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DETERMINATION OF RELATIVE IONIC MOBILITIES BY CAPILLARY ISOTACHOPHORESIS

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SUMMARY

A method for the accurate determination of relative ionic mobilities by means of isotachophoresis is described. Relative ionic mobilities of completely ionized compounds were obtained by applying suitable standard substances as the leading compound and by determining the ratio of the potential gradients in the leading and the sample zones. The relative ionic mobilities of 19 cations and 40 anions were determined with an average relative standard deviation of 0.55%.

INTRODUCTION

In separation methods based on electromigration, the electrophoretic mobility of ionic species is the basic physico-chemical constant. Its value is a qualitative parameter that can be used for the identification of substances and it is also related directly to separability as a prerequisite for quantitation. There are a number of electrochemical methods for measuring ionic mobilities, *e.g.*, the moving boundary method[1] and the measurement of conductivity and transfer numbers[2]. In these measurements, however, physico-chemical motivation prevails and very pure substances are necessary. Hence, tabulated ionic mobilities[3] are restricted to a small range of common substances.

From the viewpoint of isotachophoresis as a modern electromigration method, a wide range of ionic substances should be characterized by tabulating their ionic mobilities in order to improve the posibilities of the method for both qualitative and quantitative analysis. This requirement has led various workers to evaluate ionic mobilities from isotachophoretic experiments. The methods used were direct measurement[4,5], determination of relative values using a standard ion[6–8] and computerization of isotachophoretic records[9,10]. The various ionic mobilities obtained, however, differ substantially, because they depend on a number of experimental factors such as temperature and ionic strength and laborious corrections must be made in order to obtain standardized values. Further, the accuracy of the measurements is adversely affected by the polarization of the sensing electrodes and significant empirical corrections must be introduced in the data processing[10].

A number of calculations have been performed aimed at obtaining qualitative and quantitative information on separations, and the problems of separability, dynamics of separation and quantitation have been considered[11–14]. For such calculations, however, data of sufficient accuracy are needed, but generally are not available.

It was the aim of this work to obtain the relative ionic mobilities of a number of substances under conditions where the main factors influencing their values are well controlled or can be neglected so that no additional numerical corrections need to be made.

ANALYSIS OF THE PROBLEM

In isotachophoresis, relative ionic mobilities may be determined from the measurement of either potential gradient[7] (E) or conductivity[15] (κ) in the sample zone and the zone of a suitable standard:

$$\frac{u_S}{u_L} = \frac{E_L}{E_S} = \frac{\kappa_S}{\kappa_L} \tag{1}$$

where u is the ionic mobility and S and L indicate the sample substance and the standard, respectively. For measuring conductivity, the a.c. method is used: its disadvantages are the dependence of the measured values on frequency and the sensitivity to the coating and polarization of the sensing electrodes. The measurement of potential gradient involves d.c. measurement of conductivity. Its accuracy is mainly affected by polarization of the electrodes. In order to suppress the errors caused by this effect, higher voltages must be measured, *i.e.*, a greater distance between the sensing electrodes is needed.

As mentioned above, the relative ionic mobilities are affected by a number of other factors, *e.g.*, the degree of dissociation of the standard and the measured substance, ionic strength, temperature and the accuracy of measurement of the detection quantity.

In order to eliminate the possible influence of incomplete dissociation of the sample substances on the determined values, a standard substance (usually the leading one) must be selected that is completely dissociated over the whole pH range used. This requirement can be fulfilled by employing ions of strong acids or bases as standards. Further, sufficient dissociation of the sample substances must also be ensured. In order to provide at least 99% dissociation of weak acids and bases, for the sample the conditions $\mathrm{pH} > \mathrm{p}K_a + 2$ and $\mathrm{pH} < \mathrm{p}K_a - 2$, respectively, must hold, which may fulfilled by the choice of an appropriate buffering counter-ionic species.

The influence of the ionic strength on the ionic mobility is not negligible. If we assume, however, a relatively narrow range of working concentrations, the error in neglecting the differences in the ionic strengths between the standard and sample zone is usually less than 2%. If, moreover, relative values of ionic mobilities are considered, the error decreases to less than 1% and can be neglected to a first approximation. A further possibility for obtaining a consistent set of relative mobility values is to maintain carefully constant working concentrations in all measurements.

The production of Joule heat in the zones results in temperature differences between the zones and the cooling medium and between the zones themselves. If efficient thermostating of the capillary and detector is used, the former differences may be decreased in such a way that they do not exceed the limit of the admissible experimental error of the relative ionic mobilities obtained. The magnitude of the temperature differences between the zones depends on the operating regime used, as shown below.

(i) Maintaining a constant current during the measurement is commonly used in capillary isotachophoresis. The temperature increases from the leading to the terminating zone, being limited on one side by the thermostating temperature and on the other by the boiling point of the solvent used. If we record the potential gradient in the zones, the ratio of mobilities is inversely proportional to the ratio of the step heights (*cf.*, eqn. 1), and the length is directly proportional to the amount present. The increase in temperature in a zone is indirectly proportional to the mobility in this zone, *i.e.*,

$$\frac{u_S}{u_L} = \frac{T_L - T}{T_S - T} \tag{2}$$

where T is the thermostating temperature.

(ii) If the potential gradient in the measured zone is kept constant by changing the magnitude of the driving current I, which is simultaneously recorded, the record obtained is similar to the conductivity record. The ratio of mobilities is directly proportional to the ratio of the step heights:

$$\frac{u_S}{u_L} = \frac{I_S}{I_L} \tag{3}$$

The step lengths are inversely proportional to the mobility and directly proportional to the amount present.

The temperature of the zones decreases from the leading zone to the terminating zone, reducing to the thermostating temperature. The temperature step between the zones is therefore lower in comparison with the constant current method:

$$\frac{u_S}{u_L} = \frac{T_S - T}{T_L - T} \tag{4}$$

(iii) If the dissipated electric power P is kept constant in the measured zone by changing the driving current and the potential gradient in the zones, the temperatures of all zones are equal during the time of their measurement and no temperature differences between the zones exist. Mobilities can be evaluated from the record of the potential gradient or of the driving current. The ratio of the mobilities is equal to the ratio of the squares of the driving currents or to the reciprocal ratio of the squares of the potential gradients:

$$\frac{u_S}{u_L} = \frac{I_S^2}{I_L^2} = \frac{E_L^2}{E_S^2} \tag{5}$$

If the dissipated electric power cannot be kept exactly constant, the mobilities can be ascertained from the ratio of the conductivities, if the driving current and potential gradient are known.

EXPERIMENTAL

The measurements were performed on a simple isotachophoretic column of our own construction, consisting of two electrode compartments, sampling device, detection cell, inlet and outlet of electrolytes and a capillary of rectangular cross-section. The length of the capillary was 140 mm and its cross-section was 2.5 × 0.1 mm.

The whole capillary was efficiently thermostated, being pressed to an aluminium block with circulating water from a U 10 ultrathermostat (Prüfgerätewerk, Medingen, G.D.R.). The thermostating quotient (measured according to ref. 16) was 70°K cm W^{-1} and allowed work with maximum temperature deviations of 0.4°C from the thermostating temperature (25 ± 0.1°C).

A pair of Pt wires of 0.05 mm diameter mounted 10.7 mm apart in the column served for the measurement of the potential gradient in the zones, being connected to a laboratory-made voltmeter (range 0–100 V, input resistance 10^{12} Ω). The driving current was recorded by means of a TZ 4200 recorder (Laboratorní přístroje, Prague, Czechoslovakia) connected in parallel to a resistance in the HV circuit. An HV power supply with a regulated constant current up to 550 μA and a maximum voltage of 16 kV was used, as described earlier[7].

For the measurement of anions, ammonium chloride solution was used as the leading electrolyte and a saturated solution of $Ba(OH)_2$ as the terminator. For measuring cations, potassium acetate solution and acetic acid were used as the leading and terminating electrolytes, respectively. These systems enabled us to measure anions of weak acids with pK_a values up to 5.2 and cations of weak bases with pK_a values down to 8.3. All the chemicals in question were of analytical-reagent grade (Lachema, Brno, Czechoslovakia).

The procedure for the measurement was as follows. After filling the column with the electrolytes and injecting the sample, a constant current was applied. By changing it manually, the required constant value of the electric potential gradient in the leading zone (corresponding to a voltage of 25 V between the sensing electrodes) was adjusted. After reaching a steady state, the electric current was recorded. On entering the measured zone in the detection cell, the driving current was decreased manually in order to obtain the primary value, and the magnitude of the driving current was recorded again.

The measurements by the other two methods (keeping constant the dissipated power and/or driving current) were performed in a similar way.

RESULTS

In order to test the performance of the procedures discussed (see Analysis of the Problem), the relative ionic mobility of sodium, migrating behind potassium of a concentration of 0.01 *M* was measured. The results are shown in Table I. Comparison of the values obtained with the theoretical values (calculated from exact tabulated data[3] extrapolated to real concentrations in the zones) shows that all three methods provide satisfactory results, the measurement at constant *E* being the best (the value obtained differs by only 0.3% from the theoretical value).

By using the latter method, the relative ionic mobilities of a set of cations and

TABLE I

COMPARISON OF EXPERIMENTAL VALUES (AVERAGE OF FIVE DETERMINATIONS) OF THE RELATIVE IONIC MOBILITY, u_{Na}/u_K, OBTAINED BY THREE METHODS

Method	u_{Na}/u_K	*Standard deviation*
E = constant	0.6756	0.0039
P = constant	0.6800	0.0053
I = constant	0.6839	0.0038
Theoretical	0.6735	–

anions were determined. The values obtained, given in Tables II and III, relate to a 0.01 M ionic strength in the sample zone and therefore represent a consistent set of data where the corrections that need to be performed in order to obtain real values are eliminated as far as possible; the corrections to the mobilities of the standard ions with respect to ionic strength may be performed easily on the basis of exact tabulated values.

The concentration of the leading ions (c_L) used, given in Tables II and III, were calculated from the simple Kohlrausch equation[17] on the basis of approximate ionic mobilities obtained from a preliminary experiment with 0.01 M leading electrolyte.

In order to demonstrate the accuracy of the values obtained, a comparison with tabulated data[3] based on classical conductivity measurements is given in Table

TABLE II

RELATIVE IONIC MOBILITIES OF CATIONS

Substance	u/u_K	*Standard deviation*	c_L *(M)*
Tetramethylammonium	0.6031	0.0030	0.0125
Tetraethylammonium	0.4335	0.0023	0.0146
Tetrapropylammonium	0.3050	0.0020	0.0179
Tetrabutylammonium	0.2524	0.0016	0.0201
Phenyltrimethylammonium	0.4664	0.0030	0.0140
Ethanolamine	0.5568	0.0029	0.0127
Diethanolamine	0.4364	0.0026	0.0146
Tris	0.3836	0.0022	0.0157
Methylamine	0.7868	0.0008	0.0110
Diethylamine	0.4843	0.0018	0.0135
Triethylamine	0.4356	0.0018	0.0146
Ethylenediamine	0.5580	0.0049	0.0128
Arginine	0.3386	0.0032	0.0165
Lysine	0.3537	0.0028	0.0165
Ornithine	0.3623	0.0008	0.0156
Guanidine	0.7058	0.0015	0.0115
Ammediol	0.3950	0.0059	0.0152
Morpholine	0.5301	0.0020	0.0131
Cetyltrimethylammonium	0.1684	0.0060	0.01*

* Not sufficiently soluble.

TABLE III

RELATIVE IONIC MOBILITIES OF ANIONS

Substance	u/u_{Cl}	*Standard deviation*	c_L *(M)*
Acetylsalicylate	0.3657	0.0027	0.019
Acrylate	0.4150	0.0021	0.017
Adipate	0.5910	0.0036	0.014
o-Aminobenzoate	0.3935	0.0027	0.017
m-Aminobenzoate	0.3860	0.0035	0.018
p-Aminobenzoate	0.3899	0.0005	0.018
Azelaate	0.5258	0.0027	0.015
Acetate	0.5356	0.0011	0.015
Barbiturate	0.3983	0.0016	0.017
Benzoate	0.4134	0..0012	0.017
Chloroacetate	0.5083	0.0014	0.015
Chlorate	0.8659	0.0067	0.011
2,4-Dinitrophenolate	0.3961	0.0036	0.017
Formate	0.7185	0.0034	0.012
Fumarate	0.7199	0.0045	0.012
Gallate	0.3302	0.0052	0.020
Glutarate	0.6252	0.0017	0.013
Hypophosphite	0.5878	0.0021	0.014
Iodate	0.5390	0.0032	0.014
Glutamate	0.3484	0.0028	0.019
Lactate	0.4589	0.0031	0.016
Malate	0.6965	0.0035	0.012
Nitrate	0.9364	0.0008	0.010
Nitrobenzoate	0.4004	0.0027	0.017
Nitrite	0.9349	0.0036	0.010
Perchlorate	0.8836	0.0020	0.010
Periodate	0.7003	0.0035	0.012
Permanganate	0.8043	0.0018	0.011
Phenylacetate	0.3812	0.0021	0.018
o-Phthalate	0.6139	0.0080	0.013
Propionate	0.4656	0.0011	0.015
4-Pyridinecarboxylate	0.4182	0.0021	0.017
3-Pyridinecarboxylate	0.4214	0.0014	0.017
2-Pyridinecarboxylate	0.4213	0.0014	0.017
Sorbate	0.3736	0.0031	0.018
Sulphamidate	0.6131	0.0010	0.013
Sulphate	0.9611	0.0013	0.010
Thiocyanate	0.8623	0.0023	0.010
p-Toluenesulphonate	0.4004	0.0028	0.017
Trichloroacetate	0.4521	0.0046	0.016

IV for five examples. These five substances represent a set of strong ions, the relative ionic mobilities of which can be expected to be independent of concentration and a comparison can be made with tabulated relative limiting ionic conductivities. It is seen from Table IV that the performance of the isotachophoretic method is sufficient as far as the accuracy of the data obtained is concerned.

TABLE IV

COMPARISON OF FIVE RELATIVE IONIC MOBILITIES (u_S/u_{Cl}) FROM TABLE III WITH TABULATED[3] RELATIVE LIMITING IONIC CONDUCTIVITIES (l_{0S}/l_{0Cl})

The pair of the lowest and highest values found in the tables only is given.

S	u_S/u_{Cl}	l_{0S}/l_{0Cl}
ClO_4^-	0.8836	0.8814
		0.8965
IO_3^-	0.5390	0.5183
		0.5431
NO_3^-	0.9364	0.9306
		0.9358
CNS^-	0.8623	0.8560
		0.8704
MnO_4^-	0.8043	0.7984
		0.8049

CONCLUSION

Relative ionic mobilities of substances may be determined from isotachophoretic experiments in a simple way provided that the substances are completely ionized in their zones. In comparison with classical (*e.g.*, conductimetric) methods, only very small amounts of sample are needed and there are no special requirements on their purity. At the same time, provided that the electric potential gradient can be measured with sufficient reproducibility and accuracy, relative ionic mobilities of sufficient reproducibility and accuracy can be obtained.

By using a method where a constant potential gradient is maintained in the measured zones, the relative ionic mobilities are obtained as the ratio of the driving currents applied (eqn. 3). As the distance between the sensing electrodes was long enough, the contribution of the polarization of the electrode to the voltage measured was negligible.

Relative ionic mobilities of 19 cations and 40 anions were obtained with an average relative standard deviation of 0.55%. The values given relate to a 0.01 *M* ionic strength and represent a consistent set of values which may be used directly for qualitative and quantitative purposes. The data obtained are in good agreement with tabulated values.

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