Determination of Ultra-Traces of Anthracene in Water Samples by Solid-Phase Spectrofluorometry

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A rapid method for the determination of anthracene ultratraces in water by solid-phase fluorometry is described. Anthracene is fixed on C-18 silica to give fluorescence at λ_{ex}=357 nm and λ_{ex}=405 nm wavelengths. An anthracene–silica-gel system packed in a 1 mm quartz cell was measured directly using a solid-surface attachment. The applicable concentration range was 50 – 1000 ng dm^{-3} with a relative standard deviation of 0.7% and a detection limit of 21 ng dm^{-3}. The method was applied to the determination of anthracene in natural, tap and seawater samples. The recoveries were 100, 105 and 110%, respectively. The method is very simple and more sensitive than other methods described in the literature.

Keywords Anthracene determination, water analysis, solid-phase spectrofluorometry

Polycyclic aromatic hydrocarbons (PAHs) are substances frequently present in urban air, waste, sea and natural water[1], coal tar, *etc.*, as polluting agents. Anthracene is one PAH that is used as an important source for manufacturing anthraquinone and alizarin dyes for cotton fibers. On the other hand, the industrial production of hydrogen peroxide also requires the presence of anthracene as a raw material. In these cases the determination of anthracene in residual water can be important for monitoring its environmental impact.

In recent years numerous methods for the determination of anthracene, as one component of PAHs mixtures, have been proposed. Luminescence spectroscopy[2], synchronous excitation[3], LC, TLC and HPLC[4–7] and, more recently, laser-induced fluorescence[8], have been suggested as appropriate techniques for the determination of anthracene and PAHs in environmental samples. However, neither of these seems to be a specific method for the determination of anthracene in the presence of other PAHs.

The LC, TLC and HPLC techniques involve the use of sophisticated instrumentation, or calculations involving complex matrices.[6] During the last years we have used solid-phase spectrofluorometry (SPF) as an appropriate technique for determining different species, such as metallic ions or anions, of analytical interest.

Sephadex gels under the names C-25 (used for Ga^{3+} and Al^{3+}), DAE A-25 (for W and Mo–W mixtures), QAE A-25 (for Be^{2+}) and G-15 (for Al–Be mixtures) have been used with appropriate reactives.[9–16]

SPF has again been shown to be a technique which enables low detection limits, high selectivity, simplicity as well as the use of conventional instrumentation. Here, Sigma C-18 silica gel was used for the determination of anthracene by SPF in the presence of up to eleven PHAs as well as seven fluorescent and not fluorescent pesticides.

Experimental

Equipment and reagents

A Perkin-Elmer LS-5 luminescence spectrometer, equipped with a Xenon discharge lamp (9.9 W) pulsed at the line frequency; Monk-Gillieson F/3 monochromators; a Quantic Rhodamine 101 counter to correct the excitation spectra; a Houston Omnigraphic *X-Y* recorder; a Hamamatsu R298 photomultiplier and a Braun Melsungen Thermomix 1441 thermostat, was used.

In order to compare all of the spectrofluorometric measurements and to ensure reducible experimental conditions, the spectrometer was checked daily. A fluorescent polymer standard of *p*-terphenyl (10^{-7} M) gave a relative fluorescnece intensity of 90% at λ_{em}=340 nm with λ_{ex}=295 nm (slit widths of 2.5 and 2.5 nm; factor of 0.594).

A Crison 501 digital pH-meter with saturated calomel and glass electrodes and an Agitaser 2000 rotating agitator were also used.

Stock solutions of 1 mg dm^{-3} of anthracene (Sigma 99%) were prepared by exact weighing of the reagent and

Dedicated to Professor Fermín Capitán García on his 72th birthday.

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dissolution in ethanol. Working solutions were prepared by adequate dilution with doubly-distilled water.

Derivatized silica-gel for reverse-phase chromatography, octadecyl (C-18) HPLC sorbent (Sigma), was used for the preconcentration of analytes and subsequent relative fluorescence intensity (RFI) measurements.

Fluorescence measurements

The measured RFI of silica-gel beads containing the fluorescence product was the diffuse transmitted fluorescence emitted from the silica-gel at the unexcited surface of the cell. The optimum angle formed between the cell plane and the excitation beam was 45° in all cases[17], while the thickness of the quartz cell was 1 mm.

Procedures

Basic procedure. An adequate volume of sample water (100, 500, 1000 and 1500 cm^3) containing 100 – 10000 ng dm^{-3} for 100 cm^3, 50 – 1000 ng dm^{-3} for 500 cm^3, 40 – 600 ng dm^{-3} for 1000 cm^3 and 30 – 500 ng dm^{-3} for 1500 cm^3 of anthracene was transferred into a Pyrex-glass bottle of adequate volume, and 200 mg of C-18 gel was added. The mixture was shaken mechanically for 10 min after the silica-gel beads were collected and dried by filtration under suction. With the aid of a micro-spatula they were packed in a 1 mm quartz cell. A blank with only gel was prepared and treated in the same way as described above.

The fluorescence intensities (20.0±0.5°C) of the sample and blank were always measured at λ_{em}=405 nm with λ_{ex}=357 nm. A calibration graph was constructed in the same way using anthracene solutions of known concentration.

Procedure for water. After a volume of water sample containing an adequate amount of anthracene was levelled to 500 cm^3 with doubly-distilled water, it was placed in a Pyrex-glass bottle and 200 mg of C-18 gel were added. The mixture was shaking mechanically for 10 min and treated as described under Basic Procedure. The standard addition method was used for calibration purposes.

Treatment of the sample. Water was filtered through filter paper with a 0.45 μm size pore (Millipore) and collected in a Pyrex-glass container that had been carefully cleaned with hydrochloric acid. The sample was stored at 4°C until analysis. The usual precautions were taken to avoid contamination.[18]

Results and Discussion

Spectral characteristics

Anthracene in solution showed a native fluorescence with excitation and emission maxima located at 357 and 405 nm, respectively. Figure 1 shows the three-dimensional spectrum of anthracene fixed on gel, after subtracting the contribution of the blank, as an isometric projection, the emission spectra at stepped increments of the excitation wavelength having been recorded and plotted.

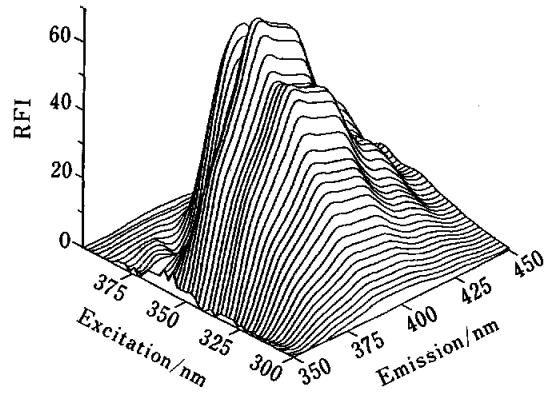

Fig. 1 Projected three dimensional spectrum of anthracene.

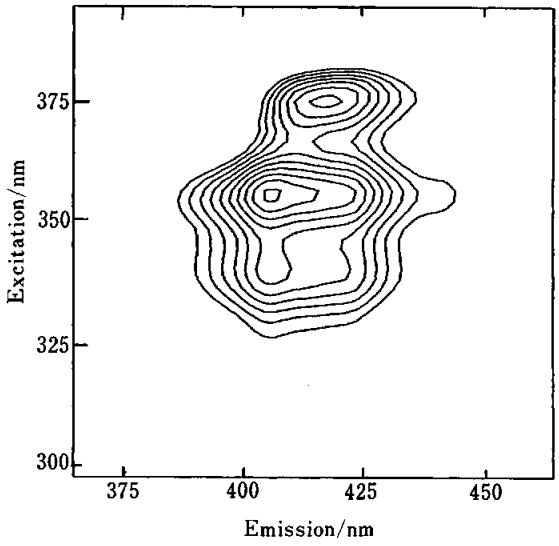

Fig. 2 Contour plot of the excitation–emission matrix of anthracene. The contour joint points show the same RFI.

In Fig. 2, the three-dimensional spectrum has been transformed into a contour plot with excitation and emission maxima located at 357 and 405 nm, respectively. For optimum excitation and emission, slit-widths of 2.5 nm were selected in all instances.

From a study of the half-life of the excited state of the system[19] in the solid-phase at different temperatures, it was concluded that the luminescence process was fluorescence with $\tau < 5\times10^{-6}$ s. The observed process agrees with similar findings for organized media, microemulsions, micellar solutions and cyclodextrins reported in other papers.[20,21]

Optimization of variables

In aqueous solutions, such as in the gel phase, the RFI of anthracene is independent of the pH values. The shaking time necessary for maximum RFI development was 10 min for 100, 500, 1000 and 1500 cm^3 volume

Table 1 Analytical parameters

Parameter	Sample volume/cm³			
	100	500	1000	1500
Intercept	3.69	9.45	5.91	4.15
Slope	0.023	0.066	0.078	0.101
Correlation coefficient	0.9997	0.9990	0.9990	0.9989
LDR/ng dm^{-3}	100 - 10000	50 - 1000	40 - 600	30 - 500
Detection limit/ng dm^{-3}	60.0	21.0	18.0	14.0
QL/ng dm^{-3}	199.0	70.0	60.0	46.0
RSD,%	0.6	0.7	0.7	0.8

LDR, linear dynamic range; QL, quantification limit.

samples without any increases for longer times. The effect of the temperature on the fixation process and, consequently, on the fluorescence emission, was studied over the range 10.0 - 70.0° C. The RFI decreases when the temperature of the system increases, the effect being totally reversible. The decrease in RFI is 0% at 10° C, 0.6% at 20° C, 3.2% at 40° C, 9.8% at 50° C, 12.0% at 60° C and 15.1% at 70° C.

In this study anthracene fixation was carried out at 20.0±0.5° C, the temperature selected for RFI measurements. The dependence of RFI with mechanical shaking speeds between 20 and 90 rpm was studied; 80 rpm was found to be the optimum speed for the development of fluorescence. The influenece of the ethanol concentration on RFI was determined, since ethanol is used in the stock solution of anthracene. Concentrations no higher than 10% are therefore recommended.

Since the use of a large amount of the gel lowered the RFI, only the amount required to fill the cell and facilitate handling around 200 mg was used in all measurements. With regard to the stability of the anthracene-C18 system, the RFI remained constant for at least 1 h.

Analytical characteristics

Table 1 shows the analytical parameters of the anthracene-C18 system.

Effect of the sample volume on sensitivity. One advantage of SPF methods is a potential increase in the sensitivity with an increase in the volume of the sample taken for analysis. This effect was assessed by measuring the RFI of identical amounts of C-18 silica-gel equilibrated with different volumes of solution containing the same concentration of anthracene. It is inferred from the experimental data that the RFI increases in proportion to the volume up to values around of 1500 cm³ showing a linear dependence over the range of 100 - 500 cm³ and being independent of the volume at higher values.

Calibration and precision. The calibration graphs for samples treated according to the procedure described above are linear for the concentration range 0.1 - 10.0 ng cm^{-3} for 100 cm³, 0.05 -1.0 ng cm^{-3} for 500 cm³, 0.04 - 0.60 ng cm^{-3} for 1000 cm³ and 0.03 - 0.50 ng cm^{-3} for 1500 cm³ sample volume. The reproducibility of the proposed method and of packing the gel in a 1-mm quartz cell was determined. The precision was measured for an anthracene concentration of 0.4 ng cm^{-3} by means of ten independent determinations. The relative standard deviation (RSD; p=0.05, n=10) were 0.6, 0.7, 0.7 and 0.8% for 100, 500, 1000 and 1500 cm³ sample volumes, respectively. The precision (RSD) of the packing operation calculated for ten measurements was 0.8%, fixed in the gel, and 0.7% for the gel only. The precision (RSD) of the fluorescence measurements (noise) was about 0.6% in all instances.

Table 2 Method for the determination of anthracene in water samples

Technique	Detection limit/ng cm^{-3}	Reference
Fluorescence[a]	0.03	2
HPLC	8.0	4
Synchronous fluorometry	170.0	22
Phosphorescence	3000.0	23
Laser fluorometry	1.0	24
This paper	0.021	—

a. Photon-counting method.

Sensitivity and detection limit. The sensitivity of this method can be enhanced by increasing the sample volume. This increase is calculated from the slope of the calibration graphs. The calculated values of the sensitivity ratio for the samples analyzed in this study are S(1500/100)=4.33, S(1500/500)=1.52, S(1500/1000)= 1.29, where the parenthesis represents the sample volume (cm³). The increase in sensitivity obtained with the proposed method is significant when compared with other solution methods (Table 2). The IUPAC detection limits (R=3), the quantification limits (R=10) and analytical sensitivities were calculated for 100, 500, 1000 and 1500 cm³ sample volumes (Table 1).

Effect of foreign species. A systematic study of the effect of foreign ions and the usual PAHs and pesticides on the determination of anthracene at the 150 ng dm^{-3} level was undertaken. Tolerance was defined as the amount of foreign ions which produces an error which

Table 3 Study of anthracene recovery in water samples

	Added/ng dm^{-3}	Found[a]	
		ng dm^{-3}	%
Sea (Motril)	200	220	110.0
	500	555	111.0
	800	875	109.4
Tap (Granada City)	200	215	107.5
	500	520	104.0
	800	845	105.6
Natural (Lanjarón)	200	205	102.5
	500	495	99.0
	800	795	99.4

a. Data are based on the average obtained from three determinations.

does not exceed ±5% in the determination of analyte. The ions tested were: Na^+, K^+, NH_4^+, Fe^{3+}, Fe^{2+}, Cu^{2+}, Cd^{2+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Al^{3+}, Mn^{2+}, Co^{2+}, Cl^-, Br^-, I^-, ClO_4^-, SO_4^{2-}, SO_3^{2-}, NO_3^-, PO_4^{3-} and CO_3^{2-}. In all cases, the tolerance level was higher than 1 M. On the other hand, the following PAHs and pesticides were also tested: fluorene, fluoranthene, dibenzo[*ah*]anthracene, crysene, acenaphthene, benzo[*a*]pyrene, pyrene, benzo[*a*]anthracene, naphthalene, phenanthrene, acenaphthylene, carbaryl, diquat, folpet, dichlone, lindane, thiabendazole, and *o*-phenylphenol. The following results were obtained: naphthalene, acenaphthylene, carbaryl, diquat, folpet, dichlone, thiabendazole, lindane and *o*-phenylphenol do not fix on the gel. Fluorene, phenanthrene, fluoranthene, crysene, pyrene and acenaphthene present interference for concentrations higher 5000 ng dm^{-3}. Dibenzo[*ah*]anthracene shows interference for concentrations higher than its solubility in water. Benzo[*a*]anthracene and benzo[*a*]pyrene shown interferences for concentrations higher than 1000 ng dm^{-3} and 200 ng dm^{-3}, respectively.

Applications. To check the accuracy of the proposed method, a recovery study was carried out on various types of water samples. Tap water from the supply to Granada (Spain), mineral water from Lanjarón (Spain) and seawater from Motril (Spain), were analyzed after adequate additions of anthracene. The volume of water used was 500 ml in all instances. The average percentages of recovery, the mean of three determinations, were acceptable within the standard conditions previously established. Table 3 shows the obtained results.

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(Received July 20, 1992)
(Accepted November 12, 1992)