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Original scientific paper

Thermodynamic characterization of the dimerization equilibrium of newly synthesized polymethine cyanine dyes

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Abstract: The monomer–dimer equilibrium and thermodynamics of three new cyanine dyes were investigated by spectrophotometric and chemometric methods. The dimerization constants of these new cyanine dyes were determined by studying the dependence of their absorption spectra on the temperature in the range 25–80 °C at concentrations of 3.0×10^{-4} , 1.9×10^{-4} and 1.1×10^{-4} M for dye **1**, **2** and **3**, respectively. The processing of the data, performed for the quantitative analysis of pure spectral profiles, was based on the simultaneous resolution of the overlapping bands in the whole set of absorption spectra. From the dimerization constant and its dependence on temperature, the values of the standard enthalpy change and entropy change of dimerization were calculated.

Keywords: chemometrics; dimerization; cyanine dyes; absorption titration; enthalpy; entropy.

INTRODUCTION

Dimerization of dyes in aqueous solution has been studied extensively as the most fundamental model of the self-aggregation of biological molecules and biomacromolecule–ligand interactions,^{1–3} and as the basis for their application as tunable lasers and other practical uses.^{4–6} Various factors have been found to affect the dimerization behavior, *i.e.*, dye structure, solvent, coexisting salt, temperature and pressure. Thermodynamic parameters, such as standard enthalpy and entropy change on dimerization, which are derived from the temperature dependence of the dimerization constant, have given some insight into the forces that maintain the dimer structure in solution.

In recent years, there has been increased interest in the field of synthesis and application as nucleic acid labels of cyanine (polymethine) dyes absorbing in different visible spectral regions. The number of both patents and scientific publica-

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tions is evidence for the commercial, scientific and practical importance of these probes in nucleic acid research, as well as clinical and environmental analysis. Representatives of this class of nucleic acid stains have fluorescence excitations and emissions covering the visible spectrum from blue to near infrared, with additional absorption peaks in the UV region, which makes them applicable in many different types of instrumentation with different light sources. Replacement of even one substituent in the dye molecule can lead to novel and sometimes better properties for nucleic acid detection.⁷ We believe that such an important research area requires more new representatives to be investigated in order to establish their new properties on interaction with nucleic acids.

Spectroscopic methods are in general highly sensitive and as such suitable for studying chemical equilibria in solution. When the components involved in the chemical equilibrium have distinct spectral responses, their concentrations can be measured directly, and the determination of the equilibrium constants is trivial.⁸

In this study, the dimerization equilibrium of newly synthesized cyanine dyes⁹ was characterized by absorption titration and chemometrics data analysis, carried out by the DATAN package developed by Kubista *et al.*^{10–15} The theory and application of the physical constraints method was discussed by Kubista *et al.* and is discussed here briefly.

Theory

Absorption spectra recorded at different temperatures are arranged as rows in an $n \times m$ matrix \mathbf{A} , where n is the number of temperature intervals and m is the number of data points in each spectrum. \mathbf{A} is decomposed into an orthogonal basis set using, for example, NIPALS.^{16,17}

$$\mathbf{A} = \mathbf{TP}^T + \mathbf{E} \approx \mathbf{TP}^T = \sum_{i=1}^r \mathbf{t}_i \mathbf{p}_i^T \quad (1)$$

where \mathbf{t}_i ($n \times 1$) are orthogonal target vectors and \mathbf{p}_i^T ($1 \times m$) are orthogonal projection vectors. These are mathematical constructs and do not correspond to any physical property of the system. r is the number of spectroscopically distinguishable components, and \mathbf{E} is the error matrix containing experimental noise, if the right value of r is selected. For a well-designed experiment, \mathbf{E} is small compared to \mathbf{TP}^T and can be neglected. Assuming linear response, the recorded spectra are also linear combinations of the spectral responses, \mathbf{v}_i ($1 \times m$), of the components:

$$\mathbf{A} = \mathbf{CV} + \mathbf{E} \approx \mathbf{TP}^T = \sum_{i=1}^r \mathbf{c}_i \mathbf{v}_i \quad (2)$$

where \mathbf{c}_i ($n \times 1$) are vectors containing the component concentrations at the different temperatures. The two equations are related by a rotation:¹⁸

$$\mathbf{C} = \mathbf{TR}^{-1} \quad (3)$$

$$\mathbf{V} = \mathbf{RP}' \quad (4)$$

where \mathbf{R} is an $r \times r$ rotation matrix. For a two-component system:

$$\mathbf{R} = \begin{bmatrix} r_{11} & r_{12} \\ r_{21} & r_{22} \end{bmatrix}$$

and

$$\mathbf{R}^{-1} = \frac{1}{r_{11}r_{22} - r_{12}r_{21}} \begin{bmatrix} r_{11} & -r_{12} \\ -r_{21} & r_{22} \end{bmatrix} \quad (5)$$

Since a single sample is studied, the total concentration must be constant, constraining the matrix \mathbf{R} .¹⁹ For a monomer–dimer equilibrium, $2X \leftrightarrow X_2$, the total concentration of monomers is constant:

$$c_X + 2c_{X_2} = c_{\text{tot}} \quad (6)$$

Combining Eq. (6) with Eq. (3), one obtains:

$$\frac{1}{r_{11}r_{22} - r_{12}r_{21}} (t_1 r_{22} - t_2 r_{21} - 2t_1 r_{12} + 2t_2 r_{11}) = c_{\text{tot}} \quad (7)$$

which can be written as:

$$f_{11}t_1 + f_{12}t_2 = c_{\text{tot}} \quad (8)$$

where

$$f_{11} = (r_{22} - 2r_{12})(r_{11} - r_{12}r_{21})^{-1} \quad (8a)$$

and

$$f_{12} = (2f_{11} - r_{21})(r_{11}r_{22} - r_{12}r_{21}) \quad (9)$$

These can be determined, for example, by fitting the target vectors to a vector with all elements equal to c_{tot} . Eqs. (8) and (9) provide two relations between the elements of the matrix \mathbf{R} , hence making two of them redundant.

In most cases, the spectra of some of the components can be determined in separate measurements. For example, the monomer–dimer equilibrium system can, in general, be diluted sufficiently to make the dimer concentration negligible. This enables the monomer spectrum to be recorded, which, of course, should be used as a constraint in the analysis. Normalizing the monomer spectrum to the same total concentration as the analyzed sample, one obtains from Eq. (4):

$$V_{\text{monomer}} = r_{11}P'_1 - r_{12}P'_2 = f_{21}P'_1 - f_{22}P'_2 \quad (10)$$

where $f_{21} = r_{11}$ and $f_{22} = r_{12}$ are determined by fitting the two projection vectors to the monomer spectrum. Equation (9) also provides two relations between the elements of matrix \mathbf{R} . These are not independent of Eq. (7) and the two equations

cannot be combined to solve for all the elements of matrix \mathbf{R} , but they can be used to express \mathbf{R} in a single element, below arbitrarily chosen to be r_{21} . Defined this way, matrix \mathbf{R} produces \mathbf{C} and \mathbf{V} matrices that are consistent with the total sample concentration and the spectral response of the monomer. The value of r_{21} :

$$\mathbf{R} = \begin{bmatrix} f_{21} & f_{22} \\ r_{21} & 2f_{22} + (2f_{21} - r_{21}) \frac{f_{11}}{f_{12}} \end{bmatrix} \quad (11)$$

determines the dimer spectrum and the monomer concentration profiles. Although all values of r_{21} produce mathematically acceptable solutions, reasonable results, in terms of spectral intensities and non-negative concentrations and spectral responses, are obtained over a relatively narrow range of r_{21} values. Still, the range is, in general, too large for a quantitative analysis.

The final constraint, which produces a unique solution, is the thermodynamic relation between temperature and the equilibrium constant. The concentrations of the components are related by the law of mass action:²⁰

$$K_D(T) = \frac{c_{X_2}(T)(c^0)^2}{c^0(c_{X_2}(T))^2} \quad (12)$$

where $c^0 \equiv 1 \text{ mol dm}^{-3}$. Assuming that the dimerization constant $K_D(T)$ depends on the temperature according to the van't Hoff equation:²¹

$$\frac{d \ln K_D(T)}{d(1/T)} = -\frac{\Delta H^0}{R} \quad (13)$$

where ΔH^0 is the molar enthalpy change, $R = 8.31 \text{ J mol}^{-1} \text{ K}^{-1}$ is the gas constant and T is the temperature. r_{21} can now be determined by requiring that matrix \mathbf{R} should rotate the target vectors to give concentration vectors (Eq. (3)) that produce an equilibrium constant whose logarithm is a linear function of $1/T$. In practice, the solution is found by a simple search procedure. r_{21} is given an arbitrary value, for which a trial rotation matrix is calculated (Eq. (11)). This is used to calculate the trial concentration profiles (Eq. (3)), which are combined to the trial equilibrium constant (Eq. (12)). A linear regression of the equilibrium constants with respect to $1/T$ is then performed (Eq. (13)), which determines the trial enthalpy change of the reaction. Each trial rotation matrix also determines the trial spectral responses (Eq. (4)). The procedure is repeated for various values of r_{21} to find a range that produces reasonable concentration profiles and spectral responses. This is done rather arbitrarily since there is no simple way to estimate r_{21} . Once a range has been found, r_{21} is varied gradually in this range and a χ^2 (regression coefficient) is calculated for each regression of $\ln K_D(T)$ with respect to $1/T$. The r_{21} that produces the best fit determines the matrix \mathbf{R} . The analysis is readily performed with the Datan program.

EXPERIMENTAL

All the employed chemicals were of analytical reagent grade. The new cyanine dyes, were synthesized by Deligeorgiev *et al.*⁹ Stock solutions were prepared by dissolving a solid dye in buffer solution containing 500 mM KCl, 15 mM MgCl₂ and 100 mM tris(hydroxymethyl) amniomethane hydrochloride (Tris-HCl). The pH of all the solutions was kept constant at 7.50 using Tris buffer. The absorption spectra were recorded using an Agilent 8453 UV–Vis Diode-Array spectrophotometer, employing Agilent UV–Visible ChemStation software for data acquisition. A quartz cuvette of 1 mm optical path was used for all measurements. The pH measurements were made using a 300 HANA model pH-meter equipped with a combined glass electrode. Data preprocessing and data analysis were carried out in Matlab, version 7, and Datan computer programs.

RESULTS AND DISCUSSION

The absorption spectra of dyes **1–3** were recorded between 400 and 600 nm, in the temperature range 25–75 °C at 2.5 °C intervals and pH 7.50. As it was expected that the monomer form would be predominant over the dimer form with increasing temperature, the spectrum of the dye at the highest temperature was chosen as an initial estimate for the monomer in the subsequent calculations. Then according to Eqs. (1)–(13), the Datan program commenced with a trial value of r_{21} , at predefined interval, and iterated all the calculation steps. The iteration ceased when all r_{21} values in the initial interval had been tested. The K_D , the dimer spectrum and the ΔH corresponding to the minimum value of χ^2 were selected as the best values. χ^2 is a goodness of fit criterion and its value indicates the predictability of the model, *i.e.*, how well the monomer spectrum and r_{21} were determined.

Two isosbestic points were found at 482 and 544 nm for dye **1** and at 498 and 550 nm for dyes **2** and **3**. This suggests an equilibrium between two spectral species, *i.e.*, a monomer–dimer equilibrium. Accordingly, the total spectra were analyzed assuming them to contain contributions of the monomer and of the dimer. With increasing temperature the absorption peak for dyes **1–3** at around 520 nm grows and the absorption peak around 460 nm for dye **1** and around 490 nm for dye **2** and **3** diminished (Fig. 1). The dimerization constants, K_D , were calculated at different temperatures.

From the dependence of $\ln K_D$ on $1/T$ (Fig. 2), ΔH^0 and ΔS^0 values were determined. The dimerization constants at 25 °C and the thermodynamic parameters of the dimerization reactions of the dyes are given in Table I. As expected, K_D decreased with increasing temperature. The values of $\Delta H^0 < 0$ and $\Delta S^0 < 0$ for dimerization mean that the dimerization reaction is enthalpy-driven. It can be seen that the dimerization reactions of these dyes are exothermic and are characterized by relatively large negative ΔH values. The latter is consistent with dispersive van der Waals interactions of aromatic chromophores being the main contributors to complex formation.^{21,22} Dispersive van der Waals interactions are characterized both by negative enthalpy and negative entropy,²³ indicating an enthalpic origin for the dimerization processes of these dyes in aqueous solution. The relative

dependence of the concentrations of the monomer and dimer of the dyes on temperature are shown diagrammatically in Fig. 3. The calculated absorption spectra of the monomer and dimer forms of the dyes are shown in Fig. 4.

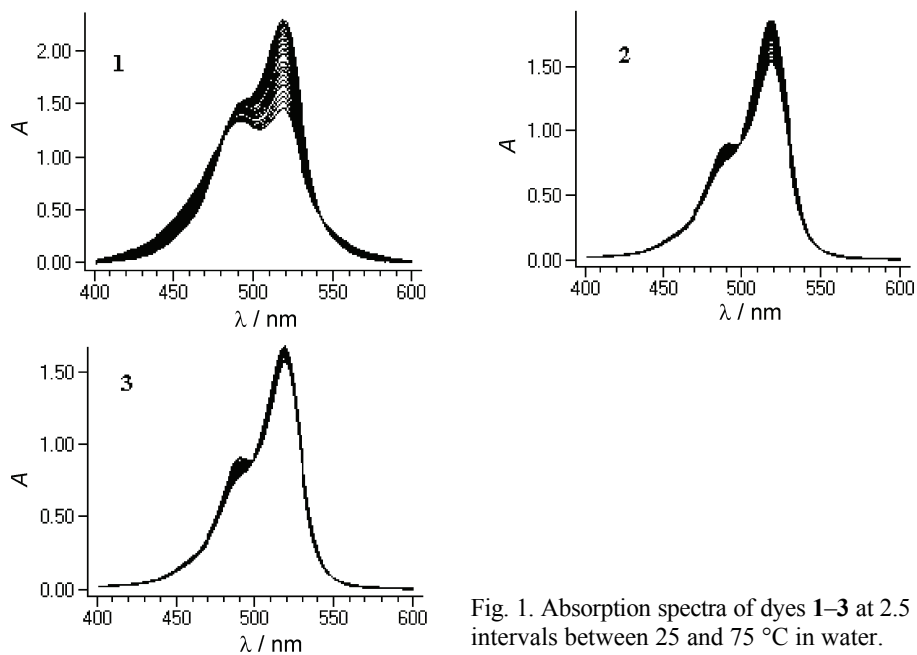


Fig. 1. Absorption spectra of dyes 1–3 at 2.5 °C intervals between 25 and 75 °C in water.

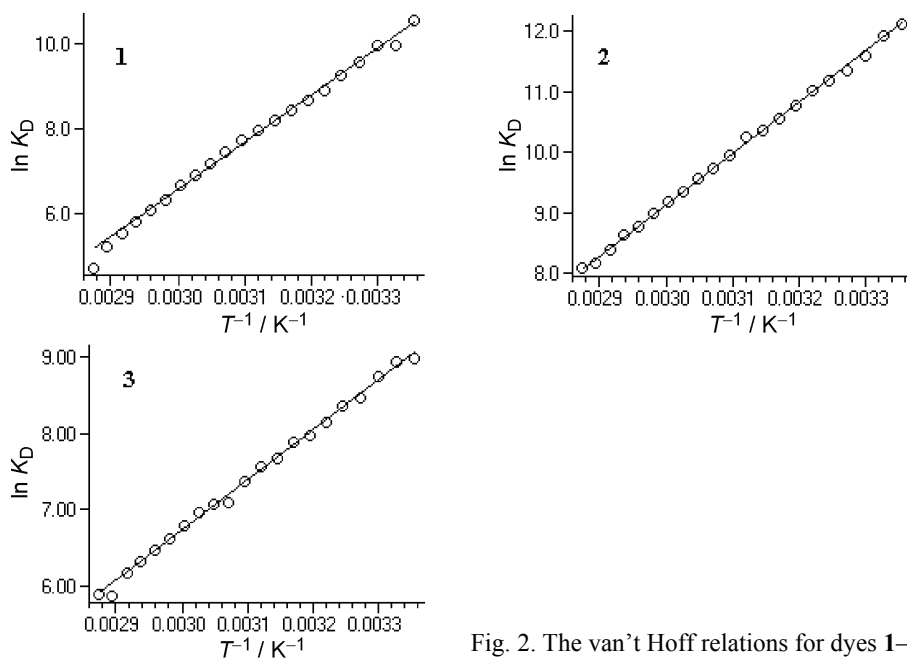
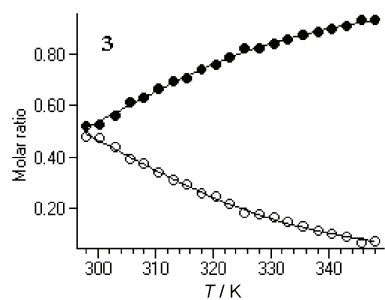
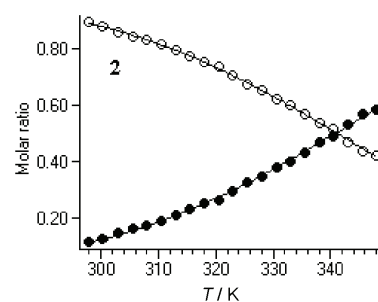
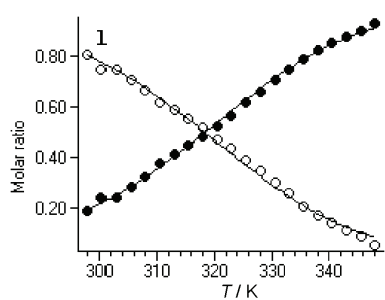
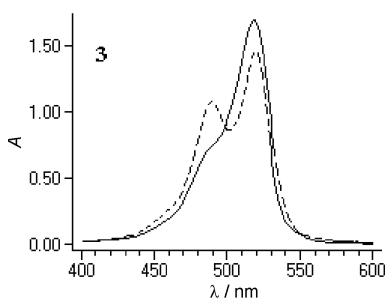
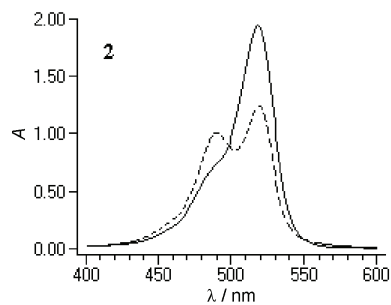
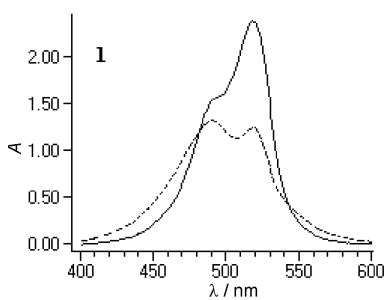


Fig. 2. The van't Hoff relations for dyes 1–3.

TABLE I. Dimeric constant and values of thermodynamic parameters for dye **1** (3×10^{-4} M), dye **2** (1.9×10^{-4} M) and dye **3** (1.1×10^{-4} M) in water

Dye	$\log K_D$ (25 °C)	$\Delta H^0 / \text{kJ mol}^{-1}$	$\Delta S^0 / \text{J mol}^{-1} \text{K}^{-1}$
1	4.57	-91.8	-220
2	5.26	-70.1	-134
3	3.90	-54.4	-107

Fig. 3. Molar ratio of monomer (●) and dimer (○) of dyes **1–3**, compared to the molar ratios predicted by the temperature dependence of the equilibrium constant (shown as line) in water.Fig. 2. Calculated monomer (—) and dimer (---) spectra of the dyes **1–3** in water.

CONCLUSION

In this paper, for the first time, a thermodynamic study of the dimerization equilibrium of three new cyanine dyes in aqueous solution using the Datan package is reported. Dimerization constants, concentration profiles for the monomer and dimer, and spectral responses of monomer and dimer were obtained by computer refinement of temperature photometric titrations. The values of K_D decreased with increasing temperature. The thermodynamic parameters, enthalpy and entropy of dimerization reaction were calculated from the dependence of the dimeric constant on temperature (van't Hoff equation). The interaction between dye molecules is attributed predominantly to enthalpic rather than entropic reasons. The dimerization forces between the dye molecules are dispersive van der Waals interactions and K_D depends on the size and rigidity of the dye molecules.

ИЗВОД

ТЕРМОДИНАМИЧКА КАРАКТЕРИЗАЦИЈА РАВНОТЕЖЕ ПРОЦЕСА ДИМЕРИЗАЦИЈЕ НОВОСИНТЕТИСНИХ ПОЛИМЕТИН-ЦИЈАНИНСКИХ БОЈА

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Равнотежа мономер–димер и термодинамика три нове цијанинске боје испитиване су спектрофотометричком и хеометријском методом. Константе равнотеже процеса димеризације ових нових цијанинских боја одређене су на основу зависности њихових апсорпционих спектра од температуре у опсегу од 25 до 80 °C при концентрацијама од $3,0 \times 10^{-4}$, $1,9 \times 10^{-4}$ and $1,1 \times 10^{-4}$ M за боју **1**, **2**, и **3**, респективно. Обрада података, у смислу квантитативне анализе спектра, заснована је на симултаном анализи преклопљених апсорпционих трака у комплетном опсегу таласних дужина. На основу температурне зависности константе равнотеже за процес димеризације одређене су промена енталпије и ентропије овог процеса.

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