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Note

Fluorescence enhancement of polycyclic aromatic hydrocarbons separated on silica gel high-performance thin-layer chromatography plates

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Silica gel thin-layer plates have been widely used for the separation of polycyclic aromatic hydrocarbons (PAHs) in environmental samples[1–4]. Two general problems have been observed in the *in situ* quantitation of PAHs on silica gel layers, namely, catalytic sample decomposition and quenching of the fluorescence signal[2,4,5]. Catalytic decomposition can generally be controlled by using reversed-phase plates and/or by adding an antioxidant to the mobile phase. With the modern commercially available high-performance thin-layer chromatographic (HPTLC) silica gel plates we have not found catalytic decomposition to be a problem on the time scale used to make densitometric measurements. PAHs adsorbed onto silica gel plates will generally show signs of decomposition if stored overnight but over a period of about one hour decomposition is usually insignificant. Quenching of fluorescence on silica gel layers is a general problem not restricted to PAHs. The mechanism of this interaction is not clearly understood but it has been observed that spraying the developed plate prior to detection with a viscous solvent such as liquid paraffin[6,7], triethanolamine[6,8,9], or Triton X-100[10–12] will enhance the fluorescence signal of many compounds to an extent sufficient for trace level analysis. Fluorescence enhancement values from 10 to 200 fold have been observed in favorable cases. It is assumed that adsorption onto silica gel provides additional nonradiative pathways for loss of the fluorescence excitation energy which are relieved by transfer of the adsorbed solute to the liquid state when the plate is sprayed with a nonvolatile liquid. In the liquid state other fluorescence enhancing mechanisms may also be important. Quenching due to atmospheric oxygen has only a small influence on the fluorescence enhancement process[13].

For trace analysis of PAHs from environmental sources it is important to maximize sample detectability without compromising resolution. To this end, three fluorescence enhancing reagents, dodecane, Triton X-100 and Fomblin Y-Vac have been evaluated for their ability to enhance the fluorescence response of a series of PAHs separated by HPTLC. Triton X-100 (isooctylphenoxypolyethoxyethanol containing an average of 10 moles of ethylene oxide) is a widely used non-ionic surface-active agent[14]. Fomblin Y-Vac is a poly(perfluoroalkyl ether) of average mol. wt. 3300 ± 150 which is used as a vacuum pump fluid[15], stationary phase in gas chromatography[16,17], and as a mass calibration standard for mass spectrometry[18].

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EXPERIMENTAL

All common solvents used during these studies were HPLC grade from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Fluoranthene, anthracene, benzo[*a*]-anthracene, benzo[*g,h,i*]perylene, dibenzo[*a,h*]anthracene, benzo[*a*]pyrene, benzo[*e*]-pyrene, pyrene, chrysene, fluorene, phenanthrene, dodecane, and 1,1,2-trichlorotri-fluoroethane were obtained from Aldrich Chemical Company, (Milwaukee, WI, U.S.A.). Benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, and indeno[1,2,3-*c,d*]pyrene were obtained from the Community Bureau of Reference (Brussels, Belgium). Triton X-100 was obtained from Cole-Parmer (Chicago, IL, U.S.A.) and Fomblin Y-Vac from Montedison (New York, NY, U.S.A.).

PAH standards were made up in hexane and their concentration adjusted individually to provide a reasonable signal-to-noise ratio while ensuring that the fluorescence signal and enhanced fluorescence signal remained within the linear range of the detector. Sample volumes of 200 nl were applied to 10 × 10 cm silica gel HPTLC plates (E. Merck, Darmstadt, F.R.G.) with fixed volume Pt-Ir dosimeters in conjunction with a Nanomat HPTLC spotter (Camag, Muttenz, Switzerland). Samples were developed for three min using the solvent system hexane–methylene chloride (99:1) in position number 4 of a short-bed continuous development (SB–CD) chamber (Regis Chemical, Morton Grove, IL, U.S.A.).

All scanning densitometric measurements were made with a Shimadzu CS-910 scanning densitometer (Shimadzu Instruments, Colombia, MD, U.S.A.). The source was a 100 Watt high-pressure mercury arc lamp with major spectral lines used for excitation at 254, 266, 313, and 365 nm. The emission signal was isolated by a series of interference filters in the range 400 to 600 nm and a broad band filter (UV-D2) having a transmittance envelope from approximately 300 to 400 nm centered at 350 nm. The peak transmittance of the UV-D2 filter was 78%. The maximum transmittance of the 400, 450, 500, 550, and 600 nm interference filters were 15, 33, 32, 42, and 43% respectively. The sensitivity of the densitometer was standardized before all measurements using a previously published procedure[19,20].

For fluorescence enhancement the dry developed plate was dipped into a solution of dodecane or Triton X-100 in hexane, or Fomblin Y-Vac in 1,1,2-trichlorotrifluoroethane. The plate was removed immediately the silica gel layer became transparent, a process requiring a few seconds. The fluorescence enhancement ratio is defined as the signal intensity of the PAH on the reagent impregnated plate compared to the signal for the same compound measured on the dry plate prior to dipping. All measuring variables being held constant for both measurements.

RESULTS AND DISCUSSION

The polycyclic aromatic hydrocarbons are naturally fluorescent substances permitting their selective determination at low levels in environmental samples. Unfortunately, when silica gel HPTLC plates are used for their separation some sacrifice in sensitivity occurs due to quenching or other molecular perturbations, resulting in a decrease in signal intensity. This diminution in signal can be restored, or even enhanced, by impregnating the HPTLC plate with a non-volatile liquid prior to determination by scanning densitometry. In this report, dodecane, Triton X-100 and

Fomblin Y-Vac are evaluated as fluorescence enhancing reagents for PAHs on silica gel HPTLC plates.

Fluorescence enhancement depends on a number of factors, of which, the most important are the PAH studied, the reagent and its concentration used for enhancement, and the time after dipping measurements are made. In practice, other considerations are equally important such as the degree of spot broadening due to maintaining the sample in a "wet" layer and shifts in the emission wavelength maxima induced by the presence of the fluorescence enhancing reagent. Generally speaking the optimum reagent for the fluorescence enhancement of PAHs on silica gel layers will be a compromise between the above considerations.

The number of reagents suitable for fluorescence enhancement of PAHs on silica gel layers is limited to non-polar reagents, or low concentrations of moderately polar reagents, to avoid washing off the sample from the plate during the dipping process. The use of reagent solutions of greater solvent strength than the developing solvent, hexane–methylene chloride (99:1), are thus inappropriate for this particular problem. We prefer plate dipping to plate spraying as viscous solutions are difficult to spray with nebulizing devices, and also, it is easier to produce an even distribution of the reagent over the plate by dipping than by spraying.

Dependence of the fluorescence enhancement ratio on reagent concentration

Using fluoranthene as a representative example of the PAHs studied, Table I indicates that the fluorescence enhancement ratio depends on the concentration of dodecane in hexane used as reagent, and to a lesser extent, on the sample amount. For dodecane concentrations greater than about 33% v/v the enhancement ratio is essentially constant. There is a small, but definite, upward trend in the value of the enhancement ratio with sample amount. Similar trends were observed with Fomblin Y-Vac. The situation with Triton X-100 is more complex for several reasons. The available concentration range with Triton X-100 is more restricted as its solubility limit in hexane is reached at about 1% (v/v). Also, there is a strong time dependence on the observed fluorescence enhancement ratio with Triton X-100. This is discussed in the following section.

Effect of the time between dipping and plate scanning on the fluorescence enhancement ratio

Ideally one would like the fluorescence enhancement process to be essentially

TABLE I

ENHANCEMENT RATIO FOR FLUORANTHENE AS A FUNCTION OF SAMPLE AMOUNT AND DODECANE CONCENTRATION

Dodecane concentration (v/v, %)	*Enhancement ratio*			
	5.0 ng	*10.0 ng*	*25.0 ng*	*40.0 ng*
1	1.38	1.40	1.48	1.52
10	1.70	1.72	1.80	1.88
33	2.04	2.06	2.18	2.28
50	2.08	2.10	2.24	2.34

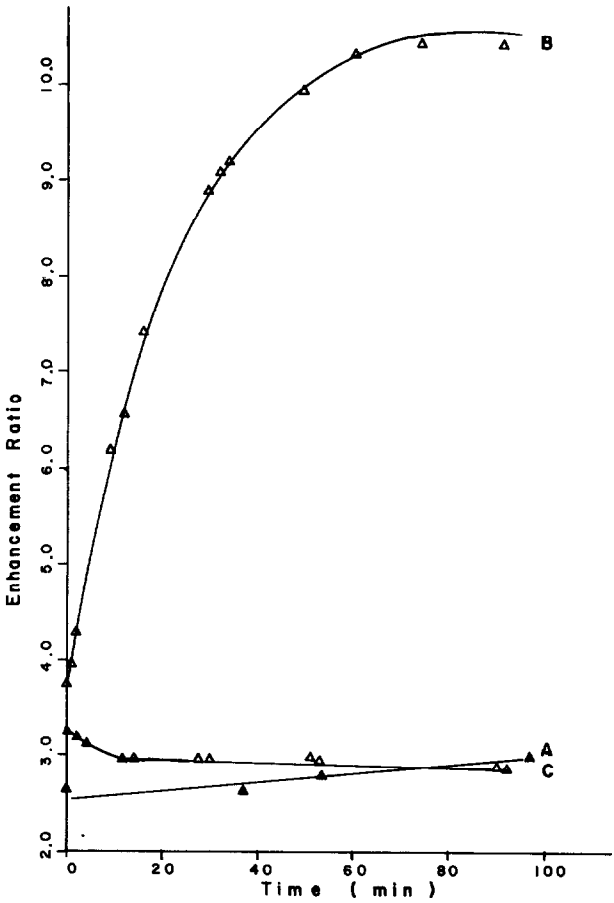

Fig. 1. Fluorescence enhancement ratio for fluoranthene as a function of the time between dipping and scanning the plate. A, 35% (v/v) Fomblin Y-Vac in 1,1,2-trichlorotrifluoroethane; B, ≈1% (v/v) Triton X-100 in hexane; C, 35% (v/v) dodecane in hexane.

instantaneous and the fluorescence enhancement ratio to be independent of the time between dipping and scanning the plate. This is approximately so for dodecane and Fomblin Y-Vac (Fig. 1). A slight increase for dodecane, and decrease for Fomblin Y-Vac, in the fluorescence enhancement ratio is observed over a period of 100 min. For Triton X-100 a very striking time dependence of the fluorescence enhancement ratio is observed. In this case the maximum in the fluorescence enhancement ratio is not reached until about 60 min after the plate was dipped. Furthermore, as shown in Fig. 2, the fluorescence enhancement ratio is a function of sample amount, concentration of Triton X-100, and the time between dipping and scanning the plate. The curves in Fig. 2 can be described mathematically by eqn. 1

$$E_R = \frac{t}{A + Bt} \tag{1}$$

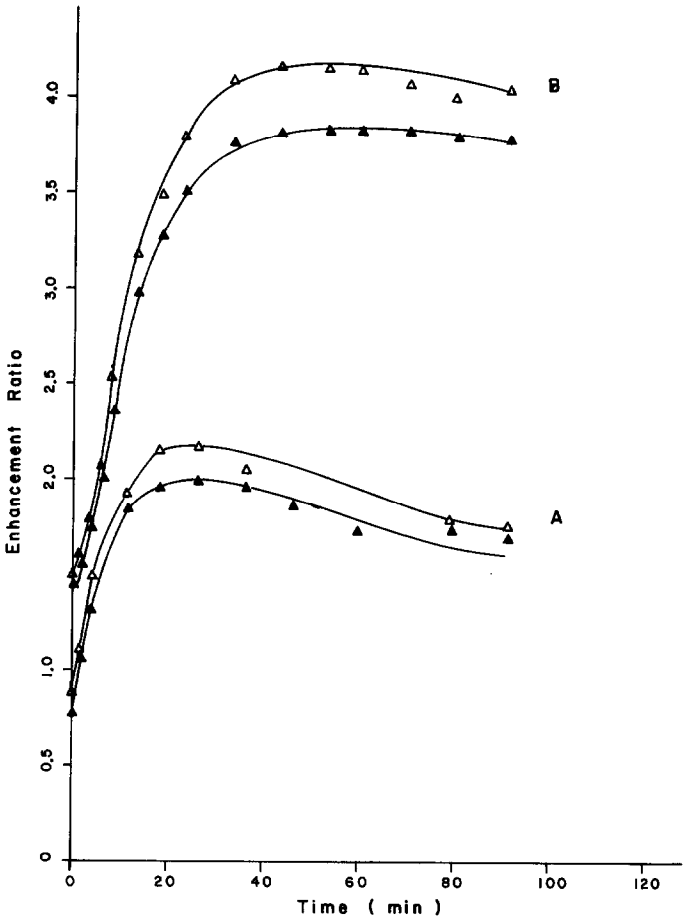

Fig. 2. Fluorescence enhancement ratio for fluoranthene as a function of time between dipping and scanning the plate. A, 0.34% (v/v) Triton X-100 in hexane and B, 0.68% (v/v) Triton X-100 in hexane. Sample amount: △, 20 ng; ▲, 40 ng.

where E_R = fluorescence enhancement ratio, t = time between dipping and scanning the plate, A and B = experimental constants which depend on the concentration of Triton X-100 and the sample amount. Optimizing the enhancement ratio for quantitative analysis of PAHs using Triton X-100 would be very difficult and probably impractical for unknown sample amounts.

Spot broadening as a function of the time between plate dipping and scanning

The object of increasing the detectability of the PAHs would be to no avail if sample diffusion in the wet layer occurred to a significant extent and resolution was severely compromised. As can be seen in Fig. 3, some spot broadening occurs due to the dipping process compared to the measured spot width at base prior to dipping. Here the zero on the time axis is not a true zero as it takes some finite time to remove the plate from the dipping chamber and position it in the densitometer. This time

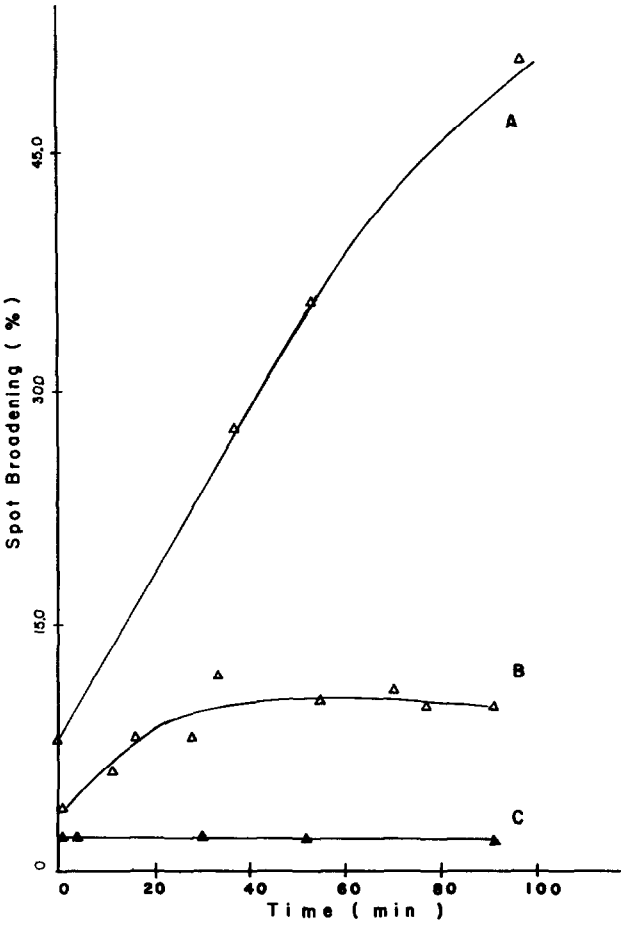

Fig. 3. Spot broadening as a function of time between dipping and scanning the plate for fluoranthene. A, 35% (v/v) dodecane in hexane; B, ≈1% (v/v) Triton X-100 in hexane; C, 35% (v/v) Fomblin Y-Vac in 1,1,2-trichlorotrifluoroethane.

was kept as short as practically possible. For Fomblin Y-Vac and Triton X-100 spot broadening at time zero is quite small and probably tolerable. In the case of dodecane, however, spot broadening is significant, and furthermore, increases fairly sharply with time. A similar trend is observed with Triton X-100, although in this case less dramatically. After about 30 min the spot size reaches a constant value. With dodecane about 200 min is required to reach a constant spot size, after which, presumably an equilibrium is obtained in the liquid-adsorbent sample distribution ratio, Fig. 4.

As far as the PAHs are concerned, if resolution is to be maintained at its highest value, then this is not possible with the fluorescence enhancement reagents and conditions studied. If fluorescence enhancement is required to improve sample detectability then all measurements should be made as soon as possible after dipping.

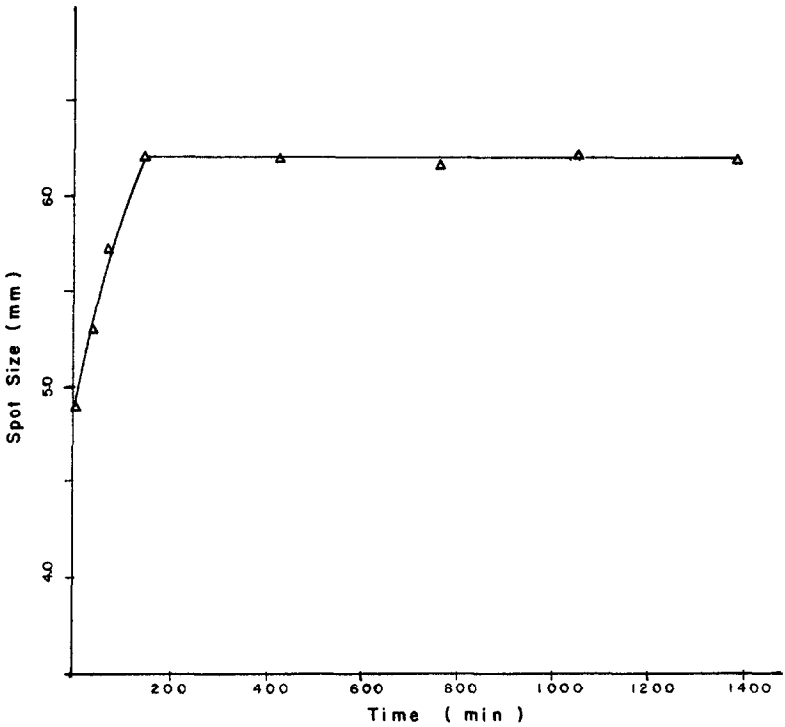

Fig. 4. Spot broadening as a function of time between dipping and scanning the plate for fluoranthene using 35% (v/v) dodecane in hexane as the fluorescence enhancing reagent.

Influence of the fluorescence enhancement reagent on the position of the fluorescence emission wavelength maximum

As well as changes in intensity, impregnating the silica gel plate with a non-volatile liquid can result in changes in the position of the emission wavelength maximum. As emission measurements with the Shimadzu CS-910 scanning densitometer are made by interchanging a series of filters separated by 50 nm increments, changes in the emission wavelength maxima have to be fairly large to be observed. No changes in the position of the emission wavelength maxima were found for benzo[*k*]fluoranthene, benzo[*b*]fluoranthene, fluoranthene, dibenzo[*a,h*]anthracene, chrysene, fluorene, and phenanthrene with the reagents studied. After treatment with dodecane the emission maxima of benzo[*g,h,i*]perylene was shifted from 500 to 450 nm, benzo[*a*]pyrene from 550 and 500 nm to 450 and 400 nm, and indeno[1,2,3-*c,d*]pyrene from 550 and 500 nm to 500 nm. Likewise, after treatment with Triton X-100, the emission wavelength maxima for benzo[*j*]fluoranthene shifted from 550 to 500 nm, benzo[*g,h,i*]perylene from 500 to 450 nm, benzo[*a*]pyrene from 550 and 500 nm to 450 and 400 nm, and indeno[1,2,3-*c,d*]pyrene from 550 and 500 nm to 500 nm. After treatment with Fomblin Y-Vac the emission wavelength maxima for benzo[*g,h,i*]perylene shifted from 500 to 450 nm, benzo[*a*]anthracene from 450 to 400 nm, indeno[1,2,3-*c,d*]pyrene from 550 and 500 to 500 nm, benzo[*e*]pyrene from 500 to 400 nm, and pyrene from 400 nm to the range covered by the UV-D2 filter. Only benzo[*g,h,i*]perylene and indeno[1,2,3-*c,d*]pyrene have their emission wavelength

TABLE II

ENHANCEMENT RATIO AS A FUNCTION OF WAVELENGTH AFTER DIPPING THE PLATE IN DODECANE–HEXANE (33:67)

Compound	*Enhancement ratio at λ_{EM} (nm)*					
	UV-D2	*400*	*450*	*500*	*550*	*600*
Benzo[*k*]fluoranthene	–	2.3	2.8	2.5	2.2	2.6
Benzo[*j*]fluoranthene	–	–	–	1.8	1.9	1.8
Benzo[*b*]fluoranthene	–	3.3	3.3	3.3	3.6	3.1
Fluoranthene	–	2.3	2.3	2.8	2.8	2.1
Anthracene	–	9.2	11.8	–	–	–
Benzo[*a*]anthracene	–	18.3	22.8	–	–	–
Benzo[*g,h,i*]perylene	–	–	17.0	1.4	–	–
Dibenzo[*a,h*]anthracene	–	2.3	1.3	0.07	–	–
Indeno[1,2,3-*c,d*]pyrene	–	–	2.6	2.2	1.9	1.3
Benzo[*a*]pyrene	–	46.4	40.4	2.4	0.3	–
Benzo[*e*]pyrene	2.2	15.7	3.1	–	–	–
Pyrene	2.5	26.2	–	–	–	–
Chrysene	10.5	4.8	0.7	–	–	–
Fluorene	6.2	–	–	–	–	–
Phenanthrene	34.2	–	–	–	–	–

maxima shifted by all three reagents. In all cases for which a wavelength shift was observed the direction was to a shorter wavelength after the plate had been impregnated with reagent.

Fluorescence enhancement ratio as a function of reagent type

The fluorescence enhancement ratio, measured for a series of PAHs as a function of emission wavelength, is given in Table II for dodecane, Table III for Triton

TABLE III

ENHANCEMENT RATIO AS A FUNCTION OF WAVELENGTH AFTER DIPPING THE PLATE IN A SATURATED SOLUTION OF TRITON X-100 IN HEXANE ($\approx$1:99)

Measurements made as close to $t = 0$ as practically possible.

Compound	*Enhancement ratio at λ_{EM} (nm)*				
	400	*450*	*500*	*550*	*600*
Benzo[*k*]fluoranthene	2.0	2.3	1.9	1.2	–
Benzo[*j*]fluoranthene	3.6	1.9	2.2	–	–
Benzo[*b*]fluoranthene	1.2	1.3	0.9	0.9	0.8
Fluoranthene	1.2	5.5	5.0	3.7	2.4
Anthracene	13.5	8.5	–	–	–
Benzo[*a*]anthracene	5.4	7.1	–	–	–
Benzo[*g,h,i*]perylene	–	4.1	0.2	–	–
Dibenzo[*a,h*]anthracene	2.7	2.0	0.2	–	–
Indeno[1,2,3-*c,d*]pyrene	–	–	2.5	1.4	0.8
Benzo[*a*]pyrene	17.8	15.7	0.9	–	–
Benzo[*e*]pyrene	3.9	0.3	–	–	–

TABLE IV

ENHANCEMENT RATIO AS A FUNCTION OF WAVELENGTH AFTER DIPPING THE PLATE IN FOMBLIN Y-VAC–1,1,2-TRICHLOROTRIFLUOROETHANE (33:67)

Compound	*Enhancement ratio at λ_{EM} (nm)*					
	UV-D2	*400*	*450*	*500*	*550*	*600*
Benzo[*k*]fluoranthene	–	6.8	6.4	5.0	3.4	2.6
Benzo[*j*]fluoranthene	–	–	*	2.4	2.5	2.3
Benzo[*b*]fluoranthene	–	4.1	3.6	3.0	2.7	2.7
Fluoranthene	*	3.6	3.0	3.0	3.0	3.3
Anthracene	8.8	6.3	6.7	*	–	–
Benzo[*a*]anthracene	–	100	40.4	16.4	10.5	*
Benzo[*g,h,i*]perylene	–	69	6.9	0.4	0.2	0.2
Dibenzo[*a,h*]anthracene	–	10	5.4	1.0	0.1	–
Indeno[1,2,3-*c,d*]pyrene	–	–	4.7	4.1	3.3	2.7
Benzo[*a*]pyrene	–	28.7	33.9	*	*	–
Benzo[*e*]pyrene	–	38.7	4.6	0.5	0.2	–
Pyrene	**	94.0	*	*	–	–
Chrysene	12.6	2.8	0.7	–	–	–
Fluorene	30.1	–	–	–	–	–
Phenanthrene	86.1	*	–	–	–	–

* No signal before dipping but a response found afterwards.
** No signal before dipping but a very large response afterwards (6.7 × value at 400 nm).

X-100, and Table IV for Fomblin Y-Vac as the fluorescence enhancement reagents. Fluorescence measurements below 400 nm could not be made with Triton X-100 due to interference from the reagent. All enhancement ratios refer to a sample size of approximately 40.0 ng except for pyrene, chrysene, fluorene, and phenanthrene, for which a sample size of approximately 100.0 ng was required to give a measurable signal prior to dipping. The fluorescence enhancement ratio varied in size by over two orders of magnitude and depends on both the fluorescence enhancement reagent and the PAH investigated. The most generally useful reagent was Fomblin Y-Vac which provided the highest fluorescence enhancement ratios with only a few exceptions.

CONCLUSIONS

Fluorescence enhancement of PAHs on silica gel HPTLC plates by impregnating the plate with non-volatile liquids prior to detection is a useful technique for imporving sample detectability. Of the reagents investigated Fomblin Y-Vac provides the highest enhancement ratios in general, the least spot broadening due to dipping, no significant spot broadening once the plate has been impregnated, and permits measurements throughout the full fluorescence emission region of the samples. Dodecane causes substantial spot broadening both during dipping and once the plate is impregnated. For this reason it is less useful than Fomblin Y-Vac. Triton X-100 produces more spot broadening than Fomblin Y-Vac, prevents measurements being

made at emission wavelengths less than 400 nm, and shows a marked time dependence for the fluorescence enhancement ratio to reach a maximum after dipping. Its use is less convenient than Fomblin Y-Vac with PAH samples.

The mechanism of the fluorescence enhancement process is not clearly understood. In fact, several probable explanations are available from studies of surface luminescence, solvate–adsorbate interactions using fluorescent probes, and phosphorescence studies[21–24]. Rather than speculate as to the likely mechanism from among several alternatives, for which definitive experimental evidence is either lacking or difficult to obtain, the interested reader is directed to the references cited above.

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