Journal of Environmental Sciences (China), Vol. 1, No. 2, pp 109–115, 1989 ISSN 1001–0742

Synchronous fluorescence technique and its use in identification of polynuclear aromatic hydrocarbons in urine samples

Zhao Zhenhua[1] and Quan Wenyi[1]

(Received January 9, 1989)

Abstract—The synchronous fluorescence spectroscopy of three polynuclear aromatic hydrocarbons was studied. It was shown that the specific $\Delta\lambda$ of the spectra were 10nm for benzo(k) fluoranthene, 25 nm for benzo(a) pyrene, and 40 nm for pyrene. The peaks of the spectra were at 407, 404, and 373 nm for the three chemicals, respectively. Benzo(k) fluoranthene, benzo(a) pyrene, and pyrene in the urine samples from smokers were identified by high pressure liquid chromatography combined with the synchronous fluorescence spectroscopy.

Keywords: polynuclear aromatic hydrocarbons; synchronous fluorescence spectroscopy; urine.

Fluorescence spectrophotometry is a photometric analysis method with high sensitivity and selectivity, especially for the analysis of trace polynuclear aromatic hydrocarbons (PAHs) commonly found in environmental samples. For complex mixed samples, however, the usage of this technique is limited. The concept of synchronous excitation was first proposed by Lloyd in 1971, followed by John, Lloyd, and Dinh *et al.*, who published a series reports on the synchronous fluorescence spectrophotometry for PAHs (Lloyd, 1971; John, 1976; Lloyd, 1977; Tuan, 1978). The PAHs in environmental samples and coal liquidation products were determined using this technique by Dinh (Tuan, 1978, 1981). However, no reports on benzo(k) fluoranthene (BkF), one of the important PAHs was found in published literatures, and the reports on benzo(a) pyrene (BaP) differed with each other. BkF is an important environmental pollution with similar fluorescent emission spectrum as BaP. So the super-trace analysis and identification of BkF and BaP has long been a very difficult analytical problem.

We studied the synchronous fluorescence spectra of BkF, BaP, and pyrene, and found these PAHs in the urine samples taken from smokers.

APPARATUS AND PROCEDURES

Apparatus

Hitachi Model MPF–4 fluorescence spectrophotometer and Hitachi Model 638–50 high pressure liquid chromatograph with Model 650–10LC fluorescence detector.

Reagents

BaP and pyrene, Aldrich Chemicals Co. (U.S.A.) BkF, made in West Germany.

Methanol and cyclohexane, analytical reagents, Beijing Chemicals Factory, redistilled when use.

Procedures

Determination of the synchronous fluorescent spectra

Keep the negative high voltage constant(-700V), select the proper relative fluorescence intensity using the sensitivity control and the slit, and scan the synchronous fluorescent spectra.

---

[1]Beijing Municipal Research Institute of Environmental Protection.

The analytical parameters should be selected according to the samples to be measured, and should not be changed after chosen. Quantitative determinations were based on the results of digital display. Cells with 1 cm optical path were used.

Determination of urine samples by HPLC

The urine samples of normal male smokers (about 20 pieces per day), taken from 8:00 to 17:00 were collected and pretreated according to the method given by Song Yanjun (Song, 1983). The cyclohexane extract of the pretreated samples was concentrated and made to graduate, and then separated by HPLC on Virian Model Micropak-MCH-5 column (4 ×150 mm). Gradient elution was performed with 100% mobile phase A (methanol/water=80/20) at the beginning and changed into 100% mobile phase C (methanol) within 10 minutes, and held 10 minutes more. The flow rate was 1 ml/min pyrene; BkF and BaP were determined by fluorescent detector at wavelength λ_{em} 320 nm and λ_{em} 380 nm; λ_{em} 294 nm and λ_{em} 404 nm, respectively. The chromatographic elutes which had the same retention times as the standard samples of BkF, BaP and pyrene were collected separately, and determined by syncronous fluorescence spectrophotometry.

RESULTS AND DISCUSSION

Synchronous fluorescence spectra of BkF, BaP and pyrene

The basic principle of synchronous fluorescence can be expressed as

$$I_L(\lambda_{em}) = K \times C \times E_x(\lambda_{em} - \Delta\lambda) \times E_m(\lambda_{em}) \quad (1)$$

where

I_L= intensity of the measured synchronous fluorescent signal;
K= a constant;
C = concentration of the analyze;
$E_m(\lambda_{em})$= contribution of the emission spectrum to fluorescence intensity;
λ_{em}=emission wavelength;
$\Delta\lambda$= difference between the emission wavelength and the excitation wavelength;
E_x= contribution of the excitation spectrum to the fluorescence intensity.

It can be seen from Equation (1) that the specific synchronous fluorescence spectra of each compound may be studied using different $\Delta\lambda$. Based on this, we investigated the synchronous fluorescence spectra of BkF, BaP and pyrene, and found the optimal $\Delta\lambda$ to obtain the specific synchronous spectra of the compounds. The measured excitation spectra, emission spectra, and synchronous fluorescence spectra of BkF, BaP and pyrene are shown in Fig. 1, 2 and 3, respectively.

It can be seen from Fig. 1 and 2 that the fluorescence spectra of BkF and BaP are very similar, and there are three similar fluorescence peaks within the range of 400–500 nm. These two compounds interfere each other, and are difficult to be identified using traditional fluorescence technique. Bap and BkF can well be separated using modern gas chromatography, the sensitivity of this method, however, is 1 to 3 orders lower than that of fluorescence spectrophotometry. Moreover, BkF can not be separated from BbF, the isomer of BkF, using gas chromatography. Modern HPLC with fluorescence detector, on the other hand, is an effective method for the analysis of trace BkF and BaP, which can be separated to the baseline using optimal inversed phase HPLC. For the analysis of real samples, however, due to the unknown peaks which have the same retention times as the standard samples of BkF and BaP, definite results can not be obtained if stepped collection or static scan is used for fluorescence spectrophotometric detection. Satisfactory results may be obtained, however, when optimal $\Delta\lambda$s for these two compounds are used for the determination of the synchronous fluorescence spectra of the collected elutes.

The experimental results showed that there was a strong and sharp spectral peak of BkF at 407 nm when the synchronous fluorescence spectrum was scanned using $\Delta\lambda$=10 nm (Fig. 1), and there was no spectral peak of BaP at the same conditions. When the synchronous spectral scan was carried out using between 19 and 25 nm, however, there was a strong and sharp spectral peak of BaP at 403–404 nm, and two spectral peaks of BkF. We determined the synchronous fluorescence spectrum of BaP using $\Delta\lambda$ = 25 nm , and a spectrum with the peak at 404 nm was obtained (Fig. 2). A characteristic synchronous fluorescence spectrum of pyrene, with the peak at 373 nm, may be obtained when the synchronous fluorescence scan was made using $\Delta\lambda$=40 nm (Fig. 3). The synchronous fluorescence spectrum was very weak when $\Delta\lambda$ < 30 nm, and there were two peaks when $\Delta\lambda$ > 50 nm, and the peaks might be shifted towards longer wavelength.

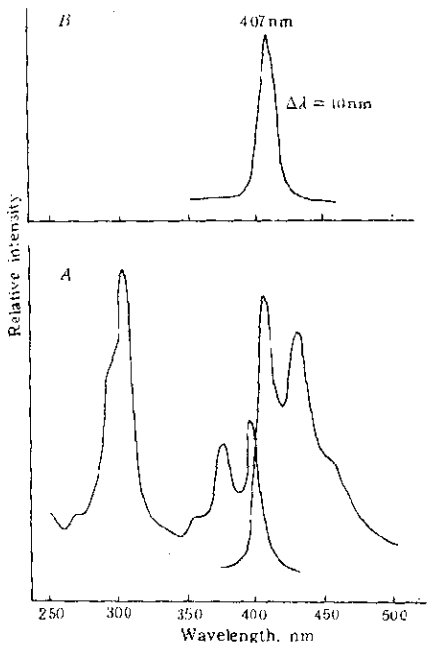

Fig. 1 Fluorescence spectra of BkF in methanol (0.08μg/L) A. excitation spectrum with fixed λ_{em} (450 nm) emission spectrum with fixed λ_{em} (350 nm) B. synchronous spectrum ($\Delta\lambda$ 10 nm)

Experiments were carried out using the mixed solution of BkF, BaP and pyrene, in order to investigate the specificities of the synchronous fluorescence spectra of the mixed solution, and the results are shown in Fig. 4. Three spectra obtained using three different $\Delta\lambda$s are shown in the figure, which indicates that the $\Delta\lambda$ specific for each compound may be used to obtain its characteristic synchronous fluorescence spectrum. These experiments also indicate that there are linear relationships between the peak values of the synchronous fluorescence spectra of the compounds and their concentrations within their respective determination ranges (0–44 ppb for Bap, 0–30 ppb for BkF, and < 170 ppb for pyrene). The linear relationship may be

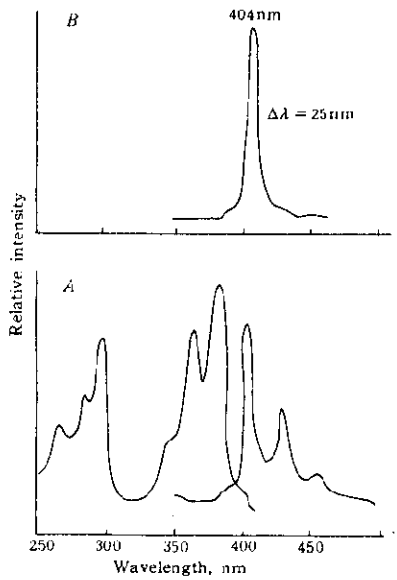

Fig. 2 Fluorescence spectra of BaP in methanol (0.04μg/L) A. excitation spectrum with fixed λ_{em} (450 nm) emission spectrum with fixed λ_{em} (280 nm) B. synchronous spectrum ($\Delta\lambda$ 25 nm)

changed at higher concentration due to the inner optical filtration effect of the solution. The relationship between the relative fluorescence intensities of the synchronous fluorescence spectra and the concentrations of the compounds (F–C curves) are shown in Fig. 5, which indicates that synchronous fluorescence spectrophotometry can be used in the quantitative analysis of trace polycyclic hydrocarbons.

Detection of pyrene, BkF and BaP in urine samples taken from smokers

The existence of proper type BaP in the urine sample taken from smokers implied the existence of other PAHs in human urine. The PAHs in urine samples were concentrated with polyurethane according to the method given by Song Yanjun (Song, 1983), and analyzed by HPLC with fluorescence detector. The existence of pyrene, BkF and BaP were primarily confirmed comparison with the retention times of standard samples (Fig. 6). The application of HPLC in trace analysis is limited, as it can not at present be combined with MS or FTIR as GC. The combination of high sensitive and selective synchronous fluorescent technique with HPLC provides a new approach for the detection of fluorescent compounds as PAHs.

The components which eluted 2 minutes ahead of and after the PAH peak to be measured were collected and detected by synchronous fluorescence spectrophotometry, as shown in Fig. 7. It can be seen that the synchronous fluorescence spectra characteristic for pyrene, BkF, and BaP may be obtained when scanned with their specific $\Delta\lambda$s, while no characteristic fluorescent peaks could be found when scanned with $\Delta\lambda$ other than the specific $\Delta\lambda$s. This result indicated the existence of the three PAHs in urine samples.

HPLC is an effective technique for the analysis of PAHs. For trace analysis, the application of fluorescent detector improves the technique greatly, in comparison with UV detector (Fig. 6). However, there exists interference to a certain degree, due to the complexity of PAH compounds.

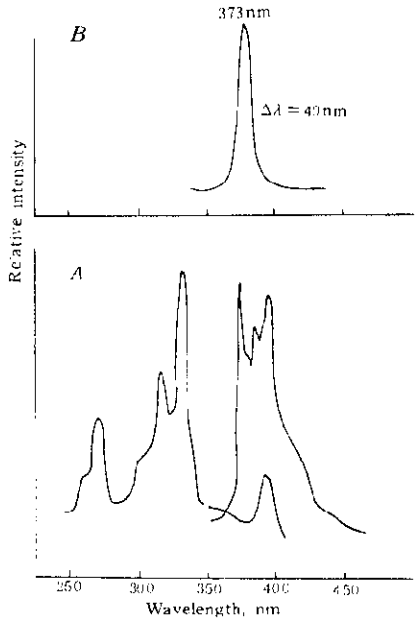

Fig. 3 Fluorescence spectra of pyrene in methanol (0.16μg/L) A. excitation spectrum with fixed λ_{em} (450 nm) emission spectrum with fixed λ_{em} (320 nm) B. synchronous spectrum ($\Delta\lambda$ 40 nm)

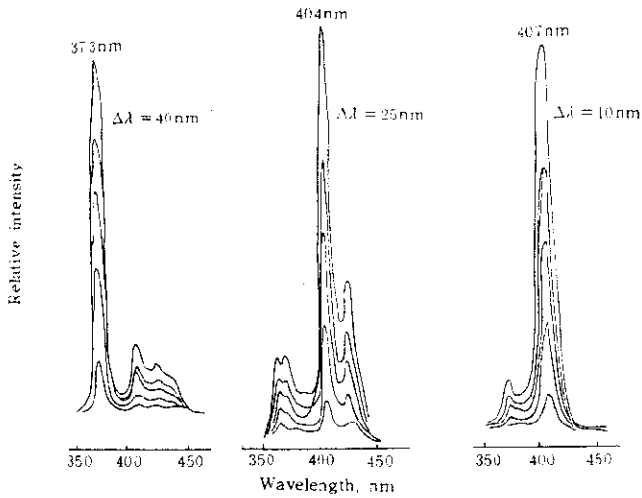

Fig. 4 Synchronous fluorescence spectra of three PAHs in methanol

Our experiments show that synchronous fluorescent technique is very specific, and will become an ideal method for the analysis of fluorescent compounds if the fluorescent detector can be modified into a synchronous fluorescent detector.

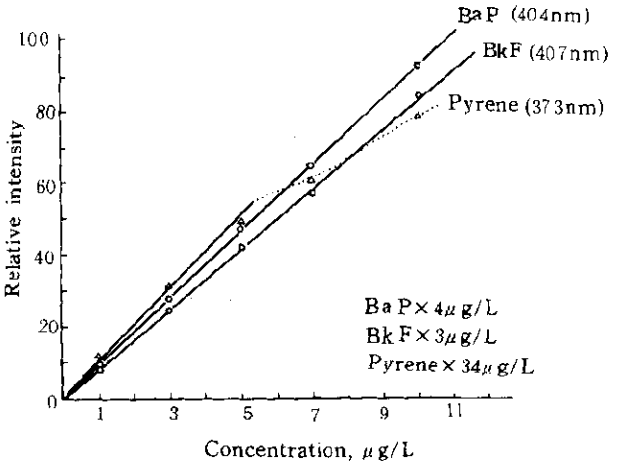

Fig. 5 F-C curve of BaP, BkF and pyrene

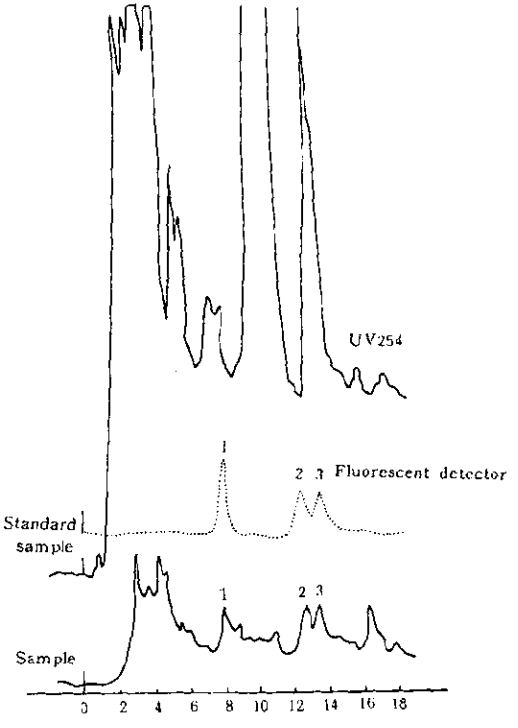

Fig. 6 HPLC chromatogram of extracts from urine of a smoker 1. pyrene 2. BkF 3. BaP

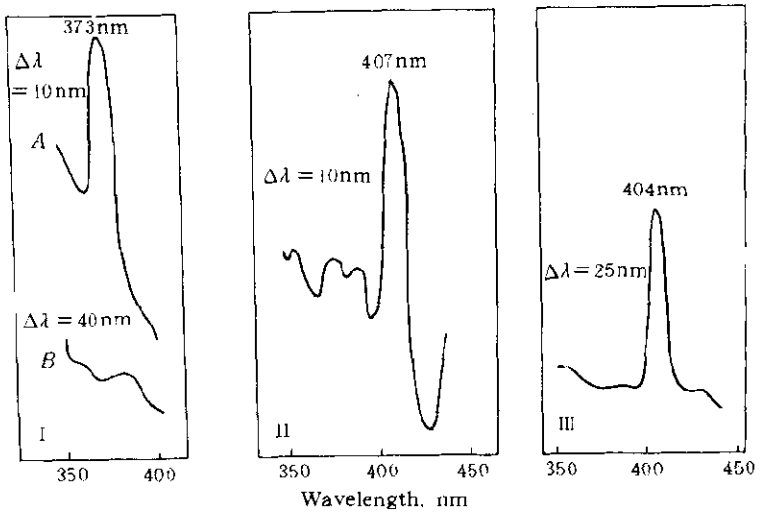

Fig. 7 Synchronous fluorescence spectra of collected eluate from HPLC of urine sample I. from peak 1 in Fig. 6 II. from peak 2 in Fig. 6 III. from peak 3 in Fig. 6

Acknowledgement—We thank Mr. Tian Dehai for his participation in the experiments and Mr. Lei Shihuan for his help in the fluorescence spectrophotometric determinations.

REFERENCES

John P. *et al.*, Anal. Chem., 1976 48 (3): 520
Lloyd J.B.F., Nature (London), 1971, 64: 231
Lloyd J.B.F. *et al.*, Anal. Chem., 1977, 49(12): 1710
Song Yanjun *et al.*, Chinese Journal of Preventive Medicine, 1983, 17 (6) : 357
Tuan Vo-Dinh, Anal. Chem., 1978, 50(3) : 396
Tuan Vo-Dinh *et al.*, Environ. Sci. Technol., 1978, 12(12) : 1297
Tuan Vo-Dinh *et al.*, Analytica Chemica Acta, 1981, 13 : 125