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DETERMINATION OF THE CHARGE OF IONS BY PARTITION MEASUREMENTS IN AQUEOUS POLYMER TWO-PHASE SYSTEMS

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SUMMARY

A method for determining the charge of ions from partition data in aqueous polymer two-phase systems containing two different electrolytes is outlined. The method was verified in polyethylene glycol–dextran systems with simple inorganic ions of known charge ranging from −4 to +2.

INTRODUCTION

During our research on the characterization of the components of Tc(Sn) diphosphonates, we noticed[1] that the results of determinations of the charge of these complexes by ion-exchange methods were considerably scattered. This is partly due to the fact that precautions were not always taken to prevent dissociation of the complexes during chromatography, by adding diphosphonate and Sn(II) to the eluent. A more serious and fundamental problem associated with these methods is, however, the impossibility of correcting for activity coefficients at the relatively high electrolyte concentrations that must be used. In a recent review, Lederer[2] also drew attention to the difficulties and the often erroneous results of determinations of ionic charge by ion-exchange methods.

Therefore, we thought it worthwhile to develop another method for the determination of ionic charge, *i.e.*, by partition in an aqueous polymer two-phase system. This partition method was originally developed by Albertsson[3] for the separation of macromolecules and cellular particles. It makes use of the phenomenon that on dissolution of two water-soluble polymers (dextran and polyethylene glycol are generally used) above certain critical concentrations in water, a separation into two immiscible aqueous liquid phases occurs (with a dextran–polyethylene glycol system the top and bottom layers are enriched in polyethylene glycol and dextran, respectively). It is well known that the partition constant of a polyelectrolyte (*e.g.*, a protein) in such a two-phase system is strongly dependent on the pH (*i.e.*, on the charge of the protein) and, at a pH different from the isoelectric point of the polyelectrolyte, on the nature of the electrolyte present in the system (and *not* on the electrolyte concentration). These effects can be understood as follows: on transfer of

a charged species (a protein or a simple inorganic ion) from one phase to the other, an equivalent amount of opposite charge, in the form of the electrolyte counter ion, provided by the electrolyte present, must be simultaneously transferred in order to maintain electroneutrality in both phases. As the affinities of the two layers of the system are different for different electrolyte counter ions (*i.e.*, the partition constants of electrolytes containing these ions are themselves different), the partition constant of a charged species must depend on the nature of the electrolyte. For a two-phase system of fixed polymer concentration it was shown[4] that the logarithm of the ratio of the partition constants of a charged species in the presence of two different electrolytes is simply proportional to its charge, the proportionality constant increasing with increasing difference in the partition constants of the electrolytes themselves. This offers, in turn, the possibility of a charge determination method.

An advantage of the proposed method is that experiments can be performed over a wide range of much lower ionic strengths than those required in ion-exchange experiments, permitting activity coefficient corrections and extrapolation of the results to zero ionic strength. A disadvantage compared with the ion-exchange chromatographic method is, however, that only the mean ionic charge of a mixture of complexes is accessible. Moreover, the method is experimentally more laborious.

THEORY

The theory of the method was given by De Ligny and Gelsema[4] for polymer systems containing the uni–univalent electrolytes MX and NY, respectively, and neglecting activity coefficient corrections. However, when the method is applied to highly charged ions [*e.g.*, Tc(Sn) diphosphonate complexes], activity coefficient corrections are imperative. Further, the electrolytes $NaClO_4$ and Na_2SO_4 (a uni–bivalent electrolyte)were chosen in this study, as the affinity of the involved aqueous polymer solutions for these electrolytes is widely different, and as ClO_4^- shows no and SO_4^{2-} little tendency to form complexes with any of the ions investigated by this method. Therefore, we present here the theory for polymer systems containing $NaClO_4$ and Na_2SO_4, taking activity coefficient corrections into account.

We define thermodynamic partition constants in these systems as

$$K = a_T/a_B \tag{1}$$

where a represents the activity and the subscripts T and B denote the top and bottom layer, respectively. Substitution of $a = m\gamma$, where m is the molality (mol kg^{-1}) and γ the activity coefficient on the molal scale, gives

$$K = \frac{m_T}{m_B} \cdot \frac{\gamma_T}{\gamma_B} = K' \cdot \frac{\gamma_T}{\gamma_B} \tag{2}$$

(owing to the high viscosity of the two layers of the polymer system, it is impossible to take accurate volumetric aliquots from both layers using a pipette. Instead, the aliquots must be weighed, which requires the use of the molality concentration scale).

Consider now the partition equilibrium of a trace amount of an ion with charge

z, P^z, in the presence of $NaClO_4$. In the equation an electroneutral combination of ions must occur:

$$P_T^z - zNa_T^+ \rightleftharpoons P_B^z - zNa_B^+ \tag{3}$$

The charge z may be either positive or negative. The equilibrium condition is

$$RT \ln\left[\frac{(m_{P^z})_T}{(m_{P^z})_B}\right] + RT \ln\left[\frac{(\gamma_{P^z})_T}{(\gamma_{P^z})_B}\right] - zRT \ln\left[\frac{(a_{Na^+})_T}{(a_{Na^+})_B}\right] =$$

$$= \mu_B^{0,P^z} - \mu_T^{0,P^z} - z(\mu_B^{0,Na^+} - \mu_T^{0,Na^+}) \tag{4}$$

where μ^0 denotes the standard chemical potential. Eqn. 4 can be simplified to

$$RT \ln K_{P^z}' + RT \ln\left[\frac{(\gamma_{P^z})_T}{(\gamma_{P^z})_B}\right] - zRT \ln K_{Na^+} = -\Delta\mu^{0,P^z} + z\Delta\mu^{0,Na^+} \tag{5}$$

For partition of the ion P^z in the presence of Na_2SO_4 an identical equation holds. When these equations are combined, the right-hand sides cancel:

$$\log\left[\frac{K_{P^z}'(NaClO_4)}{K_{P^z}'(Na_2SO_4)}\right] + \log\left[\frac{(\gamma_{P^z})_{T, NaClO_4}}{(\gamma_{P^z})_{B, NaClO_4}}\right] - \log\left[\frac{(\gamma_{P^z})_{T, Na_2SO_4}}{(\gamma_{P^z})_{B, Na_2SO_4}}\right] = z\log\left[\frac{K_{Na^+}(NaClO_4)}{K_{Na^+}(Na_2SO_4)}\right]$$

$$\tag{6}$$

This equation reflects the relationship referred to in the Introduction; also, in the presence of a 1:1 and a 1:2 electrolyte it holds that the logarithm of the ratio of the partition constants of P^z in these two electrolytes (first term on the left-hand side) is simply proportional to z, the proportionality constant, $\log[K_{Na^+}(NaClO_4)/K_{Na^+}(Na_2SO_4)]$, depending on the difference in the partition constants of the electrolytes used. The second and third terms on the left-hand side of eqn. 6 represent corrections for activity coefficients of P^z in both layers in the presence of the two electrolytes.

For the sake of brevity, we shall delete the subscript P^z on γ, and denote $NaClO_4$ and Na_2SO_4 by NX and N_2Y, respectively. Defining further $\Delta \log K \equiv \log [K(NX)/K(N_2Y)]$ and $\Delta \log K' \equiv \log [K'(NX)/K'(N_2Y)]$, eqn. 6 can be written as

$$\Delta \log K_{P^z} = \Delta \log K_{P^z}' + \log\left[\frac{\gamma_T(NX)\gamma_B(N_2Y)}{\gamma_B(NX)\gamma_T(N_2Y)}\right] = z \Delta \log K_{N^+} \tag{7}$$

Eqn. 7 predicts that a plot of $\Delta \log K_{P^z}$ for several ions of different charge (data that can be obtained by measuring $\Delta \log K_{P^z}'$ for these ions and correcting for the activity coefficient term; see below) versus z gives a straight line passing through the origin with a slope $\Delta \log K_{N^+}$. In this paper, eqn. 7 will be verified for simple inorganic ions carrying charges ranging from -4 to $+2$ in polyethylene glycol–dextran (each at a concentration of 7%, w/w) systems (using these polymer concentrations, the top and

bottom layers are almost pure polyethylene glycol and dextran solutions, respectively).

The way in which corrections of $\Delta \log K'_{P^z}$ for the activity coefficient term were made is outlined below. Clearly, the error of such corrections decreases with decreasing ionic strength of the NX and N_2Y solutions. Therefore, we performed measurements of $\Delta \log K'_{P^z}$ at three low levels of the over-all ionic strength ω ($\omega = 1/2\sum_i z_i^2 m_i$), i.e., 0.10, 0.05 and 0.01 mol kg^{-1}, in both NX- and N_2Y-containing polymer systems and we extrapolated the corrected data to zero ionic strength.

As shown in the Appendix, the activity coefficient term occurring in eqn. 7 can be written as

$$\log\left[\frac{\gamma_T(NX)\gamma_B(N_2Y)}{\gamma_B(NX)\gamma_T(N_2Y)}\right] = -z^2\sqrt{\omega}\,f(\omega,\mathring{a}_i) - f'(m) + C'\omega \tag{8}$$

Substitution in eqn. 7 yields

$$\Delta \log K_{P^z} = \Delta \log K'_{P^z} - z^2 \sqrt{\omega}\,f(\omega,\mathring{a}_i) - f'(m) + C'\omega \tag{9}$$

where $\Delta \log K'_{P^z}$ can be measured and $z^2 \sqrt{\omega}\,f(\omega,\mathring{a}_i)$ and $f'(m)$ can be calculated. Thus, linear extrapolation of $\Delta \log K'_{P^z} - z^2 \sqrt{\omega}\,f(\omega,\mathring{a}_i) - f'(m)$ to $\omega = 0$ yields $\Delta \log K_{P^z}$.

EXPERIMENTAL

Chemicals and apparatus

The following chemicals and materials were used: polyethylene glycol 6000 (Fluka, Buchs, Switzerland), Dextran T500 (Pharmacia, Uppsala, Sweden), NaClO$_4$ · H$_2$O and Na$_2$SO$_4$ (analytical-reagent grade; Merck, Darmstadt, F.R.G.), ^{22}NaCl and Na$_2$^{35}SO$_4$ (Amersham, Little Chalfont, U.K.), ^{99}Mo/^{99m}Tc generator (Mallinckrodt, Petten, The Netherlands) and Whatman DEAE-cellulose anion-exchange paper (DE 81, 2.3 cm diameter circular sheets; Balston, Maidstone, U.K.). All other chemicals were of analytical-reagent grade.

The apparatus used included: γ-counting equipment (Gamma 8000, Beckman, Irvine, CA, U.S.A.), β-counting equipment (PW 4600 series; Philips, Eindhoven, The Netherlands), an atomic absorption spectrometer (Model 1200; Varian Techtron, Melbourne, Australia) and an X-ray fluorescence spectrometer (PW 1410; Philips).

Procedures

About 40% (w/w) polyethylene glycol and 20% (w/w) dextran aqueous stock solutions were prepared by dissolution with heating of weighed amounts of the polymers. The water content of dextran, determined by thermogravimetry, was accounted for.

Two-phase polymer systems were prepared by weighing appropriate amounts of these stock solutions in order to obtain final over-all concentrations of 7.00% (w/w) for both polymers. To these mixtures 2.50 ml of NaClO$_4$ or Na$_2$SO$_4$ solutions ($\mu = 1.00, 0.50$ and 0.10 M) were added. Then the sample was added, followed by water up to a total weight of 25 g. The systems were thoroughly shaken, kept for 2 h on

a test-tube rotator and then poured into separating funnels and left overnight at room temperature. Weighed aliquots of the top and bottom layers were taken and assayed for the sample ion of interest.

The samples and assays used in the above procedure were as follows:

Mg^{2+}:   Sample: 25 μl of a 0.5 M solution of $MgCl_2$.

                   Assay: Atomic absorption spectrometry of 0.5-ml ali-
                             quots, diluted to 50 ml with water. Calibration
                             lines were obtained using solutions containing
                             (in 50 ml) standard amounts of $MgCl_2$ and 0.5
                             ml of top and bottom layers, respectively, from
                             blank $NaClO_4$- or Na_2SO_4-containing polymer
                             two-phase systems.

Na^+:   Sample: 25 μl of a 0.001 M solution of NaCl, spiked with
                             $^{22}NaCl$.

                   Assay: γ-Counting of 5-ml aliquots.

TcO_4^-:   Sample: The eluate from a $^{99}Mo/^{99m}Tc$ generator, con-
                             taining $Na^{99m}TcO_4$ in 0.15 M NaCl, was di-
                             luted 1000-fold with water. A 25-μl sample of
                             this solution was used.

                   Assay: γ-Counting of 5-ml aliquots.

SO_4^{2-}:   Sample[a]: 50 μl of a 0.005 M Na_2SO_4 solution, spiked with
                             $Na_2{}^{35}SO_4$.

                   Assay: β-Counting after collection of SO_4^{2-} on anion-
                             exchange paper[b] from 0.1–0.5-ml aliquots, di-
                             luted with water to 50 ml.

$Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$: Sample: 250 μl of a 0.05 M $K_3Fe(CN)_6$ or $K_4Fe(CN)_6$
                             solution.

                   Assay: X-ray fluorescence analysis after collection of
                             $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ on anion-exchange
                             paper[b] from 1-ml aliquots, to which 125 μl of
                             0.04 M HCl were added prior to dilution with
                             water to 50 ml.

Moreover, in order to obtain the distribution coefficient of a neutral compound, i.e., water, in these polymer systems, weighed 1-ml aliquots of top and bottom layers were evaporated to dryness at 95°C and reweighed.

Following these procedures, the final overall molalities of the sample ions and the counter ions added concomitantly with the sample ions were as summarized in Table I. Note that these molalities are all smaller and in most instances much smaller than the lowest overall Na_2SO_4 molality used ($3.3 \cdot 10^{-3}$ mol kg^{-1}). As a consequence, their contribution to the ionic strength was not taken into account.

RESULTS AND DISCUSSION

The experimental K_{P^z}' values are given in Table II. From these data, values of

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[a] This was performed only in the $NaClO_4$-containing polymer systems.

[b] This technique has been described in detail in ref. 5.

TABLE I

IONIC COMPOSITIONS OF THE TWO-PHASE POLYMER SYSTEMS

Sample ion	Molality of sample ion (mol. kg^{-1})	Molality of other species (mol. kg^{-1})
Mg^{2+}	$5 \cdot 10^{-4}$	Cl^-: 10^{-3}
Na^+	10^{-6}	Cl^-: 10^{-6}
$^{99m}TcO_4^-$	$-^a$	$NaCl$: $1.5 \cdot 10^{-7}$
SO_4^{2-}	10^{-5}	Na^+: $2 \cdot 10^{-5}$
$Fe(CN)_6^{3-}$	$5 \cdot 10^{-4}$	K^+: $1.5 \cdot 10^{-3}$
$Fe(CN)_6^{4-}$	$5 \cdot 10^{-4}$	K^+: $2 \cdot 10^{-3}$

^{a} No carrier added.

$\Delta \log K'_{P^z} = \log [K'_{P^z}(NaClO_4)/(K'_{P^z}(Na_2SO_4)]$ were calculated. These values were corrected for the two terms $f'(m)$ and $z^2 \sqrt{\omega} f(\omega, \mathring{a}_i)$ occurring in eqn. 9 as follows.

The term $f'(m)$ in eqn. 9 can be assessed by realizing that the solvents in the top and bottom layers are, essentially, aqueous 9.1% (w/w) PEG and 19.5% (w/w) dextran solutions, respectively. Using the equations

$$\overline{M_T} = \left(\frac{x_{PEG}}{M_{PEG}} + \frac{1 - x_{PEG}}{M_{H_2O}} \right)^{-1} \text{ and } \overline{M_B} = \left(\frac{x_{Dextr.}}{M_{Dextr.}} + \frac{1 - x_{Dextr.}}{M_{H_2O}} \right)^{-1}$$

with $x_{PEG} = 0.091$, $M_{PEG} = 6000$, $x_{Dextr.} = 0.195$, $M_{Dextr.} = 500\,000$, and using further $\nu_{NX} = 2$, $\nu_{N_2Y} = 3$, $K'_{NX} = 1.17$, $K'_{N_2Y} = 0.86$ and $W_B/W = 0.32$, it can be shown that this term amounts to $2 \cdot 10^{-4}$ at the highest molality used. Consequently, it was neglected.

The term $z^2\sqrt{\omega} f(\omega, \mathring{a}_i)$ in eqn. 9 was calculated by equalizing the dielectric constants of the top and bottom layers to those of aqueous 9.1% (w/w) dioxane and

TABLE II

K'_{P^z} VALUES OF DIFFERENT SPECIES P^z IN NaClO$_4$ AND Na$_2$SO$_4$ CONTAINING TWO-PHASE PEG–DEXTRAN POLYMER SYSTEMS (EACH AT A CONCENTRATION OF 7%, w/w) OF VARYING IONIC STRENGTH

The values in parentheses were not measured, but are identical to those of Na$^+$ in Na$_2$SO$_4$, as $K'_{Na^+} \equiv K'_{SO_4^{2-}}$

ω (mol kg^{-1})	NaClO$_4$			Na$_2$SO$_4$		
	0.1	0.05	0.01	0.1	0.05	0.01
Mg^{2+}	1.69	1.72	1.55	0.815	0.848	0.873
Na^+	1.16	1.17	1.17	0.827	0.843	0.870
H_2O	1.14	1.14	1.14	1.13	1.13	1.14
TcO_4^-	1.02	1.03	1.05	1.41	1.41	1.37
SO_4^{2-}	0.539	0.543	0.534	(0.827)	(0.843)	(0.870)
$Fe(CN)_6^{3-}$	1.12	1.24	1.23	2.79	2.67	2.50
$Fe(CN)_6^{4-}$	0.444	0.524	0.516	1.00	1.11	1.12

TABLE III

VALUES OF $z^2\sqrt{\omega}\,f(\omega, \mathring{a}_i)$ FOR VARIABLE ω AND z AT $\mathring{a}_i = 5$ Å

ω (mol kg^{-1})	$z^2\sqrt{\omega}\,f(\omega, \mathring{a}_i)$			
	$z = \pm 1$	$z = \pm 2$	$z = \pm 3$	$z = \pm 4$
0.1	0.012	0.046	0.104	0.186
0.05	0.010	0.041	0.092	0.163
0.01	0.006	0.026	0.058	0.102

19.5% (w/w) sucrose solutions, respectively: $D_T = 70.5$ and $D_B = 73.6^6$. This yields the Debye–Hückel constants: $A_T = 0.595$, $A_B = 0.558$, $\beta_T = 0.347$ and $\beta_B = 0.340$. Table III gives the results for several values of z at the three levels of ω used in this study.

The results obtained after applying these corrections are shown in Table IV, where the extrapolated data, representing $\Delta \log K_{P^z}$ values, are also given. In Fig. 1 a plot of $\Delta \log K_{P^z}$ versus z is shown. It can be seen that the linear relationship predicted by eqn. 7 is obtained.

Least-squares fit of the data to the equation $\Delta \log K_{P^z} = a + bz$ yields $a = 0.000 \pm 0.006$ and $b = 0.113 \pm 0.003$. The line therefore passes through the origin, within experimental error, as predicted by eqn. 7. The slope, $b = 0.113 \pm 0.003$ (5 degrees of freedom), does not differ significantly from the theoretical value, $\Delta \log K_{Na^+} = 0.125 \pm 0.005$ (1 degree of freedom) (see Table IV). Therefore, the expression $\Delta \log K_{P^z} = 0.113\, z$ can be used as a calibration line for the determination of ionic charge (of course, as the activity coefficient corrections depend on z, this determination must be done by successive approximations).

Table V (first column) gives the deviations from the real charge, calculated from the data in the last column of Table IV and the equation $\Delta \log K_{P^z} = 0.113\, z$, for the ions investigated. For a comparison with results obtained by ion-exchange methods,

TABLE IV

VALUES OF $\Delta \log K_{P^z} - z^2\sqrt{\omega}\,f(\omega, \mathring{a}_i = 5$ Å) OF DIFFERENT SPECIES P^z AT VARYING IONIC STRENGTH, AND VALUES OF $\Delta \log K_{P^z}$, OBTAINED BY EXTRAPOLATION VERSUS ω AT $\omega = 0$

Species	ω (mol kg^{-1})			
	0.1	0.05	0.01	$\rightarrow 0^a$
Mg^{2+}	0.270	0.267	0.222	0.226 ± 0.020
Na$^+$	0.134	0.134	0.124	0.125 ± 0.005
H$_2$O	0.003	0.003	0.001	0.000 ± 0.001
TcO$_4^-$	−0.154	−0.148	−0.121	−0.122 ± 0.010
SO$_4^{2-}$	−0.232	−0.234	−0.238	−0.238 ± 0.001
Fe(CN)$_6^{3-}$	−0.499	−0.427	−0.367	−0.353 ± 0.001
Fe(CN)$_6^{4-}$	−0.540	−0.490	−0.441	−0.432 ± 0.004

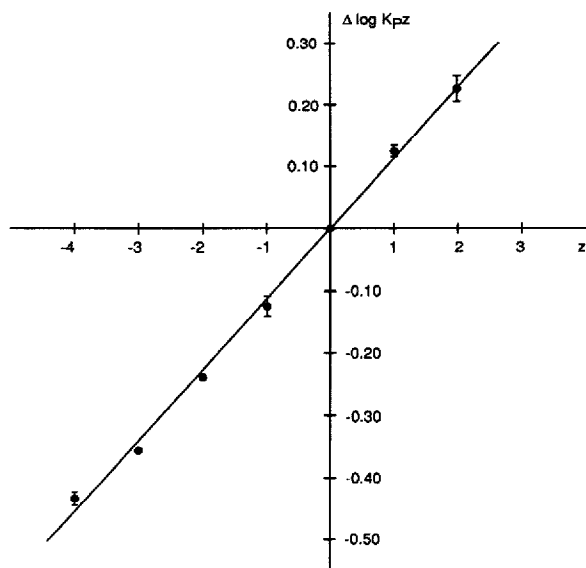^{a} With standard deviations of the extrapolated values.

Fig. 1. $\Delta \log K_{p^z}$ versus z. Data from Table IV. Regression line: $\Delta \log K_{p^z} = 0.113\ z$.

we refer to a paper by Huigen *et al.*[7]. The charges found for Cl^- and SO_4^{2-} ions by normal-pressure ion-exchange chromatography on DEAE-Trisacryl with 0.10–0.50 M $NaClO_4$ as eluent were -0.63 and -1.40, respectively; that obtained for $Fe(CN)_6^{3-}$ by experiments with 0.75–1.00 M $NaClO_4$ on the same material was -1.20. Using high-performance liquid chromatography on Aminex A-28 with 0.90–1.05 M sodium acetate as eluent a value of -2.2 for SO_4^{2-} ions was found.

These results were obtained in the usual way[2,8,9], *i.e.*, from the slope of a double logarithmic plot of the capacity factor against the electrolyte concentration. This means that corrections for activity coefficients are not applied (indeed, owing to the relatively high electrolyte concentrations in the eluents and the unknown, but also high, electrolyte concentration in the exchanger phase, such a correction is hardly feasible). One could argue that this problem can be circumvented by using a reference ion[9,10]: the double logarithmic plot of the capacity factor of an unknown ion against that of a reference ion with known charge should have a slope equal to the ratio of their charges and would provide for an "internal" correction for activity coefficients. However, this approach is valid (and feasible) only for ions that have nearly the same charge [with the data given above it is clear that the use of SO_4^{2-} as a reference ion for $Fe(CN)_6^{3-}$, or *vice versa*, is not possible owing to the non-overlapping electrolyte concentration ranges required]. Deviations from the real charge for these results from ion-exchange chromatographic experiments are also summarized in Table V. We conclude from these data that the proposed polymer two-phase partition method yields more reliable results than the ion-exchange chromatographic method.

From the standard deviation, $s_{\Delta \log K_{p^z}} = 0.0139$, of the experimental points to the calibration line and the values of the slope b and its standard deviation s_b ($b = 0.113\ \pm$

TABLE V

DEVIATIONS FROM THE REAL CHARGE FOR SOME IONS FOUND BY AQUEOUS POLYMER TWO-PHASE PARTITION (THIS WORK) AND BY ION-EXCHANGE CHROMATOGRAPHY (REF. 7)

Ion	Aqueous polymer two-phase partition	Ion-exchange chromatography	
		DEAE-Trisacryl	Aminex 28
Mg^{2+}	0.00		
Na^+	+0.11		
TcO_4^-	−0.08		
Cl^-		+0.37	
SO_4^{2-}	−0.11	+0.60	−0.20
$Fe(CN)_6^{3-}$	−0.12	+1.80	
$Fe(CN)_6^{4-}$	+0.18		

TABLE VI

STANDARD DEVIATION s_z OF AN IONIC CHARGE z, TO BE DETERMINED BY THE PROPOSED METHOD, USING THE CALIBRATION LINE OF FIG. 1

z	s_z	z	s_z
−4	0.19	0	0.16
−3	0.17	+1	0.17
−2	0.16	+2	0.19
−1	0.16		

0.003), the standard deviation s_z of an unknown ionic charge z, to be determined by the proposed method, can be estimated using[11]

$$s_z^2 = b^{-2}\left[\left(1 + \frac{1}{n}\right)s_{\Delta \log K_{P^z}}^2 + (z - \bar{z})^2 s_b^2\right]\left(1 + \frac{p}{n - 1 - p}\right)$$

where n is the number of experimental data points, p is the number of parameters used in calculating the regression line and $\bar{z}$ is the mean charge of the ions used. The resulting errors (using $n = 7$, $p = 2$ and $\bar{z} = -1$) are given in Table VI.

APPENDIX

The activity coefficient γ_{P^z} of an ion P^z, present in a trace amount in an electrolyte solution of ionic strength ω, is given by the extended Debye–Hückel equation[a]:

$$-\log \gamma_{P^z} = \frac{z^2 A \sqrt{\omega}}{1 + \beta \mathring{a}_i \sqrt{\omega}} + \log(1 + 10^{-3} v \overline{M}_{\text{solv}} m) + C\omega \tag{A1}$$

[a] An equation of this form was shown[12] to represent the activity coefficients of 1:1 and 2:1 electrolytes fairly well up to concentrations beyond 0.1 M. Here its extended use for the representation of single-ion activity coefficients of −3 and −4 ions up to 0.1 M is permitted as the correction term contains activity coefficient ratios.

where A, β and C are constants, $\mathring{a}_i$ is the distance of closest approach of the electrolyte ions to the ion P^z, v is the number of ions into which one molecule of electrolyte dissociates, $\overline{M}_{solv}$ is the mean molecular weight of the solvent and m is the molality of the electrolyte solution. The activity coefficient term occurring in eqn. 7 can then be written as:

$$\log\left[\frac{\gamma_T(NX)\gamma_B(N_2Y)}{\gamma_B(NX)\gamma_T(N_2Y)}\right] =$$

$$-z^2\left[\frac{A_T\sqrt{\omega_T(NX)}}{1+\beta_T\mathring{a}_i\sqrt{\omega_T(NX)}} - \frac{A_B\sqrt{\omega_B(NX)}}{1+\beta_B\mathring{a}_i\sqrt{\omega_B(NX)}} + \frac{A_B\sqrt{\omega_B(N_2Y)}}{1+\beta_B\mathring{a}_i\sqrt{\omega_B(N_2Y)}} - \frac{A_T\sqrt{\omega_T(N_2Y)}}{1+\beta_T\mathring{a}_i\sqrt{\omega_T(N_2Y)}}\right]$$

$$-\log\left\{\frac{[1 + 10^{-3}v_{NX}\overline{M}_Tm_T(NX)][1 + 10^{-3}v_{N_2Y}\overline{M}_Bm_B(N_2Y)]}{[1 + 10^{-3}v_{NX}\overline{M}_Bm_B(NX)][1 + 10^{-3}v_{N_2Y}\overline{M}_Tm_T(N_2Y)]}\right\}$$

$$- C_T(NX)\omega_T(NX) + C_B(NX)\omega_B(NX) - C_B(N_2Y)\omega_B(N_2Y) + C_T(N_2Y)\omega_T(N_2Y) \quad (A2)$$

The ionic strengths of the top and bottom layers are related to the overall ionic strength ω by

$$\omega_T(NX) = \omega\left[K'_{NX} + \frac{W_B}{W}(1 - K'_{NX})\right]^{-1}$$

$$\omega_B(NX) = \omega\left[1 + \frac{W_B}{W}\left(\frac{1}{K'_{NX}} - 1\right)\right]^{-1}$$

$$\omega_T(N_2Y) = \omega\left[K'_{N_2Y} + \frac{W_B}{W}(1 - K'_{N_2Y})\right]^{-1} \quad (A3)$$

$$\omega_B(N_2Y) = \omega\left[1 + \frac{W_B}{W}\left(\frac{1}{K'_{N_2Y}} - 1\right)\right]^{-1}$$

where W_B/W represents the weight fraction of the bottom layer. Analogous equations hold for the relationship between the molality in both layers and the overall molality m. Substitution in eqn. A2 gives an expression that can be written in the following simplified form:

$$\log\left[\frac{\gamma_T(NX)\gamma_B(N_2Y)}{\gamma_B(NX)\gamma_T(N_2Y)}\right] = -z^2\sqrt{\omega}f(\omega,\mathring{a}_i) - f'(m) + C'\omega \quad (A4)$$

where f and f' are known functional relationships and C' is an unknown constant.

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