Evaluation of mesoporous imprinted silicas as MSPD selective sorbents of ketoprofen in powder milk

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ABSTRACT

Two molecularly imprinted mesoporous silicas were synthesized using different ratios of ketoprofen as template molecule and amino-functionalizaed SBA-15 as support. The materials synthesized were characterized and evaluated as selective sorbents for matrix solid phase dispersion (MSPD) of ketoprofen in the presence of other nonsteroidal anti-inflammatory drugs (NSAIDs) in powder milk samples.

Keywords: Imprinted mesoporous silica; MSPD; Milk; NSAIDs, Ketoprofen.

1. Introduction

Development and application of new materials has become a very interesting research area in the field of Analytical Chemistry. In particular, ordered mesoporous silicas have attracted a great deal of attention as an inorganic support for their excellent properties, such as stable mesoporous structures, modifiable pore sizes and volumes, and large specific surface areas. These properties are of great interest for potential applications in sample treatment [1-4].

Matrix solid phase dispersion (MSPD) is a relative new methodology that allows the complete disaggregation and dispersion of the sample on a solid support, thereby generating a solid mixture having the chromatographic character sufficient to extract the

analytes [5]. The use of MSPD offers a simple sample preparation, which reduces the use of extraction solvents and length of sample preparation. Some mesoporous silicas had been used as MSPD sorbents in food sample preparation. For example, Barreto et al. [6] successfully tested MCM-41 as MSPD sorbent for the extraction of six pesticides from mango fruit. More recently, Gañán et al. [7] developed a MSPD method using SBA-15 functionalized with C18 groups as sorbent for the simultaneous determination of five steroids in milk.

In order to increase the selectivity in sample preparation by MSPD, molecularly imprinted polymers (MIPs) prepared by traditional bulky imprinting methods are commonly used [5]. Nevertheless, MIPs exhibit poor site accessibility to the target molecules, as the functional groups are totally embedded with a high cross-linking density in the polymer matrix. To overcome these drawbacks, efforts have been made to develop new selective sorbents by surface molecular imprinting (SMIP). SMIP consist of localizing the recognition sites on the surface of a supporter; this strategy greatly increases the efficiency of adsorption and desorption, reducing the leakage of the residual template by reducing the diffusion length. For SMIP process, mesoporous silicas can be used as support materials because the channels of this kind of materials facilitate the diffusion of solvent and template. In addition, these molecularly imprinted mesoporous silicas have an extremely high surface-to-volume ratio; therefore most of the template molecules are situated at the surface or in the vicinity of the material surface. This fact is expected to improve the binding capacity, binding kinetics and accessibility of the recognition sites. For these reasons, imprinted materials prepared by post-modification of mesoporous silicas, have demonstrated important advantages in food sample preparation, by combining the selectivity of MIPs and the binding capacity of mesoporous silicas [8-10]

Ketoprofen is a nonsteroidal anti-inflammatory drug (NSAID), which use is allowed only for animal species not involved in milk and egg production for human consumption. In this work, two different novel imprinted mesoporous silicas were prepared and characterized by SMIP. Both materials were evaluated as selective sorbents in MSPD to determine ketoprofen in powder milk samples in the presence of other NSAIDs.

2. Experimental

Preparation and characterization of mesoporous imprinted silicas (MIS): Mesoporous silica (SBA-15 type) was prepared according to the method of Zhao et al. [11]. Amine-functionalized mesoporous silica (SBA-15-NH_2) was obtained by modification of SBA-15 with 3-aminopropyltriethoxysilane (3-APTOS). Preparation of MIS was carried out as follows: 5 mL of 0.1 mol/L of KET solution in MeOH was added to SBA-15-NH_2 under stirring. The mixture was refluxed at 80ºC for 2 h, and then 1 or 2.5 mL of tetraethylorthosilicate (TEOS) and 5 mL of 1.0 mol/L acetic acid solution were added (in order to prepare SBA-15-MIS-1 and SBA-15-MIS-2, respectively). The mixture was stirred and refluxed for 8 h at 80ºC, filtered and dried under vacuum (100ºC, 12 h). The product was washed once with ethanol and twice with Milli Q water. The remaining solid was extracted by a mixture (1:1, v/v) of ethanol and 6 mol/L HCl in a Soxhlet for 24 h, neutralized with 0.1 mol/L KOH solution and washed by Milli Q water. Finally, the powder was dried at 100ºC for 24 h under vacuum. For characterization details see SM.

MIS-MSPD procedure: 50 mg of spiked goat milk powder (40 µg/kg) was placed into a glass mortar and gently blended with 25 mg of MIS and 50 mg Na_2SO_4 by using a glass nail until dry and homogeneous mixture was obtained. Then, the mixture was packed

into a 3 mL SPE cartridge with a plugged, with porous PTFE disks at both ends that retain the entire mixture. The target analytes were eluted using 1 mL acetonitrile, at a flow rate of 1 mL/min. Then the eluent was centrifuged at 10000 rpm during 10 min and filtered through 0.45 μm pore size disposable nylon filters. The resulting extracts were analyzed by HPLC-MS/MS (see SM for chromatographic and spectrometric conditions). Table 1 shows mass spectrum parameters and retention time for NSAIDs and Figure 1 shows the chromatogram obtained for the daughter ions. Recoveries were calculated by comparison of the areas of spiked samples with the areas of simulated samples, prepared in the same way but spiked with the analytes at the end of the MSPD process.

3. Results and discussion

Synthesis and characterization of mesoporous imprinted silicas. The synthesis MIS was performed using amine-funtionalized SBA-15, TEOS as polymerization agent and ketoprofen (KET) as template at two ratios of 1:1:5 and 1:2.5:5 (w/v/v) in order to obtain SBA-15-MIS-1 and SBA-15-MIS-2 respectively. N2 adsorption–desorption isotherms of SBA-15 and MIS are type IV according to the I.U.P.A.C. classification and have an H1 hysteresis loop that is representative of mesoporous materials with pores of constant cross-section (**Figure 1A)**. Table 1 shows the Brunauer–Emmett–Teller specific surface area (S_{BET}), pore volume and BJH (Barrett–Joyner–Halenda) pore diameter. These results indicated that S_{BET}, pore volume and pore diameter of SBA-15-MIS-1 and SBA-15-MIS-2 decreased compared with SBA-15. The difference between these materials can be attributed to the structural change that took place during the imprinting process, which occurred in the silica surface and inside of the pores. In this regards since KET (15.64 x 6.93 Å) is smaller than the pore of the SBA-15 (56.1Å),

it is possible that more than one molecule is adsorbed by amine groups of the pore wall, so after the imprinting process with the TEOS small reduction on the pore diameter was produced. Additionally, characterization results evidenced that the polymerization did not change the mesoporous structure of MIS because, as it can be seen in **Figure 1B** from TEM images of imprinted silicas, a clear arrangement of hexagonal pores with uniform size was observed, similar to SBA-15. Finally, elemental analysis of the MIS was carried out. The % N obtained in these materials indicated a functionalization degree of 1.41 mmol NH_2/g of silica.

Evaluation of MIS as selective sorbent in MSPD. MSPD efficiency depends on careful optimization of the experimental conditions affecting competition within the matrix, the dispersant sorbent or solid support, and the extraction solvent for analytes and potential matrix interferences. The nature of the solid support/dispersants will affect the retention and elution of the target analytes and interferences. In MSPD applications, conventional phases such as C18, alumina, florisil, etc. are used as dispersants. In a previous work, our group demonstrated that using a selective dispersant in MSPD, with high affinity for the target analyte, it is possible to achieve an extract sufficiently clean to be directly injected into HPLC for further analysis, without any interferences from the matrix [12]. In this work, with the aim of increasing the selectivity of the MSPD process for KET extraction in milk samples, in a first experiment SBA-15-MIS-1 was evaluated as sorbent. To achieve this 50 mg of powder milk sample spiked with KET (40 µg/kg), 25 mg of SBA-15-MIS-1 and 50 of Na_2SO_4 were gently blended until dry and homogeneous mixture was obtained. The analyte was eluted using 1 mL of acetonitrile and the extract was analyzed. Results obtained indicated recoveries near 60% for KET. In a second experiment, SBA-15-MIS-2 that prepared increasing the TEOS concentration, as described in the experimental section, was evaluated following the

same procedure. In this case, results obtained indicated better recoveries for KET (108 %). These results indicated that molecular imprinting sites are integrated successfully within long-range ordered mesoporous material and that the molecular recognition capability of the material was improved enormously using a higher amount of TEOS for the polymerization process. On the other hand, selectivity of both materials was demonstrated by comparing the binding degree of the template molecule (KET) and other NSAIDs (see SM) with similar structure such as naproxen, carprofen and vedaprofen. **Figure 2** shows the recoveries obtained for all analytes after the MIS-MSPD procedure, with both materials, of spiked milk samples (40 µg/kg of each NSAID). The results show that the SBA-15-MIS-2 exhibited significant selectivity for KET, while for other analytes recoveries were below of 52 %. In contrast, KET, naproxen and carprofen were retained rather equally on SBA-15-MIS-1 (recoveries between 50 – 60%). It is noteworthy mention that in the case of vedaprofen were observed the lowest recoveries, which can be justified since this compound has a structure more different to the rest of NSAIDs studied. This high selectivity results from the imprinting effect (imprinted cavities in the mesoporous silica) that can improve the adsorption selectivity, as a consequence of the different molecular interaction between KET and the other NAIDs with the material. Therefore the SBA-15-MIS-2 sorbent possesses excellent molecular recognition ability and high selectivity.

4. Conclusions

In summary, the results show that the MIS–MSPD procedure using SBA-15-MIS-2 as sorbent is viable to direct determination of KET in powder milk. To the best of our knowledge, this is the first example of using MIS as selective solid support for MSPD.

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Figure 1. A) N_2 adsorption-desorption isotherms; B) TEM image of SBA-15-MIS-2

Figure 2. Comparison of the recovery percentages obtained from the analysis of 25 mg of powder milk sample spiked with NSAIDs after MIS-MSPD procedure using SBA-15-MIS-1 and SBA-15-MIS-2 as sorbents.

Table 1. Characterization data of mesoporous silicas.

	S_{BET} (m^2/g)	Pore diameter (Å)	Pore Volume (cm^3/g)
SBA-15	967.30	56.1	0.973
SBA-15-MIS-1	325.45	53.2	0.543
SBA-15-MIS-2	304.99	53.8	0.486

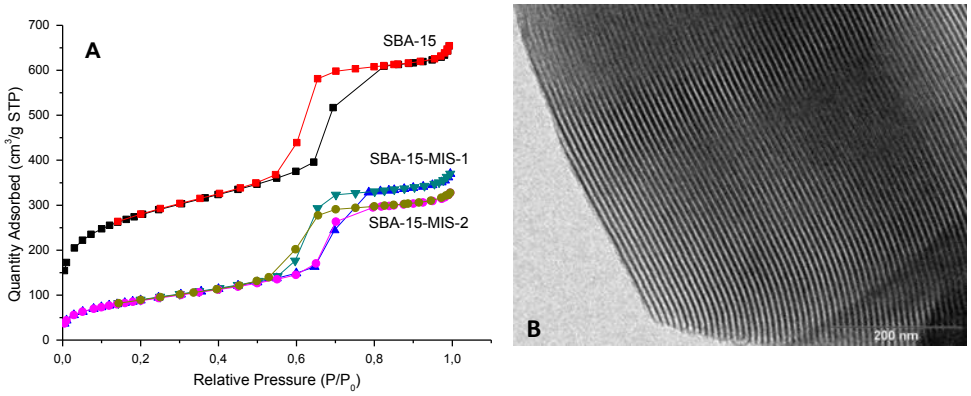
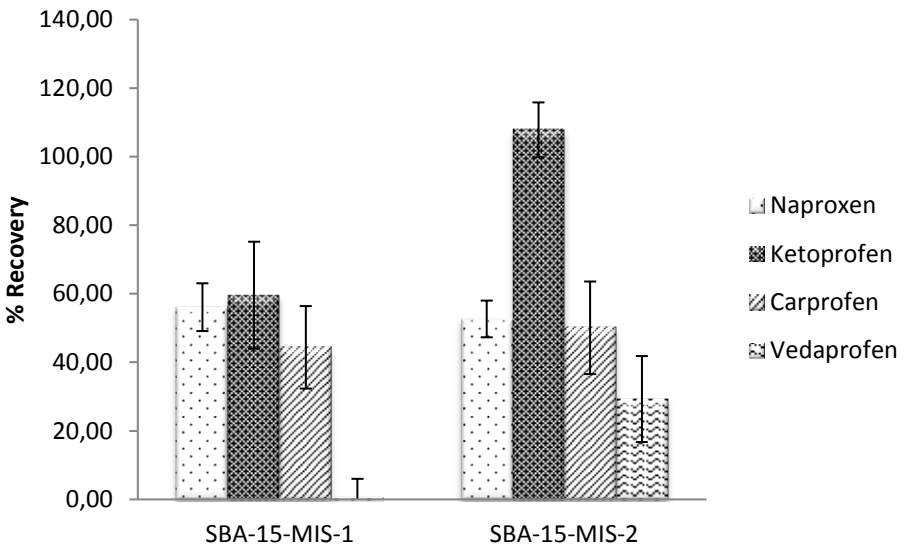

A
SBA-15
SBA-15-MIS-1
SBA-15-MIS-2
Quantity Adsorbed (cm3/g STP)
Relative Pressure (P/P0)
0
100
200
300
400
500
600
700
0,0
0,2
0,4
0,6
0,8
1,0
B
200 nm

Figure 1


140,00
120,00
100,00
80,00
60,00
40,00
20,00
0,00
% Recovery
SBA-15-MIS-1
SBA-15-MIS-2
Naproxen
Ketoprofen
Carprofen
Vedaprofen

Characterization of mesoporous imprinted silicas: N_2 gas adsorption-desorption isotherms were obtained using a Micromeritics ASAP 2020 analyzer, and pore size distributions were calculated using the Barret-Joyner-Halenda (BJH) model on the adsorption branch. Conventional transmission electron microscopy was carried out on a TECNAI 20 Philips, operating at 200 kV. Elemental analysis (% N, % C) was performed with a LECO CHNS-932 analyzer.

HPLC-MS/MS determination: The HPLC system consisted of a Varian 1200/1200L LC/MS-MS containing two solvents deliver module ProStar 210/215, a ProStar 410 autosampler (equipped with a 20-µL loop), a thermostatic column compartment, and a 1200L TQ triple cuadrupole mass spectrometry detector with an electrospray ionization (ESI) ion source (data acquisition system MS Workstation version 6.3.). The chromatographic separation of analytes was achieved on an Inertsil ODS-3-MS C18 column (150×2.1 mm I.D., 3 µm particle size) from GL Science Inc., protected by a guard column of the same type (4×3.0 mm I.D., 3 µm particle size) from Phenomenex. The mobile phase consisted of Acetonitrile (A) and water (B), both containing 2 mM ammonium acetate in gradient elution program: t = 0 min 50 % B, t =1 min 20 % B, t =10 min 15% B and t = 12 min 50% B (8 min). The total run-time of the method was 20 min. The flow rate was 0.2 $mLmin^{-1}$, the column temperature 30 ºC and the injection volume 10 µL. The TQ was operated using an ESI ion source in negative ion mode. Nitrogen was used as both drying and nebulizer gas and argon was used as collision gas under the following conditions: The nebulizer and drying gas was set at 200 ºC and 22 psi; the capillary voltage was held at -4500V and shield at -600 V. Collision gas was set at 1.90 mTorr and detector was set at 1395 V. Multiple reaction monitoring (MRM) mode was used for all analytes (mass peak width Q1 2.5; mass peak width Q3 1.5; scan

width in MRM 0.70).Table 1 shows the cone voltage and collision energy optimized for daughter ions and granddaughter ions selected for each compound during MRM acquisition each analyte and retention time with the gradient elution described above and Figure 1 shows the chromatogram obtained for the daughter ions selected for quantitation for all analytes.

Table 1. Mass spectrum parameters and retention time for NSAIDs using the HPLC-MS/MS method.

Analyte	**Ionization mode**	**Precursor ion (*m/z*)**	**Daughter ions (m/z)***	**Cone Voltage (V)**	**Collision energy (eV)**	**Retention time (min)**
Naproxen	ESI (-)	229.1	167.7	30	37	
			169.4*	30	17.5	5.55
			184.9	30	8	
Ketoprofen	ESI (-)	253.0	207.5	30	13.5	5.48
			208.7*	30	9	
Carprofen	ESI (-)	272.7	226	30	38	6.23
			227.8*	30	14.5	
Vedaprofen	ESI (-)	280.9	235.7	30	13	8.78
			236.9*	30	10.5	

* Ions used for quantitation. Chromatographic and mass spectrometric conditions are described in the text.

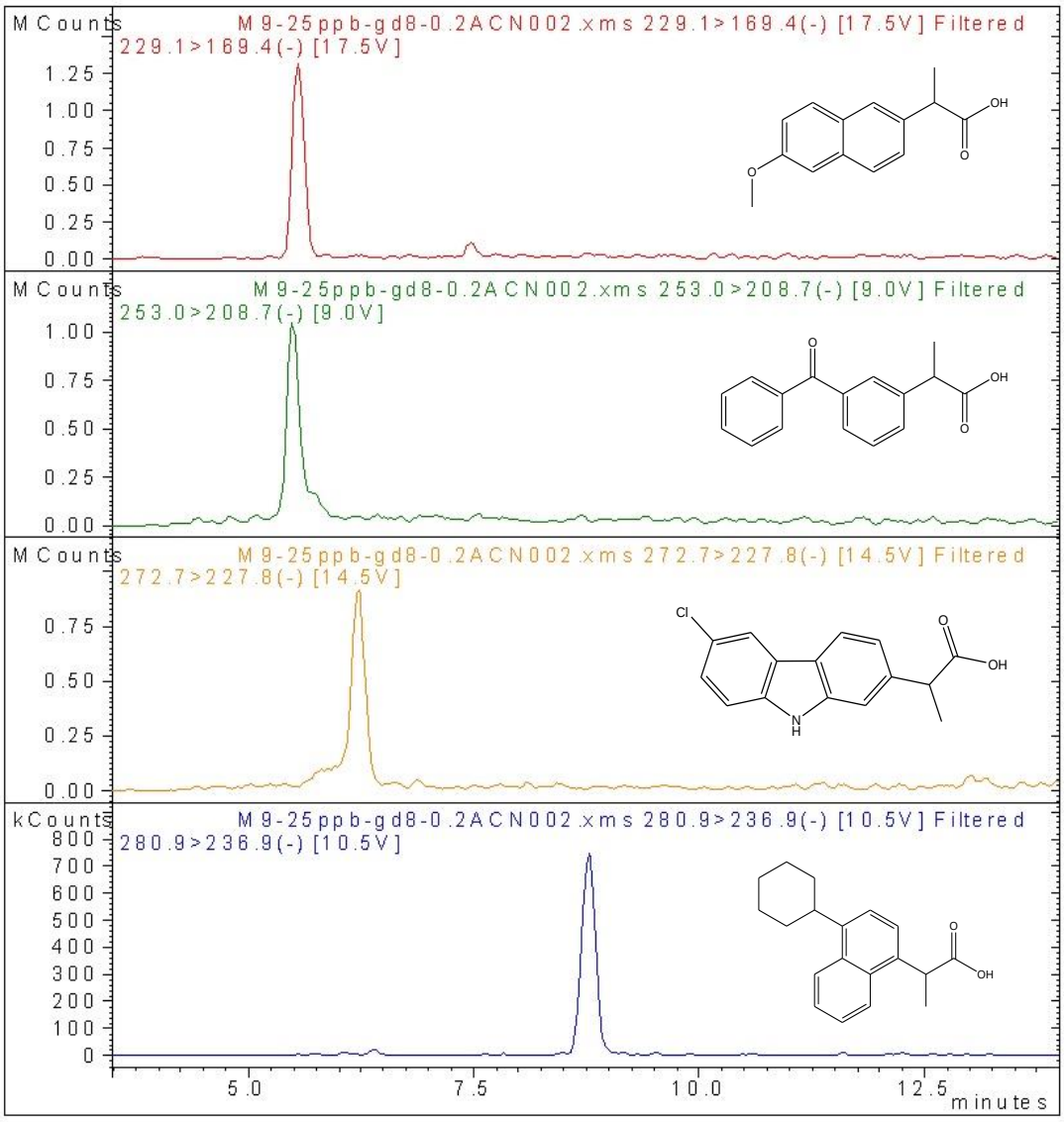


Figure 1. Chromatograms obtained for the daughter ions selected for quantitation for all analytes (25 µg/L).