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## Application of chemometrics methods in thermodynamics investigation of the dimerization equilibria of Nile blue in different concentration by spectral titration

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### ABSTRACT

The monomer-dimer equilibrium of Nile blue (NB) dye has been investigated by UV-Vis spectroscopy. The dimerization constants of Nile blue have been determined by studying of the dependence of their absorption spectra on the temperature in the range 20-80°C at different total concentrations of dye ( $8.5 \times 10^{-5}$  to  $3 \times 10^{-4}$  M). The equilibrium parameters of the dimerization of Nile blue have been determined by chemometrics refinement of the absorption spectra obtained from thermometric titrations at different concentrations. The quantitative analysis of the data of undefined mixtures, was carried out by simultaneous resolution of the overlapping spectral bands in the whole set of absorption spectra. Utilizing the van't Hoff relation, which describes the dependence of the equilibrium constant on temperature, as constraint, we determine the spectral responses of the monomer and dimer species as well as the enthalpy and entropy of the dimerization equilibrium. © 2008 Trade Science Inc. - INDIA

### KEYWORDS

Chemometrics;  
Nile blue;  
DATAN;  
Spectrophotometric;  
Equilibrium.

### INTRODUCTION

The cationic mono- and diaminophenoxazine dyes are widely used to dye acid-modified synthetic fibers, especially polyacrylonitrile and polyester fibers, containing acid group. Aminophenoxazine dyes are also suggested for dyeing polyolefin materials. The earlier areas of application, the dyeing of tanninmordanted cotton, of silk, of leather, and of lacquers, are now only of minor importance. Diaminophenoxazine dyes have found application in laser technology. They complement the emission spectrum of rhodamine on the long wavelength side. Phenoxazine dyes function as desensitizers.

Leuco phenoxazine dyes are used in acylated form as chromogenic substances in pressure-sensitive copying paper. In a photochemical oxidation step, blue dyes are formed, an acyl group being cleavage. The benzoyl derivatives are preferably used. Aminophenoxazine dyes are also suitable in the form of their leuco compounds as highly sensitive oxygen detectors. The dyes are also used for the analytical determination of ascorbic acid [1-4].

Nile blue is produced by reaction of 4-nitroso-3-hydroxydiethylaniline with  $\alpha$ -naphthylamine and derivative of the oxazine dyes class, which used in microscopy and in laser technology. It is also suitable for the

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prevention of deposits during the polymerization of vinylchloride homo-, co-, or graft polymers<sup>[5,6]</sup>.

It is well known that solutions of dyes in polar organic solvents at room temperature follow Beer's law over an extended concentration range, but in water we observed a large deviation from the law. For many classes of dye in aqueous solutions, the band of highest intensity in dilute solution becomes weaker as the concentration is increased, and new bands appear at shorter wavelengths. These spectral changes have long been attributed to aggregation of the dye molecules in water to form dimers and higher order polymers under the influence of the strong dispersion forces associated with the high polarizability of the chromophoric chain. The dominant role of water as the solvent most favorable to aggregation at room temperature is no doubt associated with the effect of its high dielectric constant in reducing the repulsive force between the similarly charged dye cations or anions in the aggregate; the absence of aggregation in organic solvents of high dielectric constant at room temperature suggests that solvation interferes with the aggregation, and in such solvents aggregates are stable only at low temperatures under conditions of high viscosity<sup>[7]</sup>.

Aggregation is one of the features of dyes in solution<sup>[8-16]</sup>, affecting their colouristic and photophysical properties and therefore being of special interest. It is well known that the ionic dyes tend to aggregate in diluted solutions, leading to dimer formation, and sometimes even higher order aggregates. In such a case the molecular nature of dye is strongly affected by, and therefore related to such parameters as dye concentration, structure, ionic strengths, temperature and presence of organic solvents<sup>[16]</sup>. Although dyes are very individualistic as structure and, of course behavior, certain broad rules are well established regarding the aggregation in general. It may increase with an increase of dye concentration or ionic strengths; it will decrease with temperature rising or organic solvents adding; addition to the dye structure of ionic solubilizing groups will decrease aggregation, whereas the inclusion of long alkyl chains increase aggregation because of higher hydrophobic interaction in solution.

The UV-Vis absorption spectroscopy is one of the most suitable methods for quantitative studying the aggregation properties of dyes as function of concentra-

tion. In the commonly used concentration range ( $10^{-3}$  to  $10^{-6}$  M) the main equilibrium is monomer-dimer reaction.

In this work, we used some physical constraints to determine the dimeric constants of Nile blue dye (SCHEME 1) in pure water and at different concentration. Data analysis was carried out by DATAN package that developed by Kubista groups<sup>[17-26]</sup>.

### Theory

Spectra recorded at different temperature are arranged as rows in an  $n \times m$  matrix  $A$ , where  $n$  is the number of temperature intervals and  $m$  is the number of data points in each spectrum.  $A$  is decomposed into an orthogonal basis set using, for example, NIPALS<sup>[13,14]</sup>:

$$A = TP^T + E \approx TP^T = \sum_{i=1}^r t_i p_i^T \quad (1)$$

where  $t_i (n \times 1)$  is orthogonal target vectors and  $p_i (1 \times m)$  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  $E$  is the error matrix containing experimental noise, if the right value of  $r$  is selected. For a well-designed experiment,  $E$  is small compared to  $TP^T$  and can be discarded.

Assuming linear response the recorded spectra are also linear combinations of the spectral responses,  $V_i (1 \times m)$ , of the components:

$$A = CV + E \approx CV = \sum_{i=1}^r c_i v_i \quad (2)$$

where  $c_i (n \times 1)$  are vectors containing the component concentrations at the different temperatures. The two equations are related by a rotation<sup>[15]</sup>:

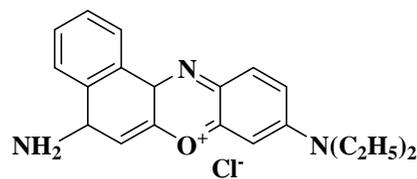
$$C = TR^{-1} \quad (3)$$

$$V = RP^T \quad (4)$$

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

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

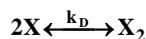
Since a single sample is studied, the total concen-



SCHEME 1

tration must be constant, constraining matrix  $R^{[27]}$ . For monomer-dimer equilibrium, the total concentration of monomers is constant:

$$c_x(T) + 2c_x(T) = c_{tot} \quad \text{or} \quad c_x + 2c_{x2} = c_{tot} \quad (6)$$



Combining Eq. (6) with Eq. (3), we obtain:

$$\frac{1}{r_{11}r_{22} - r_{12}r_{21}}(t_1r_{22} - t_2r_{21} - 2t_1r_{12} + 2t_2r_{12}) = c_{tot} \quad (7)$$

which can be written as:

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

where

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

and

$$f_{12} = (2r_{11} - r_{21})(r_{11}r_{22} - r_{12}r_{21})^{-1} \quad (9)$$

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 matrix  $R$ , hence making two of them redundant. In most cases, the spectra of some of the components can be determined in separate measurements. For example, monomer-dimer equilibrium can, in general, be diluted sufficiently to make the dimer concentration negligible. This makes it possible to record the monomer spectrum, 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. We obtain from Eq. (4):

$$\mathbf{v}_{monomer} = r_{11}\mathbf{p}_1^T + r_{12}\mathbf{p}_2^T = f_{21}\mathbf{p}_1^T + f_{22}\mathbf{p}_2^T \quad (10)$$

where  $f_{21} = r_{11}$  and  $f_{22} = r_{12}$  are determined by fitting the two projection vectors to the monomer spectrum. Eq. (9) also provides two relations between the elements of matrix  $R$ .

These are not independent of Eq. (7), and the two equations cannot be combined to solve for all the elements of matrix  $R$ , but they can be used to express  $R$  in a single element, below arbitrarily chosen to be  $r_{21}$ .

Defined this way, matrix  $R$  produces  $C$  and  $V$  matrices that are consistent with the total sample concentration and the spectral response of the monomer. The value of  $r_{21}$

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

determines the dimer spectrum and the monomer con-

centration 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 in 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 components' concentrations are related by the law of mass action<sup>[28]</sup>:

$$K_D(T) = \frac{c_{x2}(T)/c^0}{(c_x(T)/c^0)^2} \quad (12)$$

where  $c^0 = 1 \text{ mol dm}^{-3}$ . Assuming that the dimerization constant  $K_D(T)$  depends on temperature according to the van't Hoff equation<sup>[28]</sup>,

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

where  $\Delta H$  is the molar enthalpy change,  $R = 8.31 \text{ J mol}^{-1} \text{ K}^{-1}$  the universal gas constant, and  $T$  the Kelvin temperature.  $r_{21}$  can now be determined by requiring that matrix  $R$  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. (9)). This is used to calculate trial concentration profiles (Eq. (3)), which are combined to a trial equilibrium constant (Eq. (12)). A linear regression of equilibrium constants with respect to  $1/T$  is then performed (Eq. (13)), which determines a trial enthalpy change of the reaction. Each trial rotation matrix also determines 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  $\chi^2$  (a 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 matrix  $R$ . The analysis is readily performed with the DATAN program<sup>[29]</sup>. Several studies based on the application of this algorithm and program using spectrophotometric data have been reported<sup>[11,12,17-27]</sup>.

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## EXPERIMENTAL

### Materials

All the chemicals used were of analytical reagent grade. Subboiling, distilled water was used throughout. Nile blue (for microscopy grade) was purchased from Fluka and was used without additional purification. A stock solution ( $5 \times 10^{-3}$  M) was prepared by dissolving solid Nile blue in water.

### Apparatus

Absorption spectra were measured on UV S-2100 (Scinco) Spectrophotometer using 0.1 nm bandwidth and temperature controller and were digitized with ten data points per nanometer. Quartz cell (10 mm $\times$ 2 mm) were used throughout. The cuvettes were treated with repelsilane prior to measurements to avoid dye adsorption.

### Computer hardware and software

All absorbance spectra were digitized at ten data points per nanometer in the wavelength range 500-750 nm and transferred (in ASCII format) to an Athlon 2000 computer for analysis by MATLAB (Mathworks, Version 6.1) or for processing by using DATAN package<sup>[29]</sup>. The dimerization constants for MG were evaluated from DATAN program of the absorbance-temperature data to the desired equations<sup>[30,31]</sup>.

## RESULTS AND DISCUSSION

The absorption spectra of Nile blue, at different total dye concentrations, were recorded in the wavelength 500-750 nm and temperature 20-80°C at 5°C intervals. The sample absorption spectra are shown in figure 1. As it is expected, by increasing the temperature and decreasing the concentration, the monomer form would be predominant over the dimer form. So it is wise to choose the spectrum of the dye at the highest temperature and at lowest concentration as an initial estimate for the monomer in the subsequent calculations. Then according to Eqs. (1) - (13), the DATAN program start with a trial value of  $r_{21}$ , at predefined interval, and iterate all the calculation steps. The iteration stops when all  $r_{21}$  values in the preset interval are tested. The  $K_D$ , dimer spectrum,  $\Delta S$  and  $\Delta H$ , correspond to

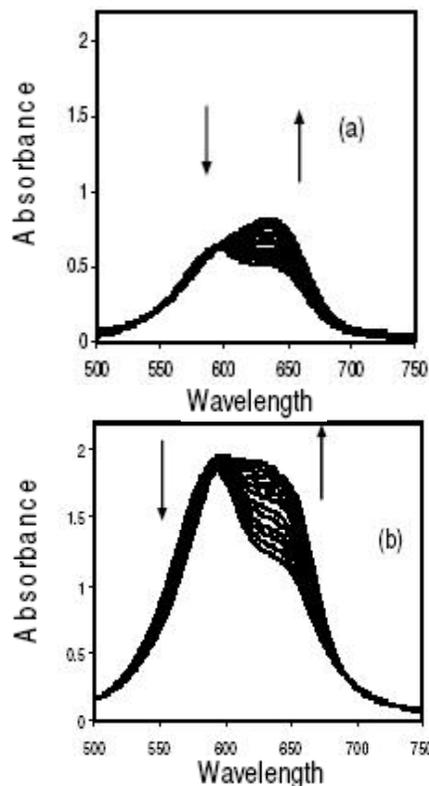


Figure 1: Absorption spectra of NB dye (a:  $8.85 \times 10^{-5}$  M, b:  $3 \times 10^{-4}$  M) in water recorded at 5°C intervals between 20 and 80°C

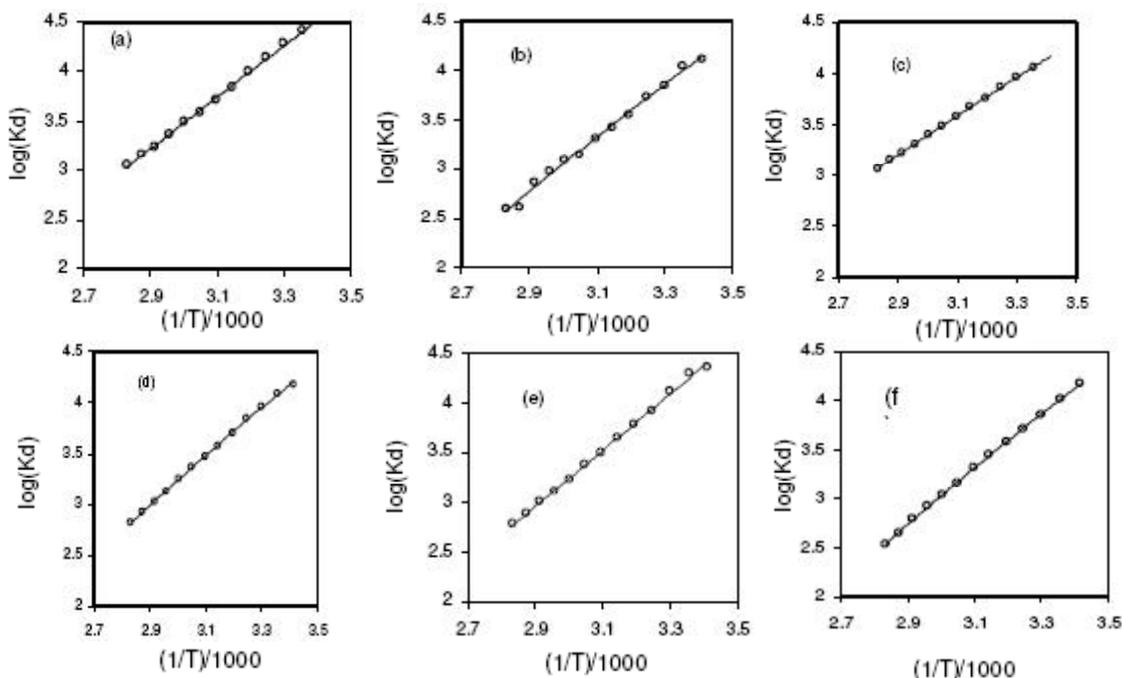
TABLE 1: Dimeric constant ( $K_D$ ) values of NB dye in various concentrations

Concentrations (M)/ Temperature(°C)	$8.85 \times 10^{-5}$	$1.2 \times 10^{-4}$	$1.62 \times 10^{-4}$	$2 \times 10^{-4}$	$2.6 \times 10^{-4}$	$3 \times 10^{-4}$
20	4.51	4.12	4.15	4.23	4.36	4.18
25	4.42	4.05	4.06	4.09	4.30	4.02
30	4.29	3.85	3.96	3.96	4.12	3.86
35	4.14	3.74	3.86	3.85	3.92	3.71
40	4.01	3.56	3.76	3.70	3.80	3.59
45	3.85	3.42	3.68	3.58	3.66	3.45
50	3.72	3.31	3.59	3.48	3.50	3.32
55	3.59	3.15	3.49	3.37	3.38	3.16
60	3.50	3.09	3.41	3.26	3.24	3.04
65	3.36	2.98	3.34	3.13	3.12	2.93
70	3.25	2.86	3.23	3.02	3.00	2.80
75	3.16	2.61	3.15	2.92	2.88	2.65
80	3.04	2.59	3.07	2.82	2.78	2.53

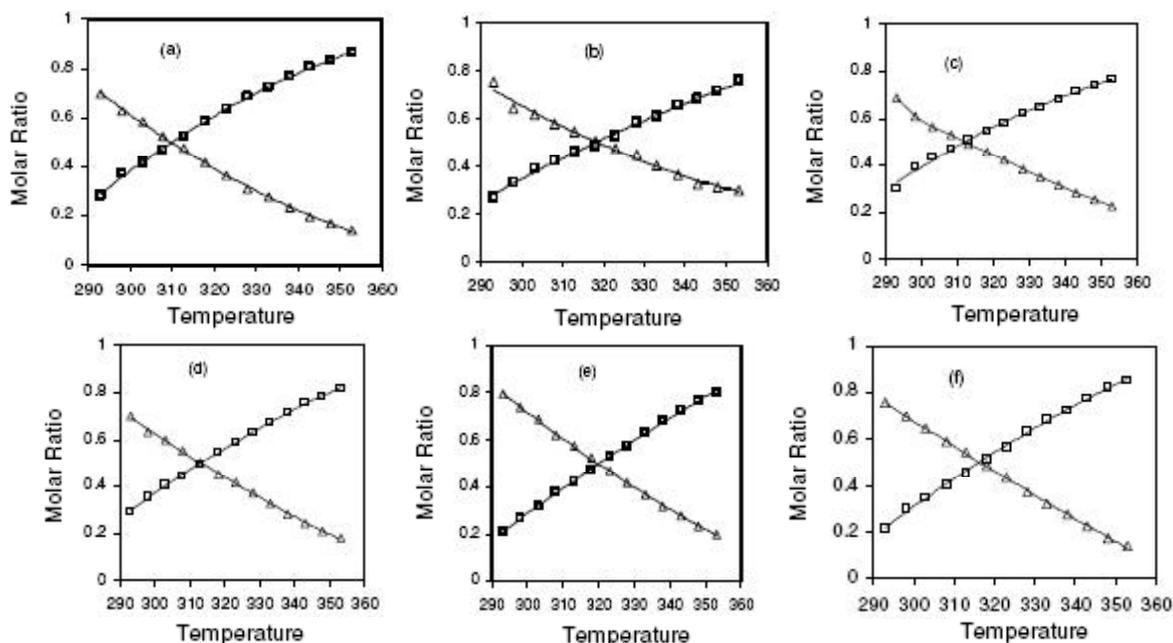
TABLE 2: Thermodynamic parameter for NB dye in various concentrations

Concentration(mol l <sup>-1</sup> )	$-\Delta H^0$ (KJ mol <sup>-1</sup> )	$-\Delta S^0$ (J mol <sup>-1</sup> K <sup>-1</sup> )
$8.85 \times 10^{-5}$	51.20	87.04
$1.2 \times 10^{-4}$	41.25	63.45
$1.62 \times 10^{-4}$	43.87	36.30
$2 \times 10^{-4}$	46.32	76.98
$2.6 \times 10^{-4}$	55.20	103.50
$3 \times 10^{-4}$	58.25	116.50

minimum value of the  $\chi^2$  statistics, are selected as the



**Figure 2:** The van't Hoff equation plot at different concentrations of NB dye: (a)  $8.85 \times 10^{-5}$  M; (b)  $1.2 \times 10^{-4}$  M; (c)  $1.62 \times 10^{-4}$  M; (d)  $2 \times 10^{-4}$  M; (e)  $2.6 \times 10^{-4}$  M; (f)  $3 \times 10^{-4}$  M



**Figure 3:** Molar ratio of NB dye monomer ( $\square$ ) and dimer ( $\Delta$ ), compared to molar ratios predicted by the temperature dependence of the equilibrium constant (shown as line) at different concentrations of Nile blue dye: (a)  $8.85 \times 10^{-5}$  M; (b)  $1.2 \times 10^{-4}$  M; (c)  $1.62 \times 10^{-4}$  M; (d)  $2 \times 10^{-4}$  M; (e)  $2.6 \times 10^{-4}$  M; (f)  $3 \times 10^{-4}$  M

final results. The  $\chi^2$  is the sum of squared residuals<sup>[18]</sup> and generally used as a goodness of fit criterion and its value indicate the predictability of the model, i.e. how well the monomer spectrum and  $r_{21}$  are determined.

With increasing temperature the absorption peak 635 nm grows and the absorption shoulder around 570

nm decreased (Figure 1). We analyzed the temperature titrations assuming monomer-dimer, monomer-dimer-trimer and even some models including higher order aggregates, and it was found that a monomer-dimer model most adequately describes the data in these ranges of dyes concentrations. The presence of exactly

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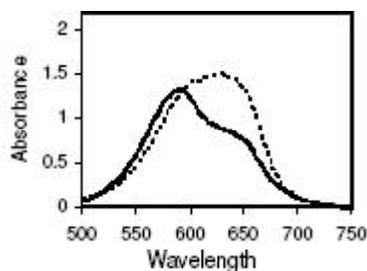


Figure 4: Calculated absorption spectra of the NB dye dimer (—) and monomer (---) in concentration of  $1.62 \times 10^{-4}$  M

two species is also evidenced by appearing of the isobestic points at 597 nm.

The dimerization constant ( $K_D$ ) was calculated at different temperatures for the six total dye concentrations (TABLE 1). As expected  $K_D$  decreased with increasing temperature, while it is virtually independent of total dye concentration. From the dependence of  $\log K_D$  on  $1/T$  (Figure 2),  $\Delta H^0$  and  $\Delta S^0$  values were determined (TABLE 2). The  $\Delta H^0$  values ranges from  $-41.25$  to  $-55.20$   $\text{kJ mol}^{-1}$  with mean  $-48.58$   $\text{kJ mol}^{-1}$ , while  $\Delta S^0$  ranges from  $-36.30$  to  $-103.50$   $\text{J mol}^{-1} \text{K}^{-1}$  with mean  $-78.38$   $\text{J mol}^{-1} \text{K}^{-1}$  for Nile blue. As described above dimerization is presumed to be the dominant form of aggregation in applied concentration ranges in aqueous Nile blue solutions. This is corroborated by the constancy of the apparent enthalpy of association. In general, the extent of aggregation depends reciprocally on the temperature of the solution and is fully reversible. The observed relationship between entropy and enthalpy reflects an electrostatic nature of the dimerization phenomenon of Nile blue dye. The relative dependence of the concentrations of the monomer and dimer of Nile blue on the temperature in different concentrations are shown diagrammatically in figure 3. These plots reveal that the concentrations of monomer and dimer are similar in a narrow range around  $50^\circ\text{C}$ . This supports the assumption of dominating monomer-dimer equilibrium.

Calculated absorption spectra of NB monomer and dimer are shown in figure 4. The spectrum of the monomer has maximum intensity at 630 nm. It is very similar to the spectrum measured in dilute solution at high temperature that was used as initial estimate. The dimer spectrum has maximum at 570 nm.

## CONCLUSION

In this paper we report a method for characterization of the monomer-dimer equilibrium of the NB dye. Dimerization constants, concentration profiles for the monomer and dimer, and spectral responses of monomer and dimer obtained by computer refinement of temperature photometric titrations. The thermodynamic parameters, enthalpy and entropy of dimerization reaction were calculated from the dependence of dimeric constant on the temperature (van'tHoff equation). To obtain more reliable estimates of the thermodynamics parameters we performed all experiments at four different total concentrations and different ionic strengths of the dye. The presented values are means of the four independent determinations.

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