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Determination of polycyclic aromatic hydrocarbons in seawater by high-performance liquid chromatography with fluorescence detection following micelle-mediated preconcentration

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Abstract

In this work, the nonionic surfactant polyoxyethylene-10-lauryl ether has been used for the extraction and preconcentration of 14 polycyclic aromatic hydrocarbons, classified as priority pollutants by the US Environmental Protection Agency, from seawater samples. The cloud-point preconcentration previous separation by HPLC and quantification using fluorimetric detection and wavelength programming allow to determine these pollutants with detection limits ranging from 1.0 to $1.5 \cdot 10^2$ ng/l with RSDs better than 10.4%. The methodology is evaluated using well-established extraction and preconcentration methods and GC–MS. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds whose mutagenic and/or carcinogenic effects are well known. These substances can be produced in natural and anthropogenic processes and they can be found in many different kinds of samples, both biological and environmental. For this reason, their detection and monitoring has become an important problem and this has led to the development of new analytical methods with improved selectivity and sensitivity.

Determination of organic pollutants identities and their concentrations in surface water, ground water, seawater and waste water is an important topic in environmental analysis. Although many man-made

organic chemicals have been detected in the aquatic environment, petroleum hydrocarbons including *n*-alkanes and PAHs represent some of the most common pollutants found in water from the industrialized countries where petroleum products are used heavily.

The determination of PAHs in aqueous samples is rather difficult as their concentration in water is extremely low due to their low solubility. Besides, because PAHs tend to adsorb on walls and surfaces with they come into contact, serious losses often occur during sampling and storage [1–4].

The preconcentration and extraction of PAHs from water can be carried out by means of liquid–liquid extraction (LLE) [1,5,6] and solid-phase extraction (SPE) [7–10] techniques. LLE is a very useful technique, which has been used by the US Environmental Protection Agency (EPA). However, it is tedious, time consuming and requires large amounts

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of solvent. SPE is a less time consuming method, but it still requires toxic solvents for the elution step.

Recently, PAH extraction with surfactants based in the cloud-point phenomenon has been proposed [11–15]. The use of this methodology offers some advantages for the extraction of PAHs compared with solvent extraction: ability to concentrate PAHs with high recoveries and very high preconcentration factors; safety and cost benefits, very small amounts of the relatively nonflammable and nonvolatile surfactant are required; compatibility with micellar or hydroorganic mobile phase, etc.

In this work, we propose the use of polyoxyethylene-10-lauryl ether (POLE) for the extraction and preconcentration of PAHs from seawater samples, using the cloud-point methodology as a prior step to their chromatographic determination with fluorimetric detection and wavelength programming. The high saline concentration in the samples facilitates the phase separation.

This surfactant was selected owing to its favourable spectroscopic characteristics and its low chromatographic retention time, allowing the determination of the more polar PAHs without the need of a clean-up step to remove the surfactant.

The effect of POLE on the PAH adsorption on the container surface used for collecting the aqueous samples and its interaction with the organic matter and/or particulate matter present in the water has been also investigated.

The impossibility to dispose a reference material with PAHs in seawater has forced to validate our methodology using well-established extraction and preconcentration methods and gas chromatography–mass spectrometry (GC–MS).

2. Experimental

2.1. Reagents

Naphthalene (Nph) was supplied by Merck (Darmstadt, Germany); fluorene (Fl), phenanthrene (Phe), anthracene (A), fluoranthene (Ft), pyrene (Py), benz[*a*]anthracene [B(*a*)A], chrysene (Chy), benzo[*b*]fluoranthene [B(*b*)Ft], benzo[*a*]pyrene [B(*a*)Py], dibenz[*a,h*]anthracene [diB(*a,h*)A] and benzo[*ghi*]perylene [(B(*ghi*)Per)] were supplied by Aldrich

(Beerse, Belgium), benzo[*k*]fluoranthene [B(*k*)Ft] was supplied by Fluka (Buchs, Switzerland) and indeno[1,2,3-*cd*]pyrene [I(1,2,3-*cd*)Py] was supplied by Supelco (Bellefonte, PA, USA). They were dissolved in ethanol (Merck).

The standard solution of PAHs used for GC–MS was the PAH mixture 64 supplied by Dr. Ehrenstorfer (Augsburg, Germany), with a concentration of 2000 mg/l for each PAH.

POLE was supplied by Sigma (St. Louis, MO, USA).

For chromatographic analysis, acetonitrile of HPLC grade (Merck) and water purified with a Milli-Q system (Millipore, Bedford, MA, USA) were used throughout.

2.2. Artificial seawater

The preparation of the artificial seawater was made by direct weighing of analytical grade salts (Merck) as follows: 3 mg NaF, 20 mg SrCl₂·H₂O, 30 mg H₃BO₃, 100 mg KBr, 700 mg KCl, 1.470 g CaCl₂·2H₂O, 4.000 g Na₂SO₄, 10.780 g MgCl₂·6H₂O, 23.500 g NaCl, 20 mg Na₂SiO₃·9H₂O and 200 mg NaHCO₃ were dissolved in 1 l of distilled water [16].

2.3. Instrumentation

All measurements were made with a Waters (Milford, MA, USA) Model 600 Multisolvant Delivery System equipped with a Waters U6K sample injector and a Waters 474 Scanning fluorescence detector. Autoanalysis 2.4 (Sciware, vcerda@p01.uib.es) software was used for acquisition data. The analytical column was a Vydac 201TP54 reversed-phase C₁₈ column, 5 μm, 300 Å (25 cm×4.6 mm I.D.) with a μBondapak C₁₈ guard column, 10 μm, 125 Å (20 mm×3.9 mm I.D.) supplied by Waters.

The thermostatic bath Comfort CB 8-30 E from Heto and a calibrated Crison thermometer 638 Pt were also used.

The validation was performed by GC–MS (Varian 3800 and Varian Saturn 2000 apparatus) on a 30 m×0.25 mm I.D. column wall-coated open tubular (WCOT) CP-SIL-8 CB supplied by Chrompack (The Netherlands) and equipped with an Autosampler

Model 8200 CX. Saturn GC–MS Workstation 5.3 software was used for the acquisition of data.

2.4. Cloud-point determination and ratio of phases

The cloud-point was determined by observing the temperature required for the onset of turbidity upon heating 3.0 ml of the surfactant, previously dissolved in artificially prepared seawater, in a small test tube that had been placed in a thermostatic bath. The slope of the temperature change of the bath was about 0.9 °C/min. The phase diagram was obtained by measuring the cloud-point temperature as a function of surfactant concentration. The reported results were the average of triplicate measurements.

The ratio of the volumes of the aqueous phase to the surfactant-rich phase for different amounts of surfactant was determined by placing the surfactant solution into a tube and conducting the extraction during 90 min at 95 °C in order to lead the phase separation. Following phase separation, the volume of each phase was measured. The reported values were the average of triplicate determinations.

2.5. Cloud-point preconcentration

Appropriate aliquots of the solutions containing the analytes in the presence of 1.0% (w/v) surfactant solution, dissolved in artificial seawater, were kept in a thermostatically controlled bath for 90 min at 95 °C. The supernatant surfactant-rich phase was withdrawn using a micro-syringe.

2.6. Liquid chromatographic analysis

After the two phases have been separated, 25 µl of the surfactant-rich phase was injected into the chromatographic system. The HPLC method used for the separation and determination of PAHs consists of a gradient elution procedure with a fluorescence detector. As mobile phase a mixture of acetonitrile and water with a linear gradient from 55 to 100% of acetonitrile over 30 min and 100% acetonitrile for 10 min was used at a flow-rate of 1 ml/min. The wavelength program of the detector is shown in Table 1.

Table 1
Wavelength program of the detector used for the PAH determination

| Time (min) | Excitation (nm) | Emission (nm) | Compound |
|------------|-----------------|---------------|--------------------|
| 0.0 | 222 | 329 | (1) Nph |
| 10.7 | 260 | 332 | (2) Fl |
| 13.9 | 248 | 370 | (3) Phe |
| | | | (4) A |
| 17.7 | 275 | 419 | (5) Ft |
| | | | (6) Py |
| 22.1 | 273 | 384 | (7) B(a)A |
| | | | (8) Chy |
| 28.5 | 254 | 451 | (9) B(b)Ft |
| 32.2 | 288 | 406 | (10) B(k)Ft |
| | | | (11) B(a)Py |
| 35.9 | 289 | 422 | (12) diB(a,h)A |
| | | | (13) B(ghi)Per |
| 40.0 | 297 | 496 | (14) I(1,2,3-cd)Py |

2.7. Fuel-oil contaminated seawater and determination of PAHs

Fuel-oil contaminated seawater was prepared by shaking natural seawater with the fuel-oil during 1 h. The sample was then allowed to ageing overnight and after reaching the phase separation, the aqueous phase was taken.

The aqueous phases are immediately mixed with an adequate POLE concentration to obtain a final solution of 1% (w/v) in the surfactant. Then, aliquots of 3.0 ml are subjected to cloud-point extraction (CPE), and chromatographed as described above.

In order to evaluate the reliability of the methodology proposed for the preconcentration and separation of PAHs, these pollutants were analysed from spiked seawater samples in the same way as unspiked samples.

2.8. Evaluation of the methodology using conventional extraction (LLE) and GC–MS

The fuel-oil contaminate seawaters were extracted as described in EPA method 3510C using 1 l of seawater and 60 ml of cyclohexane. The extract was then concentrated to 1 ml in a Turbo Vap (Zymark) working at 40 °C with a nitrogen flow between 0.54 and 1.02 bar. Aliphatic and aromatic fractions were separated using a chromatographic column packed with Florisil (Aldrich), 5 g activated at 400 °C, as

described in EPA method 3620B. Aliphatic and aromatic fractions were eluted with *n*-hexane (Scharlau suprasolv grade) and *n*-hexane–dichloromethane (1:1) (Merck suprasolv grade), respectively. The aromatic fraction was then reduced to 1 ml in the Turbo Vap working at the conditions mentioned above.

The analysis of the extracts was achieved as described in EPA method 8270C using 3,6-dimethylphenanthrene as internal standard.

GC–MS was used under the following conditions: ion source, electron impact (70 eV); acquisition, selected ion monitoring (SIM) mode with a range of mass scanned between 50 and 300 u; transfer line temperature 280 °C; ion trap manifold temperature 65 °C; carrier gas, He; flow, 0.8 ml/min; linear velocity, 32.4 cm/s; injection, split/splitless mode; injection volume, 1 μ l.

The oven temperature was programmed from 40 °C for 2 min, 10 °C/min until 280 °C, held for 26 min.

The injector was programmed from 40 °C for 0.1 min, 200 °C/min until 300 °C, held for 48 min.

The quantitative determination was carried out using the mass values corresponding to the molecular ions of the different PAHs.

3. Results and discussion

3.1. Effect of saline concentration

Several authors have shown that electrolytes increase or decrease the cloud-point temperatures of nonionic surfactants. The relevant electrolyte concentrations for the above effect are usually high concentrations (exceeding 0.1 M) [17,18]. The observed effects of the electrolytes on the cloud-points of nonionic surfactants have been explained in terms of so-called salting-in and salting-out effects [16,17].

The effect of electrolytes on the cloud-point of POLE solutions was studied. It is observed that the addition of electrolytes, including NaCl, NaNO₃, MgCl₂ and Na₂SO₄, to micellar solutions of 0.1 M POLE (critical micelle concentration, CMC=0.09 mM) decreases the cloud-point temperature, as shown in Fig. 1. The decrease of the cloud-point in such solutions by added electrolytes is nearly a linear

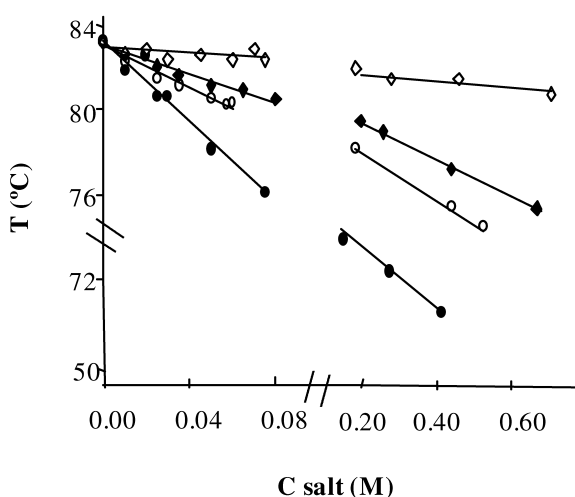


Fig. 1. Cloud-point temperature of POLE as a function of salt concentration (\diamond) NaNO₃, (\blacklozenge) NaCl, (\circ) MgCl₂ and (\bullet) Na₂SO₄.

function of the concentration. However, it is interesting to note that no significant effect on the cloud-point of POLE is detected for the addition of NaNO₃. The order in the cloud-point decrease is as follows: NaNO₃ < NaCl < MgCl₂ < Na₂SO₄.

Likewise, the results indicate that an increase in the ionic strength have not an appreciable influence on the final volume of the extracted surfactant-rich phase. Nevertheless, the addition of these salts facilitates the separation of the two phases, because it increases the density of the bulk aqueous phase.

Depending upon the density of the surfactant-rich phase in relation to that one of the aqueous phase, the surfactant-rich phase can be either the bottom or top layer. By addition of salts to the system, the density of the aqueous phase can be adjusted to some degree [19,20]. In some cases, it is found to be easier and more desirable to work with an upper surfactant-rich layer, thereby minimizing the possibility of cross-contamination of components from the corresponding aqueous phase [21]. Besides, the CPE procedure can be repeated by addition of more surfactant to the aqueous phase, in order to achieve higher extraction efficiency [22–24].

The seawater, as a result of its high saline concentration, should have this behaviour. That is, the studies carried out with different concentrations of POLE above its CMC in artificial seawater (as

described in the Experimental section) subjected to cloud-point extraction, show that the surfactant-rich phase was always the upper layer.

3.2. Phase ratio and diagrams

The cloud-point temperature of POLE solutions in artificial seawater varies between 67.5 and 74.6 °C for surfactant concentrations ranging between 0.5 and 6.0%. These values are about 14 °C less than the ones obtained with aqueous surfactant solution [25]. The cloud-point temperature is roughly constant (69.3–67.5 °C) in the range of concentrations 1–4%, thus facilitating experimentation. The phase diagram of POLE in artificial seawater is shown in Fig. 2A.

The separation of the two phases was accomplished by gravity settling. An equilibration temperature of 95 °C and an equilibration time of 90 min

were chosen as adequate for all analytes, according with our previous experiences [25].

The theoretical preconcentration factor (C_F) and the volume of the surfactant-rich phase (V_s) obtained as a function of POLE concentration are shown in Fig. 2B. The theoretical preconcentration factor was calculated as the ratio of the volume of solution used to the volume of the surfactant-rich phase. The theoretical preconcentration factor for POLE solutions at 1% (w/v) was 17.6, for which the volume of the surfactant-rich phase was 170 μl . This volume is easily handled with a micro-syringe and permits the injection of several aliquots into the chromatographic system.

3.3. Cloud-point preconcentration and liquid chromatographic analysis

Fig. 3A shows the obtained chromatogram of a standard mixture of PAHs under described experimental conditions. The different PAHs gave satisfactory retention times with RSD between 0.64 and 0.83%. The reproducibility of the chromatographic system was evaluated and relative standard deviations were between 1.50 and 8.42% for the different PAHs. Linear relationships between the peak-area and analyte concentration were found for all the studied compounds. The parameters of the least-squares fittings are shown in Table 2.

Detection limits were calculated as three times the standard deviation of the signal corresponding to a solution with PAHs concentration near to the lowest value of the linear range of each PAH to which the complete procedure (cloud-point preconcentration and chromatographic separation) was applied. As shown in Table 2, the detection limits oscillate between 1.0 ng/l for benzo[k]fluoranthene and $1.5 \cdot 10^2$ ng/l for fluoranthene. These values are in general 20 times lower than the ones obtained without the cloud-point preconcentration [26]. Furthermore, these limits can be improved considerably varying the surfactant concentration in the preconcentration step.

The relative standard deviations (RSDs) for six artificial seawater samples containing concentrations of each PAH corresponding at their limit of quantification to which the complete procedure was applied, are shown in Table 2. Fig. 3B shows the chromato-

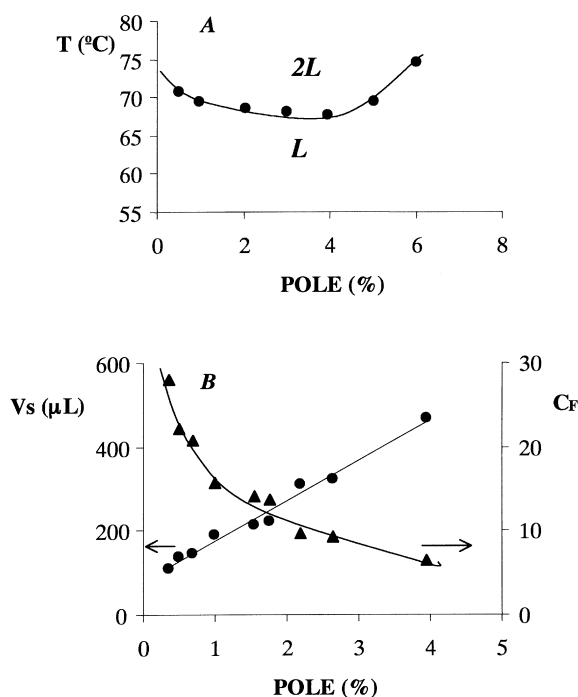


Fig. 2. (A) Experimentally measured coexistence curve for the nonionic surfactant POLE in artificial seawater solutions. (L) Denotes the single isotropic solution region whereas (2L) indicates the region where the two isotropic phases coexist. (B) Volume of surfactant-rich phase and preconcentration factor as a function of POLE concentration.

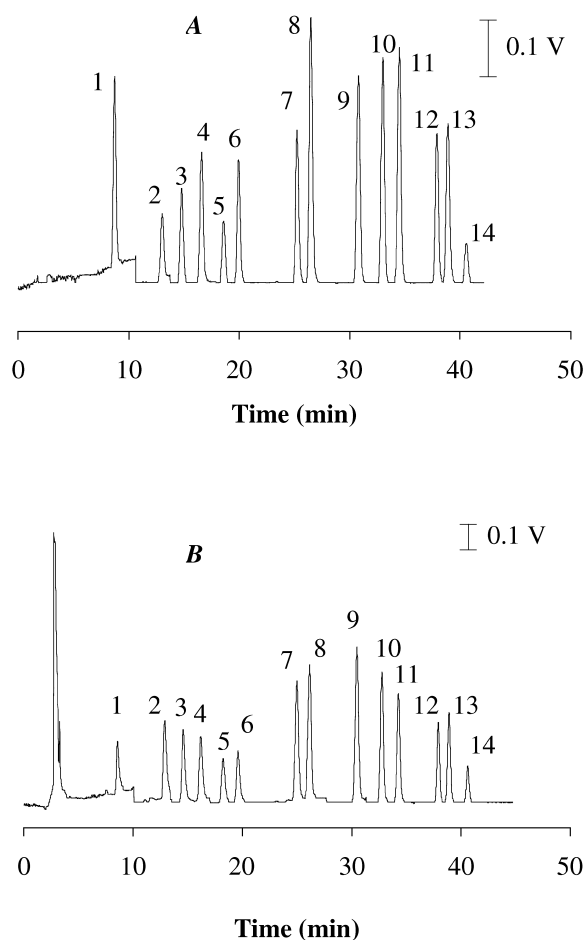


Fig. 3. (A) Chromatogram of a PAH standard mixture. (B) Chromatogram of PAHs spiked in artificial seawater subjected to the complete procedure. The assignment of peaks as in Table 1. For conditions, see text.

gram obtained for one of these samples. It should be noticed that the very low retention time of the surfactant used (first peak in the chromatogram in Fig. 3B), allows to detect the more polar PAHs without interference from the surfactant, except acenaphthene.

3.4. Effect of the analyte concentration

To determine the effect of the initial concentration of analyte in solution, solutions containing different concentrations of PAHs in artificial seawater were subjected to the CPE procedure. The results indicate

that the recovery percentages do not vary to a great extent when the analyte concentrations vary in the working limits of samples subjected to CPE, that is to say: approximately 20 times lower than the linear ranges that appear in Table 2.

Furthermore, when the added concentrations of each PAH are plotted against the ones obtained after the total process (cloud-point preconcentration and chromatographic separation), a straight regression line with $r > 0.91$ was always obtained. The t -test for $n=7$ at a confidence level of 95% shows that the slope and the intercept are not significantly different from one and zero, respectively, for most of the PAHs. Except for naphthalene which presents a proportional systematic error due to its small recoveries during the preconcentration step.

The random error for each PAH, estimated from the calculation of the standard deviation of the estimate of y on x , oscillates between 0.33 and 4.92.

This characteristic allows one to use this methodology without taking into account the initial concentration of the analyte in solution. Thus, CPE can be applied to solutions in which the initial concentration of PAHs is unknown. By the other hand, the time of analysis is lower because we can use calibration curves from standards that are not subjected to cloud-point preconcentration.

3.5. Effect of POLE on the stability of PAH solutions during storage

The sorption of PAHs onto containers during storage of water samples causes losses of these pollutants and it is a well-known phenomenon. In order to avoid this problem, it is recommended the addition of organic solvents to the aqueous sample [27–29]. The use of nonionic micellar media has also been described as an alternative to organic solvents [12,29,30]. Studies carried out with ionic surfactants such as cetylpyridinium chloride (CPC) and sodium dodecylsulfate (SDS) have revealed these surfactants to be also useful to stabilize aqueous PAH solutions [29].

On the other hand, PAHs tends to associate in some extent with the organic matter or the particulate matter suspended in seawater, either as complex or simple adsorption [30]. This kind of association leads generally to low recoveries of organic com-

Table 2
Quality parameters of the method

| PAH | Linear range ($\mu\text{g/l}$) | Slope \pm SD | Intercept \pm SD | r^2 | $S_{y/x}$ | RSD (%) | LOD (ng/l) |
|--------------------|----------------------------------|-------------------|--------------------|-------|-----------|---------|-----------------------------|
| (1) Nph | 0.9–15 | 0.861 \pm 0.027 | 0.564 \pm 0.224 | 0.990 | 0.533 | 4.04 | 81 |
| (2) Fl | 2.0–15 | 0.899 \pm 0.013 | 0.041 \pm 0.117 | 0.998 | 0.219 | 5.03 | 5.0 |
| (3) Phe | 1.0–10 | 0.798 \pm 0.021 | –0.217 \pm 0.119 | 0.991 | 0.273 | 5.24 | 54 |
| (4) A | 0.4–5.0 | 2.058 \pm 0.027 | –0.059 \pm 0.074 | 0.997 | 0.189 | 5.58 | 9.0 |
| (5) Ft | 3.5–35 | 0.197 \pm 0.002 | –0.098 \pm 0.044 | 0.998 | 0.108 | 2.91 | 1.5 \cdot 10 ² |
| (6) Py | 2.0–35 | 0.427 \pm 0.019 | –0.011 \pm 0.228 | 0.992 | 0.302 | 7.48 | 18 |
| (7) B(a)A | 0.5–5.0 | 2.548 \pm 0.086 | 0.608 \pm 0.215 | 0.990 | 0.458 | 5.06 | 5.0 |
| (8) Chy | 0.4–15 | 1.015 \pm 0.032 | 0.099 \pm 0.213 | 0.990 | 0.504 | 8.30 | 53 |
| (9) B(b)Ft | 0.6–10 | 1.743 \pm 0.029 | 0.310 \pm 0.155 | 0.996 | 0.402 | 7.78 | 14 |
| (10) B(k)Ft | 0.3–5.0 | 3.383 \pm 0.086 | 0.119 \pm 0.222 | 0.989 | 0.594 | 7.08 | 1.0 |
| (11) B(a)Py | 0.3–3.0 | 3.330 \pm 0.115 | –0.483 \pm 0.204 | 0.989 | 0.383 | 7.53 | 15 |
| (12) diB(a,h)A | 1.4–15 | 0.920 \pm 0.025 | –0.144 \pm 0.156 | 0.995 | 0.257 | 10.1 | 20 |
| (13) B(ghi)Per | 1.0–10 | 1.307 \pm 0.049 | 0.108 \pm 0.231 | 0.993 | 0.358 | 10.4 | 25 |
| (14) I(1,2,3-cd)Py | 4.0–30 | 0.179 \pm 0.003 | –0.106 \pm 0.044 | 0.997 | 0.092 | 7.69 | 96 |

r : Correlation coefficient ($n=12$). $S_{y/x}$: Standard error of the estimate. RSD: Relative standard deviation for concentrations of each PAH higher than their limit of quantification ($n=6$) with complete procedure (cloud-point preconcentration and chromatographic separation). LOD: Limit of detection calculated as described in the text.

pounds, specially when liquid–solid extractions are performed [31]. The cloud-point preconcentration of PAHs by using the nonionic surfactant Triton X-114 has been known as an useful tool to avoid the interference caused by humic acids [29].

In order to study the ability of POLE to avoid the PAH sorption onto the containers, and the interference caused by the organic and particulate matter in natural seawater, 14 PAHs in natural seawater and glass reservoirs were tested.

With this objective, two aliquots of non-filtered natural seawater and free of background PAHs levels, were spiked with levels of PAHs concentrations between 0.14 and 1.45 ng/ml. POLE was added to one of these aliquots to obtain a final solution of 1% (w/v) in the surfactant from the beginning of the experiment. At regular periods of time, previously established, 3 ml of each aliquot (the one with POLE from the beginning and the one with POLE just in the moment to carry out the CPE) were subjected to cloud-point extraction, and the surfactant-rich phases were chromatographed.

Table 3 shows the obtained results expressed by recovery percentage as a function of time. A gradual decrease in the concentration for all the tested PAHs was observed in natural seawater solution in absence of the surfactant, while the presence of POLE micelles inhibited the sorption and/or adsorption of these pollutants, at least in the period of time studied,

8 days. This decrease in the concentration of PAHs is higher during the first 48 h, and for PAHs with higher molecular mass.

Given these findings, the use of POLE as the surfactant for carrying out the preconcentration has the advantage to avoid the sorption of PAHs in containers, and it is not needed the use of other chemical reagents to prevent that sorption and/or adsorption.

3.6. Determination of PAHs in seawater

To evaluate the efficiency of the extraction and preconcentration processes using POLE, recovery studies were performed with natural seawater samples. The levels of added PAHs concentration range between (0.07 and 0.80 ng/ml) and (0.14–1.45 ng/ml). The obtained results are shown in Table 4. It can be seen that the found recoveries were highly satisfactory for most of PAHs determined. The exception is naphthalene, with recoveries lower than 40% in both cases; probably some quantities of this hydrocarbon should be lost during the extraction because of the temperatures at which the process is conducted.

In order to test the applicability of the proposed methodology for the determination of PAHs in natural seawater, seawater samples exempt of PAHs

Table 3
Stability of PAHs solutions during storage

| PAH | Recovery (%) | | | | | | | |
|--------------------|--------------|------|------|------|------|-------|------|--|
| | 0 h | 48 h | | 76 h | | 192 h | | |
| | | a | b | a | b | a | b | |
| (1) Nph | 37.7 | 43.3 | 31.2 | 33.2 | 18.9 | 40.2 | 15.5 | |
| (2) Fl | 62.1 | 60.0 | 56.6 | 53.7 | 45.4 | 63.8 | 34.4 | |
| (3) Phe | 91.6 | 87.9 | 86.4 | 78.0 | 69.9 | 95.8 | 59.2 | |
| (4) A | 63.1 | 62.4 | 55.3 | 53.1 | 34.6 | 61.2 | 47.2 | |
| (5) Ft | 79.0 | 77.6 | 73.1 | 66.0 | 63.2 | 73.5 | 45.6 | |
| (6) Py | 73.1 | 69.5 | 64.1 | 55.1 | 41.6 | 69.5 | 44.0 | |
| (7) B(a)A | 91.5 | 84.7 | 79.0 | 72.9 | 59.4 | 80.6 | 47.4 | |
| (8) Chy | 104 | 100 | 93.7 | 84.8 | 69.0 | 102 | 68.3 | |
| (9) B(b)Ft | 86.2 | 83.3 | 71.0 | 73.5 | 58.2 | 79.1 | 51.1 | |
| (10) B(k)Ft | 74.8 | 71.0 | 60.0 | 61.9 | 46.9 | 69.2 | 42.1 | |
| (11) B(a)Py | 107 | 102 | 87.8 | 90.5 | 59.7 | 94.9 | 49.9 | |
| (12) diB(a,h)A | 73.6 | 69.2 | 57.7 | 61.5 | 43.8 | 69.1 | 37.9 | |
| (13) B(ghi)Per | 80.6 | 75.2 | 59.1 | 63.4 | 46.0 | 76.2 | 39.4 | |
| (14) I(1,2,3-cd)Py | 69.3 | 68.6 | 54.0 | 61.4 | 34.2 | 63.0 | 38.6 | |
| Mean* | 81.2 | 77.8 | 69.1 | 67.4 | 51.7 | 76.7 | 46.5 | |

a=Sample stored in the presence of 1% POLE from the beginning.

b=Sample stored in the absence of surfactant.

*Mean of PAH recoveries except naphthalene.

background levels were contaminated with a fuel-oil. Fuel-contaminated seawaters were then analysed by CPE–HPLC and LLE–GC–MS for evaluating the proposed methodology. The obtained results are shown in Table 5.

PAHs of lower molecular mass show similar results with both techniques. The lower sensitivity of the applied methodology in the extraction and analysis by GC–MS do not allow to compare the results of high-molecular-mass PAHs.

Table 4
Recoveries of PAHs from spiked natural seawater

| Compound | Amount (ng/ml) | | Recovery (%) | Amount (ng/ml) | | Recovery (%) |
|---------------|----------------|--------------------|--------------|----------------|--------------------|--------------|
| | Added | Found ^a | | Added | Found ^a | |
| Nph | 0.30 | 0.098±0.034 | 32.6±11.4 | 0.58 | 0.218±0.020 | 37.7±3.5 |
| Fl | 0.20 | 0.099±0.013 | 49.5±6.5 | 0.58 | 0.360±0.035 | 62.1±6.1 |
| Phe | 0.20 | 0.167±0.011 | 83.7±5.7 | 0.46 | 0.422±0.037 | 91.6±8.1 |
| A | 0.10 | 0.054±0.010 | 53.5±9.9 | 0.23 | 0.145±0.016 | 63.1±6.9 |
| Ft | 0.80 | 0.566±0.029 | 70.8±3.6 | 1.45 | 1.145±0.121 | 79.0±8.3 |
| Py | 0.40 | 0.279±0.030 | 69.7±7.4 | 0.87 | 0.636±0.063 | 73.1±7.3 |
| B(a)A | 0.10 | 0.072±0.002 | 71.5±2.5 | 0.23 | 0.210±0.021 | 91.5±9.0 |
| Chy | 0.30 | 0.257±0.041 | 86.1±13.7 | 0.58 | 0.604±0.070 | 104±12.1 |
| B(b)Ft | 0.20 | 0.148±0.024 | 74.0±12.0 | 0.46 | 0.396±0.038 | 86.2±8.3 |
| B(k)Ft | 0.20 | 0.142±0.023 | 71.0±11.7 | 0.23 | 0.172±0.021 | 74.8±9.1 |
| B(a)Py | 0.07 | 0.074±0.004 | 106±6.2 | 0.14 | 0.150±0.018 | 107±13.2 |
| diB(a,h)A | 0.20 | 0.227±0.010 | 114±4.9 | 0.46 | 0.339±0.040 | 73.6±8.6 |
| B(ghi)Per | 0.20 | 0.230±0.029 | 115±14.5 | 0.35 | 0.282±0.033 | 80.6±9.5 |
| I(1,2,3-cd)Py | 0.60 | 0.399±0.068 | 66.5±11.4 | 1.16 | 0.804±0.082 | 69.3±7.1 |

^a Concentration of PAHs±standard deviation ($n=3$).

Table 5
PAHs found in natural seawater contaminated by a fuel-oil

| PAH | CPE–HPLC* | LLE–GC–MS* |
|---------------|-----------|------------|
| Fl | 0.32±0.30 | 0.28±0.33 |
| Phe | 1.85±0.81 | 1.28±0.13 |
| A | 0.19±0.15 | 0.28±0.15 |
| Ft | 0.21±0.15 | 0.13±0.17 |
| Py | 0.19±0.20 | 0.63±0.53 |
| B(a)A | 0.05±0.10 | <0.19 |
| B(b)Ft | 0.40±0.25 | <0.27 |
| B(k)Ft | 0.05±0.12 | <0.31 |
| B(a)Py | 0.18±0.16 | <0.28 |
| diB(a,h)A | 0.14±0.15 | <0.36 |
| B(ghi)Per | 0.35±0.30 | <0.34 |
| I(1,2,3-cd)Py | 0.20±0.15 | <0.29 |

*PAH concentrations in ng/ml± tS_{x_0} (t , $\alpha=0.05$, $n=12$ in HPLC and $n=6$ in GC–MS).

S_{x_0} =error in the predicted concentration by using the regression line.

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