ELSEVIER

Journal of Chromatography A, 680 (1994) 43–48

JOURNAL OF CHROMATOGRAPHY A

Capillary zone electrophoresis for the determination of dissociation constants

S.J. Gluck*[,a], J.A. Cleveland, Jr.[b]

[a]*Analytical Sciences, 1897B Bldg, Dow Chemical Co., Midland MI 48667, USA*

[b]*Discovery Research, DowElanco, 9410 Zionsville Rd, Indianapolis IN 46268-1053, USA*

Abstract

Automated capillary electrophoresis is an effective method for the determination of pK_a values. Advantages include having high sensitivity for poorly soluble solutes, not having to know the concentration of the solutes, and the simplicity of the method. The procedure, further investigated in this work, yielded determined pK_a values to within 0.07 units of literature values from the IUPAC database for 18 solutes having pK_a values of less than 9. The range evaluated was 2.43 to 9.99. Bases with pK_a values above 9 had significant differences with the literature values. Wall adsorption and concentration effects were not potential contributors to these differences. Spectroscopic pK_a determinations for three solutes showing large differences with the literature values agree with the capillary electrophoresis determinations.

1. Introduction

Dissociation constants (i.e., pK_a values) can be a key parameter for understanding and quantifying chemical phenomenon such as reaction rates, biological activity, biological uptake, biological transport and environmental fate [1–3]. The determination of dissociation constants of weakly acidic or basic compounds is routine using established techniques if the compound has amenable physical properties [4–7]. However, the low solubility of many pharmaceutical and agricultural compounds in water precludes convenient pK_a determinations. Indeed, many new herbicides and pesticides have poor water solubility specifically designed into the molecules for environmental concerns. Previous papers [8–10] have introduced the use of capillary electrophoresis (CE) for pK_a determination. We have used this approach because of its high sensitivity and selectivity relative to potentiometry. In this paper we continue to explore the benefits of CE for pK_a measurements and build upon the previous work in this area. In particular, this work includes more compounds of known literature pK_a values thus extending the demonstrated range.

Equations which relate electrophoretic mobility to pK_a have been adequately derived previously [10]. In brief, the thermodynamic pK_a is related in an expression similar to the Henderson–Hasselbach equation with electrophoretic

* Corresponding author.

SSDI 0021-9673(94)00369-K

mobility used to describe the state of solute ionization

$$pK_a = pH + \log\left[\frac{\mu_e}{\mu_{BH^+} - \mu_e}\right] - \frac{0.5085z^2\sqrt{I}}{1 + 0.3281a\sqrt{I}} \text{ (bases)} \quad (1)$$

$$pK_a = pH - \log\left[\frac{\mu_e}{\mu_{A^-} - \mu_e}\right] + \frac{0.5085z^2\sqrt{I}}{1 + 0.3281a\sqrt{I}} \text{ (acids)} \quad (2)$$

where μ_{BH^+} is the electrophoretic mobility of the fully protonated base, μ_{A^-} is the electrophoretic mobility of the fully deprotonated acid and μ_e is the electrophoretic mobility observed at the experimental pH. The third term in Eqs. 1 and 2 is the activity correction for buffer ionic strength, I. The variable z is the valency of the buffer and a is the ionic size parameter, 5 Å.

An alternative approach is to use linear forms of Eqs. 1 and 2 [9,11]. In theory, either approach is acceptable, but in practice, the non-linear equations shown here will give better results [11]. The K_a is linear with $1/\{H^+\}$. To cover a wide range of pH, buffers are generally chosen with equally spaced pH values. When linear regression is performed to determine the K_a, there will be highly leveraged, influential data introducing a significant error in the result. A weighted linear regression requires too many runs to determine the weights and is thus impractical. Non-linear regression of Eqs. 1 or 2 using equally spaced pH buffers about the pK_a minimizes the potential for highly leveraged, influential data.

2. Experimental

2.1. Apparatus and method

A SpectraPHORESIS 1000 CE (Thermal Separation Systems, Fremont, CA, USA) was used for all experiments. Typically, a 2-s hydrodynamic injection was performed. Since the hydrodynamic injection rate is ca. 6 nl/s for a 67 cm × 75 μm untreated fused-silica capillary (Polymicro Technology, Phoenix, AZ, USA), ca. 12 nl was loaded onto the column. The separation distance, L_d, was 59.5 cm. The temperature was set at 25°C. Absorbance was monitored at a wavelength appropriate for the solute and, usually, at 240 nm for the neutral marker, mesityl oxide. This instrument has a high speed slewing monochromator allowing multiple wavelength detection. With the instrument operating at 25 kV, typical currents were less than 20 μA.

In order to equilibrate the column and thereby minimize hysteresis effects [10,12], the following wash cycle was performed prior to each run in a sequence: (1) 5 min with 1 *M* NaOH, (2) 5 min with H_2O, and (3) 3.0 min with running buffer.

Because the SpectraPHORESIS 1000 is equipped with a single reservoir for the buffer near the detector, it is not possible to match buffers at each end of the column in a sequence. Tricine (0.02 *M*, pH 7.6 to 8.1) was used as the buffer at the detector end of the column.

Buffer pH was measured with an Orion Ross pH electrode and an Orion Model 601A pH meter and in later work a Fisher Accuphast pH electrode or an Orion Model EA940 meter. Meter calibrations were made with Fisher NIST traceable buffer solutions.

Method

All solutions were prepared using distilled, deionized, and filtered water (ASTM type I specification). A 100-μl aliquot of 10 m*M* mesityl oxide in water (neutral marker) and 900 μl analyte solution typically were combined into a 2-ml sample vial to give a final concentration of between 10 and 900 μ*M* analyte and 1 m*M* mesityl oxide.

Data pairs of the activity corrected pH and μ_e were imported into Mathcad 4.0 (MathSoft, Cambridge, MA, USA) where μ_{BH^+} or μ_{Z^-} and pK_a were determined by performing a non-linear fit to Eqs. 1 or 2.

3. Spectroscopic pK_a determination

Spectroscopic pK_a values of several bases were determined by taking UV spectra with an Hitachi Model 3101 spectrophotometer using 1-cm

cuvets at 23°C. Samples were prepared in the same buffer series as used in the CE experiments. Absorbance measurements were taken at a wavelength which showed a significant difference as a function of pH for each solute, 220 nm for 2- and 3-methylbenzylamine, 230 nm for 2-methoxybenzylamine. The pK_a values were determined using a non-linear fit of the absorbance, A, versus the pH [13]

$$pK_a = pH + \log\left[\frac{A - A_{BH^+}}{A_{BH^+} - A_B}\right] - \frac{0.5085z^2\sqrt{I}}{1 + 0.3281a\sqrt{I}} \quad (3)$$

Where A_{BH^+} and A_B are parameters also determined by the regression.

3.1. Buffer series

Different buffer series were used throughout this investigation. Most of the work was done at a constant ionic strength to enable a more consistent electroosmotic flow (EOF) between different buffers [14]. At an ionic strength of 0.01 M, the activity correction for the ionic strength of the solution is 0.05 making calculations somewhat simpler. The concentrations and the pH values of this buffer series are listed in Table 1. Buffer components were obtained from different producers and were of the highest available purity.

4. Results

4.1. Buffer capacities

A consideration in creating the buffer series in Table 1 was the buffer capacity, β, of each buffer. The β is defined as [15]

$$\beta = \frac{dC_a}{dpH} = 2.3\left(\frac{K_w}{[H^+]} + [H^+] + \frac{K_a C_t [H^+]}{(K_a + [H^+])^2}\right) \quad (4)$$

Table 1
Buffer series, pH, pK_a values, concentrations, buffer capacities and estimated pH change upon the addition of a 1-s injection of a 100 μM strong acid or base

Buffer[a]	pH	pK_a	Concentration of buffer (M)	Buffer capacity β (M/pH)	Estimated pH change
CAPS	10.96	10.4	$1.22 \cdot 10^{-2}$	$6.85 \cdot 10^{-3}$	$4.82 \cdot 10^{-3}$
CAPS	10.65	10.4	$1.71 \cdot 10^{-2}$	$1.01 \cdot 10^{-2}$	$3.27 \cdot 10^{-3}$
CAPS	10.12	10.4	$3.24 \cdot 10^{-2}$	$1.71 \cdot 10^{-2}$	$1.93 \cdot 10^{-3}$
CAPSO	9.76	9.6	$2.12 \cdot 10^{-2}$	$1.19 \cdot 10^{-2}$	$2.77 \cdot 10^{-3}$
AMPSO	9.16	9	$1.89 \cdot 10^{-2}$	$1.05 \cdot 10^{-2}$	$3.13 \cdot 10^{-3}$
TAPS	8.61	8.4	$1.71 \cdot 10^{-2}$	$9.29 \cdot 10^{-3}$	$3.55 \cdot 10^{-3}$
Tricine	7.94	8.1	$2.12 \cdot 10^{-2}$	$1.18 \cdot 10^{-2}$	$2.80 \cdot 10^{-3}$
HEPES	7.43	7.5	$1.89 \cdot 10^{-2}$	$1.08 \cdot 10^{-2}$	$3.06 \cdot 10^{-3}$
MOPS	6.94	7.2	$2.41 \cdot 10^{-2}$	$1.27 \cdot 10^{-2}$	$2.60 \cdot 10^{-3}$
ACES	6.53	6.8	$2.78 \cdot 10^{-2}$	$1.45 \cdot 10^{-2}$	$2.27 \cdot 10^{-3}$
MES	6.09	6.1	$2.12 \cdot 10^{-2}$	$1.22 \cdot 10^{-2}$	$2.71 \cdot 10^{-3}$
Acetic	5.46	4.75	$1.16 \cdot 10^{-2}$	$3.65 \cdot 10^{-3}$	$9.04 \cdot 10^{-3}$
Acetic	5.04	4.75	$1.50 \cdot 10^{-2}$	$7.75 \cdot 10^{-3}$	$4.26 \cdot 10^{-3}$
Acetic	4.45	4.75	$2.58 \cdot 10^{-2}$	$1.33 \cdot 10^{-2}$	$2.49 \cdot 10^{-3}$
Formic	4.16	3.75	$1.50 \cdot 10^{-2}$	$7.12 \cdot 10^{-3}$	$4.64 \cdot 10^{-3}$
Formic	3.54	3.75	$2.58 \cdot 10^{-2}$	$1.47 \cdot 10^{-2}$	$2.25 \cdot 10^{-3}$
Formic	3.05	3.75	$6.01 \cdot 10^{-2}$	$2.12 \cdot 10^{-2}$	$1.56 \cdot 10^{-3}$

[a] Buffer abbreviations: CAPSO = 3-[cyclohexylamino]-2-hydroxy-1-propanesulfonic acid; CAPS = 3-[cyclohexylamino]-1-propanesulfonic acid; AMPSO = 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropane sulfonic acid; TAPS = N-tris[hydroxymethyl]methyl-3-amino-propanesulfonic acid; Tricine = N-tris[hydroxymethyl]methylglycine; HEPES = N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]; MOPS = 3-[N-morpholino]propanesulfonic acid; ACES = (N-[2-acetamido]-2-aminoethanesulfonic acid; MES = 2-[N-morpholino]ethanesulfonic acid.

Where C_a is the concentration of the buffer acid, K_w is the equilibrium constant for the dissociation of water, K_a is the equilibrium constant for the dissociation of the buffer acid, and C_t is the total concentration of the buffer. Too low of a buffer capacity would result in the inability of the buffer to maintain pH control in the analyte zone. At the pH of the buffers, the actual buffer capacities are listed in Table 1 as well as the expected change in pH for the 1-s injection of a 100 μM strong acid or base. The maximum impact on the pH for a 6-nl (1-s) injection, with a typical 18-nl peak width at the detector, of a 100 μM strong acid or base is estimated to be insignificant ($\Delta pH < 0.01$) for every buffer in the series. Indeed, if the pH of the analyte zone were different than that of the run buffer, then there would be a bias in the final pK_a determination.

4.2. pK_a determinations

The pK_a values for a series of 23 acids and bases were determined (7 of these values were reported by us previously [10]). The results of those determinations are shown in Table 2. Less than a 0.07 difference of pK_a with the literature values [16,17] were obtained for pK_a values less than 9. These differences are probably reflective of the random error of the procedure. As an example, for a single compound, the random error was ± 0.071 at 95% confidence, from 10 separate determinations of 2-aminopyridine.

There were significant differences between the CE-determined pK_a values of bases with literature pK_a values greater than 9. A series of simple experiments were run to elucidate this inconsistency. In the first experiment, a weak acid with a high pK_a value, phenol, was shown to be in agreement with the literature value. This determination suggests that there is no inherent problem with the high pH buffers which could bias the result. Ionic interactions of amines with silica surfaces are a well established phenomenon in separations. At pH values greater than 8, the silica surface is fully charged. Indeed, the EOF is nearly consistent above pH 7, whereas below 7 it decreases. Thus, ion exchange with the column surface for the simple molecules in this study would be expected to correlate with α, the fractional degree of ionization. The ion-exchange wall effects would result in a proportionately decreased mobility relative to no ion exchange with the wall, and hence, would not impact the pK_a determination. Any other adsorptive effect could be measured by a non-zero mobility at a pH 2 or more units below the pK_a. There was no adsorptive effect which was measurable in our experiments. Indeed, if the electrophoretic mobility were slower due to adsorption, then the determined pK_a would be less than the actual pK_a. In the questioned data here, all of the pK_a values were higher. Also, there was excellent agreement of the literature values for nicotine, 8.08 by CE versus 8.02 literature and quinine, 8.52 by both CE and literature. The wall would be expected to have a similar effect in all the pH buffers above 7.

If there was an effect from adsorption or ion exchange, then the effect could be masked with an amine with a higher pK_a value than the solute at a higher concentration in the buffer. Indeed,

Table 2
CE pK_a determinations versus literature values

Molecule	pK_a (lit.)	pK_a (CE)	Difference
o-Bromoaniline	2.53	2.55	−0.02
Salicylic acid	2.98	2.96	0.02
p-Bromoaniline	3.88	3.85	0.03
Benzoic acid	4.2	4.18	0.02
2-Ethylaniline	4.37	4.32	0.05
Cinnamic acid	4.4	4.4	0
Aniline	4.6	4.66	−0.06
2-Ethylaniline	4.7	4.69	0.01
3-Ethylaniline	5.07	5.09	−0.02
Pyridine	5.19	5.26	−0.07
N,N′-dimethylaniline	5.99	5.96	0.03
4-Nitrophenol	7.15	7.15	0
2-Aminopyridine	6.71	6.76	−0.05
Nicotine	8.02	8.08	−0.06
Quinine	8.52	8.52	0
4-*tert.*-Butylpyridine	9.08	9.52	−0.44
4-Aminopyridine	9.11	9.25	−0.14
2-Methybenzylamine	9.19	9.4	−0.21
3-Methylbenzylamine	9.33	9.57	−0.24
2-Methoxybenzylamine	9.71	0.05	−0.35
α-Methylbenzylamine	9.83	9.94	−0.11
Phenethylamine	9.83	10.03	−0.2
Phenol	9.99	9.9	10.08

Table 3
Spectroscopic determination of pK_a values compared to literature and CE

Solute	pK_a literature	pK_a spectroscopic	pK_a CE
2-Methylbenzylamine	9.19	9.48	9.40
3-Methylbenzylamine	9.70	9.54	9.57
2-Methoxybenzylamine	9.33	9.92	10.05

the buffer series was prepared using pyrollidine ($pK_a = 11.27$) as the neutralizing base instead of sodium hydroxide. With the pyrollidine buffer series, the pK_a was determined for 2-methoxybenzylamine to be 9.96, more consistent with the previously determined CE value of 10.05 and inconsistent with the literature value of 9.70.

As calculated above, the buffers had enough capacity to prevent a significant change in the pH of the zone impacting the measurement. To prove this effect, the concentration of 4-aminopyridine was varied from 10 to 900 μM and there was no correlation with the pK_a determined.

To referee this inconsistency with the literature, the pK_a values of three bases with large errors were determined spectroscopically. (Solubility was too low for an accurate potentiometric determination.) Spectroscopic pK_a determination relies on the change in solute absorbance at a specific wavelength versus pH. As shown in Table 3, the spectroscopic determined pK_a values agree much more closely with the CE determined values.

All of the experiments to elucidate the inconsistency of the CE data with the literature data together suggest problems with the literature values for these particular bases. The method cited in the IUPAC database for these compounds was to prepare a sample at 0.02 M containing an equal concentration of salt and base and to measure the pH of that solution with a hydrogen electrode [18].

5. Conclusions

CE is an effective method for determining pK_a values. The method is particularly useful for determining the pK_a values of compounds with low water solubility. For example, compounds of limited water solubility need not be prepared in a co-solvent as is required by potentiometry, and it is not necessary to accurately know the concentration of a titrant or solute. There is no time consuming preparation of carbonate-free buffers. The detection limit for a 12-nl injection, reported previously [10] was $\epsilon^{220}bc = 1 \cdot 10^{-4}$ which, for benzoic acid, was 2 μM, 50 times lower than a typical determination limit via potentiometric titration. The procedure gave determined pK_a values to within 0.07 units of literature values from the IUPAC database for analytes below a pK_a value of 9. The range evaluated was 2.43 to 9.99. The analysis time was between 2 and 3.5 h per solute and no attempts were made to minimize this time.

Differences between CE-determined values and literature values for high pK_a bases were investigated by taking a series of 9 bases with literature pK_a values ranging between 8.02 and 9.83 and comparing the CE determined values with the literature values. Literature values above 9 were significantly lower than the CE-determined values. This difference was not due to an inherent limitation in the procedure for high pK_a determinations; a weak acid with a pK_a above 9 was accurately determined. Concentration effects and ionic interactions with the capillary wall were systematically eliminated as potential reasons for the differences also. To referee the differences between the historical data and the CE data, the pK_a values were determined spectroscopically for three compounds with large errors. The spectroscopic results agreed more closely with the CE results suggesting that the literature values of the solutes chosen may be unreliable.

Acknowledgements

The inspiration and support of Yvonne Walbroehl of the Dow Chemical Analytical Sciences Laboratory and Marguerite Benko of the DowElanco Discovery Department are gratefully acknowledged.

References

[1] L.Z. Benet and J.E. Goyan, *J. Pharm. Sci.*, 56 (1967) 665.
[2] A. Roda, A. Minutello and A. Fini, *J. Lipid Res.*, 31 (1990) 1433.
[3] R.A. Scherrer, in P.S. Magee, G.K. Kohn and J.J. Menn (Editors), *Pesticide Synthesis through Rational Approaches (ACS Symposium Series*, No. 255), American Chemical Society, Washington, DC, 1984, p. 225.
[4] R.F. Cookson, *Chem. Rev.*, 74 (1974)1.
[5] A. Albert and E.P. Serjeant, *The Determination of Ionization Constants: A Laboratory Manual*, Chapman and Hall, New York, 3rd ed., 1984.
[6] E.J. King, *Acid-Base Equilibria*, Pergamon, Oxford, 1965.
[7] J.B. Hansen and O. Hafliger, *J. Pharm. Sci.*, 72 (1983) 429.
[8] J.L. Beckers, F.M. Everaerts and M.T. Ackermans, *J. Chromatogr.*, 537 (1991) 407.
[9] J. Cai, J.T. Smith and Z. El Rassi, *J. High Resolut. Chromatogr.*, 15 (1992) 30.
[10] J.A. Cleveland, M.H. Benko, S.J. Gluck and Y.M. Walbroehl, *J. Chromatogr. A*, 652 (1993) 301.
[11] S.J. Gluck and J.A. Cleveland, *J. Chromatogr. A*, 680 (1994) 49.
[12] W.J. Lambert and D.L. Middleton, *Anal. Chem.*, 62 (1990) 1585.
[13] J.A. Cleveland, C.L. Martin and S.J. Gluck, *J. Chromatogr. A*, 679 (1994) 167.
[14] J. Vindevogel and P. Sandra, *J. Chromatogr.*, 541 (1991) 483.
[15] C.J. Nyman and R.E. Hamm, *Chemical Equilibrium*, Raytheon Education Company, 1968.
[16] Z. Rappoport (Editor), *CRC Handbook of Tables for Organic Compound Identification*, CRC Press, Cleveland., 3rd ed., 1967, pp. 428–439.
[17] D.D. Perrin (Editor), *Dissociation Constants of Organic Bases in Aqueous Solution*, Butterworths, London, 1965.
[18] W.H. Carothers, C.F. Bickford and G.J. Hurwitz, *J. Am. Chem. Soc.*, 49 (1927) 2908.