ based on signal-to-noise (S/N) ratios of 3 and 10 were determined, giving values
253 ranged 0.4-1.4 and 1.4-5.0 ng mL^{-1}, respectively. The precision (intra- and inter-day
254 reproducibilities) of the SPE combined with HPLC method was evaluated from individual
255 standards prepared at 25 µg mL^{-1}. The intra-day precision was determined by analyzing
256 four replicates within a given day, whereas the inter-day precision was estimated by
257 analyzing series of three independent experiments carried out on three different days (see
258 Table 1). The method showed a good precision with relative standard deviation (RSD)
259 values below 1.5 %.
260 The applicability of the proposed SPE method was tested by the determination of B-
261 vitamins in several fruit juices and energy drink samples. For the peak identification of
262 vitamins, individual standards were injected and retention times were compared with

those standards, and when required also by spiking the sample with standard solutions. None of the studied B-vitamins was detected in the original samples. Then, the standard solutions of B-vitamins were added (at two fortification levels) to the original samples in order to evaluate the accuracy of the presented method. Figure 3 shows a representative example of sample unspiked (dashed line) and spiked at a concentration level of 50 μg mL^{-1} (solid line). As shown in Table 2, in all instances, excellent recoveries values (ranged from 75 to 123 %) were found with RSDs below 14 %.

4. Concluding remarks

In this work, a bio-MOF containing hexagonal channels decorated with L-serine residues was successfully prepared, characterized and applied to the extraction of B-vitamins in juices and energy drink samples, followed by HPLC-DAD analysis. Thus, the bio-MOF exhibited excellent extraction performance in SPE of B-vitamins with the following merits: high extraction recovery, low detection limits and acceptable reusability. The excellent recognition ability of bio-MOF was not deemed only hydrogen bond interaction between the hydroxyl group functionalized channels within the confined space of bio-MOFs (which act as molecular recognition sites) but also to the capacity of these functional channels of bio-MOF to impart supramolecular order thus enabling the possibility of carrying out unique host-guest interactions. Besides, the synthesized bio-MOF was also successfully applied to the extraction of these vitamins by spiking in fruit juices and energy drink samples. To our knowledge, this study reports the first application of this type of materials for the extraction of biomolecules in food samples. Taking into account the well-known properties of this material, its use as SPE sorbent can offer a promising wide range of application not only for B-vitamins but also for other hydrophilic

solutes. Further exploration of this material could be also beneficial in chromatographic separation, which undoubtedly would open a new promising area for this type of MOFs.

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The authors declare no conflict of interest.

5. References

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386 Development of a validated HPLC method for the determination of B- complex vitamins

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Figure captions

Figure 1. Views of the 3D open-framework of vitB3(acid)@bio-MOF (a) and vitB3(amide)@bio-MOF (b) along the c axis (the crystallization water molecules are omitted for clarity). The 3D networks are depicted as gray sticks, with the only exception of the oxygen atoms from the serine residues, which are represented as red spheres. Views of one single channel of vitB3(acid)@bio-MOF (c) and vitB3(amide)@bio-MOF (d) in the ab (left) and bc (right) planes. The guest vitB3(acid) (a and c) and vitB3(amide)@bio-MOF (b and d) molecules are highlighted using the surface mode option. Color code for the guest molecules: oxygen: red; nitrogen: blue; carbon: yellow.

Figure 2. Effect of solvent composition (a) and (b) pH in the eluting solvent on recovery of B-vitamins using bio-MOF as SPE sorbent.

Figure 3. Chromatograms of energy drink unspiked (dashed line) and spiked with 50 µg mL^{-1} of each B-vitamin using the proposed SPE protocol (bond line). Chromatographic conditions given in Supplementary Information.

Graphical Abstract

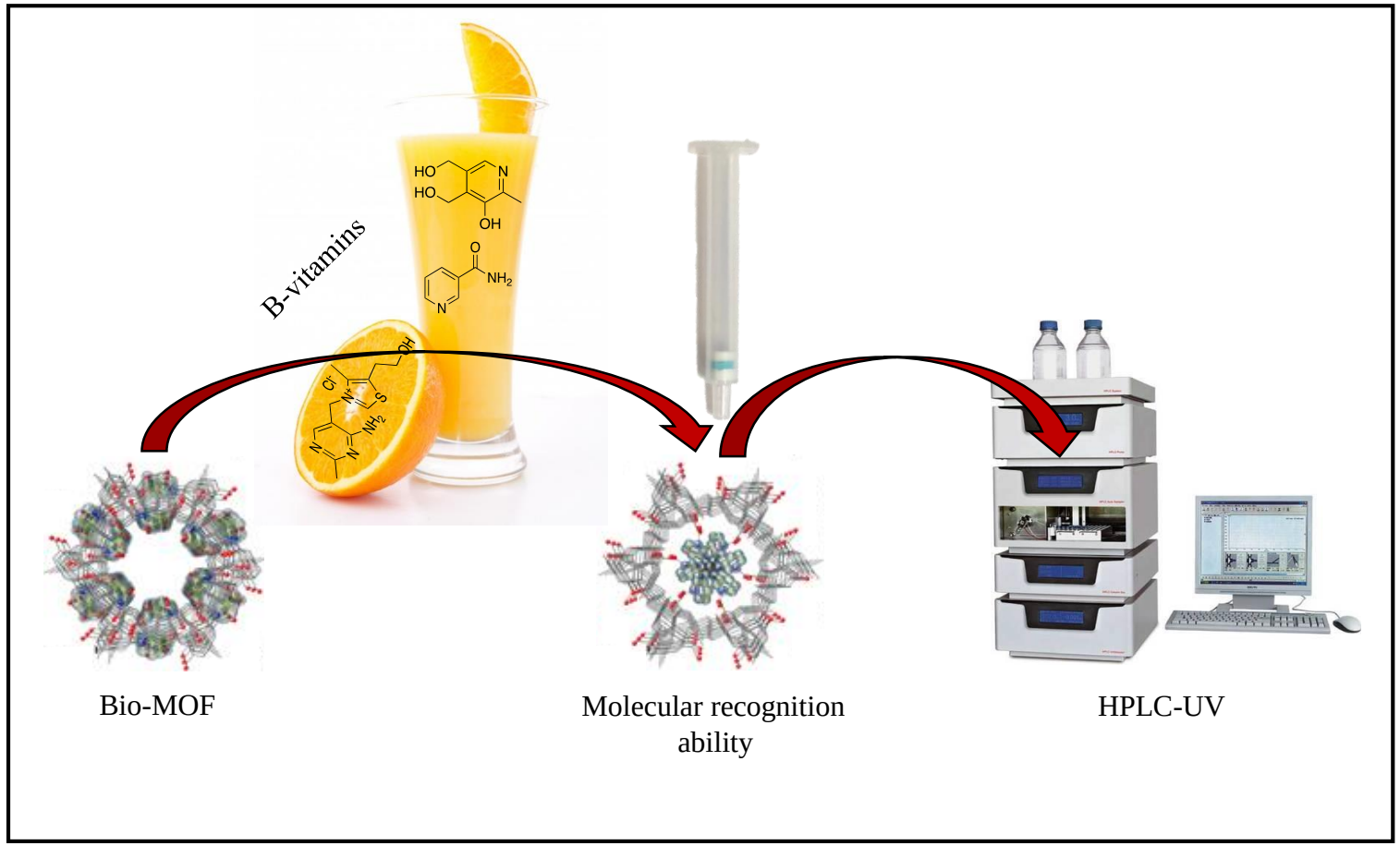

Figure 1

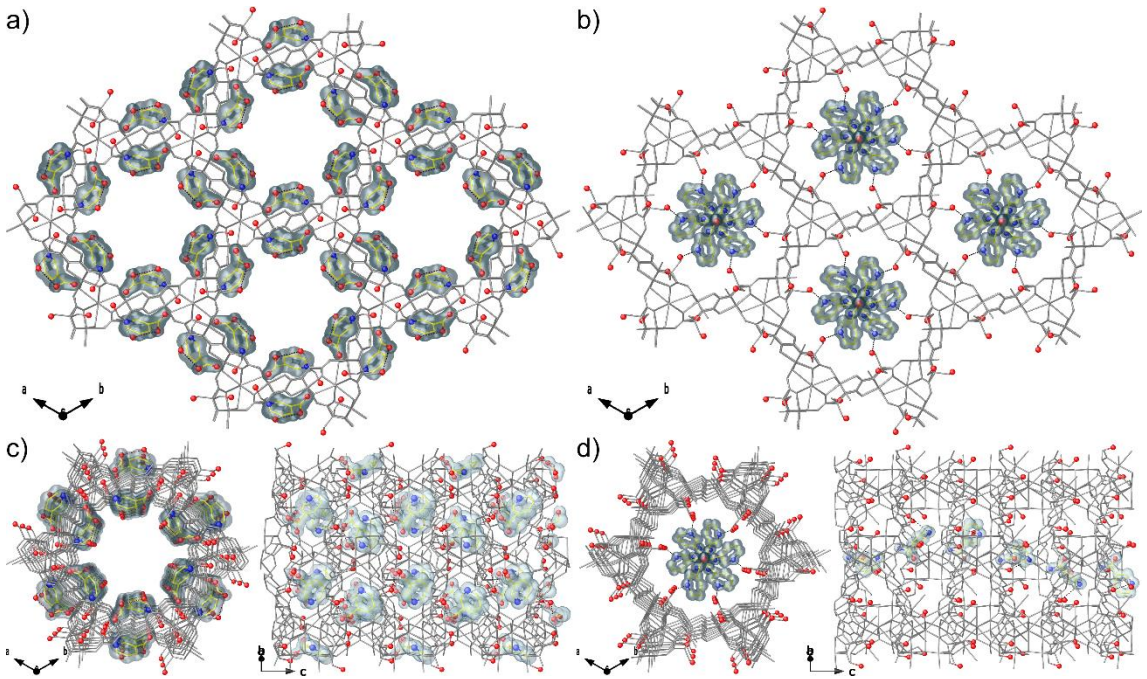

Figure 1. Martínez Pérez-Cejuela *et al.*

Figure 2

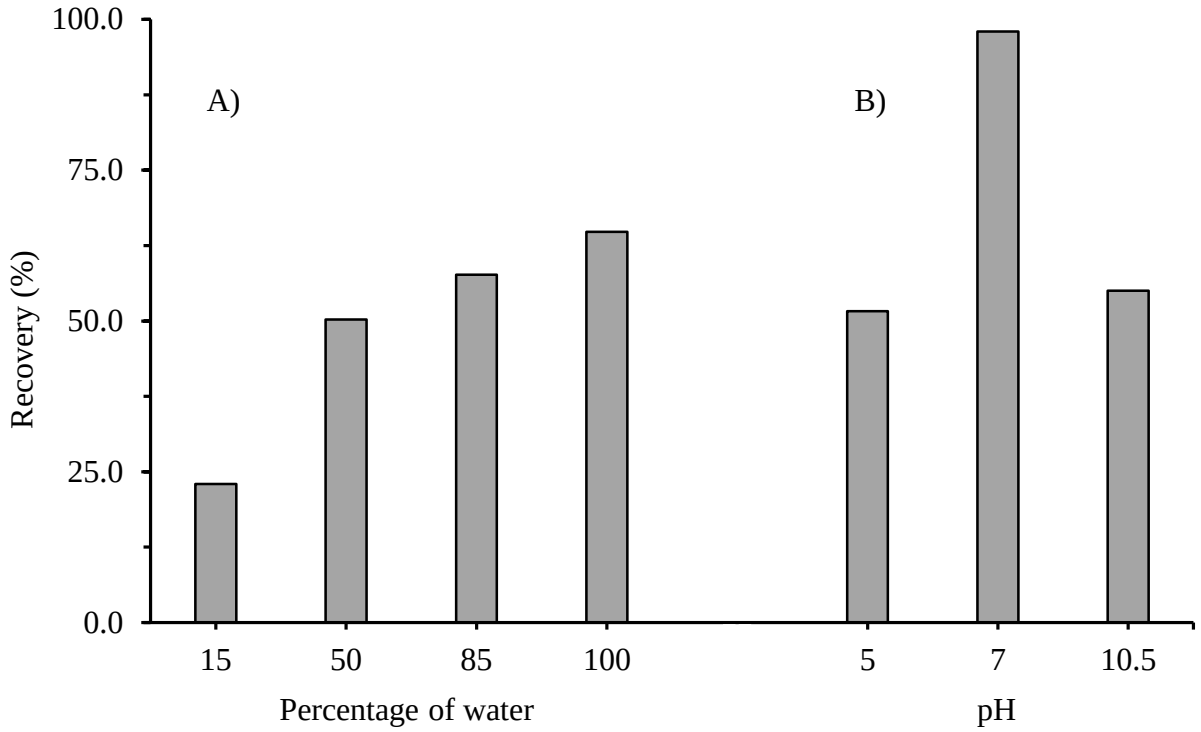

Figure 2. Martínez Pérez-Cejuela *et al.*

Figure 3

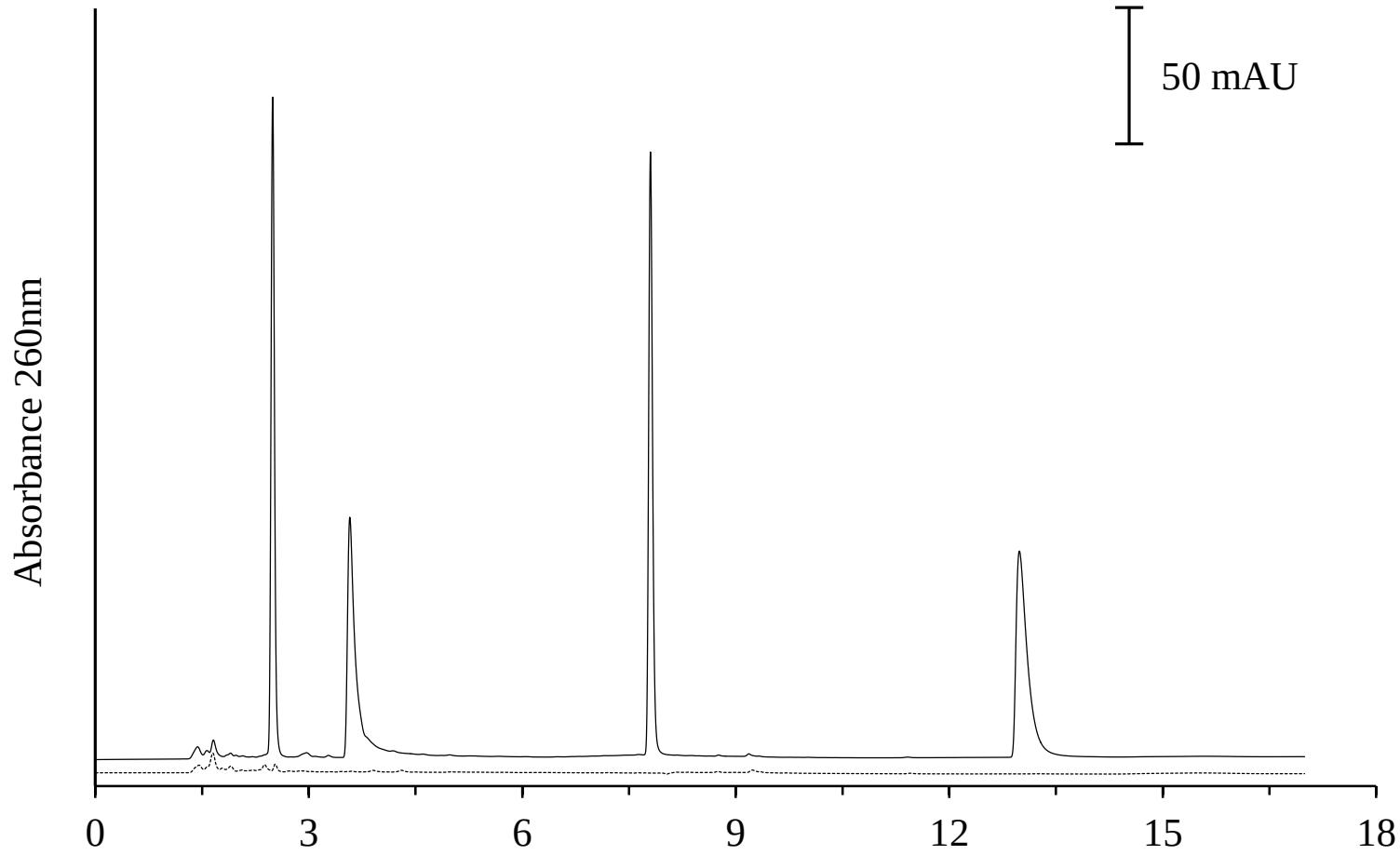

Figure 3. Martínez Pérez-Cejuela *et al.*

Table 1

Table 1. Analytical figures of merit for the proposed bio-MOF SPE protocol for determination of the target B-vitamins

Vitamin	Linear range (μg mL^{-1})	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Precision RSD (%)	
B1	0.005-500	0.5	1.6	0.5[a]	0.3[b]
B3 (amide)	0.005-250	0.4	1.4	0.12[a]	0.06[b]
B3 (acid)	0.005-500	0.8	3.0	0.6[a]	1.0[b]
B6	0.005-500	1.4	4.5	0.08[a]	1.5[b]

[a]Intra-day reproducibility values of retention time
[b]Inter-day reproducibility values of retention time

Table 2. Recoveries of target B-vitamins in spiked energy drink and juice samples (n = 3)

Sample	Analyte	Spiked conc. (mg L^{-1})	Recovery, % (RSD, %)
Energy drink	vit B1	10	86.8 ± 1.3
		50	93.1 ± 0.3
	vit B3 (amide)	10	91 ± 4
		50	84.4 ± 1.5
	vit B3 (acid)	10	94 ± 3
		50	83.2 ± 1.1
	vit B6	10	105 ± 4
		50	101 ± 5
Fruit juice	vit B1	10	111 ± 14
		50	101 ± 2
	vit B3 (amide)	10	85 ± 13
		50	93 ± 5
	vit B3 (acid)	10	84 ±12
		50	94.6 ± 0.3
	vit B6	10	75 ± 5
		50	123 ± 4

Cover Letter

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Editor-in-chiefs
Prof. Dr. O. S. Wolfbeis and Prof. Dr. A. Escarpa

Dear Editors,

I send you hereby the revised MS entitled "*Smart bio-metal-organic frameworks for selective molecular recognition of hydrophilic vitamins*" by H. Martínez-Pérez-Cejuela et al. in order to be submitted to consideration for publication in *Microchimica Acta*.

In this research, a bio metal-organic framework (bio-MOF) derived from the amino acid L-serine was prepared and the resulting material was evaluated as selective sorbent for the molecular recognition and extraction of B-vitamins. These materials showed significant features such as their tunable pore size and molecular recognition capabilities; however, these favorable properties have been scarcely explored in analytical sciences. As far as we know, this is the first time that this type of novel materials has been applied with sample pretreatment purposes. On the other hand, in most analytical studies related to the use of MOFs, the establishment of responsible mechanisms/interactions of target molecules with these materials is rarely elucidated. Here, single-crystal X-ray diffraction studies offers a unique visualization of the interactions between the bio-MOF and the guest molecules, thus governing the capture properties, and the structural flexibility of the system. Indeed, this material demonstrated a high potential as solid-phase extraction sorbent with remarkable reproducibility and low detection limits (between 0.4 to 1.4 ng mL^{-1}) in complex aqueous matrices (fruit juices and energy drinks).

We believe that the reported results are of widespread interest for the general readership of *Microchimica Acta*, due to the versatility of the presented material make it attractive sorbent with enhanced molecular recognition capabilities. Additionally, the promising material described here could be extended to several analytical methodologies such as preconcentration, separation or for flow-based analytical systems, which undoubtedly would open a new promising area for this type of MOFs.

We hope to receive a positive review from you and from the peer-review experts.

Yours sincerely,

J.M. Herrero-Martínez