

Scientific paper

A New Algorithm for the Characterization of Thermodynamics of Monomer-Dimer Process of Dye Stuffs by Photometric Temperature Titration

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Abstract

A method of data analysis for monomer-dimer equilibria employing multiwavelength detection is shown to recover both the thermodynamic constants and absorption spectra of monomer-dimer pairs in complex mixtures. Using UV-Vis spectroscopy the dimerization constants of crystal violet (CV), resazurine (R) and methylene blue (MB), that they are severely overlapped, have been determined by studying the dependence of absorption spectra on temperature in range 0–90 °C and an iterative least-squares method. The proposed method has previously proposed by Frans, S. D.; Harris, J. M.; *Anal. Chem.* 1984, 56, 466–470 for the resolving of mixtures of four acid molecules by pH titration. In contrast to the Kubista's method *Anal. Chem.* 1993, 65, 994–998 which rely on the prior knowledge of the monomer spectrum our method is free of advance any known values. The resolving power of the method is tested by studying of the mixtures of dye molecules. The multiple wavelength titration curves are used to form matrix, D, which is then factored into matrices A, containing the absorption spectra of the components, and C, containing their temperature-dependent concentrations. The correct $K_D(T)$'s and absorption spectra are derived by a least-squares minimization with $K_D(T)$'s as adjustable parameter.

Keywords: Dimerization; iterative least-squares; UV-Vis spectroscopy; crystal violet; resazurine; methylene blue.

1. Introduction

Aggregation is one of the features of dyes in solution,^{1,2} 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 and structure, ionic strength, temperature and presence of organic solvents.³ Although dyes are very individualistic as structure and, of course behaviour, certain broad rules are well established regarding the aggregation in general. It may increase with an increase of dye concentration or ionic strength; it will decrease with temperature rising or organic solvents adding; addition to the dye structure of ionic solubilizing groups (as sulphonate group) will decrease aggregation, whereas the inclusion of long alkyl chains increases aggregation because of higher hydrophobic interaction in solution. The absorption UV-Vis spectroscopy is one of

the most suitable methods for quantitative studying the aggregation properties of dyes as function of concentration, since in the concentration range used (10^{-3} – 10^{-6} M) mainly monomer-dimer equilibrium exists.⁴

There are numerous UV-Vis spectroscopy studies on the aggregation, but surprisingly their results are frequently inconsistent and sometimes contradictory,³ particularly for studies carried out on the same dye. For example the value of dimeric constant of methylene blue varies from 1500 to 55600 or in orders. This unsatisfactory situation arises from two main reasons, ill defined experimental conditions (the purified or commercial dye is used) and numerous assumptions made in the processing of the spectral data.^{3,4}

The phenomenon of aggregation of dyes, drugs, surfactants, etc., has been extensively studied by spectrophotometry, light scattering, osmometry, polarography, etc. The results of these studies have indicated that the dye molecules, in spite of repulsion due to similar charges (cationic dyes), undergo self-aggregation to form dimer, trimer, and higher aggregates, the charges being main-

tained as far as possible in the aggregate. The forces which hold the dye molecules together are believed to be hydrogen bonding, van der Waal's forces, London dispersion forces and other short-range forces. The most predominant force has been recognized to be hydrophobic interactions, which help the system to get rid of the thermodynamically unfavourable state of negative ΔS due to hydrophobic hydration. Thus the water structural features seem to play the role of catalyst in inducing aggregation.

Spectroscopic methods are in general highly sensitive and are 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 constant is trivial. However, in many cases, the spectral responses of two and sometimes even more components overlap considerably and the analysis is no longer straightforward.⁵ The analysis of complex multicomponent samples has been greatly facilitated by the use of "hyphenated" analytical methods,^{6–8} which can facilitate the identification and resolution of individual components of a mixture.^{9–13} The informing power of a two-dimensional measurement lies in the large number of information channels which increase by the generating of number of channels in each dimension, provided that the measurement dimensions uncorrelated. When one measurement dimension, which has few degrees of freedom and can be modeled, is combined with a second measurement dimension of higher informing power (more independent information channels), one can utilize the benefits of each dimension for the resolution of mixtures.^{14,15} By fitting the data along the lower informing power dimension using a physical model to provide a functional form, one can extract the richer analytical information from the other dimension. Because of the independent nature of two dimensions, considerably more overlap can be tolerated than with one-dimensional curve fitting.¹⁶ Example applications of this iterative least-squares method have included time-resolved fluorescence spectrometry¹⁴ and GC/MS.¹⁵

The iterative least-squares technique could naturally be applied to data involving chemical equilibria vs. some higher informing power dimension. For the purpose of this study, a temperature-dependent, monomer/dimer equilibrium is chosen where the second dimension is based on UV/Vis spectrophotometry. Numerous other techniques have been used to determine the dimerization constants of monomer-dimer systems.^{17–21} Generally in these studies, either more information was known in advance, the $K_D(T)$'s, or isolated absorption spectra, or a large number of parameters had to be individually optimized. The iterative least-squares approach has the unique advantage of requiring no prior information about the data to be fit. Furthermore, for a mixture of n monomer-dimer pairs, only n parameters, the $K_D(T)$'s (for each temperature), need to be optimized in order to resolve the ab-

sorption spectra of the components. The iterative least-squares method is evaluated in this work by using single and also multicomponent mixtures of dyestuff.

2. Theory of Proposed Algorithm

The temperature dependence of the absorption spectra of a mixture of monomer-dimer pairs can be expressed as a matrix, **D**. Each element of the matrix is shown as d_{ij} , where the wavelength of the measurement is the index, i , for the rows and the temperature is the index, j , for the columns. If the spectroscopic conditions are met for the Beer-Lambert relationship to hold (narrow slit width, low stray light), then the absorbance at any wavelength and temperature, D_{ij} , will be the sum of the contributions from the n components of the sample:

$$d_{ij} = \sum_{k=1}^n a_{ik} c_{kj} \quad (1)$$

where a_{ik} , in absorbance units, is the product of path length, molar absorptivity, and formal or total concentration of the k th component at the i th wavelength and a_{kj} , dimensionless, is the relative fraction of a particular conjugate form of the k th component at the j th temperature. The relationship of Eq. 1 is conveniently expressed as a matrix product:

$$\mathbf{D} = \mathbf{A}\mathbf{C} \quad (2)$$

where **A** contains the absorption spectra of the n components in its columns and **C** contains the temperature-dependent distribution curves for these components in its rows.

Given a measurement of **D**, the data analysis task is to decompose the matrix into its factors **A** and **C** which can be used for subsequent identification and quantitation of the sample components. In order to carry out this task, the rows of **C** must be linearity independent so that a unique, best fit solution exists.

For a monomer-dimer equilibrium, the total concentration of monomers is constant:

$$C_M + 2C_D = C_T \quad (3)$$



The 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:⁸

$$k_D(T) = \frac{C_D(T)/C^0}{(C_M(T)/C^0)^2} \quad (5)$$

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:

$$\ln K_D = \left(\frac{-\Delta H^0}{R} \right) \cdot \frac{1}{T} + \left(\frac{\Delta S^0}{R} \right) \quad (6)$$

where ΔH^0 is the molar enthalpy change, ΔS^0 is the molar entropy change, $R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ the universal gas constant, and T is temperature in Kelvin.

One of the peculiarities of the temperature-dependent data is an interdependence of the distribution curve of a monomer with that of its dimer, where the concentration of monomer form at a given temperature is given by:

$$C_M = \frac{-1 + \sqrt{1 + 8K_D(T)C_t}}{4K_D(T)} \quad (7)$$

And the concentration of dimer form is given by:

$$C_D = \frac{4K_D(T)C_t + 1 - \sqrt{1 + 8K_D(T)C_t}}{8K_D(T)} \quad (8)$$

To develop a set of linearly independent vectors for the \mathbf{C} matrix from a mixture of monomer-dimer pairs, one must choose a smaller "basis set" of functions which span the same measurement "space". Practically, this may be accomplished by taking as the k th row in the difference distribution curve:

$$C_{kj} = C_M - C_D = \frac{-3 - 4K_D(T)C_t + 3\sqrt{1 + 8K_D(T)C_t}}{8K_D(T)} \quad (9)$$

Combining Eq. 6 with Eq. 9, we obtain:

$$C_{kj} = \frac{-3 - 4C_t \exp((- \Delta H^0 / RT) + (\Delta S^0 / R)) + 3\sqrt{1 + 8C_t \exp((- \Delta H^0 / RT) + (\Delta S^0 / R))}}{8 \exp((- \Delta H^0 / RT) + (\Delta S^0 / R))} \quad (10)$$

The redundant vectors arising from the sum of the distribution curves, $C_M + 2C_D = C_t$, can be combined into a single constant row in the \mathbf{C} matrix. For a mixture of n monomer-dimer pairs, the elements of this constant row which preserve the area of the original distribution relationship are $C_{n+ij} = n$.

The first n columns of \mathbf{A} corresponding to the first n rows of \mathbf{C} are difference spectra given as the product of the molar absorptivity difference between monomer and dimer forms of component k , the path length, and the total concentration:

$$a_{ik} = (\varepsilon_M - \varepsilon_D)_{ik} b(C_M + 2C_D) \quad (11)$$

The last, $n + 1$, column of \mathbf{A} , corresponding to the constant row of \mathbf{C} , is the sum of all absorbing species in the solution including the contributions of any non-tem-

perature varying components.

Given a linearly independent model for the distribution matrix, \mathbf{C} , all that is required to specify the elements in this matrix are the $n \Delta H^0$'s and ΔS^0 's of the dimerization of n monomer species in the system. The minimum number of temperature points required to solve for the corresponding spectral matrix, $\hat{\mathbf{A}}$, would be $n + 1$, in which case \mathbf{C} is square and can be inverted to obtain:

$$\hat{\mathbf{A}} = \mathbf{DC}^{-1} \quad (12)$$

To reduce the impact of noise in the data matrix on the spectral data, one can overdetermine the solution by measuring many more temperature points than components. The corresponding best spectral matrix $\hat{\mathbf{A}}$ is found by multiplying the data matrix by the pseudoinverse or least-squares inverse^{22,23} of \mathbf{C} :

$$\hat{\mathbf{A}} = \mathbf{DC}^T(\mathbf{CC}^T)^{-1} \quad (13)$$

which minimizes the squared error between the actual and calculated data matrices as shown in Eq. 14.

$$\mathbf{R} = \sum_i \sum_j (d_{ij} - \hat{d}_{ij})^2 \quad (14)$$

Where \mathbf{R} is residual matrix and post multiplying of $\hat{\mathbf{A}}$ by \mathbf{C} give

$$\hat{\mathbf{D}} = \hat{\mathbf{A}}\mathbf{C} \quad (15)$$

Overdetermining the \mathbf{C} matrix provides another important advantage in that ΔH^0 's and ΔS^0 's of the dimerization processes in the under study system need not be known in advance. The value of residual matrix obtained by the above procedure will depend on the correct choice of ΔH^0 's and ΔS^0 's used to construct \mathbf{C} . The closer choice of ΔH^0 's and ΔS^0 's to the actual values for the components in the system, the smaller will the residual error in the fit indicated by \mathbf{R} . As a result, the optimum set of ΔH^0 's and ΔS^0 's to given data matrix can be found by minimizing \mathbf{R} with Levenberg-Marquardt algorithm.¹¹ The Marquardt or Levenberg-Marquardt algorithm contains elements of both the steepest descent and Gauss-Newton methods,^{24,25} but converges more rapidly than either of these. The Marquardt algorithm behaves like a steepest descent

method under conditions for which the latter is efficient, that is, far from minimum. Close to the minimum, it behaves like the Gauss-Newton method, again under conditions where the latter is efficient. The Marquardt program uses numerical differentiation so that analytical derivatives need not be provided by the user, as required in some Gauss-Newton programs.²⁶

This iterative approach can be further expanded to determine the number of components in the system. By examination of the minimum value of **R** and IND function,^{27,28} as a function of the number of components in the fit, an indication of the proper value of *n* can be realized. The IND function, developed by Malinowski, for identifying the correct dimensionality of a factor space includes a penalty for increasing the number of components in the model which is stronger than just the number of degrees of freedom, $r(c-n)$.

$$IND = \left[\frac{1}{r(c-n)^5} \sum_i \sum_j (d_{ij} - \hat{d}_{ij})^2 \right]^{1/2} \quad (16)$$

Where *r* is the number of rows or wavelengths in the data matrix and *c* is the number of columns or temperature measurements. Empirically, this results in a minimum value of the IND function when *n* has been defined correctly.

3. Experimental

3.1. Apparatus

Absorption spectra were measured on an Agilent 8453 UV-Vis Diode-Array spectrophotometer using the Agilent UV-Vis ChemStation software for data acquisition. A quartz cuvette of 1 mm optical path was used for all measurements. The temperature of the cell housing kept constant at each temperature by a Hiedolph thermo circulating bath.

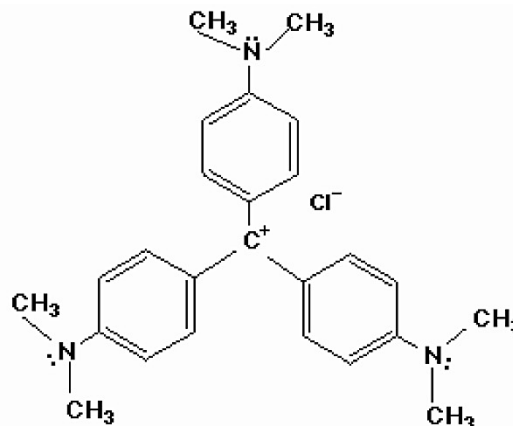
3.2. Computer Hardware and Software

All absorbance spectra were digitized at five points per nanometer in the wavelength range 350–750 nm. All calculations were run on a Dell computer with Pentium IV as central processing unit with windows XP as operating system. Other calculations were performed in the MATLAB (version 7.1, MathWorks, Inc.) environment.

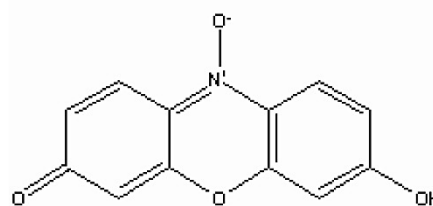
3.3. Material

The analytical reagent grade crystal violet, resazurine and methylene blue (*Scheme 1*) were all from Merck Company. Subboiling, distilled water was used throughout. Aqueous stock solutions of dyes were prepared as follows. For crystal violet, 0.0133 g, was dissolved in distilled water and diluted to the mark in a 20 ml volumetric flask. 0.0100 g, of resazurine, was dissolved in

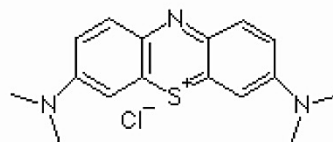
10 ml distilled water and then diluted to 40 ml. For methylene blue, 0.0101 g, was dissolved in 10 ml distilled water then diluted to a 30 ml of final volume. The final concentrations of crystal violet, resazurine and methylene blue in the mixture, were respectively: 2.71×10^{-4} , 1.66×10^{-4} and 1.57×10^{-4} mol/L.



Crystal violet



Resazurine



Methylene blue

Scheme 1

4. Results and Discussion

To assess the capabilities of the iterative least-squares method on experimental data a solution of mixture of dyes was prepared. The absorption spectra were recorded as a function of wavelength, at 5 nm intervals over a 350–750 nm range, and temperature over a temperature range of 0–90 °C at 2.5 °C intervals.

We analyzed the temperature titrations assuming monomer-dimer, monomer-dimer-trimer (there is a trimer form for crystal violet²⁰) and even models including high-

er order aggregates and found that the monomer-dimer model most adequately describes the data. The difference spectra, ΔH^0 's and ΔS^0 's determined from iterative least-squares fitting of the mixture of the dyestuffs. The results were compared with the results acquired for separate experiments which were carried out for each dye.

An example data matrix for a three-component mixture containing crystal violet, resazurine and methylene blue is shown in Fig. 1.

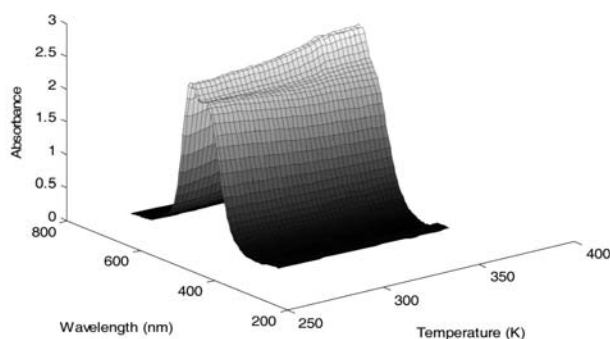


Figure 1. Three dimensional spectral data of a mixture of three dye stuffs. Detailed is given in the text.

It is clear from the figure that the data are severely overlapped. Application of the iterative least-squares procedure while increasing the value of n was successful in identifying the correct number of components, using the IND function, (Fig. 2).

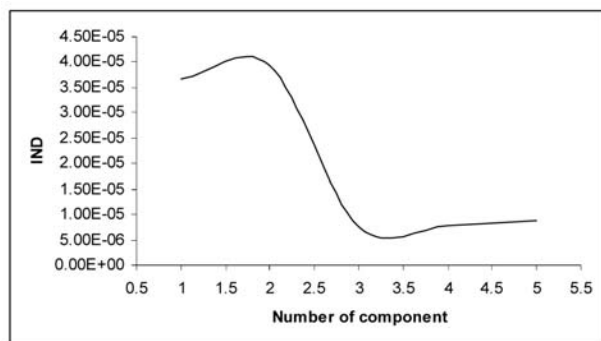


Figure 2. Determination of the number of monomer/dimer components.

Unfortunately a less than persuasive minimum typifies the behavior of this empirical function.^{27–29} A more fundamental basis for deciding the number of components based statistically on the lack of fit³⁰ requires a priori knowledge of the purely experimental error. If this error is high frequency in nature, then an autocorrelation calculation can be used to estimate its magnitude.³¹ This approach, which also utilizes an eigenvector decomposition

of the data, was tested on these results and also found to be successful in identifying the number of components.

If the correct number of components is used to construct the **C** matrix and the ΔH^0 's and ΔS^0 's adjusted to minimize residual, **R**, the difference spectra of the individual components should occupy the column of the $\hat{\mathbf{A}}$ matrix, factored from the data matrix **D** by the pseudoinverse of **C**. These column vectors are plotted in Fig. 3 for the three-component mixture along with the spectra of the individual components, scaled to equal areas to account for differences in concentration.

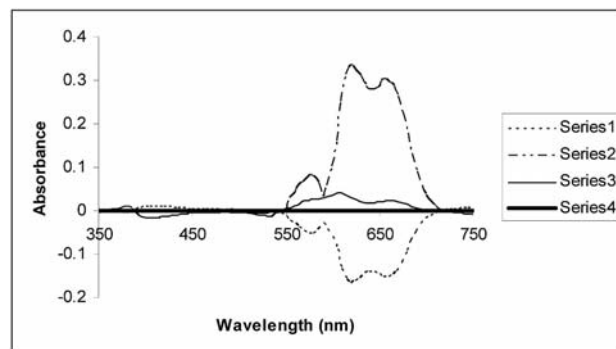


Figure 3. Difference spectra of monomer/dimer pairs. Series 1 is crystal violet; series 2 is resazurine; series 3 is methylene blue and series 4 is the plot of any non-temperature varying components.

There is little or no systematic error in the numerically resolved spectra, despite the large spectral overlapping. This result is further substantiated by the residuals plot, (Fig. 4), where no temperature or wavelength-dependent bias can be detected. There is some hint of a proportional error source since the residuals, while random, have larger amplitude in the vicinity of the maximum absorbance of the data matrix. We recorded the absorption spectra of pure potassium dichromate in different concentrations for calculating the measurement error of spectrophotometer. After applying the PCA with one component the residual plot was constructed and is shown in Fig. 5. A more quantitative summary of the quality of fit is listed in Tab. 1. The error in the resolved spectra, Tab. 1, is expressed as a standard deviation.³²

Table 1. Thermodynamic parameters for three dyes.

Dye	ΔH^0 Isolated (kJ mol ⁻¹)	ΔS^0 Isolated (J mol ⁻¹ K ⁻¹)	ΔH^0 Mixture (J mol ⁻¹)	ΔS^0 Mixture (J mol ⁻¹ K ⁻¹)	spectral error ^a std dev.
crystal violet	-46.0	-80	-48.3	-83	0.0883
resazurine	-35	-40	-37.1	-56	0.0997
methylene blue	-59.1	-112	-62.1	-116	0.2476

^a Standard deviation of difference between numerically resolved and isolated component spectra, scaled to equal areas.

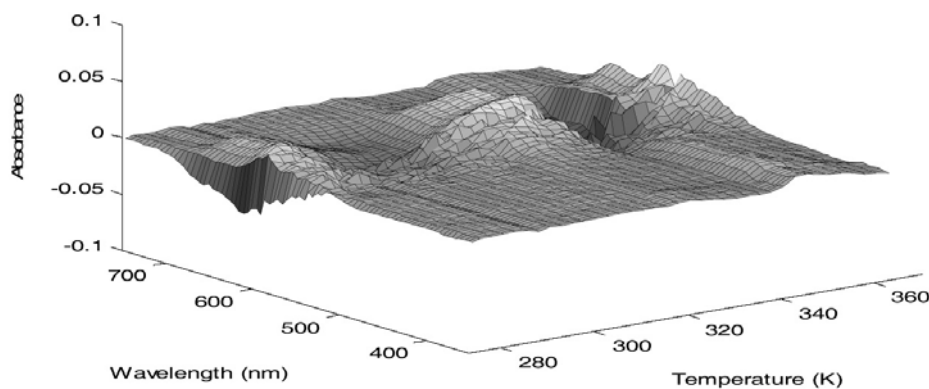


Figure 4. Residuals plot for best fit of the three-component mixture, $D-\hat{D}$ (see Eq. 15)

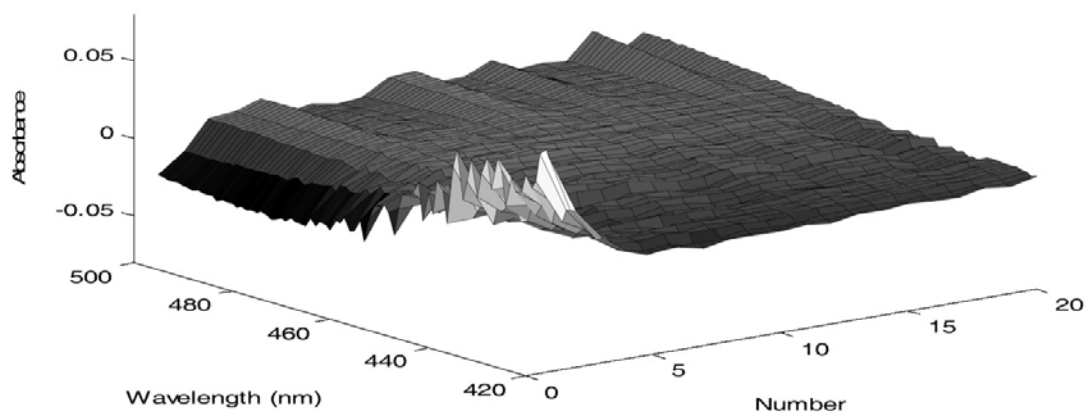


Figure 5. Residuals plot for pure potassium dichromate (noise of the instrument).

The dimerization constant according to the Eq. 6, was calculated at different temperatures for all dyes and are listed in Tab. 2. As it is expected, the dimerization constant decreases with increasing temperature. By increasing the temperature, the monomer form would be predominant over the dimer form. The row vectors of matrix C , the difference of concentration profiles of the monomer and dimer for the three-component mixture, are plotted in Fig. 6. It is not surprising to have three straight lines for three dyestuffs because it is difference of monomer/dimer

form and in all temperatures, their differences are constant. In all reported values the dimerization and self association are enthalpy favored and entropy was diminished with respect to the dimerization. Our data show more or less a similar trend. This relationship between enthalpy and entropy reflects the electrostatic nature of the dimerization phenomenon.

Table 2. Dimeric constant ($\times 10^4$) values of three dyes

Temperature (K)	crystal violet	resazurine	methylene blue
273	8.15	1.50	67.34
275.5	6.72	1.30	52.53
278	5.56	1.12	41.16
280.5	4.61	0.97	32.39
283	3.84	0.84	25.60
285.5	3.21	0.73	20.31
288	2.69	0.64	16.19
290.5	2.26	0.56	12.95
293	1.91	0.49	10.40
295.5	1.61	0.43	8.38
298	1.37	0.38	6.78
300.5	1.16	0.34	5.50
303	0.99	0.3	4.48
305.5	0.85	0.26	3.66
308	0.73	0.23	3.00
310.5	0.63	0.21	2.47
313	0.54	0.19	2.04

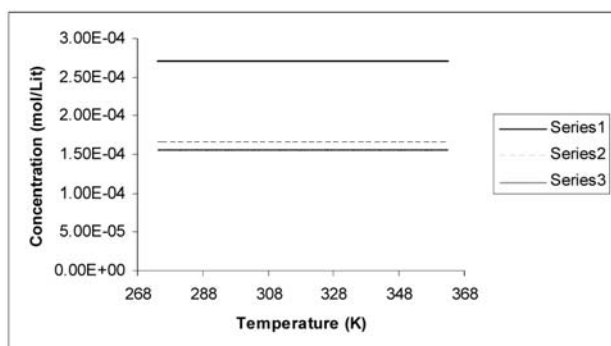


Figure 6. Difference of concentration profiles of the monomer/dimer pairs: crystal violet (Series 1), resazurine (Series 2) and methylene blue (Series 3).

Temperature (K)	crystal violet	resazurine	methylene blue
315.5	0.46	0.17	1.69
318	0.40	0.15	1.40
320.5	0.35	0.13	1.17
323	0.30	0.12	0.97
325.5	0.26	0.11	0.81
328	0.23	0.10	0.68
330.5	0.20	0.09	0.58
333	0.18	0.08	0.49
335.5	0.15	0.07	0.42
338	0.14	0.06	0.35
340.5	0.12	0.06	0.3
343	0.11	0.05	0.25
345.5	0.09	0.05	0.22
348	0.08	0.04	0.18
350.5	0.07	0.04	0.16
353	0.07	0.04	0.14
355.5	0.06	0.03	0.12
358	0.05	0.03	0.1
360.5	0.05	0.03	0.09
363	0.04	0.03	0.08

5. Conclusion

In this paper we report a new algorithm for characterization of thermodynamics of monomer-dimer process of dyestuffs by temperature titration. We determine the dimeric constant, difference of concentration profiles for the monomer-dimer, and difference spectra of monomer/dimer pairs. The thermodynamics' parameters of the dimerization reaction were calculated from the dependence of dimeric constant on the temperature (van't Hoff equation). The iterative least-squares method appears to be well suited to the spectral resolution of major components in a mixture of cationic dyes, even where the overlap in both temperature and spectral dimensions is severe. Further work will be required to characterize the performance of the method on minor components, where the IND function might be less successful. Application of the method to trimerization of dyes would require some adjustment of the model for the **C** matrix. Extension of the technique to other equilibrium-based analytical methods, complexation, redox and acid-base,^{32–35} for example, would be straightforward.

6. Supplement materials

The MATLAB code of the algorithm and original data file of a three dyes mixture. Files are deposited in the Journal log and are available upon request.

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Povzetek

V prispevku smo predstavili metodo analize UV-Vis spektroskopskih podatkov za opis ravnotežja dimerizacije v kompleksnih mešanicah, ki so jo predlagali že Frans S. D. in Harris (J. M.; *Anal. Chem.* 1984, 56, 466–470) za obravnavo p-H titracij. Medtem ko metoda, ki jo je opisal Kubista (*Anal. Chem.* 1993, 65, 994–998), zahteva poznavanje spektrov monomernih oblik, v tem delu opisan pristop ne potrebuje nobenih začetnih podatkov. Vrednosti, dobljene tekom spektrofotometrične titracije tvorijo matriko **D**, ki jo faktoriziramo v matriki **A**, ki vsebuje absorpcijske spektre komponent in **C**, ki vsebuje temperaturno odvisne koncentracije. Na ta način smo določili konstante dimerizacije za kristal vijolično, resaurin in metilensko modro v temperaturnem območju med 0–90 °C.