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Rapid simultaneous determination of four anthracene derivatives using a single non-linear variable-angle synchronous fluorescence spectrum

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Abstract A rapid, simple and inexpensive spectrofluorimetric method has been developed for the simultaneous identification and quantification of anthracene (ANT), 9,10-dimethylanthracene (DIM), 2-aminoanthracene (AMI) and dibenz*[ah]*anthracene (DIB). A well-resolved spectrum for the mixture of these four compounds is obtained based on a single non-linear variable-angle synchronous scanning. The linear concentration ranges are 10–1000, 5–500, 50–1000 and 10–200 ng mL^{-1} for ANT, DIM, AMI and DIB, respectively, at $\lambda_{ex}/\lambda_{em}$ = 358/380, 399/408, 414/465 and 298/394 nm, respectively. The analyses are performed in cyclohexane. Recoveries of 90.0–111.0% in synthetic mixtures are obtained. The detection limits are 2.0 ng mL^{-1} for DIM, 2.7 ng mL^{-1} for ANT, 15.8 ng mL^{-1} for AMI and 4.2 ng mL^{-1} for DIB. The method has also been applied to several real water samples with satisfactory results.

1 Introduction

Research on polycyclic aromatic hydrocarbons (PAHs) is a hot topic in environmental chemistry because of their mutagenic and carcinogenic properties. Due to the strong native fluorescence of PAHs, spectrofluorimetry is a good technique to analyze PAHs with high sensitivity and moderate selectivity. For highly complex samples, however, the technique is usually not sufficiently selective and combination with liquid chromatography is needed [1, 2]. The chromatographic methods are time-consuming, expensive and solvent-consuming. Synchronous fluorescence spectrometry may be the most popular solution for PAHs analysis to increase selectivity, while maintaining sensitivity [3–12]. Other fluorescence approaches include derivative techniques [13], excitation-emission matrices [14], Shpol'skii spectrometry [15] and a time-resolved technique [16].

Synchronous fluorescence spectrometry, involving the simultaneous scanning with both the excitation and emission monochromators, is capable of reducing spectral overlapping, narrowing spectral ranges and eliminating the interference of scattered light. However, conventional synchronous fluorescence with a constant-wavelength interval ($\Delta\lambda$) is a result of a 45° section cut through the contour map. The drawback of constant-energy synchronous fluorescence (CESF) is similar to constant-wavelength synchronous fluorescence (CWSF) by a fixed section cut, though it is useful in identifying PAHs with similar vibrational intervals [5, 6, 10]. Variable-angle synchronous fluorescence (VASF) spectrometry, in which the wavelength separation between the emission and excitation monochromators is varied, offers a considerable flexibility in comparison with CWSF or CESF [17, 18]. Non-linear variable-angle synchronous fluorescence (NLVASF) has been an extension of linear VASF to enhance flexibility. In this approach, the trajectory of the scan is varied continuously through the contour map. A curved trajectory can be followed, allowing optimum measurement points to be included and unwanted signals to be avoided. NLVASF takes full advantage of the information of both the excitation and emission spectra of different components. It has successfully been applied to the simultaneous determination of some overlapping PAHs [11, 12] and overlapping pharmaceutical mixtures [18, 19].

Until now, NLVASF, in its pure concept, has not been widely applied mainly because of the mechanical difficulty in the synchronization of the movements of the excitation and emission monochromators at varied scan speeds. For a complicated scan route, it has only been achieved through sophisticated software [18, 19].

In this work, rapid resolution of four spectrally-overlapping anthracene derivatives in a mixture has been achieved from a single spectrum by a single non-linear variable-angle synchronous scanning. These four anthracene derivatives have been difficult to analyze in a

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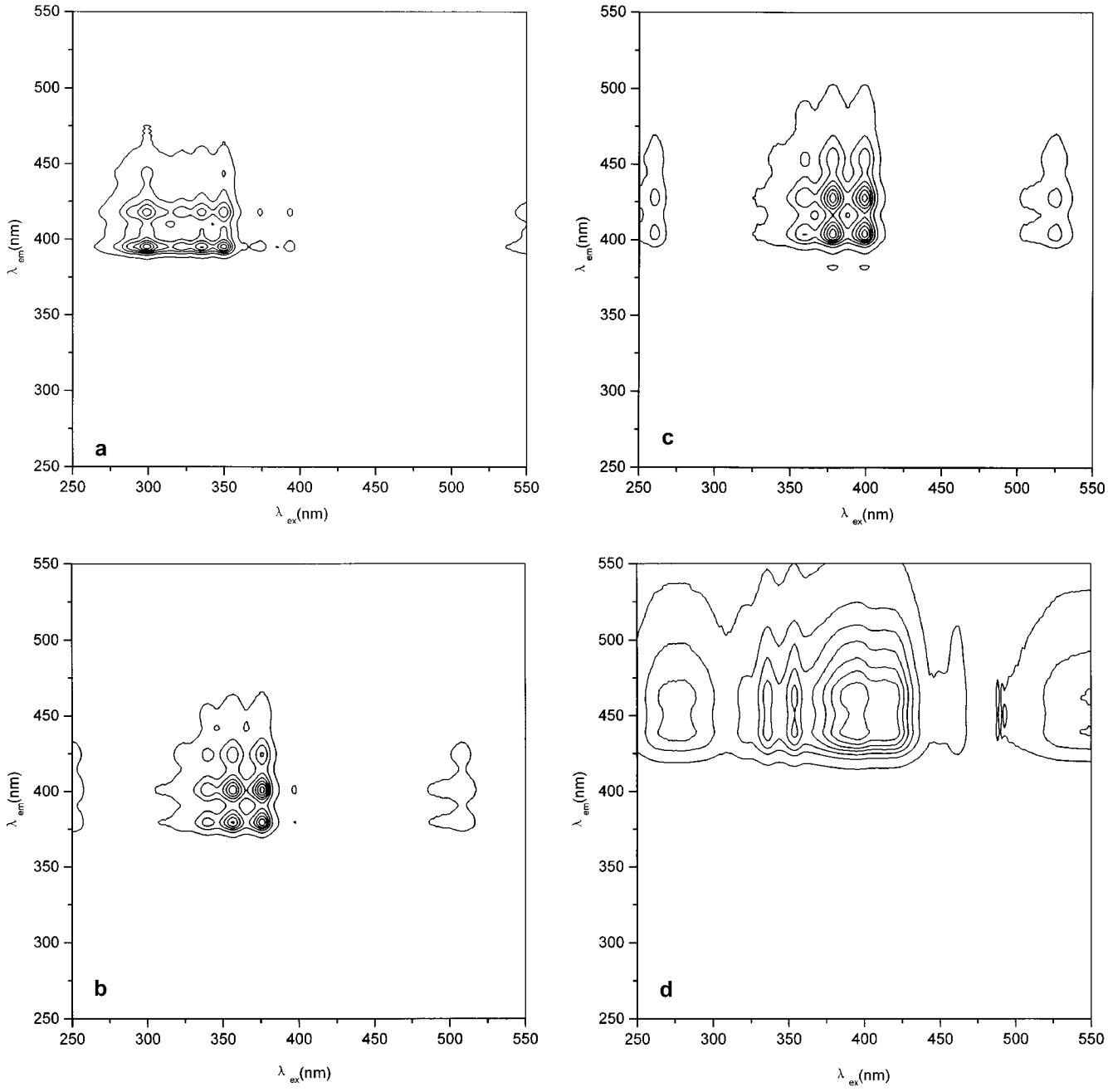
mixture by other fluorimetric methods. The application of the method to multi-component simultaneous analysis provides a good example of the high resolving power of this technique without the need to resort to expensive or time-consuming procedures. The measurement was performed on a laboratory-constructed spectrofluorimeter which could scan with any curved scan route.

Fig. 1 Contour plots for (**a**) DIB (1.00 μg mL^{-1}); (**b**) 9,10-dimethylanthracene (0.25 μg mL^{-1}); (**c**) DIM (0.10 μg mL^{-1}); (**d**) AMI (0.60 μg mL^{-1}) and (**e**) four overlaid contour plots of the individual solutions mentioned above. The bold folded line through the plot is the selected NLVASF scan route

2 Experimental

2.1 Apparatus and software All spectra were obtained on a laboratory-constructed computer (IBM)-controlled spectrofluorimeter similar to the one previously described [10, 11, 17, 20]. It was equipped with a 500 W xenon lamp. Excitation and emission grating monchromators were interfaced to the computer for controlling the instrument. A software package written in Turbo C 2.0 controlled the instrument data collection. Slit bandpasses were set at 5 nm. A quartz cuvette with a pathlength of 1 × 1 cm^2 was used throughout.

A program [20] written recently in Visual Basic for Excel was used for further data processing. The program was run on an IBM-PC/Pentium MMX 200, which was interfaced with the above computer.



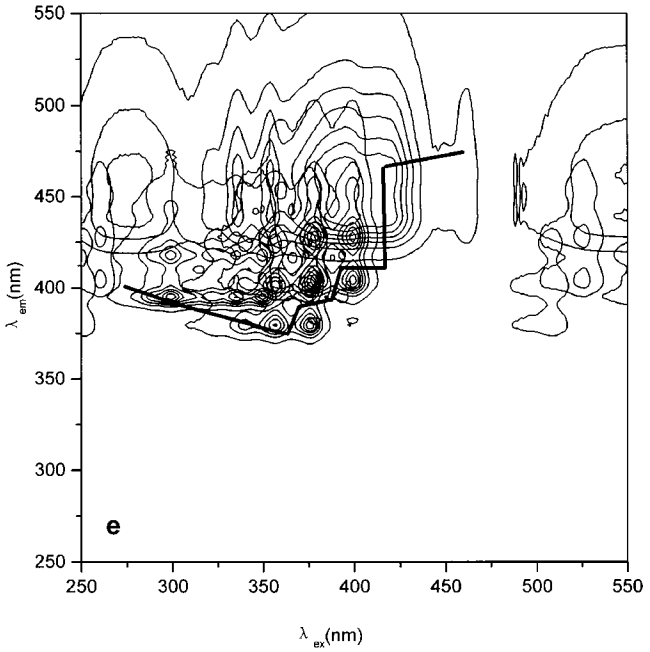


Fig. 1e

2.2 Reagent. Stock solutions of anthracene (98%, Sigma), 9,10-dimethylanthracene (99%, Aldrich), 2-aminoanthracene (99%, Wako Pure Chemical Industries, LTD) and dibenz*[ah]*anthracene (i.e. 1.2, 5.6-dibenzanthracene, 99%, Aldrich) were prepared in cyclohexane (analytical-reagent grade, Shanghai Reagent). The solutions were stored in a dark and cool place.

2.3 Procedure. Aliquots of ANT, DIM, AMI and DIB equivalent to 2.50 μg, 1.00 μg, 6.00 μg and 10.00 μg, respectively, were placed in volumetric flasks and diluted with cyclohexane to a final volume of 10 mL. The concentration levels at which each component produced similar fluorescence intensity were chosen for comparison.

The emission-excitation matrices obtained by using the excitation and emission data were used to acquire a suitable scan route by means of the program mentioned above. The NLVASF spectrum was recorded with the selected scan route.

For the preparation of calibration graphs, aliquots of ANT, DIM, AMI and DIB equivalent to 0.1–10 μg, 0.05–5 μg, 0.5–10 μg and 0.1–2 μg, respectively, were introduced into 10 mL volumetric flasks. To each flask, known amounts of the other three compounds were added and then diluted with cyclohexane to a final volume of 10 mL.

3 Results and discussion

3.1 Spectral characteristics

The full characterization of the fluorescence of compounds can be revealed by their three-dimensional spectra. The three-dimensional spectrum can be obtained either by a software method or by a mechanical scan method. In this work, we used the following program to obtain the three-dimensional spectra. Three-dimensional spectra of each component were derived from a single excitation spectrum and a single emission spectrum. For the selection of the scan route, during the acquisition of the three-dimensional spectra, it would be better if the spectral intensities of each compound were almost the same. If the intensities of the components differed very much, the densities of the iso-intensity line of each component on the contour plot differed too much to provide a guidance to choose scan route. The concentration levels used that produced similar intensities were 0.25 μg mL^{-1} for ANT, 0.10 μg mL^{-1} for DIM, 1.00 μg mL^{-1} for DIB, and 0.60 μg mL^{-1} for AMI. At these concentrations, the fluorescence intensities were suitable for the determination of the scan route.

Figure 1 (a)–(d) shows the contour plots of DIB, ANT, DIM and AMI, respectively, and Fig. 1 (e) the overlaid contour plots of the individual components. In these contour plots there was a signal at long excitation wavelengths, which resulted from the second-order light excitation at these wavelengths (the PAHs have second electronic absorption bands below 300 nm).

The spectra of these four compounds overlap badly as is clear from their contour plots in Fig. 1. Owing to spectral overlapping, it is impossible to determine them simultaneously by synchronous fluorescence spectrometry or even by linear variable-angle fluorescence spectrometry. A conventional synchronous fluorescence spectrum and a linear variable-angle fluorescence spectrum of a four-component mixture are shown in Figs. 2 and 3. As can be seen, spectral bands cannot be resolved for each compound.

3.2 Selection of the optimum route

In the application of the non-linear synchronous scan technique, it is necessary to select an optimum scan route. The goal is to produce the best NLVASF scan spectrum

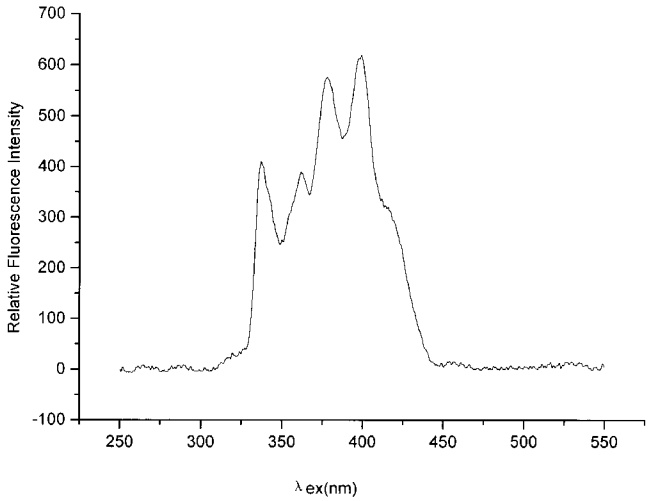


Fig. 2 Constant-wavelength (Δ λ = 60 nm) synchronous fluorescence spectrum of a mixture containing DIB (1.00 μg mL^{-1}), ANT (0.25 μg mL^{-1}), DIM (0.10 μg mL^{-1}) and AMI (0.60 μg mL^{-1}). Excitation wavelength scans from 250 nm to 550 nm at the speed of 120 nm/min

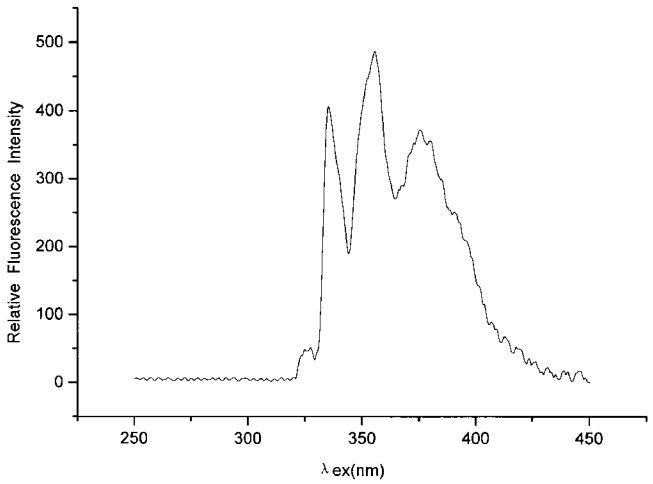
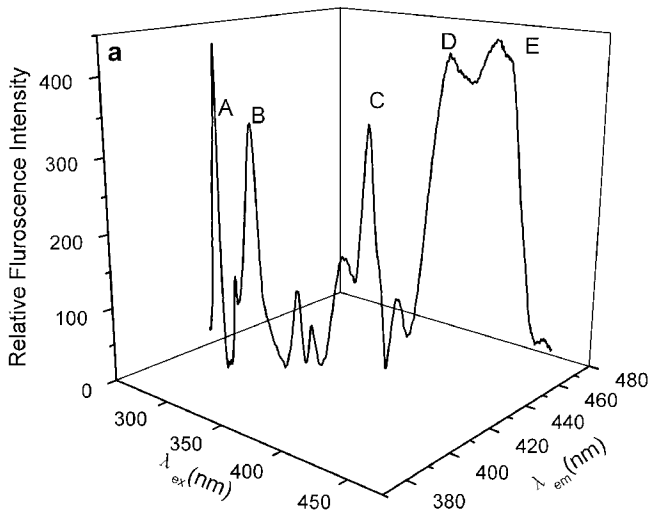


Fig. 3 Linear variable-angle (60° section cut through the contour plot) synchronous fluorescence spectrum of a mixture which contains DIB (1.00 μg mL^{-1}), ANT (0.25 μg mL^{-1}), DIM (0.10 μg mL^{-1}) and AMI (0.60 μg mL^{-1}). Excitation wavelength scans from 250 nm to 450 nm at the speed of 120 nm/min

(highest signal values, interference-free bands). In order to determine a suitable route, two procedures are needed. The first is to determine the detection point of each component, where the spectrum of each compound has a maximum signal value and a minimum interfering signal. Because a non-linear variable-angle synchronous scan is a combination of several linear variable-angle synchronous scans with different angles, in the second procedure other points chosen to make up the whole route are determined.

To find out the optimum detection points for the present system, a careful examination of the contour plots was carried out. We used our program to determine the detection points quantitatively. Rayleigh scattering should also be considered. If the detection point happens to appear on the point (λ_{ex}, λ_{em}), where the difference of the emission and the excitation wavelength is too small (for example, less than 3.0 nm), it is necessary to choose another point. For DIM, though the maximum signal value appears at the point (400, 403) nm, we chose the point (399, 408) nm where Rayleigh scattering is negligible and the fluorescence intensity is still high. In the end, we se-



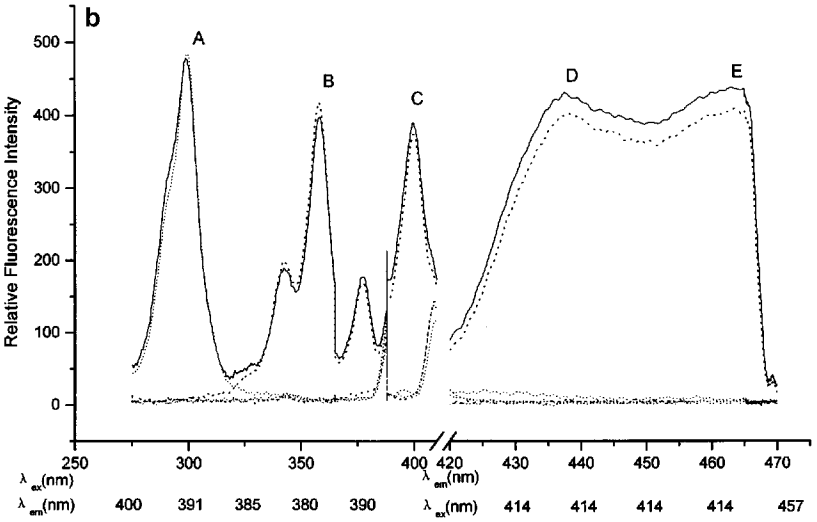


Fig. 4 **(a)** Three-dimensional NLVASF spectrum of a four-component mixture which contains DIB (1.00 μg mL^{-1}), ANT (0.25 μg mL^{-1}), DIM (0.10 μg mL^{-1}) and AMI (0.60 μg mL^{-1}); **(b)** two-dimensional NLVASF spectra of a four-component mixture which contains DIB (1.00 μg mL^{-1}), ANT (0.25 μg mL^{-1}), DIM (0.10 μg mL^{-1}) and AMI (0.60 μg mL^{-1}) (solid line) and the pure substance of DIB, ANT, DIM, AMI of the same concentrations as in the mixture (dotted lines).There are five main peaks (A-E) on either of the three-dimensional and two-dimensional spectra

Table 1 Application of least-squares regression (y = a + bx) to the data of the calibration sets obtained by the non-linear variable-angle synchronous fluorescence

Compound determined	Concentration range (μg mL^{-1})	Component present (μg mL^{-1})	Intercept(a)	Slope(b)	Correlation coefficient	SD of estimation
DIM	0.005–0.5	AMI 0.100 DIB 0.100 ANT 0.100	24	3782	0.9999	10
AMI	0.05–1	DIM 0.100 DIB 0.100 ANT 0.100	25	821	0.9996	10
DIB	0.01–0.2	AMI 0.100 DIM 0.100 ANT 0.100	9	881	0.9996	2
ANT	0.01–1	AMI 0.100 DIB 0.100 DIM 0.100	32	1585	0.9990	31

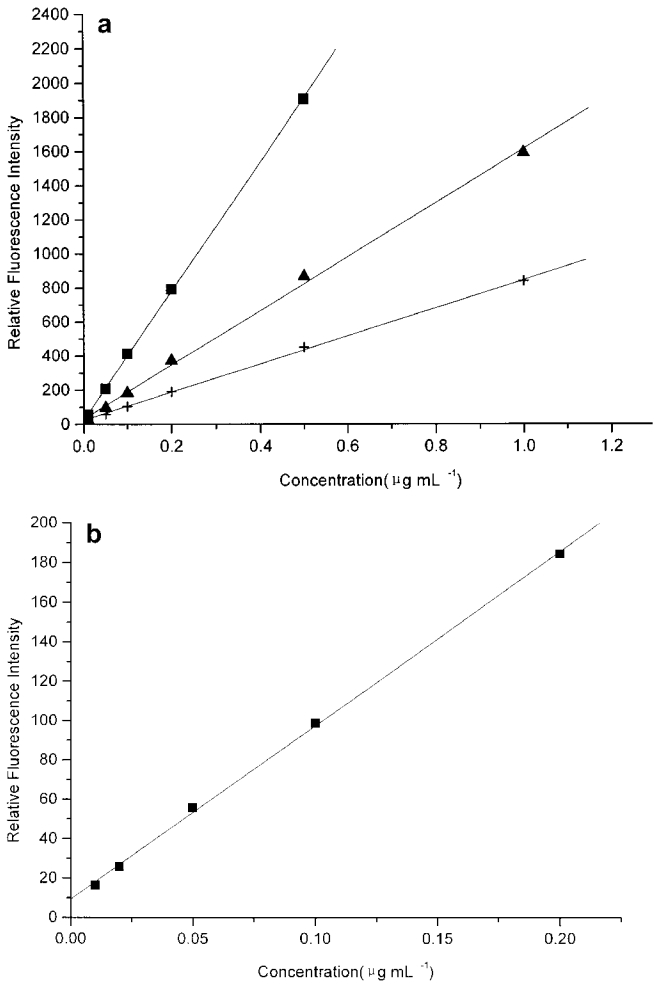


Fig. 5 Calibration curves for the four anthracene derivatives. (**a**) curves for DIM (square), ANT (up triangle) and AMI (cross); (**b**) curve for DIB

lected (358, 380) nm, (399, 408) nm, (414, 465) nm and (298, 394) nm as detection points (λ_{ex}, λ_{em}) for determining ANT, DIM, AMI and DIB, respectively. After the detection points were determined, the other points for establishing the whole route line and the route line were chosen by trial and error to simplify the NLVASF spectrum. The selected determination route is shown in Fig. 1 (e). By using the selection method, an optimum NLVASF scanning route can be defined easily.

3.3 Simultaneous determination of the four substances

Figure 4 shows the NLVASF spectrum of a mixture containing the four substances. This spectrum can be plotted either in three-dimensional (4(a)) or two-dimensional form (4(b)). In 4(b), the fluorescence intensities are plotted against the excitation wavelength axis for ANT, DIM and DIB, and against the emission wavelength for AMI. There are five main peaks (A-E) on the spectra. Peaks A, B, C belong to DIB, ANT, DIM, respectively. Peak D and peak E belong to AMI. They are all well identified. The two-dimensional NLVASF spectra of pure solutions of DIB, ANT, DIM and AMI are also shown in 4(b) for comparison. The three-dimensional NLVASF spectrum is more intentional, while the two-dimensional one is more convenient in obtaining quantitative information of the compounds. For a certain component, whether it is in pure solution or in a mixture, it has the same shape on the NLVASF spectrum. The intensities for the pure species and in a mixture are slightly different, which may be due to the deviation of the measurement or slight interaction among components.

3.4 Mutual interference

In the mixtures, the fluorescence intensities of DIB and DIM are independent of the other components within their concentration range. It has been found that the fluorescence intensity of ANT is slightly reduced by the existence of AMI and the fluorescence intensity of AMI increases slightly with the existence of DIM. These phenomena may be due to an energy-transfer. For a certain component, the interference of other components can be diminished by reducing their concentrations. ANT of

Table 2 Recoveries of the four PAHs in synthetic samples

Mixture sample	Amount added (μg/10 mL)				Amount found (μg/10 mL)				Recovery (%)			
	ANT	DIB	DIM	AMI	ANT	DIB	DIM	AMI	ANT	DIB	DIM	AMI
1	0.50	2.00	1.00	0.50	0.47	2.03	1.11	0.45	94.0	101.5	111.0	90.0
2	1.00	1.00	2.00	2.00	0.93	1.09	2.10	2.13	93.0	109.0	105.0	106.5
3	2.00	1.00	0.50	1.00	2.10	1.10	0.53	0.90	105.0	110.0	106.0	90.0
4	5.00	0.50	5.00	5.00	5.20	0.53	0.51	5.28	104.0	106.0	102.0	105.6

Table 3 Recoveries of the four PAHs in actual water samples

Source	Sample	Amount added (μg/10 mL)				Amount found (μg/10 mL)				Recoveries(%)			
		ANT	DIB	DIM	AMI	ANT	DIB	DIM	AMI	ANT	DIB	DIM	AMI
Sea water	1	2.00	0.50	2.00	0.50	1.82	0.46	2.20	0.46	91.0	92.0	110.0	92.0
	2	1.00	1.00	1.00	1.00	0.95	0.93	1.06	0.90	95.0	93.0	106.0	90.0
	3	0.50	2.00	0.50	2.00	0.50	1.95	0.49	1.80	100.0	97.5	98.0	90.0
Waste water	1	2.00	0.50	2.00	0.50	1.82	0.45	2.12	0.45	91.0	90.0	106.0	90.0
	2	1.00	1.00	1.00	1.00	0.97	0.91	0.96	0.90	97.0	91.0	96.0	90.0
	3	0.50	2.00	0.50	2.00	0.55	1.94	0.48	1.86	110.0	97.0	96.0	93.0
Tap water	1	2.00	0.50	2.00	0.50	1.92	0.46	2.15	0.50	96.0	92.0	107.5	100.0
	2	1.00	1.00	1.00	1.00	0.99	0.98	1.03	0.91	99.0	98.0	103.0	91.0
	3	0.50	2.00	0.50	2.00	0.49	2.09	0.52	1.81	98.0	104.5	104.0	90.5

0.5 μg mL^{-1} was used to test the influence of AMI. When the concentration of AMI was reduced to 1.00 μg mL^{-1}, it had a negligible effect on ANT. As for the interference of DIM on AMI, we also chose 0.50 μg mL^{-1} AMI to determine the influence of DIM. At a concentration below 0.50 μg mL^{-1} DIM had no effect on AMI. In conclusion, the interference between some of the anthracene derivatives can be reduced by lowing their concentrations to some extent.

3.5 Analytical merits

In order to test the mutual independence of the analytical signals for each component and to show that the signal produced by each component is independent of the others within the detection range, a calibration curve was measured for standards containing each component in the presence of the others. When measuring a curve for a certain component, the concentrations of the other three components were chosen within their calibration concentration ranges. At these concentrations, the other components have no or negligible effects on the component. The proposed method has been evaluated by a statistical analysis of the experimental data by fitting the least-squares line according to $y = bx + a$. The results obtained are summarized in Table 1. The calibration curves are shown in Fig. 5. Those for DIM, AMI and ANT are shown in Fig. 5 (a) and for DIB in Fig. 5 (b).

By applying the IUPAC definition, based on three times the standard deviation of the blank, detection limits of 2.0 ng mL^{-1} for DIM, 2.7 ng mL^{-1} for ANT, 15.8 ng mL^{-1} for AMI and 4.2 ng mL^{-1} for DIB were obtained.

In order to check the usefulness of this method, it has been applied to synthetic mixtures and several real water samples. The synthetic samples were prepared by mixing known amounts of the solutions of the anthracene derivatives. The recovery results for the synthetic samples are shown in Table 2. The real water samples were subjected to filtration (filter paper) to remove solid particles prior to the determination. Several kinds of water samples were spiked with different amounts of the four substances to check the recoveries. As shown in Table 3, good agreement between the amounts added and found was obtained, which proved the effectiveness of the proposed approach.

4 Conclusions

The above method allows the simultaneous determination of ANT, AMI, DIB and DIM. Their identification was accomplished in a single scan. A well resolved NLVASF spectrum was obtained for the mixture of these four anthracene derivatives. The NLVASF spectral peaks could be used to identify and quantify the four compounds at the same time. The method has been proved to be simple, rapid and inexpensive. It has been applied to the determination of the four compounds in synthetic mixtures and in several spiked water samples. Recoveries were satisfactory. The method has the potential to increase the selectivity for environment sample analysis. Further investigations are being made on samples containing more fluorescent compounds.

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References

1. Escrivá C, Viana E, Moltó JC, Picó Y, Mañes J (1994) J Chromatogr A 676:375–388
2. Chiu CP, Lin YS, Chen BH (1997) Chromatographia 44: 497–504
3. Vo-Dinh T (1978) Anal Chem 50: 396–401
4. Eiroa AA, Blanco EV, Mahía PL, Lorenzo SM, Rodriguez DP (1998) Analyst 123: 2113–2117
5. Inman EL, Winefordner JD (1982) Anal Chim Acta 138: 245–252
6. Inman EL, Winefordner JD (1982) Anal Chem 54: 2018–2022
7. Miller JS (1999) Anal Chim Acta 388: 27–34
8. Ferrer R, Beltran JL, Guiteras J (1998) Talanta 45: 1073–1080
9. Falcon MSG, Amigo SG, Yusty MAL, Lage BL, Lozano JS (1999) Talanta 48: 377–384
10. Li YQ, Huang XZ, Xu JG, Chen GZ (1992) Anal Chim Acta 256: 285–291
11. Li YQ, Huang XZ (1997) Fresenius J Anal Chem 357: 1072–1075
12. Capitan-vallvey LF, Olmo M del, Avidad R, Navalõn A, Orbe I de, Vilchez JL (1995) Anal Chim Acta 302: 193–200
13. John P, Soutar I (1976) Anal Chem 48:520–524
14. JiJi RD, Cooper GA, Booksh KS (1999) Anal Chim Acta 397: 61–72
15. Gooijer C, Kozin I, Velthorst NH (1997) Mikrochim Acta 127: 149–182
16. Furuta N (1983) Anal Chem 55: 2407–2413
17. Li YQ, Huang XZ, Xu JG, Chen GZ (1994) Talanta 41: 695–701
18. Pulgarín JAM, Molina AA (1996) Anal Chim Acta 319: 361–368
19. Pulgarín JAM, Molina AA, López PF (1998) Anal Chim Acta 370: 9–18
20. Wu C, Sui W, Li YQ (1999) Chem J Chinese Univ 20: 422