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Study of the interactions between phenolic compounds and micellar media using micellar solid-phase microextraction/gas chromatography

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Abstract

Solid-phase microextraction coupled to gas-chromatography with mass-spectrometry detection has been employed to establish the sensitivity indexes as well as to study the partition coefficients of phenols into ionic and nonionic micelles. The sensitivity indexes values can be used to estimate qualitatively the affinity between phenols and micelles. The studied phenols, some of them with high environmental interest, include chloro-, alkyl-, and methoxy-phenols. The results obtained in this work, using 85 μm polyacrylate fiber and anionic (sodium dodecyl sulphate), cationic (cetyltrimethylammonium bromide), and nonionic (Triton X-100 and polyoxyethylene-10-lauryl ether) surfactants, indicate that SPME is a viable method for estimating the micelle partition coefficients.

Keywords: Solid-phase microextraction; Micelle-partition coefficients; Phenols; Micellar media

1. Introduction

Solid-phase microextraction (SPME) is a free solvent method for the extraction and preconcentration of organic compounds from environmental samples. This method involves two steps. The first step is the partitioning of the analytes between the sample and the fused-silica fiber coated with a suitable stationary phase. The second step is the desorption of the concentrated analytes into the hot injector port of a gas-chromatograph or into an appropriate interface device for liquid chromatography or capillary electrophoresis. The sorption process implies an exposition of the coated fiber to the sample, followed by an extraction of the target analytes from the matrix to the coating. The extent of this extraction process depends on the sorbent coating used for the analyte under study. Generally, nonpolar organic compounds will be retained by nonpolar coating such as polydimethylsiloxane (PDMS), while polar coating such as polyacrylate (PA) will effectively extract polar compounds, such as phenol and its derivatives.

SPME has been successfully applied to measure the distribution of an analyte in a matrix of two or more components. In these cases, SPME appeared to be simpler and more efficient than some traditional methods like dialysis, ultradialysis and centrifugation [1,2]. Pörschmann et al. [3,4] discussed the distribution of certain organic compounds bounded to dissolved organic matter (DOM). The authors demonstrated that the amount of the analyte on the fiber could be so small that it can be neglected for semi-volatile and most volatile organic compounds. Therefore, the equilibrium can be kept virtually undisturbed, and only the freely dissolved chemical will partition into the SPME fiber. Vaes et al. [5] measured the freely available concentration of aniline, nitrobenze, 4-chloro-3-methylphenol and 4-*n*-pentylphenol, and the reduction of their concentration due to binding to biological matrixes. Pawliszyn et al. [6] demonstrated that SPME is a valid method to study the protein binding. The authors employed diazepam bound to human serum albumin as a model system to study the binding properties between bovine serum albumin and alkylbenzenes, by using SPME coupled to GC [7]. Zambonin et al. [8] determined the adsorption coefficients for six triazines in soil and sediment samples with different organic matter contents by using SPME coupled to GC–MS. In a similar way, Stempvoort et al. [9] used SPME to measure the binding of methylated naphthalenes to concentrated aqueous humic acids.

The results obtained in a previous study [10] indicate that the micellar solid-phase microextraction (MSPME) is a viable method for estimating the partition coefficients of PAHs into

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anionic and nonionic micelles, using either the PDMS or the PA fiber. The described procedure is based on the principle that by extracting only a small amount of the freely dissolved fraction, the equilibrium between the fraction of a compound bound to a matrix and the fraction dissolved in the aqueous phase is not perturbed.

The aim of the present work is the determination of partition coefficients for a wide variety of compounds and micellar media using MSPME coupled to gas-chromatography with MS detection, as well as the study of interactions that can be taking place in a solution when employing MSPME. In addition, the sensitivity index is a new and simpler parameter proposed to estimate the ability of MSPME to extract analytes from micellar media. These sensitivity indexes can also be used to establish the strength of the interactions between analytes and the micellar media.

The compounds selected for the study were phenols that, in most cases, contain between one and five chlorine atoms, differing in number and position of the chloride-substituent in the molecule. Among them, there are some with high environmental interest because they act as toxic or endocrine disrupting chemical agents for animals and human beings [11,12]. In addition, and for comparative purposes, other phenolic compounds with alkyl- and methoxy-substituents have been included. Some of these phenolic compounds present organoleptic and antioxidant characteristics in the smokes used in smoked foods [13]. Two of the most studied ionic surfactants: the anionic surfactant sodium dodecyl sulphate (SDS) and the cationic surfactant cetyltrimethylammonium bromide (CTAB) have been selected. The selected non-ionic surfactants were Triton X-100 and POLE, belonging to the polyoxyethylene surfactants family, with octyl phenyl ether and lauryl ether substituents, respectively. The POLE surfactant has been characterized extensively by our group in previous works [14,15].

2. Experimental

2.1. Reagents

The standard mixture solution of 19 chlorophenols (Phenol-Mix 10) at a concentration of 50 ng/μL in acetonitrile was supplied by Dr. Ehrenstorfer, Reference Materials (Augsburg, Germany). The standard solution of 4-chloro-3-methylphenol (4-C-3-MP) at a concentration of 10 ng/μL in acetonitrile was also supplied by Dr. Ehrenstorfer. These standards were stored at 4 °C and used for the preparation of a stock standard solution of 1 mg/L in acetonitrile. Afterwards, this stock standard solution was used for the preparation of working standard solutions. Acetonitrile of HPLC grade (Merck, Darmstadt, Germany) was used for such dilutions.

The standard mixture solution of 14 phenols (Phenol-Mix-1) at a concentration of 50 ng/μL in methanol was supplied by Dr. Ehrenstorfer. The standards of 2-ethylphenol (2-EP) and 3-ethylphenol (3-EP) were supplied by Dr. Ehrenstorfer with purity higher than 99% (w/w). These standards were used for the preparation of a stock standard solution of 2000 mg/L. The standards of 3-methoxyphenol (3-MeP), 2,6-dimethoxyphenol (2,6-DMeP) and eugenol (Eu) were supplied by Fluka (Buchs, Switzerland) with a purity higher than 98% (w/w). These standards were used for the preparation of stock standard solutions of 2000 mg/L each one. These three stock standard solutions of 2000 mg/L and the Phenol-Mix-1 were employed in the preparation of a stock standard solution of 2.5 mg/L for the 14 phenols, Eu, 2-EP and 3-EP, 100 mg/L for the 3-MeP, and 250 mg/L for the 2,6-DMeP. This stock standard solution was used in the preparation of the final working standard solutions. Acetonitrile was used for all dilutions.

Sodium dodecyl sulfate (SDS) was supplied by Merck (Darmstadt, Germany). Polyoxyethylene-10-lauryl ether (POLE) and *t*-octylphenoxypolyethoxyethanol (Triton X-100) were supplied by Sigma (St. Louis, MO, USA). Cetyltrimethylammonium bromide (CTAB) was supplied by Aldrich (Beerse, Belgium).

Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA).

2.2. Instrumentation

The SPME fiber used was a 85 μm polyacrylate (PA) (Supelco, Bellefonte, PA, USA). The fiber was conditioned in the hot injector port of the GC according to the instructions given by the manufacturer: 2 h at 300 °C.

The identification and quantification of phenols were achieved using SPME and gas-chromatography/mass-spectrometry (GC/MS). GC/MS was performed on a Varian (Varian Inc., Palo Alto, CA, USA) model 3800 Varian Saturn 2000 GC/MS system, equipped with a 30 m × 0.25 mm i.d. WCOT CP-SIL-8 CB column (Chrompack) and equipped with a Varian autosampler (model 8200 CX). The Saturn GC/MS workstation 5.3 software was used for data acquisition.

For SPME analysis, an autosampler fiber holder (model 57331) from Supelco was used. In this system, it is necessary to use a 12-vial carousel, prepared for 10 mL vials (2-7389 from Supelco). The vials are always completely filled to leave no headspace. This SPME system incorporates an agitation mechanism consisting of a small motor and a cam to vibrate the needle. The fiber in this design works as a stirrer. The amber vials were capped with PTFE-coated septa.

The GC column was employed under the following temperature program: 60 °C, 4 min isothermal, 8 °C/min to 120 °C, 2 °C/min to 135 °C, and 8 °C/min to 280 °C. The carrier gas was helium, with a flow of 1 mL/min.

The temperature of the injector was maintained at 300 °C. The desorption time for the fiber in the GC injector was always 5 min. The temperature of the transfer line was maintained at 290 °C. The ionization was performed with a kinetic energy of the impacting electrons of 70 eV. The temperature of the ion trap was 200 °C. The MS analysis was carried out in scan mode with a mass range between 60 and 280 m/z (u). The quantitative determination was carried out using the mass values corresponding to the molecular ions of the different phenols (SIM mode).

The glassware used in this study was first washed with detergent and deionized water and then rinsed with deionized water, methanol (Merck), and a mixture of acetone/ethanol (1:1), both

from Merck. Finally, the nongraduated glassware and, especially, the sample vials were dried in an oven at 550 °C and wrapped with aluminum foil before use.

Reinserting the SPME fiber after the run did not show obvious carryover (less than 0.05%). Moreover, blanks were run periodically during the analysis to confirm the absence of contaminants.

The Statgraphic (Statistical Graphics, Rockville) software package version 4.2 was used for the statistical treatment.

2.3. Procedures

The absorption-time profiles were obtained by immersion of the PA fiber into 10 mL of an aqueous or micellar solution of phenols during different lengths of time. The profiles were obtained with 400 μg/L for 3-MeP, 1000 μg/L for 2,6-DMeP and 10 μg/L for the rest of phenols in Milli-Q water, and 0.5% (w/v) for the surfactant concentration when working with POLE, SDS and Triton X-100. The concentration for CTAB was 0.08% (w/v) with double concentration of phenols. It was necessary to work with higher concentration of phenols for CTAB (and also with lower concentrations of surfactant) in order to quantify the peaks adequately. Such increase in the phenols concentration also implied an increase in the percentage of organic solvent present in the CTAB solution (from 1.5 to 3%, v/v). The surfactants concentrations were always higher than their respective critical micelle concentration (cmc).

The studies for obtaining the partition coefficients were carried out at different concentrations of surfactants, keeping the agitation time of the PA fiber for 150 min. The concentration of phenols was the same as in the experiments to obtain the absorption-time profiles. The surfactants concentrations were varied from 0.02 to 1% (w/v) for POLE, from 0.05 to 1% (w/v) for Triton X-100, from 0.04 to 1% (w/v) for SDS, and from 0.05 to 0.2% (w/v) for CTAB.

The calibration curves of phenols in Milli-Q water were also obtained with an agitation time of the PA fiber of 150 min.

3. Results and discussion

This work was focused on the study of the interactions between phenolic compounds and surfactants, with the aid of a polyacrylate SPME fiber. The chromatographic behaviour of phenols is assumed to be independent of dissolving medium. In this sense, the experimental conditions to achieve the chromatographic resolution of phenols must be the same for phenols dissolved in an aqueous or in a micellar medium.

To investigate this assumption a SPME study of phenolic compounds in water was conducted. These experiments are developed not only to enable the comparison of the results between both media but also to obtain the appropriate calibration curves in water. These calibration curves will allow obtaining the free concentration of phenols in the aqueous phase in equilibrium with the micellar phase. This free concentration is necessary for the partition coefficients calculations. In this sense, the optimum extraction time for the fiber must be carefully selected because the calibration curves will be obtained using this extraction time. Any compound that does not reach the equilibration at the selected time when using micellar media cannot be included in the partition coefficients calculations.

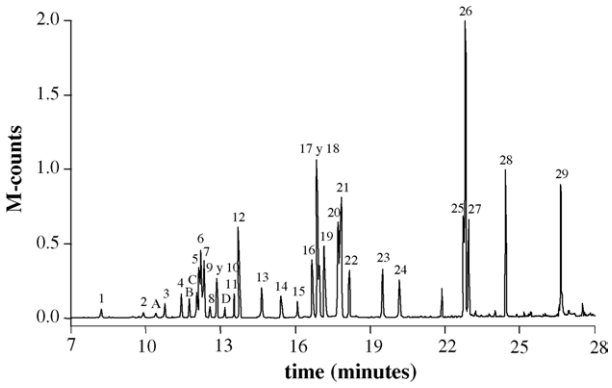

Fig. 1. Chromatogram for the studied phenols in Milli-Q water, using an agitation time of 150 min for the PA fiber. Assignment of peaks numbers as in Table 1. The overlapped compounds are: (A) 3-MP + 4-MP; (B) 2,4-DMP + 2,5-DMP; (C) 3-EP + 3,5-DMP; and (D) 3-CP + 4-CP.

3.1. SPME-GC–MS of phenols dissolved in aqueous medium

3.1.1. Chromatographic separation of phenols

The chromatographic conditions were optimized to achieve a good resolution of as many phenols as possible. Fig. 1 shows a representative chromatogram of the studied phenols under the chromatographic conditions described in Section 2. The chromatogram was obtained with an agitation time of 150 min for the PA fiber and with a concentration of 7.5 μg/L for 2-EP and Eu, 12 mg/L for 3-MeP, 64 mg/L for 2,6-DMeP and 10 μg/L for the rest of phenols, being all of them dissolved in Milli-Q water.

It was not possible to achieve a chromatographic resolution between the 3-chlorophenol (3-CP) and the 4-chlorophenol (4-CP). In addition, these compounds generate the same ions into the detection system and therefore, the peak is expressed as 3-CP + 4-CP. The same problems appeared with the pairs of 2,4-dimethylphenol (2,4-DMP) and 2,5-dimethylphenol (2,5-DMP), with 3-ethylphenol (3-EP) and 3,5-dimethylphenol (3,5-DMP), and with 3-methyphenol (3-MP) and 4-methylphenol (4-MP). All these overlapped compounds coming from standard-mix solutions generate the same ions and therefore, they were not included in this study. On the other hand, the phenol (P) did not have enough sensitivity to be accurately detected under these experimental conditions.

3.1.2. Characterization of the SPME of phenols in aqueous medium

In SPME, the determination of the equilibration time for a given analyte is done by constructing an absorption-time profile by exposing the fiber to an analyte solution of the same concentration for different lengths of time. It is considered that the equilibration has been reached when the amount of an analyte adsorbed by the fiber (in terms of chromatographic response) stops increasing. The fiber was immersed into aqueous stan-

Table 1
SPME equilibration times for the studied phenols in different media

Compound	Equilibration time (min)				
	Water	POLE	Triton X-100	SDS	CTAB
1. 2-Chlorophenol (2-CP)	120	100	90	90	100
2. 2-Methylphenol (2-MP)	150	110	90	90	110
3. 2,6-Dimethylphenol (2,6-DMP)	100	110	100	90	130
4. 2-Ethylphenol (2-EP)	120	120	100	90	120
5. 2,5-Dichlorophenol (2,5-DCP)	100	150	N.D.	90	N.D.
6. 2,3-Dichlorophenol (2,3-DCP)	120	120	N.D.	120	N.D.
7. 2,4-Dichlorophenol (2,4-DCP)	120	120	N.D.	120	N.D.
8. 3,4-Dimethylphenol (3,4-DMP)	120	120	110	N.D.	90
9. 2,4,6-Trimethylphenol (2,4,6-TMP)	120	110	120	90	120
10. 2,6-Dichlorophenol (2,6-DCP)	120	120	120	90	100
11. 2,3,6-Trimethylphenol (2,3,6-TMP)	120	120	110	90	120
12. 3-Methoxyphenol (3-MeP)	150	120	110	80	120
13. 2,3,5-Trimethylphenol (2,3,5-TMP)	120	100	150	90	150
14. 4-Chloro-3-methylphenol (4-C-3-MP)	120	120	100	80	120
15. 3,4,5-Trimethylphenol (3,4,5-TMP)	140	120	150	90	150
16. 2,6-Dimethoxyphenol (2,6-DMeP)	180	120	120	90	120
17. 2,3,5-Trichlorophenol (2,3,5-TCP)	120	120	150	90	N.D.
18. Eugenol (Eu)	120	110	150	90	120
19. 2,4,5-Trichlorophenol (2,4,5-TCP)	120	120	100	120	N.D.
20. 2,4,6-Trichlorophenol (2,4,6-TCP)	140	150	120	80	N.D.
21. 2,3,4-Trichlorophenol (2,3,4-TCP)	120	150	150	120	N.D.
22. 2,3,6-Trichlorophenol (2,3,6-TCP)	>250	100	100	90	N.D.
23. 3,5-Dichlorophenol (3,5-DCP)	120	120	120	100	90
24. 3,4-Dichlorophenol (3,4-DCP)	100	120	120	90	90
25. 2,3,5,6-Tetrachlorophenol (2,3,5,6-TeCP)	>250	150	150	90	N.D.
26. 2,3,4,5-Tetrachlorophenol (2,3,4,5-TeCP)	120	>250	150	90	N.D.
27. 2,3,4,6-Tetrachlorophenol (2,3,4,6-TeCP)	>250	100	120	90	N.D.
28. 3,4,5-Trichlorophenol (3,4,5-TCP)	120	>250	150	90	90
29. Pentachlorophenol (PCP)	>250	>250	>250	90	N.D.

N.D., non-detected. The compound cannot be accurately quantified in this medium.

dard solutions of phenols with stirring at room temperature ($25 \pm 2\,^{\circ}C$) for a period of time that was increased from 10 to 250 min, as described in the experimental section. Table 1 shows the obtained equilibration times for the studied compounds. In general, phenols showed equilibration times between 120 and 150 min. An extraction time of 150 min was finally selected to ensure that all compounds will reach equilibration within this time with the exception of 2,3,6-TCP, 2,3,5,6-TeCP, 2,3,4,6-TeCP and PCP, which require longer equilibration times. It should be noticed that these four phenols have low values of p*K*a (between 4.68 and 6.06). It is possible that a considerable fraction of these phenols is present as ionic form in solution.

Table 2 illustrates the retention times, linearity of the calibration curves and the limits of detection for the studied phenols in water using an extraction time of 150 min. For quantitative analysis, it is not necessary for the analytes to reach equilibrium. It should be remarked that all these phenols were extracted without adjustment of the pH or the ionic strength. It is well-known that the efficiencies of these compounds are affected by the solution pH or the ionic strength [16]. Nevertheless, the obtained detection limits (LODs) fulfil our purposes and no extra effort was made to improve them, in order not to introduce more experimental variables that can affect to the equilibria.

3.2. SPME of phenols in micellar media

Similar studies were then carried out with micellar media. Four different surfactants were selected in order to compare the results obtained between cationic (CTAB), anionic (SDS), and nonionic (Triton X-100 and POLE) micellar media.

3.2.1. Equilibration times and sensitivity indexes

The absorption-time profiles were obtained for the studied phenols in micellar media as it has been described in the experimental section. Fig. 2 shows representative profiles obtained with Triton X-100 and CTAB for some phenols. This study was carried out to determine the equilibration time for each compound in each micellar media. Table 1 summarizes the obtained results. For some compounds, the extraction efficiency was dramatically deteriorated so they could not be quantified appropriately.

A high decrease in the equilibration times with micellar media was observed when studying PAHs [10]. Nonetheless, this effect was not so noticeable with phenols, except when using the anionic surfactant SDS. The equilibration times are around 90 min for the SDS, with extreme values between 80 and 120 min, showing a higher decrease if compared with water (up to 90 min). However, the equilibration times are around 120–150 min for CTAB, Triton X-100 and POLE.

Table 2
Retention times, linearity, limits of detection, and p*K*a values for the studied phenols in water

Compound	Retention time (min) ± SD[a]	R^2	Linearity range (ng/mL)	LOD[b] (ng/mL)	p*K*a[c]
2-CP	8.22 ± 0.05	0.995	1–12	0.8	8.50
2-MP	9.95 ± 0.17	0.997	2–30	1.8	10.31
2,6-DMP	10.76 ± 0.06	0.998	2–30	1.3	10.66
2-EP	11.53 ± 0.13	0.997	2–30	1.4	10.27
2,5-DCP	12.22 ± 0.05	0.996	1–12	0.7	7.53
2,3-DCP	12.28 ± 0.05	0.995	1–12	0.5	7.53
2,4-DCP	12.31 ± 0.04	0.991	1–12	1.2	8.05
2,6-DCP	12.81 ± 0.03	0.990	1–12	1.3	7.02
3,4-DMP	12.83 ± 0.16	0.996	2–30	1.8	10.38
2,4,6-TMP	12.84 ± 0.07	0.998	2–30	1.3	10.97
2,3,6-TMP	13.51 ± 0.08	0.998	2–30	1.5	10.77
3-MeP	13.72 ± 0.14	0.997	20–250	21.8	9.58
2,3,5-TMP	14.70 ± 0.14	0.997	2–30	1.7	10.53
4-C-3-MP	15.47 ± 0.07	0.994	1–12	1.3	9.63
3,4,5-TMP	16.12 ± 0.15	0.998	2–30	1.5	10.51
2,6-DMeP	16.67 ± 0.09	0.996	30–350	16.0	9.97
2,3,5-TCP	16.79 ± 0.13	0.990	1–12	0.8	6.57
Eu	16.90 ± 0.10	0.997	2–30	1.7	10.29
2,4,5-TCP	17.11 ± 0.05	0.993	1–12	1.0	7.10
2,4,6-TCP	17.68 ± 0.13	0.994	1–12	1.0	6.59
2,3,4-TCP	17.82 ± 0.12	0.993	1–12	0.9	7.10
2,3,6-TCP	18.17 ± 0.06	0.994	1–12	0.8	6.06
3,5-DCP	19.48 ± 0.19	0.995	1–12	1.1	8.04
3,4-DCP	20.18 ± 0.16	0.992	1–12	0.9	8.55
2,3,5,6-TeCP	22.84 ± 0.07	0.994	1–12	0.8	5.09
2,3,4,5-TeCP	23.01 ± 0.05	0.991	1–12	1.1	6.15
2,3,4,6-TeCP	23.14 ± 0.11	0.992	1–12	1.1	5.63
3,4,5-TCP	25.09 ± 0.10	0.992	1–12	1.1	7.61
PCP	26.88 ± 0.13	0.988	1–12	0.8	4.68

[a] $n = 150$.
[b] LODs calculated by three times the error of the estimate divided by the slope.
[c] Calculated using Advanced Chemistry Development (ACD/Labs) Software Solaris V4.67 (©1994–2005 ACD/Labs).

In order to ensure that the equilibration times were not dependent on the concentration of the surfactant, different absorption-time profiles were carried out with two concentrations of surfactants (both concentrations higher than the cmc). Fig. 3 shows a representative example for 2,6-DCP in presence of 0.02 and 0.50% (w/v) of POLE. These experiments ensured that the equilibration times for all phenols were the same independently of the surfactant concentration used when constructing the absorption-time profile plots. The only difference emerged from the sensitivity, that is, the lower concentration of surfactant presents the higher peak areas.

Several experiments have been carried out to establish the influence in the extraction efficiency of phenols by the surfactant concentration in solution. The chromatographic signals of phenols have been measured at different surfactant concentrations in solution, keeping constant the rest of experimental conditions (as have been described in Section 2).

There was an exponential decrease in the extraction efficiency of phenols for the fiber as the surfactant concentration increases in solution. This behaviour was also observed when extracting PAHs [17]. Such decrease of the free analyte concentration in the presence of each surfactant is attributed to a partitioning

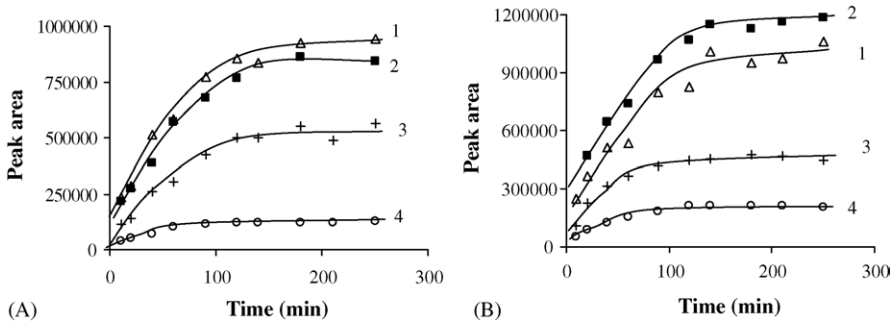

Fig. 2. Absorption-time profiles with micellar media. (A) Triton X-100 and (B) CTAB. The represented phenols are: (1) 4-C-3-MP; (2) 2,4,6-TMP; (3) 3,4-DCP; and (4) 2-MP. Experimental conditions as described in the text.

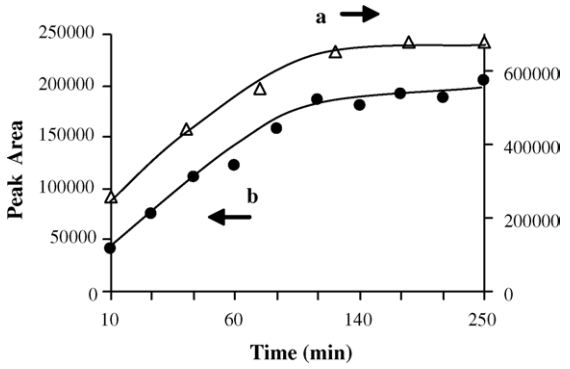

Fig. 3. Comparison of the absorption-time profiles for 2,6-DCP in POLE (A) 0.02% (w/v) and (B) 0.50% (w/v).

of the analyte between two phases, the water and the micellar phase [10]. Fig. 4 shows some examples. It is observed that the magnitude of the decrease depends on the surfactant and the compound studied. This decrease in the extraction efficiency is not too significant for some phenols, and there are exponential decreases for other phenols, which can be related to the characteristics of the phenols and to the surfactant nature. Obviously, the different behaviours are highly dependent on the different interactions phenols-micelle, which will condition the free phenol concentration in solution available for the SPME fiber. In addition, these results may also indicate that the potential of the fiber to extract phenols decreases as the surface of the coating fiber is occupied by monomers of surfactant, and therefore, it is surfactant- and fiber coating-dependent.

The comparison of sensitivities between water and the micellar media can be expressed by the sensitivity index SI = (maximum area in water/μg phenol)/(maximum area in the micellar media/μg phenol), both areas obtained at the equilibration time or at the maximum extraction time studied. Table 3

Table 3
Sensitivity indexes for phenols in different media

Compound	(Maximum area/μg phenol)$_{water}$/(maximum area/μg phenol)$_{micellar\ media}$			
	POLE 0.5%	Triton X-100 0.5%	SDS 0.5%	CTAB 0.08%
2-CP	2.62	1.07	1.82	1.53
2-MP	1.47	1.08	1.49	1.31
2,6-DMP	1.78	0.82	1.50	1.32
2-EP	–	1.14	1.64	1.53
2,5-DCP	12.93	–	6.74	15.39
2,3-DCP	7.61	–	3.44	–
2,4-DCP	9.09	–	1.28	–
3,4-DMP	1.48	0.97	–	1.41
2,4,6-TMP	–	1.14	2.29	1.63
2,6-DCP	2.79	1.35	1.81	19.28
2,3,6-TMP	–	1.09	2.06	1.52
3-MeP	0.91	0.85	1.17	1.00
2,3,5-TMP	3.19	1.14	2.34	1.75
4-C-3-MP	10.85	1.43	7.97	2.54
3,4,5-TMP	–	1.01	2.26	1.71
2,6-DMeP	0.76	0.82	1.03	0.93
2,3,5-TCP	36.00	4.12	8.56	398.26
Eu	3.13	1.72	3.88	2.38
2,4,5-TCP	8.68	1.47	1.95	57.98
2,4,6-TCP	40.29	7.90	9.74	–
2,3,4-TCP	25.11	2.46	5.49	–
2,3,6-TCP	9.20	1.48	2.05	–
3,5-DCP	17.15	2.19	3.73	7.38
3,4-DCP	13.44	2.13	2.96	5.37
2,3,5,6-TeCP	11.61	2.58	5.01	–
2,3,4,5-TeCP	24.50	7.94	9.79	–
2,3,4,6-TeCP	35.68	4.37	2.90	–
3,4,5-TCP	56.20	4.22	8.80	25.74
PCP	10.38	–	6.20	–

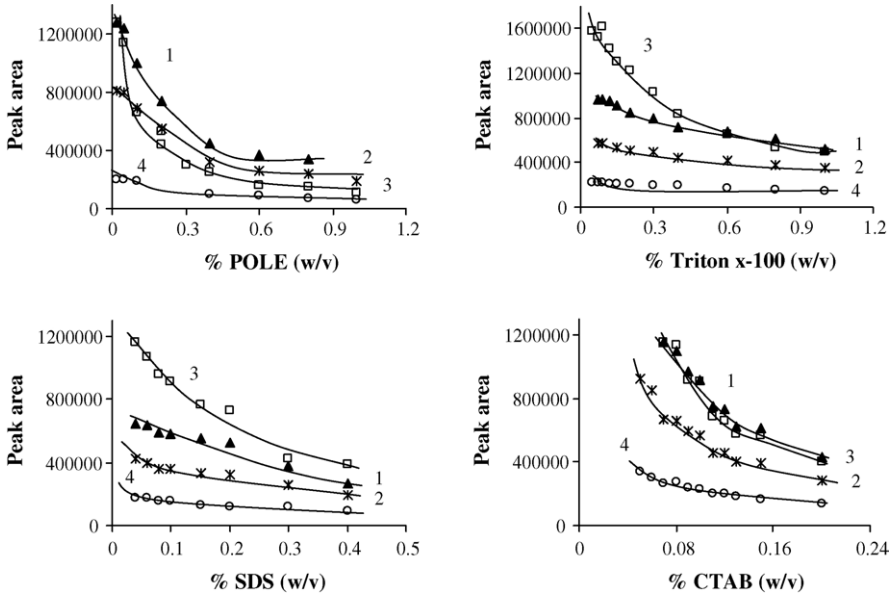

Fig. 4. Extraction efficiencies of some phenols when using different micellar media. (1) 2,4,6-TMP; (2) 2-EP; (3) 4-C-3-MP; and (4) 2-CP. Experimental conditions as described in the text.

shows those relationships. It should be noticed that the experiments with CTAB were carried out with a surfactant concentration of 0.08% (w/v), whereas the experiments with POLE, Triton X-100 and SDS were carried out with a surfactant concentration of 0.50% (w/v). All these surfactant concentrations are higher than the cmc.

The SI values vary considerably depending on the nature and position of the substituents in the phenolic compounds, as well as the nature of the surfactant. The methoxy-phenols have SI values similar or lower than the unity, independently of the surfactant used. For these compounds, the MSPME generates the same or higher signals than the ones obtained in conventional aqueous SPME. The alkyl-phenols have SI values between 0.8 and 3.2, being the lowest values the corresponding to Triton X-100.

The maximum differences in the sensitivity indexes correspond to the chloro-phenols. These compounds have values between 1 and 2 for mono- and di-chlorosubstitued phenols in Triton X-100, and values higher than 20 for the majority of tri- and tetra-chlorosubstitued phenols in POLE and CTAB. In general, the lowest SI values are obtained with Triton X-100, and the highest values are obtained with POLE. Considering the number of substituents, the SI values tend to increase when increasing the number of chlorine atoms in the molecule. For the same surfactant and the same number of chlorine atoms in the compound, some differences can also be observed depending on the position of the chlorine atom in the molecule. In this sense, the lowest SI values for dichlorophenols correspond to 2,6-DCP for the nonionic surfactants, whereas the lowest SI values for trichlorophenols correspond to 2,4,5- and 2,3,6-TCP, for all surfactants.

These SI values show the competitions that are being established in the equilibria where the phenols are taking part. The amount of phenols extracted by the SPME fiber strongly depends on the affinity of the phenol by the micellar media. That is to say, it depends on the interactions that are occurring between the phenols and the cores or the head-groups of the micelles. It is especially significant the results obtained with the cationic surfactant CTAB. The decrease in the extraction efficiency when using this medium is so high that avoids the adequate identification of a high number of phenols. Therefore, the high affinity for such phenols and the CTAB can be justified by electrostatic interactions phenolate-micelle in the head groups, altogether with hydrophobic interactions with the core of the micelles. Not only must the cationic nature of the surfactant be considered but also the acid–base equilibria of phenols. The obtained results can be analyzed using the p*K*a values in water [18]. The twelve phenols non-detected in this study when using CTAB, have p*K*a values between 4.68 and 8.05, that is, they have a strong acidity between phenols. On the other hand, phenols with the lowest acidity, with p*K*a values higher than 8.05, are extracted in quantities higher than the quantification limit. The phenols that are only ionized in alkaline media are the ones that less strongly interact with the CTAB, and therefore are easily extracted by the PA fiber. The exceptions are 3,5-DCP, 2,6-DCP and 3,4,5-TCP, which are detected despite their p*K*a values are between 7.02 and 8.04.

The predominant interactions must be others with the rest of surfactants. The interactions can include electrostatic repulsion when using SDS and consequently, less affinity solute-micelle. For nonionic surfactants, the predominant interactions must include the influence of the hydrocarbon chains of the micelle cores. According to the equilibration times, there are no significant differences between the values obtained in Triton X-100 or POLE. However, the SI values indicate a higher affinity of phenols for the POLE micelles.

3.2.2. Obtaining partition coefficients

In a micellar medium, the partition coefficient of an analyte is defined as the relationship between its concentrations in the micellar and in the aqueous phase. It is assumed that in the presence of micellar media, the analytes retained in the fiber are coming from the aqueous phase, and the micellar concentration is substituted by the surfactant concentration in solution. It is also assumed that the concentration of the analyte in the headspace is negligible. So, to determine the free concentration of an analyte ($C_{W,m}$ values), we must use the calibration curves of phenols in aqueous solution at a fixed exposure time of the SPME fiber.

In a previous work [10], we proposed the following equation:

$$\frac{1}{C_{W,m}} = \frac{1}{C_{total,m}} + \frac{K_{M,m}}{C_{total,m}} C_M$$

to determine the analyte partition coefficients between water and micellar media, being C_M the surfactant concentration in solution; $C_{W,m}$ the analyte concentration in water (measured by SPME); and $C_{total,m}$ the total analyte concentration.

Plotting $1/C_{W,m}$ versus C_M (surfactant concentration) must be a straight line, with the slope related to the phenol-micelle partition coefficient ($K_{M,m}$). Some of these plots are shown in Fig. 5. For all phenols, we obtained a linear relationship with correlation coefficients (R) varying between 0.92 and 0.99, as observed from the data in Table 4. The obtained intercepts were close to the theoretical values, that is, the inverse of the initial total concentration of each phenol, in practically all cases. The correlations between the theoretical and the experimental intercepts were statistically significant at the 99% level for all surfactants ($R^2 = 0.934$). Considering each surfactant independently, these correlations were significant statistically at the 99% level for each one, having correlation coefficients (R) of 0.999, 0.999, 0.999 and 0.830 for POLE, Triton X-100, SDS and CTAB, respectively. These good correlations support the theoretical considerations adopted.

The obtained $K_{M,m}$ values are shown in Table 5. The presence of an empty space in this table can be due to two reasons: the equilibration time was not reached by the phenol during the exposition time of the fiber (and so, the model cannot be applied), or the peak-area was not high enough to quantify the compound adequately. The agreement between these $K_{M,m}$ values and the available bibliographic values [19] is adequate. We found a reduced number of phenols with reported bibliographic values, and even in these cases, not for all the surfactants studied. These bibliographic values are included in Table 5. In general, the reported bibliographic values for phenols were obtained at

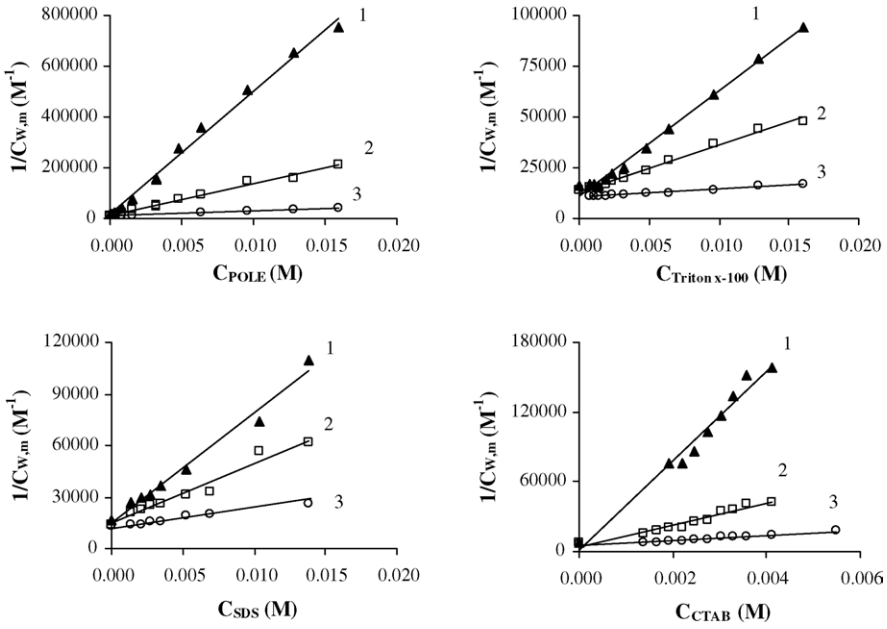

Fig. 5. Plots obtained when representing $1/C_{W,m}$ vs. C_M for different micellar media being (1) 3,5-DCP; (2) 4-C-3-MP; and (3) 2-CP.

Table 4
Correlations and intercepts obtained when plotting $1/C_{W,m}$ vs. C_M for different micellar media and the phenols studied

Compound	Theoretical intercept	SDS		Triton X-100		POLE		Theoretical intercept	CTAB	
		Intercept ± SD[a]	*R*	Intercept ± SD[a]	*R*	Intercept ± SD[a]	*R*		Intercept ± SD[a]	*R*
2-CP	12800	11627 ± 558	0.99	10443 ± 129	0.99	11429 ± 482	0.99	6400	4830 ± 467	0.98
2-MP	1.08×10^7	$(1.46 \pm 0.10) \times 10^7$	0.99	$(1.10 \pm 0.01) \times 10^7$	0.99	$(1.11 \pm 0.01) \times 10^7$	0.99	0.54×10^7	$(0.51 \pm 0.02) \times 10^7$	0.99
2,6-DMP	1.22×10^7	$(1.09 \pm 0.12) \times 10^7$	0.99			$(1.06 \pm 0.05) \times 10^7$	0.98	0.61×10^7	$(0.49 \pm 0.05) \times 10^7$	0.97
2-EP	1.27×10^7	$(0.73 \pm 0.21) \times 10^7$	0.98	$(0.98 \pm 0.14) \times 10^7$	0.99	$(0.78 \pm 0.11) \times 10^7$	0.97	0.64×10^7	$(0.13 \pm 0.05) \times 10^7$	0.99
2,5-DCP	16200	12895 ± 3531.7	0.99			13037 ± 2976	0.99			
2,3-DCP	16200	21279 ± 2018.6	0.99			20399 ± 4585	0.99			
2,4-DCP	16200	12332 ± 1060.5	0.97			16890 ± 5461	0.99			
3,4-DMP	1.22×10^7			$(1.48 \pm 0.58) \times 10^7$	0.96	$(1.04 \pm 0.12) \times 10^7$	0.98	0.61×10^7	$(0.30 \pm 0.09) \times 10^7$	0.97
2,4,6-TMP	1.36×10^7	$(1.49 \pm 0.25) \times 10^7$	0.95	$(1.40 \pm 0.31) \times 10^7$	0.99	$(1.11 \pm 0.13) \times 10^7$	0.99	0.68×10^7	$(0.22 \pm 0.14) \times 10^7$	0.97
2,6-DCP	16200	11971 ± 4093.9	0.99	14719 ± 405	0.99	13806 ± 1115	0.99			
2,3,6-TMP	1.36×10^7	$(1.58 \pm 0.12) \times 10^7$	0.98	$(1.42 \pm 0.25) \times 10^7$	0.98	$(1.14 \pm 0.12) \times 10^7$	0.99	0.68×10^7	$(0.31 \pm 0.11) \times 10^7$	0.97
3-MeP	309923	396949 ± 13984	0.99	340844 ± 4408	0.96	298450 ± 11087	0.95	154961	148220 ± 7498	0.97
2,3,5-TMP	1.36×10^7	$(1.50 \pm 0.20) \times 10^7$	0.97	$(1.36 \pm 0.03) \times 10^7$	0.99	$(1.15 \pm 0.13) \times 10^7$	0.99	0.68×10^7	$(0.04 \pm 0.19) \times 10^7$	0.97
4-C-3-MP	14200	14493 ± 1698.5	0.98	13039 ± 515	0.99	14094 ± 3388	0.99	7100	3390 ± 1971	0.97
3,4,5-TMP	1.36×10^7	$(1.60 \pm 0.17) \times 10^7$	0.97	$(1.36 \pm 0.02) \times 10^7$	0.99	$(1.17 \pm 0.11) \times 10^7$	0.99	0.68×10^7	$(0.17 \pm 0.16) \times 10^7$	0.97
2,6-DMeP	154000	199964 ± 10306	0.99			156150 ± 2315	0.97	77000	84305 ± 4513	0.96
2,3,5-TCP	19600			4720 ± 2755	0.98	16227 ± 25209	0.99			
Eu	1.57×10^7	$(1.39 \pm 0.08) \times 10^7$	0.96			$(1.00 \pm 0.15) \times 10^7$	0.98	0.78×10^7	$(0.33 \pm 0.13) \times 10^7$	0.97
2,4,5-TCP	19600			12282 ± 1434	0.97	18656 ± 4079	0.99			
2,4,6-TCP	19600	12286 ± 5151	0.99	7993 ± 3230	0.99	30770 ± 11154	0.99			
2,3,4-TCP	19600	12836 ± 5413	0.96	11703 ± 2119	0.97	4132 ± 16330	0.99			
2,3,6-TCP	19600	20854 ± 1940	0.92	11426 ± 1551	0.96	18780 ± 4232	0.99			
3,5-DCP	16200	15164 ± 2278	0.99	10885 ± 924	0.99	15254 ± 10225	0.99	8100	1170 ± 6944	0.98
3,4-DCP	16200	9499 ± 3626	0.98	10577 ± 1414	0.99	18181 ± 5024	0.99	8100	2921 ± 3209	0.97
2,3,5,6-TeCP	23000	9299 ± 8504	0.99	8596 ± 5998	0.97	23808 ± 1726	0.99			
2,3,4,5-TeCP	23000	33806 ± 34887	0.99	3949 ± 1309	0.99					
2,3,4,6-TeCP	23000	11901 ± 8678	0.99	7015 ± 4578	0.99					
3,4,5-TCP	19600	14467 ± 3827	0.98	8496 ± 3058	0.98			9800	2150 ± 7873	0.99
PCP	26400	30085 ± 6117	0.99							

[a] $n = 9$.

Table 5
Obtained partition coefficients for the phenols studied in different micellar media

Compound	$K_{M,m} \pm SD$[a]				$K_{bibliography}$[b]
	SDS	CTAB	Triton X-100	POLE	
2-CP	101.6 ± 2.9	356.2 ± 24.5	32.4 ± 1.4	143.6 ± 4.5	26_{SDS}[c]
2-MP	160.1 ± 6.5	532.3 ± 14.5	35.4 ± 1.7	51.3 ± 1.4	84_{SDS}[d], 120.2_{SDS}[i]
2,6-DMP	167.8 ± 6.9	348.1 ± 26.3		89.0 ± 5.6	
2-EP	175.3 ± 11.2	549.3 ± 24.6	34.5 ± 1.4	113.1 ± 11.1	$33_{Brij\ 30}$[e]
2,5-DCP	532.3 ± 15.5			1129.1 ± 25.9	
2,3-DCP	328.2 ± 9.0			788.4 ± 33.8	
2,4-DCP	109.2 ± 9.8			651.8 ± 47.0	97_{SDS}[f], 3847_{CTAB}[g]
3,4-DMP		689.9 ± 48.3	109.1 ± 11.1	150.8 ± 11.9	685_{CTAB}[h]
2,4,6-TMP	229.1 ± 27.4	845.2 ± 67.8	67.7 ± 3.1	235.2 ± 15.0	
2,6-DCP	365.6 ± 17.9		121.4 ± 4.3	264.1 ± 9.3	
2,3,6-TMP	159.2 ± 13.1	709.0 ± 56.4	43.3 ± 2.5	265.7 ± 13.8	
3-MeP	57.6 ± 3.1	234.4 ± 16.2	11.9 ± 1.6	37.8 ± 4.5	41.7_{SDS}[i] (4-MeP)
2,3,5-TMP	247.0 ± 22.0	1155.0 ± 91.7	73.1 ± 2.8	379.0 ± 12.2	
4-C-3-MP	245.0 ± 17.9	1351.3 ± 105.4	162.8 ± 5.1	878.3 ± 32.4	
3,4,5-TMP	219.3 ± 18.4	1037.9 ± 77.6	55.2 ± 2.3	246.3 ± 10.1	
2,6-DMeP	106.2 ± 4.6	231.1 ± 20.5		20.8 ± 2.4	
2,3,5-TCP			628.3 ± 54.3	4929.5 ± 174.5	
Eu	80.5 ± 9.7	688.8 ± 56.9		196.9 ± 15.5	
2,4,5-TCP			150.7 ± 12.6	934.3 ± 28.2	138_{SDS}[f]
2,4,6-TCP	670.4 ± 39.5		1013.9 ± 52.7	2384.0 ± 75.1	
2,3,4-TCP	437.3 ± 57.5		358.2 ± 34.6	6458.4 ± 251.9	
2,3,6-TCP	83.4 ± 14.5		135.1 ± 13.7	716.9 ± 29.3	
3,5-DCP	393.8 ± 21.2	4752.1 ± 303.6	319.7 ± 8.1	2996.1 ± 85.6	122_{SDS}[f]
3,4-DCP	499.6 ± 33.7	1820.0 ± 162.6	362.3 ± 12.3	2030.8 ± 42.1	
2,3,5,6-TeCP	448.2 ± 20.1		1001.8 ± 114.1	1760.9 ± 22.7	
2,3,4,5-TeCP	1895.5 ± 81.3		272.9 ± 17.1		
2,3,4,6-TeCP	747.1 ± 50.6		951.5 ± 39.6		
3,4,5-TCP	434.4 ± 40.7	7466.1 ± 383.3	696.4 ± 49.9		
PCP	2318.4 ± 43.6				

[a] $n = 9$.
[b] Obtained by micellar liquid chromatography (MLC) [19].
[c] Obtained by MLC with 3% (v/v) of 2-propanol at 40 °C.
[d] Obtained by MLC with no additives in the mobile phase at 25 °C.
[e] Obtained by MLC with 60% acetonitrile in the mobile phase.
[f] Obtained by MLC with no additives in the mobile phase at 40 °C.
[g] Obtained by MLC with no additives in the mobile phase at 35 °C.
[h] Obtained by MLC with no additives in the mobile phase for 3,5-DMP.
[i] From ref. [20].

temperatures higher than 25 °C and using micellar media with organic modifiers. Therefore, these bibliographic values are not strictly comparable with our values.

In general, considering the surfactant nature, when developing a statistical ordering of the obtained partition coefficients by magnitude (specifically, for all partition coefficients of the mono-, di- and tri-substituted phenolic compounds), the following order is found: CTAB ∼ POLE > Triton X-100 ∼ SDS (the ">" symbol denoting statistical differences at the 95% confidence level). A similar trend is observed for the average partition coefficients obtained for all the chlorinated phenols. On the other hand, the statistical order was CTAB > POLE ∼ SDS > Triton X-100 for the methylated phenols.

The partition coefficients for the chlorinated phenols are significantly higher than the partition coefficients for the methylated phenols at the 99% confidence level when including all the partition coefficients values obtained with all the surfactants studied. There was not a statistical increase of the partition coefficient in a specific surfactant when increasing the number of the substituents in the phenolic compound.

Considering the position of the substituent and keeping constant the number of substituents, the chlorophenols without the chlorine-substituent in an *ortho*-position had significant higher partition coefficients than those with the chlorine-substituent in an *ortho*-position, at the 95% confidence level for practically all the surfactants studied (the exception was the SDS). That is, 3,4- and 3,5-DCP have higher partition coefficients than 2,5-, 2,3-, 2,4- and 2,6-DCP for each surfactant studied.

It was possible to find two classifications when developing a discriminant analysis with the partition coefficients of the eleven phenols that have partition coefficients values in all the surfactants. Considering the number of substituents in the phenolic compound, mono-, di- and tri-substituents were

Table 6
Comparison between the partition coefficients and the SI values for the studied phenols ($K_{M,m}$ vs. SI)

Surfactant	R	Intercept ± SD	Slope ± SD	n
POLE	0.97	−213.37 ± 101.74	143.54 ± 8.81	19
CTAB	0.84	109.00 ± 137.48	347.95 ± 64.35	14
SDS	0.81	95.02 ± 33.36	49.90 ± 8.29	21
Triton X-100	0.95	−83.34 ± 35.87	167.79 ± 13.10	20

separated and correctly classified at the 72.73% level, independently of the kind of substituent in the phenolic compound. Taking into account the kind of substituent in the phenolic compound, chloro-, methylated- and methoxylated- substituents were separated and correctly classified at the 90.91% level.

Finally, it is possible to obtain some kind of relationship between the sensitivity indexes values and the micelle-partition coefficients, despite their analytical meaning. Both parameters give information related with the equilibria between the analytes and the micellar media. In addition, both parameters make use of the chromatographic signals obtained from a SPME fiber introduced in a micellar solution. In this sense, the correlations between these parameters have been established, as it can be observed from Table 6. The obtained correlation coefficients were higher than 0.81. These correlations can indicate that, in each studied micellar media, there is a close relationship between both parameters. The obtained positive slopes support the fact that the analytes retained by the fiber in MSPME are coming from the aqueous phase. In general, it can be verified that high values of SI are related to high values of partition coefficients, and vice-versa.

It can also be observed that the obtained slopes are significantly higher than the unity. So, important differences can exist between the $K_{M,m}$ values for the different phenols in a micellar medium, whereas the differences in the SI values are not that high. In general, the extreme values for the phenols-micelles partition coefficients are related to CTAB and SDS, whereas there is not such a differentiation between the SI values. It can also be noted the similarities between the slopes of the nonionic surfactants.

4. Conclusions

The MSPME is an analytical technique that can be used for the determination of partition coefficients in micellar media. It has been successfully applied to the determination of phenols-micelles partition coefficients including 9 alkyl-, 18 chloro-, and 2 methoxy-phenols, and ionic or nonionic micelles. In addition, it should be noted the use of GC as an analytical tool to study the different equilibria in micellar media.

The obtained partition coefficients are dependent on the nature of the surfactant as well as the nature and position of the substituents in the phenolic compound. In general, the obtained partition coefficients were higher for chlorophenols, and always higher for CTAB when considering the surfactant nature.

The sensitivity indexes were proposed as an alternative parameter to measure the ability of MSPME to extract phenols from micellar media. These indexes were well correlated to the partition coefficients and therefore, they can be used to express the intensity of the interactions analytes-micelles. The sensitivity index is a parameter less rigorous than the partition coefficient itself, but its calculation is simpler.

The Micellar SPME is a technique that can provide, for compounds like methoxyphenols, similar sensitivities than the ones obtained in conventional SPME. In addition, it is possible to obtain selective determination of methoxyphenols over alkyl- and chloro-phenols in any surfactant, or selective determination of non-acidic phenols over acidic phenols when working with CTAB. In general, the modification of experimental variables like pH, and nature and concentration of surfactants, can be the key to obtain sensitive and selective determinations.

The main applications of the proposed Micellar SPME method are expected to result from the combination of the micellar extraction of solid matrixes with the gas-chromatography. Therefore, solutes that are initially bound to solid matrixes can be solubilized in a micellar medium followed by a separation by SPME, and injection in a GC without further clean-up steps.

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