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Resolution of 13 Polycyclic Aromatic Hydrocarbons by Constant-wavelength Synchronous Spectrofluorometry

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A method capable of determining 13 PAHs (acenaphthene, anthracene, benzo[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenzo[*ah*]anthracene, fluoranthene, fluorene, indene[1,2,3-*cd*]pyrene, phenanthrene and pyrene) in a mixture of 16 EPA PAHs by second derivative synchronous spectrofluorometry in the constant wavelength mode was developed. It has not been possible to determine the following PAHs in the mixture: acenaphthylene, benzo[*ghi*]perylene and naphthalene. The approach studied allows the sensitive, rapid and inexpensive identification and quantitation of 13 PAHs in a solution of hexane. The detection limits are <1 μg L^{-1} (except for chrysene and phenanthrene).

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds whose mutagenic and/or carcinogenic effects are well known. These substances can be produced in natural and anthropogenic processes and they can be found in many different kinds of samples, both biological[1-5] and environmental.[7-9] For this reason, their detection and monitoring has become an important problem and these needs have led to the development of new analytical methods with improved selectivity and sensitivity.

The interest in monitoring PAHs has increased in recent years because of their potential as carcinogens and their frequent occurrence in the environment.

The USEPA[10] has recognized 16 PAHs as priority contaminants. PAHs have been included in the CERCLA priority list of hazardous substances, and in the CEP list (substances most frequently found in Completed Exposure Pathways at hazardous waste sites).[11] IARC[12] classifies those compounds according to their carcinogenic actions in the groups 2A, 2B and 3.

Spectrofluorometry, because of its high sensitivity and selectivity, is a powerful analytical technique for the analysis of chemical pollutants in environmental samples. The selectivity is based on the facts that relatively few compounds show intrinsic fluorescence and that emission intensity depends on two variables: excitation and emission wavelengths. Therefore, fluorescent compounds can be determined by means of either their excitation or their emission spectra, or even by their synchronous spectra.

In conventional luminescence spectrometry, an emission spectrum can be monitored by scanning the emission wavelength (λ_{em}) while the luminescent compound is excited at fixed excitation wavelength (λ_{ex}). On the other band, an excitation spectrum can be obtained by scanning the excitation wavelength (λ_{ex}) while the emission is monitored at a given emission wavelength (λ_{em}). Another possibility suggested consists of simultaneously varying both the excitation and emission wavelengths. This technique has several variants, depending on the scan-rates of the two monochromators. If the scan-rate is constant for both monochromators, and therefore a constant wavelength interval ($\Delta\lambda$) is kept between λ_{em} and λ_{ex}, the technique is known as synchronous excitation fluorescence spectroscopy and was introduced by Lloyd.[13]

The combination of synchronous excitation and second derivative technique was suggested by Green and O'Haver in 1974[14,15] for enhancing minor spectral features and allowing more reliable identification of spectra. The main advantages of this technique are selectivity, sensitivity, rapidity, simplicity and low cost. Synchronous fluorescence spectroscopy has been applied to the identification and determination of PAHs[3,4,14,16-29] and other fluorescent molecules, since their synchronous spectra give considerably more structural information than the conventional spectra.

Liquid chromatography with a fluorescence detector is perhaps the technique more used for PAH determination,[7,3,30-32] but chromatographic methods are more expensive, more time-consuming and require large amounts of organic solvents.

According to these analytical features and technical advantages, we directly studied a defined mixture of 16 EPA PAHs with highly overlapping spectra using second derivative synchronous spectrofluorometry in the constant wavelength interval ($\Delta\lambda$) mode.

Experimental

Apparatus

All spectrofotometric measurements were performed in a Perkin-Elmer LS-50 luminescence spectrometer equipped with

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Table 1 Wavelength intervals ($\Delta\lambda$) selected for each PAH and λ_{exc} used for their identification

PAH	$\Delta\lambda$	λ_{exc}	PAH	$\Delta\lambda$	λ_{exc}
Ana	90	232	Ch	115	269
Any	65	281	DBA	95	299
A	120	255	F	155	289
BaA	95	290	Fl	40	263
BaP	15	387	In	190	316
BbF	160	302	N	115	222
BkF	25	402	Pe	110	253
Bghi	115	301	Py	50	334

Ana (acenaphthene), Any (acenaphthylene), A (anthracene), BaA (benzo[*a*]anthracene), BaP (benzo[*a*]pyrene), BbF (benzo[*b*]fluoranthene), BkF (benzo[*k*]fluoranthene), Bghi benzo[*ghi*]perylene), Ch (chrysene), DBA (dibenzo[*ah*]anthracene), F (fluoranthene), Fl (fluorene), In (indene[1,2,3-*cd*]pyrene), N (naphthalene), Pe (phenanthrene), Py (pyrene).

Table 2 Ranges of concentration and spectral regions (excitation wavelength in direct spectra or intervals between maximum–minimum in second derivative), selected for the detection limit (LD) and quantification limit (LQ) of the resolved PAHs

PAH	Range/ μg L^{-1}	Spectral region	LD/ μg L^{-1}	LQ/ μg L^{-1}
Ana	1 – 12	$\lambda_{232} - \lambda_{237}$	0.10	0.80
A	1 – 12	λ_{255} (direct spectra)	0.77	1.08
BaA	1 – 12	$\lambda_{284} - \lambda_{290}$	0.12	1.26
BaP	1 – 12	λ_{387} (direct spectra)	0.41	1.08
BbF	1 – 12	$\lambda_{298} - \lambda_{302}$	0.71	1.85
BkF	0.1 – 1.2	$\lambda_{397} - \lambda_{402}$	0.14	0.33
Ch	1 – 12	$\lambda_{265} - \lambda_{269}$	2.48	8.34
DBA	1 – 12	$\lambda_{299} - \lambda_{303}$	0.1	0.68
F	1 – 12	$\lambda_{284} - \lambda_{289}$	0.65	1.95
Fl	1 – 12	$\lambda_{258} - \lambda_{263}$	0.92	1.81
In	2.5 – 30	$\lambda_{316} - \lambda_{321}$	0.1	2.06
Pe	8 – 99	$\lambda_{249} - \lambda_{253}$	29.4	84.9
Py	1 – 12	$\lambda_{334} - \lambda_{340}$	0.41	1.78

See footnote of Table 1 for abbreviations.

a xenon lamp, Monk-Gillieson monochromators and 1 cm quartz cuvettes with teflon stoppers. Spectral data acquisition and processing were carried out by means of the program Fluorescence Data Manager (Ver. 3.5) and FL WinLab (Ver. 2.01) serially interfaced (RS232C) to the LS-50.

Reagents and standards

Residue analysis grade *n*-hexane was purchased from Merck. Benzo[*a*]pyrene (BaP), benzo[*a*]anthracene (BaA), chrysene (Ch), fluoranthene (F) and pyrene (Py) were purchased from Aldrich. Acenaphthene (Ana), acenaphthylene (Any), anthracene (A), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*ghi*]perylene (Bghi), dibenzo[*ah*]anthracene (DBA), fluorene (Fl), indene[1,2,3-*cd*]pyrene (In), naphthalene (N) and phenanthrene (Pe) were from Sugelabor.

Preparation of standards

Stock solutions containing 100 mg L^{-1} and 100 μg L^{-1} of PAH individuals were prepared in *n*-hexane and were stored at 4°C in glass vials wrapped in aluminium foil to avoid possible light degradation. Concentrations (individual component solution and in the mixture) of working solutions were 8 μg L^{-1} for all the PAHs, except for BkF (0.8 μg L^{-1}), Bghi and In (30 μg L^{-1}), Any, Pe and N (100 μg L^{-1}), because of the fluorescence efficiency of these molecules. Other mixture solutions were also studied.

Procedure

Forty synchronous fluorescence spectra with a wavelength interval of 10 nm between the excitation and emission monochromators and an increment of 5 nm (range $\Delta\lambda$ from 10 nm to 205 nm) of the 16 PAH individual hexane solutions and their mixtures, have been recorded between 200 and 500 nm at a scan speed of 240 nm/min and with excitation and emission slit-widths set to 2.5 nm and 5 nm respectively.

Second derivative spectra were then generated by the data handling system of the spectrometer.

Results and Discussion

According to different investigators[15,33] the synchronous fluorescence spectroscopy allows: narrowing of spectral bands, simplification of emission spectra and contraction of the spectral range. This method increases the analysis sensitivity, essentially by avoiding different perturbing effects (Rayleigh and Raman scatter).

If the synchronous technique is used, the resulting spectrum consists of a series of exceptionally well-resolved peaks. Each band corresponds unequivocally to one component in the mixture and can be correlated perfectly with its synchronous signal in each individual spectrum.

The first correlation between the structure of a PAH compound and its fluorescence spectrum is reflected by the dependence of the energy of the 0-0 band with the ring size of the compound.[17] The spectrum of a higher ring number linear cyclic compound occurs generally at a longer wavelength than that of a lower ring number compound. Non-linear PAHs also follow, to a certain extent, this basic rule. With conventional spectrometry, because of severe spectral overlap, this simple rule cannot be efficiently applied, especially when a large number of components in a mixture have to be analyzed.

With the synchronous technique, however, the effect of limiting each individual spectrum to a definite spectral band provides the most useful feature to locate the presence of specific compounds in a mixture. The synchronous technique can achieve some sort of spectral confinement or spectral separation into individual components without requiring any actual physical separation process.

The slit widths for excitation and emission used in the most of the references are of 5 nm for both slits.[18,21] The most intense and resolved peaks in this work were obtained for excitation and emission slit widths set to 2.5 nm and 5 nm, respectively.[14]

The solutions were realized in *n*-hexane, an excellent solvent for use in qualitative and quantitative spectrofluorometric analysis, it offers many advantages over alternative solvents: it is commercially available in the grade of purity necessary and it is essentially free of interfering Raman bands.[34]

The range from 10 nm to 205 nm (40 synchronous spectra) was studied for the PAH individuals and their mixtures. Any $\Delta\lambda$ values higher than 205 nm present Raman bands in the solvent and can not be utilized. The perfect coincidence of the peaks when the spectra of the mixture and the individual compound in the same interval are overlapped reveals the resolution of the compounds. It was necessary to obtain

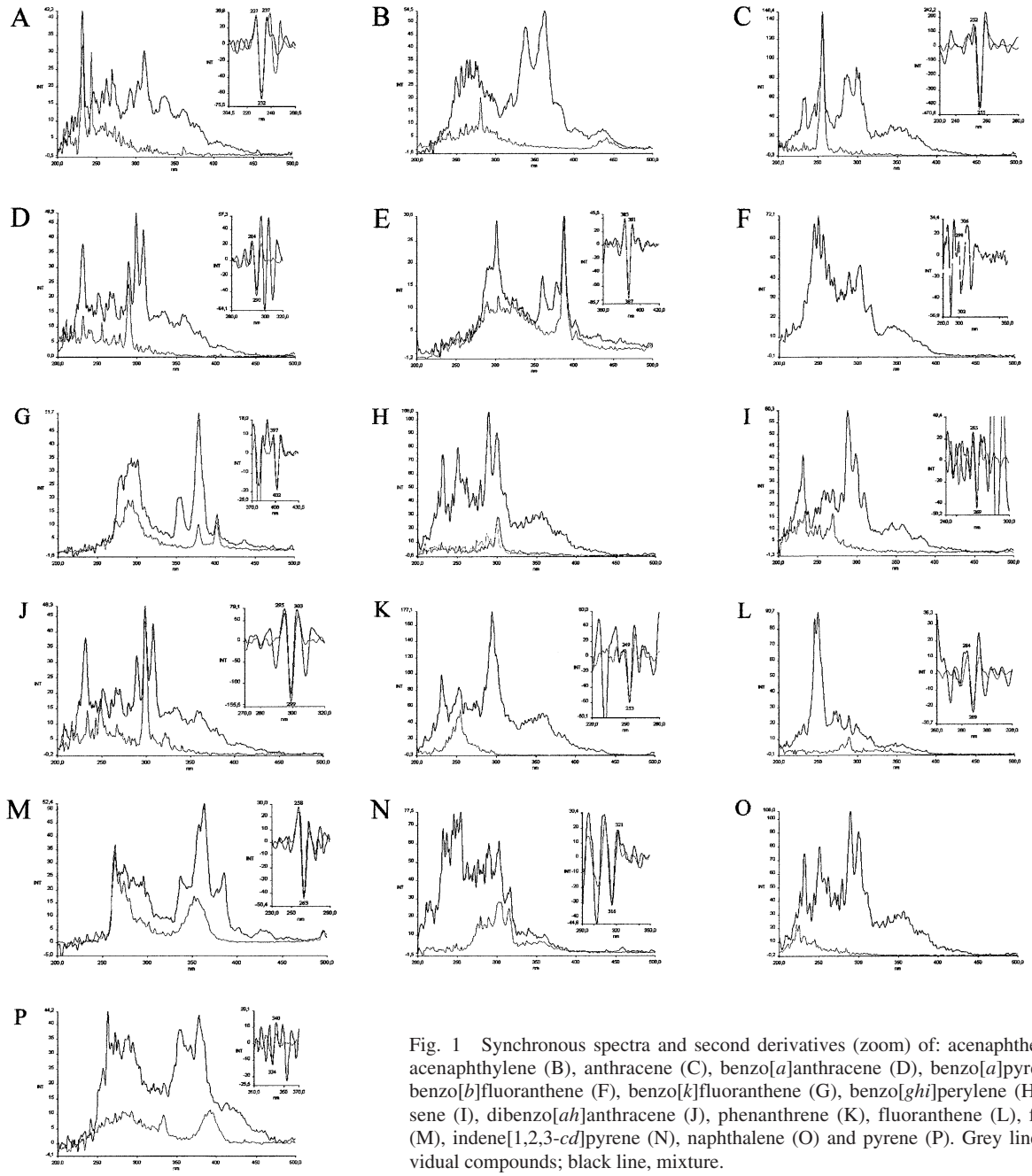

Fig. 1 Synchronous spectra and second derivatives (zoom) of: acenaphthene (A), acenaphthylene (B), anthracene (C), benzo[*a*]anthracene (D), benzo[*a*]pyrene (E), benzo[*b*]fluoranthene (F), benzo[*k*]fluoranthene (G), benzo[*ghi*]perylene (H), chrysene (I), dibenzo[*ah*]anthracene (J), phenanthrene (K), fluoranthene (L), fluorene (M), indene[1,2,3-*cd*]pyrene (N), naphthalene (O) and pyrene (P). Grey lines, individual compounds; black line, mixture.

second-derivative spectra since complete identification and quantification of the compounds using direct spectra was impossible. The second-derivative technique is a mathematical operation which eliminates constant background. In these cases the perfect coincidence was with the minimum, or the maximum–minimum interval in the second-derivative spectra.

The resolution of 13 PAHs (of the 16 PAHs EPA list) was carried out in the working mixture. The compounds identified, the intervals ($\Delta\lambda$) where the resolution was achieved and the maximum excitation wavelength found in the direct spectra are given in Table 1. Acenaphthylene and naphthalene have very little sensitivity and benzo[*ghi*]perylene is overlapped with dibenzo[*ah*]anthracene (both present maxima at 301 and 299 nm, respectively, with $\Delta\lambda = 115$ (Fig. 1)).

The calibration line (with determination coefficients approaching 1.000), constructed by regression peak-to-through height in the direct spectra, or the maximum–minimum interval in the second derivative spectra, permit one to determine the detection and quantification limits[35] for each PAH of the 13 identified (Table 2).

In Fig. 1 are shown the selected synchronous spectra and second derivative (zoom) for each PAH and their mixtures in hexane.

Conclusions

The resolution of 13 PAHs was achieved in the working mixture of a solution of 16 EPA PAHs in hexane. Using eleven different $\Delta\lambda$, 13 PAHs were identified and quantified. Limits of detection < 1 μg L^{-1} and quantification < 2 μg L^{-1}, were obtained except for chrysene and phenanthrene.

The method proposed is simple and rapid (2.5 min for a fluorescence report) and the fact that several spectra with

different $\Delta\lambda$ values can be successively recorded on the same sample makes the synchronous fluorescence method suitable for routine analysis for determining PAHs.

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