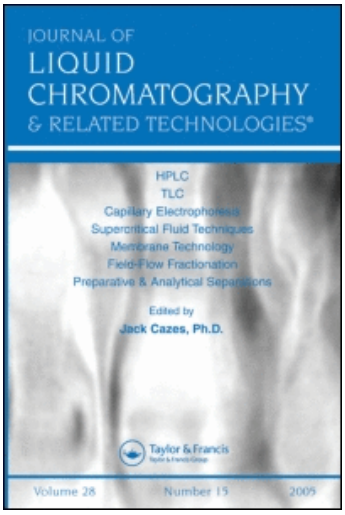

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Determination of PAHs in Particulate Air by Micellar Liquid Chromatography

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DETERMINATION OF PAHs IN PARTICULATE AIR BY MICELLAR LIQUID CHROMATOGRAPHY

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ABSTRACT

An acetonitrile / 0.20M SDS mobile phase was used to determine PAHs by HPLC with fluorimetric detection.

Because the peak area is greater the method is more sensitive than using an acetonitrile/water mobile phase. The method was applied to determine PAHs in particulate air samples and the results are in good agreement with those found by GC.

INTRODUCTION

The carcinogenic power of polyaromatic hydrocarbons (PAHs) and their importance in environmental studies is well accepted[1-6].

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Due to the low concentration of PAHs in environmental samples and their complexity, the analytical techniques used most often for their determination are gas chromatography (GC) and high performance liquid chromatography (HPLC) with fluorimetric detection[7-10]; sensitivity has been increased by using a program of selected excitation and emission wavelength pairs[11], but the need to increase this sensitivity justifies further study.

Moreover, the sensitivity of the fluorimetric determination of PAHs has been increased using micellar solutions of anionic surfactants[12]. We have found no references to the behavior of PAHs in micellar liquid chromatography (MLC). However, information about other compounds is available[13,14]. To decrease the large retention times found using aqueous micellar phases the presence of small percentages (3%) of low molecular weight modifiers, such as alcohols, has been recommended for anthracene and other compounds containing one aromatic ring[15,16].

In this paper we present the results of separating 13 PAHs by HPLC using a sodium dodecyl sulfate (SDS) micellar solution as mobile phase. The possibility of using surfactants to decrease the percentage of organic eluents such as acetonitrile in the mobile phase is also evaluated.

EXPERIMENTAL

Apparatus and material

A HPLC system equipped with a high pressure gradient Milton Roy CM 4000 pump, a Rheodyne 7125 samples injector

with a 20μl loop, a Waters 420 fluorimetric detector with 254 and 375 nm (long-pass) excitation and emission filters, respectively and a Milton Roy CI 4100 integrator were used. The column was a C_{18} Nucleosil 5μm particulate size (150 x 4.6 mm, Phenomenex) thermostated in a P-Selecta Precisterms bath. An MCV high volume sampler equipped with a 15cm diameter Watman GF/A glass fibre filter was installed for collecting particulate air samples. A P-Selecta Ultrasons bath was used to extract samples and Heidoph W 2000 vacuum rotary evaporator to concentrate them. A P-Selecta Meditronic centrifuge capable of 4800 rpm (3700g) was used; Lida nylon membrane filters with 0.45μm pore size were used to filter the sample extracts and the eluents used to prepare the mobile phase.

Reagents

Standard stock methanolic solutions of different PAHs from Sigma at concentrations within the range 10^{-3}-10^{-4}M were prepared by weighing and dissolving the solid products in methanol (Carlo Erba). More dilute solutions were prepared by dilution with methanol.

An aqueous micellar solution of sodium dodecyl sulfate (SDS) ($C_{12}H_{15}NaSO_4$, FW=288.38 from Fluka) was prepared by weighing and dissolving the solid product in 1.0 l of water to give a final concentration of 0.20M; SDS critical micellar concentration (cmc)=8.1×10^{-3}M. More dilute solutions were prepared by dilution with water.

HPLC purity acetonitrile and methanol were from Carlo Erba. Water was obtained from a Milli-Q system (Millipore). All chemicals were of analytical reagent grade. Before use, all eluents were degassed and filtered under vacuum.

Procedure

1. Calibration graphs

They were prepared using the standard methanolic solutions of PAHs in the range 0.002-85.6 ng/μl. The injected volume was 20 μl. The PAHs were separated and quantified under the following conditions: the acetonitrile/0.20M SDS gradient specified in Table 1 was employed with a flow rate of 0.65 ml/min at 22°C; for fluorimetric detection the 254 and 375 nm (long-pass) excitation and emission filters were used; the areas of the peaks were used for quantification of PAHs. During the chromatographic analysis He was used to degas the mobile phase. The column was conditioned by applying the gradient specified in Table 2.

2. Air sample collection, extraction and clean-up

The sampler was placed in the open air in Madrid city center where 105,000 cars pass every day. The samples were taken for 24 hours each time at a flow-rate of 30 m^3/h in winter 1993.

The filters were treated twice with 50 ml of methylene chloride in the ultrasonic bath for 20 minutes and then

TABLE 1

Gradient Program

Time (min)	A (%)	B (%)
0.0	55	45
2.0	55	45
30.0	85	15
38.0	85	15
40.0	55	45

A: acetonitrile; B: 0.20M SDS or Milli-Q water

TABLE 2

Conditioning of the Nucleosil C_{18} Column

Time(min)	A(%)	B(%)	C(%)	Flow-rate(ml/min)
0.0	0	1	99	1
60.0	0	100	0	1
90.0	0	100	0	1
90.1	0	100	0	0.1
690.0	0	100	0	0.1
691.0	55	45	0	1
730.0	55	45	0	1

A: acetonitrile; B: 0.20M SDS; C: Milli-Q water; temperature 22°C

centrifugated at 4000 rpm. The extracts were collected and the solvent was evaporated in the vacuum rotary evaporator and finally the volume was reduced to 0.5 ml with a N_2 stream. Clean-up was carried out in a glass column (25 cm long and 1 cm internal diameter) containing 8g of silica gel, previously treated with methylene chloride, activated at 130°C for 24 hours and deactivated with 5% water. Elution was carried out first with 25 ml of n-hexane and then with 40 ml of n-hexane: methylene chloride (4:1). This second fraction was concentrated in a rotary vacuum evaporator and the solvent was eliminated under a N_2 stream. The residue was dissolved in 10 ml of methanol and the above calibration procedure was applied.

RESULTS AND DISCUSSION

Preliminary studies

As mentioned in the introduction, small amounts of alcohols such as propanol and butanol have been used as modifiers to decrease the retention time of anthracene[15,16].

According to our results, using 0.05-0.20M SDS mobile phases containing 3% of n-propanol, the naphthalene capacity factors were above 30, which are useless for practical purposes. Increasing the percentages of n-butanol to 20% decreased the naphthalene capacity factor to 21, which is still not practical.

However, the presence of acetonitrile in the micellar mobile phase drastically decreased the capacity factors. Compared with the results obtained using the acetonitrile/water mobile phase, both lower acetonitrile percentages and lower flow-rates were necessary to get the same retention factors when acetonitrile/SDS mobile phases were used.

The effect of methanol is similar to that of acetonitrile; however as the baseline drift found in the gradient technique is significantly higher, acetonitrile was preferred.

Chromatographic parameter optimization

a.- Conditioning of the column

The reproducibility of the capacity factors and resolution was clearly influenced by the previous chromatographic run. Thus, the column was conditioned using a 0.20M SDS mobile phase for 30 minutes, followed by acetonitrile/0.20M SDS: 55/45 (v/v) for 40 minutes at a flow-rate of 1 ml/min. The equilibrium was taken to have been reached when the retention time for naphthalene remained constant in the ±0.20 s range. In these conditions the behavior of the column was quite reproducible for about 200 runs. For practical reasons the column was conditioned over night once a month (see Table 2). This treatment seems to saturate the column with SDS retained by apolar interactions.

b.- Effect of SDS concentration in the mobile phase

Figure 1 shows the influence of SDS concentration on the PAH capacity factors. Retention factors decrease significantly with increasing SDS concentration. The capacity factor for dibenzo(ah)anthracene, which is most strongly retained in the column, decreases from 25 when using a mobile phase without SDS, to 18 when the mobile phase was 0.20M in SDS. Likewise, the retention times fell from 37 to 27 minutes.

As Figure 2 shows, lower acetonitrile/SDS flow-rates were necessary to achieve the same analysis time found when an acetonitrile/water mobile phase was used. It should be noted that the noise increases slightly in SDS although the resolution is good down the base line; on the other hand, the peak areas also increase significantly. The increase of sensitivity through an increase of the peak areas should be attributed to the sensitization of the fluorimetric detection in SDS mobile phase. This sensitization could not be explained by the change of flow rate because the opposite effect should be expected. In these experimental conditions SDS behaves as a modifier, decreasing the retention of PAHs with increasing concentration. According to the literature this behavior is typical of systems using micellar mobile phases[17]. The acetonitrile/SDS gradient was optimized in search of a compromise between resolution and analysis time. The recommended gradient is shown in Table 1. Lower acetonitrile concentrations considerably increase the

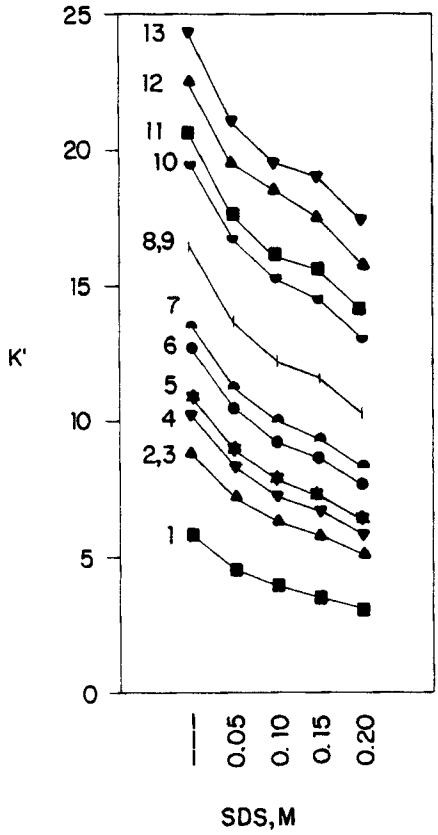

FIGURE 1. Effect of SDS concentration on the capacity factors of PAHs. The PAHs identified are indicated in Table 3.

capacity factors. On the other hand, the elution order of PAHs did not change with respect to that obtained with an acetonitrile/water mobile phase.

The use of methanol as a modifier on the SDS/acetonitrile mobile phase in percentages of 5-20% decreased the resolution of the following pairs: benzo(e)pyrene-benzo(a)pyrene and benzo(ghi)perylene-

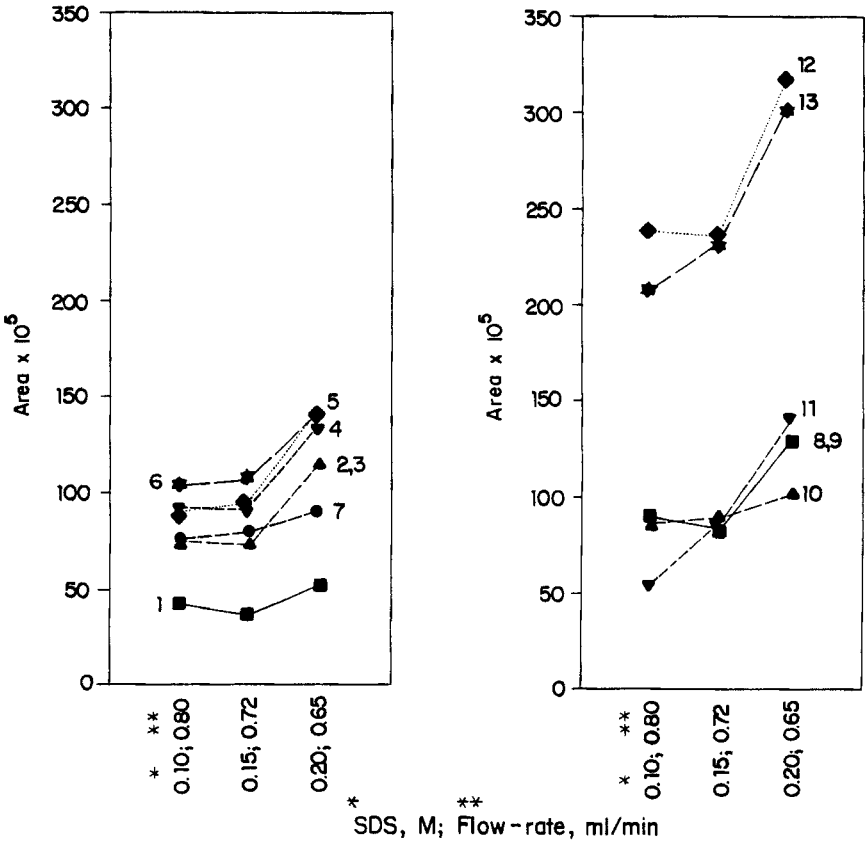

FIGURE 2. Effect of the SDS concentration and flow-rate on the chromatography peak area. The PAHs identified are indicated in Table 3.

dibenzo(ah)anthracene. Similar effects were found with n-propanol; percentages above 5-10% decreased resolution of the phenanthrene-anthracene and benzo(e)pyrene-benzo(a)pyrene pairs.

TABLE 3

Analytical Characteristics

Nº	PAH	x(ng/µl)	RSD,%		m_1/m_2*	DL(ng/µl)	
			1	2		1	2
1	Naphthalene	26	3.1	2.6	1.2	5.86	10.2
2	Fluorene	5.8	2.3	6.9	1.1	1.74	2.08
3	Acenaphthene	6.5	5.0	0.3	1.3	1.38	2.11
4	Phenanthrene	0.12	1.2	0.8	1.9	0.022	0.033
5	Anthracene	0.01	0.6	1.5	1.9	0.002	0.003
6	Fluoranthene	0.16	4.1	1.2	1.7	0.031	0.047
7	Pyrene	0.06	6.0	3.3	1.1	0.016	0.035
8	Chrysene	0.14	4.6	9.5	1.4	0.024	0.042
9	Benzo(a)anthracene	0.06	7.4	3.8	1.4	0.009	0.015
10	Benzo(e)pyrene	0.15	5.1	5.2	1.2	0.022	0.049
11	Benzo(a)pyrene	0.03	4.2	5.0	1.2	0.005	0.010
12	Benzo(ghi)perylene	0.25	1.3	3.8	1.4	0.019	0.039
13	Dibenzo(ah)anthracene	0.42	0.1	5.4	1.2	0.033	0.069

1: mobile phase acetonitrile/0.20M SDS; Flow-rate 0.65 ml/min.
2: mobile phase acetonitrile/ Milli-Q water; Flow-rate 1 ml/min.
* ratios of calibration graph slopes

c.- Effect of the flow-rate

As indicated above, a flow-rate of 0.65 ml/min gave an analysis time of 37 minutes, which is the same as is found for an acetonitrile/water mobile phase at 1 ml/min flow-rate. Flow-rates above 1 ml/min decreased the analysis time to 27 minutes and decreased resolution of the phenanthrene-

anthracene and benzo(e)pyrene-benzo(a)pyrene pairs. This means that the percentages of acetonitrile in the mobile phase can be decreased by exchanging to an SDS aqueous micellar phase, which decreases the waste of acetonitrile, with inherent advantages in cost and environmental protection.

The simultaneous effect of flow-rate and SDS concentration is shown in Figure 2, which shows that the peak areas increase quite significantly.

d.- Effect of temperature

According to the literature the viscosity of micellar mobile phases makes it necessary to work at above room temperature to avoid high pressures[16,18]. In this case there is no need to increase the working temperature because the pressure drop for the gradient specified in Table 1 is about 1600 psis.

Analytical characteristics

As shown in Table 3, the peak areas in acetonitrile/0.20M SDS mobile phase give higher slope ratios for the calibration graphs than in acetonitrile/water for the same analysis time, the flow rate being lower in micellar phase. As indicated in the table, the precision of the areas is about the same in both mobile phases. However detection limits are reduced in micellar phase, in inverse proportion

TABLE 4

Linearity of Calibration Graphs and Retention Times

PAH	Highest limit tested* (ng/µl)	t_r(min)**		RSD(%)***	
		1	2	1	2
Naphthalene	59	9.24	9.85	0.13	0.28
Fluorene	10	13.68	14.01	0.72	0.20
Acenaphthene	17	13.83	14.24	0.32	0.22
Phenanthrene	0.27	15.13	16.27	0.21	0.42
Anthracene	0.03	16.18	17.27	0.19	0.34
Fluoranthene	0.39	18.54	19.88	0.19	0.41
Pyrene	0.20	20.20	21.02	0.31	0.25
Chrysene	0.34	23.40	24.92	0.18	0.26
Benzo(a)anthracene	0.12	23.60	25.18	0.12	0.32
Benzo(e)pyrene	0.28	29.03	29.76	0.38	0.31
Benzo(a)pyrene	0.06	31.04	31.28	0.87	0.22
Benzo(ghi)perylene	0.53	33.86	34.07	0.28	0.23
Dibenzo(ah)anthracene	0.94	36.81	36.86	0.30	0.29

1: acetonitrile/ 0.20M SDS mobile phase; Flow-rate 0.65 ml/min.
2: acetonitrile/ Milli-Q water mobile phase; Flow-rate 1 ml/min.
* for both mobile phases
** t_r = retention time
*** average of 4 determinations

to the increase area ratios. Noteworthy are the cases of pyrene, benzo(e)pyrene and benzo(a)pyrene, whose detection limits are clearly lower in micellar solution.

The precision of the retention times is also similar in both mobile phases.

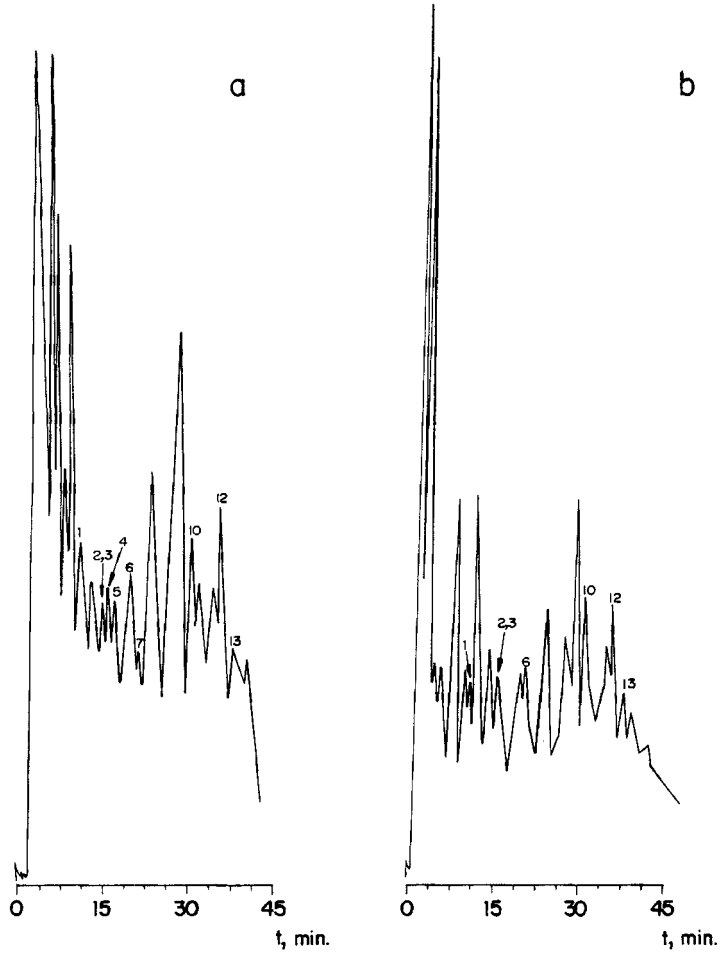

FIGURE 3. Chromatograms of a particulate air sample
a: acetonitrile/0.20M SDS mobile phase; Flow-rate= 0.65 ml/min.
b: acetonitrile/Milli-Q water mobile phase; Flow-rate= 1 ml/min.
The PAHs identified are indicated in Table 3.

TABLE 5

Determination of PAHs in Particulate Air Samples.

PAH	Concentration of PAHs (ng/m^3 air)* Sample 1		2		3	
	MLC**	GC***	MLC**	GC***	MLC**	GC***
Phenanthrene	1.0	1.3	3.3	6.0	1.5	1.8
Anthracene	0.4	0.2	0.7	1.2	0.3	0.3
Fluoranthene	2.1	11.3	1.5	12.7	0.5	6.1
Pyrene	5.2	11.9	3.5	16.5	---	7.9
Chrysene		6.5		13.3		8.2
	16		11.8		9.9	
Benzo(a)anthracene		5.0		7.3		6.9
Benzo(e)pyrene	19	2.0	15.8	6.3	14.7	2.1
Benzo(a)pyrene	3.9	3.9	7.8	7.6	3.2	6.0
Benzo(ghi)perylene	0.6	---	1.0	0.8	0.9	0.9
Dibenzo(ah)anthracene	4.8	2.8	3.3	3.5	9.3	9.9

* samples were collected in the city of Madrid in winter 1993
** n=3; average of 3 determinations of the same sample. Relative standard deviation (RSD) in the range 1 - 10%
*** Gas chromatography[19]

The linearity of the calibration graphs extends up to the higher concentration tested (Table 4) also in both mobile phases, the correlation coefficients being higher than 0.99.

The selectivity of the method was evaluated by comparing chromatograms obtained from the same particulate air sample. As shown in Figure 3, it is possible to detect more PAHs e.g. phenanthrene, anthracene and pyrene using a micellar mobile phase due to its higher sensitivity.

Determination of PAHs in urban particulate air

The MLC method was applied to determine PAHs in three particulate air samples from the city of Madrid. Results are shown in Table 5, which includes the results obtained by gas chromatography, applying the EPA method TO-13[19]. The results obtained by both methods agree well for phenanthrene, anthracene, benzo(a)pyrene, benzo(ghi)perilene and dibenzo(ah)anthracene. On the other hand, naphthalene, fluorene and acenaphthene are not detected by either of the two methods. The differences observed may be attributed to a different resolution of the two methods; for example chrysene and benzo(a)anthracene overlap in the HPLC method.

CONCLUSIONS

The use of the acetonitrile/SDS mobile phase to determine PAHs by HPLC with fluorimetric detection gives a higher sensitivity than that obtained with an acetonitrile/water mobile phase, due to the increase of the area ratios; moreover, lower flow-rates are necessary to achieve the same analysis time. This decreases the waste of acetonitrile and consequently, the cost and pollution. SDS modifies the surface of the stationary phase which then behaves as a modifier.

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