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Determination of polycyclic aromatic hydrocarbons in marine sediments by high-performance liquid chromatography after microwave-assisted extraction with micellar media

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Abstract

A simple and rapid method is developed for extraction and determination of polycyclic aromatic hydrocarbons (PAHs) in marine sediments. The procedure was based on the microwave-assisted extraction of PAHs in marine sediment samples using a micellar medium of Polyoxyethylene 10 lauryl ether as extractant. Two-level factorial designs have been used to optimize the microwave extraction process. The analysis of extracts has been carried out by HPLC with UV detection. Fortified sediments gave an average recovery between 85.70 and 100.73%, with a relative standard deviation of 1.77–7.0% for PAHs with a ring number higher than three. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: Microwave-assisted extraction; Extraction methods; Marine sediments; Polynuclear aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants. Such persistent compounds damage the entire ecosystem and especially the aquatic environment. Persistence of these organic contaminants in sediments is possible owing to their low solubilities and also to their tendency to associate with suspended particulate matter. Analysis of PAHs in marine sediments is particularly important because they are considered pollution indicators, since they present a view of the spatial distribution of the pollutants [1] as well as providing a constant source of food for a number of aquatic organisms.

Extraction of pollutants in sediments by conven-

tional techniques has several disadvantages, like great sample preparation costs, high risk of laboratory pollution and high extraction times. Microwave-assisted extraction (MAE) is a viable alternative to the conventional techniques [2]. At present, it appears that MAE exhibit many substantial improvements in analytical sample preparations because it requires much lower volumes of organic solvent, reduces extraction time and increases sample throughput through extraction of multiple samples [3–8].

The use of micellar systems, to extract and/or preconcentrate organic compounds from aqueous and solid samples, as alternative extractants to organic solvents presents advantages such as safety, cost, compatibility with micellar and aqueous–organic mobile phases in HPLC, etc. [9–13].

The contact of the solid sample with a nonionic surfactant micellar solution below (or above for zwitterionic surfactant systems) its critical tempera-

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ture for some time period allows desorption of the organic substances from the solid matrix and their solubilization in the micellar aggregate. Using this approach, anthracene has been extracted from a coal sample using the zwitterionic surfactant C_9APSO_4 [14]; polychlorinated biphenyls (PCBs), anthracene, pyrene, phenanthrene and benzopyrenes have been extracted from spiked solid samples with recoveries being in the range of 65–90% [9]. In addition chrysene and benzo[*a*]pyrene removal from coal tar-contaminated soil was using C_8E_3 [9]. Benzo[*a*]pyrene and benzo[*k*]fluoranthene were reportedly solubilized and recovered from soil suspension using the nonionic surfactant Genapol X-80 [15].

García et al. [16] have reported the extraction of fluoranthene, pyrene and benzo[*k*]fluoranthene from smoke particulates and spiked wood ash samples with Triton X-114 at 5%, the time of contact of the solution with the solid was 5 h.

However, although well established, the extraction of the organic substances from the solid matrix with micellar media is not particularly suited for automatization, because of the time required to carry out extraction is too lengthy. We have, therefore, developed a new method to reduce the time required to carry out the extraction of PAH from sediments.

In the present work, we propose for the first time the extraction of PAHs from marine sediments by microwave-assisted extraction with a micellar medium as a prior step to their determination by HPLC with UV detection. The use of factorial experimental designs to optimize four experimental variables, pressure, extraction time, surfactant concentration and radiation power supplied, allows us to find an optimal set of operational conditions. Optimization was targeted at the maximum recovery of each PAH from spiked sediment sample prepared in the laboratory.

2. Experimental

2.1. Chemical and reagents

Naphthalene (Nph) was supplied by Merck; acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), 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; and indene[1,2,3-*cd*]pyrene [I(1,2,3-*cd*)Py] was supplied by Supelco. They were dissolved in ethanol (Merck).

Polyoxyethylene 10 lauryl ether (POLE) was supplied by Sigma.

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. Equipment

All measurements were made with a Waters 600 Multisolvant Delivery System equipped with a Waters U6K sample injector and a Waters Lambda-Max 481 LC variable-wavelength spectrophotometric detector operating at 254 nm. Autoanalysis 2.4 (Sciware, vcerda@p01.uib.es) software was used for acquisition data. The analytical column was a Vydac 201TP54 reversed-phase C_{18} , 5 μm , 300 Å (25 cm×4.6 mm) with a μ Bondapak C_{18} guard column, 10 μm , 125 Å (20 mm×3.9 mm) supplied by Waters.

The microwave extraction system was a CEM (Matthews, NC, USA) MDS 2000 microwave digestion system. The system delivers approximately 630 W (100%) of microwave energy at a frequency of 2450 MHz at full power and provides constant feedback control of extraction conditions through continuous monitoring of pressure data on a control vessel. The system is provided with method and data storage capabilities and a printer. The microwave extraction vessels were CEM PFA PTFE-lined advanced composite digestion vessels used for sediments extraction. The vessels are constructed with a PFA PTFE liner and a cap for high purity analysis that are capable of sustaining temperatures up to 200°C and pressures of 13.9 bar.

For the statistical treatment the Statgraphics software package was used.

2.3. Marine sediments and spiked procedure

Marine sediments were collected from the coast of S/C de La Palma (Islas Canarias) at 8 m in depth.

These sediments have 9.2% of organic matter and the following size grain distribution: >250 μm , 82.4%; 125–250 μm , 15.1%; 74–125 μm , 0.96%; 31–74 μm , 0.25%; 15–31 μm , 0.62%, 8–15 μm , 0%; 4–8 μm , 0% and <4 μm , 0.69%.

Fractions of sediment under 250 μm were taken and spiked with PAHs as follows. A portion of the sediment was accurately weighed in the PTFE-lined extraction vessel. Then, a volume of the PAHs dissolved in ethanol is added and allowed to equilibrate with the matrix, stored in the dark and allowed to dry. The amounts of PAHs added to 0.8 g of sediment were always the same: 17 μg of naphthalene, 27 μg of acenaphthene, 5 μg of fluorene, 2 μg of phenanthrene, 6 μg of fluoranthene, pyrene, benz[*a*]anthracene and chrysene, and 7 μg of benzo[*b*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, benzo[*ghi*]perylene and indene[1,2,3-*cd*]pyrene.

2.4. Microwave-assisted extraction method

Once the sediment has been spiked, 10 ml aqueous micellar solution of POLE are added, (the concentration dependent on the particular experiment to be carried out). After ensuring that a new rupture membrane was in place, the extraction vessel was closed and introduced into the microwave cavity. Extraction was performed at a fixed maximum pressure inside the vessels and at a level of microwave oven power (dependent both on the particular experiment). After fixed extraction time was completed, the vessels were allowed to cool at room temperature before they were opened. The supernatant was quantitatively transferred, filtered through a 0.45- μm HV Durapore membrane filter and an aliquot of 25 μl was directly injected in the HPLC system.

3. Results and discussion

3.1. Chromatographic conditions

The PAHs selected, which are all US Environmental Protection Agency (EPA)-priority pollutants [17,18], were prepared with the quantities of PAH and ethanol necessary to reach the desired con-

centration of hydrocarbons and 0.5% (v/v) of organic solvent, to which was added the volume of POLE solution required so that the final solution was $2.0 \times 10^{-2} M$, a concentration of surfactant higher than its critical micellar concentration.

Different solvent mixtures and gradient programs were tried to reduce the analysis time while keeping a good resolution of all PAH peaks. Good results were obtained using 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, at a flow-rate of 1 ml/min. The different PAHs gave satisfactory retention times (Fig. 1) with RSD between 0.706 and 0.857%. The surfactant is not retained and gives a peak, which does not interfere with studied hydrocarbons.

Calibration graphs were constructed by plotting the peak-area against the PAH concentration. A linear relationship with $r^2 > 0.99$ was always obtained, and detection limits [19] were between 0.01 and 0.14 ppm for the different PAHs studied. The reproducibility of the chromatographic system was evaluated. Quality parameters for the chromatographic method are reported in Table 1.

3.2. Optimization of the microwave extraction: factorial design

The use of a factorial design to explore the

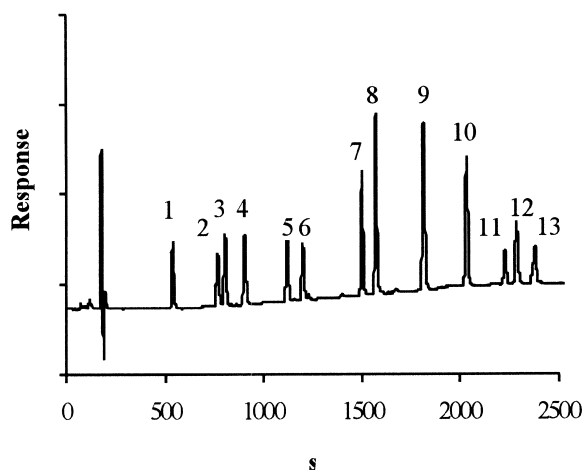


Fig. 1. Chromatogram of a standard mixture of PAHs in micellar medium of POLE. For peak assignment, see Table 1. For conditions, see text.

Table 1
Quality parameters for the chromatographic method

PAH	Working range (ppm)	LOD (ppm)	Reproducibility ^a		
			Added (ppm)	Found (ppm)	RSD (%)
(1) Naphthalene	0.8–2.0	0.14	2.00	2.06	1.08
(2) Acenaphthene	1.8–3.0	0.11	3.00	2.97	1.56
(3) Fluorene	0.1–0.6	0.05	0.60	0.57	1.02
(4) Phenanthrene	0.05–0.3	0.01	0.30	0.30	1.44
(5) Fluoranthene	0.2–0.8	0.02	0.75	0.73	1.72
(6) Pyrene	0.2–0.8	0.05	0.75	0.76	2.81
(7) Benz[<i>a</i>]anthracene	0.2–0.8	0.03	0.75	0.76	0.30
(8) Chrysene	0.2–0.8	0.03	0.75	0.76	1.22
(9) Benzo[<i>b</i>]fluoranthene	0.2–0.9	0.04	0.90	0.90	0.85
(10) Benzo[<i>a</i>]pyrene	0.2–0.9	0.04	0.90	0.88	2.02
(11) Dibenzo[<i>a,h</i>]anthracene	0.2–0.9	0.06	0.90	0.90	2.28
(12) Benzo[<i>ghi</i>]perylene	0.2–0.9	0.06	0.90	0.91	1.42
(13) Indene[1,2,3- <i>cd</i>]pyrene	0.2–0.9	0.07	0.90	0.93	1.45

^a Mean of six determinations.

variables that affect the microwave extraction allows a consideration of the overall number of experiments and possible interaction effects between the variables. The application of a statistical approach using a factorial design can both reduce the development time and provide less ambiguous data.

Several variables could potentially affect the extraction efficiency: surfactant concentration, maximum pressure inside the vessels, time at which the maximum pressure is maintained constant (so-called TAP), and microwave oven power. A two-level full factorial design, 2⁴, involving 16 runs was used as a first approach to the response surface of the microwave extraction process [20]. The upper and lower values given to each factor were: 10⁻⁴ and 10⁻¹ M; 1.35 and 3.38 bar; 0 and 10 min and 20 and 80%, respectively. Other variables implicated in the extraction were kept constants: volume of surfactant (10 ml), amount of sediment (0.8 g) and amount of PAHs spiked.

The experimental design parameters and their elemental response (recovery of each PAH is the response value used in the design) are shown in Tables 2 and 3. The chronological listing of the experimental design parameters represents the statistically randomized order in which the experimental treatments were undertaken. Nph, Ace and Fl are not included in tables of response values of designs because their recoveries are near zero.

Main effects and interactions obtained are shown in Table 4. In all cases, the main effect is the POLE concentration, while the other factors and the interactions between them are not statistically significant ($\alpha=0.5$). However, for most of studied hydrocarbons, the pressure inside the vessels is the second factor in importance. The estimated response surface obtained for the experimental model using these two variables shows that the region, where the experiment was carried out, did not include the optimum.

Table 2
Design matrix in the screening design (2⁴)

Run	Power (%)	P_{\max} (bar)	TAP (min)	$C_{\text{POLE}} \times 10^3$ (M)
1	20	3.38	0	0.1
2	80	3.38	10	0.1
3	20	1.35	10	100
4	80	3.38	10	0.1
5	80	1.35	10	0.1
6	20	3.38	10	100
7	20	1.35	0	100
8	80	1.35	0	100
9	80	1.35	10	100
10	20	3.38	0	100
11	20	3.38	10	0.1
12	20	1.35	10	100
13	80	3.38	0	100
14	80	3.38	0	0.1
15	80	1.35	0	0.1
16	20	1.35	0	0.1

Table 3
Response values in the screening design (2⁴)

Run	Phe	Ft	Py	B[a]A	Chy	B[b]Ft	B[a]Py	diB[a,h]A	B[ghi]Per	I[1,2,3-cd]Py
1	0.0	0.0	0.0	0.0	13.3	7.0	0.0	14.3	20.6	0.0
2	0.0	0.0	0.0	12.4	27.5	34.8	21.9	52.5	59.1	40.1
3	51.0	61.2	60.3	68.8	77.2	64.6	65.0	67.6	74.3	41.5
4	29.9	26.0	29.8	26.5	46.1	30.9	13.4	33.9	42.6	17.7
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	10.0	2.9	0.0	7.9	16.1	0.0
7	0.0	0.0	0.0	2.8	33.1	15.3	0.0	4.9	26.4	0.0
8	48.3	84.3	101.8	104.7	110.4	103.7	89.7	93.7	104.8	90.8
9	10.6	33.6	33.6	0.0	56.0	57.5	0.0	20.9	0.0	0.0
10	47.9	60.2	60.2	62.0	67.8	63.5	43.2	61.6	68.4	46.2
11	56.2	61.9	61.9	42.3	77.8	64.2	21.0	52.0	64.7	22.5
12	0.0	0.1	0.1	0.0	10.0	4.4	0.0	0.0	7.7	0.0
13	20.8	32.1	32.1	0.0	49.9	43.2	0.0	13.6	0.0	0.1
14	0.0	0.1	0.1	0.0	8.4	9.0	0.0	4.3	0.0	0.0
15	4.4	3.8	3.8	13.1	12.1	25.0	24.9	7.1	17.4	11.8
16	2.7	1.4	1.4	10.1	22.9	11.1	13.2	15.7	19.7	1.0

Table 4
Estimation effects and interactions

Effects	Phe	Ft	Py	B[a]A	Chy	B[b]Ft	B[a]Py	diB[a,h]A	B[ghi]Per	I(1,2,3-cd)Py
Power (%)	-5.47	-0.61	1.69	-3.66	-0.21	8.89	0.94	0.25	-9.25	6.28
P_{\max} (bar)	4.73	-0.51	-4.79	-7.04	-2.61	-3.26	-11.66	3.78	2.65	-2.20
TAP (min)	2.94	0.11	-6.14	-5.34	-1.66	-2.31	-6.21	2.45	0.90	-3.40
C_{POLE} (M)	32.19	44.24	39.71	33.94	51.76	43.59	21.54	30.80	30.08	20.85
Power $\times P_{\max}$	-7.88	-15.36	-15.91	-12.69	-9.04	-13.81	-8.16	-8.12	-7.78	-8.98
Power \times TAP	-11.19	-15.29	-17.06	-14.39	-11.14	-12.11	-13.61	-5.30	-6.02	-7.82
Power $\times C_{\text{POLE}}$	-8.89	-1.21	-1.56	-7.51	1.84	-1.96	-7.46	-6.25	-12.35	-6.68
$P_{\max}\times$ TAP	1.41	-1.24	4.91	10.14	7.16	4.84	9.49	10.68	22.48	11.90
$P_{\max}\times C_{\text{POLE}}$	6.51	0.79	-0.14	-4.34	-6.16	-6.56	-7.61	-10.28	-10.10	-9.25
TAP $\times C_{\text{POLE}}$	4.72	1.41	2.61	-2.64	0.64	0.19	-2.16	-2.30	-5.40	-10.45

So, a new factorial design shifted in the direction of the maximum gradient would be established.

To do this, we developed a two-level factorial design shifted relative to the previous one. Microwave power and TAP are kept constants. Microwave power was fixed at 60%, which was sufficiently high to minimize the total time of extraction. The TAP

was fixed at 5 min, to make sure of the quantitative extraction of PAHs. We used a central composite design, 2²+star with three central points, resulting in 11 randomized runs. Table 5 shows the levels used. The results obtained and the corresponding design matrix are shown in Table 6.

Fig. 2 shows the response surface for the chrysene

Table 5
Extraction parameters and factor levels used in the central composite design and the optimum values for the microwave extraction of PAHs

Factor	Fixed	Low	High	Center	Optimum
Power (%)	60				60
P_{\max} (bar)		203	541	372	203
TAP (min)	5				5
C_{POLE} (M)		5	1	525	2

Table 6
Design matrix and response values in the central composite design

Design	C_{POLE} (M)	P_{max} (bar)	Recovery (%)								
			Phe	Ft	Py	B[a]A	Chy	B[b]Ft	B[a]Py	diB[a,h]A	B[ghi]Per
0.0525	3.72	42.9	65.8	32.3	64.2	74.8	57.5	40.1	66.5	76.2	67.9
0.005	5.41	0.0	0.0	0.1	63.5	0.0	32.4	0.1	47.6	47.1	0.0
0.0525	2.03	58.4	67.5	61.2	70.1	72.8	52.6	62.7	68.3	66.7	72.4
0.1	2.03	52.4	43.8	41.0	32.7	56.8	12.8	10.5	46.4	47.9	28.9
0.1	3.72	29.7	31.8	0.1	12.9	44.8	4.7	0.1	30.1	27.7	24.2
0.0525	3.72	56.0	60.6	55.9	60.4	69.1	46.3	43.5	67.5	63.8	57.4
0.005	3.72	17.0	0.0	19.1	13.0	52.6	28.7	0.1	60.6	37.9	35.2
0.0525	5.41	29.0	36.9	0.0	17.3	50.9	20.5	1.5	38.4	32.4	31.9
0.1	5.41	35.4	31.9	21.6	26.6	43.0	5.5	2.6	35.8	31.0	26.4
0.005	2.03	38.5	62.6	58.4	74.7	86.2	74.7	56.2	86.9	85.3	84.4
0.0525	3.72	59.2	98.3	33.4	73.4	105.8	101.1	0.1	98.5	91.0	85.8

with this new model, where we can see the maximum in the region of low concentrations of surfactant and low pressures inside the vessels. At high pressures, the recoveries are smaller, possibly due to degradation of PAHs, because of temperatures reached inside the vessels.

For the different hydrocarbons studied, optimum concentrations of POLE are between 0.01 and 0.04 M, while the optimum pressure is always 2.03 bar. In order to select the best surfactant concentration, the recoveries of PAHs were tested with four different

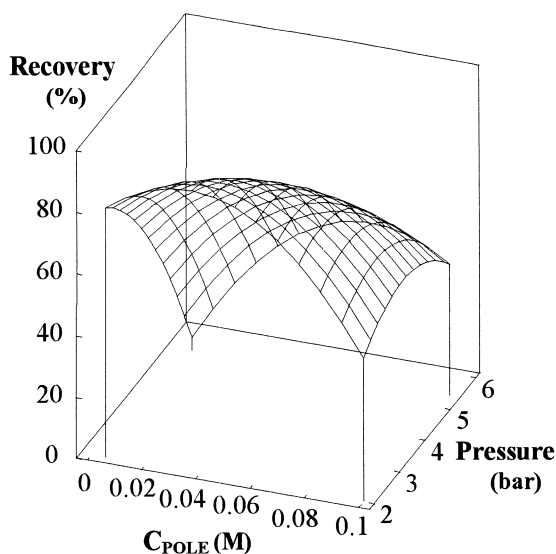


Fig. 2. Response surface estimated by the central composite design.

concentrations: 0.04, 0.03, 0.02 and 0.01 M. For these concentrations, values of recoveries with differences between 3 and 12% are obtained. Results indicated that the best recoveries in all cases, were obtained with a concentration of surfactant 0.02 M.

Given these findings, we decide to work with the experimental conditions given in Table 5, as optimum values.

To determine the accuracy of the extraction method, six independent extractions were carried out keeping the established experimental conditions. Table 7 shows the average results obtained. It must emphasize that those hydrocarbons with a ring number higher than three gave recoveries between 85.70 and 100.73%. The obtained RSD values show

Table 7
Average recoveries and relative standard deviations for studied PAHs^a

PAH	Recovery (%)	RSD (%)
Indene[1,2,3-cd]pyrene	95.97	4.02
Benzo[ghi]perylene	98.45	3.93
Dibenz[a,h]anthracene	99.43	3.48
Benzo[a]pyrene	84.37	5.77
Benzo[b]fluoranthene	100.73	3.51
Chrysene	95.04	1.77
Benzo[a]anthracene	85.70	3.78
Pyrene	87.81	6.58
Fluoranthene	87.79	7.00
Phenanthrene	49.45	29.37
Fluorene	11.51	62.13
Acenaphthene	6.42	115.91

^a Mean of six extractions.

the reproducibility of the proposed extraction method.

However, the more volatile PAHs such as phenanthrene, fluorene and acenaphthene, gave low recoveries, 14.52, 7.15 and 7.44%, respectively, and RSDs very high, becoming higher than 100% for acenaphthene. Hydrocarbons such as naphthalene show recoveries near zero. Different reasons could contribute to these low recoveries, the degradation of PAH, which may occur when a microwave energy is being used, due to the temperature and pressure inside the microwave extraction vessel [21] and the loss of these hydrocarbons during the fortification process of the sediment.

Studies carried out with PAHs solubilized in a micellar medium of POLE and subjected to the same established extraction conditions show the stability of PAHs versus microwave energy. It is proper to think that during the fortification process, as it is indicated in the experimental part, some hydrocarbons could volatilize.

3.3. Sample amount and number of simultaneous extractions

Changes in the sample amount could make it necessary to vary the optimum extraction conditions. To study these factors, a set of experiments was performed working with sample amounts ranging between 0.3 and 5 g, and with the optimum conditions established. The results obtained for a sample amount less than 3 g, do not show significant differences in the recoveries. For higher sample amounts, higher extraction times will be necessary.

One of the advantages of microwave extraction devices is the possibility of conducting many simultaneous extractions. When several extractions are carried out simultaneously, optimum experimental conditions can be maintained. The obtained recoveries are similar to that ones obtained with a single extraction. In this case, the extraction time has to be prolonged until 10 min for six vessels in comparison with 6 min for a single reactor. Fig. 3 shows the variation of the pressure inside the vessels, when one or six vessels are used. To reduce the extraction time, as the number of vessels increases higher powers can be used, which permit to reach the maximum pressure of 2.03 bar in a lower time.

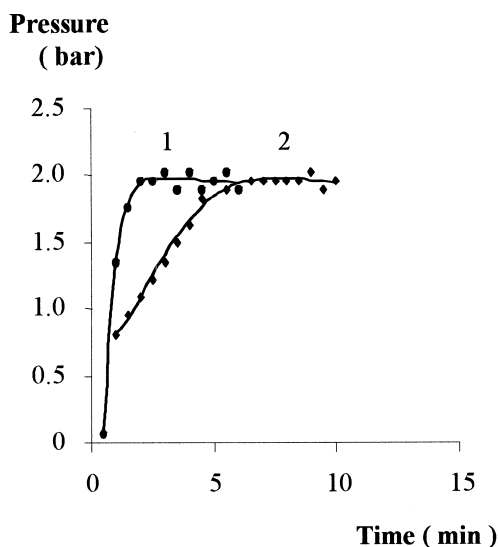


Fig. 3. Variation of the pressure inside the vessels versus extraction time, when one (1) or six (2) vessels are used.

4. Conclusions

This study is the first to present a method for the extraction of PAHs from marine sediments by MAE with a micellar medium.

MAE with a micellar medium seems to be a viable alternative to other extraction techniques. The main advantages are shorter extraction time (typically 5 min for extraction and 20 min for pre- and post-extraction sample handling), a reduction in the amount of sample required for analysis, higher sample throughput (up to 12 samples can be extracted simultaneously) and do not require the use of potentially hazardous organic solvents.

The methodology developed permits the simple, fast and selective (non-interference in the PAHs chromatographic peaks from the surfactant was observed) determination of the PAHs, considered priority pollutants by the EPA, in sediment samples.

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