Journal of Chromatography, 320 (1985) 33–43
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,302

DETERMINATION OF DISSOCIATION CONSTANTS OF WEAK ELECTROLYTES BY CAPILLARY ISOTACHOPHORESIS

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SUMMARY

The method described for the determination of the dissociation constants of weak electrolytes is based on the fact that the effective mobility of a weak electrolyte is a function of pH. Dissociation constants (together with actual mobilities of ionic forms of the given weak electrolyte) are parameters of this function. On the basis of a mathematical model of the isotachophoretic (ITP) steady state, a set of appropriate pairs of pHs and effective mobilities is calculated and dissociation constants are obtained from them by a non-linear least-squares procedure.

The method was validated by calculation of the dissociation constants and absolute ionic conductivities of anions of weak organic acids (formic, acetic, propionic, oxalic, malonic, succinic, adipic and citric) from the data obtained by their ITP analysis using fourteen different electrolyte systems in the pH range 3.1–7.9. Good agreement with literature values was achieved.

INTRODUCTION

In tandem with the growing number of applications of capillary isotachophoresis (ITP) in many fields of analysis of all types of ionogenic substances, the theory of ITP has been developed[1–5] and ITP has also been used in theoretical studies. The tendency to obtain from ITP analysis more general data on separated substances, such as effective, actual and absolute mobilities[6–8], relative molecular mass[7], dissociation constants of weak acids[9–11] and stability constants of complexes[12]; is apparent.

The first attempt to calculate dissociation constants from ITP data was made by Vozková[9]. Her calculation was based on a simplified mathematical model of the ITP steady state; electrophoretic and relaxation effects were not considered, temper-

0021-9673/85/$03.30

ature influences were neglected and the results were unsatisfactory. Our method[10] was based on the more precise mathematical model of the ITP steady state. In this model the concentration mobility dependence and the temperature corrections of all temperature-dependent quantities were included[13] and the pH of the leading electrolyte was calculated.

The method for the determination of dissociation constants by ITP developed by Kiso and Hirokawa[11] is based on a comparison of calculated and observed values of the ratio E_V/E_L in different electrolyte systems, where E_V and E_L are the intensities of the electric field in the zone of sample and leading electrolyte, respectively.

THEORY

The determination of the dissociation constants of weak electrolytes by capillary ITP is based on the fact that the effective mobility of weak electrolyte is a function of pH. Dissociation constants (together with actual mobilities of ionic forms) are parameters of this function. If a set of appropriate pairs of pH and effective mobilities is obtained from ITP data, the dissociation constants can be calculated from them by a non-linear approximation by the least-squares procedure. The set of appropriate pairs of pHs and effective mobilities is calculated from the known and measured ITP data on the basis of the mathematical model of the ITP steady state.

Mathematical model of the ITP steady state[1,3,13]

The mathematical model involves the following:

(1) mass balances (total substance concentration equals the sum of the concentrations of its ionic forms, including the zero charge form);

(2) acid–base equilibria;

(3) electroneutrality condition;

(4) Ohm's law;

(5) Kohlrausch regulation function;

(6) ITP condition;

(7) concentration dependence of mobilities (Debye–Hückel and Onsager–Fuoss theories);

(8) Joule heat generation;

(9) temperature corrections of temperature-dependent magnitudes;

(10) relationship between specific electric conductivity and electrophoretic mobilities;

(11) relationship between specific electric conductivity and voltage of potential gradient detector or relationship between specific electric conductivity and resistance of conductivity detector.

Non-linear approximation by least-squares procedure[14]

This method allows the determination of unknown parameters $a_1, \ldots, a_n$ of a non-linear function:

$$y = f(x, a_1, \ldots, a_n) \tag{1}$$

if a set of pairs (x_i, y_i), $i = 1, \ldots, s$, $y_i = f(x_i, a_1, \ldots, a_n)$ is given.

A non-linear function f(x) is approximated by Taylor's expansion, where the terms of order higher than the first are ignored:

$$\begin{aligned} f(x_i, a_1, \ldots, a_n) &= f(x_i, {}^0a_1, \ldots, {}^0a_n) + \frac{\partial f}{\partial a_1}(a_1 - {}^0a_1) \\ &\quad + \ldots + \frac{\partial f}{\partial a_n}(a_n - {}^0a_n) \\ &= \mathrm{f}(x_i, {}^0a_1, \ldots, {}^0a_n) + \sum_{j=1}^{n} \frac{\partial f}{\partial a_j} \Delta a_j \end{aligned} \tag{2}$$

$$\Delta a_j = a_j - {}^0a_j \tag{3}$$

${}^0a_1, \ldots, {}^0a_n$ are the initial estimates of parameters $a_1, \ldots, a_n$. Δa_j can be calculated from the general condition of least-squares methods:

$$S = \sum_{i=1}^{s} [f(x_i) - y_i]^2 = \min. \tag{4}$$

i.e.,

$$\frac{\partial S}{\partial \Delta a_j} = 0, \qquad j = 1, \ldots, n \tag{5}$$

Δa_j are obtained as a solution of a set of linear equations 5. Then the new estimates ${}^1a_j = {}^0a_j + \Delta a_j$ are used for repeated calculation of Δa_j. This iterative procedure continues until the following condition is fulfilled:

$$\Delta a_j = ({}^ra_j - {}^{r-1}a_j) < \varepsilon \tag{6}$$

where ε is the required accuracy and ${}^ra_j$ are taken as a good approximation of a_j.

Effective mobility of weak electrolyte

The effective mobility, m_X, of weak electrolyte (including ampholyte) X is defined as:

$$m_X = \sum_{J=N}^{M} \mathrm{sgn}\,(J) m_{X(J)} D_{X(J)} \qquad \mathrm{sgn}\,(J) = \begin{cases} 1 \text{ for } J > 0 \\ 0 \text{ for } J = 0 \\ -1 \text{ for } J < 0 \end{cases} \tag{7}$$

where N (M) is the minimal (maximal) charge (in elementary units including sign) of substance X (*e.g.*, for oxalic acid $N = -2$, $M = 0$; for imidazole $N = 0$, $M = 1$; for glycine $N = -1$, $M = 1$), $m_{X(J)}$ are actual mobilities of different ionic forms X(J), J is effective charge (in elementary units including sign) and $D_{X(J)}$ is the molar fraction of ionic form X(J) in substance X:

$$D_{X(J)} = c_{X(J)}/c_X = c_{X(J)}/\sum_{J=N}^{M} c_{X(J)} \tag{8}$$

where $c_{X(J)}$ is the concentration of ionic form X(J) and c_X is the total concentration of substance X (including the form with zero effective charge).

From the acid–base equilibrium we can derive the equation

$$D_{X(J)} = \frac{\left[\prod_{l=0}^{J-1} K(l)/H^{|J|}\right]^{-1} \cdot (J > 0) + \left[\prod_{l=J}^{-1} K(l)/H^{|J|}\right] \cdot (J < 0)}{1 + \sum_{i=N}^{-1} \left[\prod_{l=i}^{-1} K(l)/H^{|i|}\right] + \sum_{i=1}^{M} \left[\prod_{l=0}^{i-1} K(l)/H^{|i|}\right]^{-1}} \tag{9}$$

where $J \in \langle N, M \rangle$, $J \neq 0$, H is the concentration of H^+ ions, i and l are auxiliary variable indices and $K(J)$ are the dissociation constants corresponding to the equilibrium

$$X(J + 1) \rightleftharpoons X(J) + H^+ \tag{10}$$

$$K(J) = X(J) \cdot H/X(J + 1) \tag{11}$$

By combination of eqns. 7 and 9 we obtain

$$m_X = \frac{\sum_{J=N}^{M} \mathrm{sgn}(J) \cdot m_{X(J)} \cdot \left\{\left[\prod_{l=0}^{J-1} K(l)/H^{|J|}\right]^{-1} \cdot (J > 0) + \left[\prod_{l=J}^{-1} K(l)/H^{|J|}\right] \cdot (J < 0)\right\}}{1 + \sum_{i=N}^{-1} \left[\prod_{l=i}^{-1} K(l)/H^{|i|}\right] + \sum_{i=1}^{M} \left[\prod_{l=0}^{i-1} K(l)/H^{|i|}\right]^{-1}} \tag{12}$$

The general relationship 12 for the effective mobility of a weak electrolyte is for the divalent acid

$$m_X = \frac{-m_{X(-2)} \cdot K(-2) \cdot K(-1) - m_{X(-1)} \cdot K(-1) \cdot H}{K(-2) \cdot K(-1) + K(-1) \cdot H + H^2} \tag{13}$$

A graph of the function $m_X = f(\mathrm{pH})$ for hypothetical values $m_{X(-1)} = 3 \cdot 10^{-8}$, $m_{X(-2)} = 6 \cdot 10^{-8}$ (m^2 sec^{-1} V^{-1}), p$K(-1) = 3$, p$K(-2) = 6$ is shown in Fig. 1.

From Fig. 1 is evident that: (1) pH changes have the greatest influence on effective mobility near the pK values and (2) pH changes in the region pH > p$K(-2) + 2$ have only a very small influence on effective mobility because almost all of the substance is dissociated to a degree -2 and the effective mobility is approximately equal to the actual mobility of ionic form X(-2).

These facts are important for estimating values of p$K(J)$ and $m_{X(J)}$ in the first cycle of the iterative procedure of their calculation by the non-linear least-squares procedure. As the initial estimates of p$K(J)$, values of pH are chosen at which the greatest slope of the dependence of effective mobility on pH is observed. As the initial estimate of the actual mobility $m_{X(N)}$, a value of the effective mobility from a pH region is chosen at which the effective mobility is almost independent of pH.

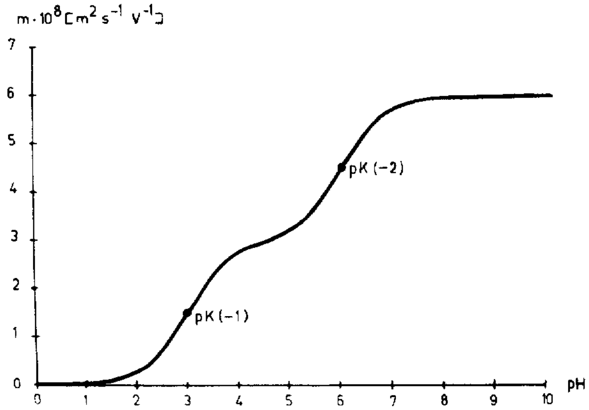

Fig. 1. Graph of the dependence of effective mobility, *m*, of a divalent weak acid on pH for hypothetical values of parameters [p$K(-1)$ = 3, p$K(-2)$ = 6, $m_{X(-1)} = 3 \cdot 10^{-8}$, $m_{X(-2)} = 6 \cdot 10^{-8}$ (m² sec⁻¹ V⁻¹)].

Calculation of dissociation constants

Eqn. 12 shows that effective mobility m_X is a function of pH. Dissociation constants p$K(J)$ and actual mobilities $m_{X(J)}$ are parameters of this function. If we have several pairs of corresponding values m_X and pH, then the calculation of dissociation constants can be converted into the general problem of the determination of parameters $a_1, \ldots, a_n$ of a function $y = f(x, a_1, \ldots, a_n)$ if a set of pairs (x_i, y_i) is given. Solution of this problem by the non-linear least-squares procedure is briefly shown above.

The procedure for the calculation of dissociation constants can be divided into three parts, as follows.

(1) Calculation of the characteristics of the first (leading) zone and the effective mobility of the sample substance for some different electrolyte systems in the pH range where the pK values of the sample substance are assumed.

The input data are concentration, minimal and maximal charge and pK values of the leading ion and counter ion, estimate of the pH of the leading electrolyte, absolute mobility of the leading ion, electric current, voltage of the potential gradient (PG) detector in the first zone and in the sample zone, ambient temperature (temperature at zero current), resistance capacity of the PG detector, cross-section of the capillary, coefficients of temperature corrections of temperature-dependent quantities and general constants (Faraday, Avogadro, Boltzmann, etc.). Based on these data and above mathematical model of the ITP steady state, the conductivity, electric field intensity, Joule heat per unit length and temperature of the leading zone and sample zone are calculated. Temperature-dependent quantities (mobilities, pK of leading ion and counter ion, viscosity, relative permittivity) are recalculated to the values corresponding to the temperature of the zone and these corrected values are used for calculation of the characteristics of the zone of leading electrolyte (actual and effective mobilities of leading ion and counter ion, concentration of ionic forms, pH) and the effective mobility of the sample substance, which is recalculated from the temperature of the zone to the standard temperature of 25°C. In this way, a set of values

$[{}^1H, m_X]_i$ is obtained, where ${}^1H$ is the concentration of H^+ ions in the leading zone, m_X is the effective mobility of the sample substance, $i = 1, ..., s$ and s is the number of electrolyte systems. Calculated values that will be used later are stored in auxiliary arrays.

(2) In the second part, the non-linear least-squares procedure is applied to the set of values $[{}^1H, m_X]_i$ and the first values of ${}^1K(J)$ and ${}^1m_{X(J)}$ are obtained. At the first input in this part of the calculation the initial estimates of the calculated parameters must be given.

(3) In the third part, the values of $K(J)$ (obtained in the second part) and magnitudes stored in the auxiliary arrays (obtained in the first part) are used for the iterative calculation of pH in the zone of the sample substance. During this procedure, other important magnitudes such as actual and absolute mobilities, steady-state concentrations and molar conductivities of the ionic forms present are calculated. In this way, more accurate values of pH corresponding to effective mobilities are obtained and the non-linear least-squares procedure is applied again to the set of pairs $[{}^2H, m_X]_i$, *i.e.*, we come back to the second part of the calculation where more accurate values of $K(J)$ are obtained. These new values of $K(J)$ are used for new calculations of the pH of the sample zone in the third part of the calculation. This iterative procedure continues until the following condition is fulfilled:

$$({}^r\text{p}K(J) - {}^{r-1}\text{p}K(J)) < \varepsilon \tag{14}$$

where ${}^r\text{p}K(J)$ and ${}^{r-1}\text{p}K(J)$ are values of $\text{p}K(J)$ calculated in the rth and $(r - 1)$th cycle of the iterative procedure and ε is the required accuracy. ${}^r\text{p}K(J)$ are accepted as resulting values of dissociation constants $\text{p}K(J)$.

EXPERIMENTAL

ITP analyses were performed in apparatus of our own construction[15]. This consists of a PTFE capillary tube (I.D. 0.45 mm), PTFE sample valve (dosing volume 2 μl) and PTFE electrode compartments. It is equipped with two PG detectors[16] and a specific UV-photometric detector at 254 nm. The electric current was 30–50 μA and ambient temperature was 24–26°C.

The resistance capacity of the PG detector was determined by measurement of the voltage of the detector in a solution of KCl of known specific conductivity at a low input power where the Joule heat can be neglected.

The specific conductivity of the zones was calculated from the voltage of the PG detector in this zone, the electric current used and the resistance capacity of the PG detector.

The temperature (T) of the zone was calculated from the equation

$$T = T_0 + KQ \tag{15}$$

where T_0 is ambient temperature (temperature at zero current), Q is the Joule heat generated per unit length of the zone and K is a proportionality constant (obtained by measuring the dependence of the temperature inside the capillary on the generated

TABLE I

COMPOSITION OF ELECTROLYTE SYSTEMS

BALA = β-alanine; EACA = ε-aminocaproic acid; PYR = pyridine; HIS = histidine; IMID = imidazole; TRIS = tris(hydroxymethyl)aminomethane. Leading ion: Cl^- (0.01 mol l^{-1}). Terminating electrolyte: propionic acid (0.01 mol l^{-1}).

Counter ion	*Concentration (mol l^{-1})*	*pH*
BALA	0.015	3.13
	0.020	3.40
	0.030	3.70
EACA	0.015	4.08
	0.020	4.38
	0.030	4.65
PYR	0.015	4.75
	0.020	5.10
	0.030	5.40
HIS	0.015	5.60
	0.020	5.92
	0.030	6.26
IMID	0.020	6.95
TRIS	0.020	7.85

Joule heat; the temperature inside the capillary was determined from the known temperature dependence of the specific conductivity of KCl solution, which was measured with the PG detector at different Joule heats).

Chemicals

All chemicals were of analytical-reagent grade. β-Alanine was obtained from Calbiochem (San Diego, CA, U.S.A.), ε-aminocaproic acid and imidazole from Koch-Light (Colnbrook, U.K.), histidine from Pierce (Rockford, IL, U.S.A.), histidine–HCl from Merck (Darmstadt, F.R.G.), Tris [tris(hydroxymethyl)aminomethane] from Serva (Heidelberg, F.R.G.) and other chemicals from Lachema (Brno, Czechoslovakia).

ITP analyses of some weak acids (formic, acetic, propionic, oxalic, malonic, succinic, adipic and citric) were performed with fourteen different electrolyte systems. The composition of the electrolyte systems is given in Table I.

RESULTS

On the basis of the above mathematical model and computation procedure, a program for the calculation of dissociation constants from ITP data was developed. The program is written in Basic (version for the Hewlett-Packard 9830) and consists of two parts: the subprogram ITP allows the calculation of the characteristics of the ITP steady state and the subprogram Regression allows the calculation of dissociation constants from the set of appropriate pH and effective mobility pairs. A schematic flow chart of the program is shown in Fig. 2.

The developed program was used for the calculation of the dissociation con-

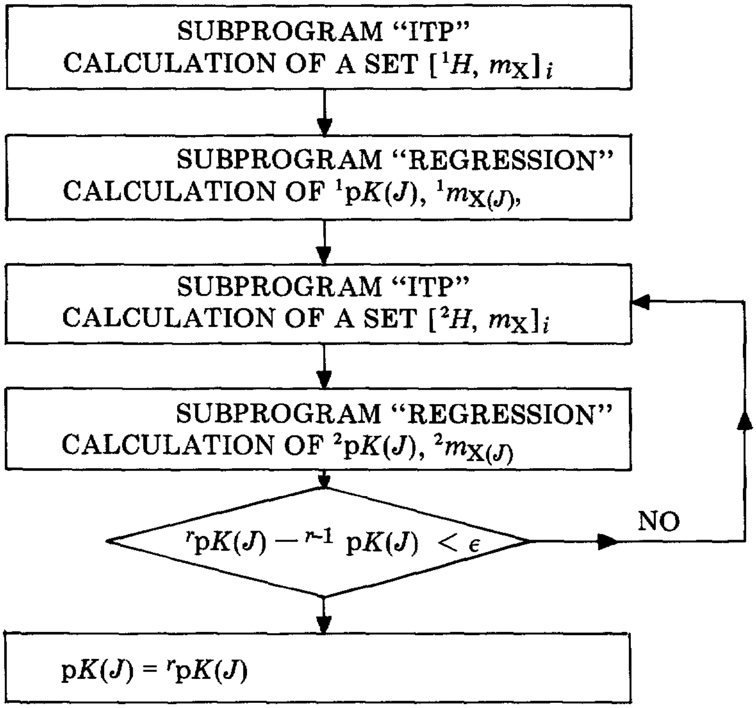

Fig. 2. Schematic flow chart of the program. ${}^1H$ = concentration of H^+ ions in the first zone (leading electrolyte); ${}^2H$ = concentration of H^+ ions in the second zone (sample zone); ${}^rpK$ = pK value calculated in the rth cycle, ${}^rm_{X(J)}$ = actual mobility of ionic form X(J) calculated in the rth cycle of the iteration procedure; ε = required accuracy of calculation; $i = 1, \ldots, s$; s = number of electrolyte systems.

TABLE II

CALCULATED AND LITERATURE[17] VALUES OF DISSOCIATION CONSTANTS AT 25°C

Acid	*Dissociation constants*					
	Calculated			*Literature*		
	pK(−1)	*pK(−2)*	*pK(−3)*	*pK(−1)*	*pK(−2)*	*pK(−3)*
Formic	3.72			3.75		
Acetic	4.70			4.76		
Propionic	4.82			4.87		
Oxalic	–	4.15		1.27	4.27	
Malonic	2.83	5.59		2.85	5.70	
Succinic	4.15	5.55		4.21	5.64	
Adipic	4.38	5.26		4.43	5.28	
Citric	2.89	4.55	6.50	3.13	4.76	6.40

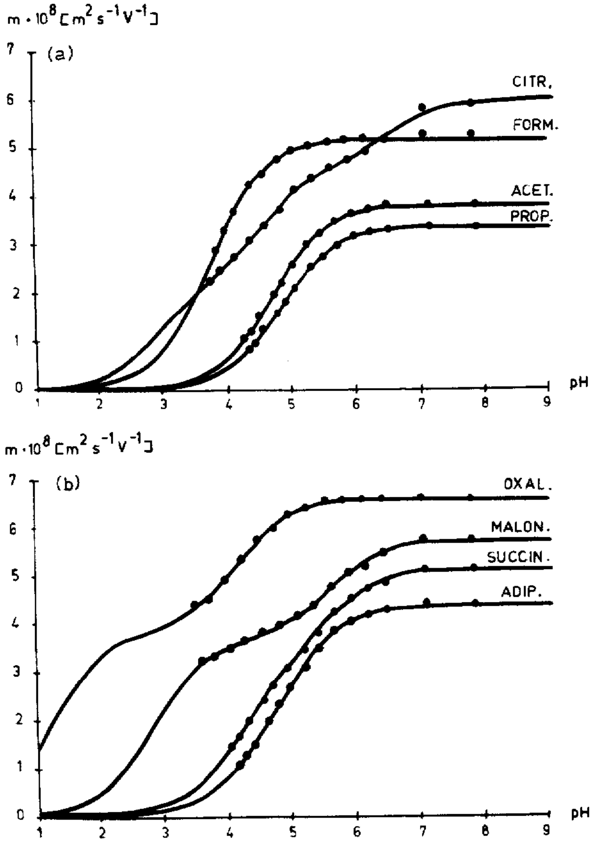

Fig. 3. Dependence of effective mobility of weak acids on pH. (a) Citric, formic, acetic and propionic acid; (b) oxalic, malonic, succinic and adipic acid. Experimental points were obtained from ITP data and the curves were fitted by the non-linear least-squares procedure.

stants of weak acids from the data obtained by their ITP analysis with different electrolyte systems. The calculated values (see Table II) are in good agreement with the literature values.

Fig. 3. shows graphs of the dependence of the effective mobility of the acids on pH as obtained from ITP data. Experimental points [pH, effective mobility] were fitted to the theoretical curve by the non-linear least-squares procedure and in this way the values of p*K*(*J*) and actual mobilities were obtained.

The average values of the absolute ionic conductivities of different ionic forms that were also calculated by the developed program are given in Table III.

DISCUSSION

The method presented allows the determination of the dissociation constants of weak electrolytes provided that the ITP analysis of an electrolyte is performed with some different electrolyte systems in the pH range where the p*K* value is expected to lie and in this way the dependence of effective mobility on pH is measured. This

TABLE III

CALCULATED AND LITERATURE[18] VALUES OF ABSOLUTE IONIC CONDUCTIVITIES AT 25°C

Ionic forms	*Absolute ionic conductivity (Ω^{-1} m^2 mol^{-1} · 10^4)*		
	Calculated	*Standard deviation*	*Literature*
Formates(−1)	54.4	0.5	54.6
Acetates(−1)	41.0	0.6	40.9 (41.4)
Propionates(−1)	36.5	0.7	35.8
Oxalates(−1)	42.3	0.9	40.2
Oxalates(−2)	74.1	2.0	72.7(74.0)
Malonates(−1)	38.3	1.4	–
Malonates(−2)	69.2	3.7	58.9
Succinates(−1)	33.2	0.7	–
Succinates(−2)	61.4	2.8	60.9 (55.1)
Adipates(−1)	27.4	0.4	–
Adipates(−2)	51.1	1.4	–
Citrates(−1)	28.4	0.7	–
Citrates(−2)	51.7	1.8	–
Citrates(−3)	73.4	3.4	70.2

dependence must be measured over a sufficiently wide pH range, so that the initial values of pK and actual mobilities (used in the non-linear least-squares procedure) could be estimated and so that the iterative calculation procedure could converge.

Dissociation constants can be determined even if the pK value is out of the pH region where the dependence of effective mobility on pH is measured (see data for malonic acid in Fig. 3b and Table II), but the difference between the calculated pK and the pH of the leading electrolyte must not be too great, because then the dependence of effective mobility on pH in the measured pH range is only very slightly influenced by the pK value and this pK value cannot be calculated. This fact was confirmed with oxalic acid: its dissociation constant p$K(-1) = 1.27$ is too far from the pH range where the dependence of effective mobility on pH was measured (see Fig. 3b), which is why it could not be determined (the iterative procedure did not converge). This implies that our method is not suitable for the determination of low pK values (p$K \lesssim 2.5$) because the dependence of effective mobility on pH cannot be measured in this pH range by ITP owing to the disturbances of H^+ ions at low pH.

The calculated pK values are apparent dissociation constants because the concentration of appropriate components instead of their activities are used. Another simplification of our method is that pK values are considered to be independent of ionic strength changes when the dependence of effective mobility on pH is measured with different ITP electrolyte systems (see Fig. 3). In order to minimize these differences in ionic strength the same monovalent leading anion of the same concentration and the same concentration ratios of counter ions and leading ion (see Table I) were used in all electrolyte systems. With respect to relatively low ionic strength (0.007–0.016) and small ionic strength changes (*e.g.*, 0.002 for formic acid, 0.005 for malonic acid, 0.009 for citric acid) during ITP analysis with different electrolyte systems, our simplification does not influence the calculated pK values significantly and they can be related to the average ionic strength of the leading electrolytes used (0.01). Nevertheless, our simplifications may explain the differences between calculated and literature pK values, especially with trivalent citric acid.

Because effective mobilities in the set of pairs $[^2H, m_X]_i$ relate to 25°C, the calculated pK values also relate to 25°C. Recalculation of mobilities from values corresponding to the temperature of the zone to a temperature of 25°C is more accurate for small temperature differences, which is why ITP analyses were performed at a low electric current at which the temperature increase in the zone was not greater than 5°C.

The proposed method represents a new way of determining dissociation constants of weak electrolytes. Compared with other methods (potentiometry, spectrophotometry, conductimetry), the advantage of our method is the small sample consumption (of the order of nanomoles) and the possibility of the simultaneous determination of dissociation constants of electrolytes present in the mixtures. In addition to pK values, other general characteristics of sample substances such as absolute ionic mobilities and conductivities are obtained.

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