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Substituent effects on aggregation phenomena in aqueous solution of thiacarbocyanine dyes

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Abstract

Visible absorption and fluorescence spectra of seven thiacarbocyanine dyes with varying substituents at 5,5′- and *meso*-positions have been studied suggesting J-aggregate formation in aqueous solutions. The molar extinction coefficients of monomers and dimers (ε_M and ε_D), the dimer association constant (K_D) and the dimerization enthalpy (ΔH_D) were determined studying dye concentration and temperature dependencies of absorption spectra. In 0.01 mol/dm^3 KCl aqueous solutions, J-aggregates were observed due to the association of dimers at a critical dye concentration, which correlated with the K_D value. In addition, the aggregation number, the molar extinction coefficient of the J-aggregate (ε_J) and the J-aggregate association constant (K_J) were quantitatively estimated from a modified Harris–Hobbs equation. The J-aggregates were found to be oligomers composed of 20–80 monomers. K_D and ΔH_D suggested that substituents at the 5,5′-position influenced dimer formation and played an important role in aggregates formation.

Keywords: Thiacarbocyanine dyes; J-aggregates; Aggregation number; Dimerization enthalpy; Dimerization constant; Visible spectroscopy; Fluorescence spectroscopy

1. Introduction

Cyanine dyes, which have been used as sensitizers in photography, have recently attracted interest for a number of other uses. They are being investigated for use as laser dyes, Q-switching and mode-locking dyes, red and near-IR fluorescent dye probes for polymeric, micellar and biological systems, and as spectral sensitizers for photoreceptors and organic photoconductors. Jelley has suggested that the appearance of these functions is affected by the formation of aggregates, most notably J-aggregates. J-aggregation is characterized by the appearance of a sharp and bathochromically shifted absorption band relative to monomer and dimer absorption bands [1]. These spectral changes have been used to determine various properties, including the aggregation number and thermodynamic quantities of aggregation such as aggregation constants and free energies and enthalpies of aggregation. Previous studies have used methods such as UV–VIS spectroscopy, fluorescence spectroscopy, and X-ray spectroscopic analysis to examine the aggregation phenomena in aqueous solutions and the effects of changes in dye concentration, temperature, solvent, and added organic or inorganic salts [1–5]. However, in spite of the numerous studies on aggregates function, there have been few studies on the mechanism of aggregates formation.

Knowledge of the spectroscopic properties of cyanine dye aggregates in silver halide, polymer matrices, and in solution is important in order to understand the

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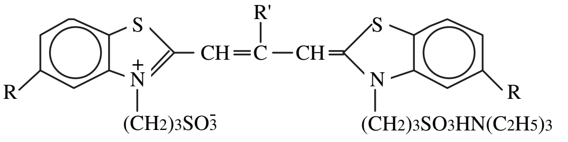
relationship between the molecular structure of the aggregates and the mechanism of aggregation from the viewpoint of molecular design and molecular control. Herein, the mechanism of cyanine dye J-aggregate formation and the aggregation number were investigated in order to clarify the relationship between the molecular structure of the dyes and aggregates formation. J-aggregation was spectrophotometrically analyzed by UV–VIS and fluorescence absorption spectra of the aqueous solutions of seven cyanine dyes in the presence of KCl. Spectroscopic characteristics were utilized to determine the fundamental thermodynamic parameters of the aggregation. These findings are of great interest for controlling the molecular aggregates of functional dyes.

2. Experimental

The molecular structures of cyanine dyes reported here are shown in Fig. 1. All dyes were carbocyanine dyes with a trimethine bridge between two benzothiazole moieties, and showed different substituents at the 5,5′-positions of heterocyclic rings (Dyes 1–4) and at the *meso*-position of the trimethine group (Dyes 1, 5–7). All dyes were obtained from Hayashibara Biochemistry Research Institute Co. Ltd. (the purity of dyes; above 95% by HPLC measurements) and KCl (reagent grade) was purchased from Wako Pure Chemical Industry Co. Ltd. These materials were used without further purification. UV–VIS absorption spectra were recorded on a DU640 spectrophotometer (Beckman Instruments Inc.). The fluorescence spectra were measured with a Hitachi model F-4500 spectrophotometer for aqueous solutions. Most solutions were bubbled with nitrogen gas before preparation of samples in order to minimize the effects of oxygen on the fluorescence spectra. All water used in this study was deionized, distilled, and filtered through a Gelman 0.22 μm filter.



Dye	Substituent	
	5,5'-position (R)	meso-position (R')
1	Cl	C_2H_5
2	H	C_2H_5
3	CH_3	C_2H_5
4	OCH_3	C_2H_5
5	Cl	C_6H_5
6	Cl	CH_3
7	Cl	H

Fig. 1. Chemical structures of cyanine dyes 1–7.

3. Results and discussion

Fig. 2 shows the absorption spectra of cyanine dye in aqueous solutions containing 0.01 mol/dm^3 KCl. Two

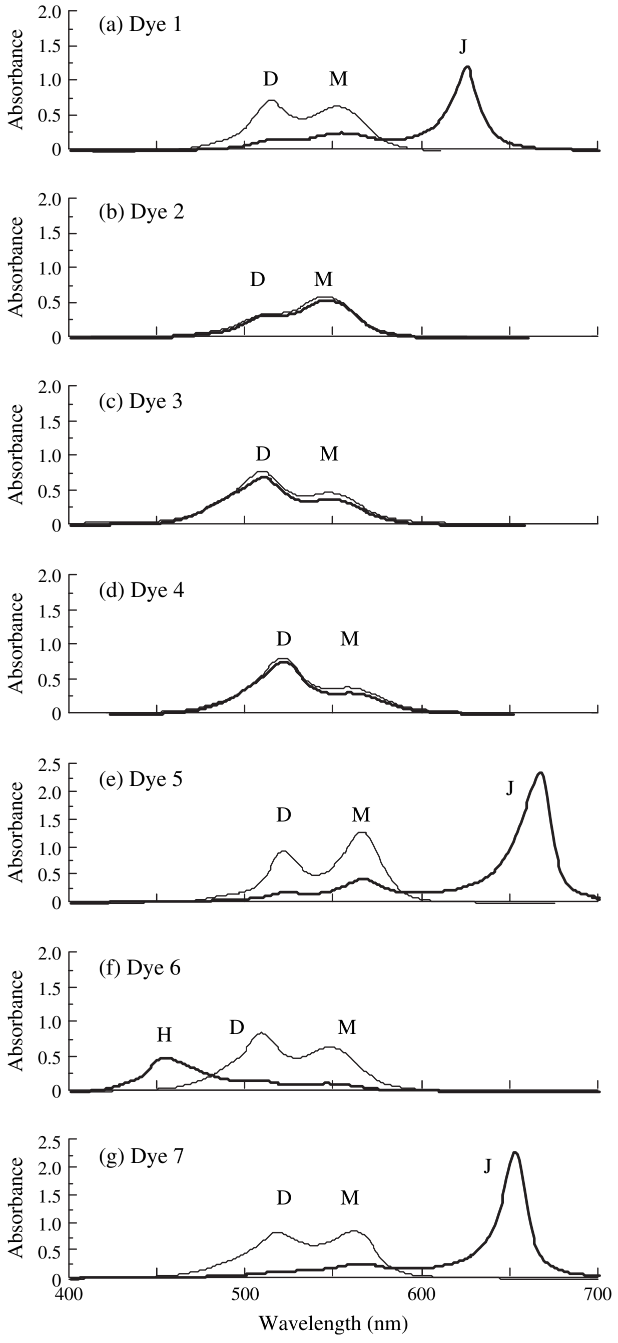


Fig. 2. Absorption spectra of cyanine dyes 1–7 (10 μmol/dm^3) in aqueous solutions without KCl (—) and in the presence of 0.01 mol/dm^3 KCl (—) at 25 °C and stabilized state.

absorption peaks at 540–570 nm and 510–520 nm ranges were observed generally in aqueous solution without addition of KCl, assigned to the monomeric and dimeric species, respectively. The peak at 510–520 ranges is partially overlapped by the dimer band. In aqueous solution containing 0.01 mol/dm^3 KCl, in addition to the monomer (M) and dimer (D) bands, Dye 1 exhibited an extra peak at 625 nm (J-aggregates), that became stable after about 10 min. This indicates a three-component equilibrium among M, D, and J-aggregates. Similar aggregation trends were observed in solutions of Dyes 5 and 7. In the case of Dye 6, the addition of KCl (0.01 mol/dm^3) determined the appearance of a novel broad absorption band at 508 nm, hypsochromically shifted than dimer band, assignable to H-aggregates (H-bands). The other dyes (Dyes 2–4) did not exhibit spectral changes upon addition of 0.01 mol/dm^3 KCl. Increasing the concentration of dye and KCl at 40 μmol/dm^3 and 0.1 mol/dm^3, respectively, Dyes 3 and 4 showed H- and J-bands, respectively, while no variations of absorption bands were observed for Dye 2. These results clearly suggested that, except Dye 2, the aggregation of dyes is affected by the dye concentration and the molecular structure, in particular substituents at 5,5′-positions.

Fig. 3 shows the time dependence of absorption spectra for the aqueous solution of Dye 6 containing 0.01 mol/dm^3 KCl. A J-band, similar to that of aqueous solutions of Dyes 1, 5, and 7, was observed in the presence of 0.01 mol/dm^3 KCl, that decreased with the time while a H-band appeared to increase. This observation indicates that association occurred as a result of strong dipole–dipole interactions among dye molecules in KCl solution. After a few days, the simultaneous disappearance of the H-band was observed with the solid precipitation. Further investigation will be devoted to clarify spectroscopic characteristics and the mechanism of H-aggregate formation.

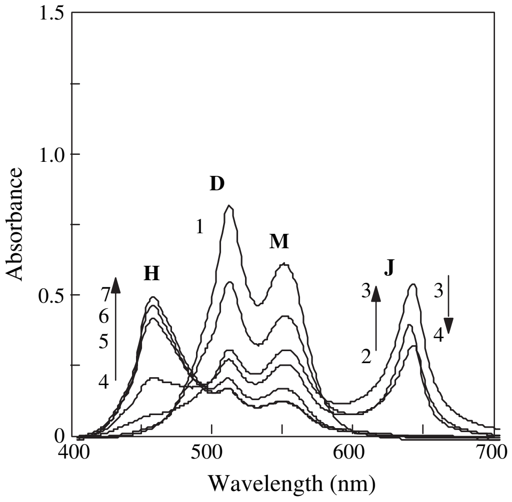


Fig. 3. Time dependence of absorption spectra for 10 μmol/dm^3 aqueous Dye 6 solutions in the presence of 0.01 mol/dm^3 KCl at 25 °C: time (min): 1,0; 2,1; 3,10; 4,30; 5,60; 6,90; 7,120.

The sensitization efficiencies of dyes in thermally processed silver film were previously reported to correlate with the equilibrium constants of their dimeric association (K_D) in aqueous solution [6]. In addition, aggregate formation was not observed for dyes with low K_D values. Therefore, examination of the spectroscopic characteristics of the dimer is important in order to clarify the mechanism of formation and the structures of the J-aggregates.

Fig. 4a shows absorption spectra of Dye 1 at different concentrations and 25 °C in aqueous solutions, taken as

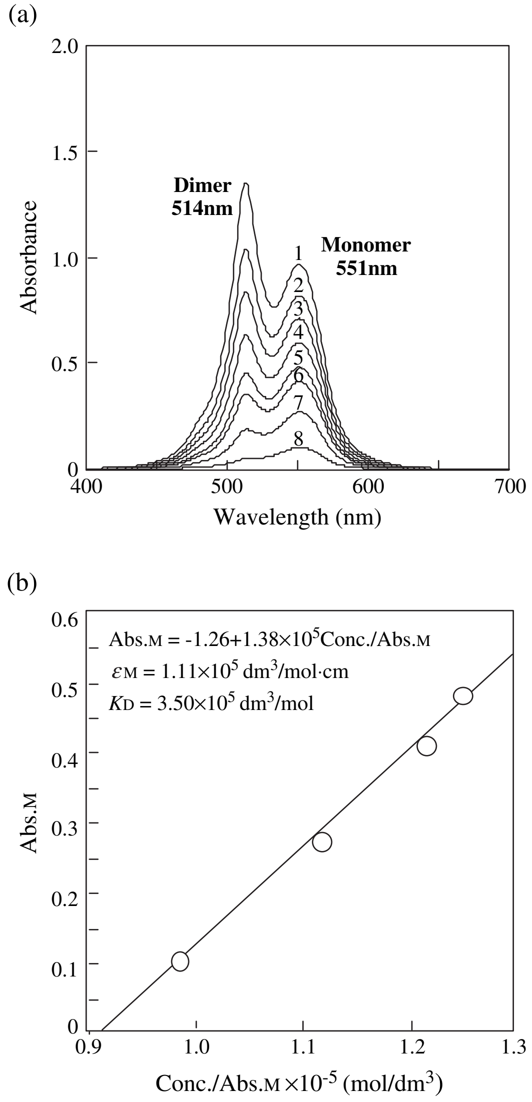


Fig. 4. Absorption spectra of aqueous Dye 1 solutions with different concentrations without KCl at 25 °C; (a) Dye 1 Conc. (μmol/dm^3): 1,15.0; 2,12.0; 3,10.0; 4,8.0; 5,6.0; 6,5.0; 7,4.0; 8,3.0; (b) plots of Abs.$_M$ versus Conc./Abs.$_M$ for the estimation of dimer association constant (K_D) and the molar extinction coefficient of the monomer and dimer (ε_M and ε_D) for Dye 1.

an example. As shown, the absorbance of the D- and M-band peaks increased with increasing dye concentration. Furthermore, the monomer–dimer equilibrium shifted in favor of formation of the dimer as the concentration (C) of the dye increased.

The dimer association constant (K_D) and the molar extinction coefficient of the monomer (ε_M) were determined based on the dependence of the monomer absorbance band (Abs.$_M$) at C using the Eq. (1) developed by Harris and Hobbs [7]

$$\mathrm{Abs.}_M = \left(\varepsilon_M^2 l^2/2K_D\right)\left(C/\mathrm{Abs.}_M\right) - \varepsilon_M l/2K_D \tag{1}$$

where l is the solution thickness.

If monomers and dimers are the only solute species, then a plot of Abs.$_M$ against C/Abs.$_M$ would give a straight line with slope $\varepsilon_M{}^2 l^2/2K_D$ and intercept $-\varepsilon_M l/2K_D$, from which both K_D and ε_M can be determined. The following Eq. (2) can be used to calculate the monomeric and dimeric concentrations (C_M and C_D in mol/dm^3).

$$C_D = (C - C_M)/2 \tag{2}$$

Furthermore, K_D may be expressed as follows:

$$K_D = C_D/C_M^2 \tag{3}$$

From the logarithm of Eq. (3), Eq. (4) is obtained as follows:

$$\log C_D = 2\log C_M + \log K_D \tag{4}$$

The accuracy of the estimated values from Eq. (1) for ε_M, the molar extinction coefficient of the dimer ε_D, and K_D can thus be confirmed by the slope of the plot of the log C_D versus log C_M. As can be seen in Fig. 4b, the plot of Abs.$_M$ against C/Abs.$_M$ for Dye 1 gave a straight line, from which the values $K_D = 3.50 \times 10^4$ dm^3/mol and $\varepsilon_M = 1.11 \times 10^5$ dm^3/mol cm were determined. A plot of log C_D versus log C_M gave a straight line of slope 2.0, confirming the predominance of monomer–dimer solute species. Similarly, the slopes of the plots of the log C_D versus log C_M for the other dyes were nearly equal to 2.0.

The values of ε_M, ε_D, and K_D recorded for seven dyes are listed in Table 1, together with the enthalpies of dimerization (ΔH_D) (estimated from the temperature dependence of K_D using the van't Hoff equation) and the Stokes shift (ΔS) of fluorescence spectra.

Dyes 1, 5, and 7, which have electron-withdrawing chlorine groups at 5,5′-positions, formed aggregates at lower concentration than Dyes 3 and 4, which have electron-donating substituents methyl and methoxy at 5,5′-positions, respectively. However, there were few differences in the ε_M and ε_D values for all Dyes. Furthermore, large K_D and ΔH_D values were observed in all dyes with the exception of Dye 2. These results suggest that the substituents at the 5,5′-positions affected not only dimer formation, but also the tendency to form J-aggregates. Herz [3] reported that the introduction of chlorine groups at the 5,5′-positions lead to a somewhat large free energy of dimerization. Iwasaki et al. [8] reported that the introduction of larger substituents at 5,5′ (4,4′) and 6,6′-positions in anionic thiacyanine dye increased the tendency of dye molecules to dimerize. Furthermore, they reported that dyes with chlorine groups facilitate the formation of J-aggregates in the presence of KBr. Dyes with methoxy groups, however, did not form J-aggregates. These findings were in good agreement with the results found in this study. Thus, in addition to promoting dimerization, it appears that introduction of electron-withdrawing substituents such as chlorine groups and phenyl groups at the 5,5′-positions may play an important role in the molecular design of dyes that readily form aggregates.

In order to further develop the molecular design of useful dyes, it is necessary to consider the ability to control the methine chain length [9] and introduce larger substituents at the *meso*-position [10]. In an effort to investigate the effects of the substituent at the *meso*-position on J-aggregation, the dye concentration dependence of absorption spectra in 0.01 mol/dm^3 KCl aqueous solutions at 25 °C was examined; in Fig. 5 the results for Dye 1 are reported as an example. The absorbance of the J-band for Dye 1 increased as the

Table 1
The molar extinction coefficient of monomer and dimer (ε_M, ε_D) and equilibrium constants for dimerization (K_D) of cyanine dyes

Dye	Monomer		Dimer		K_D (dm^3/mol)	ΔH_D (kJ/mol)	λ_{EM} (nm)	ΔS(nm)	Intensity
	λ_{max} (nm)	ε_M (dm^3/mol cm)	λ_{max} (nm)	ε_D (dm^3/mol cm)					
1	551	1.11×10^5	514	4.67×10^5	3.50×10^4	−33.8	570.2	19.2	37.6
2	546	1.05×10^5	505	1.82×10^5	1.24×10^4	−14.3	566.6	20.6	25.7
3	548	9.19×10^4	510	1.77×10^5	4.58×10^4	−43.1	571.4	23.4	15.1
4	557	8.74×10^4	521	4.85×10^5	1.30×10^5	−59.6	579.6	22.6	12.1
5	566	3.02×10^5	523	1.28×10^5	3.69×10^4	−21.0	579.8	13.8	222.8
6	547	1.09×10^5	508	2.01×10^5	8.96×10^4	−40.6	567.6	20.6	20.0
7	562	1.69×10^5	518	5.10×10^5	1.14×10^5	−31.0	574.8	12.8	489.8

In this table, ε_M, ε_D, and ΔS represent the molar extinction coefficient of monomer and dimer, and Stokes shift, respectively.

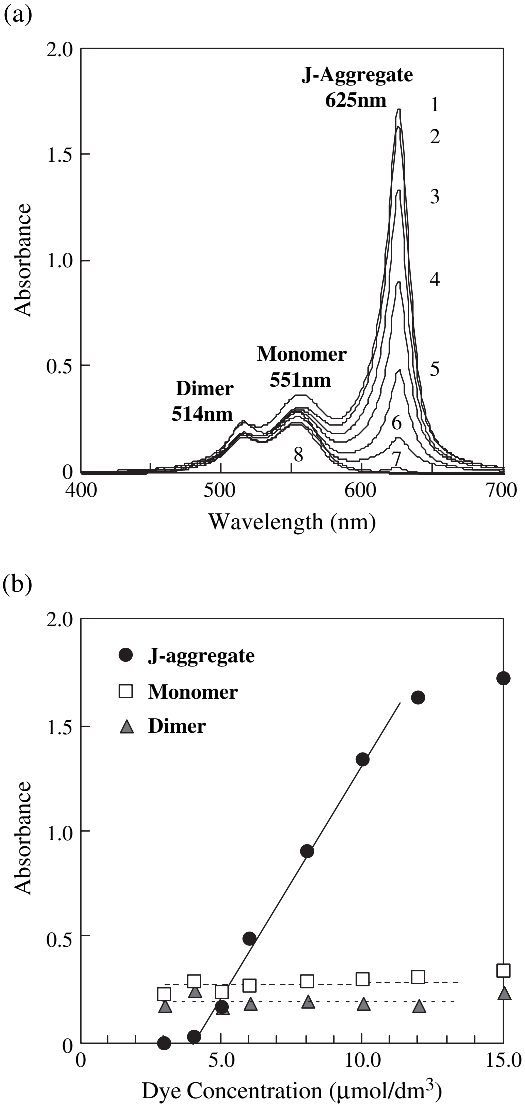


Fig. 5. Absorption spectra of aqueous Dye 1 solutions with different concentrations in the presence of 0.01 mol/dm^3 KCl at 25 °C: (a) Dye 1 Conc. (µmol/dm^3): 1,15.0; 2,12.0; 3,10.0; 4,8.0; 5,6.0; 6,5.0; 7,3.0; 8,1.0; (b) variation in absorbance of monomer, dimer, and J-aggregate with dye concentration.

concentration of the dye increased, whereas the absorbance of the D- and M-bands remained constant. In addition, J-aggregation occurred at a dye concentration of 4.0 µmol/dm^3, but was not observed at concentrations lower than 3.8 µmol/dm^3. Daltrozzo et al. reported that the threshold concentration for J-aggregation of 1,1′-diethyl-2,2′-cyanine chloride lies between 3.0 and 4.0 mmol/dm^3 at temperatures down to 0 °C [11]. Similarly, Tanaka et al. reported that J-aggregation of 1,1′-diethyl-2,2′-cyanine chloride occurred at dye concentrations higher than about 0.3 mmol/dm^3 in the presence of 0.5 mol/dm^3 sodium sulfate [12]. Thus, a critical concentration seems necessary for the formation of precursors of J-aggregates. The critical concentration of J-aggregation (C^*) for Dye 1 was determined to be 3.84 µmol/dm^3 by extrapolating to zero absorbance (Fig. 5b). The C^* for Dyes 5 and 7 were analogously estimated at 2.70 and 1.41 µmol/dm^3, respectively.

As shown in the spectra of Fig. 5a, J-aggregates coexisted with monomers and dimers in solutions containing KCl. If J-aggregates are formed by the association of dimers (m dimers $\rightleftarrows$ J-aggregate), then the equilibrium constant for J-aggregation (K_J) may be expressed as follows:

$$K_J = C_J / C_D^m \tag{5}$$

where C_J denotes the molar concentration of J-aggregates and m is the dimer number and $2m$ the aggregation number. Eq. (6) was obtained from Eq. (5) as follows:

$$\log C_J = m \log C_D + \log K_J \tag{6}$$

Under the assumption of J-aggregate formation, C_J is expressed as

$$C_J = (C - C_M - 2C_D)/2m \tag{7}$$

Substitution of Eq. (7) into Eq. (6) gives

$$\log(C - C_M - 2C_D) = m \log C_D + \log 2mK_J \tag{8}$$

Therefore, the value of m may be obtained from the slope of the plot of $\log(C - C_M - 2C_D)$ versus $\log C_D$. Fig. 6 shows the plot of $\log C_D$ versus log $(C - C_M - 2C_D)$ for Dye 1, from which the aggregation number of J-aggregates for Dye 1 was determined to be 80 ($m = 40$).

Characteristics of the J-aggregates for Dyes 1, 5, and 7, including the aggregation number ($2m$), the maximum absorption wavelength, and the molar extinction

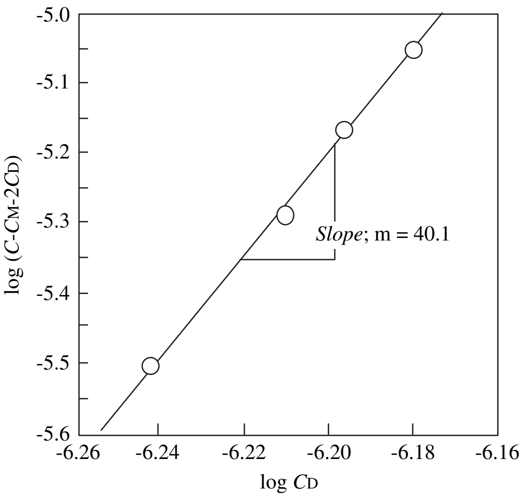


Fig. 6. Plots of $\log C_D$ versus $\log(C - C_M - 2C_D)$ for the estimation of aggregation number of J-aggregates for Dye 1.

Table 2
Spectroscopic parameters of J-aggregates

Dye	Aggregation number (2*m*)	λ_{max} (nm)	Molar extinction coefficient (dm^3/mol cm)			C^* (mol/dm^3)	K_D (dm^3/mol)	λ_{EM} (nm)	Intensity
			ε_J	ε_D	ε_M				
1	80	625	5.59×10^6	4.67×10^5	1.11×10^5	3.84×10^{-6}	3.50×10^4	625.0	1025
5	32	667	8.87×10^6	1.28×10^5	3.02×10^5	2.70×10^{-6}	3.69×10^4	667.4	153.9
7	20	652	6.69×10^6	5.10×10^5	1.69×10^5	1.41×10^{-6}	1.14×10^5	652.0	278.0

In this table, C^* represents the critical concentration of J-aggregation.

coefficient of the J-aggregates (ε_J) are listed, together with the C^* and K_D values, in Table 2. As a result, the aggregation number of J-aggregates for Dyes 5 and 7 were estimated at 32 and 20, respectively. As can be seen in Table 2, the aggregation number of the J-aggregates appears to be correlated with both C^* (higher C^* yields a higher aggregation number) and K_D (higher K_D yields a lower aggregation number). In aqueous solutions, low ΔS values and high fluorescence intensities were observed for Dyes 5 and 7, but were not observed for Dyes 1–4 and 6 (see Table 1). These observations suggest that there was little difference in the degree of polarization between ground and excited states of Dyes 5 and 7 [13,14]. The energy transfer among molecules in high-structured J-aggregates applauds more importance than the energy transfer occurring in fluorescence.

4. Conclusions

Spectroscopic characteristics of seven carbocyanine dyes with different substituents at 5,5′- and *meso*-positions were evaluated as a function of dye concentration and temperature in aqueous solution with 0.01 mol/dm^3 KCl. The results obtained are summarized as follows:

(i) The substituents at the 5,5′-positions affect the ease of dimerization and is important in aggregate formation. Electron-withdrawing groups such as chlorine appear to promote both dimerization and aggregation.
(ii) Formation of J-aggregate precursors obtained by association of dimers at a critical concentration correlate with the K_D values.
(iii) The J-aggregates formed were oligomers composed of 20–80 monomer units.

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