Measurement of Mobilities and Dissociation Constants by Capillary Isotachophoresis

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Received June 9, 1988 (Revised Manuscript Received November 28, 1988)

Contents

I. Introduction

For almost 20 years, analytical isotachophoresis has presented itself as a useful method for the analysis of a number of materials for ionogenic components.[1,2] The growing number of interesting applications[3] as well as the recent appearance of advanced instrumentation on the market[4] show the important place of isotachophoresis among modern analytical methods.

In isotachophoresis, substances are separated on the basis of different effective mobilities, $\bar{u}$. The sample zone is placed between the zones of leading (L) and terminating (T) electrolyte (see Figure 1a), and an electric current is applied. After complete separation has been reached, the individual substances migrate in individual zones with sharp boundaries (Figure 1b,c). The velocity of all these zones (including the leading and terminating zones) is the same:

$$v = E_A\bar{u}_A = E_B\bar{u}_B = E_i\bar{u}_i \quad (1)$$

where E is the electric field strength (potential gradient) and i denotes any zone of individual substance.

The effective mobility is the fundamental quantity that determines the isotachophoretic behavior of substances and the properties of isotachophoretic zones, including properties that are used for detection. There exists therefore an implicit possibility to obtain from an isotachophoretic anaysis knowledge of effective mobilities of substances. The primary data obtained from the experiment are the actual values of this mobility (which are related to the actual ionic strength). For totally ionized species, these actual effective mobilities are equal to the actual ionic ones and can simply be recalculated to limiting ionic mobilities (which are related to zero ionic strength). When substances that are not fully dissociated are involved, obtaining the ionic mobilities is more complicated, but additionally the appropriate dissociation constants may be evaluated.

If analytical isotachophoresis is used for the abovementioned purpose, it gains the status of a method of physical chemistry. A great advantage of isotachophoresis lies in its separation possibilities, which enable one to determine the mobilities of substances that are not pure or in mixtures, without any sample pretreatment. That is why papers on the use of isotachophoresis for evaluation of mobilities and other data are not at all rare at present, forming a well-founded independent trend. The aim of this review is to give complete information on the basic methods used for obtaining mobilities and dissociation constants by isotachophoresis, as well as a survey of data published to date.

II. Relations between Effective Mobility and Physicochemical Constants of Substances (u_i, pK_a)

The effective mobility characterizes the migration of a substance as a whole; i.e., it describes the behavior of an electrophoretic zone of the substance or of a migrating front of this substance. The appropriate substance may consist of several ionic or neutral species that are in dynamic equilibrium with each other. The effective mobility of a partially dissociated substance is a function of the ionic mobilities:

$$\bar{u} = \sum_i x_i u_i$$

$$x_i = c_i / \sum_j c_i \quad (2)$$

where x_i are the molar fractions and c_i are the concentrations of the individual ionic forms of substance j present in the solution. The value of the effective mobility therefore depends also on the dissociation constants of the chemical equilibria and on the concentration of the component of the solution that influences these equilibria, i.e., the concentration of H^+ in acid–base equilibria and ligand concentration in complex-forming equilibria.

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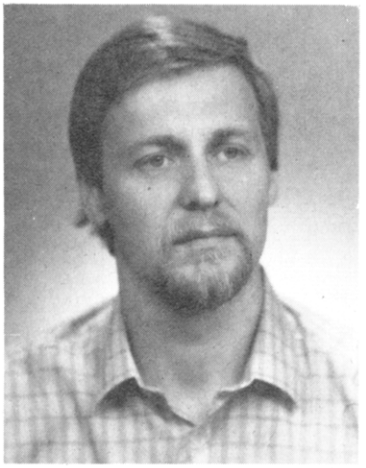
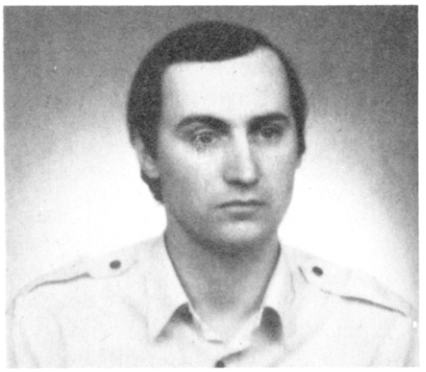


Jan Pospíchal studied at the University of Chemical Technology, Pardubice, Czechoslovakia, where he was interested in syntheses of phthalocyanine dyes. After obtaining his M.Sc. degree in 1981, he began working in the Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, Brno, where he received his Ph.D. His field of interest is analytical capillary isotachophoresis.



Petr Gebauer studied at the Faculty of Science, University of Brno, Brno, Czechoslovakia, where he was involved in the research of inorganic S–N heterocycles. After obtaining his M.Sc. in 1978, he started his research career in the field of isotachophoresis at the Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, Brno, and received his Ph.D. in this field. At present, he is engaged in theoretical and methodological aspects of analytical isotachophoresis.

Petr Boček, born in Brno, Czechoslovakia, graduated from the University of Brno in 1964. After obtaining his M.Sc. in 1967 for work in analytical spectrophotometry, he obtained his Ph.D. in the field of gas chromatography and his D.Sc. in the field of analytical electrophoresis, both at the Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, Brno. He currently works at this institute and is head of the department of electromigration methods. He worked with Prof. C. A. Cramers, Technological University, Eindhoven, The Netherlands, in 1971 and with Dr. A. Chrambach, National Institutes of Health, Bethesda, MD, in 1985. He has published over 100 scientific papers, mostly in the *Journal of Chromatography* and *Electrophoresis*, where he is also member of the editorial boards.

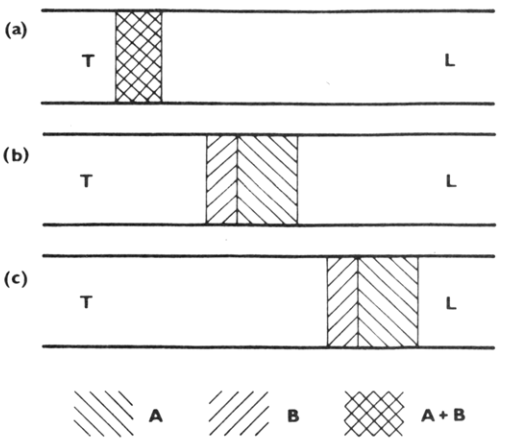


Figure 1. Scheme of isotachophoretic separation of a two-component mixture A + B: (a) initial state; (b, c) steady-state migration after complete separation of A and B has been reached.

For a monohydric weak acid HA, e.g., the molar fraction of A^- is given by

$$x_A = K_{HA}/(c_H + K_{HA}) \tag{3}$$

K_{HA} is the dissociation constant of HA, and c_H is the concentration of H^+. The effective mobility of acid HA is then obtained by combining eq 2 and 3:

$$\bar{u}_{HA} = u_A \frac{K_{HA}}{c_H + K_{HA}} \tag{4}$$

Analogously, for the molar fractions and effective mobility of a dihydric weak acid H_2A, one can write

$$x_{HA} = \frac{K_1 c_H}{c_H^2 + K_1 c_H + K_1 K_2} \tag{5}$$

$$x_A = \frac{K_1 K_2}{c_H^2 + K_1 c_H + K_1 K_2} \tag{6}$$

$$\bar{u}_{H_2A} = \frac{K_1 c_H u_{HA} + K_1 K_2 u_A}{c_H^2 + K_1 c_H + K_1 K_2} \tag{7}$$

where K_1 and K_2 are the dissociation constants of the acid and the subscripts HA and A denote the species HA^- and A^{2-}, respectively.

These relationships make the determination of ionic mobilities and dissociation constants from the measurement of effective mobilities possible.

III. Methods of Determination of Effective Mobility

In contemporary analytical capillary isotachophoresis, three techniques are commonly used for on-line detection of migrating zones, namely, potential-gradient detection, conductivity detection, and UV-absorption detection. The former two, which measure electric zone properties, are convenient for the evaluation of effective mobilities.

A. Potential-Gradient Detection

The potential-gradient detector provides a signal directly proportional to the electric field strength in the

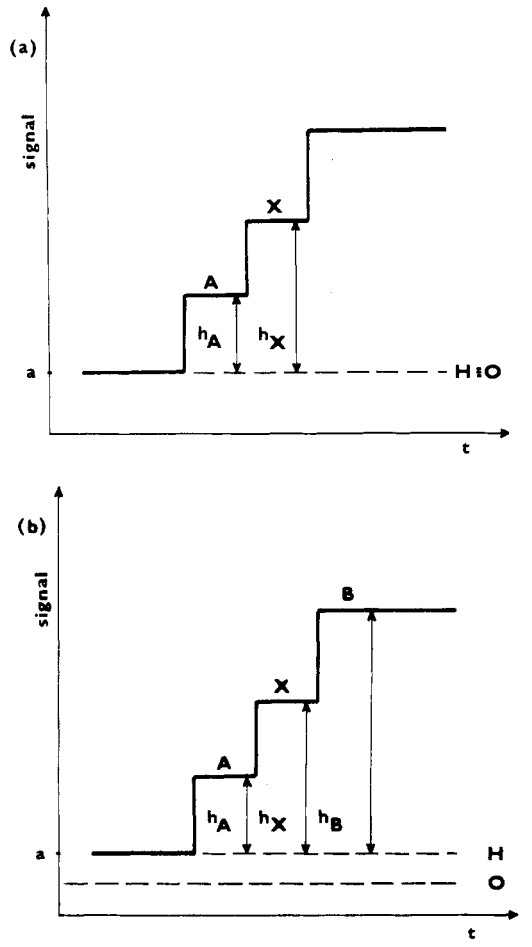


Figure 2. Evaluation of potential-gradient detection record: (a) actual base line (*H*) identical with ideal zero line (0); (b) actual base line (*H*) shifted against ideal zero line (0); *a* is the signal at zero driving current.

zone.[5,6] Assuming constant cross-section of the detection cell and known distance between the electrodes, the real value of the electric potential gradient is measured. In order to obtain an absolute scale, the detection signal may be calibrated by an external power source.

When performing exact measurements, one must take into account the polarization of the sensing electrodes. This contributes an extra voltage across the electrodes, which becomes part of the detection signal. As a result, the base line shows some drift from the real zero. In Figure 2a we can see a record of a potential-gradient detector signal; the actual base line (*H*) is identical with the ideal zero (0). Figure 2b shows the case where electrode polarization causes a displacement of the base line. The disturbing effect of polarization voltage can be suppressed by a suitable experimental arrangement, or it can be calculated and used for correction of experimental values (see below).

An experimental solution of the problem consists in the use of nonpolarizable electrodes.[7] Alternatively, it is possible to increase the measured voltage values so that the contribution of the polarization voltage to the signal magnitude becomes negligible. In this case it is also necessary to increase the distance between the measuring electrodes; otherwise too great of a driving current would be needed.

In potential-gradient detection, the zero is usually taken equal to the actual base line. The effective mobility of substance X can be expressed[6] as

$$\bar{u}_X = \bar{u}_A(h_A/h_X) \tag{8}$$

where h is the step height measured from this line and A is a suitable standard (e.g., the leading ion); cf. Figure 2a. For the elimination of the influence of electrode polarization, two standard substances are necessary

$$\bar{u}_X = \bar{u}_A\bar{u}_B\frac{h_B - h_A}{\bar{u}_A(h_X - h_A) - \bar{u}_B(h_X - h_B)} \tag{9}$$

where the step heights, h, are measured from any arbitrary base line (e.g., from the actual base line; cf. Figure 2b). If this base line is shifted into the step height of the leading zone and if the leading substance (A) is one of the standards, eq 9 reduces to

$$\frac{1}{\bar{u}_X} = \frac{1}{\bar{u}_A} + \left(\frac{1}{\bar{u}_B} - \frac{1}{\bar{u}_A}\right)\frac{h_X}{h_B} \tag{10}$$

Alternatively, the correction to the ideal zero can be done by calculating the magnitude of the polarization voltage.[9] This is performed by comparison of the measured and simulated ratios of potential gradients in the zones of a pair of standard substances whose mobilities and dissociation constants are known with sufficient precision. Hirokawa et al. derived the following equation:[9]

$$\Delta h = \frac{h_S - h_L R_{E,S}}{R_{E,S} - 1} \tag{11}$$

where $R_{E,S}$ is the simulated ratio of potential gradients in zones S and L, E_S/E_L, and Δh is the magnitude of the polarization voltage expressed as the contribution to the step height. The so-obtained value of Δh is then added to the measured step heights of all zones. For the R_E value of substance X this gives

$$R_{E,X} = \frac{h_X + \Delta h}{h_L + \Delta h} = \frac{\bar{u}_L}{\bar{u}_X} \tag{12}$$

All of the foregoing expressions neglect the influence of temperature differences between the zones on the effective mobilities. For extrapolation of the measured values to zero driving current (i.e., to the thermostating temperature), the following equation can be used:[10]

$$\bar{u}_X = \bar{u}_A\frac{h_{A1}}{h_{X1}}\frac{h_{A2}}{h_{X2}}\frac{h_{X1}^2 - h_{X2}^2}{h_{A1}^2 - h_{A2}^2}\frac{h_{A1}/I_2 - h_{A2}/I_1}{h_{X1}/I_2 - h_{X2}/I_1} \tag{13}$$

where the subscripts 1 and 2 relate to two measurements at two different driving currents I_1 and I_2 and where the h values are assumed to be measured from the ideal zero.

B. Conductivity Detection

In conductivity detection we measure the specific resistance of the zone; the sensing electrodes are usually in contact with the electrolyte. The measurement is carried out by using alternating current of a suitable frequency.[1] The disadvantage of the isotachophoretic conductivity measurement over conventional conductometry stems from the small surface area of the sensing electrodes. This may result in a bias in the measured

values, arising from the adsorption of substances in the zones onto the surface of the sensing electrodes. Such adsorption effects occur especially when macromolecular substances are measured.

For conductivity detectors, the signal magnitude usually does not express the absolute value of the measured quantity, and the signal magnitude at zero specific resistance is not experimentally available. The absolute value must be obtained by calibration of the detection cell with solutions of known conductivity, e.g., 0.01–0.001 M KCl.[11]

In isotachophoretic practice, more often the detection signal is calibrated by the evaluated quantity,[1] which is usually mobility. The evaluation of effective mobilities from the conductivity detector signal is analogous to the previous case; since the zero value is generally not available, two standards must be always used and the evaluation is made by using eq 9 or 10 (see also Figure 2b).

C. Thermocouple Detection

Formerly, the thermocouple detector signal was also used for evaluation of effective mobilities. This detector measures the temperature increase on the surface of the separation capillary. This is proportional to the electric output dissipated in a unit zone volume, corresponding to the rate of the Joule heat production. The thermocouple signal is linearly proportional to the effective mobility of the substance in its individual zone; thus, by using a suitable standard, the mobility may be evaluated. The thermocouple detector is not used in contemporary commercial instrumentation since it is not very suitable for the detection of short zones.

D. Determination of Effective Mobility from Migration Time

In some cases, the effective mobility has been evaluated from other data than described above. Most important is the evaluation from migration time. Here the measured substance must form either the leading or the terminating zone; i.e., the advantage of isotachophoresis as a separation technique is lost to a great extent.

The method of Kenndler et al.[12] is useful when there is no suitable standard ion of known mobility; this is especially the case for nonaqueous solvents. In this method, the migration speed of the rear boundary of the leading zone containing the given substance is measured at a constant driving current. From the voltage across the ends of the column at the beginning of the experiment, the value of E is determined (see Figure 3a) and the effective mobility is then calculated directly from eq 1; see Figure 3b.

Another method for evaluating mobility from migration time has been described by Carchon and Eggermont.[13] The method consists in measuring the time of passage of the front boundary of the terminating zone (containing the measured substance) over a given distance in the column, with constant working voltage across the ends of the column. In the course of such a measurement, the boundary between the terminating zone and leading zone migrates into the column, causing an increase in resistance. This causes a decrease of the driving current and thus also a decrease of the thermocouple detection signal. From the record we may

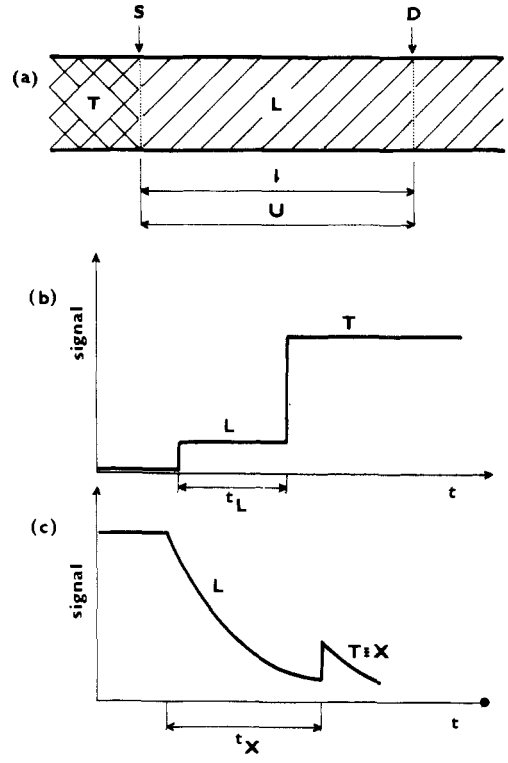


Figure 3. Determination of effective mobility from migration time. (a) Starting situation: the column of length l is filled with leading electrolyte, the voltage across this length is U, and thus $E_L = U/l$; the boundary T–L starts to migrate at point S and it is detected at point D. (b) Evaluation of migration time t_L at constant driving current. (c) Evaluation of migration time t_X at constant voltage.

read out the time of passing the boundary through the detector, as depicted in Figure 3c. This time can be expressed as

$$t_X = \frac{u_L + \bar{u}_X}{2u_L\bar{u}_X}\frac{l^2}{U} \tag{14}$$

where u_L and $\bar{u}_X$ are the mobilities of the leading and measured ions, respectively, l is the migration distance, and U is the applied voltage. The relative apparent mobility was defined as

$$\mathrm{RAM} = (t_S/t_X) \times 100 \tag{15}$$

where S denotes a suitable standard of known mobility. The effective mobility of the measured substance X is then given by

$$\bar{u}_X = \frac{(\mathrm{RAM})u_S u_L}{100(u_L + u_S) - (\mathrm{RAM})u_S} \tag{16}$$

IV. Procedures for Determination of u_i and pK_a

A. Measurement of u_i at Full Ionization

If the experimental conditions are selected so that the given substance is fully ionized in its zone, then its effective mobility is equal to the actual value of its ionic mobility. The choice of working conditions is easy in the case of strong electrolytes, where nearly any electrolyte system is convenient. For the measurement of ionic mobilities of weak electrolytes the choice of a system is more difficult. Calculations may help in the

choice of an electrolyte system where the electrolytes are completely ionized. Obviously, for this, the ionization constant must be known.

Owing to the dependence of mobility on temperature and ionic strength, the experimental conditions must always be specified. Concerning temperature effects, the measurement is usually carried out at low driving current where heating of the zone is negligible (<1 K), and the temperature is thus taken to be equal to the thermostating one. The ionic strength in the zone of a given substance depends on the parameters of this substance and on the composition of the leading electrolyte used. It may be calculated with sufficient precision after the measurement.

Values of ionic mobilities of alkylammonium and carboxylate ions were obtained by Kiso and Hirokawa.[14] The zone pH was calculated to show that the electrolytes were completely ionized at the experimental conditions.

The ionic strength in the zone was calculated and used for the calculation of the limiting ionic mobility. The measurements were performed at various temperatures that were determined directly in the zone by a miniature thermocouple. The reproducibility of the obtained values was estimated to be $\pm 1.5 \times 10^{-9}$ m^2 V^{-1} s^{-1}.

For precise measurement of actual ionic mobilities a method based on the measurement of the electric potential gradient in the zones has been developed.[8] This method eliminates the effect of polarization of the sensing electrodes by increasing the magnitude of the measured voltage owing to increased distance between these electrodes (10.5 mm). Moreover, the measurements were performed at a constant potential gradient in all zones (obtained by varying the driving current); this suppressed the temperature differences between the zones and eliminated the influence of nonlinearity of the detector on the accuracy of the measurement. The obtained values are determined at 0.01 M ionic strength.

B. Evaluation of u_i and pK_a from Effective Mobility Measurements

From section II it follows that the evaluation of data from measurements at partial ionization of the substances is not easy. In practice, one should consider such experiments in only two cases: (i) if no isotachophoretic system in which the given substance is fully ionized is available and (ii) if one also needs to evaluate the values of the respective ionization constants from the measurements. In the latter case it is advantageous to evaluate the values of the ionic mobilities first from measurements performed at full ionization; this leads to increased precision of the determined values of the ionization constants.

The process of evaluation is depicted in Figure 4. Measurements must be performed in several electrolyte systems selected in such a way that the measured substance shows varied values of its effective mobility in each system. In this way, a series of effective mobilities and of the corresponding parameters of the leading electrolytes are obtained.

In the next step, the effective mobilities of the given substance are calculated for the leading electrolyte compositions used, with ionic mobility and pK_a taken as the parameters. Usually, the well-known RFQ method[1] is used for this purpose. The best fit of the calculated data with the experimental data is used.

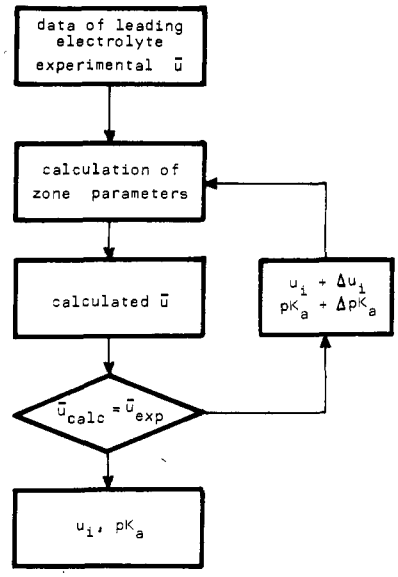


Figure 4. Scheme of evaluation of u_i and pK_a from measured effective mobilities ($\bar{u}$).

The described method has been extensively used especially by Hirokawa et al., who obtained large sets of limiting ionic mobilities and thermodynamic dissociation constants of anions,[15] nucleotides, phosphorus oxoacids,[16] amino acids,[17] and dipeptides.[18] Their calculation program is based on nonlinear regression by the least-squares method of the measured and simulated R_E values of the given substance. The best results were obtained by first determining the actual ionic mobility. For the simulation, only the pK_a value was taken as the variable parameter.[19]

A separate paper[20] was aimed at the accuracy of the resulting values of u_i and pK_a obtained by computer simulation (see section VI).

The method described above was used also for the measurement of mobilities and apparent dissociation constants of anionogenic substances in methanol.[21]

Kašička et al.[11] published a program for the calculation of concentration values of dissociation constants and of limiting ionic mobilities, including corrections to temperature and ionic strength. They used effective mobilities obtained by potential-gradient detection as input experimental values. From the known thermal resistance of the detection cell, the conductivity of the electrolyte, and the magnitude of the driving current, the zone temperature could be calculated and used for correction of all temperature-dependent quantities to standard temperature. In order to reach sufficient precision of the measured data, 14 different electrolyte systems were used.

The determination of stability constants of complexes by Kiso and Hirokawa[22] is based on the same principle as in the case of weak protolytes (see above). The R_E values are measured at different concentrations of the ligand in the leading electrolyte and then compared with the calculated ones. The values of mobilities of some complex ions that were not known could be estimated by calculation from their molecular mass. By the mentioned method the authors obtained the stability constants of some complexes of tartaric and citric

TABLE I. Mobilities and pK_a Values of Anions in Water

substance	u, 10^{-9} m^2 V^{-1} s^{-1}	pK_a	I, M	ref	substance	u, 10^{-9} m^2 V^{-1} s^{-1}	pK_a	I, M	ref
ACES	−31.3	6.84	0.00505	32	cyanoacetate	−42.1		?	13
acetate	−42.7		?	13	cysteine (1)	−27.0	8.405		17
	−39.4		0.01	8	cysteine (2)	−53.9	9.845		17
acetylsalicylate	−26.7	3.485[a]		9	dichloroacetate	−39.4	1.257[a]		15
	−26.7		0.01	8	dichromate (1)	−58.9	0.745[a]		15
acrylate	−30.4		0.01	8	dichromate (2)	−82.1	6.723		15
adipate (2)	−43.5		0.015	8	diethylbarbiturate	−26.2	7.91	0.00602	32
ADP (1)	−19.2			16	2,4-dihydroxybenzoate	−31.8	3.239		15
ADP (2)	−36.7	4.101		16	3,5-diiodotyrosine (1)	−21.0	6.5		17
ADP (3)	−53.7	7.056		16	3,5-diiodotyrosine (2)	−42.0	9.469		17
α-alanine	−32.2	9.857		17	2,3-dimethylbenzoate	−27.1	3.738[a]		9
β-alanine	−30.8	10.241		17	2,4-dimethylbenzoate	−27.1	4.182[a]		9
alanylalanine	−27.0	8.490		18	2,5-dimethylbenzoate	−27.1	3.977[a]		9
alanyl-α-amino-*n*-butyrate	−25.8	8.495		18	2,6-dimethylbenzoate	−27.1	3.246[a]		9
alanylasparagine	−25.5	8.470		18	3,4-dimethylbenzoate	−27.1	4.408[a]		9
alanylglycine	−28.8	8.390		18	3,5-dimethylbenzoate	−27.1	4.301[a]		9
β-alanylhistidine	−24.4	9.664		18	2,4-dinitrophenolate	−29.0		0.01	8
alanylleucine	−23.9	8.505		18	diphenylacetate	−23.8		?	13
alanylmethionine	−24.2	8.463		18	dithionite (2)	−67.7		?	13
alanylphenylalanine	−23.9	8.502		18	dodecyl sulfate	−23.1		?	13
alanylserine	−26.2	8.297		18	enanthate	−28.7	4.887		15
alanylvaline	−25.2	8.500		18	*o*-ethoxybenzoate	−26.6	4.208[a]		9
m-aminobenzoate	−28.2		0.01	8	*p*-ethoxybenzoate	−26.6	4.796[a]		9
o-aminobenzoate	−28.8		0.01	8	*o*-ethylbenzoate	−26.5	3.793[a]		9
p-aminobenzoate	−32.3	4.939		15	*p*-ethylbenzoate	−26.5	4.353[a]	?	9
	−28.9		?	13	fluoride	−58.5		?	13
	−28.5		0.01	8	formate	−57.1	3.796		15
α-amino-*n*-butyrate	−30.5	9.827		17		−58.4		?	13
AMP	−22.6	3.981		16		−53.2		0.01	8
	−39.5	6.791		16	fumarate (1)	−35.1	3.02[a]		15
asparagine	−31.6	9.030		17		−31.0	3.019[a]		9
aspartate (1)	−30.1	3.900		17	fumarate (2)	−60.5	4.384[a]		15
aspartate (2)	−55.4	10.002		17		−61.2	4.384[a]		9
ATP (2)	−37.5			16		−53.3		0.015	8
ATP (3)	−49.2	4.418		16	gallate	−24.0	4.469[a]	0.01	8
ATP (4)	−64.7	7.064		16	GDP (1)	−18.7[b]			16
azide	−71.9		?	13	GDP (2)	−37.3	2.958		16
azelaate (2)	−38.7		0.015	8	GDP (3)	−52.9	7.116		16
barbiturate	−29.2		0.01	8	GDP (4)	−68.5[b]	9.5[b]		16
benzoate	−32.9	4.166		15	glucuronate	−26.6	3.516		15
	−31.9		?	13	glutamate (1)	−28.7	4.302		15
	−30.3		0.01	8		−27.0	4.324		17
DL-benzyl aspartate	−25.7	4.885[a]		15		−25.4		0.01	8
BES	−24.0	7.16	0.00602	32	glutamate (2)	−54.3	9.960		17
5-bromo-2,4-dihydroxybenzoate (1)	−27.6	3.0[b]		17	glutamine	−28.8	9.244		17
5-bromo-2,4-dihydroxybenzoate (2)	−50.7	7.60		17	glutarate (2)	−46.2		0.015	8
o-*tert*-butylbenzoate	−23.2	3.535[a]		9	glycerate	−36.3	3.656		15
p-*tert*-butylbenzoate	−23.2	4.400[a]		9	glycine	−37.4	9.7796		17
n-butyrate	−33.7	4.807		15	glycolate	−42.3	3.850		15
	−32.4		?	13	glycylalanine	−28.8	8.435		18
cacodylate	−29.9	6.182		15	glycyl-α-amino-*n*-butyrate	−27.5	8.412		18
caprate	−22.1	5[a]		15	glycylasparagine	−27.2	8.388		18
n-caproate	−30.5	4.913		15	glycylglycine	−31.5	8.400		18
	−28.4		?	13	glycylisoleucine	−25.2	8.412		18
caprylate	−27.4	4.930		15	glycylleucine	−25.1	8.432		18
CDP (1)	−19.5			16	glycylphenylalanine	−24.8	8.235		18
CDP (2)	−40.0	4.782		16	glycylproline	−27.8	8.746		18
CDP (3)	−57.3	7.349		16	glycylserine	−28.1	8.350		18
chlorate	−66.6	−2.7[a]		15	glycylthreonine	−26.3	8.334		18
	−64.3		0.01	8	glycyltryptophan	−23.6	8.359		18
chloroacetate	−41.9	2.865[a]		15	glycyltyrosine (1)	−19.7	8.211		18
	−37.4		0.01	8	glycyltyrosine (2)	−39.4	9.981		18
3-chloropropionate	−36.8	3.804		15	glycylvaline	−26.0	8.385		18
chromate (1)	−59.3	0.745[a]		15	GMP (1)	−21.7	2.845		16
chromate (2)	−81.1	6.49[a]		15	GMP (2)	−38.0	6.512		16
cinnamate	−28.3	4.438[a]		9	GMP (3)	−54.3[b]	9.5[b]		16
cis-cinnamate	−28.3	3.879[a]		9	GTP (2)	−34[b]			16
citrate (1)	−31.0	3.128[a]		15	GTP (3)	−49.8	3.044		16
citrate (2)	−53.4	4.761[a]		15	GTP (4)	−64.1	7.158		16
citrate (3)	−70.8	6.396[a]		15	GTP (5)	−78.4	9.5[b]		16
CMP (1)	−23.7	4.468		16	HEPES	−21.8	7.51	0.00545	32
CMP (2)	−40.6	6.705		16	HEPPSO	−22.0	7.51	0.00520	32
crotonate	−33.5	4.705[a]		9	hippurate (1)	−25.9	2.5		15
CTP (2)	−36.0			16	hippurate (2)	−55.3	7.346		15
CTP (3)	−54.4	5.255		16	histidine	−28.3	9.330		17
CTP (4)	−66.8	7.35[b]		16	2-hydroxybutyrate	−34.3	3.979[a]		9

TABLE I (Continued)

substance	u, 10^{-9} m^2 V^{-1} s^{-1}	pK_a	I, M	ref
3-hydroxybutyrate	−34.3	4.519[a]		9
4-hydroxybutyrate	−34.4	4.721[a]		9
m-hydroxycinnamate	−27.0	4.397[a]		9
o-hydroxycinnamate	−27.0	4.613[a]		9
p-hydroxycinnamate	−27.0	4.678[a]		9
2-hydroxyisobutyrate	−33.5	3.971[a]		9
p-hydroxyphenylacetate	−26.9	3.171[a]		9
hydroxyproline	−30.1	9.816		17
hypophosphite	−45.1	1.1		16
	−43.3	1.230[a]	0.01	8
IDP (2)	−38.3			16
IDP (3)	−54.2	7.165		16
IDP (4)	−70.2[b]	9.5[b]		16
iodate	−41.9	0.772[a]		15
	−39.7		0.01	8
	−40.3		?	13
IMP (1)	−22.6	2.575		16
IMP (2)	−38.2	6.545		16
IMP (3)	−53.9[b]	9.5[b]		16
isoleucine	−26.7	9.765		17
o-isopropylbenzoate	−24.7	3.635[a]		9
p-isopropylbenzoate	−24.7	4.354[a]		9
ITP (3)	−52.8			16
ITP (4)	−65.0	7.2		16
ITP (5)	−77.3	9.5[b]		16
α-ketoglutarate (1)	−37.5	2.800		15
α-ketoglutarate (2)	−59.0	5.272		15
lactate	−35.8	3.854		15
	−33.7		0.01	8
leucine	−26.4	9.728		17
leucylglycine	−25.0	8.269		18
leucylleucine	−21.6	8.397		18
leucylphenylalanine	−21.8	8.413		18
L-leucyl-L-tyrosine (1)	−18.2	7.828		18
L-leucyl-L-tyrosine (2)	−36.4	10.065		18
leucylvaline	−22.3	8.364		18
levulinate	−33.4	4.594		15
lysine	−26.4	10.79		17
maleate (1)	−42.5	1.943[a]		15
maleate (2)	−62.0	6.225[a]		15
malate (1)	−34.9	3.46[a]		15
malate (2)	−58.5	5.05[a]		15
	−51.6		0.015	8
malonate (1)	−42.4	2.847[a]		15
malonate (2)	−65.4	5.696[a]		15
	−62.2		?	13
mandelate	−28.3	3.411[a]		9
MES	−26.8	6.13	0.005	32
	−28.0	6.095[a]		9
methacrylate	−36.6	4.458		15
methionine	−29.3	9.344		17
m-methoxybenzoate	−28.3	4.088[a]		9
o-methoxybenzoate	−28.3	4.094[a]		9
MOPS	−24.4	7.16	0.006	32
MOPSO	−23.8	6.79	0.0062	32
naphthalene-2-sulfonate	−31.3	−2[a]		15
nicotinate	−34.6	4.819		15
nitrate	−75.4	−1.37[a]		15
	−68.3		?	13
	−69.7		0.01	8
nitrite	−68.8		?	13
	−69.6		0.01	8
p-nitrobenzoate	−32.1	3.391		15
m-nitrobenzoate	−29.3		0.01	8
ornithine	−28.4	10.755		17
orotate	−32.9	2.519		15
oxalate (1)	−44.9	1.271[a]		15
oxalate (2)	−74.6	4.266[a]		15
	−73.4		?	13
pelargonate	−26.7	4.678		15
perchlorate	−70.0	−2[a]		15
	−71.9		?	13
	−65.8		0.01	8
periodate	−51.8		0.01	8
permanganate	−59.7		0.01	8
phenoxyacetate	−27.8	3.171[a]		9
phenylacetate	−31.5	4.351		15
	−29.2		?	13
	−27.8		0.01	8
phenylalanine	−26.9	9.262		17
4-phenylbutyrate	−24.7	4.757[a]		9
2-phenylpropionate	−26.5	2.237[a]		9
3-phenylpropionate	−26.5	4.664[a]		9
phosphate (1)	−34.1	2.148[a]		15
phosphate (2)	−58.3	7.22[a]		15
phosphate (3)	−71.5[a]	11.5[a]		15
phosphite (1)	−40.0	1.3		16
phosphite (2)	−65.9	7.086		16
o-phthalate (1)	−35.3	2.95[a]		15
o-phthalate (2)	−52.7	5.408[a]		15
	−52.5		?	13
	−45.4		0.015	8
picrate	−31.7	0.708[a]		15
	−30.5		?	13
pimelate (1)	−27.6	4.509[a]		15
pimelate (2)	−48.4	5.312[a]		15
pivalate	−31.6	5.007		15
proline	−25.4	10.640		17
propionate	−36.9	4.779		15
	−36.1		?	13
	−34.2		0.01	8
pyrazine-2,3-dicarboxylate (1)	−36.6	1[a]		15
pyrazine-2,3-dicarboxylate (2)	−55.7	4.308		15
pyrazole-3,5-dicarboxylate (1)	−25.4	1[a]		15
pyrazole-3,5-dicarboxylate (2)	−56.9	3.894		15
2-pyridinecarboxylate	−29.6		0.01	8
3-pyridinecarboxylate	−30.6		0.01	8
4-pyridinecarboxylate	−30.6		0.01	8
pyrophosphate (1)	−29.0	1		16
pyrophosphate (2)	−57.9	1.9		16
pyrophosphate (3)	−76.4	6.6		16
pyrophosphate (4)	−89.4	9.6		16
pyruvate	−42.3	2.490[a]		9
salicylate	−35.3	2.937		15
	−33.9		?	13
selenate (1)	−41.0	0.000[a]		9
selenate (2)	−80.5	2.050[a]		9
selenite (1)	−41.2	2.620[a]		9
selenite (2)	−60.5	8.450[a]		9
serine	−33.6	9.302		17
sorbate	−27.3	4.770[a]	0.01	8
succinate (1)	−35.2	4.207[a]		15
succinate (2)	−57.5	5.638[a]		15
	−61.5		?	13
sulfamate	−50.3	−2[a]		15
sulfamidate	−45.5		0.01	8
sulfanilate	−33.7	3.127		15
sulfate (1)	−45[a]	−2[a]		15
sulfate (2)	−79.5	1.921[a]		15
	−71.6		0.015	8
sulfite (1)	−49.3	1.78[a]		15
sulfite (2)	−67.1	6.991[a]		15
TAPS	−25.0	8.300[a]		9
tartrate (1)	−34.6	3.036[a]		15
tartrate (2)	−60.5	4.366[a]		15
tartronate (1)	−38.9	2.366[a]		15
tartronate (2)	−67.8	4.735[a]		15
taurine	−37.9	9.182		17
terephthalate (1)	−28.0	3.540[a]		9
terephthalate (2)	−53.0	4.460[a]		9
TES	−22.4	7.43	0.00566	32
tetrametaphosphate (3)	−75.6			16
tetrametaphosphate (4)	−94.7	2.74		16
thiocyanate	−68.0		?	13
	−64.2		0.01	8
threonine	−30.9	9.200		17
m-toluate	−29.1	4.272[a]		9
o-toluate	−29.1	3.908[a]		9
p-toluate	−29.1	4.373[a]		9
p-toluenesulfonate	−29.3		0.01	8
trichloroacetate	−36.2	0.635[a]		15
	−37.9		?	13

TABLE I (Continued)

substance	u, 10^{-9} m^2 V^{-1} s^{-1}	pK_a	I, M	ref	substance	u, 10^{-9} m^2 V^{-1} s^{-1}	pK_a	I, M	ref
	−33.1		0.01	8	tyrosine (2)	−40.0	10.189		17
trimetaphosphate (1)	−28.9	−2.00[a]		9	UDP (2)	−39.4			16
trimetaphosphate (2)	−57.8	−1.00[a]		9	UDP (3)	−56.0	7.088		16
trimetaphosphate (3)	−87.6	2.050[a]		9	UDP (4)	−72.6	9.5[b]		16
	−87.7	2.05		16	UMP (1)	−23.4	2.499		16
2,4,6-trimethylbenzoate	−24.7	3.437[a]		9	UMP (2)	−39.7	6.529		16
triphosphate (2)	−48[b]	1.1		16	UMP (3)	−56[b]	9.5[b]		16
triphosphate (3)	−74.7	2.3		16	UTP (3)	−54.9			16
triphosphate (4)	−89.3	6.5		16	UTP (4)	−66.8	7.1		16
triphosphate (5)	−113.0	9.24		16	UTP (5)	−78.8[b]	9.5[b]		16
tryptophan	−25.4	9.594		17	*n*-valerate	−30.3		?	13
tungstate (2)	−74.6		?	13	valine	−28.4	9.710		17
tyrosine (1)	−20.0	9.099		17	vanilate	−27.1	4.523[a]		9

[a] Data taken from the literature.[33,34] [b] Data estimated by authors of the respective paper. ACES, BES, HEPES, HEPPSO, MES, MOPS, MOPSO, TAPS, and TES are abbreviations of Good's buffers; ADP, AMP, ATP, CDP, CMP, CTP, GDP, GMP, GTP, IDP, IMP, ITP, UDP, UMP, and UTP are abbreviations of nucleotides.

acids,[23] complexes of acetic and α-hydroxyisobutyric acid with lanthanoids,[24] and complexes of hydroxycarboxylic acids.[25]

In the papers,[24,25] also the coordination numbers of complexes were determined. Also here the best-fit method of calculation was used. Since the calculation can be performed only for integer values of coordination numbers, it was necessary to estimate its noninteger value from two neighboring fits. In this way, the coordination numbers of complexes of Ba, Sr, Ca, Mg, Cd, Co, Zn, and Ni with glycolate, α-hydroxybutyrate, β-hydroxybutyrate, α-hydroxyisobutyrate, and lactate were determined.[25]

The same authors also determined the ion-pair formation constants of phosphorus oxoacids with histidine.[26]

The method of calculation of ionic mobilities and dissociation constants of monovalent ions by Beckers[27] is based on the idea that to a given value of zone conductivity corresponds an infinite number of pairs of u_i and pK_a values. If these pairs are plotted in a u_i–pK_a coordinate system, for a given value of conductivity and given electrolyte system a curve, called an isoconductor, is obtained. In another electrolyte system, the zone of a substance of a given u_i and pK_a may have another conductivity and thus another corresponding isoconductor. The intersection of two such isoconductors then determines the proper values of u_i and pK_a of the given substance.

A paper[28] on the evaluation of limiting ionic mobilities and dissociation constants presented a program including temperature and ionic strength correction of mobilities and dissociation constants. The input data for the calculation were thermostating temperature, thermal resistance coefficients of the column, driving current, the resistance capacity of the conductivity detection cell, the capillary cross-section, the pH of the leading electrolyte and its conductivity, the chart speed, the ionic mobilities and concentrations of the leading ion and the counterion in the leading electrolyte, and the coefficients of the temperature dependence of the dissociation constant of the counterion.

A simplified theory for the calculation of stability constants of neutral ligand complexes was presented by Stover[29,30] with the aim of describing the separation of alkali metals with neutral 18-crown-6 ether. The values of effective mobilities of these metal cations obtained from conductivity detection data served for the calculation of the respective stability constants.

A paper by Jokl et al.[31] was aimed at the determination of ionic mobilities and dissociation constants of cations of pharmaceutically interesting substances. The method described here is based on the calculation from the linearized dependence of effective mobility on pH. The respective zone pH is calculated from the known composition of the leading electrolyte and from the u_i and pK_a values of the given substance. As input parameters for the iteration procedure the following are necessary: two different effective mobilities measured in two electrolyte systems, the parameters of the two electrolyte systems, and an estimate of u_i and pK_a.

C. Evaluation of u_i and pK_a from Simultaneous Measurement of $\bar{u}_i$ and pH$_i$

In the previous section, we described the methods of obtaining u_i and pK_a values based on calculation of zone properties from the known composition of the leading electrolyte. The values obtained in such a way involve naturally the bias of the input values, including the parameters of the leading electrolyte. From the viewpoint of accuracy and precision, use of specialized apparatus[32] that allows direct micropreparative measurement of zone pH is preferable. The needed pair of $\bar{u}_i$ and pH$_i$ values is measured experimentally, and from these it is easy to evaluate the respective u_i and pK_a values. The relative bias of the method is <3% in the determination of mobilities and 0.05 unit in the determination of pK_a.

V. Survey of Data

We present a list of data for anions in water (Table I) and methanol (Table II), for cations in water (Table III) and mixed water/methanol/dimethyl sulfoxide medium (Table IV), and for metal–ligand complexes (Tables V and VI). The data are corrected to zero ionic strength, unless another value of ionic strength is given in the column labeled "I".

VI. Precision and Accuracy of the Methods

The reproducibility of the resulting values of u_i and pK_a is strongly affected by the reproducibility of the input experimental values. For potential-gradient

TABLE II. Mobilities and Apparent pK_a Values (pK_a*) of Anions in Methanol[21]

substance	u, 10^{-9} m^2 V^{-1} s^{-1}	pK_a*
acetate	−43.8	8.472
acrylate	−43.5	8.134
p-anisate	−38.9	8.669
benzoate	−41.4	8.299
bromate	−56.6	
bromide	−58.3	
bromoacetate	−45.3	6.716
2-bromopropionate	−43.1	7.002
5-bromosalicylate	−44.4	
butyrate	−41.6	8.745
caproate	−39.6	8.767
caprylate	−38.4	8.760
chlorate	−63.9	
chloroacetate	−45.6	6.636
m-chlorobenzoate	−41.3	7.780
o-chlorobenzoate	−40.0	7.182
p-chlorobenzoate	−40.9	7.978
2-chloropropionate	−43.9	6.876
cinnamate	−38.5	8.291
crotonate	−41.7	8.587
dehydroacetate	−40.8	8.771
2,3-dibromobutyrate	−41.1	6.643
2,3-dibromopropionate	−42.5	6.961
dichloroacetate	−47.1	
enanthate	−39.0	8.752
fluoride	−41.6	
fluoroacetate	−45.7	4.3
formate	−48.4	7.064
glucuronate	−32.6	7.331
glutamate	−32.2	7.884
glycerate	−41.0	7.396
glycolate	−45.0	7.549
2-hydroxybutyrate	−42.9	7.724
3-hydroxybutyrate	−41.4	8.323
2-hydroxyisobutyrate	−43.2	7.873
iodate	−40.9	7.149
iodide	−65.6	
iodoacetate	−43.6	7.152
isovalerate	−41.7	8.830
lactate	−43.8	7.749
levulinate	−41.2	8.582
mandelate	−40.2	7.413
MES	−40.6	7.410
methacrylate	−42.8	8.616
2-naphthalenesulfonate	−41.3	
nicotinate	−40.3	7.334
nitrate	−62.9	
nitrite	−57.6	7.128
palmitate	−32.6	8.793
pelargonate	−37.1	8.753
picrate	−49.3	
propionate	−43.4	8.781
pyruvate	−47.4	7.649
salicylate	−45.1	6.728
sorbate	−40.2	8.592
sulfosalicylate	−45.3	6.661
thiocyanate	−64.3	
trichloroacetate	−47.3	
trifluoroacetate	−52.9	
valerate	−40.8	8.754

measurements, the reproducibility of the R_E values by Hirokawa et al.[15] lies between ±0.02 and ±0.05 unit; the average relative standard deviation (rsd) of the relative effective mobilities by Pospíchal et al.[32] was 0.55%. The average rsd of the apparent mobility by Carchon and Eggermont[13] was 1.2%. The rsd of the relative step heights of potential-gradient measurements by Kenndler et al.[12] was 2%; the average rsd of direct mobility measurement was 0.4%. For conductometric detection, Everaerts et al.[1] give a precision of the relative step heights of better than 4%. It can be concluded from

TABLE III. Mobilities and pK_a Values of Cations in Water

substance	u, 10^{-9} m^2 V^{-1} s^{-1}	pK_a	I, M	ref
β-alanine	+37.5	3.552		16
4-aminoantipyrine	+25.0	4.41		31
p-aminobenzoic acid	+32.3	2.38		15
ε-aminocaproic acid	+29.8	4.374		16
	+30.0	4.44		31
aminophenazone	+24.2	5.18		31
ammediol	+32.0	8.78[a]		17
	+27.7		0.01	8
ammonium	+72.2	9.25[a]	?	12
n-amylammonium	+32.4			14
aniline	+38.7	4.67		31
arginine	+26.9	8.919		17
	+23.6		0.01	8
benzylammonium	+35.8	9.33		16
n-butylammonium	+34.6			14
	+39.0	10.61[a]	?	12
4-chloroaniline	+31.7	4.17		31
chlorodiazepoxide	+20.8	4.89		31
creatinine	+36.8	4.848		16
	+33.1	4.89	0.0068	32
cyclohexylammonium	+33.6	10.66		16
di-*n*-butylammonium	+27.2	11.25[a]	?	12
diethanolamine	+30.6		0.01	8
diethylammonium	+34.1		0.01	8
	+37.9	11.04[a]	?	12
dimethylammonium	+53.1	10.73[a]	?	12
di-*n*-propylammonium	+31.6	11.00[a]	?	12
ethanolamine	+44.3	9.498[a]		17
	+39.3		0.01	8
ethylammonium	+48.6	10.70[a]	?	12
	+44.8			14
ethylenediammonium (1)	+39.4		0.015	8
glutamic acid	+28.7	2.162		15
guanidine	+50.1		0.01	8
hexamethylenetetramine	+36.8	4.90		31
n-hexylammonium	+30.6			14
histidine	+29.6	6.04		17
	+29.2	6.03		31
	+26.7	6.13	0.0062	32
imidazole	+52.0	7.15[a]		17
	+42.5	7.15		16
lysine	+26.4	9.127		17
	+24.7		0.01	8
medazepam	+23.1	5.61		31
methylammonium	+58.5	10.66[a]	?	12
	+55.0			14
	+55.9		0.01	8
2-methylpyridinium	+41.5	6.42		31
4-methylpyridinium	+42.8	6.08		16
morpholine	+35.8	8.33		16
	+37.4		0.01	8
n-octylammonium	+27.8			14
ornithine	+25.4		0.01	8
	+28.4	10.755		17
1,10-phenanthroline	+32.3	5.07		31
phenyltrimethylammonium	+32.8		0.01	8
n-propylammonium	+37.3			14
	+42.3	10.69[a]	?	12
pyridinium	+51.1	5.13		31
tetra-*n*-butylammonium	+18.5		?	12
	+17.5		0.01	8
tetraethylammonium	+32.9		?	12
	+30.5		0.01	8
tetramethylammonium	+42.6		0.01	8
	+44.9		?	12
tetra-*n*-propylammonium	+23.0		?	12
	+21.2		0.01	8
tri-*n*-butylammonium	+22.0	9.93[a]	?	12
triethylammonium	+30.6		0.01	8
	+33.8	10.72[a]	?	12
trimethylammonium	+47.6	9.80[a]	?	12
tri-*n*-propylammonium	+25.9	10.66[a]	?	12
Tris	+29.5	8.076		17
	+26.9		0.01	8

[a] Data taken from the literature.[33-35]

TABLE IV. Mobilities and Apparent pK_a Values of Cations (pK_a*) in Mixed Solvents[12]

substance	M/W[a]		D/M/W[b]	
	u[d]	pK_a*[c]	u[d]	pK_a*[c]
ammonium	+49.9	8.76	+25.9	9.73
methylammonium	+51.3	9.89	+27.3	10.69
dimethylammonium	+51.1	9.86	+29.2	10.47
trimethylammonium	+49.0	8.38	+27.5	8.79
tetramethylammonium	+46.5		+26.4	
ethylammonium	+45.6	9.81	+25.5	10.59
diethylammonium	+40.8	10.07	+24.5	10.68
triethylammonium	+36.5	9.49	+22.4	9.81
tetraethylammonium	+37.8		+23.9	
n-propylammonium	+41.0	9.55	+23.5	10.72
di-*n*-propylammonium	+34.0	11.08	+20.9	10.46
tri-*n*-propylammonium	+29.0	9.29	+18.7	9.45
tetra-*n*-propylammonium	+26.6		+18.0	
n-butylammonium	+37.7	9.69	+22.3	10.46
di-*n*-butylammonium	+29.4	9.92	+18.7	10.46
tri-*n*-butylammonium	+23.3	9.24	+16.0	9.38
tetra-*n*-butylammonium	+22.4		+14.9	

[a] M/W = 7:3 (mol/mol) methanol/water. [b] D/M/W = 3:4:3 (mol/mol/mol) dimethyl sulfoxide/methanol/water. [c] Data obtained by potentiometric measurement. [d] 10^{-9} m^2 V^{-1} s^{-1}.

the given data that the precision of the data by other authors lie within the same reproducibility interval.

When u_i and pK_a are evaluated by computer simulation, a number of constants (e.g., u_i and pK_a of the counterion) must be involved in the computation procedure. Obviously, the accuracy of these data also has influence on the accuracy of the resulting data. Fortunately, these constants are mostly known with sufficient precision.

The problem of the accuracy of the resulting values of u_i and pK_a obtained by computer simulation was also the subject of a separate paper.[20] On the basis of the reproducibility of the experimental R_E values being ±0.02 unit, the accuracy of the obtained quantities was calculated for each of the electrolyte systems used. The conclusion was that the accuracy of the results depends on the mobility and ionization constant of the measured substance, on the pH of the leading electrolyte, and on the ionization constant of the counterion used. The best accuracy that could be reached was (0.2–1.2) × 10^{-9} m^2 V^{-1} s^{-1} in mobility and ±0.01 in pK_a measurements.

The accuracy of the methods described may be discussed on the basis of comparison of the obtained data with tabulated data from conductometric and potentiometric measurements. The precision of the methods may be estimated from the values of the standard deviation (if given by the authors). Table VII enables comparison of the various methods by presenting u_i, pK_a, and the respective standard deviation (σ) of three selected anions (acetate, formate, and benzoate). It can be seen from the table that the accuracy of all the methods described is sufficient; the measured values mostly coincide with the tabulated ones within the interval $\pm 3\sigma$.

Concerning the precision of the methods, there are greater differences between the various methods. In the method of Carchon and Eggermont[13] the rsd of 338 values ranged from 0 to 9.1% with a median of 1.2%. The method of Pospíchal et al.[8] provided an average rsd of 59 measured values equal to 0.55%. The computational method of Beckers[27] presents its results without data on rsd; the precision of the method strongly depends on the number of experimental data taken for the evaluation of u_i and pK_i. The method of Hirokawa et al.[15] used for the evaluation of a set of data measured

TABLE V. Mobilities and Stability Constants of Complexes

substance[a]	u, 10^{-9} m^2 V^{-1} s^{-1}	log β	ref
Ba-Ac(+)	+21.7	−3.76	22
Ca-Ac(+)	+28.9	−0.24	22
Zn-Ac(+)	+26.2	0.83	22
Cd-Ac(+)	+23.0	1.35	22
Cu-Ac(+)	+26.4	2.12	22
Mg-Tar	0	2.349	23
Ca-Tar	0	2.895	23
Sr-Tar	0	2.690	23
Ba-Tar	0	2.686	23
Cd-Tar	0	2.913	23
Co-Tar	0	3.225	23
Mg-HCit	0	1.672	23
Mg-Cit(−)	−21.1	4.917	23
Ca-HCit	0	2.786	23
Ca-Cit(−)	−22.3	4.850	23
Sr-HCit	0	2.815	23
Sr-Cit(−)	−23.3	4.410	23
Ba-HCit	0	2.686	23
Ba-Cit(−)	−22.0	4.150	23
Ba-Glyc(+)	+21.6	0.935	25
Sr-Glyc(+)	+24.8	1.079	25
Ca-Glyc(+)	+29.7	1.612	25
Mg-Glyc(+)	+32.0	1.441	25
Cd-Glyc(+)	+23.0	1.841	25
Co-Glyc(+)	+27.4	2.216	25
Ni-Glyc(+)	+27.5	2.230	25
Ni-Glyc$_2$	0	3.544	25
Zn-Glyc(+)	+26.8	2.286	25
Zn-Glyc$_2$	0	3.684	25
Ba-Lac(+)	+20.9	0.540	25
Sr-Lac(+)	+23.7	0.777	25
Ca-Lac(+)	+28.0	1.381	25
Mg-Lac(+)	+29.9	1.255	25
Cd-Lac(+)	+22.2	1.662	25
Co-Lac(+)	+26.1	2.027	25
Fe-Lac(+)	+26.3	1.877	25
Ni-Lac(+)	+26.1	2.191	25
Zn-Lac(+)	+25.5	2.262	25
Ba-βHB(+)	+20.2	0.512	25
Sr-βHB(+)	+22.8	0.819	25
Ca-βHB(+)	+26.5	1.235	25
Mg-βHB(+)	+28.2	0.491	25
Ni-βHB(+)	+24.9	1.038	25
Zn-βHB(+)	+24.3	1.163	25
Ba-αHB(+)	+20.2	0.357	25
Sr-αHB(+)	+22.8	0.723	25
Ca-αHB(+)	+26.5	1.471	25
Mg-αHB(+)	+28.2	1.297	25
Cd-αHB(+)	+21.4	1.800	25
Co-αHB(+)	+24.8	2.131	25
Ni-αHB(+)	+24.9	2.155	25
Ni-αHB$_2$	0	3.426	25
Zn-αHB(+)	+24.3	2.209	25
Zn-αHB$_2$	0	3.572	25
Ba-HIB(+)	+20.2	0.414	25
Sr-HIB(+)	+22.8	0.666	25
Ca-HIB(+)	+26.5	1.495	25
Mg-HIB(+)	+28.2	1.350	25
Mg-HIB$_2$	0	2.733	25
Cd-HIB(+)	+21.4	1.581	25
Cd-HIB$_2$	0	3.110	25
Co-HIB(+)	+24.8	2.130	25
Co-HIB$_2$	0	3.033	25
Ni-HIB(+)	+24.9	2.008	25
Ni-HIB$_2$	0	3.865	25
Zn-HIB(+)	+24.3	2.145	25
Zn-HIB$_2$	0	3.862	25

[a] Ac = acetate, Tar = tartrate, Cit = citrate, Glyc = glycolate, Lac = lactate, βHB = β-hydroxybutyrate, αHB = α-hydroxybutyrate, HIB = α-hydroxyisobutyrate.

TABLE VI. Mobilities and Stability Constants of Lanthanoid Complexes[a]

	u, 10^{-9} m^2 V^{-1} s^{-1}					log β
Ln^{3+}	Ln-Ac(2+)	Ln-Ac(+)	Ln-HIB(2+)	Ln-HIB(+)	Ln-HIBAc(+)	Ln-HIBAc(+)
La	47.4	23.7	41.3	20.7	22.3	5.18
Ce	47.1	23.5	41.1	20.5	22.2	5.42
Pr	47.1	23.6	39.7	19.9	21.9	5.54
Nd	47.1	23.6	39.6	19.8	21.9	5.64
Sm	47.2	23.6	39.6	19.8	21.9	5.89
Eu	47.2	23.6	40.5	20.2	22.1	5.95
Gd	47.2	23.6	39.9	20.0	22.0	5.84
Tb	47.1	23.6	39.7	19.8	21.9	5.90
Dy	47.0	23.5	37.8	18.9	21.5	5.87
Ho	47.1	23.5	38.6	19.3	21.6	5.88
Er	47.1	23.5	39.6	19.8	21.9	5.89
Tm	47.0	23.5	38.7	19.3	21.6	5.96
Yb	47.0	23.5	38.1	19.1	21.5	5.93
Lu	47.1	23.5	39.7	19.8	21.9	5.98

[a] Reference 24. Ac = acetate, HIB = α-hydroxyisobutyrate.

TABLE VII. Comparison of Precision and Accuracy of the Various Methods of Measurement of u (10^{-9} m^2 V^{-1} s^{-1}) and pK_a[a]

	acetate		formate		benzoate		
	u	pK_a	u	pK_a	u	pK_a	ref
tabulated values	42.4	4.755	56.6	3.752	33.6	4.203	
	42.9				33.4	4.200	33
measurement at complete ionization	42.7		58.4		31.9		13
	43.3 (0.09)		57.4 (0.27)		33.9 (0.10)		8
measurement at incomplete ionization	43.0	4.745	57.5	3.65	33.9	4.465	
					33.6	4.164	27
	42.5 (0.6)	4.70[b]	56.8 (0.5)	3.72[b]			11
			57.1 (0.58)	3.796 (0.030)	32.9 (1.44)	4.166 (0.106)	15
	41.9 (1.23)	4.736[b] (0.015)	56.5 (0.76)	3.705[b] (0.005)	33.9 (0.62)	4.179[b] (0.013)	32

[a] The numbers in parentheses are the corresponding σ values. [b] Concentration values of pK.

in six electrolyte systems. The average rsd was 1.5% and 2.4% in the determination of mobility for 37 monobasic acids and 17 dibasic acids, respectively; the average value of σ in the determination of pK_a was 0.055 and 0.32 unit for mono- and dibasic acids, respectively. The method of Kašička et al.[11] was based on data from 14 electrolyte systems, and the rsd of mobility for monobasic acids was 2%. The method of Pospíchal et al.[32] provided an average rsd of 10 values of mobilities of 2% (from measurements in 3 electrolyte systems). The computer-aided slope–intercept method of Jokl et al.[31] used data from two measurements at two different pHs; the resulting average rsd of mobility was 0.8% and the average value of pK_a was 0.03 (n = 12).

The conclusion can be made that the isotachophoretic method of determination of u_i and pK_a provides accurate results. In comparison with other (potentiometric, conductometric) methods, it shows lower precision, which is, however, compensated by the short analysis time and by the possibility of evaluating data of several substances simultaneously by merely injecting a couple of microliters of a solution of the respective mixture.

VII. Acknowledgments

We are grateful to Dr. Andreas Chrambach, National Institutes of Health, Bethesda, MD, for initiating our interest in mobility measurements and to Dr. Charles B. Cuono, Yale University, New Haven, CT, for kindly providing samples of some Good's buffers.

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