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New Analytical Method of Dye Aggregation using PCA Method

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ABSTRACT

The use of several cells of different length is required for the analysis of dye aggregation by the spectral method, and the length of the cell must be calibrated before measurement of the absorption spectra of the dye solution. A new analytical method for dye aggregation in solution using the principal component analytical (PCA) method is suggested. Since calibration of the length of cell is not required, this new spectral method can be more easily used for the analysis of the aggregation of a dye in solution.

1 INTRODUCTION

Characteristics of the aggregation of a dye, such as the aggregation number, the aggregation equilibrium constants and the spectra of the monomer and the aggregate(s), are obtained from the relationships between physical properties and the dye concentration in solution.[1] The physical properties used for this purpose are the absorption spectra, the diffusion constant and light scattering; UV and visible absorption spectra are widely used because of their facile availability and good precision.

On the basis of the Lambert–Beer rule, the apparent absorptivity (ε) is denoted by eqn (1)

$$\varepsilon = A/(C_0 l) \tag{1}$$

where A, C_0 and l are the absorbance, the dye concentration and the length of the light path of the cell (the length of cell), respectively. In order to determine the characteristics of the aggregation, the dye concentration is,

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necessarily, in the range 10^{-5}–10^{-2} mol litre^{-1}. Several cells of different path-length must be adopted to cover these ranges of dye concentration, because the absorbance is usually limited below approximately two. Thus, the length of the cell must be calibrated using a standard solution such as $KCrO_4$ in aqueous KOH before the measurement of the spectra of the dye solution.[2] This makes the spectroscopic method tedious, but it is an easy method to use. If the length of the cell can be calibrated by calculation using only the spectra of the dye solution, the absorption spectra could be more easily used for analysing the dye aggregation. In this paper, a calculation method in which it is feasible to calibrate the length of cell using only the spectra of dye solution is postulated, and its usefulness tested in the case of a binary aggregation system containing monomer and dimer.

2 THEORIES

When a component number is p, the absorbance at dye concentration C_{0i} (i = 1–m) and wavelength λ is expressed by

$$A_{i\lambda} = l_k \sum_{j=1}^{p} C_{ij}\varepsilon_{j\lambda} \tag{2}$$

where C_{ij}, $\varepsilon_{j\lambda}$ and l_k are the concentration of the j-mer, its absorptivity and the cell length, respectively. The total dye concentration C_{0i} is given by eqn (3).

$$C_{0i} = \sum_{j=1}^{p} jC_{ij} \tag{3}$$

Even though $A_{i\lambda}$ and C_{0i} are known, all amounts on the right-hand side of eqns (2) and (3) cannot be determined, since the absorptivities of the monomer and the aggregate(s) are not orthogonal, as expressed by eqn (4).

$$\sum_{\lambda} \varepsilon_{i\lambda} \times \varepsilon_{j\lambda} \neq 0 \tag{4}$$

If these absorptivities are linearly transformed to the normalized and orthogonal vectors (unit vectors) as in eqn (5), the absorbance can be rewritten by eqn (6)

$$\sum_{\lambda} e_{i\lambda} \times e_{j\lambda} = \delta_{ij} \tag{5}$$

where $\delta_{ij} \neq 0$ for $i \neq j$, but $\delta_{ii} = 1$.

$$A_{i\lambda} = \sum_{h=1}^{q} f_{ih}e_{h\lambda} \tag{6}$$

$e_{h\lambda}$ is the hth unit vector at wavelength λ, f_{ih} is its developing coefficient and the number of terms is q. The vector ($e_{h\lambda}$), the matrix (f_{ih}) and the number of the term (q) can all be easily obtained by the usual principal component analytical method (PCA method or PCM).[3] The equality of the number of the term (q) to the component number (p) can be easily verified. The calibrated length of cell, the equilibrium constant(s) and the spectra of the monomer and the aggregate(s) all are obtained from the vectors ($e_{h\lambda}$) and the matrix (f_{ih}). Thus, the number of components, the length of cell and the characteristics of the aggregation are obtained using only the spectra of the dye solution by the PCA method (as detailed below).

2.1 Principal component analytical method

A matrix (**D**) of dimension $n \times n$ is composed by eqn (7) using the observed absorbances at m dye concentrations and n wavelengths.

$$(\mathbf{D})_{\lambda\lambda'} = \sum_{i=1}^{m} A_{i\lambda} \times A_{i\lambda'} \tag{7}$$

The n eigen-vectors (**e**) and the n eigen-values (V) are obtained by diagonalization of the matrix **D**.

$$\mathbf{eD} = \mathbf{Ve} \tag{8}$$

When the n eigen-values are arranged in decreasing order, all values of $V_j (j > q)$ can be ignored.

$$V_1 > V_2 > \cdots > V_q > V_{q+1} = V_{q+2} = \cdots = V_n = 0 \tag{9}$$

Therefore the number of term (q) in eqn (6) is determined so that it equals the number of non-zero eigen-values. Actually the values of $V_j (j > q)$ are not necessarily eliminated by virtue of the error in the observed absorbances or the calculations. Thus a small enough value of V_j, such as one less than 10^{-4} V_1, can be assumed to be zero (PCM assumption). The developing coefficients (f_{ih}) are given by eqn (10).

$$f_{ih} = \sum_{\lambda} A_{i\lambda} \times e_{h\lambda} \tag{10}$$

2.2 The calibration of the length of cell

The absorptivity ($\varepsilon_{j\lambda}$) of the jth comonent in the dye solution can be developed by the unit vectors ($e_{h\lambda}$) as eqn (11).

$$\varepsilon_{j\lambda} = \sum_{h=1}^{p} a_{jh} \times e_{h\lambda} \tag{11}$$

Comparing eqn (6) with eqn (12), obtained by inserting eqn (11) into eqn (2), eqn (13) can be derived.

$$A_{i\lambda} = l_k \sum_{h=1}^{p} \sum_{j=1}^{p} C_{ij} a_{jh} e_{h\lambda} \tag{12}$$

$$f_{ih} = l_k \sum_{j=1}^{p} C_{ij} a_{jh} \tag{13}$$

Eliminating the concentrations of the monomer and the aggregates $\{C_{ij}$ $(j = 1\text{–}p)\}$ from eqns (3) and (13), eqn (14) is obtained

$$\sum_{h=1}^{p} (-1)^{h+1} \Delta_{1,h} f_{ih} + (-1)^{p+2} \Delta_{1,p+1} l_k C_{0i} = 0 \tag{14}$$

where $\Delta_{1,h}$ is the small determinant of Δ.

$$\Delta = \begin{vmatrix} 1 & 1 & \cdots & 1 & 1 \\ a_{11} & a_{12} & \cdots & a_{1p} & 1 \\ a_{21} & a_{22} & \cdots & a_{2p} & 2 \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ a_{j1} & a_{j2} & \cdots & a_{jp} & j \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ a_{p1} & a_{p2} & \cdots & a_{pp} & p \end{vmatrix}$$

When L_k and R_k $(k = 1$ to $r)$ denote the nominal length of the used cell and its relative calibration, respectively, eqn (14) can be rewritten by m simultaneous equations in $(p + r - 1)$ unknowns as eqn (15)

$$\sum_{h=1}^{p} B_h F_{jh} + R_k = 0 \qquad (i = 1\text{–}m,\ k = 1\text{–}r) \tag{15}$$

where

$$F_{ih} = f_{ih}/(C_{0i} L_k) \tag{16}$$

$$B_h = (-1)^{j-p-1} \Delta_{1,j}/\Delta_{1,p+1} \tag{17}$$

and R_1 is taken to be unity. If the number of the dye concentration (m) is larger than $(p + r - 1)$, the unknowns, $B_h(h = 1$ to $p)$ and $R_k(k = 2$ to $r)$, can be obtained by solving eqn (15) using the least squares method.

2.3 Application to analysis of the monomer–dimer system

When the PCA method concludes that the number (p) of components in the dye solution equals 2, the system can be treated as a monomer–dimer system. Then eqns (13) and (3) give rise to eqns (18) and (19) in two unknowns, respectively.

$$f_{ih} = l_k(C_{i1}a_{1h} + C_{i2}a_{2h}) \tag{18}$$

$$C_{0i} = C_{i1} + 2C_{i2} \tag{19}$$

$$C_{i2} = K_h(C_{i1})^2 \tag{20}$$

Equation (20) is the law of mass action between the concentrations of the monomer and the dimer. Elimination of the concentrations of the monomer (C_{i1}) and the dimer (C_{i2}) from eqns (18), (19) and (20) gives rise to eqn (21), where a_{1h} is the estimate of F_{ih}/R_k at the infinite diluted dye concentration.

$$a_{1h} = \lim_{C_{0i}\to 0} (F_{ih}/R_k)$$

$$\{(F_{ih}/R_k - a_{1h})/C_{0i}\}^{1/2} = S_h(F_{ih}/R_k - a_{2h}/2) \tag{21}$$

Using the nonlinear least squares method, eqn (21) is solved to give sets of S_h and a_{2h} (h = 1 and 2), from which two sets of the equilibrium constant and the spectra of the monomer and the dimer are calculated by eqns (22)–(24).

$$K_h = (S_h)^2(a_{1h} - a_{2h}/2)/2 \tag{22}$$

$$\varepsilon_{1\lambda} = a_{11}e_{1\lambda} + a_{12}e_{2\lambda} \tag{23}$$

$$\varepsilon_{2\lambda} = a_{21}e_{1\lambda} + a_{22}e_{2\lambda} \tag{24}$$

3 TEST OF ACCURACY AND UTILITY OF THE CALCULATION METHOD

The accuracy and utility of the calculation method mentioned above were examined by assuming the dye concentration and the length of cell as shown in Table 1. For simplicity, a binary system of the monomer and the dimer was assumed, where the PCA method calculation will conclude that the number of components (p) equals 2. The spectra of the monomer and the dimer were assumed as listed in Table 2.

Using these absorbances, the PCA calculations correctly concluded that the number of the components is 2. As compared in Table 3, the agreement between the assumed and the calculated results of the length of cell and the equilibrium constant is complete. A small discrepancy in the larger equilibrium constant may be caused by an error in the calculations.

Since the observed absorbance usually includes error from three to four decimal places, the random numbers of the normal distribution $\{N(0, \sigma^2)\}$ are added to the assumed values, where the standard deviation (σ) was taken to be $2{\cdot}5 \times 10^{-4}$ or $2{\cdot}5 \times 10^{-3}$. The calculated results

TABLE 1
The Assumed Values of the Dye Concentration and the Length of Cell

k	*Dye concentration* C_0 ($\times 10^4$ *mol* l^{-1})	*Length of cell* *Nominal length* L_k (*cm*)	*Calibration*
1	0·01, 0·03, 0·05	5	1·000
2	0·08, 0·10	2	0·972
3	0·15, 0·20, 0·25, 0·30	1	0·990
4	0·50, 0·60	0·5	1·079
5	0·80, 1·00, 1·50	0·2	0·841
6	2·00, 3·00	0·1	0·801
7	5·00, 8·00, 10·00	0·05	0·747
8	15·00, 20·00, 30·0	0·01	0·744

listed in Tables 4(a) ($\sigma = 2{\cdot}5 \times 10^{-4}$) and 4(b) ($\sigma = 2{\cdot}5 \times 10^{-3}$) show good agreement with the assumed values. Hence the calculation using the PCA method can be reasonably applied to the calibration of the length of cell and to the determination of the equilibrium constant. The equilibrium constants K_1 and K_2 showed fairly good agreement with each other.

When the third component is added to the binary system, but its absorbance is negligibly small, then the PCA method will show that the number of components is 2, by virtue of PCM assumption. In this case eqn (15) should be rewritten as eqn 25

$$B_1F_{i1} + B_2F_{i2} + R_k(1 - X) = 0 \tag{25}$$

where X denotes a fraction of the third component, which is a function of the dye concentration. In the case of the true binary system without the

TABLE 2
The Assumed Spectra of the Monomer and the Dimer

Wavelength (nm)	*Absorptivity* (10^5 *dm*^{2} *mol*$^{-1}$) *Monomer*	*Dimer*
450	0·0132	0·4826
460	0·0912	0·5178
470	0·2334	0·5373
480	0·3850	0·5285
490	0·5186	0·4901
500	0·6117	0·4221
510	0·6285	0·3450
520	0·6254	0·2613
530	0·5667	0·1855
540	0·5124	0·1274
550	0·4319	0·0823

TABLE 3
The Calculated Results of the Length of Cell and the Equilibrium Constant

Equilibrium constant[a] K	*Calibration* $[k]R_k$	*Calculated equilibrium constant*[a] K_1	K_2
0·0155	(1) 1·000, (2) 0·972, (3) 0·990, (4) 1·079, (5) 0·841, (6) 0·801, (7) 0·747, (8) 0·744	0·0155	0·0155
1·183	(1) 1·000, (2) 0·972, (3) 0·990, (4) 1·079, (5) 0·841, (6) 0·801, (7) 0·747, (8) 0·744	1·183	1·183
9·874	(1) 1·000, (2) 0·972, (3) 0·990, (4) 1·079, (5) 0·841, (6) 0·801, (7) 0·747, (8) 0·744	9·877	9·877

[a] Units of 10^4 l mol^{-1}.

TABLE 4
The Calculated Results in the Case Including an Error of (a) Four and (b) Three Decimal Places in the Absorbance

	Equilibrium constant[a] K	*Calibration* $[k]R_k$	*Calculated equilibrium constant*[a] K_1	K_2
	0·0155	(1) 1·000, (2) 0·972, (3) 0·990, (4) 1·079, (5) 0·841, (6) 0·801, (7) 0·747, (8) 0·745	0·0156	0·0156
	1·183	(1) 1·000, (2) 0·973, (3) 0·991,	1·187	1·188
(a)		(4) 1·080, (5) 0·842, (6) 0·802, (7) 0·748, (8) 0·746		
	9·874	(1) 1·000, (2) 0·972, (3) 0·989, (4) 1·078, (5) 0·840, (6) 0·799, (7) 0·745, (8) 0·743	9·842	9·872
	0·0155	(1) 1·000, (2) 0·972, (3) 0·992, (4) 1·080, (5) 0·843, (6) 0·805, (7) 0·749, (8) 0·748	0·0156	0·0154
	1·183	(1) 1·000, (2) 0·975, (3) 0·993,	1·235	1·224
(b)		(4) 1·085, (5) 0·849, (6) 0·807, (7) 0·755, (8) 0·753		
	9·874	(1) 1·000, (2) 0·976, (3) 0·995, (4) 1·088, (5) 0·849, (6) 0·808, (7) 0·754, (8) 0·753	9·443	9·872

[a] Units of 10^4 l mol^{-1}.

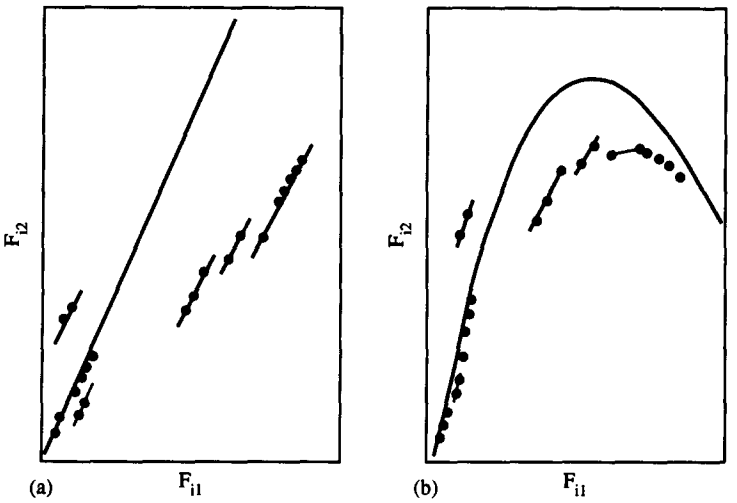

Fig. 1. Plots of F_{i2} against F_{i1}.

third component, a plot of F_{i2} against F_{i1} is a group of lines of the same slope, while in case of a pseudo binary system, in which the third component having small absorbance coexists, the plot is a group of curves. Figure 1 illustrates plots of F_{i2} against F_{i1} in two cases; the one is the true binary system, the other is the pseudo binary system, where the third component is a trimer.

Therefore, by using these plots we can easily judge whether the system is a true or pseudo binary system. This judgment is more rigorous than that based upon the existence of an isosbestic point.

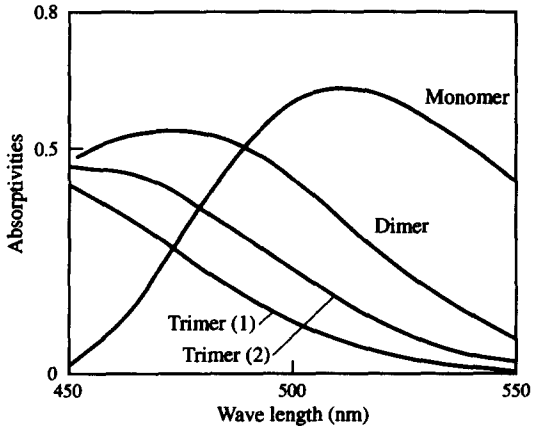

Fig. 2. The assumed spectra of the monomer and aggregates (absorptivity unit = 10^5 dm^2 mol^{-1}).

TABLE 5
The Calculated Results of the Pseudo Binary Systems[a]

	Equilibrium constant[b] *K*	*Calibration* $[k]R_k$ *(1)*	*(2)*	*(3)*	*(4)*	*Calculated equilibrium constant*[b] K_1	K_2	*mean*
	0·444 (0·148)	1·000	0·979	1·005	1·122	0·531	0·588	0·595
(a)	1·5 (0·333)	1·000	0·978	0·997	—	1·467	1·676	1·572
	0·15 (0·0033)	1·000	0·973	0·991	1·115	0·151	0·154	0·152
	0·150 (0·0033)	1·000	0·973	0·991	1·115	0·146	0·148	0·147
(b)	0·444 (0·148)	1·000	0·976	0·998	1·119	0·435	0·464	0·449
	1·500 (0·333)	1·000	0·976	0·997	1·117	1·522	1·589	1·556
	2·5 (0·926)	1·000	0·978	0·998	1·158	2·516	2·654	2·585
	3·75 (2·083)	1·000	0·979	0·999	1·114	3·769	4·071	3·920
(c)	7·5 (8·33)	1·000	0·981	0·998	1·106	7·334	8·470	7·902
	7·5 (15)	1·000	0·985	1·000	1·102	7·495	9·175	8·335
	15 (33·33)	1·000	0·981	0·991	1·091	13·52	17·59	15·56
	15 (45)	1·000	0·983	0·991	1·087	13·62	18·47	16·05

(a) Illustrates the case in which the trimer absorbs no light in the wavelength region measured. (b) and (c) illustrate the case including the timer (1) and trimer (2), respectively, illustrated in Fig. 2.

[a] The formation constants of the trimer ($K \times 10^{-8}$ mol^2) are shown in parentheses.

[b] Units of 10^4 l mol^{-1}.

When the system is concluded to be a pseudo binary system, eqn (21) can be used only in the range of the dye concentration corresponding to the part of lines having nearly the same slope. The method was tested for three cases of the pseudo binary system. In the first case, it was assumed that the trimer absorbs no light in the wavelength region of the spectral measurements, while in the other two cases the absorptivities of the trimer were assumed as illustrated by Fig. 2. As shown in Table 5 the accuracy of the calculated results and agreement between two obtained equilibrium constants were not necessarily good, but were also not so bad. Thus, the analytical method using PCA can be applied even in the case of a pseudo binary system, if a suitable concentration range can be selected.

In Table 6 the spectra of the monomer and the dimer calculated by eqns (23) and (24), respectively, are compared with those assumed. Agreement is excellent in the true binary system, but only fair in the pseudo binary system.

Summarizing the above results it can be concluded that the method of calibration of the length of cell using the PCA method can be reasonably used for both the true and pseudo systems.

TABLE 6
The Calculated Spectra of the Monomer and the Dimer

	True binary system ($K = 0{\cdot}0155 \times 10^4$)[a] absorptivities[c]		*Pseudo binary system ($K = 15 \times 10^4$)[a,b] absorptivities[c]*	
Wavelength (nm)	*Monomer*	*Dimer*	*Monomer*	*Dimer*
450	0·013	0·489	—	0·443
460	0·092	0·525	0·081	0·461
470	0·236	0·544	0·231	0·457
480	0·390	0·536	0·392	0·427
490	0·525	0·497	0·532	0·376
500	0·620	0·428	0·630	0·306
510	0·637	0·350	0·649	0·236
520	0·634	0·265	0·646	0·166
530	0·574	0·188	0·585	0·108
540	0·519	0·129	0·529	0·063
550	0·439	0·083	0·446	0·033

[a] Units of mol^{-1}.
[b] The formation constant of the trimer was assumed to be 45×10^8 mol^{-2}.
[c] Units of 10^5 dm^2 mol^{-1}.

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