ISOTACHOPHORETIC DETERMINATION OF MOBILITY AND pK_a BY MEANS OF COMPUTER SIMULATION

I. ESTIMATION OF ACCURACY OF THE EVALUATED CONSTANTS

TAKESHI HIROKAWA* and YOSHIYUKI KISO

Applied Physics and Chemistry, Faculty of Engineering, Hiroshima University, Shitami, Saijo, Higashi-hiroshima 724 (Japan)

(Received July 14th, 1982)

SUMMARY

The accuracy has been estimated for the absolute mobility, m_0, and thermodynamic acid dissociation constants, pK_a, evaluated by analysing a qualitative index, R_E, obtained by means of isotachophoresis. For several model mono- and divalent anions, m_0 could be determined within *ca.* $\pm 0.2 \cdot 10^{-5}$ to $\pm 1.2 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1} and pK_a to *ca.* ± 0.01 or less, when the reproducibility of a R_E value was ± 0.02. The accuracy, especially that of pK_a, was closely influenced by the pH of the leading electrolyte, the pK_a of the buffer used and the m_0 and pK_a of the sample itself. Statistically, the accuracy may be improved by the use of a set of different R_E values obtained under different electrolyte conditions.

INTRODUCTION

In isotachophoresis, an observable qualitative index, R_E, the ratio of a potential gradient of sample zone to that of the leading zone ($R_E = E_S/E_L$), can be simulated for the sample zone in an isotachophoretically steady state if the absolute mobilities and thermodynamic acid dissociation constants of the zone constituents are available[1,2]. This is quite an important feature of isotachophoresis since it suggests that, besides the analytical utility of the method, another usage could be to measure the physico-chemical constants of samples by analysing the R_E values observed under different electrolyte conditions. The sample characteristics from the obtained isotachopherograms can be used for qualitative analysis, without the need for a standard sample. A computational method using the least squares method for the evaluation of absolute mobilities and thermodynamic acid dissociation constants has already been described for several amines and organic acids[3,4]. The proposed method has been shown to be a powerful technique especially for mixtures. However, in the previous papers, the accuracy of the evaluated constants achievable by the proposed method has not been discussed.

In this study, the accuracy of the evaluated m_0 and pK_a was estimated by

simulation of the isotachophoretic equilibria for model mono- and divalent anions, taking into account the reproducibility of an R_E value obtained under electrolyte conditions. The effect of the nature of the leading electrolytes used and of the m_0 and pK_a of the sample on the accuracy of the evaluated constants is discussed and the preferred electrolyte conditions given for the evaluation of m_0 and pK_a with high accuracy. It should be noted that the present treatment is not concerned with the accuracy of the simultaneous determination of m_0 and pK_a using a set of different R_E values obtained under different electrolyte conditions, since an exact statistical treatment would be too complicated.

THEORETICAL

A theoretical treatment for samples in an isotachophoretically steady state has been described by Everaerts et al.[1] and we have recently extended their theory to complex-forming equilibria[2]. The validity of these treatments has already been confirmed experimentally, therefore the details of the fundamental theory used will not be repeated.

In isotachophoresis, the observable qualitative index, R_E, is the ratio of the effective mobility of the sample ion to that of the leading ion, or the ratio of the conductivity of the sample zone to that of the leading zone. It can be obtained, by the use of a potential gradient detector or a conductometric detector, as the ratio of the step heights of the detected zones in isotachopherograms. A thermometric detector may be not suitable for this purpose, since the sharp steps of isotachopherograms cannot usually be observed, due to thermal diffusion between the adjacent zones, and strict linearity of step heights cannot be expected due to the complicated radiation problem. As a temperature increase in a subsequent zone is inevitable, it is preferable to suppress this as much as possible by applying a reasonably low driving current.

From the equality of the speed of the migrating zones in the steady state, one can obtain the following equation

$$R_E = h_S/h_L = E_S/E_L = \kappa_L/\kappa_S = \bar{m}_L/\bar{m}_S \tag{1}$$

where h_L is the step height of the leading zone, h_S that of the sample zone, $\bar{m}_L$ the effective mobility of leading ion, $\bar{m}_S$ that of the sample ion, E_L the potential gradient of the leading zone, E_S that of the sample zone, κ_L the specific conductivity of the leading zone and κ_S that of the sample zone. Usually, the leading ions used are Cl^- and K^+, the pK_a values of which are out of the pH range and the absolute mobilities are larger than almost all other ions. The values of the absolute mobility have been extensively studied[5]. Since the absolute mobility can easily be corrected to the effective mobility in the electrolyte at a finite ionic strength using Onsager's equation[6], the effective mobility of the sample can be determined by measurement of the R_E value. The effective mobility of a sample ion without any complex-forming interaction with the zone constituents can be expressed using the mobility and acid dissociation constants and the pH

$$\bar{m}_S = \frac{m_{Z_S} + \sum_{i=1}^{n_S} m_{Z_S-i}\left(\prod_{j=1}^{i} K_{S,j}/C_{H,S}^i\right)}{1 + \sum_{i=1}^{n_S}\left(\prod_{j=1}^{i} K_{S,j}/C_{H,S}^i\right)} \tag{2}$$

where m_{Z_S}, $m_{Z_S - i}$ are the mobilities of the sample ion components with charges Z_S and $Z_S - i$ corrected to a finite ionic strength, K_S the successive dissociation constants corrected also to a finite ionic strength using the activity coefficients estimated from the Debye–Hückel equations[7,8] and $C_{H,S}$ the hydrogen ion concentration in the sample zone. The absolute mobility and/or the thermodynamic dissociation constants can be evaluated if the ionic strength and the pH of the sample zone can be measured or estimated theoretically.

In isotachophoresis, the ionic strength and the pH values of the separated zones including the leading zone are generally different. The measurement of the ionic strength and the pH in a narrow-bore tube under a high potential gradient is very difficult, therefore, we must estimate them theoretically. In earlier work by Everaerts and Routs[9] the validity of the estimated pH was confirmed by the measured value (off-line) using equipment on a preparative scale. In our computational program, their treatment has been extended by taking into account the ionic strength correction to the mobility and pK_a. Thus, the simulation of the isotachophoretically steady state is much more reliable and the pH and ionic strength of a separated zone can be calculated to a good approximation. The difference between the pH of the sample zone, pH_S, and that of the leading zone, pH_L, was sometimes unexpectedly large when an unsuitable electrolyte was used and this caused a low accuracy of the determined constants, especially for pK_a, as discussed subsequently.

To show how the electrolyte conditions affect the isotachophoretic equilibria, a typical weak acid, acetic acid, is considered. The results of the following simulation may be useful for the selection of the optimum experimental condition for the m_0 and pK_a determination. Fig. 1 shows the dependency of the effective mobility of acetate ion on pH_L. The buffers were β-alanine (β-Ala: pK_a 3.55), ε-aminocaproic acid (ε-AMC: 4.43), histidine (His: 6.04) and tris(hydroxymethyl)aminomethane (Tris: 8.08),
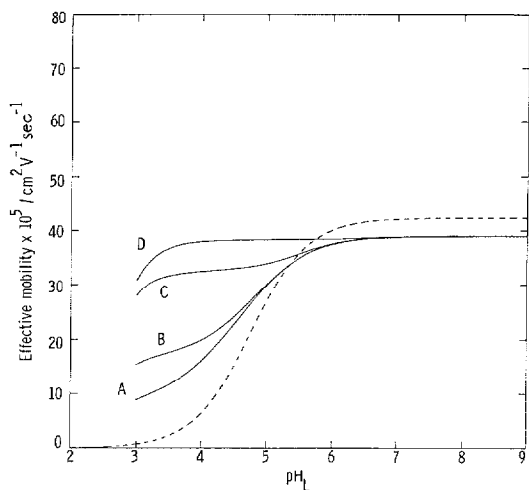

Fig. 1. The effect of the pH of the leading electrolyte, pH_L, and the buffer on the effective mobility of acetate ion at the isotachophoretically steady state. The leading ion was 10 mM Cl$^-$. The pH buffers were β-alanine (β-Ala: A), ε-aminocaproic acid (ε-AMC: B), histidine (His: C), and tris(hydroxymethyl)amino-methane (Tris: D).

which have previously been used in isotachophoresis[1]. In the present simulation, it is noted that the individual buffers in the pH range 3–9 act only as counter ions of the acetate ion. The concentration of the leading ion, Cl^-, was 10 mM and the ionic strength of the leading zone was 0.01. The physico-chemical constants used in the simulation are summarized in Table I.

TABLE I

PHYSICO-CHEMICAL CONSTANTS USED IN SIMULATION (25°C)

m_0 = Absolute mobility (cm^2 V^{-1} sec^{-1}) × 10^5. pK_a = Thermodynamic acidity constant, assumed values being used for Cl^-. The absolute mobilities of the cations were obtained isotachophoretically; the other constants were taken from ref. 5. Ac$^-$ = Acetate.

Cation	m_0	pK_a	Anion	m_0	pK_a
β-Ala$^+$	31.0	3.55	Cl$^-$	79.08	−3
ε-AMC$^+$	28.8	4.43	Ac$^-$	42.4	4.756
His$^+$	29.6	6.04			
Tris$^+$	29.5	8.08			

The dotted curve in Fig. 1 was plotted using the absolute mobility and thermo-dynamic dissociation constants and does not apply to the isotachophoretically steady state. The difference between this curve and the solid ones in the higher pH range of *ca.* 6–9 is due to the correction to the mobility in the sample zone at a finite ionic strength (*ca.* 0.008). On the other hand, the difference in the lower pH range is caused by the fact that the pH of the acetate zone is different from the pH_L, although the ionic strength correction to the dissociation constants still slightly contributes to this difference.

In anionic analysis, pH_S is higher than pH_L and *vice versa* in cationic analysis. The pH shift, $\Delta pH_L = pH_S - pH_L$, in these electrolyte systems is shown in Fig. 2. The pH shift in the low pH_L range is especially large for the counter ions of His and Tris. When the Tris buffer was used for the pH_L adjustment to 4, the pH_S of the acetate zone was 6.2. Such a large pH shift is closely related to the concentration of the buffer in the sample zone, which can be described by the mass balance equation[1]; the larger the effective mobility of the buffer in the leading zone the larger the pH shift. The concentrations and the effective mobilities of the buffers in the acetate zone are shown in Fig. 3. Apparently, the effective mobilities of Tris and His were larger than those of β-Ala and ε-AMC. Usually, it is recommended that the pH_L should be chosen to be similar to the pK_a of the buffer. This ensures not only a high buffer capacity but also a small pH shift, which is important especially for the measurement of the R_E values, used for the estimation of the pK_a of a sample. For this purpose, R_E values which are affected by the pK_a are necessary, *i.e.*, pH_S should be nearly equal to or less than the pK_a of the sample. Such a condition cannot be achieved by the use of an unsuitable buffer. Fig. 4 shows the effects of pH_L and the buffer on the observable R_E values. The satisfactory change in R_E values for the electrolyte systems buffered by ε-AMC and β-Ala in the low pH_L region suggest relatively low pH_S values. In contrast, in the electrolyte systems pH-adjusted by Tris and His, even in the low pH_L range, there is little change in the R_E values, due to the large pH shift. To obtain an appropriate
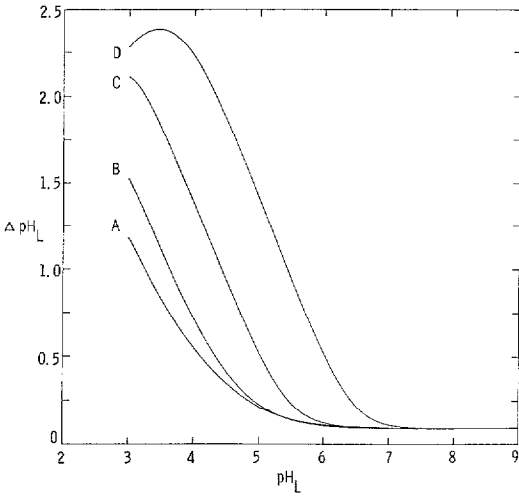
Fig. 2. The effect of pH$_L$ and the buffer on the pH shift, ΔpH$_L$ = pH$_S$ − pH$_L$, of the acetate zone at the isotachophoretically steady state. Buffers as in Fig. 1.

value of R_E, a small degree of dissociation of acetic acid is desirable at the pH of the sample zone.

For the measurement of the absolute mobility, it seems that the pH$_L$ should be selected in the high pH range, where acid is in its fully charged state. This is valid in cases where the determination of pK_a is not necessary. However, when the pK_a of the sample is well defined, the R_E value at the lower pH$_L$ may give a more accurate absolute mobility, as discussed below.
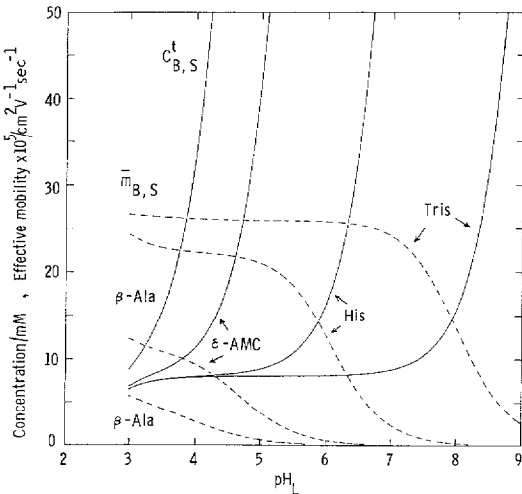
Fig. 3. The effect of pH$_L$ and the buffer on the effective mobility, $\bar{m}_{B,S}$, and the concentrations of the buffers of the acetate zone ($C^t_{B,S}$) at the isotachophoretically steady state.
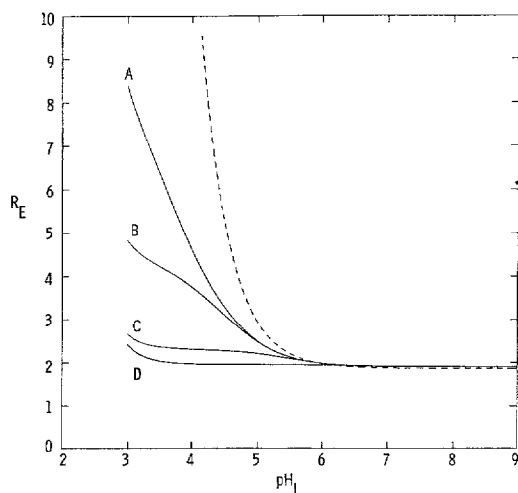
Fig. 4. The effect of pH_L and the buffer on the qualitative index, R_E, for acetate ion at the isotachophoreti-
cally steady state. For electrolytes, see Fig. 1.

RESULTS AND DISCUSSION

Four factors may affect the accuracy of the constants determined by means of a
computer simulation: (1) the reproducibility of the observed R_E values in different
determinations; (2) the difference in the temperatures of the separated zones in
narrow bore tubes, which is inevitable in isotachophoresis; (3) the precision of the m_0
and pK_a of the leading and buffer ions; (4) for a pK_a determination, the precision of
m_0 may also affect the accuracy of the evaluated pK_a (this is the method used to
correct the thermodynamic values). With respect to the fourth factor, we use
Onsager's equation for the mobility correction[6] and the Debye–Hückel equations for
the pK_a correction[7,8] which are valid in the usual electrolyte systems employed in
isotachophoresis at ionic strengths less than 0.01. For the third factor, the pK_a of the
buffer is especially important.

The values of the constants used in the simulation are shown in Table I.
Different pK_a values from those given may be found in literature, and the use of them
may yield slightly different results. A defect of the proposed method in comparison
with conventional methods may be that the pK_a value cannot be determined without
the absolute mobility, however, from a different viewpoint, an unique feature is that
the simultaneous evaluation of m_0 and pK_a is possible. Concerning the second factor,
the undesirable temperature increase can be suppressed using an effective temperature-
controller and suitable coolant. According to Boček et al.[10], a driving current of 50
μA (capillary cross-section $= 2 \cdot 10^{-3}$ cm^2) causes an increase of $0.13°$K for a zone of
$R_E = ca.$ 2. While such an increase of such magnitude is not very troublesome,
however it is proportional to the R_E values.

According to preliminary calculations taking account of the above factors, it
seems that the main factor affecting the accuracy of m_0 and pK_a is the reproducibility
of the R_E values. Thus, the following discussion will be restricted to this factor.
Although the exact value of a potential gradient or a conductivity cannot be

measured by the two-electrode method, which has been used as a detector in isotacho-phoresis, this is not a problem when the R_E index is used. However, a recurrent problem in the use of such a qualitative index is that the exact null point of the step heights cannot easily be found due to undesirable asymmetric potentials. To estimate the step height caused by the asymmetric potential, we use an internal standard for the exact R_E measurements, of which the m_0 and pK_a are well defined and the exact R_E values can be simulated at the thermostatted temperature, usually 25°C. The step height corresponding to an asymmetric potential was estimated to reproduce the simulated R_E value of the internal standard, and by subtracting this from the step heights of the leading and sample zones the corrected R_E values of samples can be obtained precisely. If the effect of the temperature increase is serious the simultaneous use of internal standards with low and high R_E values may be useful. In the method of Boček et al.[10] it was assumed that the null point of the step heights can be obtained, however, in our opinion, it is difficult to determine this experimentally. The detector must exhibit linearity over a wide signal range. A considerable number of samples with different R_E values can be used as the internal standard and in the subsequent study, we used acetic acid as the internal standard to analyse the step heights reported by Everaerts et al.[1]. In our experience, careful experimentation and the use of a detec-tor with high linearity may afford R_E values having a reproducibility of less than ca. ± 0.02, at most, ± 0.05. It is again stressed that, for the precise measurement of R_E values, a reasonably low driving current should be applied.

The question then is how accurately can m_0 and pK_a be evaluated taking into account the above reproducibility of R_E. Several mono- and divalent anions were considered. At first, monovalent anions with pK_a = 4.5 were taken. The absolute mobilities of the model anions were varied in the range of $20 \cdot 10^{-5}$–$70 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}, which includes those of almost all real monovalent anions. Fig. 5 shows the dependency on pH$_L$ of the simulated R_E values for the model anions in the iso-tachophoretically steady state. The concentration of the leading ion, Cl$^-$, was 10 mM
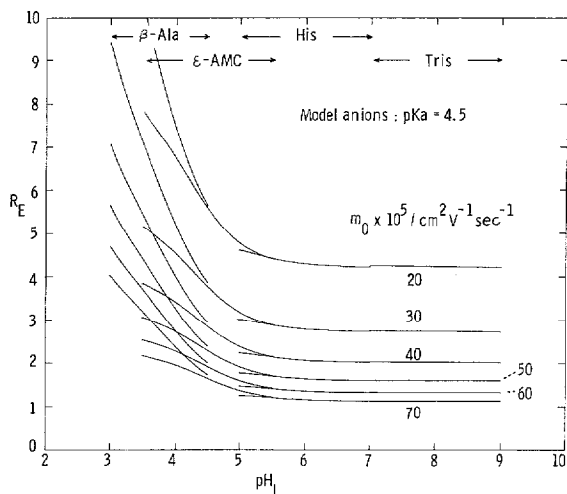
Fig. 5. The effect of pH$_L$ and the buffer on the R_E values of model monovalent anions (pK_a = 4.5) at the isotachophoretically steady state.
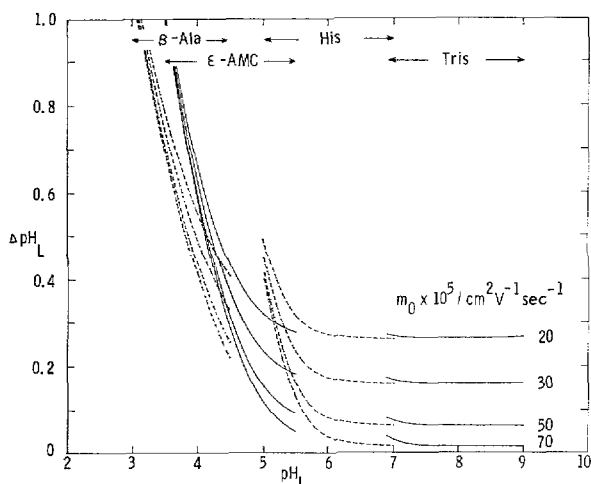
Fig. 6. The effect of pH_L and the buffer on the pH shift of model monovalent anions ($pK_a = 4.5$) at the isotachophoretically steady state.

and the buffers used were β-Ala (pH_L: 3–4.5), ε-AMC (3.5–5.5), His (5–7) and Tris (7–9). It is seen that the curves in Fig. 5 are not continuous, because the pH_S is not the same in the different buffers used, even if pH_L is the same. The R_E values of the models did not exceed 10 even in the low pH_L range, except for a sample of $m_0 = 20 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}. The pH shift is shown in Fig. 6. Apparently, the shift was small for all of the model anions, since the appropriate buffers were used for the individual pH_L ranges. Therefore, a pH_S nearly equal to or less than pK_a (4.5) can easily be achieved by the use of β-alanine buffer (maximum pH_L *ca.* 4), and in the corresponding pH_L range the variation of R_E was remarkable in proportion to the decrease in the degree of dissociation. In the pH_L range buffered by ε-AMC the coefficient of variation of R_E was smaller. In the higher pH_L range, R_E values were not affected by pH_L, but governed by the absolute mobilities of the model anions.

Since the observable R_E value is the ratio of the effective mobilities, the accuracy estimated by the same reproducibility of R_E value may be different for anions with different absolute mobilities. The coefficients of variation of R_E for m_0 and pK_a, $\Delta R_E/\Delta m$ and $\Delta R_E/\Delta pK_a$ were calculated for the model anions. These coefficients can be approximated for kth m_0 and jth pK_a as follows

$$\Delta R_{E,i}/\Delta m_{0,k} = [R_{E,i}(m_{0,k} + 1.0) - R_{E,i}(m_{0,k} - 1.0)]/2$$

$$\Delta R_{E,i}/\Delta pK_{a,j} = [R_{E,i}(pK_{a,j} + 0.1) - R_{E,i}(pK_{a,j} - 0.1)/0.2$$

(3)

where $R_E (m_0 \pm 1)$ denotes the calculated R_E values using the absolute mobility $m \pm 1 \times 10^{-5}$ cm^2 V^{-1} sec^{-1} fixing pK_a at 4.5, and R_E ($pK_a \pm 0.1$) the calculated R_E values using $pK_a \pm 0.1$.

Fig. 7 shows the dependency on the pH_L and the absolute mobility of the calculated $\Delta R_E/\Delta m$. The negative signs of the coefficients indicate that the R_E values decrease with increasing absolute mobility. The large value of the coefficient $\Delta R_E/\Delta m$
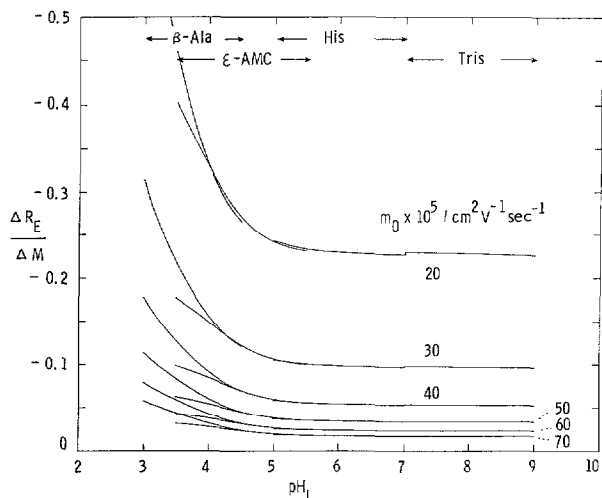
Fig. 7. The effect of pH_L and the buffer on the coefficients of variation, $\Delta R_E/\Delta m$, of model monovalent anions (pK_a = 4.5).

suggests that the observable R_E values are sensitive to m_0. For example, a change of $m = \pm 1 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1} at pH_L = 3 resulted in a change in the R_E values of the model anions with $m_0 = 30 \cdot 10^{-5}$ and $50 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1} of ± 0.25 and ± 0.1, respectively. In other words, anions with $m_0 = 30 \cdot 10^{-5}$ and $31 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1} can easily be distinguished if the reproducibility of the observable R_E values is ± 0.02. From Fig. 7, the coefficients are large in the low pH_L range for all model anions, and also for anions with low mobilities. In the higher pH_L range the coefficients are not affected by pH_L, only by m_0 of the sample itself. Fig. 8 shows the dependency on pH_L and the absolute mobility of the calculated $\Delta R_E/\Delta pK_a$. The positive signs mean that
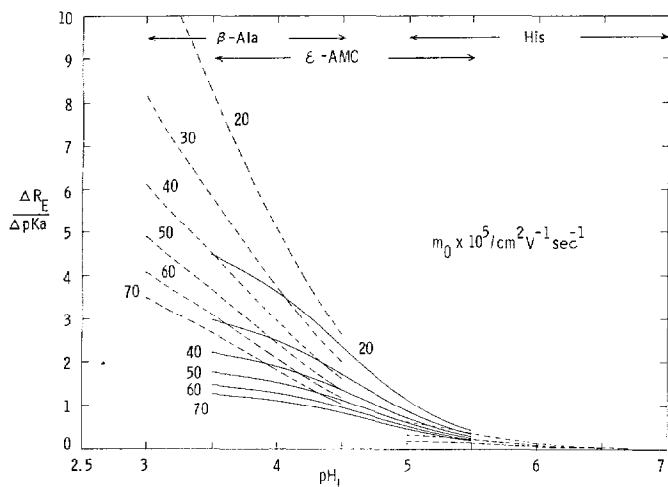

Fig. 8. The effect of pH_L and the buffer on the coefficients of variation, $\Delta R_E/\Delta pK_a$, of model monovalent anions (pK_a = 4.5).

the R_E value increases with increasing pK_a. In contrast to Fig. 7, the coefficient $\Delta R_E/\Delta pK_a$ quickly approaches to zero in the high pH_L range, confirming the fact that R_E values are not affected by pK_a when the substances are in the fully charged state. For example the coefficient for the model ion with $m_0 = 40 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1} at $pH_L = 3.5$ is 5 which means that the R_E value changes by ± 0.05 for a variation in pK_a of ± 0.01. This suggests that samples with $pK_a = 4.5$ and 4.51 or 4.49 and 4.50 ($m_0 = 40 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}) can be distinguished with difficulty at the pH_L.

The accuracy of m_0 and pK_a may be expressed by use of the above coefficients of variation and the reproducibility of the R_E values, $R_E(\text{rep})$, as follows:

$$\text{Accuracy of } m_0 = R_E(\text{rep})/(\Delta R_E/\Delta m_0)$$

$$\text{Accuracy of } pK_a = R_E(\text{rep})/(\Delta R_E/\Delta pK_a) \tag{4}$$

Figs. 9 and 10 show the expected accuracy of m_0 and pK_a for the model anions, assuming $R_E(\text{rep})$ constant within ± 0.02. As expected from Fig. 7, the accuracy of the mobility is high when m_0 is small and the experimental pH_L is low. In percentage terms, the absolute mobility of the samples with $m_0 = 70 \cdot 10^{-5}$ and $20 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1} was accurate to ± 1.7 and $\pm 0.4\%$ at the fully charged state, and ± 0.5 and $\pm 0.2\%$, respectively, at $pH_L = 3$. Using the R_E values in the low pH_L range, pK_a may be accurate to within ± 0.01. However, the accuracy of pK_a quickly decrease if the R_E values at high pH_L are used. Thus, it can be concluded that in the range of $1.5 > pK_a - pH_L > 0.5$ the absolute mobility may be accurate to $ca. \pm 0.1$ to ± 0.6 and the pK_a to $ca. \pm 0.003$ to ± 0.007 for the present model ions, $pK_a = 4.5$. In the pH range, the R_E values vary from $ca.$ 4 to 7 for the sample having $m_0 = 40 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}; the reproducibility of ± 0.02 corresponds to ± 0.5 and $\pm 0.3\%$ of the R_E values 4 and 7, respectively. To obtain the accuracy for different reproducibilities of R_E, eqn. 4 can be used.
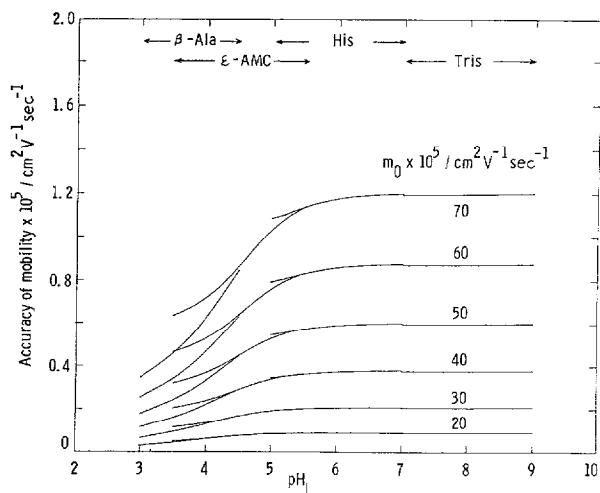

Fig. 9. The effect of pH_L and the buffer on the accuracy of the absolute mobility of model monovalent anions ($pK_a = 4.5$).
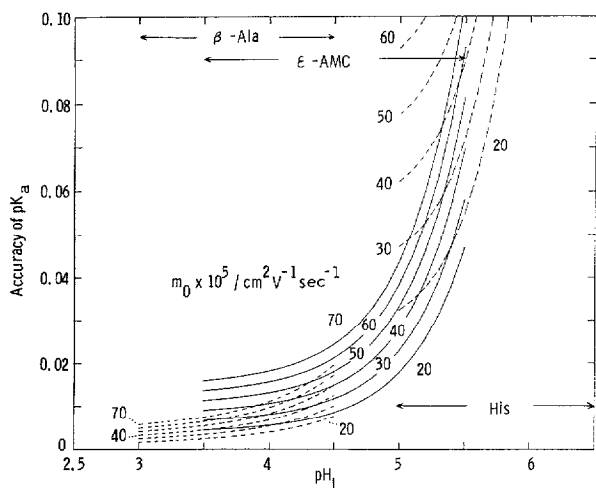
Fig. 10. The effect of pH_L and the buffer on the accuracy of the thermodynamic dissociation constants of model monovalent anions (pK_a = 4.5).
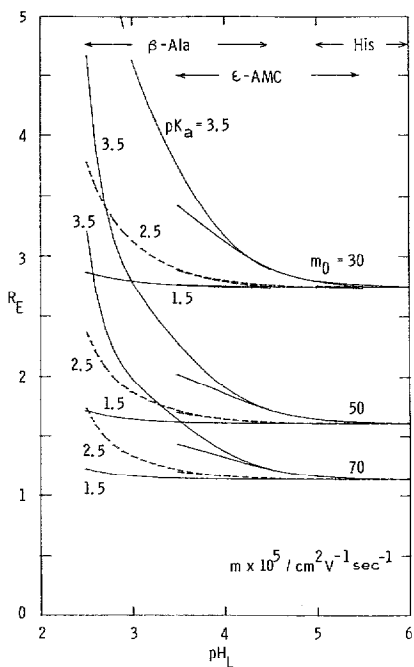

Fig. 11. The effect of pH_L, the buffer and m_0 and pK_a of model monovalent anions on the qualitative index, R_E, at the isotachophoretically steady state. The leading ion was 10 mM Cl$^-$.
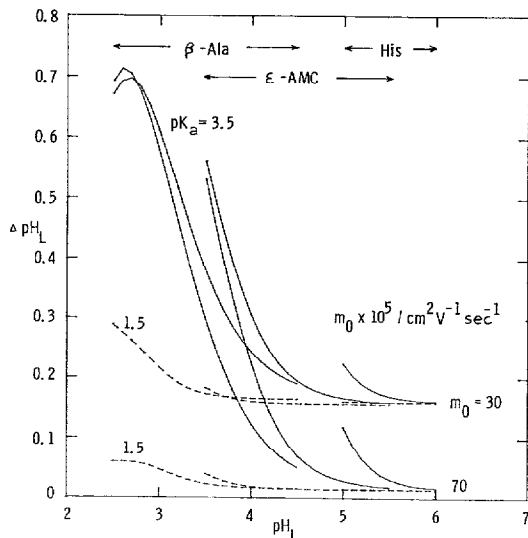
Fig. 12. The effect of pH_L and the buffer on the pH shift of model monovalent anions at the isotachophoretically steady state.

According to the above discussion, for samples with $pK_a > 4.5$, the accuracy of m_0 and pK_a may be expected to be as high as for the anions with $pK_a = 4.5$ when an appropriate buffer is used. On the other hand, if the pK_a of the sample is lower than that of the above anions, the accuracy may be lower. This is caused by the fact in isotachophoresis the steady state is limited to the pH region $ca.$ 2.5–11 when the leading ion is Cl^- and the concentration is $ca.$ 10 mM. Therefore, the pK_a of strong acids cannot be estimated due to the constancy of the effective mobility in the above pH region, although m_0 can be evaluated. To demonstrate the decrease in accuracy of pK_a, model anions with $pK_a = 1.5$, 2.5 and 3.5 were considered. The absolute mobilities of the anions were $30 \cdot 10^{-5}$, $50 \cdot 10^{-5}$ and $70 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}. Fig. 11 shows the effects of pH_L, m_0 and pK_a on the R_E indexes. The simulation conditions were the same as those of Fig. 5. Apparently, in the high pH_L range, the R_E values are the same as those of the model anions of the same mobility shown in Fig. 5. In the lower pH_L range a significant difference in R_E is seen, caused by the smaller pK_a. Fig. 12 shows the pH shift of the anions having $m_0 = 30 \cdot 10^{-5}$ and $70 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}. For the anions of $pK_a = 3.5$, pH_S can be nearly equal to or less than pK_a when pH_L is set below 3; however, for the anions of $pK_a = 1.5$ and 2.5 (not shown in Fig. 12) such a pH_S could not be achieved. Fig. 13 shows the accuracy of m_0 for the anions assuming $R_E(\text{rep}) = \pm 0.02$. In the high pH_L range the accuracy was the same as with the model anions of $pK_a = 4.5$, confirming again that the accuracy in the fully charged state is
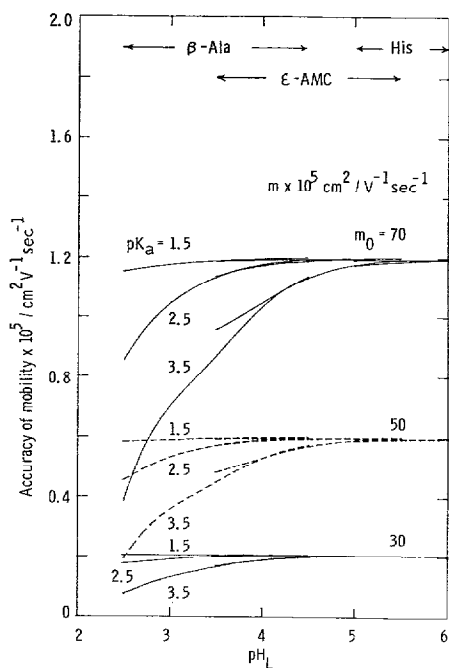

Fig. 13. The effect of pH_L and the buffer on the accuracy of the absolute mobility of model monovalent anions.
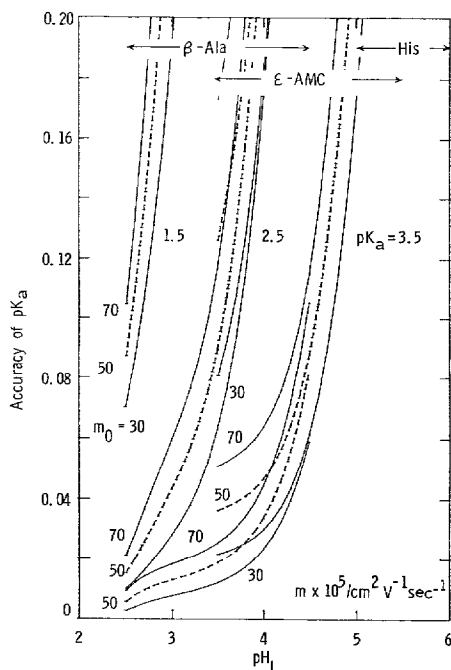
Fig. 14. The effect of pH_L and the buffer on the accuracy of the thermodynamic dissociation constants of model monovalent anions.
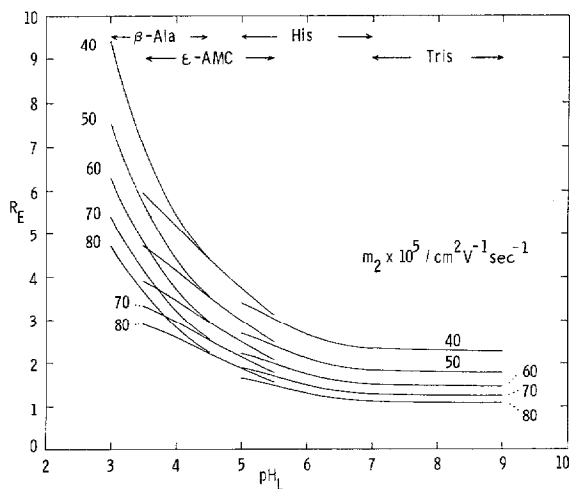
Fig. 15. The effect of pH_L and the buffer on the R_E values of model divalent anions ($pK_a = 4$ and 6) at the isotachophoretically steady state.

not affected by the value of pK_a. In the lower pH_L range the accuracy is a function of both pK_a and m_0; however, the expected accuracy of m_0 is low when the pK_a is small and m_0 is large. This is also valid for the evaluation of pK_a. Fig. 14 shows the dependencies on pH_L, m_0 and pK_a of the accuracy of pK_a [R_F(rep) $= \pm 0.02$]. Apparently, the expected accuracy is low in comparison with the model anions with $pK_a = 4.5$, even in the low pH_L range.

In conclusion, for monovalent anions, the exact R_E values in the low pH_L region may give accurate m_0 and pK_a values when the pK_a of a sample is sufficiently high and a suitable buffer is used. When the pK_a of the sample is small, the pK_a cannot be determined accurately. The accuracy of m_0 is determined by the mobility of
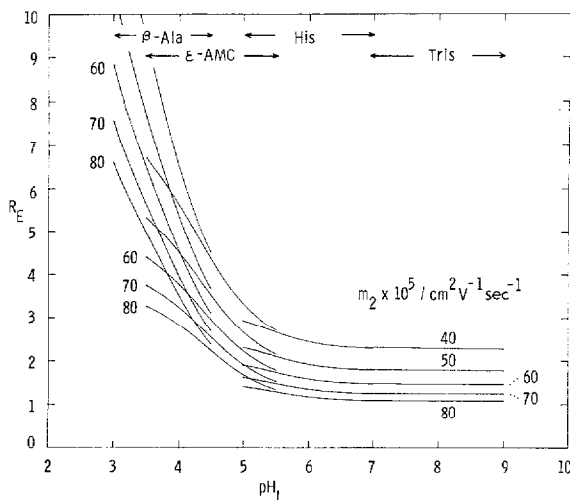

Fig. 16. The effect of pH_L and the buffer on the R_E values of model divalent anions ($pK_a = 4.5$ and 5.5) at the isotachophoretically steady state.
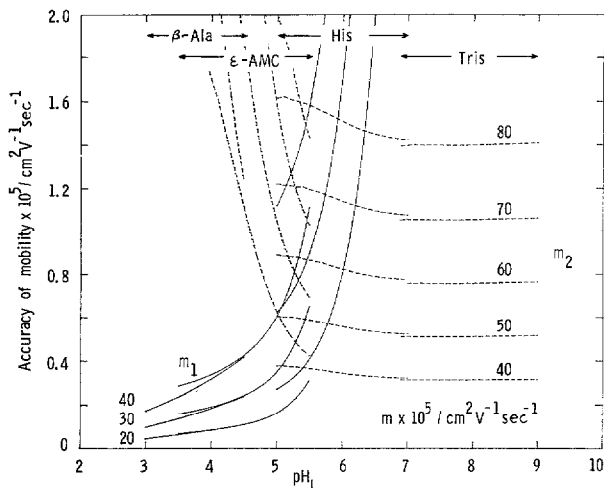
Fig. 17. The effect of pH_L and the buffer on the accuracy of the absolute mobilities, m_1 and m_2, of model divalent anions ($pK_a = 4$ and 6) at the isotachophoretically steady state.

the sample itself and the reproducibility of R_E values, and is not affected by pH_L. The determination of the m_0 values of acids such as chloroacetic and chloric acids will be reported in Part II[11].

For divalent anions the accuracy problem is more complicated since the difference between pK_1 and pK_2 strongly affects the expected accuracy of both m_0 and pK_a. Many combinations can be assumed for pK_a of divalent anions; we limited the above treatment to two sets of model anions with $pK_a = 4$ and 6 (abbreviated as 4–6) and $pK_a = 4.5–5.5$. Similar pK_a values can be found among organic acids, the absolute mobility of which will be reported in Part II[11]. The absolute mobility of
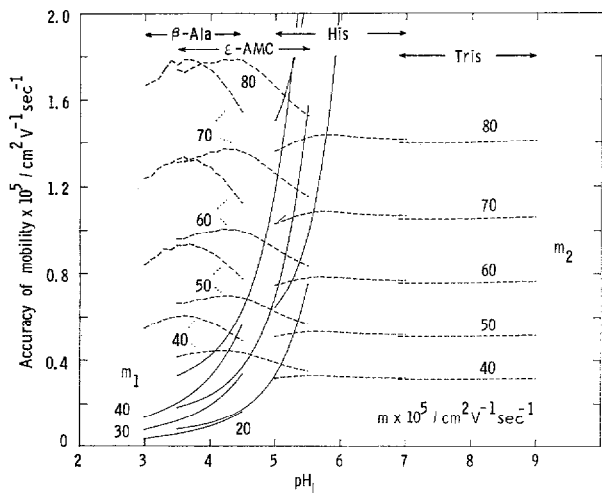

Fig. 18. The effect of pH_L and the buffer on the accuracy of the absolute mobilities, m_1 and m_2, of model divalent anions ($pK_a = 4.5$ and 5.5) at the isotachophoretically steady state.

divalent component (m_2) varied in the range $40 \cdot 10^{-5}$–$80 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}. The absolute mobility of monovalent component (m_1) was assumed to be $m_2/2$. The simulation conditions were the same as those for the model monovalent anions.

Figs. 15 and 16 show the dependencies on pK_a, m_0 and pH$_L$ of the R_E values. Since the differences between pK_1 and pK_2 are small, the curves are similar to those of monovalent anions. The simulated pH shifts are sufficiently small and a pH$_S$ near to pK_1 or pK_2 can easily be achieved by the buffers used. Figs. 17 and 18 show the accuracy of m_1 (solid curves) and m_2 (dotted curves). The former curves were plotted on the assumption that the values of m_2 are known and *vice versa* for the latter curves. Although the R_E variation is not very different between the anions of p$K_a = 4$–6 and p$K_a = 4.5$–5.5, the accuracy of the mobility m_2 is distinctly different in the low pH$_L$ range. Namely, for the anions of p$K_a = 4$–6, the accuracy of m_2 quickly decreases with decreasing pH$_L$; on the other hand, for the anions of p$K_a = 4.5$–5.5, the accuracy is little affected by pH$_L$, *i.e.*, R_E values at any pH$_L$ may afford m_2 with similar accuracy. Such behaviour can easily be understood in terms of the dependency of the abundance of the divalent component on pH$_L$. The difference in the accuracy between m_1 and m_2 can be explained in a similar manner. For accurate determination of m_1, a low pH$_L$ is desirable for divalent anions; on the other hand, for the determination of m_2, a high pH$_L$ is desirable. It should be noted that the accuracy of m_2 cannot exceed that of m_1 when the reproducibility of R_E in both cases is the same, even if the optimal electrolyte conditions are selected for R_E measurements. If m_1 is to be determined, the value of m_2 is necessary in the case where the difference between pK_1 and pK_2 is small. For independent determination of m_1, this difference should be greater than *ca.* 4. On the other hand, m_2 can always be determined independently using the R_E values in the high pH$_L$ range. The "safe" pH$_L$ range is pH$_L > $ p$K_a + 2$. The simultaneous determi-
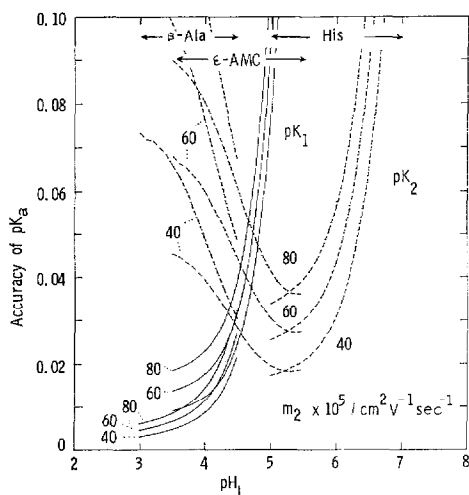

Fig. 19. The effect of pH$_L$ and the buffer on the accuracy of the dissociation constants, pK_1 and pK_2, of model divalent anions (p$K_a = 4$ and 6) at the isotachophoretically steady state.
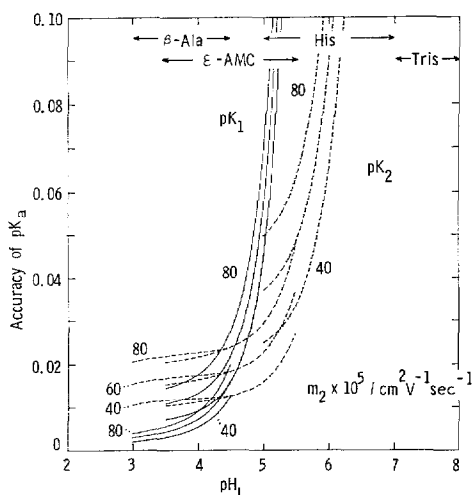
Fig. 20. The effect of pH$_L$ and the buffer on the accuracy of the dissociation constants, pK_1 and pK_2, of model divalent anions (p$K_a = 4.5$ and 5.5) at the isotachophoretically steady state.

nation of m_1 and m_2 is possible when the R_E values are obtained over a wide pH_L range using a suitable buffer.

Figs. 19 and 20 show the expected accuracy of pK_1 and pK_2. For the curves of pK_1, pK_2 is fixed, and *vice versa* for pK_2. In both figures, the accuracy curves for pK_1 resemble those for monovalent anions. On the other hand, the curves for pK_2 differ from those of pK_1, and also between the two sets of model anions with different pK_a values. For the model anions with $pK_a = 4$–6 the maximum accuracy of pK_2 can be obtained with R_E values measured in the pH_L range 5–5.5. Outside of this pH_L range the accuracy quickly decreases. The accuracy of pK_2 for the model anions of $pK_a = 4.5$–5.5 is relatively high even in the low pH_L range, although the accuracy is lower than that of pK_1. If the difference between pK_1 and pK_2 becomes smaller than in the present model anions, the accuracy curves of m_0 and pK_a approach those of monovalent anions. Among real divalent anions, many samples with low pK_1 can be found, such as oxalic acid ($pK_a = 1.271$–4.266). For these, as well as for monovalent anions, pK_1 cannot be determined accurately. Excluding these cases, pK_1 and pK_2 can be determined simultaneously when a sufficient number of different R_E values are measured at different pH_L using appropriate buffers. For other multivalent ions, the proposed method can be adopted similarly, although a careful selection of the electrolyte systems is necessary for the precise determination of mobility and pK_a.

The above discussion of the accuracy of the evaluated m_0 and pK_a using one R_E value obtained by means of isotachophoresis reveals that three significant figures may be allowed for the expression of the values of monovalent anions, but for divalent anions the evaluated m_2 and pK_2 are less accurate in principle. However, the accuracy is comparable with those of conventional methods, such as conductivity and pH measurements. It should be noted that we have restricted the accuracy problem to the reproducibility of one R_E value under a certain electrolyte condition. Namely, the above discussion was not based on an exact statistical treatment. If a set of precise R_E values obtained under different electrolyte conditions is used in the least squares method for the evaluation of m_0 and pK_a the accuracy can be improved. Practical examples of the least squares method and the statistical treatment of the accuracy of the determined m_0 and pK_a of many anions will be reported in Part II[11].

REFERENCES

1 F. M. Everaerts, J. L. Beckers and Th. P. E. M. Verheggen, *Isotachophoresis*, Elsevier, Amsterdam, 1976.
2 T. Hirokawa and Y. Kiso, *J. Chromatogr.*, 242 (1982) 227.
3 Y. Kiso and T. Hirokawa, *Chem. Lett.*, (1979) 891.
4 Y. Kiso and T. Hirokawa, *Chem. Lett.*, (1980) 745.
5 *Landolt-Börnstein, Zahlenwerte und Funktionen*, 6 Aufl. Bd. II, Teil 7, Springer, Berlin, 1960.
6 L. Onsager, *Phys. Z.*, 28 (1927) 277.
7 P. Debye and E. Hückel, *Phys. Z.*, 24 (1923) 185.
8 P. Debye and E. Hückel, *Phys. Z.*, 24 (1923) 305.
9 F. M. Everaerts and R. J. Routs, *J. Chromatogr.*, 58 (1971) 181.
10 P. Boček, M. Deml and J. Janák, *J. Chromatogr.*, 106 (1975) 283.
11 T. Hirokawa and Y. Kiso, *J. Chromatogr.*, 252 (1982) 49.