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Spectrophotometric determination of ionization constants by capillary zone electrophoresis

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Abstract

One of the historical means of determining the ionization constant of a solute relies on the ionized and neutral states of a molecule having different absorption coefficients at an analytical wavelength. An ionic distribution curve can be constructed by directly determining the ratio of neutral species to ionized species in a series of buffer solutions of differing pH. This method is complementary to a recently developed procedure which utilizes electrophoretic mobilities to determine ionization constants. The same data system used to generate mobilities at different pH values also includes information on peak areas, which correlate with the absorbance of a species. Thus, absorbances regressed against pH yields ionization constants which can corroborate those derived from electrophoretic migration.

1. Introduction

Aqueous-phase potentiometric titration, the most widely used method for determining pK_a values requires that the concentration of an analyte be at least 0.1 m*M*; otherwise, interferences are caused by the presence of dissolved CO_2. A common alternative for determining the pK_a at low concentrations is UV–Vis spectrophotometric titration, which relies on the neutral and ionic states of a molecule having different absorption coefficients at an analytical wavelength [1]. Recently, capillary electrophoresis (CE) has been introduced as a method for convenient and precise aqueous pK_a determination [2–4]. The method takes advantage of the relation between electrophoretic mobility and fractional ionization of a compound at different pH values. In this paper we demonstrate that the spectrophotometric method can also be used in conjunction with CE by relating peak areas to absorbance. Moreover, it is possible to determine pK_a by the two methods using the same CE data set, thereby providing an internal check on each method.

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2. Theoretical

2.1. Relating pK_a to mobility

The thermodynamic equilibrium constant, K_a^{th}, for the dissociation of a weak acid e.g.

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$$HZ \Leftrightarrow H^{+} + Z^{-} \quad (1)$$

is defined as

$$K^{th} = \gamma_{Z^-} \gamma_{H^+} \cdot \frac{[H^+][Z^-]}{[HZ]} \quad (2)$$

where γ is the activity coefficient and γ_{HZ} is assumed to be 1. Activities can be calculated from Debye–Hückel theory according to the relation

$$-\log \gamma = \frac{0.509z^2\sqrt{\mu}}{1 + 0.328a\sqrt{\mu}}; \quad \mu = \frac{1}{2} \cdot \sum_{i=1}^{m} C_i z_i^2 \quad (3)$$

where a is the hydrated diameter of an ion in Å [5], z is the valency of the ion and μ is the ionic strength of the solution. In general, the exact value of the parameter a, which can range from 1–11 Å, will not be known; in this study, the value 5 Å was assumed.

A relation between pH versus electrophoretic mobility plots and titration curves has been known for quite some time [6,7]. Morris and Morris [8] derived an equation for calculating mobilities at different pH values when the pK_a of an acid was known. Similarly, it has recently been shown that the pK_a of an acid/base can be determined when the mobilities are known at different pH values [2–4].

Since $m_e = \alpha m_a$, where m_e is the net electrophoretic mobility, m_a is the electrophoretic mobility of the fully deprotonated species Z^-, and α is the fraction ionized ($0 \leqslant \alpha \leqslant 1$), Eq. 2 can be rearranged to give (at 25°C):

$$pK_a^{th} = pH - \log\left(\frac{m_e}{m_a - m_e}\right) + \frac{0.509z^2\sqrt{\mu}}{1 + 0.328a\sqrt{\mu}} \quad \text{(acids)} \quad (4)$$

The analogous equation for a base, B, is given as:

$$pK_a^{th} = pH + \log\left(\frac{m_e}{m_b - m_e}\right) - \frac{0.509z^2\sqrt{\mu}}{1 + 0.328a\sqrt{\mu}} \quad \text{(bases)} \quad (5)$$

where m_b is the electrophoretic mobility of the fully protonated species, BH^+.

2.2. Relating pK_a to peak area

Assuming that Beer's law is valid, the absorbance, A, of an acid HZ at some analytical wavelength is the sum of the absorbances of the molecular species, A_m, and the ionized species, A_I [1]:

$$A = A_m + A_I \quad (6)$$

Provided the same total concentration is used for measurements at different pH values, the absorbance at each pH can be expressed in terms of α, A_I and A_m:

$$A = \alpha(A_I - A_m) + A_m \quad \text{for } A_I > A_m \quad (7)$$

$$A = \alpha(A_m - A_I) + A_I \quad \text{for } A_m > A_I \quad (8)$$

From these expressions, it follows directly that

$$\frac{[Z^-]}{[HZ]} = \frac{\alpha}{1-\alpha} = \frac{A - A_m}{A_I - A} \quad \text{for } A_I > A_m \quad (9)$$

$$\frac{[Z^-]}{[HZ]} = \frac{\alpha}{1-\alpha} = \frac{A_m - A}{A - A_I} \quad \text{for } A_m > A_I \quad (10)$$

While in CE it is peak area, A, not absorbance, that is directly measured, these parameters are related through the expression $A \propto A/t$, where t is migration time required for the solute band to travel the distance from the capillary end to the detector [9–11]. Using this relation with Eqs. 9 and 10, Eq. 2 can be rearranged to give:

$$pK_a^{th} = pH - \log\left(\frac{\frac{A}{t} - A_m}{A_I - \frac{A}{t}}\right) + \frac{0.509z^2\sqrt{\mu}}{1 + 0.328a\sqrt{\mu}} \quad (\text{acids}, A_I > A_m) \quad (11)$$

$$pK_a^{th} = pH - \log\left(\frac{\frac{A}{t} - A_I}{A_m - \frac{A}{t}}\right) + \frac{0.509z^2\sqrt{\mu}}{1 + 0.328a\sqrt{\mu}} \quad (\text{acids}, A_m > A_I) \quad (12)$$

Analogous expressions for bases are as follows:

$$\mathrm{p}K_{\mathrm{a}}^{\mathrm{th}} = \mathrm{pH} + \log\left(\frac{A_{\mathrm{I}} - \frac{A}{t}}{\frac{A}{t} - A_{\mathrm{m}}}\right) - \frac{0.509z^2\sqrt{\mu}}{1 + 0.328a\sqrt{\mu}} \quad (\text{bases, } A_{\mathrm{I}} > A_{\mathrm{m}}) \tag{13}$$

$$\mathrm{p}K_{\mathrm{a}}^{\mathrm{th}} = \mathrm{pH} + \log\left(\frac{\frac{A}{t} - A_{\mathrm{I}}}{A_{\mathrm{m}} - \frac{A}{t}}\right) - \frac{0.509z^2\sqrt{\mu}}{1 + 0.328a\sqrt{\mu}} \quad (\text{bases, } A_{\mathrm{m}} > A_{\mathrm{I}}) \tag{14}$$

3. Experimental

All buffer and sample solutions were prepared in filtered and deionized water. The zwitterionic buffers shown in Table 1 were generated by titrating 20 m*M* solutions with 1 *M* NaOH until the desired pH was reached, as determined with an Orion EA 940 pH meter equipped with a Ross combination electrode. The series was designed so that the maximum gap between data points is 0.6 pH units.

Where applicable, electrophoretic mobilities were calculated according to the relation $m_{\mathrm{e}} = m_{\mathrm{app}} - m_{\mathrm{eof}}$, where m_{app} is the apparent mobility of an analyte and m_{eof} is the electroosmotic mobility. The electroosmotic mobility was determined by monitoring a species added to the sample vial, in this study 200 μM mesityl oxide, which remains neutral throughout the entire pH range.

A SpectraPHORESIS 1000 (Thermo-Separation Products, Fremont, CA, USA) CE instrument was used for all experiments. Instrument parameters included the following: voltage 25 kV, temperature 25°C and hydrodynamic injection time 2 s. Since the hydrodynamic injection rate for this instrument is nominally 6 nl/s for a 70 cm × 75 μm untreated fused-silica capillary (Polymicro Technology, Phoenix, AZ, USA), approximately 12 nl were loaded onto the column. Absorbance was monitored at 237 nm. The wash cycle performed prior to each run in a sequence consisted of 2.5 min with each of 0.1 *M* NaOH and deionized water followed by a 5-min rinse with the running buffer.

The buffers given in Table 1 were run in sequence from high to low pH. The Spec-

Table 1
Buffer series spanning pH range 6 to 11

Component	pH	[HZ]	$[Z^-]$	μ	$-\log\gamma$ [a]
MES, Morpholinoethanesulphonic acid	6.10	0.010	0.010	0.010	0.044
ACES, 2-[(2-amino-2-oxoethyl)amino]ethanesulphonic acid	6.65	0.012	0.008	0.008	0.040
MOPS, 3(4-morpholino)propanesulphonic acid	7.20	0.010	0.010	0.010	0.044
HEPES, N-(2-hydroxyethyl)piperazine-N′-ethanesulphonic acid	7.65	0.008	0.012	0.012	0.047
HEPPSO, N-(2-hydroxyethyl)piperazine-N′-2-hydroxypropanesulphonic acid	7.90	0.009	0.011	0.011	0.046
Tricine	8.10	0.010	0.010	0.010	0.044
TAPS, N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid	8.40	0.010	0.010	0.010	0.044
TAPS	8.70	0.007	0.013	0.013	0.049
AMPSO, 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulphonic acid	9.00	0.010	0.010	0.010	0.044
CHES, 2-(N-cyclohexylamino)ethanesulfonic acid	9.30	0.010	0.010	0.010	0.044
CAPSO, 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid	9.60	0.010	0.010	0.010	0.044
CAPSO	10.0	0.006	0.014	0.014	0.050
CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid	10.4	0.010	0.010	0.010	0.044
CAPS	11.0	0.004	0.016	0.016	0.053

[a] Sign of correction is + for acids and − for bases.

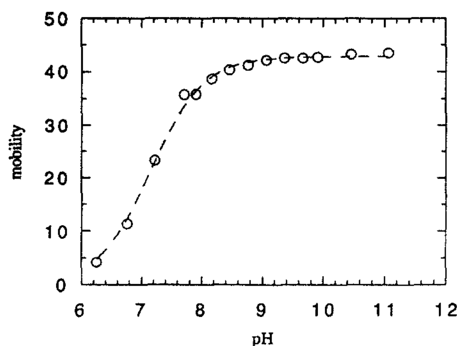

Fig. 1. Plot of net electrophoretic mobility (m_e, $\times 10^5$ cm^2/V s) vs. pH for 200 μM *p*-nitrophenol with superimposed curve fit.

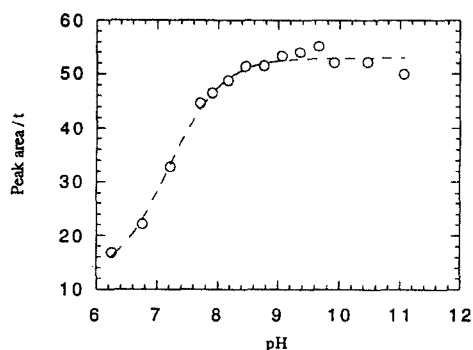

Fig. 2. Plot of peak area divided by migration time (arbitrary units) vs. pH for 200 μM *p*-nitrophenol with superimposed curve fit.

traPHORESIS 1000 is equipped with a single reservoir for the buffer at the detector end of the column. This reservoir was filled with 20 m*M* N-[tris(hydroxymethyl)methyl]glycine (Tricine) at a pH of 8.1. Since each run was 10 min in length, the total analysis time per sample was just under 5 h.

Peak areas and migration times were computed using the SpectraPHORESIS 1000 software package. The nonlinear regression was performed using Kaleidagraph (Synergy Software, Reading, PA, USA).

4. Results

Fig. 1 shows a plot of net electrophoretic mobility vs. pH for 200 μM *p*-nitrophenol along with the superimposed curve fit to Eq. 4. Values of pK_a and m_a as determined from the two-parameter non-linear regression are given in Table 2. For an independent data set acquired using the same sample, Fig. 2 shows a plot of peak area divided by migration time vs. pH with superimposed curve fit to Eq. 11. The three-parameter non-linear regression yields values of the parameters pK_a, A_I and A_m given in Table 2. The pK_a values determined by both methods have precisions of better than 1% and agree with the literature to within experimental error.

5. Discussion

An assumption of the spectroscopic method is that the total concentration of molecular and ionic species of a solute remain constant. As such, the method as described herein is strictly valid only with respect to hydrodynamic injections, where a constant amount of solute can be loaded onto the column in each run by simply specifying the injection period. In an electrokinetic injection, the total amount of an analyte loaded onto a column in a given period of time is not necessarily constant but depends on such factors as the relative ionic strengths and pH values of the sample solutions and running buffers.

Table 2
Comparison of CE-determined pK_a values with literature value for *p*-nitrophenol at 25°C [1]

Data source	pK_a	m_a ($\times 10^5$ cm^2/V s)	A_I (arb. units)	A_m (arb. units)
Literature	7.15	–	–	–
Mobility	7.15 ± 0.02	42.8 ± 0.3	–	–
Area	7.19 ± 0.07	–	53.0 ± 0.5	11.9 ± 1.8

Errors are given as standard deviations, where $n = 14$.

The spectroscopic method is not appropriate for an analyte at the solubility limit. Solubility, S'_0, is described according to the relations [1]:

$$S'_0 = S_i[1 + 10^{(pH-pK_a)}] \text{ (acids)} \quad (15)$$

$$S'_0 = S_i[1 + 10^{(pK_a-pH)}] \text{ (bases)} \quad (16)$$

where S_i, the intrinsic solubility, is usually determined in 0.01 *M* HCl for an acid and in 0.01 *M* NaOH for a base. For a saturated solution of an analyte, the relation between peak area and pH is governed not only by the relative absorption coefficients of the neutral and ionized species, but also by the relative solubilities of these species. Any attempt to apply the spectroscopic analysis to such a system must account for both of these effects.

The limitations of the spectroscopic method characterized above stem directly from the method's reliance on amplitude data. In contrast, mobility-based analysis relies essentially on frequency data and as such is not limited by the above consideration. In addition, mobilities are generally more reproducible than peak areas [12]. All these factors support the conclusion that mobility-based analysis should be more universally applicable and precise than the spectroscopic method when detection limit is not an issue.

Variation in temperature is undoubtedly a source of error for both methods, since mobility varies by 2.7% per °C [13] and buffer pH is temperature-dependent. However, the air-based cooling system of the instrument is quoted as being stable to $< \pm 1$°C, so that this effect is minimized. It is also possible that the temperature inside the capillary is higher than the surroundings due to the effects of Joule heating. Several studies have recently attempted to examine the magnitude of this difference both experimentally [14–19] and computationally [13]. Because of the low ionic strengths of the buffers used in this study, the current is so low (<20 μA) that internal heating is presumed to be insignificant.

6. Conclusions

In the determination of the ionization constant of *p*-nitrophenol from electrophoretic mobility, spectroscopic analysis based on peak areas was used as corroborating data. Peak areas divided by migration time versus pH for a constant solute concentration gave an ionic distribution plot which was fit using a non-linear regression. In order to emphasize the independence of the mobility- and spectroscopic-based methods of analysis, each was applied here to a different data set. However, the information required for both methods is supplied in each CE data set. As such, it is possible to perform two independent analyses concurrently, thereby providing an internal check on each method.

References

[1] A. Albert and E.P. Serjeant, *The Determination of Ionization Constants: A Laboratory Manual*, Chapman & Hall, New York, 3rd ed., 1984.

[2] J.L. Beckers, F.M. Everaerts and M.T. Ackermans, *J. Chromatogr.*, 537 (1991) 407.

[3] J. Cai, J.T. Smith and Z. El Rassi, *J. High Resolut. Chromatogr.*, 15 (1992) 30.

[4] J.A. Cleveland, Jr., M.H. Benko, S.J. Gluck and Y.M. Walbroehl, *J. Chromatogr. A*, 652 (1993) 301.

[5] J. Kielland, *J. Am. Chem. Soc.*, 59 (1937) 1675.

[6] H.A. Abramson, *J. Gen. Physiol.*, 15 (1931) 575.

[7] L.G. Longsworth, *Ann. NY Acad. Sci.*, 41 (1941) 267.

[8] C.J.O.R. Morris and P. Morris, *Separation Methods in Biochemistry*, Pitman Publ., London, 2nd ed., 1976, p. 714.

[9] D.M. Goodall, S.J. Williams and D.K. Lloyd, *Trends Anal. Chem.*, 10 (1991) 272.

[10] D. Demoret and J. Dubrow, *J. Chromatogr.*, 559 (1991) 43.

[11] S. Hjertén, K. Elenbring, F. Kilar, J.-L. Liao, A.J.C. Chress, C.J. Sierbert and M.-D. Zhu, *J. Chromatogr.*, 403 (1987) 47.

[12] J.A. Lux, H.F. Yin and G. Schomburg, *Chromatographia*, 30 (1990) 7.

[13] S. Hjertén, *Electrophoresis*, 11 (1990) 665.

[14] M.S. Bello, M. Chiari, M. Nesi and P.G. Righetti, *J. Chromatogr.*, 625 (1992) 323.

[15] T.L. Rapp, W.K. Kowalchyk, K.L. Davis, E.A. Todd, K.-L. Liu and M.D. Morris, *Anal. Chem.*, 64 (1992) 2434.

[16] H. Watzig, *Chromatographia*, 33 (1992) 445.

[17] S. Hjertén, *Chromatogr. Rev.*, 9 (1967) 122.

[18] T.T. Lee and E.S. Yeung, *Anal. Chem.*, 63 (1991) 2842.

[19] M.S. Bello, E.I. Levin and P.G. Righetti, *J. Chromatogr. A*, 652 (1993) 329.