Available online at www.sciencedirect.com

ScienceDirect

Spectrochimica Acta Part A 65 (2006) 73–78

SPECTROCHIMICA ACTA PART A

www.elsevier.com/locate/saa

Spectrophotometric and thermodynamic study on the dimerization equilibrium of ionic dyes in water by chemometrics method

Ali Niazi [a,*], Ateesa Yazdanipour [a], Jahanbakhsh Ghasemi [b], Mikael Kubista [c]

[a] Department of Chemistry, Faculty of Sciences, Azad University of Arak, Arak, Iran
[b] Department of Chemistry, Faculty of Sciences, Razi University, Kermanshah, Iran
[c] Department of Chemistry and Biosciences, Chalmers University of Technology, Gothenburg, Sweden

Received 15 June 2005; accepted 22 September 2005

Abstract

The monomer–dimer equilibrium and thermodynamic of several ionic dyes (Neutral Red, Nile Blue A, Safranine T and Thionine) has been investigated by means of spectrophotometric and chemometrics methods. The dimerization constants of these ionic dyes have been determined by studying the dependence of their absorption spectra on the temperature in the range 20–75 °C at concentrations of Neutral Red (1.73×10^{-5} M), Nile Blue A (3.94×10^{-5} M), Safranine (6.59×10^{-5} M) and Thionine (6.60×10^{-5} M). The monomer–dimer equilibrium of these dyes has been determined by chemometrics refinement of the absorption spectra obtained by thermometric titrations performed. The processing of the data carried out for quantitative analysis of undefined mixtures, based on simultaneous resolution of the overlapping 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 to the temperature (van't Hoff equation).

Keywords: Dimerization; Chemometrics; Spectrophotometric; Neutral Red; Nile Blue A; Safranine T; Thionine; Ionic dyes

1. Introduction

Many dyes demonstrate ability for self-association which has applications in the photographic and dye industry [1]. The self-association phenomenon plays an important role in biological processes. The photoprocesses of aggregated molecules play an important role for the photosynthesis in green plants and photosynthetic bacteria [2]. Dimers as the simplest aggregates are the subject of many studies concerned with the thermodynamics of monomer–dimer equilibrium and photo-physical properties, and therefore being of special interest [3–10]. 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 [6–12]. 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.

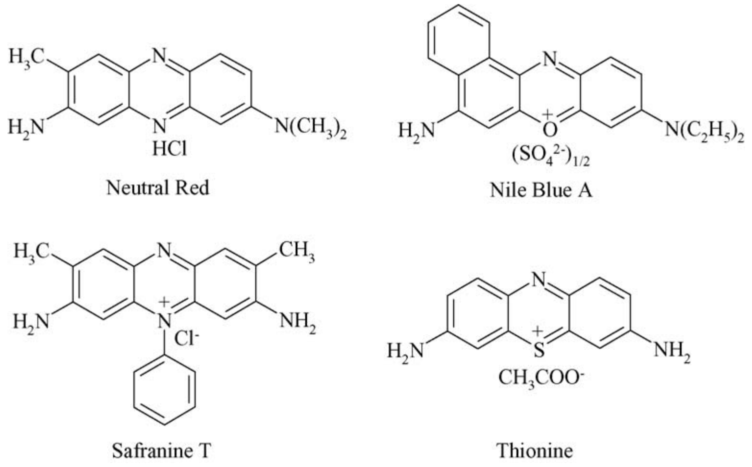
Spectroscopic methods are in general highly sensitive and as such suitable for studying chemical equilibria in solution. When the components involved in the chemical equilibrium have distinct spectral responses, their concentrations can be measured directly, and the determination of the equilibrium constants is trivial. The absorption UV–vis spectroscopy is one of the most suitable methods for quantitative studying the aggregation phenomena of dyes as function of concentration [13–17]. In the commonly used concentration range (10^{-3} to 10^{-6} M) the main equilibrium is monomer–dimer reaction. In this work, we used physical constraints to determine the dimeric constants of ionic dyes (Scheme 1) in pure water.

Data analysis was carried out by DATAN package that developed by Kubista et al. [18–23]. The theory and application of physical constraints method was discussed by Kubista et al. in

---

* Corresponding author. Tel.: +98 8613663041x370; fax: +98 8613670017.
E-mail address: ali.niazi@gmail.com (A. Niazi).

1386-1425/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.saa.2005.09.030



Scheme 1.

several papers [18–26]. However, the general principal will be outlined briefly.

The absorption spectra are digitized and arranged as rows in a matrix $\boldsymbol{A}$. Matrix $\boldsymbol{A}$ is then decomposed into an orthogonal basis set using, for example, the NIPALS routine [18]:

$$\boldsymbol{A} = TP + \boldsymbol{E} \approx TP = \sum_{i=1}^{r} \boldsymbol{t}_i \boldsymbol{p}'_i \tag{1}$$

where $\boldsymbol{t}_i$ are orthogonal target vectors and $\boldsymbol{p}'_i$ are orthogonal projection vectors, $\boldsymbol{E}$ is the error matrix, and r the number of spectroscopically distinguishable components, which is two in this case. Assuming linear response the recorded spectra are also linear combinations of the spectral responses, $\boldsymbol{v}_i$, of the components:

$$\boldsymbol{A} = CV + \boldsymbol{E} \approx CV = \sum_{i=1}^{r} \boldsymbol{c}_i \boldsymbol{v}_i \tag{2}$$

where $\boldsymbol{c}_i$ are vectors containing the component concentrations at the different temperatures. Eqs. (3) and (4) are related by a rotation:

$$C = T\boldsymbol{R}^{-1} \tag{3}$$

$$V = \boldsymbol{R}P \tag{4}$$

where $\boldsymbol{R}$ is an $r \times r$ rotation matrix, for which a two-component system has the element:

$$\boldsymbol{R} = \begin{bmatrix} r_{11} & r_{12} \\ r_{21} & r_{22} \end{bmatrix} \tag{5}$$

Two constraints are used to determine three of the element in $\boldsymbol{R}$. The first is the spectrum of monomer, which is measured separately, and the second is the constant total concentration of the dye:

$$c_x(T) + 2c_{x_2}(T) = c_{\text{tot}} \tag{6}$$

Matrix $\boldsymbol{R}$ can now be described by a single scalar r_{21}, and other factors that are determined by the constraints. The value of r_{21} determines the dimer spectrum and the monomer concentration profiles. Although many values of r_{21} produces a mathematically acceptable solution, 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 [31]:

$$K_{\text{D}}(T) = \frac{c_{x_2}(T)/c^\circ}{(c_x(T)/c^\circ)^2} \tag{7}$$

where $c^\circ = 1\ \text{mol/dm}^3$. Assuming that the dimerization constant $K_{\text{D}}(T)$ depends on temperature according to the van't Hoff equation [31],

$$\frac{\text{d} \ln K_D(T)}{\text{d}(1/T)} = \frac{-\Delta H^\circ}{R} \tag{8}$$

where ΔH° is the molar enthalpy change, $R = 8.31\ \text{J mol}^{-1}\ \text{K}^{-1}$ 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 a trial enthalpy change of the reaction. Several studies based on the application of this method to spectrophotometric data have been reported [11,12,27–29]. The analysis is readily performed with the DATAN program [30].

2. Experimental

2.1. Material

All the chemicals used were of analytical reagent grade. Subboiling distilled water was used throughout. Neutral Red, Nile Blue A, Safranine T and Thionine (for microscopy grade) were purchased from Fluka and were used without additional purification. A stock solution (5×10^{-4} M) was prepared by dissolving these solid ionic dyes in water. The pH of the all solutions was kept constant at 7.00 using Tris buffer.

2.2. Apparatus

Absorption spectra were measured on CARY 100 UV–vis spectrophotometer (Varian) equipped by 6×6 multi-cell and temperature controller and were digitized with five data points/nm. Conventional quartz cells (10 mm × 10 mm) and (10 mm × 1 mm) 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.

2.3. Computer hardware and software

All absorption spectra where digitized at five data points/nm in the wavelengths 400–650 nm, 500–720 nm, 400–650 nm and 450–650 nm for Neutral Red, Nile Blue A, Safranine T and Thionine, respectively, and transferred (in ASCII format) to an Athlon 2000 XP computer for analysis by MATLAB (Mathworks, Version 6.5) or for processing by using DATAN package [30].

3. Results and discussion

The absorption spectra of Neutral Red, Nile Blue A, Safranine T and Thionine, were recorded between 400 and 650 nm, 500 and 720 nm, 400 and 650 nm, and 450 and 650 nm, respectively, in the temperature range 20–75 °C at 5 °C intervals and pH 7.00; absorption spectra are shown in Fig. 1. As it is expected, by increasing the temperature, 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 as an initial estimate for the monomer in the subsequent calculation.

According to Eqs. (1)–(8) 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 and ΔH, correspond to minimum value of the χ^2 statistics, are selected as the final results. The χ^2 is the sum of squared residuals [19] 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. The general formula of the χ^2 is:

$$\chi^2 = \sum_{i=1}^{n} \frac{(\boldsymbol{A}_{\mathrm{exp}} - \boldsymbol{A}_{\mathrm{calc}})^2}{\boldsymbol{A}_{\mathrm{exp}}} \qquad (9)$$

where $\boldsymbol{A}_{\mathrm{exp}}$ is the expected value and $\boldsymbol{A}_{\mathrm{calc}}$ is calculated form experimental data over n data point.

With increasing temperature the absorption peak for Neutral Red, Nile Blue A, Safranine T and Thionine, around 535, 635, 520 and 595 nm grows, and the absorption around 500, 590, 495 and 560 nm decrease (Fig. 1). We analyzed the temperature titrations assuming monomer–dimer, monomer–dimmer–trimer and even some tested models including higher order aggregates, and found that the monomer–dimer model most adequately describes the data in these range of used concentrations. The presence of exactly two species is also evidenced by appearing of the isobestic points at 515, 605, 508 and 570 nm for Neutral Red, Nile Blue A, Safranine T and Thionine, respectively.

The dimerization constants (K_D) were calculated at different temperatures. 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 $1/T$ (Fig. 2) ΔH°

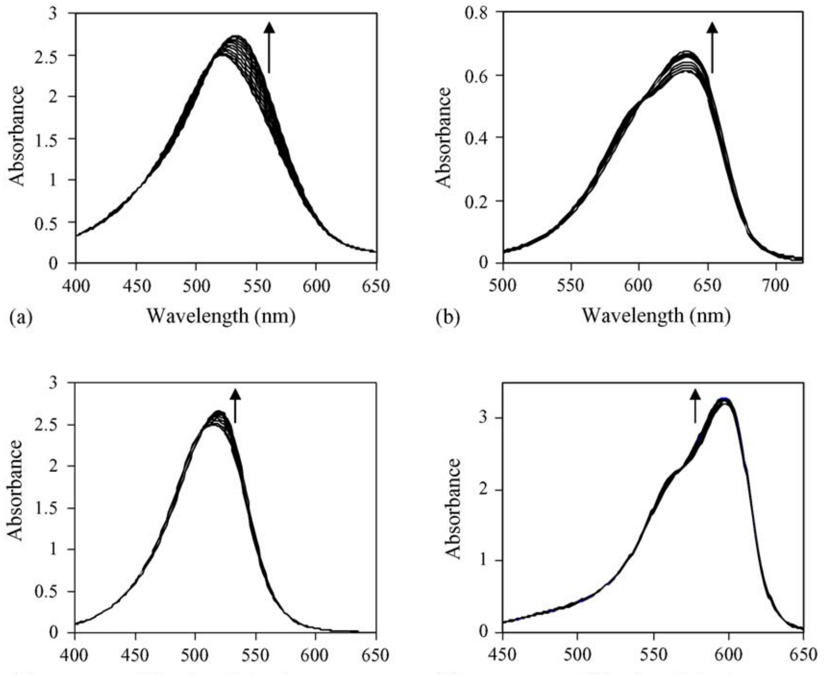


Fig. 1. Absorption spectra of: (a) Neutral Red (1.73×10^{-5} M), (b) Nile Blue A (3.94×10^{-5} M), (c) Safranine (6.59×10^{-5} M) and (d) Thionine (6.60×10^{-5} M) in 5 °C intervals between 20 and 75 °C at pH 7.00 in water.

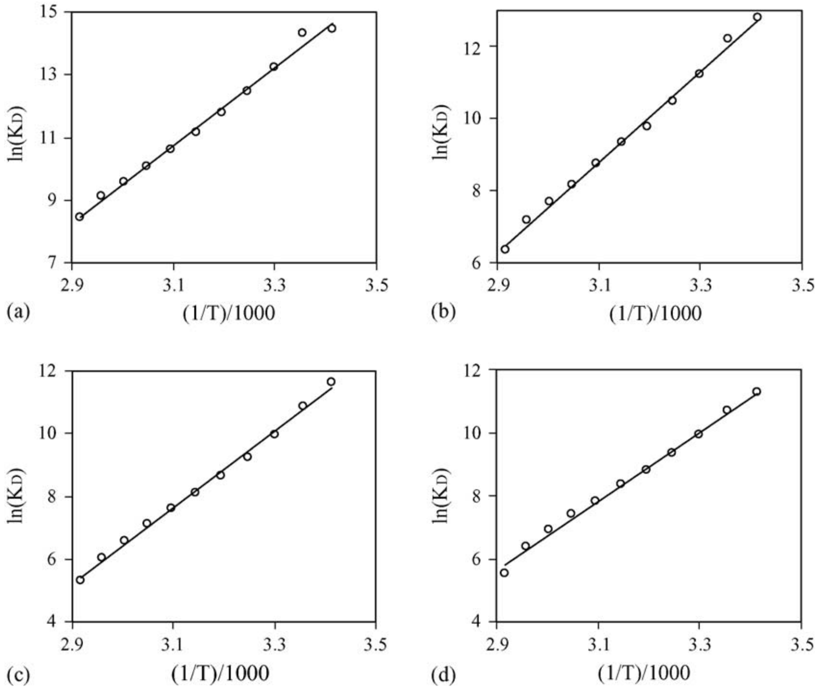


Fig. 2. The van't Hoff equation plot of: (a) Neutral Red, (b) Nile Blue A, (c) Safranine T and (d) Thionine dyes.

and ΔS° values were determined. The dimerization constants at 25 °C and thermodynamic parameters of the dimerization reactions of the Neutral Red, Nile Blue A, Safranine T and Thionine dyes are listed in Table 1. As described above dimerization is presumed to be the dominant form of aggregation in applied concentration in aqueous Neutral Red, Nile Blue A, Safranine T and Thionine. 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 these ionic dyes. The relative dependence of the concentrations of the monomer and dimer of Neutral Red, Nile Blue A, Safranine T and Thionine dyes on the temperature in

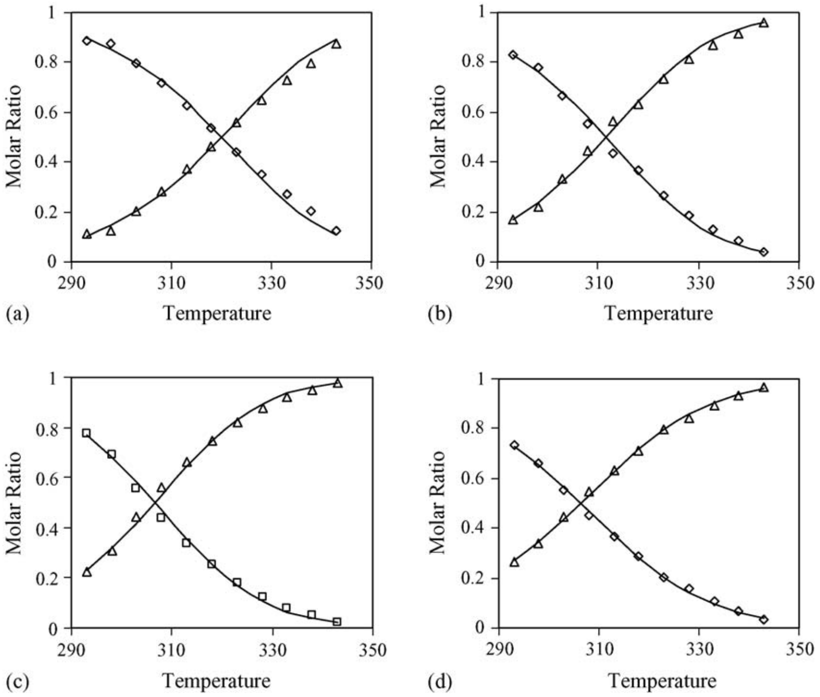


Fig. 3. Molar ratio of: (a) Neutral Red, (b) Nile Blue A, (c) Safranine T and (d) Thionine dyes monomer (△) and dimer (□), compared to molar ratios predicted by the temperature dependence of the equilibrium constant (shown as line) in water (temperature unit is °K scale).

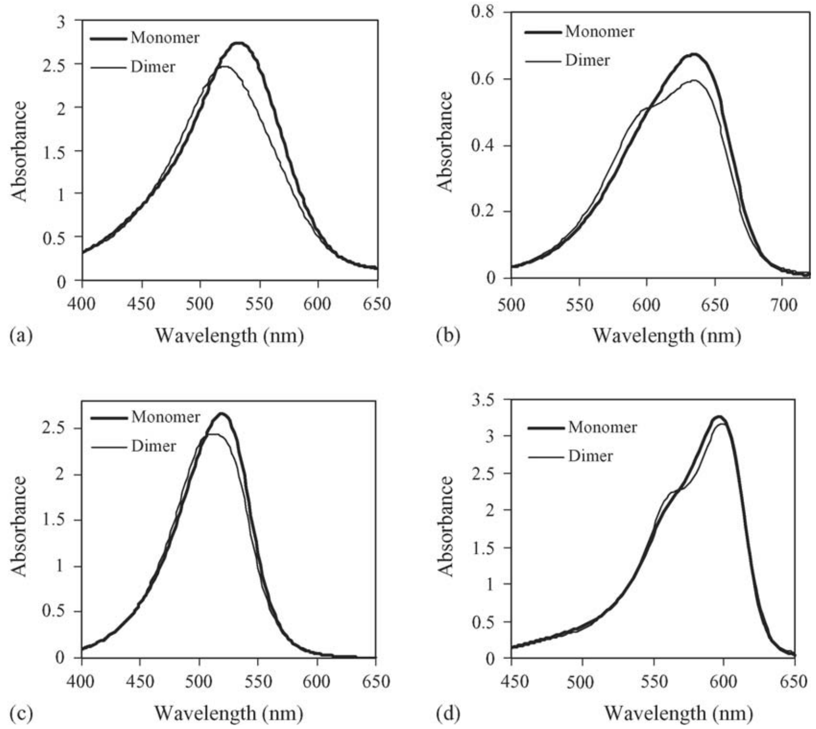


Fig. 4. Calculated absorption spectra of: (a) Neutral Red, (b) Nile Blue A, (c) Safranine T and (d) Thionine dyes.

Table 1
Dimeric constant and thermodynamic parameters values of Neutral Red (1.73×10^{-5} M), Nile Blue A (3.94×10^{-5} M), Safranine (6.59×10^{-5} M) and Thionine (6.60×10^{-5} M) dyes in water

Dye	Neutral Red	Nile Blue A	Safranine T	Thionine
log K_D (25 °C)	6.21	5.31	4.73	4.64
ΔH° (kJ mol^{-1})	−108.5	−109.1	−106.7	−90.8
ΔS° (J mol^{-1} K^{-1})	−244.5	−265.4	−267.9	−216.4

different concentration are shown diagrammatically in Fig. 3. The calculated absorption spectra of Neutral Red, Nile Blue A, Safranine T and Thionine dyes in monomer and dimer forms are shown in Fig. 4.

4. Conclusion

In this study we report for the first time to our knowledge a thermodynamics study of the dimerization equilibrium of the Neutral Red, Nile Blue A, Safranine T and Thionine dyes in aqueous solution. 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't Hoff equation).

References

[1] A.K. Chibisov, T.D. Slavnova, H. Gorner, Chem. Phys. Lett. 386 (2004) 301.
[2] T. Miyatake, H. Tamiaki, A.R. Holzwarth, K. Schaffner, Helv. Chim. Acta 82 (1999) 797.
[3] B.C. Burdett, Aggregation of Dyes, in Studies in Physical and Theoretical Chemistry, vol. 2, Elsevier, 1983.
[4] R. Pepperkok, R. Saffrich, Microinjection and Detection of Probes in Cells, EMBL, Heidelberg, 1999.
[5] S. Miljanic, Z. Cimerman, L. Frkanec, M. Zinic, Anal. Chim. Acta 468 (2002) 13.
[6] N.O. Mchedlov-Petrossyan, Y.V. Kholin, Russ. J. Appl. Chem. 77 (2004) 414.
[7] J.C. Micheau, G.V. Zakharova, A.K. Chibisov, Phys. Chem. Chem. Phys. 6 (2004) 2420.
[8] L. Antonov, G. Gergov, V. Petrov, M. Kubista, J. Nygren, Talanta 49 (1999) 99.
[9] L. Evans III, G. Patonay, Talanta 48 (1999) 933.
[10] G.J. Su, S.X. Yin, L.J. Wan, J.C. Zhao, C.L. Bai, Surf. Sci. 551 (2004) 204.
[11] J. Ghasemi, A. Niazi, G. Westman, M. Kubista, Talanta 62 (2004) 835.
[12] J. Ghasemi, A. Niazi, M. Kubista, Spectrochim. Acta Part A, in press.
[13] I. Baraldi, M. Caselli, F. Momicchioli, G. Pontereini, D. Vanossi, Chem. Phys. 275 (2002) 149.
[14] A.G. Gilani, R. Sariri, K. Bahrpaima, Spectrochim. Acta Part A 57 (2001) 155.
[15] M.T.M. Choi, P.P.S. Li, D.K.P. Ng, Tetrahedron 56 (2000) 3881.
[16] A.K. Chibisov, V.I. Prokhorenko, H. Gorner, Chem. Phys. 250 (1999) 47.
[17] K. Patil, R. Pawar, P. Talap, Phys. Chem. Chem. Phys. 2 (2000) 4313.
[18] M. Kubista, R. Sjoback, J. Nygren, Anal. Chim. Acta 302 (1995) 121.
[19] M. Kubista, J. Nygren, A. Elbergali, R. Sjoback, Crit. Rev. Anal. Chem. 29 (1999) 1.
[20] M. Kubista, R. Sjoback, B. Albinsson, Anal. Chem. 65 (1993) 994.
[21] I. Scarminio, M. Kubista, Anal. Chem. 65 (1993) 409.
[22] A. Elbergali, J. Nygren, M. Kubista, Anal. Chim. Acta 379 (1999) 143.
[23] J. Nygren, J.M. Andrade, M. Kubista, Anal. Chem. 68 (1996) 1706.

[24] N. Svanvik, J. Nygren, G. Westman, M. Kubista, J. Am. Chem. Soc. 123 (2001) 803.
[25] J. Nygren, N. Svanvik, M. Kubista, Biopolymers 46 (1998) 39.
[26] J. Nygren, A. Elbergali, M. Kubista, Anal. Chem. 70 (1998) 4841.
[27] J. Ghasemi, A. Niazi, M. Kubista, A. Elbergali, Anal. Chim. Acta 455 (2002) 335.
[28] J. Ghasemi, Sh. Ahmadi, M. Kubista, A. Forootan, J. Chem. Eng. Data 48 (2003) 1178.
[29] A. Rouhollahi, F.M. Kiaie, J. Ghasemi, Talanta 66 (2005) 653.
[30] http://www.multid.se.
[31] L.V. Levine, Physical Chemistry, McGraw-Hill, New York, 1988.