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Ionic strength and charge number correction for mobilities of multivalent organic anions in capillary electrophoresis

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Abstract

An empirical expression was derived for the actual mobility, μ , valid in an extended range of ionic strength, I , between 0.001 and 0.1 mol/l, using a three-parameter exponential fit for the ratio of actual and absolute mobilities (μ_0 at $I = 0$), which consists of two variables: ionic strength of the buffer and charge number, z , of the solute. The charge numbers of the solute anions (21 aromatic sulfonates) ranged between 1 and 6, and the ionic strength was varied in the range mentioned using a sodium acetate buffer. The mobilities were determined at 25.0°C in a coated capillary with suppressed electroosmotic flow. The resulting fitting equation, valid under these conditions, is $\mu/\mu_0 = \exp(-0.77\sqrt{zI})$. The difference between measured mobilities and mobilities calculated from a tenfold higher or lower ionic strength, using this equation, is within 5% for all experiments. For multivalent ions, μ values can be derived from μ_0 with comparable accuracy.

1. Introduction

The effective mobility, μ_{eff} , of the separands is the physico-chemical property of key importance in capillary electrophoresis (CE). It is given by $\mu_{\text{eff}} = \mu_0 f \alpha$ (for monovalent ions), where μ_0 is the absolute mobility (at infinite dilution), α is the degree of dissociation and f is a correction factor counting for the deviation from ideality. The actual mobility, μ , is that of the fully protonated or deprotonated ion at ionic strength I .

The ionic strength includes contributions from all ionic species in solution, including H^+ and OH^- , and with increased contribution of multivalent ions: $I = \frac{1}{2} \sum c_i z_i^2$, where c_i is the equilib-

rium concentration of species i and z_i the charge. The effective mobility depends on the pH and the ionic strength of the background electrolyte. Whereas its dependence on the pH through α is well defined, the influence of the ionic strength on the correction factor, f , is still under discussion, at least for those concentrations between 0.001 and 0.1 mol/l, which are commonly used in capillary zone electrophoresis (CZE).

Long before CE was introduced, a number of workers published papers on ionic strength and temperature dependence of molar conductivity and transport numbers; readers are referred to standard works on the subject [1]. Data were obtained from conductivity and other measurements under different conditions. In general, these models include \sqrt{I} . Their validity is limited to ionic strengths of up to 0.001 mol/l, without

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additional data on the sample components. A number of corrections were introduced to increase the concentration range, requiring additional sample-specific data, such as ionic radii. These data are, in general, difficult to obtain.

A further complication is that all of these models refer to the molar conductivity of electrolytes, dissolved at different concentrations, rather than to mobilities of individual ionic species, dissolved in a background electrolyte.

Concerning CE, in most cases some information will be available on the electrophoretic mobility of the solutes in a certain operational system, either from the values of absolute mobilities from the literature, or obtained by measurements with CE at a certain buffer composition. In order to optimize a separation, it will certainly be useful to have some idea of how the mobility will change if the pH or the ionic strength of the background electrolyte is altered. The effect of pH in selectivity tuning is fairly elementary from theory of isotachopheresis [2,3] and has been treated in a number of textbooks on capillary electrophoresis [4,5] and in previous publications [6,7]. The effect of ionic strength on mobility and selectivity in capillary electrophoresis is mentioned in the same textbooks. It is, however, still a matter of discussion, which is reflected by the number of contradictory contributions published on the subject. In some of them, the "overall" mobility (including electroosmosis) is measured as a function of buffer concentration [8,9]. An exponential decrease with increasing ionic strength was found, attributed mainly to a change in electroosmosis. Another publication [10] gives the mobility as being proportional to $1/\sqrt{I}$. In a computational study [11], the data taken from a textbook on the subject [1] are summarized in stating that the mobility (rather than the molar conductivity) is a non-linear function of $-\sqrt{I}$ for 1:1 electrolytes. In a more recent study [12], mobilities of a number of monovalent components were determined in a series of buffers with a limited range of ionic strength and the results were fitted to a linear model. Finally, in a publication on computational simulation of migration and dispersion in zone electrophoresis [13,14], a straightforward

empirical model for the ionic strength dependence of mobilities was introduced:

$$\mu/\mu_0 = \exp(-0.5z^{1.78}\sqrt{I}) \quad (1)$$

The idea behind the necessity for such an empirical relationship was that a 10% predictability for certain experimental conditions is preferred over a 1% accuracy in a concentration range where zone electrophoretic experiments are commonly not carried out (≤ 0.001 mol/l).

In dealing with these kinds of models presented in the literature [1], one has to be careful in applying them to other systems [10–13]. In addition, the conductivity and mobility data in the literature refer to ionic strengths originating from the ions themselves, not from a buffer with a relatively high concentration (even if ion pairing and complexation can be excluded). Finally, literature data are mostly from inorganic anions and metal ions, which often have rotational symmetry and a high charge density. From the practical point of view, the larger, organic ions with a lower charge density are more interesting for CE. This is another reason why the present study was undertaken. For simplicity, strong acids were chosen as reference compounds in order to avoid effects caused by incomplete protolysis: the separands used are fully dissociated at the operating pH. A choice was made for μ/μ_0 as an exponential function, because it makes it possible to obtain μ_0 by extrapolating $\ln \mu$ to $I=0$. In addition, a linearized function (see the \sqrt{I} models above) at higher concentration always leads to an exponential deviation: a linear function is only a special case of the expansion series of an exponential function.

2. Experimental

2.1. Apparatus

The instrument used for the measurements was a P/ACE System 2100 (using System Gold 6.01) (Beckman, Palo Alto, CA, USA) equipped with a UV absorbance detector at 214 nm. The separation capillary was made from fused silica,

75 μm I.D. and 363 μm O.D. (Supelco, Bellefonte, PA, USA). The total length was 0.269 m and the effective length (the distance from the injector to the detector) was 0.202 m. The capillary was thermostated at 25.0°C. The operating voltage was chosen between 2000 and 5000 V, depending on the ionic strength of the background electrolyte. Injection of the sample was carried out by pressure for 1 s.

2.2. Reagents

Chemicals used for the preparation of the buffer solutions were analytical-reagent grade acetic acid and sodium hydroxide (Merck, Darmstadt, Germany). A number of different dyes with 1–6 sulfonic acid groups were used as model components (see Table 1). The azo dyes were prepared [16] by coupling the diazotized 1-naphthylamine sulfonic acid to the 2-naphtholsulfonic acid. All other components were obtained from commercial sources (Aldrich, Steinheim, Germany; Fluka, Buchs, Switzerland; Merck) in the highest available purity.

2.3. Procedures

The analytes were first titrated in order to verify that the sulfonic acid groups are fully dissociated at the working pH and that no other protolysis is likely to occur in the background electrolyte system used.

The background electrolyte was prepared by adding acetic acid and sodium hydroxide (at a molar concentration ratio of 2:1), resulting in buffers with ionic strengths of 0.1, 0.05, 0.01, 0.005 and 0.001 mol/l and a pH of about 4.75 (which was not further adjusted). This background electrolyte was chosen because complexation or ion pairing between sample and buffer ions was least likely, an obvious requirement for the present investigation. For the same reason, no additives were used.

In order to prevent systematic errors in migration time due to stacking, the sample components were dissolved in background electrolyte. The sample concentration was as low as possible in order to prevent concentration overload.

The mobility was calculated from the migration time t_m using the equation $\mu = L_d L_t / V t_m$, where L_d is the length of the capillary to the detector, L_t the total length and V the voltage. The quality of the determination of the migration times was determined with two anions (iodide and 2,4,5-trichlorobenzenesulfonate) as external reference compounds. The migration times of these standards were constant during the measurements in each particular buffer system to within about 0.2%.

The voltage was chosen in such a way that the temperature rise due to heat development inside the capillary was only 0.1°C or less. This temperature rise was calculated using the previously mentioned simulation program [13]. The coating procedure described by Hjertén [15] was applied.

3. Results and discussion

Some examples of the dependence of the actual mobility on the ionic strength for ions of different charge are shown in Fig. 1. It can be seen that the mobility decreases with increasing I in all cases. The slope of the graphs (especially at low I) is, however, strongly dependent on the charge number; it becomes increasingly steeper with increasing charge number. This leads to the fact that, within the range of measurement, the mobilities of the more highly charged species are reduced nearly by a factor of 2. The monovalent ions behave differently, however: between 0.001 and 0.01 mol/l the mobility remains nearly constant and is finally reduced at 0.1 mol/l by only about 20%. This unexpectedly deviant behaviour of the monovalent solutes will also be reflected in subsequent graphical representations.

3.1. Determination of model parameters

The basic approach to describe the dependence of the actual mobility, μ , on the ionic strength, I , and on the charge number, z , is the following three-parameter fitting equation:

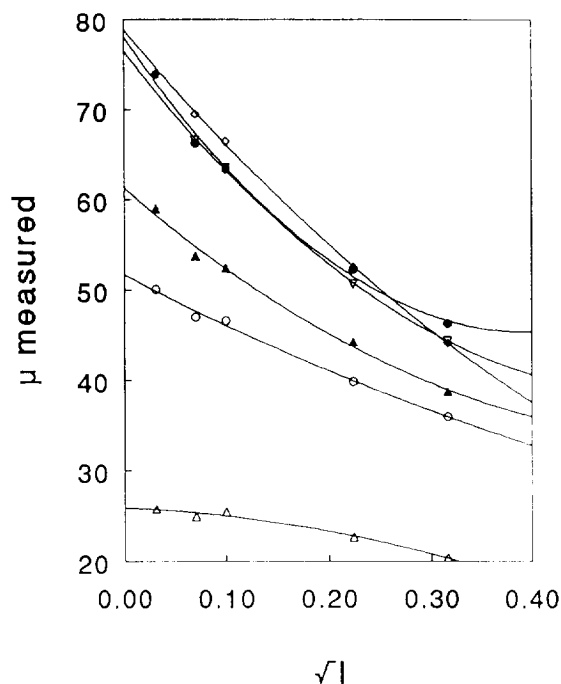


Fig. 1. Dependence of the measured actual mobility, μ , on the square root of the ionic strength, I , used for the determination of the absolute mobility, μ_0 , by extrapolating a second-order polynomial fit of μ to $I=0$. I is given in mol/l and the mobilities are given in $10^{-9} \text{ m}^2/\text{V}\cdot\text{s}$. Examples of six components with different charge numbers are shown: Δ = 1AN2; \circ = 2NA36; \blacktriangle = 3IN; \bullet = 4IN; ∇ = 5AZ11; \diamond = 6AZ13. See Table 1 for component codes.

$$\mu = \mu_0 f = \mu_0 \exp(-az^b I^c) \quad (2)$$

As the value of the constant c is expected to be close to 0.5, $\ln \mu$ was plotted vs. \sqrt{I} for each individual component to obtain μ_0 using a second-order polynomial fit. The μ_0 values determined in this way are listed in Table 1. The correlation coefficients are always 0.990 or better.

Conversion of the model Eq. 2 leads to

$$\ln[-\ln(\mu/\mu_0)] = \ln(az^b) + c \ln I \quad (3)$$

All values of μ/μ_0 were subsequently calculated. The slope of the plot of $\ln[-\ln(\mu/\mu_0)]$ vs. $\ln I$ for each individual solute gives c . The correlation coefficients were 0.997 or better. Some examples of these plots are shown in Fig. 2. The lines corresponding to different charge numbers z are

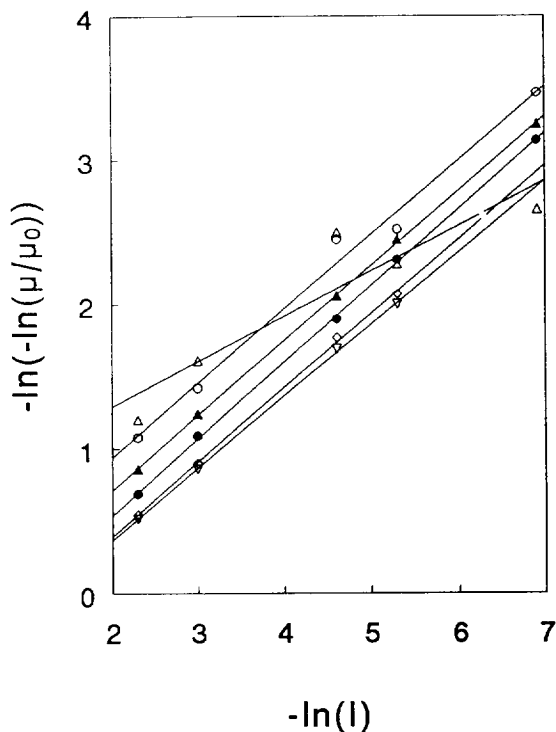


Fig. 2. Examples of the determination of c using components with different charge numbers, according to Eq. 3. See text and the caption of Fig. 1 for further details.

approximately parallel, but they have different intercepts, which are equal to $\ln(az^b)$.

The values of c obtained in this way are shown in Fig. 3. It can be seen that the three monovalent solutes are clearly outliers with c values of about 0.3, in contrast to all other compounds. This deviating behaviour of the monovalent ions (which was mentioned above) is not fully understood. Nevertheless, for the calculation of the parameters, the data from the monovalent solutes were further excluded. An overall average value of 0.52 for c is determined, with a standard deviation σ_c of 0.04 and a 95% confidence interval of 0.02. This value of c is close to that corresponding to the square-root equation, mentioned in the literature [1,13]. Based on the value for c , the model equation can be converted into

$$\ln[-\ln(\mu/\mu_0)/I^{0.52}] = \ln a + b \ln z \quad (4)$$

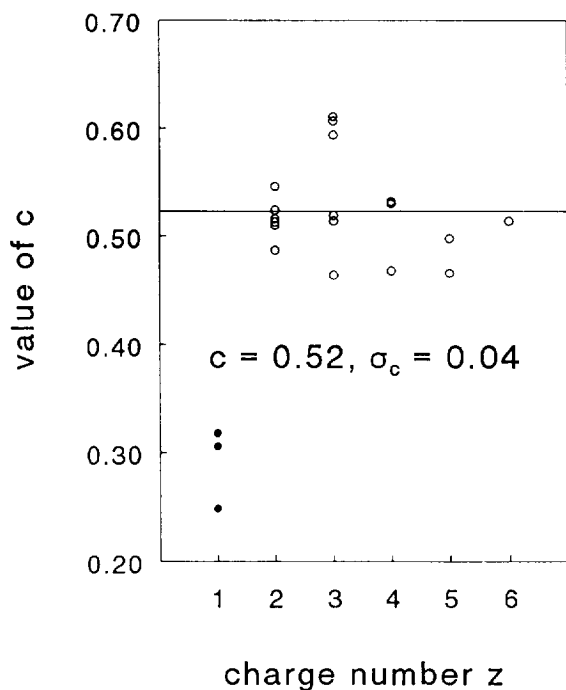


Fig. 3. Value of c from all components ($n = 21$) versus charge number z . ● = Outliers.

A plot of $\ln[-\ln(\mu/\mu_0)/I^{0.52}]$ vs. $\ln z$ is given in Fig. 4. After removing only two obvious outliers, the correlation coefficient is fair (0.78). The slope of the plot in Fig. 4 is $b = 0.49$ with a standard deviation σ_b of 0.04. The intercept is $\ln a$, from which $a = 0.85$ is obtained with a standard deviation σ_a of 0.13. The overall equation, valid for ionic strengths between 0.001 and 0.1 mol/l and charge numbers from 2 to 6, thus becomes

$$\mu/\mu_0 = \exp(-0.85z^{0.49}I^{0.52}) \quad (5)$$

Simplification of the fitting equation

As both parameters c and b are relatively close to 0.5, a further simplification of Eq. 5 seems reasonable:

$$\mu/\mu_0 = \exp(-d\sqrt{zI}) \quad (6)$$

In order to evaluate the effect of this simplification on the predictive value of the model, d was determined from all experiments with multivalent ions. The average value of d was 0.77 and

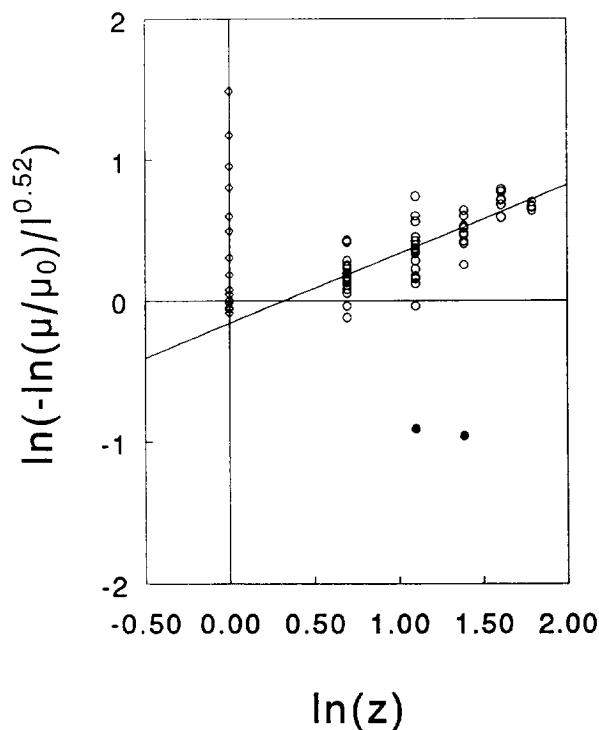


Fig. 4. Determination of b and a (from the slope and the intercept, respectively, according to Eq. 5) for all ionic strengths and all multivalent ions (○). Monovalent ions (◇) and some outliers (●) were omitted from the linear curve fit.

the standard deviation σ_d was 0.11. This is not surprising because both c and b in the model of Eq. 2 do not deviate significantly from 0.5, as seen from their respective standard deviations. In addition, the relative standard deviation of d in Eq. 6 is not larger than that of a in Eq. 5. In view of its inherent simplicity, preference should therefore be given to the model of Eq. 6, which now becomes

$$\mu/\mu_0 = \exp(-0.77\sqrt{zI}) \quad (7)$$

3.2. Evaluation of the model

Two different approaches were applied to evaluate the model by calculating μ values for different ionic strengths and charge numbers from Eq. 7 and to compare the results with the measured data: (i) from the values of the absolute mobilities, μ_0 (Table 1) and (ii) from one

actual mobility determined at a particular ionic strength. Both approaches are of practical interest, because in practice either the absolute mobility can be found in the literature, or a single experiment in a particular buffer gives a certain mobility value.

The mobilities calculated in these ways were compared with the data measured at the same ionic strength.

Approximation of μ from μ_0

For this approximation, μ_0 values determined with the five-point second-order polynomial fit of all experiments were used (Table 1). From this value, actual mobilities μ were calculated for each component at all ionic strengths. The agreement between measured and calculated mobility was excellent, as shown in Fig. 5. The linear correlation coefficient of the line in Fig. 5 is high (0.996). Differences are of the order of a few

Table 1
Model components investigated

Code	$-\mu_0$	Name
1AN2	27.7	Anthraquinone-2-sulfonic acid
1NA2	33.7	2-Naphthalenesulfonic acid
1TO4	34.6	4-Toluenesulfonic acid
2AN15	48.1	Anthraquinone-1,5-disulfonic acid
2AN26	49.5	Anthraquinone-2,6-disulfonic acid
2NA15	55.6	Naphthalene-1,5-disulfonic acid
2NA26	54.9	Naphthalene-2,6-disulfonic acid
2NA36	51.7	Naphthalene-3,6-disulfonic acid
2BE13	63.1	Benzene-1,3-disulfonic acid
3IN	61.3	Indigo-5,5',7'-trisulfonic acid
3NA136	67.5	Naphthalene-1,3,6-trisulfonic acid
3AZ05	54.6	AZO-4,3',6'-trisulfonic acid
3AZ06	55.2	AZO-3,6,6'-trisulfonic acid
3AZ07	56.3	AZO-3,6,8-trisulfonic acid
3AZ08	54.8	AZO-3',6',8'-trisulfonic acid
4IN	74.7	Indigo-5,5',7,7'-tetrasulfonic acid
4AZ09	67.1	AZO-3,6,8,6'-tetrasulfonic acid
4AZ10	68.0	AZO-3,6,3',6'-tetrasulfonic acid
5AZ11	76.5	AZO-3,6,8,3',6'-pentasulfonic acid
5AZ12	77.3	AZO-3,6,3',6',8'-pentasulfonic acid
6AZ13	78.9	AZO-3,6,8,3',6',8'-hexasulfonic acid

The first digit of the code is the charge number z . The absolute mobility μ_0 (in sodium acetate buffer) determined at 25°C by extrapolation to $I = 0$ (see text for further details) is given in $10^{-9} \text{ m}^2/\text{V}\cdot\text{s}$. AZO = (2-hydroxy-1-naphthylazo)naphthalene.

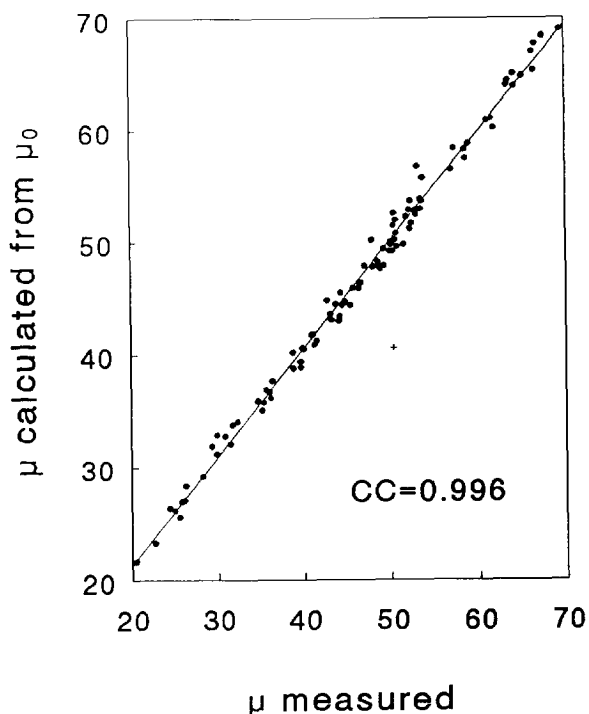


Fig. 5. Correlation between the measured actual mobility and the actual mobility calculated from μ_0 , using the model described in the text. In this case μ_0 was obtained with a five-point second-order polynomial fitted to the square-root model from all ions and all ionic strengths ($n = 100$). There is one outlier (+). The mobilities are given in $10^{-9} \text{ m}^2/\text{V}\cdot\text{s}$.

percent. With the purpose of quantifying the predictability of the model, the results are presented in a different way.

In Fig. 6, the relative difference between measured and calculated mobility is shown plotted against the charge number. What we observe is a random error of 5% or less for charge numbers $z = 2-6$. A systematic deviation (the average of the values is about 5%) and a slightly larger scatter is observed only for monovalent ions, which is not surprising for the reasons mentioned above.

As mentioned in the Introduction, it is not advisable to derive μ from μ_0 values obtained from conductivity measurements and literature transport numbers: the predictive value of mobilities in buffers obtained in this way is inherently doubtful. Although calculation of μ_0 is not

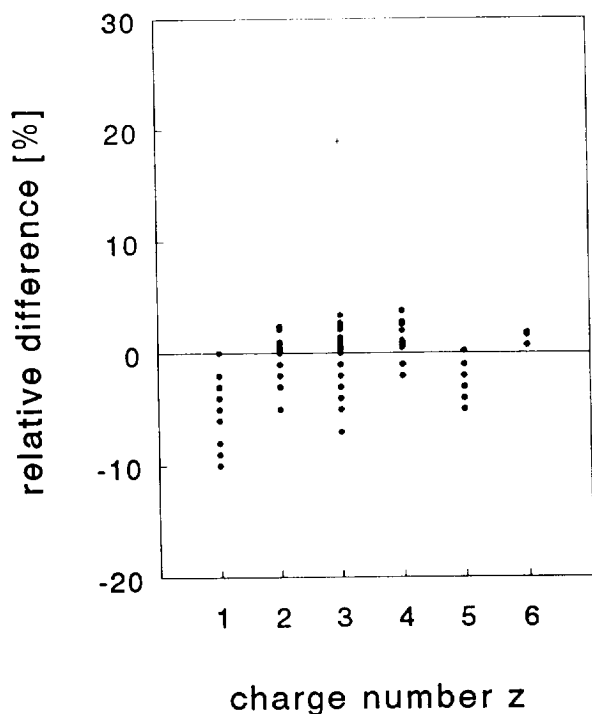


Fig. 6. Relative difference between measured and calculated actual mobility (see caption of Fig. 5) as a function of charge number z , using Eq. 5. The mean relative difference is 0.8%; there is one outlier (+).

necessary in order to calculate the mobility at one ionic strength from experiments at another ionic strength in the same buffer system, μ_0 may be useful in comparing results in different laboratories using the same buffer at different ionic strengths. For each condition, only a single experiment is needed. Further electrophoretic behaviour in the chosen buffer can then be predicted using the Henderson–Hasselbach equation and Eq. 7 for the effects of pH and ionic strength, respectively.

Approximation of μ from a different ionic strength

The model can also be used to calculate the mobility at a certain ionic strength from that obtained experimentally at another ionic strength. Obviously, one has to be careful not to go beyond the valid ionic strength range. The form of Eq. 5 makes it possible to do so without

the need to know μ_0 because μ/μ_0 is written explicitly. The mobility calculated from a tenfold higher ionic strength is plotted against the measured mobility in Fig. 7. The correlation is fairly satisfactory (correlation coefficient = 0.995). Comparable or even better results are obtained if μ is calculated from a 2–5-fold lower ionic strength. Here also the relative differences are of the order of a few percent. The deviation is random, not systematic, and the relationship seems valid for charge numbers 2–6.

Comparison with a former approach

When comparing the model Eq. 7 with the previously published model [13,14] (Eq. 1), the following differences can be seen. The square root of I was a good estimate, even at the ionic strengths used in the present investigation. The effect of the charge number was previously

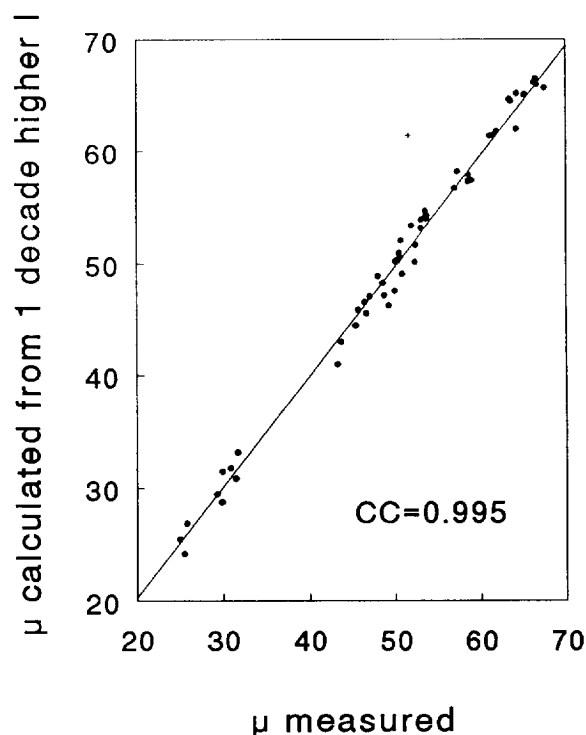


Fig. 7. Correlation between measured actual mobility and actual mobility calculated from experimental data at a tenfold higher ionic strength ($n = 100$), using the model of Eq. 7. Mobilities are given in 10^{-9} $\text{m}^2/\text{V}\cdot\text{s}$ units. There is one outlier (+).

overestimated, although for low charge numbers it was compensated for by the lower value of a . Previous experimental verification [14] of Eq. 1 was satisfactory, however, probably because these experiments were limited to charge numbers 1–3 and an ionic strength of ca. 0.01 mol/l.

4. Conclusions

An empirical model was developed that makes it possible to calculate mobilities of strong, multivalent ions with charge numbers between 2 and 6 in the ionic strength range 0.001–0.1 mol/l either from a single experiment or from the absolute mobility. Calculation of mobilities within one decade of ionic strength in the range mentioned can be carried out with an error of 5% or less, for charge numbers 2–6. Extrapolation from zero ionic strength is also possible, leading to a comparable accuracy, at least for all multivalent ions. Further experiments will have to show if the present model is also valid for other components and weak solutes.

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