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Determination of acidity constants of monoprotic and diprotic acids by capillary electrophoresis

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Abstract

Capillary electrophoresis as a tool to determine thermodynamic pK_a values of monoprotic and diprotic acids has been extended to the pK_a range between 1.5 and 3.4. Previously developed procedures for determinations of pK_a values in this range have been difficult to implement using conventional fused-silica capillaries. The improved procedure uses a dynamic coating of a positively charged polymer to impart a positive charge to the capillary thus making the electroosmotic flow stable and less dependent on pH than is generally found with uncoated fused-silica capillaries. The dynamic coating reverses the electroosmotic flow allowing the acids to elute before neutral solutes. The procedure was evaluated using five monoprotic and three diprotic acids. For multiprotic solutes, a general model was presented. The pK_a values in all cases were easily determined by nonlinear regression with the appropriate equation. It was found that for diprotic cases, where the two pK_a values differ by approximately one pK_a unit, the determination of the pK_a from the migration data is less precise. This is an inherent limitation of this method that is also shared with any other method for determining the pK_a values for multi-protic solutes.

Keywords: Dissociation constants; Monte Carlo calculations; Electrophoretic mobility; Organic acids

1. Introduction

Dissociation constants (i.e., pK_a values) can be a key parameter for understanding and quantifying chemical phenomena such as reaction rates, biological activity, biological uptake, biological transport and environmental fate [1]. The discovery of new, systemic xenobiotics requires accurate pK_a value determinations. As an example, the mobility of weak acids in the phloem of a plant has been described as a function of the membrane permeabilities and the pK_a [2]. Hence, it is necessary to screen new compounds for their pK_a values. A concern of the analysis process in the discovery role is the amount and number of materials to be analyzed. Since new compounds only exist in small quantities, a step towards a significant improvement in discovery productivity is achieved if the pK_a of less than a milligram of material can be determined. The low solubility of many pharmaceutical and agricultural compounds in water precludes convenient, classical pK_a determinations. Indeed, many new xenobiotics have poor water solubility specifically designed into the molecules for environmental concerns. These new material challenges have thus resulted in new challenges for the analyst.

Recently [3–9] capillary electrophoresis (CE) has been introduced as a method for convenient and precise aqueous pK_a determination. Its features allow unattended sample analysis of microgram material quantities. The method relies on the principle that a

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solute exhibits an electrophoretic mobility continuum versus pH. In its neutral state, the solute has no mobility, in its fully charged state, it has its maximum mobility. Intermediate mobilities are a function of dissociation equilibrium and can be solved for by regression analysis. The parameters of the regression of mobility versus pH are the pK_a and the electrophoretic mobility of the fully charged solute. We have used this approach because of its high sensitivity and selectivity relative to potentiometry and spectrophotometry.

This paper deals with limitations with the previous efforts in pK_a determination by CE. At low pH, both the mobility of acids and the electroosmotic flow decreases. The practical manifestations are first recognized as an increase in analysis time. Indeed, for acids with a pK_a less than 4, the solute will generally move in a direction opposite to the electroosmotic flow. This phenomenon results in several practical limitations including necessitating a reversal of instrument polarity, injection of the neutral marker at the detector end of the column and the inability to detect the solute when the electroosmotic flow is nearly equal to the negative of the electrophoretic mobility and hence resulting in a region where mobilities cannot be determined accurately. In a worst case situation, we have seen diprotic acids move opposite to the direction of the electroosmotic flow at high pH, at intermediate pH the direction will be aligned with the electroosmotic flow, at lower pH it will be in the opposite direction and at the lowest pH it will be aligned. In the region of pH 2–7, the slow hysteresis of electroosmotic flow [10] can thus turn a 2 h pK_a determination into a problem which may require several weeks of frustrating work. Indeed, pK_a determination below 4 for weak acids cannot be done reliably with fused-silica capillaries.

This paper presents the first practical means to determine acid pK_a values less than 4. In brief, the solution has been to impart a positively charged surface on the CE column to reverse the direction of the electroosmotic flow [11,12]. In this mode, using negative high voltage, the acids elute first, before the neutral marker, overcoming all of the problems mentioned above. We also briefly investigate the average internal temperature of the background electrolyte within the column.

2. Theory

The solutions for the determination of the dissociation constant for acids and bases by CE were developed and evaluated in earlier reports [3–7]. The principle behind these solutions is that at a pH where the solute is neutral, its electrophoretic mobility (μ) is equal to zero. At a pH where the solute is fully ionized, μ is equal to the limiting electrophoretic mobility (μ_L). At all other pHs where the solute is partially ionized, the μ can be described by Eq. 1 or Eq. 2 for an acid. Similar equations can be derived for bases.

$$\mu = \frac{\mu_L \cdot 10^{pH_c - pK_a}}{1 + 10^{pH_c - pK_a}} \quad (1)$$

$$\frac{1}{\mu} = \frac{\{H^+\}\gamma}{K\mu_L} + \frac{1}{\mu_L} \quad (2)$$

The γ is the activity coefficient and $\{H^+\}$ is the activity of the hydrogen ion. The pH_c is the activity corrected pH

$$pH_c = pH - (-\log(\gamma)) \quad (3)$$

The activity coefficient is solved by either the extended Debye–Hückel equation (EDHE) [13] or the Davies modification to the EDHE [14] and it is used to correct for effect of solution ionic strength on solute ionization. This correction gives thermodynamic pK_a values as opposed to concentration dependent values or mixed constants [15]. Eq. 1 is solved by non-linear regression of the mobilities versus the pH. Eq. 2 is solved by linear, or weighted linear regression. Non-linear regression using Eq. 1 will give more precise results if the pH values are spaced evenly about the pK_a thus leading to a better balanced experimental design relative to what can be accomplished using Eq. 2.

Our approach for handling multi-protic solutes is also based on equilibrium expressions and regression analysis as follows:

The dissociation of a diprotic solute is described in the following steps for the concentrations of the individual species and the hydrogen ion activity

$$H_2A \Leftrightarrow HA + H \quad (4)$$

and

$$HA \Leftrightarrow H + A \quad (5)$$

For a diacid, H_2A is neutral, HA is singly charged and A is doubly charged. For a dibase, H_2A is doubly charged (positive), HA is singly charged and A is neutral. For a zwitterionic or an amphoteric solute[1], H_2A is positively charged, HA is neutral and A is negatively charged. Thus the mixed dissociation constants, K_1 and K_2 are determined by

$$K_1 = \frac{H \cdot HA}{H_2A} \quad (6)$$

$$K_2 = \frac{H \cdot A}{HA} \quad (7)$$

The total concentration of all the species, C_t, can be expressed in terms of the dissociation constants, the hydrogen ion activity and the diprotonated species concentration.

$$C_t = (H^2 + K_1H + K_1K_2)\frac{H_2A}{H^2} \quad (8)$$

From Eq. 8, the fractions, F, of the individual species are

$$F_{H_2A} = \frac{H^2}{(H^2 + K_1H + K_1K_2)} \quad (9)$$

$$F_{HA} = \frac{K_1H}{(H^2 + K_1H + K_1K_2)} \quad (10)$$

$$F_A = \frac{K_1K_2}{(H^2 + K_1H + K_1K_2)} \quad (11)$$

The observed electrophoretic mobility is the sum of the contributions of the mobilities of each species

$$\mu = \mu_1F_{H_2A} + \mu_2F_{HA} + \mu_3F_A \quad (12)$$

By substituting and simplifying we obtain

$$\mu = \frac{(\mu_1H^2 + \mu_2K_1H + \mu_3K_1K_2)}{(H^2 + K_1H + K_1K_2)} \quad (13)$$

There are two μ parameters to solve for in Eq. 13, one is equal to 0, and two dissociation constants; hence a minimum of four runs would be required for a solution to this problem. Thermodynamic dissociation constants are determined using the hydrogen ion activity corrected for solution ionic strength as in Eqs. 1 and 3.

Similar expressions may be derived for triprotic (Eq. 14) and tetraprotic (Eq. 15) species

$$\mu = \frac{\mu_3H^3 + \mu_2H^2K_1 + \mu_1HK_1K_2 + \mu_0K_1K_2K_3}{H^3 + H^2K_1 + HK_1K_2 + K_1K_2K_3} \quad (14)$$

$$\mu = \frac{\mu_4H^4 + \mu_3H^3K_1 + \mu_2H^2K_1K_2 + \mu_1HK_1K_2K_3 + \mu_0K_1K_2K_3K_4}{H^4 + H^3K_1 + H^2K_1K_2 + HK_1K_2K_3 + K_1K_2K_3K_4} \quad (15)$$

The general solution to the mobility of polyprotic species is

$$\mu = \frac{\sum_{i=0}^{n} H^{n-i}\mu_{n-i}\prod_{j=1}^{i} K_j}{\sum_{i=0}^{n} H^{n-i}\prod_{j=1}^{i} K_j} \quad (16)$$

where n is the number of ionization states plus one.

3. Experimental

3.1. Apparatus

A Beckman (Fullerton, CA, USA) model 5510 P/ACE capillary electrophoresis unit with a photodiode array detector scanning from 190 to 290 nm and monitoring at 240 nm was used for this study. A 50 μm I.D.×365 μm O.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with a length of 19.9 cm to the detector and 26.5 cm overall was used at electric fields ranging from −10 kV to −1 kV depending on the conductivity of the background electrolyte. Buffer pH was measured on an Orion EA940 ion analyzer (ATI, Boston, MA, USA)

[1]To distinguish the sometimes misunderstood difference between a zwitterionic and an amphoteric solute, a zwitterion is a solute wherein both the protonated and deprotonated species can exist at the same pH, an amphoteric solute will have the pK_a values of the acid and the base separated by at least 2 units. Thus amphoteric solutes can only exist with a single charge state.

Table 1
Buffer preparation for background electrolytes

Acid	pK_a	Ionic strength	$-\log \gamma$	Concentration of acid (mM)	pH measured
Acetic	4.75	0.01	0.05	10	5.94
Acetic	4.75	0.01	0.05	11	5.83
Acetic	4.75	0.01	0.05	12	5.53
Acetic	4.75	0.01	0.05	15	5.03
Acetic	4.75	0.01	0.05	26	4.53
Formic	3.75	0.01	0.05	15	4.26
Formic	3.75	0.01	0.05	12	3.81
Formic	3.75	0.01	0.05	26	3.53
Formic	3.75	0.01	0.05	60	3.04
Phosphoric	2.12	0.01	0.05	14	2.60
Formic	3.75	0.01	0.05	170	2.59
Phosphoric	2.12	0.01	0.05	22	2.45
Phosphoric	2.12	0.01	0.05	34	2.24
Phosphoric	2.12	0.01	0.05	47	2.02
Phosphoric	2.12	0.02	0.07	130	1.65
Phosphoric	2.12	0.04	0.09	150	1.45
Phosphoric	2.12	0.07	0.12	440	1.15
Phosphoric	2.12	0.26	0.16	1480	0.59

All buffers were prepared from the free acid dissolved in 0.01 M NaOH except for those below pH 1.5 which were prepared directly in water.

using a Ross pH electrode. A prerinse of 1 min of Micro-Coat (Perkin-Elmer, Foster City, CA, USA) at 20 p.s.i.g. (1 p.s.i. = 6894.76 Pa) was followed by a 2 min rinse of background electrolyte. Samples of about 100 ppm concentration with 1% acetone as the neutral marker were dissolved in background electrolyte and injected for 2 s at 0.5 p.s.i.g. After each run, the column was rinsed with 0.01 M NaOH for 0.5 min at 20 p.s.i.g. The buffers were the same at each end of the column.

3.2. Buffer and reagent preparation

Buffers were prepared as shown in Table 1. The reagent for creating a positively charged column surface, Micro-Coat, was prepared according to the vendor's directions as a 2% aqueous ethylene glycol solution.

4. Results

4.1. Temperature determination inside of column

Acid dissociation constants have varying degrees of temperature dependencies. In our work we wanted to have reasonable assurance that the background electrolyte temperature was close to the thermostated temperature, 25°. We chose to investigate three simple methods for measuring the temperature of the background electrolyte under high voltage conditions. The methods of measuring the conductivities at a low (σ_1) and high voltage (σ_2) [16], measuring the difference in electroosmotic flow at low (μ_{eof1}) and high voltages (μ_{eof2}) [16], and monitoring the absorbance of a Co(II) solution at different voltages [17] were attempted. In the first method, the temperature inside the column, T_2, is

$$T_2 = \frac{\frac{\sigma_2}{\sigma_1} - 1}{0.0205} + 25°C \quad (17)$$

In the second method, the T_2 is

$$T_2 = \frac{1820K}{\ln(\mu_{eof1}) - \ln(\mu_{eof2}) + 6.11} \quad (18)$$

In the third method, a calibration curve was constructed based on the absorbance at 495 nm of a 25 mM solution of $CoCl_2$ in the pH 2 buffer. The solution was pumped at 0.5 p.s.i.g. while the temperature was thermostated from 25 to 50°C. The calibration curve is in Fig. 1. The voltage was then

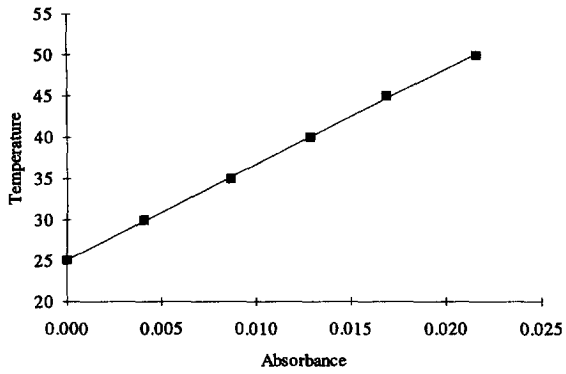

Fig. 1. Thermostated temperature versus absorbance of Co(II) in a 50 μm column.

varied at a thermostated temperature of 25°C and, from the resulting absorbance, the temperature was determined. A plot of the temperature versus the power is in Fig. 2 for this system. Based on this plot and the powers applied for every background electrolyte, the temperatures were always between 25 and 26°C. The other two methods failed to give reproducible results in this particular system which uses a dynamic column coating procedure.

4.2. Determination of pK_a values

Nonlinear optimization was used to determine the parameter values in either Eq. 1 or Eq. 13 from the measured mobility data. For simple determinations,

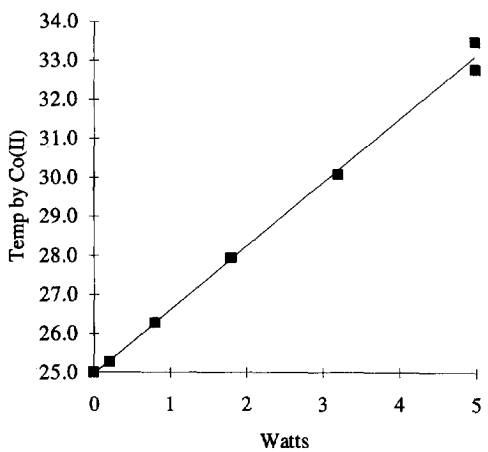

Fig. 2. Temperature of the background electrolyte, determined by the absorbance procedure, versus power.

we use the Levenburg–Marquardt algorithm and the PC program Mathcad 5.0 (Math Soft, Cambridge, MA, USA). For more in-depth studies of the goodness of the fit, we use the modelling and simulation software SimuSolv (Dow Chemical, Midland, MI, USA). The pK_a values determined by this procedure are listed below in Table 2.

Good agreement between the values obtained by this procedure and the literature values of monoprotic acids was found in all cases expect for benzenesulfinic acid. The Medchem database reference cited it as being 1.84, [18] references their own poor agreement with two other pK_a references for this compound [19,20] being 1.29 and 1.46. Indeed, our value of 1.54 does not seem so much out of agreement when the literature values are controversial.

The determination of pK_a values for the diprotic acids can be difficult. Difficulties occur when the two pK_a values are relatively close. In the case of phthalic acid, the two reported pK_a values are about two units apart, and standard nonlinear regression tools easily find the optimum set of parameters for Eq. 13. This is illustrated in Fig. 4. Fig. 3 shows the mobility as a function of pH, and Fig. 4 shows the contour plot of the optimization objective function with respect to pK_{a1} and μ_1. The contours at the optimum are tight, and the standard deviations of the estimates for all of the parameters are less than 2.5% of their values. Table 3 shows the sensitivity of the parameter estimation to the initial value of μ_1 used in the optimization. For phthalic acid, the optimization is relatively insensitive to the initial value of μ_1.

For terephthalic and isophthalic acids, the pK_a values are closer, and the optimum sets of parame-

Table 2
Results of pK_a value determination by CE

Solute	pK_a (literature)	pK_a (CE)
2,4-dinitrobenzoic acid	1.42	1.49
2,6-dimethoxybenzoate	3.44	3.39
3,6-dichloro-2-anisic acid	1.90	1.95
Benzenesulfinic acid	1.84	1.54
3,5-dinitrobenzoic acid	2.82	2.80
Terephthalic acid	3.54, 4.46	3.25, 4.51
Isophthalic acid	3.62, 4.6	3.63, 4.58
Phthalic acid	2.95, 5.41	2.73, 4.78

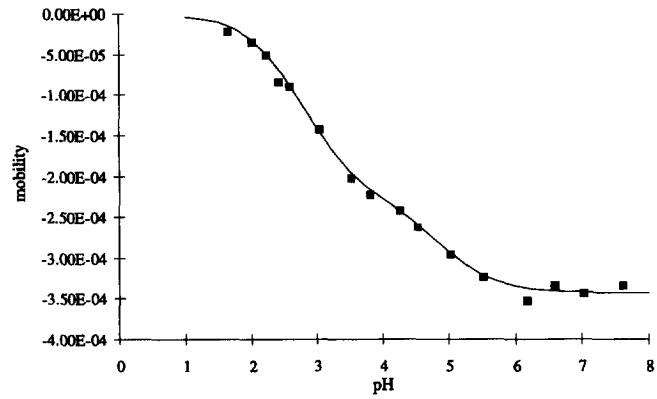

Fig. 3. Electrophoretic mobility of phthalic acid as a function of pH.

ters for Eq. 13 are more difficult to find. For isophthalic acid, the two reported pK_a values are only one unit apart, and the electrophoretic mobility curve with pH appears to have only one inflection point as shown in Fig. 5. As a result, it is difficult to estimate all four of the parameters, which are highly correlated, of Eq. 13 simultaneously. The contour plot shown in Fig. 6, drawn for a specific initial value of μ_1 ($-1.5 \cdot 10^{-4}$), shows broad contours, and the optimization objective function can take on the same maximum value for a wide range of values of pK_{a1} and μ_1. The data in Table 3 shows that the parameter estimates for isophthalic acid are very sensitive to the initial value of μ_1 used in the optimization. For isophthalic acid, the standard deviations of the parameter estimates range between 7 and 20%.

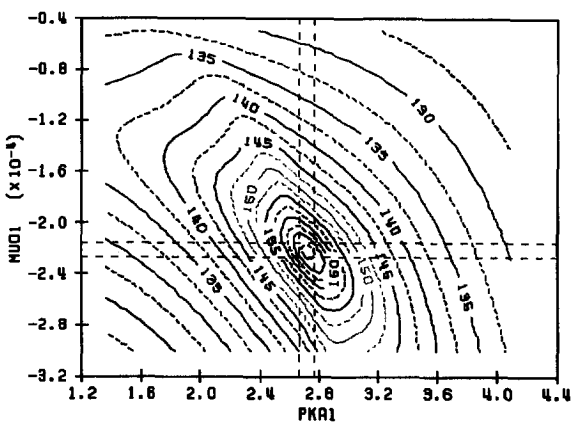

Fig. 4. Contour plot of the objective function for the four parameter (pK_{a1}, μ_1, pK_{a2}, μ_2) optimization of phthalic acid mobility data.

Parameter optimization for the terepthalic acid is somewhere between phthalic and isophthalic acids in difficulty. The mobility curve (Fig. 7) does not show as much definition as the phthalic acid mobility curve, but both the contour plot (Fig. 8) and the data in Table 3 reflect this intermediate case. The standard deviations of the parameter estimates are less than 5%.

Further simulation work using Monte Carlo techniques was done to evaluate the sensitivity of parameter estimation to uncertainty in the migration values. These simulations were done with an error with standard deviation of 2% of the migration value added at random to each of the 16 values in the phthalic acid data set. With this level of proportional uncertainty in the migration values, the results show that at the 95% confidence level pK_{a1} is between 2.70 and 2.77, and pK_{a2} is between 4.63 and 4.98. The significant difference between the literature values of pK_{a1} and pK_{a2} and those determined in this work is unexplained at this point. It is clear from this work that more precise parameter optimization does not necessarily mean better agreement with literature data.

5. Conclusions

Prior to this work, the determination of pK_a values for weak acids by CE was limited to a pK_a of around 4. A procedure was presented here which overcomes

Table 3
Sensitivity of optimum parameter values to initial value of μ_1

μ_1 initial	μ_1 final	pK_{a1}	pK_{a2}	μ_2	Objective Function
Phthalic acid					
$-0.11 \cdot 10^{-4}$	$-0.12 \cdot 10^{-4}$	0.26	3	$-3 \cdot 10^{-4}$	152
$-0.5 \cdot 10^{-4}$	$-0.54 \cdot 10^{-4}$	1.86	3.29	$-3.23 \cdot 10^{-4}$	154
$-1 \cdot 10^{-4}$	$-2.22 \cdot 10^{-4}$	2.72	4.78	$-3.43 \cdot 10^{-4}$	169
$-1.5 \cdot 10^{-4}$	$-2.24 \cdot 10^{-4}$	2.73	4.8	$-3.43 \cdot 10^{-4}$	169
$-2 \cdot 10^{-4}$	$-2.24 \cdot 10^{-4}$	2.73	4.79	$-3.43 \cdot 10^{-4}$	169
$-2.5 \cdot 10^{-4}$	$-2.24 \cdot 10^{-4}$	2.73	4.8	$-3.43 \cdot 10^{-4}$	169
$-3 \cdot 10^{-4}$	$-2.24 \cdot 10^{-4}$	2.73	4.81	$-3.43 \cdot 10^{-4}$	169
$-3.5 \cdot 10^{-4}$	$-2.24 \cdot 10^{-4}$	2.73	4.8	$-3.43 \cdot 10^{-4}$	169
Isophthalic acid					
$-0.11 \cdot 10^{-4}$	$-0.11 \cdot 10^{-4}$	2.45	3.75	$-3.6 \cdot 10^{-4}$	102
$-0.5 \cdot 10^{-4}$	$-0.51 \cdot 10^{-4}$	2.86	3.9	$-3.7 \cdot 10^{-4}$	102
$-1 \cdot 10^{-4}$	$-1.03 \cdot 10^{-4}$	3.11	4.05	$-3.73 \cdot 10^{-4}$	103
$-1.5 \cdot 10^{-4}$	$-1.53 \cdot 10^{-4}$	3.29	4.16	$-3.74 \cdot 10^{-4}$	103
$-2 \cdot 10^{-4}$	$-2.01 \cdot 10^{-4}$	3.44	4.32	$-3.76 \cdot 10^{-4}$	104
$-2.5 \cdot 10^{-4}$	$-2.46 \cdot 10^{-4}$	3.55	4.52	$-3.76 \cdot 10^{-4}$	104
$-3 \cdot 10^{-4}$	$-2.9 \cdot 10^{-4}$	3.65	4.79	$-3.77 \cdot 10^{-4}$	104
$-3.5 \cdot 10^{-4}$	$-2.9 \cdot 10^{-4}$	3.65	4.78	$-3.79 \cdot 10^{-4}$	104
Terephthalic acid					
$-0.11 \cdot 10^{-4}$	$0.11 \cdot 10^{-4}$	1.4	3.8	$-3.66 \cdot 10^{-4}$	138
$-0.5 \cdot 10^{-4}$	$0.52 \cdot 10^{-4}$	2.63	3.99	$-3.7 \cdot 10^{-4}$	141
$-1 \cdot 10^{-4}$	$1.03 \cdot 10^{-4}$	2.98	4.21	$-3.78 \cdot 10^{-4}$	144
$-1.5 \cdot 10^{-4}$	$1.5 \cdot 10^{-4}$	3.18	4.4	$-3.82 \cdot 10^{-4}$	147
$-2 \cdot 10^{-4}$	$1.95 \cdot 10^{-4}$	3.32	4.61	$-3.85 \cdot 10^{-4}$	147
$-2.5 \cdot 10^{-4}$	$1.71 \cdot 10^{-4}$	3.25	4.5	$-3.83 \cdot 10^{-4}$	147
$-3 \cdot 10^{-4}$	$1.78 \cdot 10^{-4}$	3.27	4.53	$-3.84 \cdot 10^{-4}$	147
$-3.5 \cdot 10^{-4}$	$1.7 \cdot 10^{-4}$	3.25	4.5	$-3.84 \cdot 10^{-4}$	147

this limitation. The direction of the electroosmotic flow was reversed by using a dynamic coating of a positively charged polymer to impart a positive charge to the capillary. Acids elute first followed by the neutral marker over the entire pH range of the experiment. The method was demonstrated over the pK_a range of 1.5 to 3.4 for a set of five acids.

Models were presented for multi-protic solute

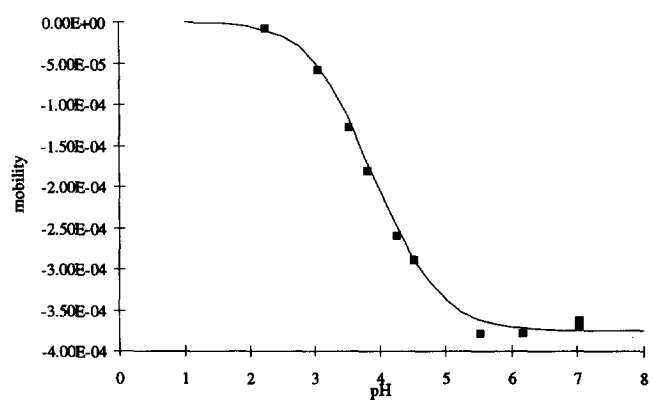

Fig. 5. Electrophoretic mobility of isophthalic acid as a function of pH.

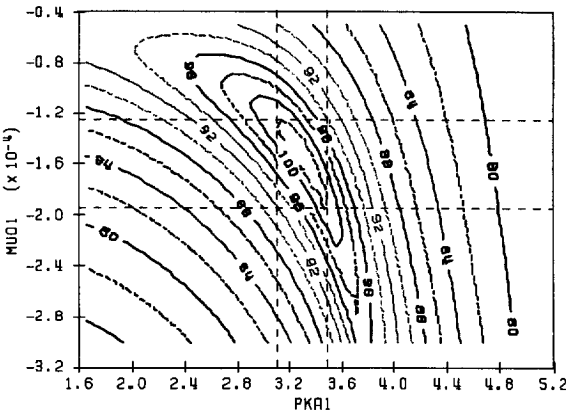

Fig. 6. Contour plot of the objective function for the four parameter (pK_{a1}, μ_1, pK_{a2}, μ_2) optimization of isophthalic acid mobility data.

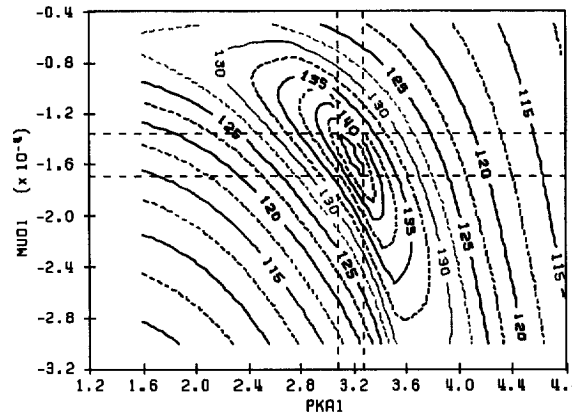

Fig. 8. Contour plot of the objective function for the four parameter (pK_{a1}, μ_1, pK_{a2}, μ_2) optimization of terephthalic acid mobility data.

determinations and tested with three dicarboxylic acids. The closer the pK_a values are for a diprotic solute, the more difficult the pK_a (and other) parameter estimation. This problem is an inherent limitation of CE determined pK_a values. Previously reported data on ampholytes (4, 9) may have given investigators the impression that it may be simple to determine pK_a values of multiprotic solutes. This is certainly not true for diprotic acids whose pK_a values are close. However, other techniques such as spectroscopic or potentiometric, must also rely on nonlinear parameter optimization for pK_a determinations and hence suffer a similar limitation.

Acknowledgments

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References

[1] C. Hansch and A. Leo, Exploring QSAR, Vol. 1, American Chemical Society, Washington DC, 1995.
[2] B.T. Grayson and D.A. Kleier, Pestic. Sci., 30 (1990) 67.
[3] J.M. Beckers, F.M. Everaerts and M.T. Ackermans, J. Chromatogr., 537 (1991) 407.

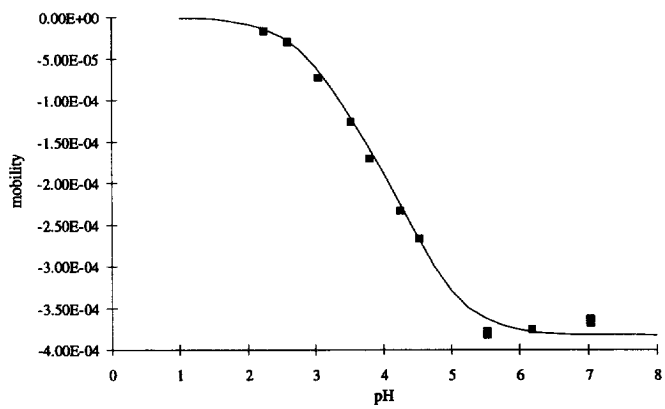

Fig. 7. Electrophoretic mobility of terephthalic acid as a function of pH.

[4] J. Cai, J.T. Smith and Z.E. Rassi, J. High Resolut. Chromatogr., 15 (1992) 30.
[5] J.A. Cleveland, M.H. Benko, S.J. Gluck and Y.M. Walbroehl, J. Chromatogr. A, 652 (1993) 301.
[6] S.J. Gluck and J.A. Cleveland, J. Chromatogr. A, 680 (1994) 43.
[7] S.J. Gluck and J.A. Cleveland, J. Chromatogr. A, 680 (1994) 49.
[8] J.A. Cleveland, C.L. Martin and S.J. Gluck, J. Chromatogr. A, 679 (1994) 167.
[9] Y. Ishihama, Y. Oda and N. Asakawa, J. Pharm. Sci., 83 (1994) 1500.
[10] W.J. Lambert and D.L. Middleton, Anal. Chem., 62 (1990) 1585.
[11] R.W. Chiu, J.C. Jimenez and C.A. Monnig, Anal. Chim. Acta., 307 (1995) 193.
[12] J.E. Wiktorowicz, US Pat., 5 015 350.
[13] J. Kielland, J. Am. Chem. Soc., 59 (1937) 1675.
[14] J.F. Zemaitis, Jr., D.M. Clark, M. Rafal and N.C. Scrivner, Handbook of Aqueous Electrolyte Thermodynamics, American Institute of Chemical Engineers, New York, 1986, p. 59.
[15] A. Albert and E.P. Serjeant, The Determination of Ionization Constants, Chapman and Hall, New York, 3rd ed., 1984, pp. 28–53.
[16] D.S. Burgi, K. Salomon and R.L. Chien, J. Liq. Chromatogr., 14 (1991) 487.
[17] H. Wätzig, Chromatographia, 22 (1992) 445.
[18] R.K. Burkhard, D.E. Sellers, F. DeCou and J.L. Lambert, J. Org. Chem., 24 (1959) 767.
[19] P. Rumpf and J. Sadet, Bull. Soc. Chim. Fr., (1958) 447.
[20] R.R. Coats and D.T. Gibson, J. Chem. Soc., (1940) 442.