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The development of solid-surface fluorescence characterization of polycyclic aromatic hydrocarbons for potential screening tests in environmental samples

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Abstract

This paper presents the characterization of polycyclic aromatic hydrocarbons (PAHs) in solid-surface fluorescence as the first step for obtaining new optical sensors for PAHs screening. The fluorescence properties of the EPA-PAHs (naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[a]anthracene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[a]pyrene, indeno [1,2,3-cd]pyrene, benzo[g,h,i]perylene and dibenzo[a,h]anthracene) on five types of solid-surfaces were evaluated. The experimental variables (pH and percentage of organic solvent in samples) were studied, obtaining different possibilities for making individual sensors for some of these PAHs and the best conditions for developing sensors for PAH screening were also studied.

Keywords: Solid-surface fluorimetry; Polycyclic aromatic hydrocarbons; Screening test; Luminescence sensor

1. Introduction

The analysis of polycyclic aromatic hydrocarbons (PAHs) have been of great interest because of their carcinogenicity and the general ubiquity of these compounds in the environment.

The two main sources of PAHs in the environment arise from incomplete combustion of fossil fuels for energy production and from incomplete combustion of refuse [1], certain technological processes and cooking procedures can also cause elevated levels of PAHs in some foods [2] and an increase of PAH levels in smokers' urine have been detected by several researchers [3,4]. So, human exposure to PAHs can occur through contaminated air and water, ingestion of food, and for different life styles.

The high percentage of probability of human exposure to PAHs and their cancer-inducing activity has led to the necessity to identify and determine PAHs and to the establishment of numerous methods for their determination [5,6] and national and international governments have

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developed different laws to control these pollutants [7,8].

Identification and quantification is usually by HPLC with UV–Visible, fluorimetric or amperometric detection or by means of GC–MS or GC–FID [9–13] and most of them include a preconcentration step. These steps are time-consuming and require great deal of effort, thus making the analysis unsuitable for routine control analysis.

Fluorescence methodologies offer the advantage of wide linear dynamic ranges, low detection limits and good selectivity. The formation of luminescent species on the surface can be explained due to the room temperature solid-surface luminescence phenomenon. The basic difference between solution luminescence and solid-surface luminescence is that in solid luminescence, the luminescent species are usually adsorbed on a solid substrate. Since the molecules are isolate and collision-restricted, this technique enables very sensitive determinations for many organic and inorganic substances at room temperature without cryogenic conditions [14–16].

A goal for the analysis of PAHs in solution is to develop screening techniques that obviate expensive, time consuming and laborious chromatographic procedures [17–19]. The development of a screening test method leads to a shorter turnaround analysis time and reduces costs for environmental controls. So, as a large part of the samples prove to be non-polluted, rapid analytical methods such as a screening test that provides reliable 'yes/no' responses are of increasing interest. These systems can usually be described as systems that 'filter' samples to select those with analyte content levels 'similar to' or 'higher than' a previously established threshold. These 'probably polluted' samples must then be examined with more exact instrumental methods [20,21].

In this paper, a characterization of PAHs by solid-surface fluorescence is presented as the first step for developing selective sensors for one PAH or screening test sensors for different EPA-PAHs of environmental interest (Table 1).

Table 1
EPA-PAH names, chemical structures, abbreviations and fluorescence characteristics

Name	Chemical Structure	Abbreviations	$\lambda_{exc/em}$ (nm)
Naphthalene		NAPH	286/322
Acenaphthylene		ACEN	292/324
Acenaphthene		ACE	292/322
Fluorene		FLU	290/304
Phenanthrene		PHE	294/364
Anthracene		ANT	358/402
Fluoranthene		FLT	358/460
Pyrene		PYR	300/406
Chrysene		CHRY	322/386
Benzo(a)anthracene		BaA	288/388
Benzo(k)fluoranthene		BkF	308/414
Benzo(b)fluoranthene		BbF	358/446
Benzo(a)pyrene		BaP	386/406
Indeno(1,2,3-cd)pyrene		IcdP	362/496
Benzo(ghi)perylene		BghiP	336/394
Dibenzo(a,h)anthracene		DBahA	304/404

2. Experimental

2.1. Chemicals and materials

Analytical reagent grade chemicals were used for the preparation of all the solutions.

EPA-PAHs (naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[a]anthracene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[a]pyrene, indeno [1,2,3-cd]pyrene, benzo-[g,h,i]perylene and dibenzo[a,h]anthracene) were purchased from Sigma and used as received.

Table 2
Characterization of PAHs by solid-surface fluorescence

	Silica gel Davisil	Silica gel Merck	Amberlite XAD 2	Amberlite XAD 4	Amberlite XAD 7
NAPH	**	**	**	**	**
ACEN	**	**	**	**	**
ACE	**	**	**	**	*
FLU	**	**	**	**	*
PHE	**	**	**	**	*
ANT	**	**	*	*	*
FLT	**	**	*	*	*
PYR	**	**	**	**	*
CHRY	**	**	**	**	**
BaA	**	**	**	**	*
BkF	**	**	**	**	*
BbF	*	*	*	*	*
BaP	**	**	*	*	*
IcdP	**	**	**	**	**
BghiP	**	**	**	**	**
DbahA	**	**	**	**	*

*The analyte interacts with the reagent phase and emits fluorescence on solid-surface. **The analyte does not interact with the reagent phase and/or dose not emit fluorescence.

50 μg ml^{-1} solutions of each PAH (Sigma) were prepared in 1,4-dioxane, methanol, ethanol, dimethylformamide, acetonitrile and acetone.

Samples of each PAH at 600 ng ml^{-1} were prepared in organic solvent/water (10:90). Water was distilled twice and prepared with a Milli-Q System (Millipore, Bedford, MA).

The non-ionic resins (Amberlite XAD 2, Amberlite XAD 4, Amberlite XAD 7, Silica Gel Davisil and Silica Gel Merck) (Sigma) were sieved and then used at 80–120 μm grain size.

2.2. Fluorescence measurements

A Hellma Model 176.052-QS flow-through cell of 25 μl volume was used in all solid-surface fluorescence measurements. No attempts were made to remove oxygen from PAH solution.

All fluorescence measurements (relative fluorescence intensity, R.F.I.) were carried out with an Aminco Bowman Series 2 luminescence spectrometer equipped with a 150 W continuous high-power xenon lamp, two monochromators with a resolution of 0.2 nm and a high performance R928 photomultiplier detector. The system was controlled with a personal computer with 4 MB RAM memory, OS/2 version 2.0, and a GPIB (IEEE-488) interface card for computer-instrument communication.

2.3. General procedure

In a single-line-flow-injection system, a Hellma Model 176.052-QS flow-through cell of 25 μl volume was packed with the corresponding resin and placed in the conventional sample compartment of the detector. A rotatory valve (Supelco 5020) was used for sample introduction. PTFE tubing (0.8 mm i.d.) and fittings were used for connecting the flow-through cell. A Gilson Miniplus-3 peristaltic pump was used to generate the flow stream (Fig. 1).

When the flow-through cell was packed with the corresponding resin, the fluorescence background was measured at the excitation and emission wavelengths of each PAH (Table 1). Then, 500 μl of sample was passed through the cell, and the fluorescence signal was recorded at the excitation and emission wavelengths chosen for each PAH, detector voltage of 600 V and slit width of 4 nm for excitation and emission.

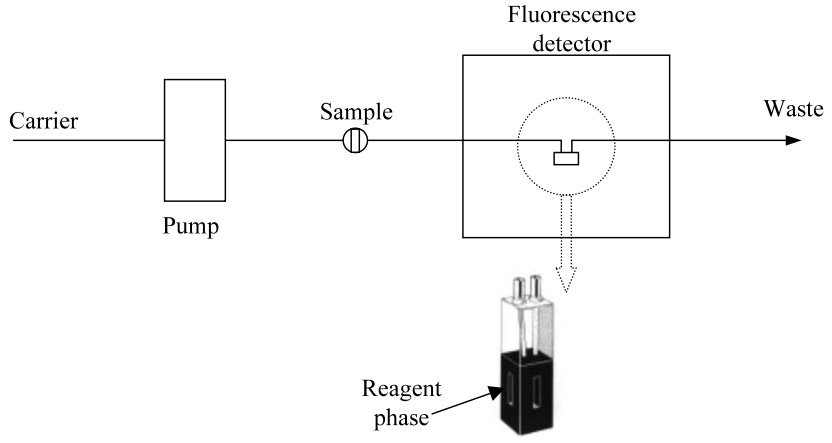

Fig. 1. Flow manifold used.

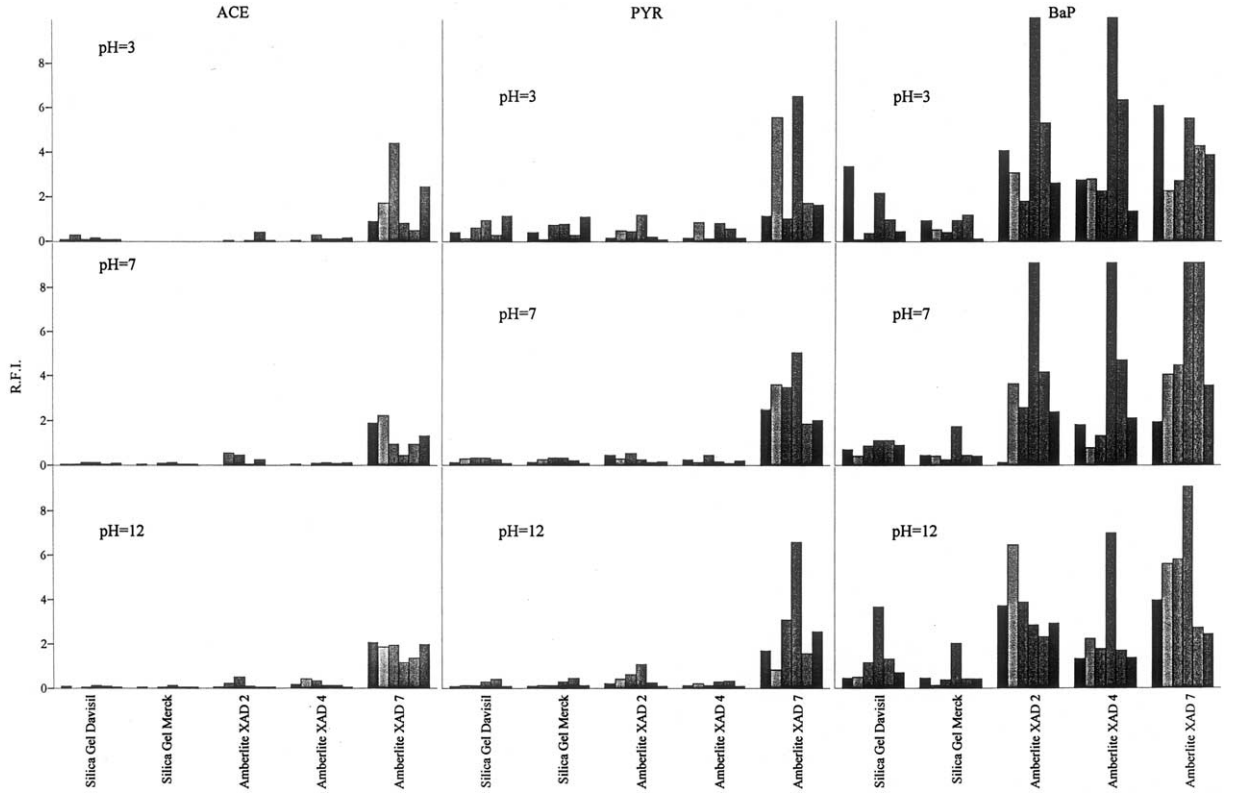

Fig. 2. Effect of pH on the solid-surface fluorescence intensity in (■) ethanol, (▒) acetone, (●) acetonitrile, (▨) dimethylformamide, (▨) 1,4-dioxane and (▒) methanol. [PAH] = 600 ng ml^{-1}, 10% of organic solvent.

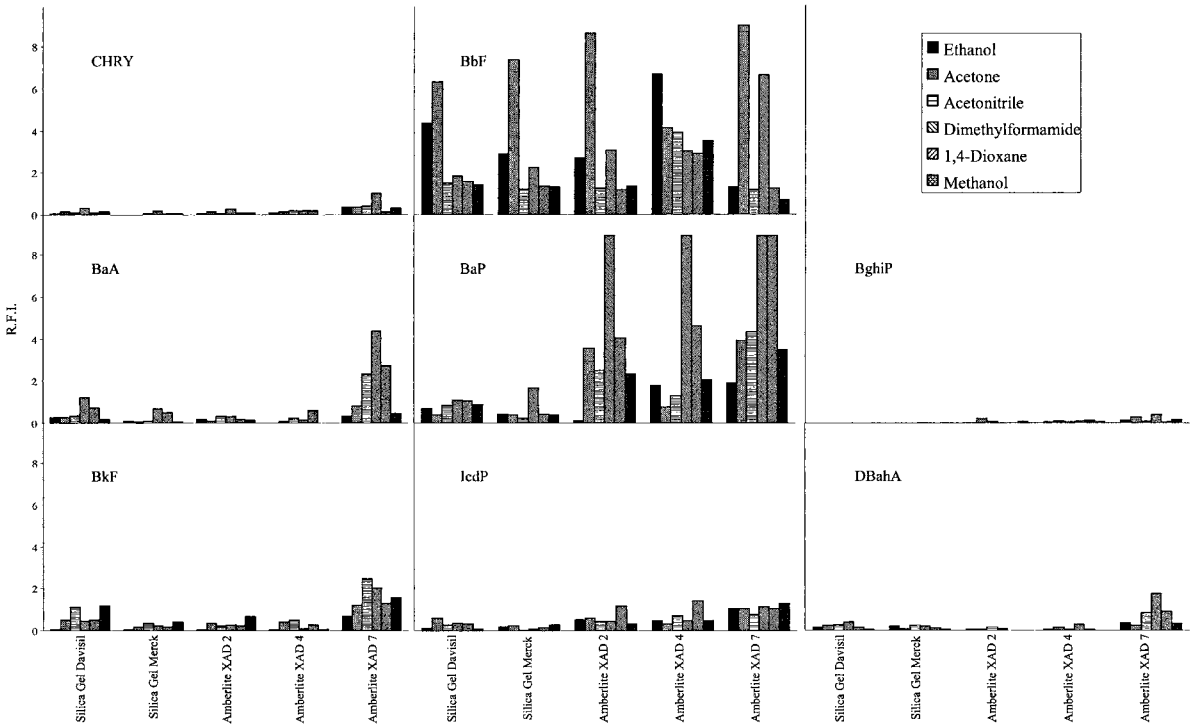

Fig. 3. Characterization of PAHs by solid-surface fluorescence. [PAH] = 600 ng ml^{-1}, 10% of organic solvent.

3. Results and discussion

The most important experimental variables which can affect solid-surface fluorescence emission are: pH, type of solid support and the organic solvents present in the medium.

The effect of pH will be related to the dissociation of the ionisable groups of the analytes and also affect the capacity of the resins to interact with analytes [22]. In the case of PAHs, in the presence of the resins assayed, the pH does not affect the dissociation of the compounds because they do not have dissociable groups in the molecules; consequently, the pH will only affect the interaction analyte–exchanger resins.

Three PAHs (ACE, PYR and BaP) have been selected as examples to show the influence of pH over the interaction analyte–resin. As can be seen in Fig. 2 there are no significant differences in the interaction between the three analytes at the three pHs tested (an acid value (pH 3), a neutral value (pH 7) and a basic value (pH 12)) because the graph shapes at the three pH values for each analyte are very similar so we can conclude that the interaction of the three analytes with the five resins tested is independent of the pH values.

Bearing these conclusions in mind, we have chosen pH 7 for the rest of the experimental work to simplify the methodology.

In solid-surface room temperature fluorescence a solid support to carry out the interaction with the analyte is necessary to concentrate the emission fluorescence intensity in a small area for measurement. Many resins could be used as a solid-surface, however, as the analytes do not have ionisable groups, non-ionic exchangers (Amberlite XAD 2, Amberlite XAD 4, Amberlite XAD 7, Silica Gel Davisil and Silica Gel Merck) were selected to carry out the characterization of PAHs by solid-surface fluorescence. These solid-surfaces have a high capacity to pre-concentrate the PAHs when they are at low concentrations. Thus the

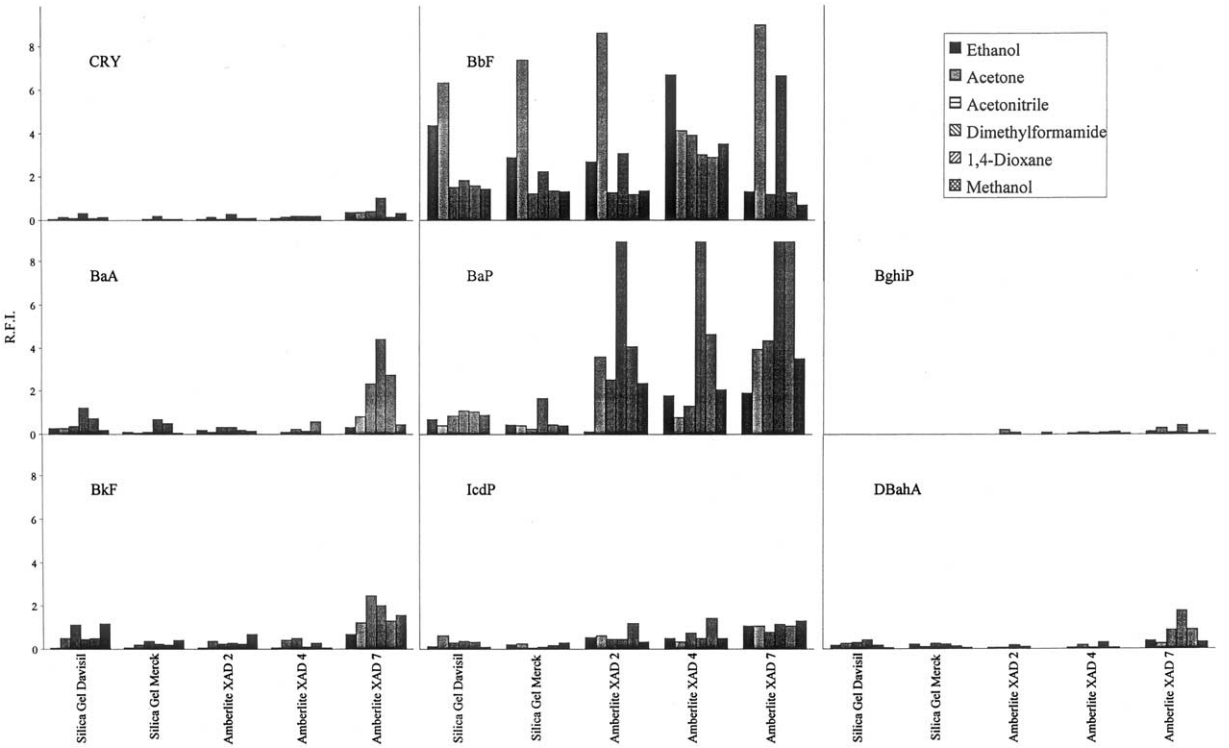

Fig. 3 (*Continued*)

retention capacity of the solid-surfaces tested has been considered the same as the experimental conditions used.

The organic solvent presents in the sample is an important experimental variable because it helps to dissolve the analyte and impedes the retention of the analyte in the flow system, guaranteeing that the all injected analyte reaches the solid-surface. Moreover, in the development of opto-sensors, the carrier and the samples must be as identical as possible, so organic solvents miscible with water (ethanol, methanol, acetonitrile, acetone, dimethylformamide and 1,4-dioxane) are selected in the study of the effect of the organic solvents over the fluorescence emission intensity of sixteen EPA-PAHs at pH 7 in solid-surface (Silica Gel Davisil, Silica Gel Merck, Amberlite XAD 2, Amberlite XAD 4 and Amberlite XAD 7).

In general, the presence of organic solvents in the samples affects the excitation and emission wavelengths, but in our case, the analyte is retained by the solid-surface and the organic solvent passes through the flow cell. Therefore, the presence of organic solvents in the samples does not significantly affect the maximum excitation and emission wavelengths of the PAHs.

The organic solvents affect the emission fluorescence intensity, e.g. when dimethylformamide is used with BaP at pH 7, the emission solid-surface fluorescence is four times higher than in ethanol or methanol and when acetonitrile is used with ACE at pH 3, the emission solid-surface fluorescence is five times higher than dimethylformamide or 1,4-dioxane (Fig. 2).

Under these conditions, the differences between the fluorescence signal of PAHs and the background of the resins were measured at the maxima excitation and emission wavelength of each PAH. Fig. 3 shows the results obtained.

To consider whether the interaction analyte–exchanger resin is effective or ineffective, we have taken that if the signal-background difference is higher than 10% of the highest fluorescence intensity, the PAH–resin interaction is effective,

while if is less than 10% the interaction is considered to be ineffective. The results obtained as yes/no for all the analytes under study is summarised in Table 2.

NAPH, ACEN, CHRY, IcdP and BghiP do not interact with any tested solid support. ACE, FLU, PHE, PYR, BaA, BkF and DbahA interact only with Amberlite XAD 7. ANT, FLT and BaP interact only with Amberlite XAD 2, Amberlite XAD 4 and Amberlite XAD 7, and BbF interacts with all the resins tested.

In general, we have observed that when an analyte interacts with Amberlite XAD 2 it also interacts with Amberlite XAD 4 and the emission intensities on Amberlite XAD 2 and Amberlite XAD 4 are very similar. However, the interaction with Amberlite XAD 2 or Amberlite XAD 4 does not imply an interaction with Amberlite XAD 7. This is due to Amberlite XAD 2 and Amberlite XAD 4 having a styrene and divinylbenzene matrix and Amberlite XAD 7 having a polymethacrylate matrix [23].

4. Conclusion

The results show, it is possible to develop future sensors for a screening test of ANT, FLT, BbF and BAP using non-ionic exchanger Amberlite XAD 2 or Amberlite XAD 4 and another for the screening of ACE, FLU, PHE, ANT, FLT, PYR, BaA, BkF, BbF, BaP and DbahA with Amberlite XAD 7 as the solid support. The detection of limits obtained in the screening test of ANT, FLT, BbF and BAP using Amberlite XAD 4 as solid-surface and in presence of 25% of 1,4-dioxane as organic solvent are between 7 and 18 ng ml^{-1} [24]. The development of selective solid-surface fluorescence sensor would be a easy task for environmental control.

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