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Determination of the dissociation constants of sulfonated azo dyes by capillary zone electrophoresis and spectrophotometry methods

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Abstract

The dissociation constants of 10 sulfonated azo dyes, six of the most common food colours used as additives (Food Yellow 4, Food Yellow 3, Food Red 9, Food Red 7, Food Red 17 and Food Blue 5), and four commonly used as textile dyes (Acid Orange 7, Acid Orange 12, Acid Red 26 and Acid Red 88), have been determined by two different systems, one by using capillary electrophoresis (CE) with diode array detection and the other by using UV–visible absorption spectrophotometry, which has been used as reference method to obtain the pK_a values. The pK_a values obtained by CE were determined in two ways, first on the basis of the electrophoretic mobilities (calculated from the migration times), and after we propose a new methodology, in which the dissociation constants are determined from the spectra corresponding to the maxima of electrophoretic peaks. The pK_a values obtained by using these CE methods have been compared with those obtained by using the spectrophotometric method. The results show that the pK_a values obtained by the CE proposed method are in general closer to the reference values than those obtained from the electrophoretic mobilities. Moreover, the proposed method retains the advantages of CE, as the possibility of working with small amounts of sample, despite its purity.

Keywords: Dissociation constants; Azo dyes

1. Introduction

Capillary electrophoresis (CE) has been used in the last decade as a method for pK_a determination [1–9], which allows calculations independent of solute purity, since impurities can be separated from the solutes of interest [2,3]. The determination of dissociation constants by CE has been based on the difference of electrophoretic mobilities of the different species. Thus, CE has been proposed as a method that offers several advantages over the two most commonly used methods for pK_a determination: potentiometric titration and ultraviolet–visible spectroscopy [10–12]. In CE low solute concentrations can be used (between 10^{-4} and 10^{-5} *M*), whereas in potentiometric titrations the useful concentration range is between 10^{-2} and 10^{-3} *M*. Determination of pK_a values by UV–Vis spectrophotometry depends on the species having different spectra, and it is assumed that the solute of interest is pure or that its impurities do not absorb in the UV–Vis range, since impurities cannot be separated from the solutes of interest. This effect can be overcome by using CE, as the impurities can be effectively separated.

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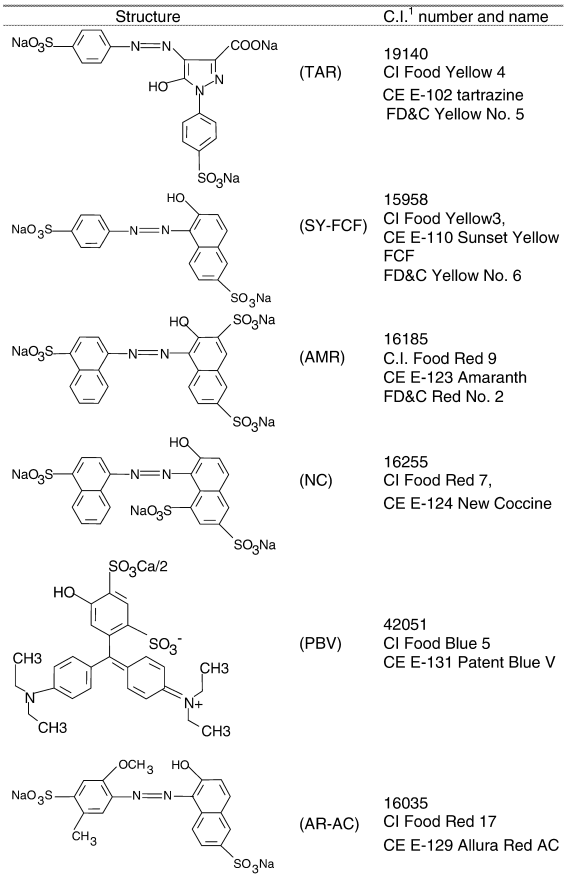
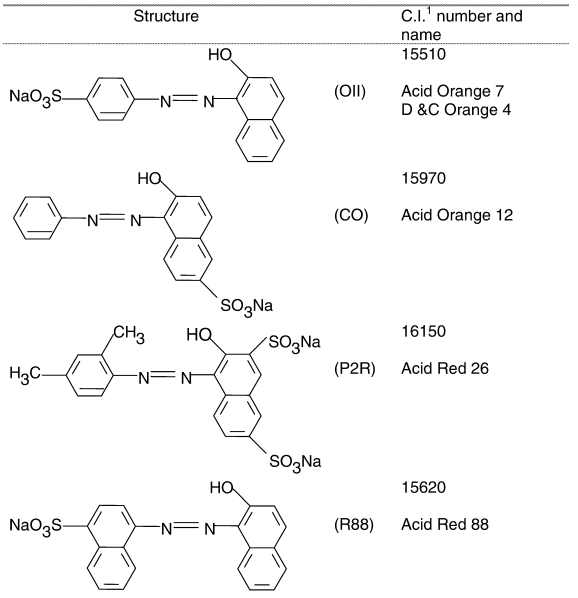
The aim of this work was to determinate the dissociation constants of 10 sulfonated azo dyes and to compare the values obtained from a standard methodology (spectrophotometry) with those obtained from CE procedure. Moreover, a new method is proposed, which is based in the absorbance spectra in the maxima of the electrophoretic peaks. This new procedure can be applied to data obtained from CE with diode array detection (DAD) instruments.

The proposed method has been applied to the determination of the dissociation constants of 10 sulfonated azo dyes, which are widely used in textile (Acid Orange 7, Acid Orange 12, Acid Red 26 and Acid Red 88) and food (Food Yellow 4, Food Yellow 3, Food Red 9, Food Red 7, Food Red 17 and Food Blue 5) industries among others. Figs. 1 and 2 show structures, names and CI numbers for food and textile dyes, respectively.



[1]Colour Index

Fig. 1. Structures, numbers and names of the food dyes studied.



[1]Colour Index

Fig. 2. Structures, numbers and names of the textile dyes studied.

pK_a values for textile dyes were determined previously spectrophotometrically [13]. Other literature data were found for Food Red 7 (pK_a=11.29) and Tartrazine (pK_a=9.43 and ca. 10) [14,15] by potentiometric methods.

2. Experimental

2.1. Reagents

Ultrapure Milli-Q water from Millipore was used for the preparation of solutions. Dyes were obtained from Aldrich and Fluka. Stock standard solutions between 300 and 630 μg/ml were prepared in Ultrapure Milli-Q water and diluted to obtain 30 μg/ml of each dye. The chemicals used for the preparation of the buffers [succinic acid, sodium dihydrogenphosphate, tris(hydroxymethyl)aminomethane, boric acid and hydrochloric acid] were of analytical-reagent grade. Other reagents used were

orthophosphoric acid (Fluka) and sodium hydroxide (Merck).

2.2. Apparatus

The spectrophotometric determinations of dissociation constants were done using a Perkin-Elmer Lambda 19 scanning spectrophotometer, and a Hewlett-Packard HP8453 DAD spectrophotometer. The pH values were measured on a Radiometer PHM 84 pH meter, equipped with an Orion 81-02 Ross glass combination electrode. Solutions were thermostated at 25°C on a Selecta Tectron thermostated bath.

Capillary zone electrophoresis measurements were carried out with a P/ACE System 5500 (Beckman, Palo Alto, CA, USA) equipped with a DAD system. An untreated fused-silica capillary (Supelco, Bellefonte, PA, USA) of 57 cm (50 cm effective length)× 75 μm I.D. was used. The capillary was activated by pressure injection of 1.0 *M* sodium hydroxide solution for 20 min followed by a 10-min rinse with Ultrapure Milli-Q water and a 10-min rinse with the run electrolyte.

2.3. Procedures

2.3.1. Spectrophotometry

Absorbance data were obtained by batch experiments by measuring the spectra of several series of solutions, in which the dye concentration was kept constant and the pH varied by the addition of the appropriate buffer solution [succinic acid, sodium dihydrogenphosphate, tris(hydroxymethyl)aminomethane, boric acid, hydrochloric acid and sodium hydroxide], according to Dempsey and Perrin [16]. The temperature was kept constant at 25±0.2°C, and the ionic strength was 0.1 *M*. The final concentration of the dyes was about $2\cdot10^{-5}$ *M*. Absorbance data were recorded between 270 and 600 nm and then they were processed by using the STAR program [17]. This program calculates the equilibrium constants using the Gauss–Newton non-linear least-squares algorithm by numerical differentiation, until a minimum in the sum of squares residuals (U) is attained:

$$U = \sum_{i=1}^{ns}\sum_{j=1}^{nw}(A_{i,j,\mathrm{exp}} - A_{i,j,\mathrm{calc}})^2 \quad (1)$$

where ns and nw indicate the number of solutions and wavelengths, respectively. $A_{i,j,\mathrm{exp}}$ and $A_{i,j,\mathrm{calc}}$ are the experimental and calculated absorbance values corresponding to the j-wavelength of the i-solution. The minimization process is repeated until the relative change of the sum of squares residuals between two consecutive iterations is less than 0.01%.

2.3.2. CE

The electrophoresis buffers covering the pH range between 5.6 and 12 were prepared by adding the required amount of sodium hydroxide to phosphoric acid in order to obtain the desired pH values. The concentration of the buffer was 0.02 *M*. Each dye solution contained acetone at 3% (v/v) as electroosmotic flow (EOF) marker. Separation was performed at 25°C and the applied voltage was 15 kV. Injections were done by triplicate in hydrodynamic mode for 4 s. The absorbance from 275 to 550 nm was monitored with an on-column photodiode-array detector.

The pK_a determination is based on the principle that a solute has its maximum electrophoretic mobility when it is fully ionized, has the lowest mobility in its protonated form, and has an intermediate mobility in the pH region surrounding its pK_a [2,3]. Electrophoretic mobility (m_e) is calculated from the migration time of a neutral marker, t_{eof}, the migration time of the solute, t, the length of the column L_c, the length of the column between the injection end and the detector L_d, and the applied voltage, V, according to the following equation where m_e is given in $cm^2\ V^{-1}\ s^{-1}$:

$$m_e = \left(\frac{L_c L_d}{V}\right)\cdot\left(\frac{1}{t} - \frac{1}{t_{eof}}\right) \quad (2)$$

The relationship between dissociation constant, pH and electrophoretic mobilities can be derived from Ref. [1] to give the following equation:

$$\mathrm{p}K_a = -\log\left[\mathrm{H}^+\right] - \log\left(\frac{m_e - m_a}{m_b - m_e}\right) \quad (3)$$

where pK_a is the stoichiometric pK_a (this is by using concentrations of the different species instead of activities), m_e, the electrophoretic mobility at the pH of the buffer in the CE column, m_a the electro-

phoretic mobility of the fully ionized acid and m_b the electrophoretic mobility of the protonated species. The pH values measured were transformed to $-\log [H+]$ values according to the Davies equation [15]:

$$-\log f_{H^+} = \frac{A\sqrt{I}}{1+\sqrt{I}} - 0.1 \quad (4)$$

where f_{H+} is the activity coefficient of the hydrogen ion, I is the ionic strength of the buffer solution, and A is the Debye–Hückel parameter. In water, A has a value of 0.5103 at 25°C. The ionic strength of the buffers used was between 0.08 and 0.12. We have taken $I=0.1$ as a mean value, which corresponds to an error less than 0.02 units in the pK_a value.

The pK_a values were determined by performing a non-linear fit to Eq. (3) using the NLREG program [18]. In this case, the program minimizes the sum of squares residuals (U_{CE}), defined as:

$$U_{CE} = \sum_{i=1}^{n} \left\{ pK_a + \log [H^+]_i + \log \left(\frac{m_{e,i} - m_a}{m_b - m_{e,i}} \right) \right\}^2 \quad (5)$$

where n is the number of experimental data pairs of $-\log [H^+]_i$ and $m_{e,i}$. The unknown parameters [pK_a and mobility of the fully dissociated species (m_a)] are refined by the program to give a minimum in the U_{CE} function. This was done because the m_a values could not be determined directly owing the high pK_a values (except in the cases of PBV and TAR); on the other hand, the mobility corresponding to protonated species were obtained by direct measurements in all cases (see Fig. 3A and B).

2.3.3. CE–DAD

The absorbance spectra in the maxima of the electrophoretic peaks were used to obtain the pK_a values, and all spectra were processed by STAR program as described in Section 2.3.1.

In order to evaluate the reproducibility of the pK_a values obtained by using CE–DAD method, two independent series of analysis were done and the pK_a values varied between 0.01 and 0.1 pK_a units. In the first batch, the analyses at each pH were done in duplicate and in the second batch in triplicate. The pK_a values and their standard deviations given in Table 1 have been obtained by combining the results of these two batches.

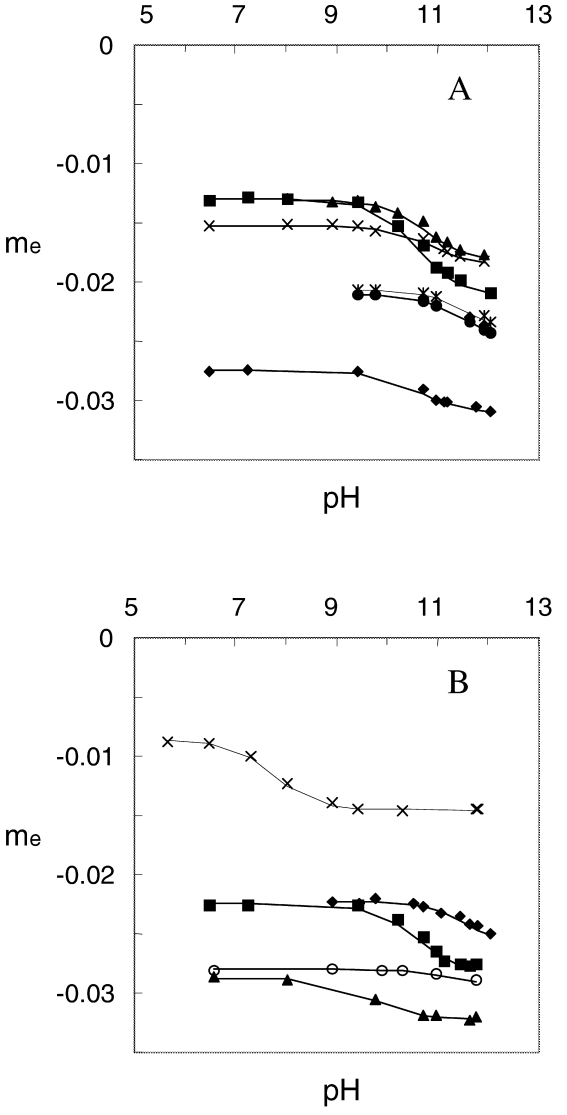


Fig. 3. Plot of electrophoretic mobility, m_e (cm^2 V^{-1} s^{-1}) vs. pH, with superimposed curve fits. (A) AMR (♦), CO (■), OII (▲), R88 (×), P2R-1 (*) and P2R-2 (●). (B) AR-AC (♦), SY-FCF (■), TAR (▲), NC (○) and PBV (×).

The uncertainty in the absorbance values at peak maxima of the dyes has been evaluated for the different pH values in triplicate. The uncertainty varied between 3 and 12% (as RSD) depending on

Table 1
pK_a values obtained by using capillary zone electrophoresis and spectrophotometry methods

Analyte	pK_a value[a]		
	CE		Spectrophotometry
	1[b]	2[c]	
OII	10.65 (0.08)	10.68 (0.12)	10.83 (0.03)
CO	10.46 (0.04)	10.43 (0.07)	10.43 (0.01)
P2R			11.59 (0.02)
P2R-1	11.42 (0.13)	11.26 (0.14)	
P2R-2	11.53 (0.11)	11.61 (0.21)	
AMR	10.47 (0.08)	10.49 (0.10)	10.36 (0.02)
R88	10.70 (0.06)	11.06 (0.08)	11.00 (0.03)
TAR	9.49 (0.08)	9.43 (0.10)	9.40 (0.01)
SY-FCF	10.44 (0.08)	10.36 (0.09)	10.36 (0.01)
NC	11.04 (0.13)	11.19 (0.13)	11.24 (0.01)
PBV	7.63 (0.03)	7.58 (0.09)	7.67 (0.02)
AR-AC	11.45 (0.14)	11.38 (0.12)	11.35 (0.01)

[a] At 0.1 *M* ionic strength, the stimate standard deviation is placed in parentheses.
[b] By using migration times.
[c] By using DAD spectra.

the analyte and the pH values. The uncertainty in the pK_a values given in Table 1 (between 0.07 and 0.2 pK_a units) reflects the variation in the spectra.

3. Results and discussion

The dissociation constants determined by using CE, CE–DAD and spectrophotometry are shown in Table 1. Values of electrophoretic mobilities against the pH for the studied dyes are given in Fig. 3A and B. The values of m_e for all dyes studied were negative and were detected after the EOF marker. The variation of the m_e values in triplicate measurements was between 0.1 and 0.9%.

The determination of the dissociation constants by using CE–DAD offers many advantages: the determination can be done in aqueous solution, the analytes are separated from its impurities, as is shown by P2R in this work. Moreover, small amounts of sample (pmol in our case) are required and the final concentrations can be very low. The use of CE with diode array absorptiometric detection allows the determination of pK_a values by using migration times or absorbance spectra. The comparison of both results is a valuable tool in order to obtain better precision and confirm the results obtained. Fig. 4 show the spectra obtained for Acid Orange 12 by CE–DAD.

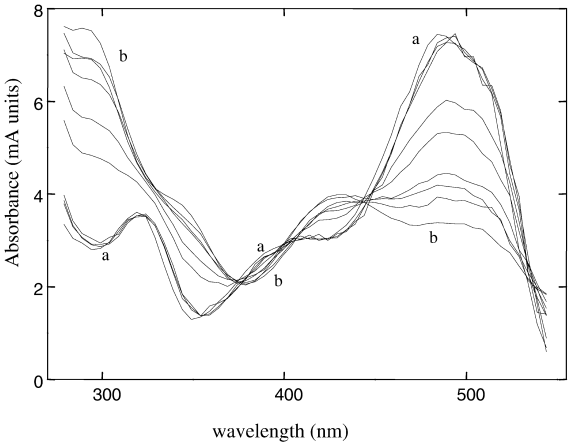


Fig. 4. Spectra obtained for Acid Orange 12 in the maxima of CE–DAD peaks. The pH of the buffers were from 6.51 (a) and 11.98 (b).

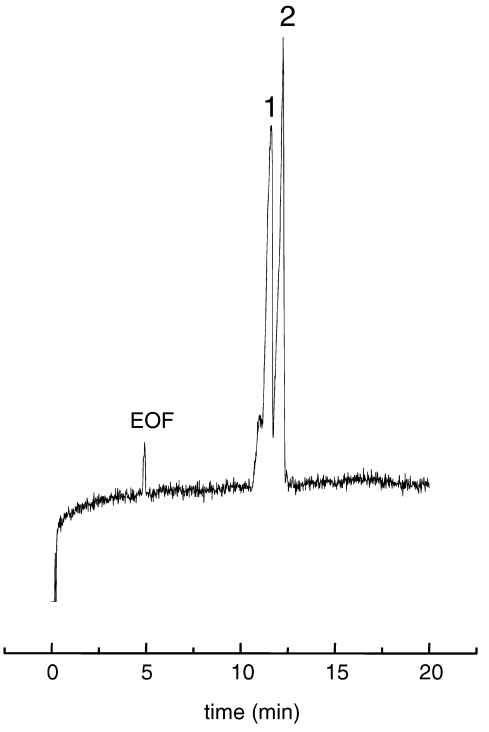


Fig. 5. Electropherogram of P2R at pH 11.9.

On the other hand, the results obtained by using spectrophotometry are very similar to those obtained by CE when the analyte of interest does not have impurities which absorb in the UV–visible range.

Interesting results were obtained for P2R: as it is known, commercial dyes are mixtures of compounds including dyes and impurities. Appropriate isolation procedures and recrystallization can purify some of them; however, this is not possible in all cases [19]. P2R (dye content≈70%) shows two peaks with similar peak area in the electropherograms with most of the electrophoretic buffers used as is shown in Fig. 5. Thus, it is possible to determine the pK_a values for both peaks by CE, but only one pK_a value when the determination is made by classical spectrophotometric procedures. As is shown in Table 1, the pK_a value determined by spectrophotometry for P2R is in good agreement with the obtained for the second CE peak.

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References

[1] J.L. Beckers, F.M. Everaerts, M.T. Ackermans, J. Chromatogr. A 537 (1991) 407.
[2] J.A. Cleveland Jr., M.H. Benko, S.J. Gluck, Y.M. Walbroehl, J. Chromatogr. A 652 (1993) 301.
[3] J. Cai, J.T. Smith, Z.E. Rassi, J. High Resolut. Chromatogr. 15 (1992) 30.
[4] S.J. Gluck, J.A. Cleveland Jr., J. Chromatogr. A 680 (1994) 49.
[5] S.J. Gluck, K.P. Steele, M.H. Benko, J. Chromatogr. A 745 (1996) 117.
[6] J. Barbosa, D. Barrón, E. Jiménez-Lozano, J. Chromatogr. A 839 (1999) 183.
[7] S. Bellini, M. Uhrova, Z. Deyl, J. Chromatogr. A 772 (1997) 91.
[8] J.-P. Mercier, P. Morin, M. Dreux, A. Tambute, Chromatographia 48 (7/8) (1998) 529.
[9] Y. Ishihama, Y. Oda, N. Asakawa, J. Pharm. Sci. 83 (1994) 1500.
[10] F.J.C. Rossotti, H. Rossotti, The Determination of Stability Constants, MacGraw-Hill, London, 1961.
[11] M. Meloun, J. Havel, E. Högfeldt, Computation of Solution Equilibria: A Guide To Methods in Potentiometry, Extraction and Spectrophotometry, Ellis Horwood, Chichester, 1988.
[12] F.R. Hartley, C. Burgess, R.M. Alcock, The Determination of Stability Constants, Ellis Horwood, Chichester, 1980.
[13] M. Pérez-Urquiza, J.L. Beltrán, J. Chromatogr. A 871 (2000) 227.
[14] X. Gang, Shangqiu Shizhuan Xuebao 15 (6) (1999) 78.
[15] E.P. Mazzola, S.J. Bell, S.A. Turujman, J.N. Barrow, J.E. Bailey Jr., J.B. McMahon, T.K. Germann, W.F. Reynols, Can. J. Chem. 77 (1999) 1941.
[16] D.D. Perrin, B. Dempsey, Buffer for pH and Metal Ion Control, Chapman and Hall, London, 1974.
[17] J.L. Beltrán, R. Codony, M.D. Prat, Anal. Chim. Acta 276 (1993) 441.
[18] P.H. Sherood, NLREG, Nonlinear Regression Analysis and Curve Fitting Program, 1997, www.nlreg.com
[19] R. Straub, R. Voyksner, J.T. Keever, J. Chromatogr. 627 (1992) 173.