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Application News

No.A500

Spectrophotometric Analysis

Separation Analysis by Synchronous Fluorescence Spectroscopy

Separation analysis of multicomponent samples can be performed by synchronous fluorescence spectroscopy using a spectrofluorophotometer. Normally, excitation spectra measurements are performed by fixing the emission wavelength and then scanning wavelengths with an excitation monochromator. Also, emission spectra measurements are normally performed by fixing the excitation wavelength and then scanning wavelengths with a emission monochromator. Compared to those methods, synchronous fluorescence spectra measurements are performed by scanning both the excitation and emission wavelengths simultaneously while maintaining a constant wavelength difference between them. This wavelength shift ($\Delta\lambda$) remains constant while an excitation monochromator and a emission monochromator scan spectra wavelengths at identical scanning speeds. Light received by the detector is excited by the wavelength of the excitation monochromator, while only fluorescent light of this wavelength shifted by $\Delta\lambda$ is emitted. Appropriate selection of $\Delta\lambda$ allows for separation analysis of the target components.

In this article we explain synchronous fluorescence spectroscopy, and also introduce an example separation analysis of a mixed sample of polycyclic aromatic compounds performed by synchronous fluorescence spectroscopy using the RF-6000 spectrofluorophotometer shown in Fig. 1.

Fig. 1 RF-6000 Spectrofluorophotometer

Synchronous Fluorescence Spectroscopy

The principles of synchronous fluorescence spectroscopy are shown below[1]. Where λ' is the wavelength of excitation light shone on the fluorescent material, and $E_M(\lambda)$ is the intensity distribution pattern of emission (emission spectrum). The photometric value $I(\lambda)$, which is the amount of fluorescent light measured at emission wavelength λ, is dependent on $E_M(\lambda)$, and is also proportional to $R_{\lambda'}$, which is the spectral radiance of the light emitted by the fluorescent material excited by light of wavelength λ'.

$$I(\lambda) = k_1 R_{\lambda'} E_M(\lambda) \quad (1)$$

Where k_1 is a coefficient. When the concentration of the fluorescent material is sufficiently low, $R_{\lambda'}$ can be expressed as shown in equation (2).

$$R_{\lambda'} = k_2 \varepsilon(\lambda') c d I_0(\lambda') \varphi(\lambda') \quad (2)$$

Where k_2 is a coefficient, $\varepsilon(\lambda')$ is the absorption coefficient, c is concentration of the fluorescent material, d is optical path length, $I_0(\lambda')$ is intensity of excitation light, and $\varphi(\lambda')$ is the quantum yield of the fluorescent material.
The product of $\varepsilon(\lambda')$, $I_0(\lambda')$, and $\varphi(\lambda')$ is defined as the excitation function, and if the excitation spectrum is taken as $E_X(\lambda')$, it can be represented in equation (3).

$$E_X(\lambda') = k_3 \varepsilon(\lambda') I_0(\lambda') \varphi(\lambda') \quad (3)$$

Where k_3 is a coefficient.
From equations (1), (2) and (3), the intensity $I_S(\lambda', \lambda)$ of synchronous fluorescence spectra can be represented by equation (4).

$$I_S(\lambda', \lambda) = K c d E_X(\lambda') E_M(\lambda) \quad (4)$$

Where $K = k_1 k_2 k_3^{-1}$.

For synchronous fluorescence spectrum measurement, the difference between excitation wavelength and emission wavelength ($\Delta\lambda$) is constant.

$$\lambda - \lambda' = \Delta\lambda \text{ (constant)} \quad (5)$$

From equations (4) and (5),

$$I_S(\lambda', \lambda) = K c d E_X(\lambda') E_M(\lambda' + \Delta\lambda) \quad (6)$$

Or,

$$I_S(\lambda', \lambda) = K c d E_X(\lambda - \Delta\lambda) E_M(\lambda) \quad (7)$$

In the above equations, the synchronous fluorescence spectrum is represented as the relationship between λ and λ', showing that the intensity pattern changes depending on $\Delta\lambda$ selection.

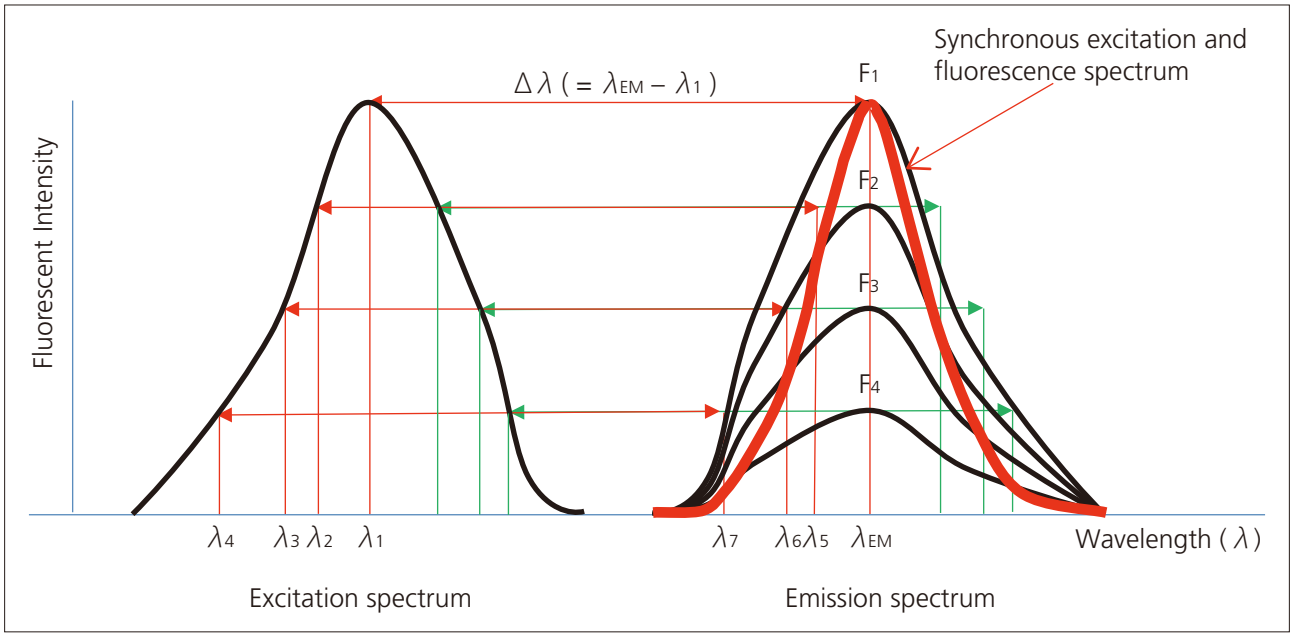

Fig. 2 Schematic Diagram of Synchronous Fluorescence Spectra

For a better visual understanding of synchronous fluorescence spectroscopy, see the hypothetical excitation spectrum and emission spectrum shown in Fig. 2.
When excitation occurs at λ_1, at a wavelength of λ_{EM} the emission spectrum of intensity F_1 is obtained. Analogously, when the excitation wavelength is set at λ_2, the emission spectrum of intensity F_2 is obtained.
The synchronous fluorescence spectrum is measured by scanning the excitation wavelengths and emission wavelengths simultaneously, using the peak wavelength difference between the spectra for $\Delta\lambda$, where $\Delta\lambda=\lambda_{EM}-\lambda_1$. When the excitation wavelength is λ_4, at a wavelength of λ_{EM} the fluorescence intensity is F_4, but when measuring the synchronous fluorescence spectrum, the emission intensity at λ_7 ($=\lambda_4+\Delta\lambda$) shifted by $\Delta\lambda$ on the F_4 intensity emission spectrum becomes the intensity of the synchronous fluorescence spectrum. Similarly, when the excitation wavelength is λ_3, at a wavelength of λ_{EM} a emission spectrum of intensity F_3 is obtained, where the emission intensity at λ_6 ($=\lambda_3+\Delta\lambda$) becomes the intensity of the synchronous fluorescence spectrum. When scanning is performed in this way while a constant $\Delta\lambda$ interval is maintained between the excitation wavelength and emission wavelength, the synchronous fluorescence spectrum shown by the red line is obtained.
As is clear from Fig. 2, the FWHM (Full Width at Half Maximum) of the synchronous fluorescence spectrum is narrower than the original emission spectrum, and setting the wavelength difference between the peak wavelengths on the excitation spectrum and emission spectrum for $\Delta\lambda$ produces the maximum peak intensity in the synchronous fluorescence spectrum.

Synchronous Fluorescence Spectra of Polycyclic Aromatic Compounds

We prepared cyclohexane solutions of pyrene (2.9 µg/mL), benzo [a] pyrene (1.6 µg/mL), and benzo [k] fluoranthene (2.0 µg/mL), and measured the emission spectrum of these solutions using the RF-6000 spectrofluorophotometer. Analytical conditions are shown in Table 1, and measured results are shown in Fig. 3. The excitation wavelength of pyrene, benzo [a] pyrene, and benzo [k] fluoranthene was 335, 385, and 308 nm respectively. At the above concentrations, the emission intensity of benzo [k] fluoranthene was the strongest at approximately 40 times the strength of the smallest emission intensity, which was measured from the pyrene solution. In order to display clearly the emission spectra of pyrene and benzo [a] pyrene, the vertical axis in Fig. 3 has been expanded and shown in Fig. 4.
In order to maximize the peak intensity for pyrene with synchronous fluorescence spectroscopy, the difference (47 nm) between the wavelength (382 nm) with the highest peak intensity on the pyrene emission spectrum and the excitation wavelength (335 nm) of pyrene was chosen as the difference in wavelength between the excitation monochromator and emission monochromator. The synchronous fluorescence spectra measured for pyrene, benzo [a] pyrene, and benzo [k] fluoranthene using this wavelength difference are shown in Fig. 5, with the analytical conditions shown in Table 2. The intensity ratio between the maximum peaks of pyrene and benzo [k] fluoranthene is reduced by approximately 8 times on the synchronous fluorescence spectra, where the pyrene peak intensity has increased relative to the benzo [k] fluoranthene peak intensity. The pyrene peak is also fully separated on the synchronous fluorescence spectra.

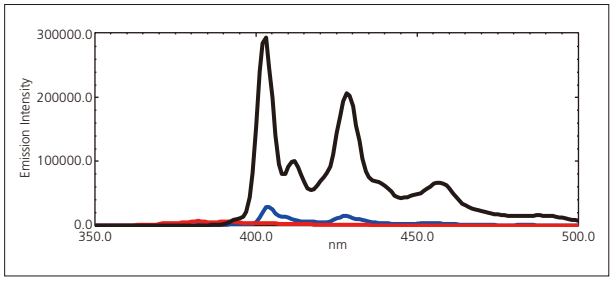

Fig. 3 Emission Spectra of Pyrene (Red), Benzo [a] pyrene (Blue) and Benzo [k] fluoranthene (Black)

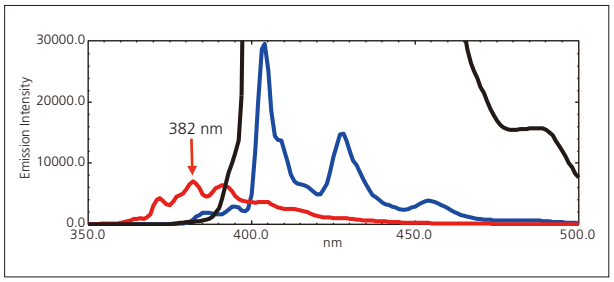

Fig. 4 Expanded Emission Spectra from Fig. 3

Table 1 Analytical Conditions

Analytical Instrument	: FR-6000 spectrofluorophotometer
Spectrum Type	: Emission spectrum
Data Interval	: 1.0 nm
Scanning Speed	: 600 nm/min
Bandwidth	: Ex 3 min, Em 3 nm
Sensitivity	: Low

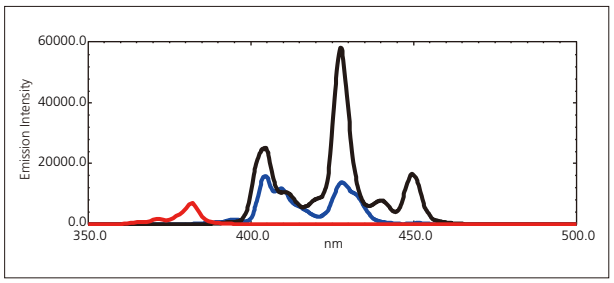

Fig. 5 Synchronous Fluorescence Spectra of Pyrene (Red), Benzo [a] pyrene (Blue) and Benzo [k] fluoranthene (Black)

Table 2 Analytical Conditions

Analytical Instrument	: FR-6000 spectrofluorophotometer
Spectrum Type	: Synchronous fluorescence spectrum
Data Interval	: 1.0 nm
Scanning Speed	: 200 nm/min
Bandwidth	: Ex 3 min, Em 3 nm
Sensitivity	: Low
Difference Between Excitation and Emission Wavelengths	: 47 nm

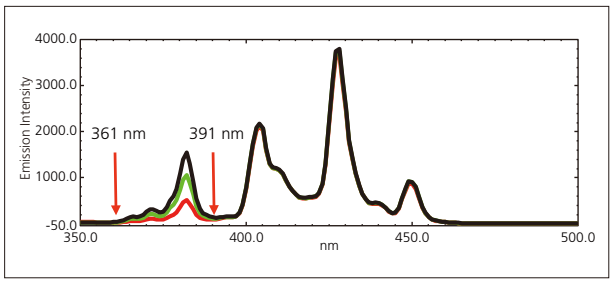

Fig. 6 Synchronous Fluorescence Spectra of Pyrene Measured at Three Concentrations
Red: 0.145 µg/mL, Green: 0.29 µg/mL, Black: 0.435 µg/mL

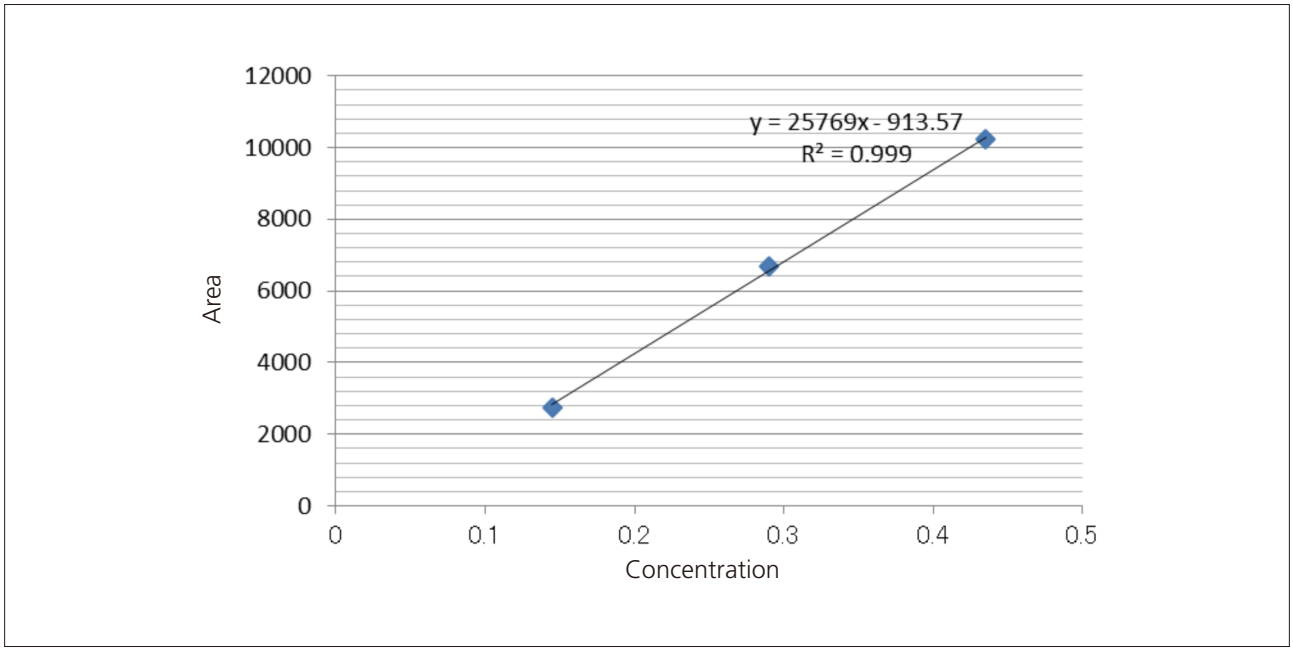

Fig. 7 Relationship Between Peak Area and Concentration for Pyrene

Next, 1 mL of each cyclohexane solution of benzo [a] pyrene and benzo [k] fluoranthene used for the measurements shown in Fig. 3 was added to a container of 1 mL, 2 mL, and 3 mL of the cyclohexane solution of pyrene also used for measurements shown in Fig. 3, after which cyclohexane was added to each mixture to make up 20 mL. The final concentration of benzo [a] pyrene in all mixtures was 0.08 µg/mL, and of benzo [k] fluoranthene was 0.1 µg/mL. The final concentrations of pyrene used were 0.145 µg/mL, 0.29 µg/mL, and 0.435 µg/mL.

As shown in Fig. 5, 47 nm was set as the difference between the excitation and emission monochromator wavelengths, and the solutions were analyzed by synchronous fluorescence spectroscopy. Measured results are shown in Fig. 6. Analytical conditions were the same as those shown in Table 2.

Next, a baseline was drawn between 361 nm and 391 nm in Fig. 6, and the peak areas above this baseline were calculated.

The relationship between peak area and pyrene concentration is shown in Fig. 7. Good linearity was obtained, showing that separation and quantitation is possible.

Conclusion

We confirmed that the synchronous fluorescence spectrum measurement function on the RF-6000 can be used to performed separation analysis of a mixture. Mixture separation is possible using synchronous fluorescence spectroscopy when an appropriate wavelength interval is chosen, and this technique shows promise for application in a variety of areas.

[References]
1) T. Vo-Dinh, Anal. Chem. 50 (1978) 396.

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