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Spectrophotometric determination of concentration profiles and thermodynamic parameters of aggregation phenomena of rhodamine B by application of multivariate curve resolution

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Abstract

The dimerization constants of rhodamine B have been determined by studying the dependence of its absorption spectra on the temperature in the range 30–70 °C at different total concentrations of rhodamine B (5.15×10−5 to 6.35×10−5 M). The monomer–dimer equilibrium of rhodamine B have been determined by applying MCR-ALS method on the absorption spectra. 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. The enthalpy and entropy of the dimerization reactions were determined from the dependence of the equilibrium constants on the temperature (van't Hoff equation)

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Introduction

Rhodamine B is derivative of the xanthene dyes class, which are among the oldest and most commonly used of all synthetic dyes that, of their applications were using in cloth and food colouring. They are applied as probes in biochemistry, monitoring of membrane fusion, for determination of the aggregations distance in biology, as fluorescent probes of protein in detecting protein orientation because of their high time-zero anisotropy, photostability and also red emission making them ideal for use in microscope. The special photophysics properties of these types of molecules cause the vast and increasing up applications in chemistry and physics (Visser *et al.*, 1988; van Zandvoort *et al.*, 1999).

The monomer–dimer equilibrium of the rhodamines plays a substantial role in biochemical research. The advantage is the drastically change in fluorescence intensity accompanying the monomer–dimer transition (Toptygin *et al.*, 1997). In addition to the above-mentioned unique optical properties rhodamines have significant role in several fields of scientific researches. Nowadays, they serve as water tracing agents, as photosensitizers, fluorescent markers for microscopic studies of complex cellular processes and structure, laser dyes, and since recently, as chromoionophores in optical chemical sensors (Miljanic *et al.*, 2002).

Traditionally rhodamine dyes have high light absorbing potential large molar absorptivity (≈105 lmol $^{-1}$ cm^{-1}) in the visible region of the electromagnetic spectrum which is attributed to a $\pi\rightarrow\pi^*$ transition (Miljanic *et al.*, 2002). Like all other dyestuff the absorption and emission are influenced by subsistent on the nitrogen of the amino groups of the xanthene moiety. When the phenyl carboxylic group is not etherified, the acid–base equilibrium has an effect on the spectroscopic properties of them. Since the probability of the intersystem crossing is very small, in addition to fluorescence, internal conversion is also a way of deactivation. Depending on proton activity, concentration, temperature and solvent, rhodamine dyes exist in solution in several forms: as ionized forms, neutral species and lactones or as aggregates. Each form is characterized by emission spectra and appropriate absorption which are further influenced by specific medium effect, i.e. ionic strength. Therefore, spectroscopic properties of rhodamine are still the subject of many research studies and controversies (Miljanic *et al.*, 2002; Micheau *et al.*, 2004). On the other hand, their equilibria can be very useful for analytical application in optical sensors.

Aggregation is one of the features of dyes in solution (Miljanic *et al.*, 2002; Micheau *et al.*, 2004; Antonov *et al.*, 1999; Ghasemi *et al.*, 2004; Su *et al.*, 2004; Chibisov *et al.*, 1999), affecting their colouristic and photophysical properties and therefore being of special interest. It is famous 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 structure, concentration, ionic strengths, presence of organic solvents and temperature. 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 ionic strengths or dye concentration; 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. Mchedlov-Petrossyan *et al.* recently reported several comprehensive studies on the rhodamine dyes (Mchedloy-Petrossyan *et al.*, 1994; Mchedloy-Petrossyan *et al.*, 2003; Mchedloy-Petrossyan *et al.*, 2003). They studied the effects of the several experimental parameters and also the effect of the addition of some additives like crown ethers, β-cyclodextrin and inert salts as ionic strength modifiers. In spite of the presence of these interesting reports in one hand there are real vacancies on the thermodynamics parameters and on the other hand there are demands to determine the dimerization constants for the cited dyes in dilute solutions.

The absorption UV–Vis spectroscopy is one of the most suitable methods for quantitative studying the aggregation phenomena of dyes as function of concentration. In the commonly used concentration range (10^{-3} to 10^{-6} M) the main reaction is monomer–dimer equilibrium. In this paper, the application of the multivariate curve resolution (MCR) method for the analysis of UV-Vis data is proposed. The goal is to calculation of the thermodynamic parameters and equilibrium constants of monomer-dimer equilibria of rhodamine B.

Experimental

Material

All the chemicals used were of analytical reagent grade. Distilled water was used throughout of experiments. Rhodamine B were purchased from Fluka and were used without additional purification. A stock solution (2×10^{-4} M) was prepared by dissolving solid rhodamine B in water. UV-Vis data were recorded for three concentration of rhodamine solutions ($5.15\text{x}10^{-5}$M, $5.75\text{x}10^{-5}$M, $6.35\text{x}10^{-5}$M). In all experiments the ionic strengths were maintained constant by KCl (Fluka) at 1.0 mol l^{-1} solutions. The pH of the all solutions was kept constant at 7.10 using Tris buffer.

Apparatus

Absorption spectra were measured on CARY 100 UV–vis Spectrophotometer (Varian) equipped by temperature controller. Conventional quartz cell (10mm×10mm) were used throughout. The cuvettes were treated with repel-silane prior to measurements to avoid dye adsorption. The pH values were measured by a Metrohm 692 furnished with combined calomel Ag/AgCl electrode.

Computer hardware and software

All absorption spectra where digitized at five data points per nanometer in the wavelength 450–620 nm for rhodamine B and transferred to a computer for subsequent analysis by MATLAB (Mathworks, Version 7.8).

Data treatment: Multivariate analysis

Multivariate UV-Vis data were analyzed with the softmodeling MCR procedure to evaluate pure spectra and concentration profiles of spectroscopically active components present in the system from decomposition of experimental data matrix D according to the equation:

$$D = CS^T + E \qquad (1)$$

where C and S^T are data matrices containing concentration profiles and pure spectra for each one of these components or conformations present in the experiment, respectively. E contains residual noise not explained by the proposed components or conformations in C and S^T. The MCR procedure applied in this work consisted of the following steps (for a more extensive explanation, see references (Tauler *et al.*, 1995).

Data arrangement

For an experiment monitored by UV-Vis, the recorded spectra were collected in a table or matrix D. The dimensions of this matrix were N_r rows×λ_m columns, where N_r represents the spectra recorded at successive temperature values and λ_m the number of wavelengths measured.

Determination of the number of conformations, N

The number of spectroscopically active conformations N was estimated by applying several methods, like singular value decomposition (SVD) or SIMPLISMA (Tauler *et al.*, 1995).

ALS optimization

The ALS optimization procedure is an iterative method used to solve Eq. (1) for the proposed number of conformations N. This iterative process is started with an initial estimation of the pure spectra S_T for each one of the N components or conformations proposed (Tauler *et al.*, 1995).

Concentration profiles C and pure spectra S_T resolved for each conformation in the analysis of individual data matrices may differ from the true ones because of possible unresolved underlying factor analysis ambiguities (Tauler *et al.*, 1995). This means that concentration profiles and pure spectra may be only one solution within a band of feasible solutions that are bounded by the constraints applied in the calculation. Some of these ambiguities are more easily solved by means of the simultaneous MCR analysis of multiple experiments under different conditions. The model used for MCR simultaneous analysis of several dimerization systems at different total concentrations is described by Eq. (2):

$$\begin{bmatrix} D_{5.15x10^{-5}M} \\ D_{5.75x10^{-5}M} \\ D_{6.35x10^{-5}M} \end{bmatrix} = \begin{bmatrix} C_{5.15x10^{-5}M} \\ C_{5.75x10^{-5}M} \\ C_{6.35x10^{-5}M} \end{bmatrix} S^T + \begin{bmatrix} E_{5.15x10^{-5}M} \\ E_{5.75x10^{-5}M} \\ E_{6.35x10^{-5}M} \end{bmatrix}$$

This simultaneous analysis of several matrices has already been shown to be more powerful than the separate individual analysis, and allows improvement of the resolution of complex experimental data structures. MCR analysis of column-wise augmented data matrices has been shown to give more reliable solutions, eventually removing rotational ambiguities and rank deficiency problems (Tauler *et al.*, 1995).

All MCR calculations were performed using in MATLAB (version 7.8, The Mathworks Inc., Natick, MA, USA) routines, which can be downloaded from the MCR webpage (ASCII files).

Results and discussion

The absorption spectra of rhodamine B, at different total dye concentrations, were recorded in the wavelength 440–560 nm and temperature 30–70 °C at 5 °C intervals and pH 7.10. The sample absorption spectra are shown in Fig. 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. The experiments were carried out at three concentrations ($5.15x10^{-5}M$, $5.75x10^{-5}M$, $6.35x10^{-5}M$).

Table 1. Dimeric constant (KD) and thermodynamic parameters values of rhodamine B dye at different concentrations.

Concentration	$5.15x10^{-5}$ mol l^{-1}	$5.75x10^{-5}$ mol l^{-1}	$6.35x10^{-5}$ mol l^{-1}
Log KD (30°C)	5.42	5.51	5.30
ΔH° (kJ mol^{-1})	-90.3	-90.6	-89.9
ΔS° (J mol^{-1} K^{-1})	-194.1	-193.4	-195.1

The MCR analysis was repeated with two and three components, and with several combinations of constraints, i.e. non-negativity, equality, unimodality and closure. The best (chemically meaningful) results were obtained when only two components were considered, all of them included in the closure constraint, and when the non-negativity constraint was applied to both spectral and concentration profiles. The lack of fit was 3.02% of the experimental data matrix D, which was considered good taking into account the instrumental technique and the large number of matrices simultaneously analyzed, recorded in independent experimental conditions.

The resolved concentration profiles (Fig. 2) and spectra profiles (Fig. 3) show the presence of two components (monomer and dimer).

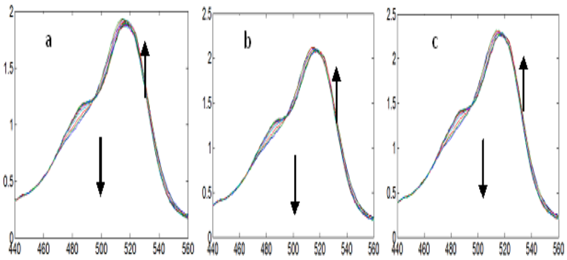

Fig. 1. Absorption spectra of rhodamine B: a) $5.15x10^{-5}M$, b) $5.75x10^{-5}M$ and c) $6.35x10^{-5}M$ in5 °C intervals between 30 and 70 °C at pH 7.10.

In the present paper, by utilizing the Vant-Hoff relation (Ghasemi *et al.*, 2004), which describes the dependence of equilibrium constant on temperature, thermodynamic parameters of dimerization equilibrium have been determined:

$$lnK = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} \qquad (3)$$

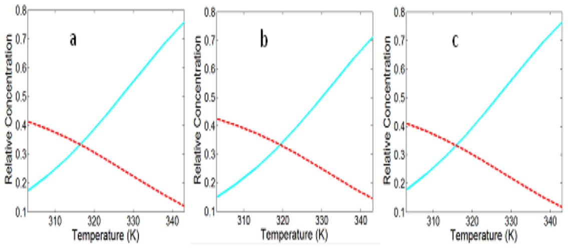

Fig. 2. Concentration profiles of monomer (—) and dimer (---): a) 5.15x10^{-5}M, b) 5.75x10^{-5}M and c) 6.35x10^{-5}M in 5 °C intervals between 30 and 70 °C.

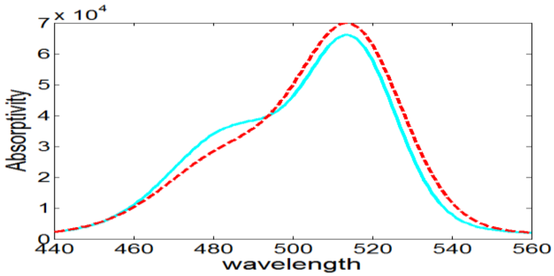

Fig. 3. Spectra profiles of monomer (—) and dimer (---).

where H° is the molar enthalpy change, S is the molar entropy change , R = 8.31 Jmol^{-1} K^{-1} the universal gas constant, and T the Kelvin temperature. A linear regression of equilibrium constants with respect to 1/T is then performed, which determines enthalpy and entropy change of the reaction (Table 1).

Conclusions

Multivariate analysis has been shown to be a useful tool for the analysis of UV-Vis data recorded during thermal change of monomer-dimer equilibria. The application of MCR improved the results obtained by classical univariate analysis of UV-Vis data. The simultaneous analysis of several data matrices, corresponding to different experimental conditions, has allowed the resolution of several systems which are difficult to solve by individual analysis.

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