Synchronous Fluorescence Spectroscopic Technique: The Tool for Rapid Identification of Polycyclic Aromatic Hydrocarbons (PAHs) at Sub-ppm Level in Liquid Samples

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ABSTRACT

Various polycyclic aromatic hydrocarbons (PAHs) at sub-ppm level were identified qualitatively by synchronous scanning fluorescence technique at various wavelength intervals ($\Delta\lambda$). Due to the difference in chemical structure, each PAHs gives specific characteristic spectrum for each wavelength intervals ($\Delta\lambda$). This work demonstrated that the synchronous scanning fluorescence method can be used as a tool for the rapid identification of PAHs in ethanolic sample which contain three or six types of PAHs such as fluorene, truxene, benzo(k)fluoranthene, carbazole, chrysene, anthracene, acenaphthene and indeno(1,2,3,cd)pyrene in mixture.

Key words: polycyclic aromatic hydrocarbons, synchronous scanning, spectrofluorometry

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants in environment, consisting of two or more fused benzene rings in linear, angular or cluster arrangement. Substitution of carbon in benzene ring with nitrogen, sulfur, oxygen or other elements gives heterocyclic compounds which are also classified as PAHs. Crystalline solid of PAHs has high melting points and low vapor pressures. Unlike most hydrocarbons, PAHs are usually colored. PAHs are produced naturally by combustion processes, e.g. forest fires, volcanic activity, etc., and anthropogenically via industrial processes, particularly the combustion of fossil fuels for heating, power and transport. There was also strong evidence indicating that PAHs may be produced by bacteria and plants (Gibson, 1995).

PAHs in the atmosphere can be polluted in many kinds of environmental samples such as soil, rain, river, underground water, plants, etc., which many are known to be carcinogenic agents. Figure 1 shows the chemical structure of the sixteen unsubstituted PAHs identified by The Environmental Protection Agency (EPA) as potential carcinogens (Boonyatumanond, 1999).

The relation between PAHs and human cancer is strongly suggested by their occurrence in environment and their carcinogenic pathways. For example, lung cancer mortality that related to PAHs has been increasing in many countries. It is not only necessary to distinguish between acute and chronic toxicity thresholds, but also important to know the toxic chemical species (Lakowicz, 1999).

The toxicity of the PAHs in environment depends on the types and quantity of each PAHs. Environmental samples, usually consist of more than one PAHs. Therefore, a rapid and simple qualitative analysis for preliminary identification of PAHs in samples is important especially when a

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large number of samples need to be analyzed. The structures of polycyclic aromatic hydrocarbons (PAHs) are have aromatic system that electron can delocalize from $\pi \rightarrow \pi *$ which cause fluorescence phenomena (Skoog, 1985). Therefore, the spectrofluorometric method was considered to be simple and time saving method. Thus, the

development of the spectrofluorometric method to make it more suitable for the determination of PAHs in sample is necessary (Schulman, 1999).

Vo-Dinh (1978) used synchronous excitation technique to improve the selectivity of luminescence spectrometry. This approach offered several advantages, including narrowing of spectral bands,

Naphthalene	Acenaphthene	Acenaphthylene
Fluorene	Phenanthene	Anthracene
Fluoranthene	Pyrene	Benz{a}anthracene
Chrysene	Benzo{b}fluoranthene	Benzo{k}fluoranthene

Benzo{a}pyrene	Dibenz{ah}anthracene	Benzo{ghi}perylene	Indeno{1,2,3,cd}pyrene
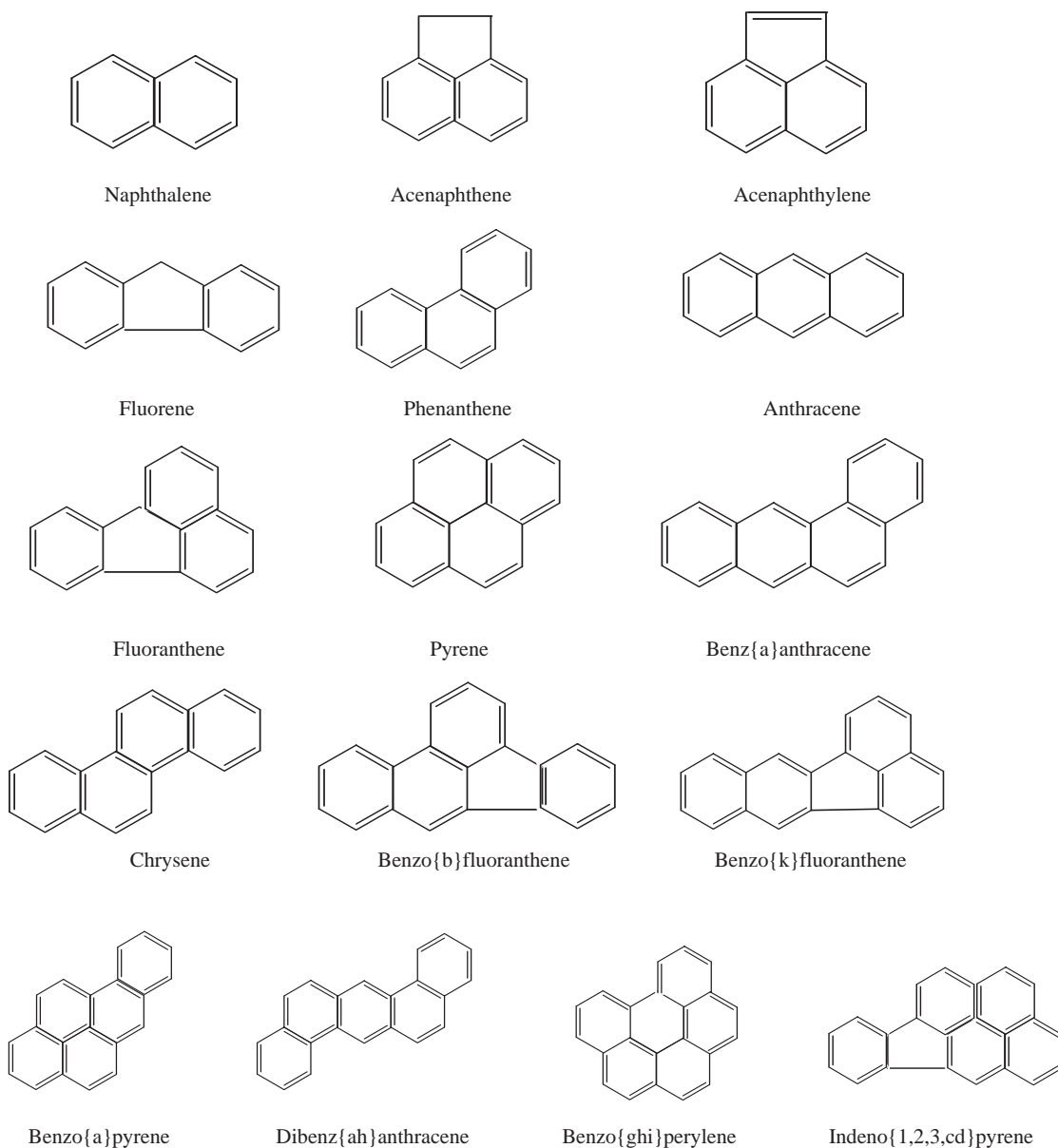
Figure 1 Chemical structure of the sixteen PAHs which were identified by The Environmental Protection Agency (EPA) as priority pollutants.

an enhancement in selectivity by spectral simplification, and a decrease of measurement time in multicomponent analysis. Vo-Dinh *et al*. (1980) used fluorometric method to identify polycyclic aromatic compounds in a synthetic fuel. Several trace and major components including benzo[a]pyrene, chrysene, fluorene, fluoranthene, phenanthrene and pyrene has been identified and determined at a concentration range of 10 - 1,000 ppm. It was found that the selectivity of the fluorometric analysis is greatly improved by synchronous excitation scanning. In this work, synchronous scanning technique was used for qualitative analysis of PAHs mixture in liquid samples at a sub-ppm level.

MATERIALS AND METHODS

Reagents

All reagents used were analytical reagent grade. Truxene, fluorene and carbazole were purchased from Chem Service (West Chester, USA). Benzo(k)fluoranthene, anthracene and chrysene were purchased from Fluka (Steinheim, Switzerland). Acenaphthene and indeno(1,2,3,cd)pyrene were purchased from BDH (Poole, England). Ethanol was purchased from Merck (Darmstadt, Germany). Hydrochloric acid was purchased from Lab-Scan (Bangkok, Thailand).

Experimental procedures
- Preparation of standard solution
Stock standard solution (10 ppm)

Five hundred micrograms of each PAHs standard was accurately weighed. Each PAHs standard was dissolved with ethanol and then made up to the volume of 50 ml in a volumetric flask. The stock solution was kept at the temperature close to 0°C and absence of light.

Intermediate standard solution

Each of the intermediate standard solution of PAHs was diluted from stock standard solution (10 ppm) with ethanol to 1 ppm.

Working standard solution

Working standards were prepared by diluting the intermediate standard solution (1 ppm) to sub-ppm levels (0.005-0.04 ppm). The dilution of each PAHs standard depended on the sensitivity of the synchronous signal obtained from the spectrofluorometer in the presence of ethanol as solvent. Each of the working standard solution and pure ethanol were measured by spectrofluorometer using synchronous scanning technique at various wavelength interval ($\Delta\lambda$) from 1-300 nm.

- Preparation of synthetic samples
A mixture consisting of benzo(k)fluoranthene, fluorene and truxene standard, a mixture consisting of chrysene, acenaphthene and indeno(1,2,3,cd)pyrene standard and a mixture consisting of benzo(k)fluoranthene, carbazole, chrysene, anthracene acenaphthene and indeno(1,2,3,cd)pyrene standard

Each mixture was prepared by diluting each of the intermediate standard solution (1 ppm in ethanol) to 0.01 ppm in the presence of ethanol as solvent. Each mixture was analyzed by spectrofluorometer using synchronous scanning technique at various wavelength interval ($\Delta\lambda$) from 1-300 nm.

Apparatus
Spectrofluorometer

The samples were analyzed by using a Varian Cary Eclipse spectrofluorometer. The spectrum was recorded at the wavelength interval ($\Delta\lambda$) of 1-300 nm, excitation wavelength of 200-500 nm for each standard PAHs and synthetic samples.

Electrical balance

An analytical balance (PERKIN ELMER AD-4 Autobalance, 0.05 mg-200 mg, USA.) was used for preparation of standard solution and synthetic samples.

RESULTS AND DISCUSSION

Table 1 shows the wavelength interval (Δλ) which give characteristic synchronous scanning fluorescence spectrum for each PAHs in ethanol. Some of these spectra are shown in this article. The results suggested that synchronous fluorescence spectroscopic method can be used as a tool for the qualitative analysis for the PAHs in mixtures. Each PAHs gave characteristic spectrum at each Δλ due to the difference in chemical structure of each PAHs. The characteristic peaks of fluorene, truxene, benzo(k)fluoranthene, carbazole and anthracene begin to appear when Δλ is less than 10 nm. For acenaphthene and chrysene, the characteristic peaks appear at Δλ equal to 10 nm and higher than 30 nm, respectively. The characteristic peaks of indeno (1,2,3,cd)pyrene begin to appear at Δλ equal to 110 nm. These suggested that six PAHs including benzo(k)fluoranthene, carbazole, chrysene, anthracene, acenaphthene and indeno(1,2,3,cd) pyrene can be separated qualitatively by synchronous scanning fluorescence.

Synthetic sample between benzo(k) fluoranthene, fluorene and truxene

To identify each PAHs in the synthetic mixture of benzo(k)fluoranthene, fluorene and truxene, the synchronous fluorescence spectrum at Δλ 20 nm of synthetic sample at the concentration of 0.01 ppm for each PAHs (Figure 2) was compared with the synchronous fluorescence spectra of each standard PAHs at the same Δλ (Figure 2 to Figure6).

From Figure 2, there were three groups of peak in the spectrum which indicated the presence of benzo(k)fluoranthene, fluorene and truxene. The triplet peaks at the excitation wavelength of 277.96, 289.06 and 299.07 nm indicated the presence of fluorene. The peak at the excitation wavelength of 336.00 nm indicated the presence of truxene. The doublet peaks at the excitation wavelength of 381.07 and 401.07 nm indicated the presence of benzo(k)fluoranthene. Thus, this showed that synchronous scanning fluorescence method at only Δλ of 20 nm can be used to identifiy the species of each PAHs in the mixture consisting of 0.01 ppm of benzo(k)fluoranthene, fluorene and truxene.

Synthetic mixture of acenaphthene, chrysene and indeno(1,2,3,cd)pyrene

To identify each PAHs in synthetic mixture of acenaphthene, chrysene and indeno(1,2,3,cd) pyrene by synchronous scanning fluorescence technique, Δλ at 95 and 110 nm have to be considered. Acenaphthene and chrysene in the sample can be identified by comparing the synchronous fluorescence spectra at the Δλ of 95 nm of synthetic sample at the concentration of 0.01

Table 1   Wavelength interval (Δλ) which give characteristic synchronous scanning fluorescence spectrum for each PAHs when ethanol was used as solvent.

| Type of PAHs	Wavelength interval (Δλ)
Truxene	5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65
Fluorene	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35 40, 45, 50, 55, 60
Benzo(k)fluoranthene	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70,75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200
Carbazole	2, 3, 4, 5, 6, 7, 8, 9, 10, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110
Anthracene	2, 3, 4, 5, 6, 7, 8, 9, 10, 120, 130, 140, 170
Acenaphthene	10, 15, 20, 25, 30, 35, 45, 90, 95
Chrysene	50, 90, 95, 100, 110, 120
Indeno(1,2,3,cd)pyrene	110, 120, 180,190
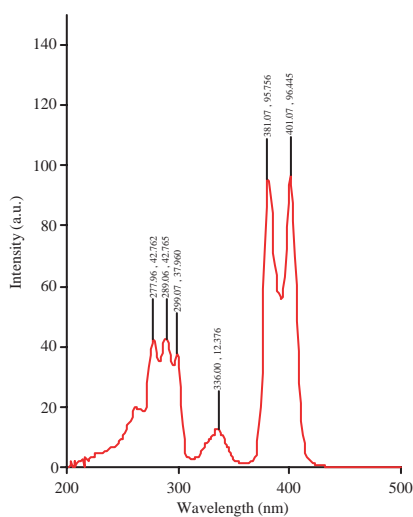
Figure 2 The synchronous spectrum of benzo(k) fluoranthene, fluorene and truxenein ethanol at Δλ = 20 nm.

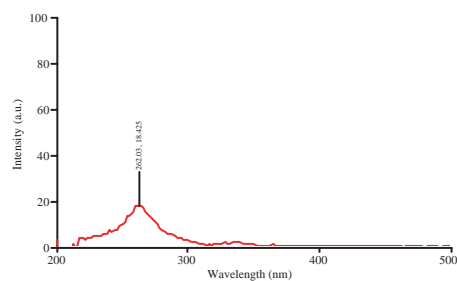**Figure 3** The synchronous spectrum of solvent (ethanol) at Δλ = 20nm.

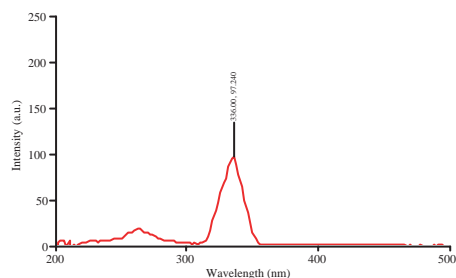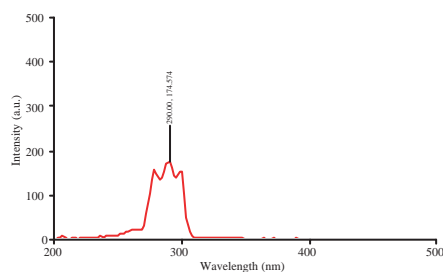**Figure 4** The synchronous spectrum of truxene at Δλ = 20nm.

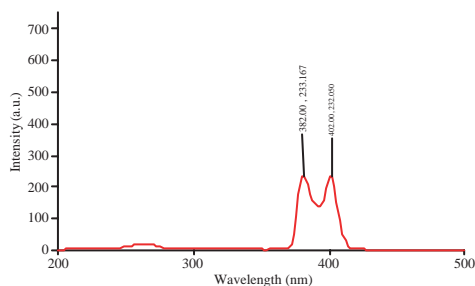**Figure 5** The synchronous spectrum of fluorene at Δλ = 20nm.

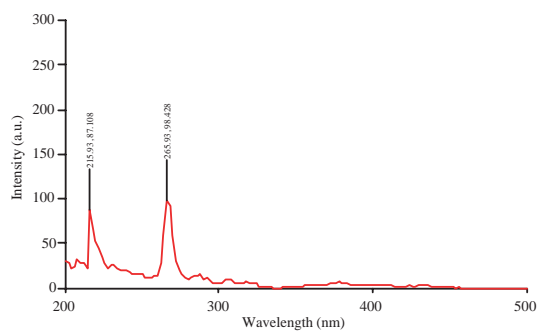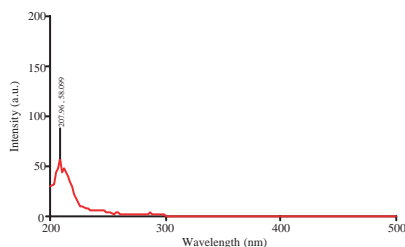**Figure 6** The synchronous spectrum of benzo(k) fluoranthene at Δλ = 20 nm. 2220 nm.

Figure 7 The synchronous spectrum of chrysene, acenaphthene and indeno(1,2,3,cd) pyrene.

ppm for each PAHs (Figure 7) with the synchronous fluorescence spectra of ethanol, acenaphthene and chrysene at the same Δλ (Figure 8 to Figure 10).

The peak at the excitation wavelength of 215.93 and 265.93 nm indicated the presence of acenaphthene and chrysene in the sample respectively. However, the peak of acenaphthene was shifted by 10 nm from the standard acenaphthene (excitation wavelength of 225.93 nm). Nevertheless, this still confirms that there was acenaphthene in the sample because acenaphthene is the only PAHs that can give the peak at the excitation wavelength around 225.93 nm. The presence of indeno(1,2,3,cd)pyrene in the sample was identified by considering the synchronous spectra of synthetic mixture, ethanol, indeno (1,2,3,cd)pyrene and the other two PAHs in the mixture at the Δλ of 110 nm (Figure 11 to Figure 15). At the Δλ of 110 nm, only indeno(1,2,3,cd)

pyrene give characteristic peak at the excitation wavelength higher than that of 350 nm. This showed that the synchronous scanning fluorescence technique can be used to identify each PAHs from the synthetic mixture of acenaphthene, chrysene and indeno(1,2,3,cd)pyrene.

Synthetic mixture of benzo(k) fluoranthene, carbazole, chrysene, anthracene acenaphthene and indeno(1,2,3,cd)pyrene at the concentration of 0.01 ppm for each PAHs

To identify each PAHs in the synthetic mixture of benzo(k)fluoranthene, carbazole, chrysene, anthracene acenaphthene and indeno (1,2,3,cd)pyrene by synchronous scanning fluorescence technique, the Δλ at 6 nm, 20 nm, 95 nm and 110 nm have to be considered. Carbazole, benzo(k)fluoranthene and anthracene in the sample can be identified by comparing the synchronous fluorescence spectra at the Δλ of 6 nm of synthetic

Figure 8 The synchronous spectrum of solvent (ethanol) at Δλ = 95nm.
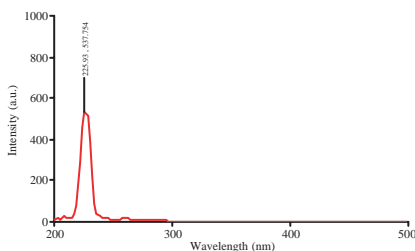
Figure 9 The synchronous spectrum of acenaphthene at Δλ = 95nm.
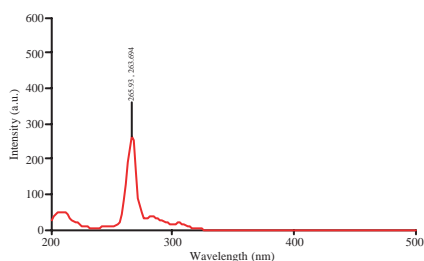
Figure 10 The synchronous spectrum of chrysene at Δλ = 95 nm.
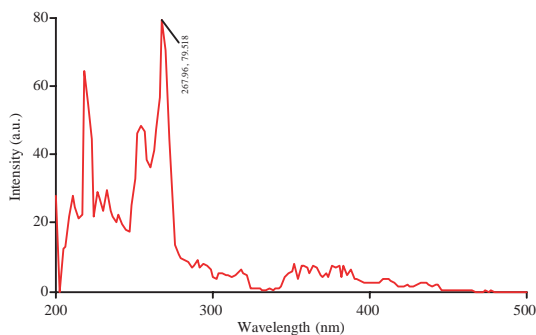
Figure 11 The synchronous spectrum of chrysens, acenaphthene and indeno(1,2,3,cd) pyrene in ethanol at Δλ = 110 nm.

sample at the concentration of 0.01 ppm for each PAHs (Figure 16) with the synchronous fluorescence spectra of ethanol, carbazole, benzo(k)fluoranthene and anthracene at the $\Delta\lambda$ of 6 nm (Figure 17 to

Figure 20).

The peaks at the excitation wavelength of 336.00, 374.00 and 400.00 nm indicated the presence of carbazole, anthracene and benzo(k)fluoranthene
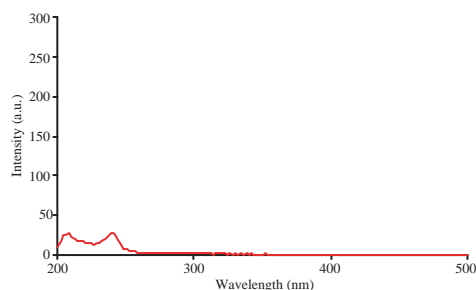

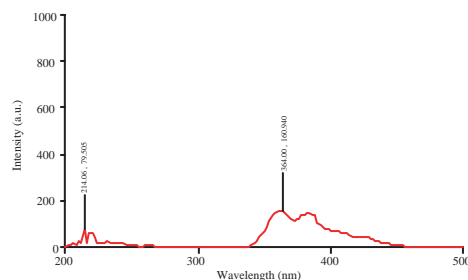**Figure 12** The synchronous spectrum of solvent (ethanol) at $\Delta\lambda = 110$ nm.

Figure 13 The synchronous spectrum of indeno (1,2,3,cd)pyrene at $\Delta\lambda = 110$ nm.
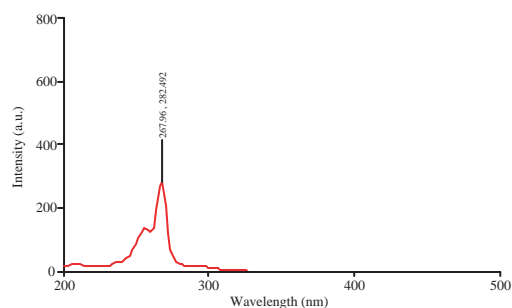
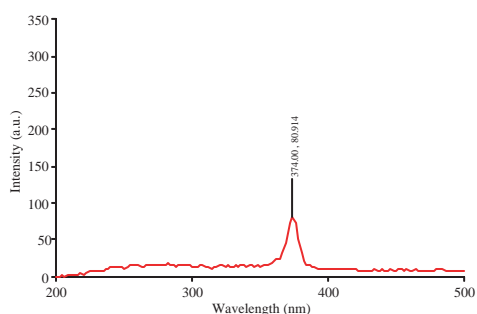**Figure 14** The synchronous spectrum of chrysene at $\Delta\lambda = 110$ nm.
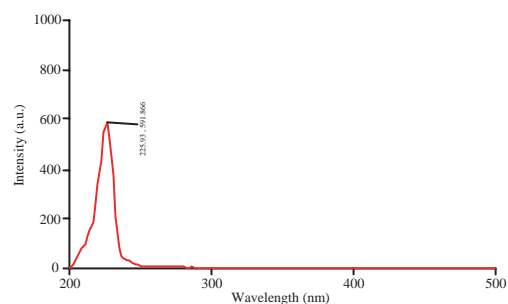
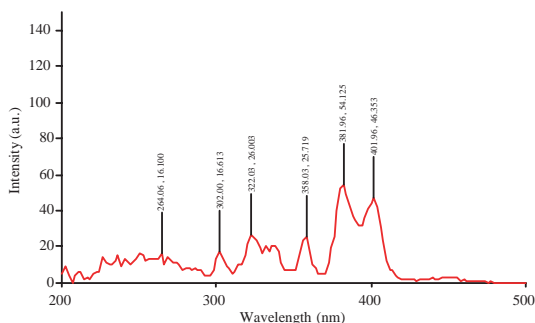**Figure 15** The synchronous spectrum of acenaphthene at $\Delta\lambda = 110$ nm.
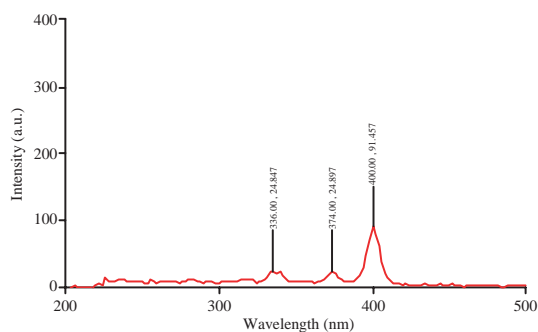
Figure 16 The synchronous spectrum of benzo(k) fluoranthene, carbazole, chrysene, anthracene, acenaphthene and indeno (1,2,3,cd)pyrene in ethanol at $\Delta\lambda = 6$ nm.
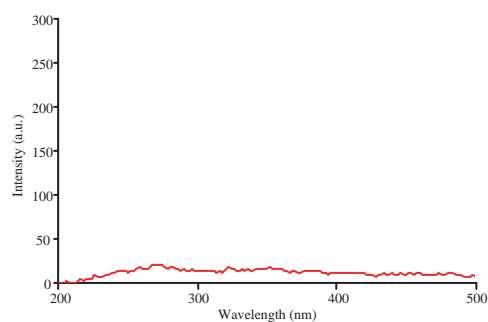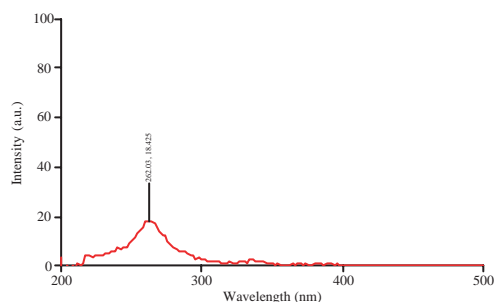

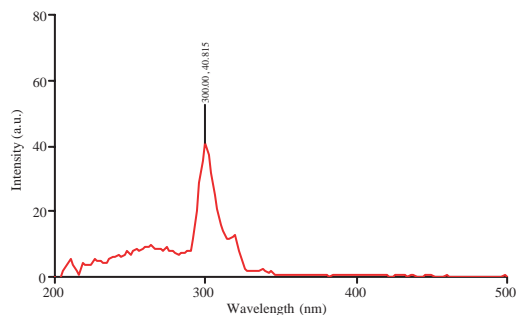**Figure 17** The synchronous spectrum of solvent (ethanol) at $\Delta\lambda = 6$ nm.

in the sample respectively. For the identification of acenaphthene, the synchronous fluorescence spectra at the Δλ of 20 nm have to be considered (Figure 21 to Figure 23).

The presence of acenaphthene in the sample was suggested by a peak at the excitation wavelength of 302.00 nm. However, the peak of acenaphthene was shifted by 2 nm from the standard acenaphthene
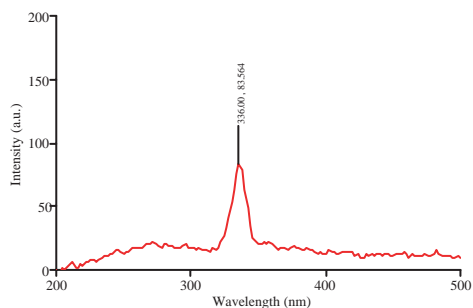
Figure 18 The synchronous spectrum of carbazole at Δλ = 6 nm.
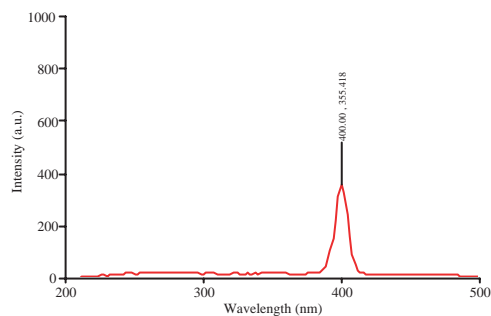
Figure 19 The synchronous spectrum of benzo(k) fluoranthene at Δλ = 6 nm.

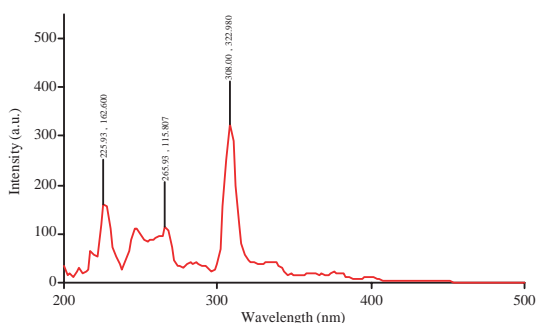**Figure 20** The synchronous spectrum of anthracene at Δλ = 6 nm.

Figure 21 The synchronous spectrum of benzo(k) fluoranthene, carbazole, chrysene, anthracene, acenaphthene and indeno (1,2,3,cd)pyrene in ethanol at Δλ = 20 nm.

Figure 22 The synchronous spectrum of solvent (ethanol) at Δλ = 20 nm.

Figure 23 The synchronous spectrum of acenaphthene at Δλ = 20 nm.

(excitation wavelength of 300.00 nm). Nevertheless, this still confirms that there was acenaphthene in the sample because acenaphthene is the only PAHs that can give the peak at the excitation wavelength around 300.00 nm. The presence of chrysene in the sample was proved by comparing the chrysene of synthetic mixture, ethanol and chrysene at the $\Delta\lambda$ of 95 nm (Figure 24 to Figure 26).

The peak at the excitation wavelength of 265.93 nm indicated that there was chrysene in the sample. The presence of indeno(1,2,3,cd)pyrene in the sample was identified by considering the synchronous spectra of synthetic mixture, ethanol, indeno(1,2,3,cd)pyrene and other PAHs in the mixture at the $\Delta\lambda$ of 110 nm (Figure 27 to Figure 34). At the $\Delta\lambda$ of 110 nm, only indeno(1,2,3,cd) pyrene gives the characteristic peak at the excitation wavelength higher than that of 350 nm. These results suggested that the synchronous scanning fluorescence technique can be used to identify each PAHs from synthetic mixture of benzo(k) fluoranthene, carbazole, chrysene, anthracene acenaphthene and indeno(1,2,3,cd)pyrene.

Figure 24 The synchronous spectrum of benzo(k) fluoranthene, carbazole, chrysene, anthracene, acenaphthene and indeno (1,2,3,cd)pyrene in ethanol at $\Delta\lambda$ = 95 nm.
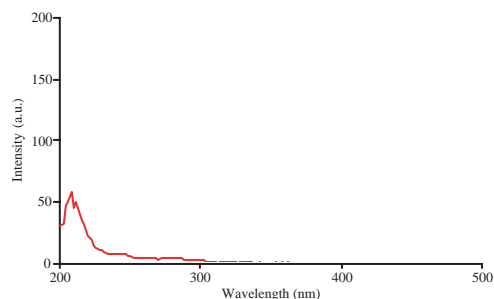

Figure 25 The synchronous spectrum of solvent (ethanol) at $\Delta\lambda$ = 95 nm.
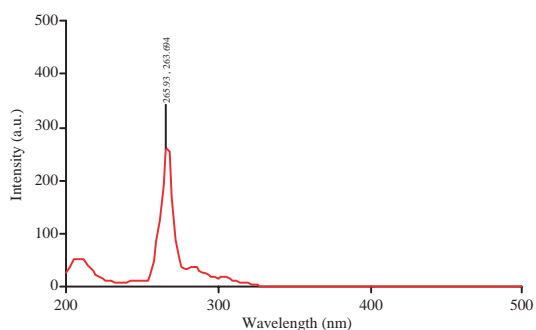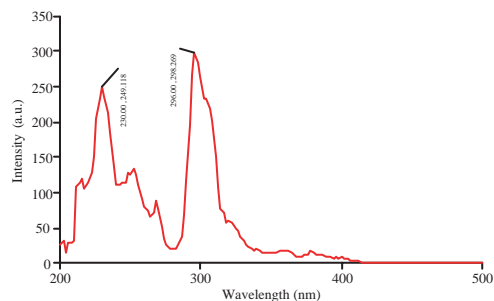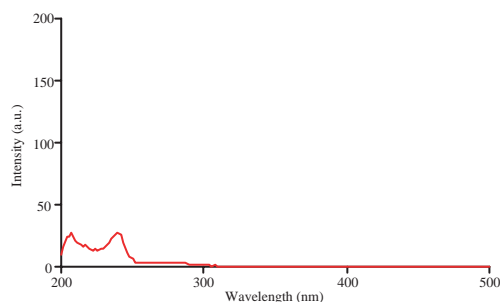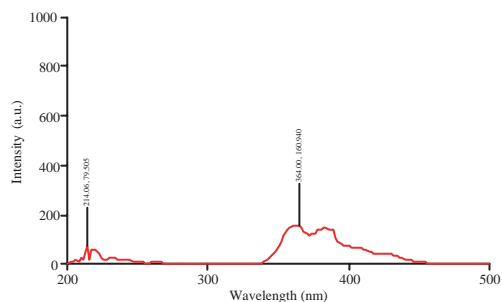

Figure 26 The synchronous spectrum of chrysene at $\Delta\lambda$ = 95 nm.

Figure 27 The synchronous spectrum of benzo(k) fluoranthene, carbazole, chrysene, anthracene, acenaphthene and indeno (1,2,3,cd)pyrene in ethanol at $\Delta\lambda$ = 110 nm.

Figure 28 The synchronous spectrum of solvent (ethanol) at Δλ = 110 nm.

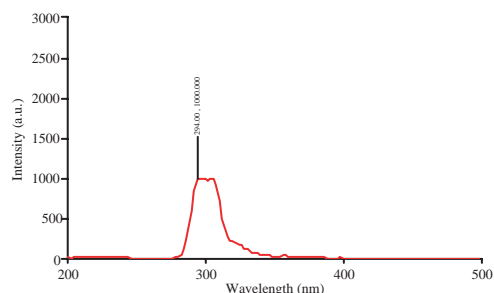**Figure 29** The synchronous spectrum of indeno (1,2,3,cd)pyrene at Δλ = 110 nm.

Figure 30 The synchronous spectrum of benzo(k) fluoranthene at Δλ = 110 nm.

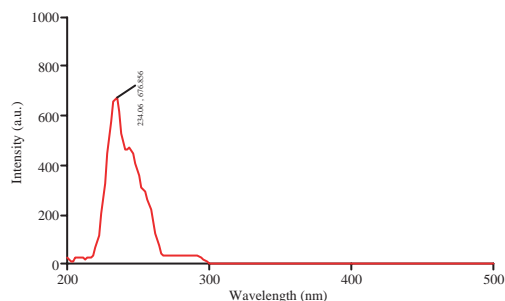**Figure 31** The synchronous spectrum of carbazole at Δλ = 110 nm.

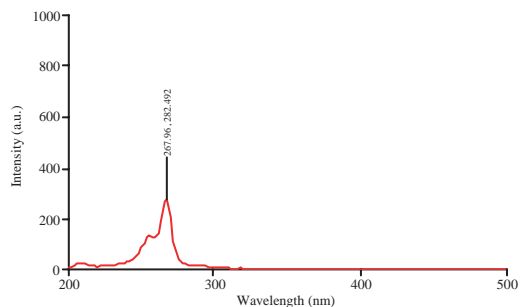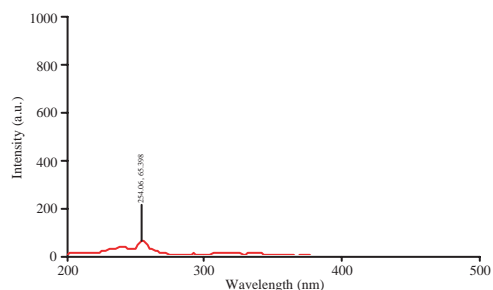**Figure 32** The synchronous spectrum of chrysene at Δλ = 110 nm.

Figure 33 The synchronous spectrum of anthracene at Δλ = 110 nm.

CONCLUSION

The results of the three synthetic mixtures from the previous section suggested that the synchronous scanning fluorescence method can be used to identify each PAHs in synthetic mixtures. The number of wavelength interval (Δλ) to be considered depending on the species and numbers of PAHs presence in the analytical mixture. Comparing with other methods, such as gas chromatography and high-performance liquid chromatography, the synchronous scanning fluorescence method is more convenient than other methods because of less time consuming and the simplicity in preparation of samples. This works shown that the synchronous scanning fluorescence
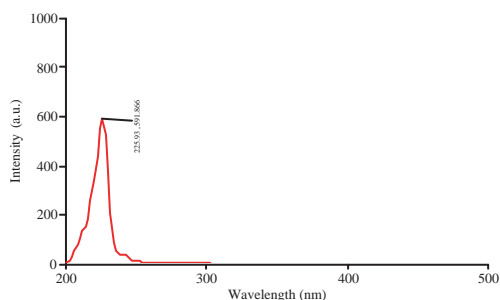
Figure 34 The synchronous spectrum of acenaphthene at $\Delta\lambda = 110$ nm.

technique can be applied for the qualitative analysis of PAHs from the environmental samples which were extracted by ethanol. However, the use of synchronous scanning fluorescence technique for the qualitative analysis of PAHs in others organic solvents is also possible and would be more useful for the identification of PAHs in the environmental samples.

ACKNOWLEDGEMENT

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