Journal of Fluorescence, Vol. 10, No. 4, 2000

Direct Fluorometric Analysis of PAHs in Water and in Urine Following Liquid Solid Extraction

M. Algarra,[1] C. Radin,[2] Ph. Fornier de Violet,[3] M. Lamotte,[3,5] Ph. Garrigues,[3] M. Hardy,[4] and R. Gillard[4]

Received October 25, 1999; revised April 14, 2000; accepted April 17, 2000

A method for the determination of PAHs in aqueous media based on synchronous fluorescence analysis directly on a solid phase after extraction was evaluated. Tests were conducted for (i) the simultaneous analysis of naphthalene, phenanthrene, anthracene, and pyrene in water at a parts per billion concentration level using a 0V1 sorbing phase block and (ii) the analysis of 1-hydroxyphenanthrene (1-OHPh) and 1-hydroxypyrene (1-OHPy) in human urine using a small sheet cut from a C18 silica fiberglass extraction disk (ENVI-Disk). Because the linear dynamic concentration range is dependent on both the immersion time and the concentration range, in all cases, an optimum immersion time must be determined to preserve linearity of intensity versus concentration. Calibration curves were determined for a concentration lower than 15 ppb in the case of 1-OHPh and at a concentration lower than 1.5 ppb in the case of 1-OHPy. In all cases the limits of detection were estimated to be lower than 0.2 ppb, i.e., for 1-OHPh and 1-OHPy, in the lowest concentration limit these metabolites were found in the urine of persons unexposed or weakly exposed to PAHs.

KEY WORDS: SPE; PAH analysis; urine.

INTRODUCTION

Due to their low solubility in water, polycyclic aromatic hydrocarbons (PAH) are found to be present in aqueous media at very low concentrations [a few parts per billion (ppb) or even less]. Most of the methods developed so far for their analysis [1] include an extraction/concentration step, followed by elution with an appropriate solvent and chromatographic analysis. However, all these steps are subject to introducing some loss of the analytes and errors in the measurements [2]. Moreover, extraction requires the use of organic solvents whose utilization is more and more subject to law limitation.

Previous works [3,4] have shown that it is possible to avoid the elution and chromatographic steps by performing a direct analysis of PAH pollutants from the aqueous medium at the ppb level, directly on a polydimethylsiloxane phase after extraction by either absorption [3] or fluorescence spectroscopy [4].

In this work we have evaluated further the potential of the method using two types of solid sorbents which are normally used in the SPE method to extract nonpolar pollutants from water: a OV1 polydimethylsiloxane phase and a C18 silica fiberglass phase (ENVI-Disk), both provided by Supelco.

Tests for analysis were performed both with plain water spiked with naphthalene, phenanthrene, anthracene, or pyrene and with human urine spiked with pyrene, 1-

[1] Departamento Quimica Analitica, Universidad de Malaga, Campus de Teatinos s/n, S29071 Malaga, Spain.
[2] Département de Mesures Physiques, IUT, Université de Bordeaux I, F33405 Talence, France.
[3] Laboratoire de Physico-Toxico-Chimie, UMR CNRS 5472, Université de Bordeaux 1, 351 cours de la Libération, F33405 Talence, France.
[4] Supelco, Sigma-Aldrich, F38297 Saint-Quentin Fallavier, France.
[5] To whom correspondence should be addressed at LPTC, Université de Bordeaux 1, 351 cours de la Libération, F33405 Talence, France. e-mail: m.lamotte@lptc.u-bordeaux.fr

1053-0509/00/1200-0355$18.00/0

hydroxyphenanthrene (1-OHPh), and 1-hydroxypyrene (1-OHPy), which are known markers for occupationally PAH-exposed workers [2,5–8].

EXPERIMENTAL

With both phases the extraction/adsorption process is based on the immersion of a piece of the sorbent into a given volume (150 ml) of the water to be analyzed for a fixed immersion time. In the case of the gum-like phase OV1, the sorbent is shaped as a 4 × 4 × 8-mm block. After adsorption, the block is introduced into a 10 × 10-mm spectroscopic cell for fluorescence measurements as shown in Fig. 1a. In the case of the ENVI-Disk, a 5 × 10-mm sheet is cut from a extraction disk and set on a sample holder for both adsorption process and front-face fluorescence measurement (Fig. 1b).

In this work the measurements were performed using only synchronous fluorometry, which is based on synchronous scanning of both excitation and emission wavelengths with a constant wavelength interval (offset). The choice of offsets was made either from the difference between characteristic wavelength maxima selected in the absorption and emission spectra or by selecting a mean value which allows the simultaneous observation of signals from several PAHs. This technique, which associates both good sensitivity and selectivity, is widely used [9,10]. The fluorometer we utilized was either a highly sensitive SPEX Fluorolog or an F-4500 Hitachi. The latter is a less expensive and more commonly used instrument than the former. We used it to demonstrate that the method can be applied with an affordable fluorometer.

Special attention was paid to avoiding adsorption onto the flask walls by using only stainless-steel vessels.

RESULTS

Detection of PAHs in Water

Figure 2 shows the synchronous fluorescence spectrum obtained from a mixture of naphthalene, phenanthrene, anthracene, and pyrene, whose concentrations in water were 1.1, 1.6, 1.6, and 1.4 ppb, respectively. The experiment was made with a OV1 phase according to the scheme in Fig. 1a with a wavelength offset of 70 nm, which allows these four PAHs to be detected simultaneously.

The linear dynamic range for an immersion time of 120 min was estimated to be in the 0.2- to 15-ppb range, which is similar to that determined for 2,6-dimethylnaphthalene [4]. The limits of detection were estimated to be of the order of 0.2 ppb, but still lower limits, as explained below, could be expected if the sizes of the sorbent block and of the spectrofluorometer beams were more closely fit with each other to take into account the nonhomogeneous distribution of the analytes within the sorbent phase.

Concentration Gradient Within the Sorbent

Taking pyrene as the probe molecule we first immersed a 4 × 4 × 8-mm OV1 block in a saturated water solution for 120 min. The block was then cut to give eight 4 × 4 × 1-mm-thick slices and the pyrene absorbance at 335 nm was measured for each of them. As shown in Fig. 3, a decreasing concentration gradient of the pyrene molecules from the surface to the inner part of the polymer block was evident. This result indicates that a good fit of the sorbent block section and of the fluorometer beam section is required for optimizing the signal. In our case, the block section is larger than the beam section, and as a consequence, the signal is

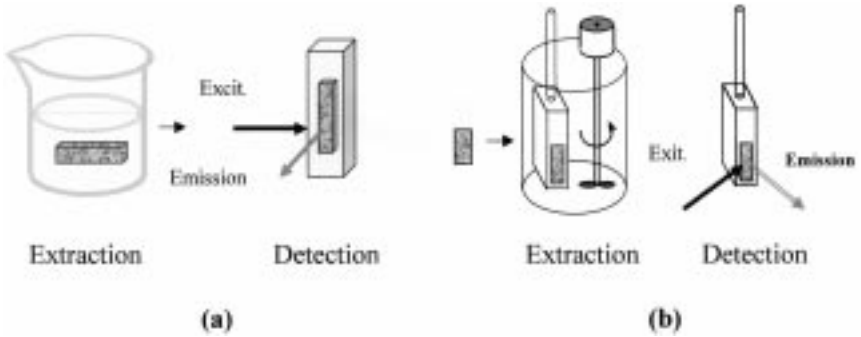

Fig. 1. Schematized experimental procedure used for the fluorometric analysis of PAHs in water after adsorption into solid phases: (a) 0V1 polydimethylsiloxane as the sorbent phase; (b) fiberglass C18 ENVI-Disk as the sorbent phase.

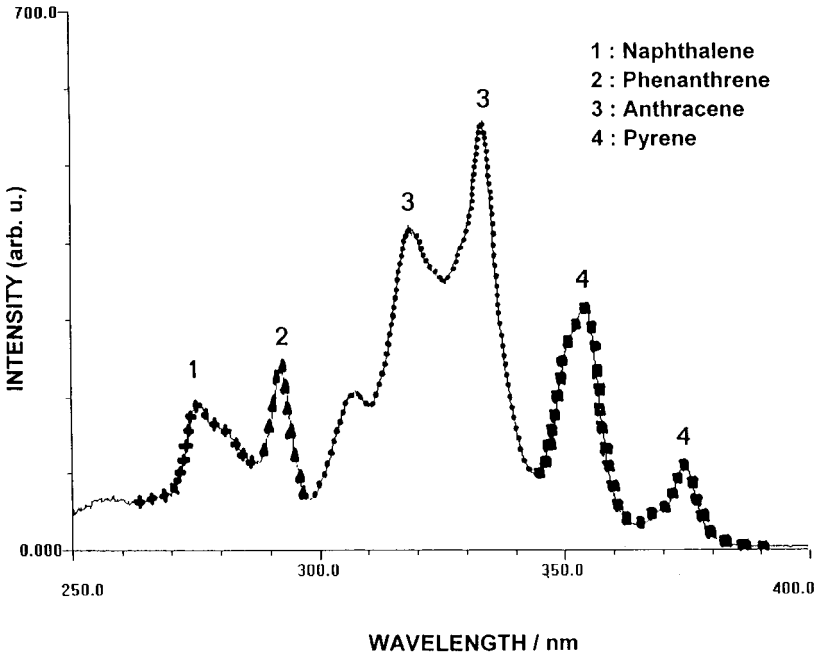

Fig. 2. Synchronous fluorescence spectra recorded from a OV1 sorbent block after immersion for 50 min in a dilute water solution containing naphthalene (1.1 ppb), phenanthrene (1.6 ppb), anthracene (1.6 ppb), and pyrene (1.4 ppb). Offset ($\Delta\lambda$ = 70 nm). The bands associated with each analyte are indicated. The wavelength abscissa refers to the excitation wavelength.

weaker than the signal that would have been recorded with a good fitting. In practice, because of the lack of firmness of the gum-like OV1 phase, a compromise must be found to satisfy the requirements for shaping reproducibility, mechanical resistance, and good sensitivity.

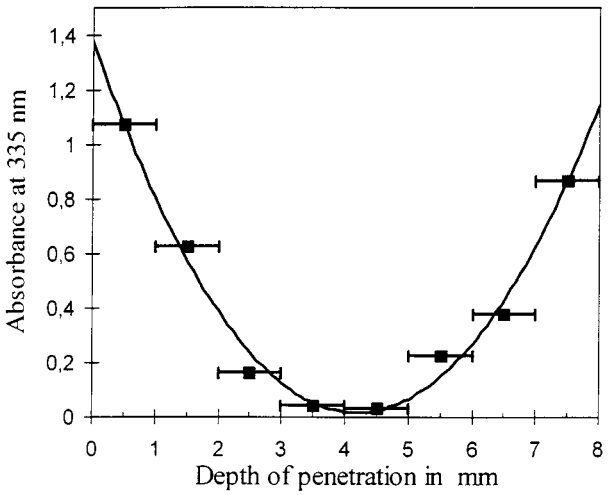

Fig. 3. Gradient concentration of adsorbed pyrene within an 8-mm-thick OV1 polydimethylsiloxane block. Immersion time: 120 min. Pyrene concentration in the water solution: $\cong 7.2 \times 10^{-7}$ *M*.

Detection of Pyrene, 1-Hydroxypyrene, and 1-Hydroxyphenanthrene in Human Urine

For a few years, numerous works have stated the possibility to control human exposures to PAHs by analyzing specific metabolites in urine [8]. In this respect, it has been shown that 1-hydroxyphenanthrene (1-OHPh), 1-hydroxypyrene (1-OHPy), and their conjugated glucoronides are reliable markers for PAH exposure [2,5–8]. The development of a simple and rapid method for estimating the content of such metabolites in human urine is thus of interest.

Figure 4a shows the synchronous fluorescence spectrum ($\Delta\lambda$ = 54 nm) obtained from an ENVI-Disk sample which was first immersed for 50 min in a water solution of 1-OHPh at a concentration of 10 ppb. The same figure also shows the signal recorded under the same conditions from a water solution. From these results, an enhancement factor of about 260 in the solid phase was estimated. Figure 4b compares the calibration curves obtained after adsorption for 30 min, from pure water solutions and from spiked urine samples. With urine samples, a smaller slope was obtained, pointing to some interference effect with compounds present in the urine complex medium, which appears to affect the amount of adsorbed analyte.

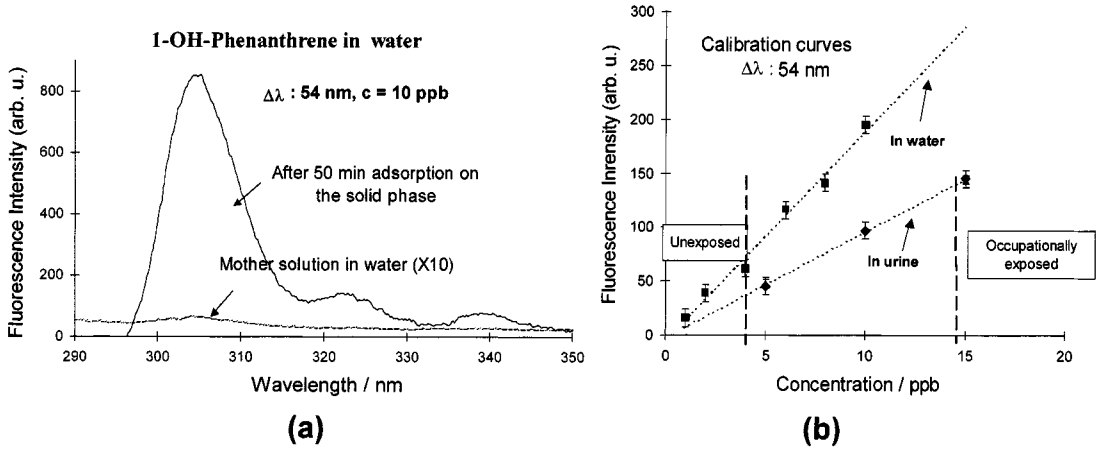

Fig. 4. Detection of 1-hydroxyphenanthrene adsorbed on a C18 ENVI-Disk by synchronous fluorescence. (a) Comparison of the fluorescence signals recorded from the water solution and from the C18 ENVI-Disk after extraction. Immersion time: 50 min. The wavelength abscissa refers to the excitation wavelength. (b) Calibration curves after adsorption from pure water solutions and from spiked samples of human urine. Immersion time: 30 min.

According to this observation, it appears that, for quantitative analysis, the use of a standard addition method is preferable to that of an external calibration method. The limit of detection is estimated to be around 0.1–1.2 ppb, which is about the range of concentration found among nonexposed persons [2,7].

Figure 5a shows the synchronous fluorescence spectra of pyrene and 1-OHPy, also recorded after adsorption on an ENVI-Disk after a 30-min adsorption in human urine spiked with increasing amounts of these analytes. These spectra were recorded with the SPEX Fluorolog instrument. The selected wavelength offset ($\Delta\lambda$ = 37 nm) allowed their simultaneous detection with a good sensitivity and selectivity. Similar spectra were obtained with the Hitachi fluorometer. The calibration curves shown in Fig. 5b were built from the results obtained with this instrument.

The detection limit is estimated to be slightly below 0.1 ppb, i.e., as for 1-OHPh, in the lowest limit of the concentration range usually found among unexposed or weakly exposed persons [2,7].

As for nonsubstituted PAH, the linear dynamic concentration ranges (LDR) were found to be dependent on both the immersion time and the concentration range.

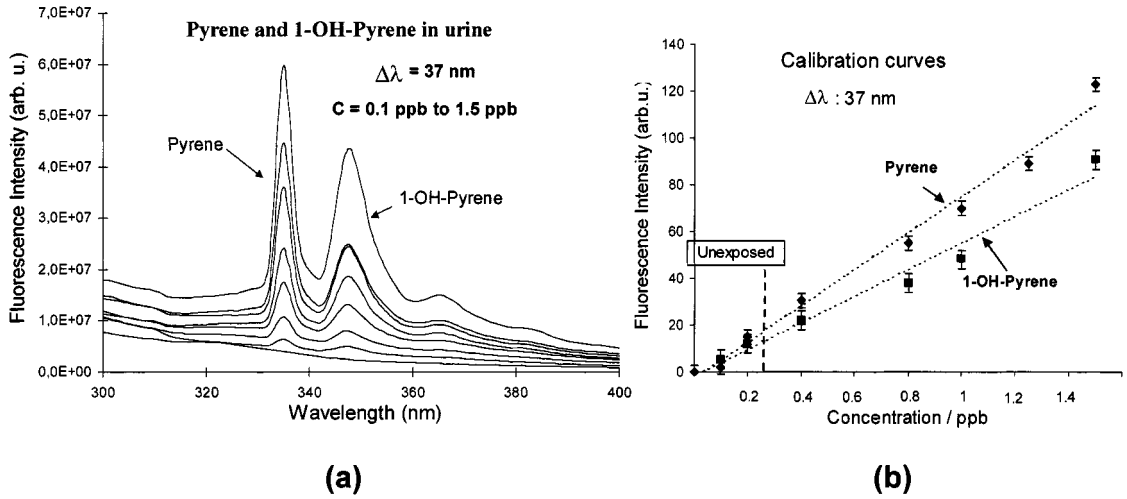

Fig. 5. Detection of pyrene and l-hydroxypyrene adsorbed on a C18 ENVI-Disk by synchronous fluorescence spectra after extraction from spiked samples of human urine. (a) Synchronous fluorescence spectra recorded with the SPEX Fluorolog. The wavelength abscissa refers to the excitation wavelength. (b) Calibration curves built from data obtained with the Hitachi 4500 fluorometer. Immersion time: 30 min.

In the case of 1-OHPy at a concentration of 50 ppb, equilibrium is attained after about 50 min of immersion. For a 30-min immersion time, which appears to be a good compromise, the upper limit of the LDR was estimated to be 15 ppb. For higher concentrations the immersion time must be shortened. As a rule, the kinetics of adsorption must be recorded as a function of the nature of the sorbent phase and the concentration to determine the optimum immersion time for quantitative analysis.

CONCLUSION

The preliminary results presented above show clearly that direct fluorometric detection of PAHs adsorbed from an aqueous medium on specific nonpolar phases is able to detect the presence of PAHs with sufficient sensitivity and selectivity with a commonly used type of fluorometer. Moreover, because of its simplicity, it appears to be an alternative method for rapid evaluation of PAH water contamination or human exposure to PAHs and deserves further development.

REFERENCES

1. Fed. Regist. (1984) USEPA Method 624, p. 141.
2. K. H. Schaller, J. Angerer, and N. Hausmann (1991) in Ph. Garrigues and M. Lamotte (Eds.), *Polycyclic Aromatic Compounds. Proceedings of the 13th International Symposium on PAHs*, Bordeaux, France, Gordon and Breach Science, pp. 1023–1030.
3. B. L. Wittkampt and D. C. Tilotta (1995) *Anal. Chem.* **67**, 600–605; B. L. Wittkampt, S. B. Hawthorne, and D. C. Tilotta (1995) *Anal. Chem.* **69**, 1197–1210.
4. P. Fornier de Violet, P. Garrigues, and M. Hardy (1998) SETAC meeting, Bordeaux, France.
5. M. Lipniak, M. Gawlik, J. Madej, and P. Niezabitowski (1994) *Chem. Anal. (Warsaw)* **39**, 29–32.
6. S. M. Ramasamy, R. J. Hurtubise, and A. Weston (1997) *Appl. Spectrosc.* **51**, 1377–1383.
7. G. Grimmer, G. Dettman, K.-W. Naujack, and J. Jacob. (1991) *Int. J. Environ. Anal. Chem.* **43**, 177–186.
8. S. Øvrebø, A. Haugen, P. Farmer, and D. Anderson (1995) *Occup. Environ. Med.* **52**, 750–756.
9. T. Vo-Dinh and R. B. Gammage (1978) *Anal. Chem.* **50**, 2054–2058.
10. T. Vo-Dinh (1981) In E. L. Wehry (Ed.), *Modern Fluorescence Spectroscopy, Vol. 4*, Plenum Press, New York, Chap. 5.