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Study on total luminescence spectra. Application of the Monte-Carlo method to three-dimensional synchronous fluorescence spectrometry

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Abstract

Three-dimensional synchronous fluorescence spectrometry (TDSFS, a combination of synchronous fluorescence spectrometry and three-dimensional fluorescence spectrometry) is a new method which has been developed recently. The method has usually been used as an efficient tool to select the best $\Delta\lambda$ value for synchronous fluorescence spectra. This paper studies the sensitivity of the method, which was not been done in the past. The total fluorescence intensity has been used instead of the conventional single point intensity, calculated by the Monte-Carlo method, as the experimental parameter to determine fluorescein and tryptophan. The sensitivity of the total fluorescence method is nearly one hundred times better than that of the single point method. The new method has been used to simultaneously determine naphthalene, pyrene and perylene successfully. The mechanism of the method has also been studied.

Keywords: Fluorimetry; Fluorescein; Tryptophan; PAHs; Monte-Carlo method

1. Introduction

It has been shown that three-dimensional luminescence spectra have many uses in analytical chemistry [1–4]. Warner et al. carried out the quantitative analysis of polynuclear aromatic compounds in environmental samples. Wolfbeis and Leiner [4] proved that three-dimensional fluorescence spectrometry (TDFS) of normal and tumour sera is useful as a rapid screening method for cancerous diseases. Some computational methods such as least squares [5], rank annihilation [6], eigenanalysis [7], ratio [8], rank estimation [9], pattern recognition [10] and neural networks [11] have been applied in TDFS.

Synchronous fluorescence spectra enhance the selectivity of fluorescence spectra greatly because they combine the characteristics of excitation and emission spectra. It has become an efficient tool for the analysis of mixtures of fluorescent compounds in solutions which have overlapping fluorescence bands [12]. The best $\Delta\lambda$ for synchronous spectrometry, however, is difficult to select. The remedy for this situation is three-dimensional synchronous fluorescence spectrometry (TDSFS).

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Although TDSFS has been widely used, its characteristics such as fingerprinting, sensitivity, etc. have not been fully studied. In this paper we take the total fluorescence intensity as an experimental parameter to make use of the sensitivity of TDSFS.

We have already used the Monte-Carlo method to calculate the total fluorescence intensity of TDFS [14] and discovered that it can greatly improve the sensitivity of TDFS by using the total fluorescence intensity as the experimental parameter. In this paper, this method has been examined by determining fluorescein and tryptophan by TDSFS and the results are compared with those of TDFS.

2. Theory

The fluorescence of the fluorophore can be expressed by the following equation:

$$F = K_0 \phi_0 I_0 (1 - e^{-abc}) \quad (1)$$

Where ϕ_0 is the quantum efficiency, K_0 is a constant, I_0 the incident intensity, a the molar absorptivity, b the length of the cuvette and c the concentration of the fluorescent substance.

In extremely dilute solution ($abc \ll 0.05$), fluorescence intensity is routinely calculated according to the following equation:

$$F = K_1 I_0 abc \quad (2)$$

Where K_1 is a constant equal to $2.303\phi_0$.

TDSFS can be simulated by F-distribution [13]:

$$F = K_2 C f(\lambda_{\Delta\lambda}) f(\lambda_{em}) \quad (3)$$

In Eq. 3, K_2 is a constant, $f(\lambda_{\Delta\lambda})$ and $f(\lambda_{em})$ are the synchronous spectra associated with $\Delta\lambda$ and the emission spectra, respectively.

When determined by the total fluorescence intensity:

$$\Sigma F = \int_{\Delta\lambda_0}^{\Delta\lambda_1} \int_{em_0}^{em_1} F \, d\lambda_{\Delta\lambda} \, d\lambda_{em} \quad (4)$$

where $\Delta\lambda_0$, em_0, $\Delta\lambda_1$ and em_1 are the initial and terminal wavelength differences and emission wavelengths, respectively, for the integral region of the total fluorescence intensity.

It was found in our experiment that the relationship between the total fluorescence intensity, F, and the concentration, C, was not linear since the TDSFS was best simulated by F-distribution rather than Gaussian distribution.

The relationship can be described as follows.

$$F = KC^{\alpha} \quad (5)$$

In Eq. 5, K is a constant concerned with quantum efficiency, absorptivity and instrumental conditions and α is a parameter depending on the shape of peak in TDSFS.

Since an integration approach is adopted in this method, the value of K is increased and the random error is suppressed, thus, the sensitivity of determination is greatly increased because of the increase in signal to noise.

3. Experimental

3.1. Instrumentation

TDSFS were performed on a Hitachi-650 10S fluorescence spectrometer connected with an IBM/PC XT 386 microcomputer through a 12 bit A/D converter and plotted by SURF software (Golden Software, Golden, CO). All data were dealt with by the QuickBasic program (Microsoft).

3.2. Reagents

Buffers, fluorescein and tryptophan were of analytical grade or better.

3.3. Determination procedure for fluorescein

0.2 ml of NaOH solution (1 mol l^{-1}) and 0–1.0 ml of fluorescein solution (10 ppb) were added to a 10-ml flask and diluted to the mark. TDSFS data of the solution were collected as a series of emissions with a 5-nm scanning interval for wavelength differences and a 1-nm interval for the emission wavelength on the fluorescence spectrometer (a 1 cm quartz cell and 5 nm slits for both excitation and emission were used).

3.4. Determination procedure for tryptophan

2.5 ml of Tris buffer solution (pH 8.0) and 0–1.0 ml of tryptophan solution (5 ppm) were added to a

10-ml flask and diluted to the mark. TDSFS data of the solution were collected under the same instrumental conditions as those for the determination of fluorescein.

3.5. Determination procedure for naphthalene, pyrene and perylene

2 ml of Tris buffer solution (pH 7.0), some naphthalene solution (10^{-6} mol/l), pyrene solution (10^{-6} mol/l), perylene solution (6 ppm) or their mixtures were added to a 25-ml flask and diluted to the mark. TDSFS data were collected under the same instrumental conditions as those for the determination of fluorescein.

3.6. Data processing

The integration of TDSFS data was calculated by the Monte-Carlo technique. The total fluorescence intensity was obtained by adding the intensities of the random points produced by simulation. The trials and sample numbers of the Monte-Carlo method were experimentally chosen to be 256 and 5000, respectively.

4. Results and discussion

4.1. Determination of fluorescein

Fig. 1 shows the TDSF spectra of fluorescein at a concentration of 40 ppb. The spectral peak is at $\Delta\lambda/\lambda_{em} = 25/517$ nm. It can be seen that the TDSF spectra of fluorescein are less influenced by scattered light than its TDF spectra.

Different integration regions have been selected to examine the new method. The block diagram of the selected integration regions is described in Fig. 2. Fig. 3 shows the experimental results that describe the relationship between concentration and the total fluorescence intensity of fluorescein. This relationship is not linear in this occasion and can be described by the equation $F = KC^{\alpha}$, where α is equal to 1.88. α is related to the shape of the peak in the TDSF spectra of fluorescein, having no relation to the selected regions.

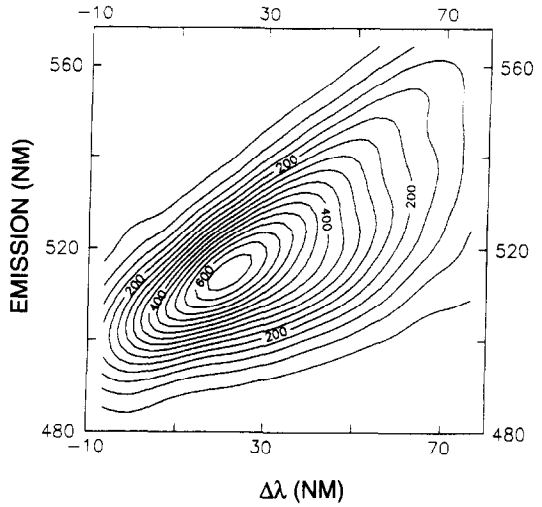

Fig. 1. Contour map of the $\Delta\lambda$ emission matrix for 40 ppb fluorescein in 0.02 M NaOH.

The results of the fluorescein detection by the Monte-Carlo method are shown in Table 1. Table 1 illustrates clearly that the detection limit of fluorescein can be increased about fifty times by using the total fluorescence intensity measurement over that by using the single point measurement, because the signal-to-noise (S/N) ratio is greatly increased from 0.118×10^3 to 5.83×10^3. The best detection limit for fluorescein in Region 1 is 0.51 ppt. It is also

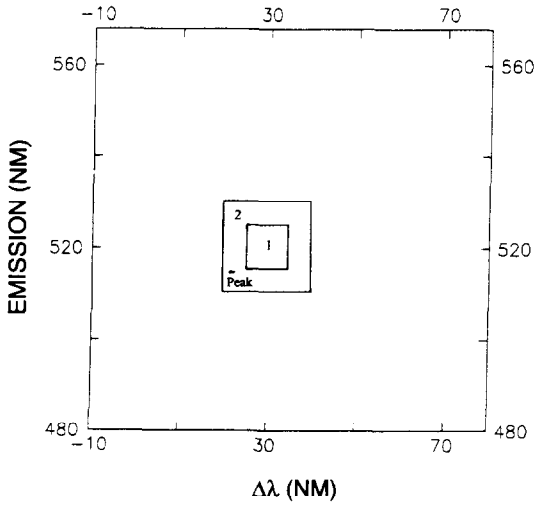

Fig. 2. Block diagram of the integration regions chosen for the determination of fluorescein.

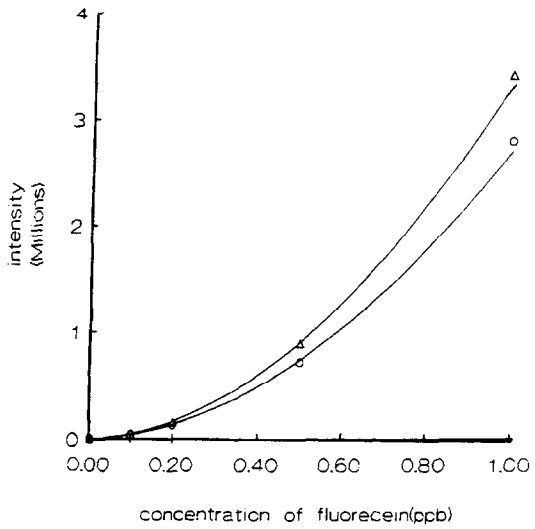

Fig. 3. Fitting of the relation between the total fluorescence intensity and the concentration of fluorescein ($F = KC^{\alpha}$). Key to symbols: Region 1 (△), Region 2 (○), Peak point (+).

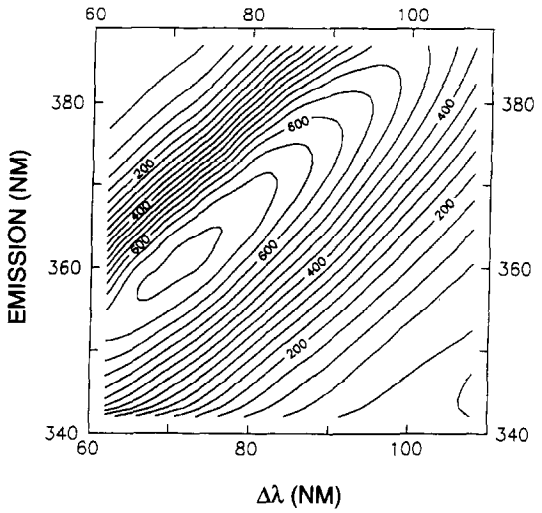

Fig. 4. Contour map of the $\Delta\lambda$ emission matrix for 0.25 ppm tryptophan at pH 8.0.

shown that the selectivity cannot be lost by using the total fluorescence intensity measurement, for the integration region is very small (3×10 grid) when compared to the whole TDSF spectrum (17×90 grid).

The detection limit of fluorescein by TDSFS is better than that by TDFS [14] for TDSFS can diminish the influence of scattered light. This indicates that the method based on the total luminescence intensity of TDSFS is superior when the Stoke's shift of the fluorescent substance is small.

4.2. Determination of tryptophan

The TDSF spectrum for 0.25 ppm tryptophan is shown in Fig. 4 and the spectral peak is located at $\Delta\lambda/\lambda_{em} = 73/360$ nm. Also, the TDSF spectrum of tryptophan is almost not influenced by scattered light.

The marked regions in Fig. 5 represent the selected integral regions to which the Monte-Carlo method is applied. Different integration regions, as seen in Fig. 5, fully examine the method for the determination of tryptophan. Fig. 6 shows the relationship between the concentration of tryptophan and the total fluorescence intensity can also be described by the equation $F = KC^{\alpha}$. Here α is about 1.89. As described above, α has almost no relation to the selected integration region.

The analytical results of tryptophan in different integration regions are shown in Table 2. The results in Table 2 indicate clearly that the detection limit of tryptophan (0.09 ppb in Region 1) can be increased for about 100 times by using the total fluorescence intensity measurement over that by using the single

Table 1
Analytical results for fluorescein obtained by TDSFS using the Monte-Carlo method

Integration region	Data points ($a \times b$)	S/N ($\times 10^3$)	α	γ	Detection limit (ppt) [a]
1	3×10	5.83	1.88	0.9997	0.51
2	4×20	3.69	1.87	0.9997	0.82
Peak point		0.118	0.96	0.9998	25.4

a and b represent the data points of wavelength differences and emission spectra, respectively.
S/N is the signal-to-noise ratio and γ is the correlation coefficient.
[a] Calculated in terms of $S/N = 3$.

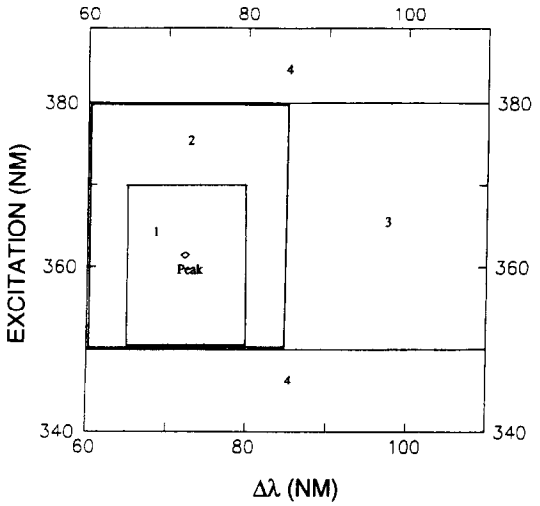

Fig. 5. Block diagram of the integration regions chosen for the determination of tryptophan.

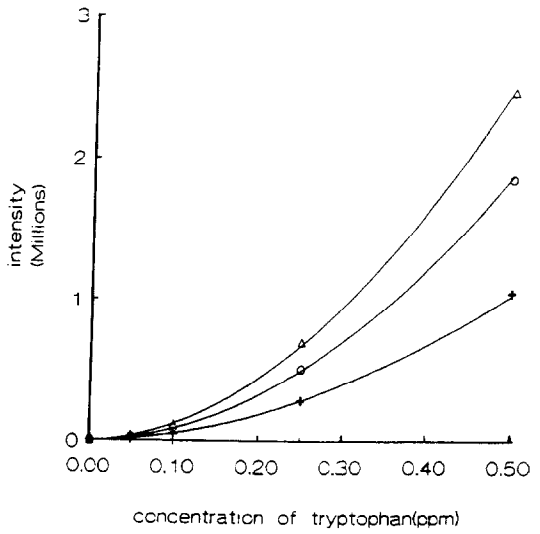

Fig. 6. Fitting of the relation between the total fluorescence intensity and the concentration of tryptophan ($F = KC^{\alpha}$). Key to symbols: Region 1 (△), Region 2 (○), Region 3 (+), Peak point (+).

point measurement (7.3 ppb at peak point), for S/N is greatly increased from 0.041×10^4 to 3.41×10^4. The selectivity would not be lost for the integration region is small (4×20 grid) when compared with the whole TDSF spectrum (11×50 grid).

Compared with TDFS (detection limit 0.05 ppb), the detection limit for tryptophan is not improved by TDSFS since the Stoke's shift for tryptophan is so large that the effect of scattering light on the fluorimetric determination of tryptophan is inherently small.

Table 2
Analytical results for tryptophan obtained by TDSFS using the Monte-Carlo method

Integration region	Data points ($a \times b$)	S/N ($\times 10^4$)	a	γ	Detection limit (ppb) [a]
1	4×20	3.41	1.89	0.9998	8.8×10^{-2}
2	6×30	3.40	1.93	0.9999	8.8×10^{-2}
3	11×30	3.03	1.87	0.9998	9.9×10^{-2}
4	11×50	2.94	1.88	0.9998	0.10
Peak point		0.041	0.97	0.9997	7.3

[a] Calculated in terms of $S/N = 3$.

Table 3
Some data for the determination of naphthalene, pyrene and perylene by TDSFS

Substance	Single point		Integration region		
	$\Delta\lambda/\lambda_{em}$ (nm)	Detection limit (ppb)	$\Delta\lambda$ (nm) range	λ_{em} (nm) range	Detection limit (ppb)
Naphthalene	50/335	1.6	45–65	325–345	0.063
Pyrene	60/400	1.0	50–65	395–405	0.035
Perylene	190/450	1.0	185–195	440–455	0.029

Table 4
Analytical results for mixtures of naphthalene, pyrene and perylene obtained by TDSFS using the total fluorescence intensity method

Sample number	Added (ppb)			Found (ppb)		
	Naphthalene	Pyrene	Perylene	Naphthalene	Pyrene	Perylene
1	10.0	2.0	2.0	9.7 (97%) [a]	1.7 (85%)	2.0 (100%)
2	8.0	8.0	4.0	8.1 (101%)	7.9 (99%)	3.9 (98%)
3	6.0	6.0	6.0	6.0 (100%)	6.1 (102%)	5.8 (97%)
4	4.0	4.0	8.0	3.8 (95%)	3.8 (95%)	7.9 (99%)
5	2.0	10.0	10.0	2.3 (115%)	9.6 (96%)	9.7 (97%)

[a] Recovery.

4.3. Simultaneous determination of naphthalene, pyrene and perylene

This method has been used to simultaneously determine naphthalene, pyrene and perylene. The integration regions for calculating the total fluorescence intensity and the points for detecting intensity were selected as shown in Table 3. Table 3 also shows that the detection limits of naphthalene, pyrene and perylene have been improved 20–50 times by detecting the total fluorescence intensity using TDSFS than by detecting the single point. Table 4 shows the results for simultaneous analysis of naphthalene, pyrene and perylene by detecting the total fluorescence intensity using TDSFS. This also proves that the sensitivity of TDSFS can be greatly improved by detecting the total fluorescence intensity, and the selectivity is almost not influenced.

5. Conclusions

From above experiment the following conclusions can be drawn. (1) The sensitivity of determination can be greatly increased and the selectivity will not be decreased by using the total fluorescence intensity measurement. (2) Compared with the three-dimensional fluorescence spectra, the three-dimensional synchronous fluorescence spectra are less influenced by scattered light and the method shows its merits when used to detect the fluorescent substances with small Stoke's shifts. (3) The random error in quantitative analysis can be efficiently suppressed by using this method. (4) α in the fitting equation, $F = KC^{\alpha}$, influences the peak shape in TDSFS, hardly even having any relation to the integration region selection. However, the optimal detection limit can be obtained by selecting the region nearby the peak position. (5) This method may be widely applied to other determinations of fluorescent compounds.

Acknowledgements

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