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## Determination of the charge of ions by partition coefficient measurements in gel permeation chromatography

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### SUMMARY

A method is presented for determining the charge of ions from data on their gel permeation chromatographic partition coefficients with two eluents containing different electrolytes over a range of ionic strengths. The method was verified by measurements on Bio-Gel P-4 with simple inorganic ions of known charge ranging from  $-4$  to  $+2$ .

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### INTRODUCTION

In a recent paper<sup>1</sup> we proposed a method for the determination of the charge of ions from partition measurements in aqueous polymer two-phase systems, containing two different electrolytes. Briefly, the concept of this method is as follows. As the two phases of such a system have different polarities, the partition constant of any substance is not exactly unity. This is particularly true for (highly) charged ions, leading to partition constants of electrolytes (strongly) dependent on their chemical nature. Therefore, if a trace amount of an ion  $P^z$  is added to such a two-phase system containing an electrolyte  $NX$ , its partition coefficient can be expected to depend on  $K_{NX}$  and on its charge  $z$ , as electroneutrality must be maintained in both phases. From the combined results in the presence of two different electrolytes ( $NX$  and  $NY$ ), the charge  $z$  can be derived. The method was verified in sodium perchlorate and sodium sulphate containing polyethylene glycol–dextran systems with simple inorganic ions of known charge. It was argued that this method has distinct advantages over charge determination by ion-exchange chromatography, mainly because measurements can be performed at much lower ionic strengths, permitting activity coefficient corrections and extrapolation of the experimental data to zero ionic strength.

However, a few drawbacks of the method should be mentioned. As a result of the high viscosity of the two polymer layers, concentrations must be expressed as molalities ( $\text{mol kg}^{-1}$ ). Further, the calculation of activity coefficients requires assumptions about the dielectric constants of the two polymer layers.

Gel permeation chromatography can be considered as a special case of partition

in an aqueous polymer two-phase system: the gel particles and the eluent represent two phases of different polymer concentration and therefore different polarity. Hence, also in this case, when using an eluent containing an electrolyte NX, the transfer of an ion  $P^{z+}$  from the eluent to the gel phase must be accompanied by a simultaneous transfer of  $z$  ions  $X^-$  from the eluent to the gel phase (or of  $z$  ions  $N^+$  from the gel phase to the eluent). Therefore the partition coefficient of  $P^z$  depends on  $K_{NX}$  and  $z$ .

The analogy between the two methods was described in previous papers<sup>2,3</sup>. This means that gel permeation chromatographic partition experiments using two eluents containing different electrolytes can also be used for charge determination. It also means that the advantage mentioned above for the aqueous polymer two-phase partition method holds for the gel permeation chromatographic method. Moreover, the latter is simpler. First, as the partition coefficients are derived from elution volumes, a single universal detector can be used for all the ions investigated. Further, there is no need for the use of a molality concentration scale. Finally, if eluents of equal ionic strength are used, activity coefficient corrections are simpler and the dielectric constant of water can be used for the solvent in the gel phase.

In this work, the gel permeation chromatographic method was verified by experiments on Bio-Gel P-4, using sodium perchlorate and sodium sulphate as the eluent electrolytes, with simple inorganic ions of known charge ranging from  $-4$  to  $+2$ .

## THEORY

For the partition of a trace amount of an ion with charge  $z$ ,  $P^z$ , in an aqueous polymer two-phase system, containing the electrolytes NX and  $N_2Y$ , respectively, an equation was derived (eqn. 7 in ref. 1) showing that  $\Delta \log K_{P^z} \equiv \log [K_{P^z}(NX)/K_{P^z}(N_2Y)]$  and the charge  $z$  are proportionally related, where  $K_{P^z}$  represents the thermodynamic partition constant of the ion  $P^z$ .

For gel permeation chromatography, using the eluent electrolytes NX and  $N_2Y$ , this equation can be written as

$$\Delta \log K_{P^z} = \Delta \log K'_{P^z} + \log \left[ \frac{y_S(NX) y_M(N_2Y)}{y'_M(NX) y'_S(N_2Y)} \right] = z \Delta \log K_{N^+} \quad (1)$$

where the subscripts S and M denote the stationary and mobile phase, respectively,  $K'$  is the partition coefficient, *i.e.*, the ratio of the concentrations in the two phases ( $K' = c_S/c_M$ ),  $y$  is the activity coefficient of  $P^z$  on the molar scale and  $\Delta \log K'$  represents  $\log [K'(NX)/K'(N_2Y)]$ . For NX- and  $N_2Y$ -containing eluents of equal ionic strength, the activity coefficients of  $P^z$  in the two eluents can be taken as equal, *viz.*,  $y_M(NX) = y'_M(N_2Y)$ , which simplifies eqn. 1 to

$$\Delta \log K_{P^z} = \Delta \log K'_{P^z} + \log \left[ \frac{y_S(NX)}{y'_S(N_2Y)} \right] = z \Delta \log K_{N^+} \quad (2)$$

Eqn. 2 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. 2 will be verified.

The way in which corrections of  $\Delta \log K_p^+$  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  $\Delta \log K_p^+$  at three low levels of the ionic strength  $I$  of the eluent ( $I = 1/2 \sum_i z_i^2 c_i$ ), *viz.*, 0.30, 0.10 and 0.03 mol  $l^{-1}$ , and we extrapolated the corrected data to zero ionic strength. As shown in the Appendix, the activity coefficient term occurring in eqn. 2 can be written as

$$\log \left[ \frac{y_s(NX)}{y_s(N_2Y)} \right] = -z^2 A \sqrt{I} f(I, \hat{a}_i) - f'(c) + C'I \quad (3)$$

Substitution in eqn. 2 yields

$$\Delta \log K_p^+ = \Delta \log K_p^+ - z^2 A \sqrt{I} f(I, \hat{a}_i) - f'(c) + C'I \quad (4)$$

where  $f$  and  $f'$  are known functional relationships and  $C'$  is an unknown constant. This equation shows that linear extrapolation of  $[\Delta \log K_p^+ - z^2 A \sqrt{I} f(I, \hat{a}_i) - f'(c)]$  to  $I = 0$  yields  $\Delta \log K_p^+$ .

## EXPERIMENTAL

### *Chemicals and apparatus*

The following chemicals and materials were used: Bio-Gel P-4 (200–400 mesh) (Bio-Rad Labs., Richmond, CA, U.S.A.),  $NaClO_4 \cdot H_2O$  and  $Na_2SO_4$  (Analyzed, Baker, Deventer, The Netherlands),  $^{22}NaCl$  (Amersham, Little Chalfont, U.K.),  $^{99}Mo/^{99m}Tc$  generator (Mallinckrodt, Petten, The Netherlands), human serum albumin (Sigma, St. Louis, MO, U.S.A.) and  $K_4Mo(CN)_8$  (gift from the laboratory of inorganic chemistry, University of Utrecht, The Netherlands). All other chemicals [ $MgCl_2 \cdot 6H_2O$ , methanol,  $NaCl$ ,  $K_3Fe(CN)_6$  and  $K_4Fe(CN)_6 \cdot 3H_2O$ ] were of analytical-reagent grade.

The chromatographic equipment consisted of a column (C 10/40), flow adapter (AC10), laboratory valve (LV4), gel and eluent reservoir (RC 10), polyethylene tubing (Pharmacia, Uppsala, Sweden), peristaltic pump (Minipuls-2; Gilson, Villiers-le-Bel, France), differential refractometer (R 403; Waters Assoc., Milford, MA, U.S.A.) and a well-type  $NaI(Tl)$  scintillation crystal, HV supply and ratemeter (Baird Atomic, Bedford, MA, U.S.A.).

### *Sample preparation*

Sample solutions of  $MgCl_2$ , methanol,  $NaCl$ ,  $Na_2SO_4$ ,  $K_3Fe(CN)_6$ ,  $K_4Fe(CN)_6$  and  $K_4Mo(CN)_8$  were prepared by dissolution at a concentration of 3–5 mM in the eluents. Sample solutions of  $^{22}NaCl$  in the eluents were prepared by adding 50  $\mu l$  of a 1 mM solution of  $NaCl$ , spiked with  $^{22}NaCl$ , to 5 ml of eluent. Sample solutions of  $Na^{99m}TcO_4$  in the eluents were prepared by addition of 50  $\mu l$  of the generator eluate in

0.15 M NaCl to 5 ml of eluent. For human serum albumin, 0.2% solutions in the eluents were used.

### Chromatography

The pretreatment of Bio-Gel P-4 and the packing of the column (38 × 1.0 cm I.D.) were performed as recommended by the manufacturer. Aliquots of about 0.15 ml of the samples were applied and eluted at a flow-rate of 8.5–9.5 ml h<sup>-1</sup>. Flow-rates were accurately determined by weighing the column effluent. The eluents used were NaClO<sub>4</sub> and Na<sub>2</sub>SO<sub>4</sub> solutions of ionic strength 0.30, 0.10 and 0.03 mol l<sup>-1</sup>.

### Calculation of partition coefficients

Elution volumes were corrected for the extra-column dead space. Partition coefficients,  $K'_{P^z}$ , were calculated from

$$K'_{P^z} = \frac{V_e - V_0}{V_1 - V_0}$$

where  $V_e$  is the corrected elution volume of P<sup>z</sup>,  $V_0$  is the corrected elution volume of human serum albumin and  $V_1$  is the total liquid volume in the column, obtained from  $V_1 = V_{\text{bed}} - W/\rho$ , where  $V_{\text{bed}}$  is the bed volume,  $W$  is the weight of Bio-Gel P-4 used in packing the column and  $\rho$  is its density. For  $\rho$  an arbitrary value of 1.1 g ml<sup>-1</sup> was taken; this value results in a mean value of  $\Delta \log K'_{\text{CH}_3\text{OH}} = 0$ , averaged over four levels of the ionic strength ( $I = 0.005$  mol l<sup>-1</sup> was also used).

## RESULTS AND DISCUSSION

The experimental  $K'_{P^z}$  values are given in Table I. Note, that  $K'_{\text{SO}_4^{2-}}(\text{Na}_2\text{SO}_4)$ , measured by eluting a small excess of SO<sub>4</sub><sup>2-</sup> in Na<sub>2</sub>SO<sub>4</sub> eluents, is not equal to  $K'_{\text{Na}^+}(\text{Na}_2\text{SO}_4)$ , measured by eluting a small amount of <sup>22</sup>Na in Na<sub>2</sub>SO<sub>4</sub> eluents. This may be due to some ion-exchange behaviour of the gel; it is well known that Bio-Gel

TABLE I

PARTITION COEFFICIENTS,  $K'_{P^z}$ , OF DIFFERENT SPECIES IN THE PRESENCE OF NaClO<sub>4</sub> AND Na<sub>2</sub>SO<sub>4</sub> OF IONIC STRENGTH 0.3, 0.1 AND 0.03 mol l<sup>-1</sup>

Species	NaClO <sub>4</sub>			Na <sub>2</sub> SO <sub>4</sub>		
	0.3	0.1	0.03	0.3	0.1	0.03
Mg <sup>2+</sup>	1.962	2.191	2.301	1.044	1.211	1.428
Na <sup>+</sup>	1.321	1.382	1.553	1.016	1.069	1.235
MeOH	1.016	0.951	1.014	0.979	1.018	0.998
TcO <sub>4</sub> <sup>-</sup>	1.903	1.934	2.037	2.712	2.668	2.503
Cl <sup>-</sup>	0.942	0.916	0.935	1.194	1.158	1.120
SO <sub>4</sub> <sup>2-</sup>	0.694	0.663	0.657	0.928	1.018	1.029
Fe(CN) <sub>6</sub> <sup>3-</sup>	0.861	1.032	1.037	1.889	1.913	1.968
Fe(CN) <sub>6</sub> <sup>4-</sup>	0.621	0.724	0.776	1.415	1.529	1.725
Mo(CN) <sub>6</sub> <sup>4-</sup>	0.933	1.149	1.351	2.255	2.793	2.933

contains some carboxylate groups that are negatively charged at neutral pH. This leads to a slight increase in  $K'_{\text{Na}^+}(\text{Na}_2\text{SO}_4)$  by ion-exchange and to a slight decrease in  $K'_{\text{SO}_4^{2-}}(\text{Na}_2\text{SO}_4)$  by ion exclusion (measurements at  $I = 0.005 \text{ mol l}^{-1}$  showed these effects to a large extent and are therefore not reported). A second effect that may be responsible for this discrepancy arises if the sorption isotherm of the eluent electrolyte is not linear. In that case, the measurement of the elution volume of a slight concentration disturbance does not yield  $K'_{\text{SO}_4^{2-}} = (c_{\text{SO}_4^{2-}})_s / (c_{\text{SO}_4^{2-}})_M = (c_{\text{Na}^+})_s / (c_{\text{Na}^+})_M$  but the derivative of the sorption isotherm,  $K'_{\text{SO}_4^{2-}} = d(c_{\text{SO}_4^{2-}})_s / d(c_{\text{SO}_4^{2-}})_M = d(c_{\text{Na}^+})_s / d(c_{\text{Na}^+})_M$ . On the other hand, the measurement of the elution volume of a  $^{22}\text{Na}^+$  spike does yield  $K'_{\text{Na}^+} = (c_{\text{Na}^+})_s / (c_{\text{Na}^+})_M$ . Obviously  $K'^* \neq K'$  if the sorption isotherm is not linear. This effect has been clearly demonstrated<sup>4</sup> in gel chromatographic results on Sephadex G-10.

From the data in Table I, values of  $\Delta \log K'_{\text{P}^z} = \log [K'_{\text{P}^z}(\text{NaClO}_4) / K'_{\text{P}^z}(\text{Na}_2\text{SO}_4)]$  were calculated. For the ions the latter data were corrected for the terms  $z^2 A \sqrt{I} f(I, \hat{a}_i)$  and  $f'(c)$  occurring in eqn. 4. The correction terms are given in Table II for several values of  $z$  at the three levels of  $I$  considered. They were calculated using  $\hat{a}_i = 5 \text{ \AA}$ ,  $A = 0.506$  and  $\beta = 0.329$  (the values<sup>5</sup> of the Debye-Hückel constants of water at 25°C), values of  $K'_{\text{NX}}$  and  $K'_{\text{NY}}$  from Table I [ $K'_{\text{NX}} = K'_{\text{Na}^+}(\text{NaClO}_4)$  and  $K'_{\text{NY}} = K'_{\text{Na}^+}(\text{Na}_2\text{SO}_4)$ ],  $v_{\text{NX}} = 2$ ,  $v_{\text{NY}} = 3$  and values of  $d$  from ref. 6.

The results obtained after applying these corrections are given in Table III, together with the extrapolated data, representing  $\Delta \log K'_{\text{P}^z}$  values. In Fig. 1 a plot of  $\Delta \log K'_{\text{P}^z}$  versus  $z$  is shown. It is seen that the linear relationship predicted by eqn. 2 is obtained. Least-squares fitting of the data to the relationship  $\Delta \log K'_{\text{P}^z} = a + bz$  yields  $a = -0.010 \pm 0.006$  and  $b = 0.106 \pm 0.002$ . The line thus passes through the origin within the 90% confidence interval of the intercept ( $a = -0.010 \pm 0.011$ ). The slope,  $b = 0.106 \pm 0.002$  (7 degrees of freedom), is not significantly different from the theoretical value,  $\Delta \log K'_{\text{Na}^+} = 0.095 \pm 0.005$  (1 degree of freedom) (see Table III). The standard deviation of the experimental points to the calibration line,  $s(\Delta \log K'_{\text{P}^z}) = 0.0148$ , is even smaller than the pooled standard deviation of the data in the last column of Table III,  $s_p = 0.0205$ . Therefore, the expression  $\Delta \log K'_{\text{P}^z} = 0.106 z - 0.010$  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 IV (first column) gives the deviations from the real charge, calculated from the data in the last column of Table III and the relationship  $\Delta \log K'_{\text{P}^z} = 0.106 z - 0.010$ , for the ions investigated. For comparison, deviations from

TABLE II

VALUES OF  $z^2 A \sqrt{I} f(I, \hat{a}_i)$  FOR VARIABLE  $I$  AND  $z$  AT  $\hat{a}_i = 5 \text{ \AA}$  AND VALUES OF  $f'(c)$  FOR VARIABLE  $I$

$I \text{ (mol l}^{-1}\text{)}$	$z^2 A \sqrt{I} f(I, \hat{a}_i = 5 \text{ \AA})$				$f'(c)$
	$z = \pm 1$	$z = \pm 2$	$z = \pm 3$	$z = \pm 4$	
0.3	0.013	0.052	0.118	0.210	0.024
0.1	0.009	0.037	0.083	0.147	0.008
0.03	0.007	0.027	0.060	0.107	0.003

TABLE III

VALUES OF [ $\Delta \log K_{pz} - z^2 A \sqrt{I} f(I, \bar{a}_i = 5 \text{ \AA}) - f(c)$ ] OF DIFFERENT SPECIES  $P^z$  AT VARYING IONIC STRENGTH, VALUES OF  $\Delta \log K_{pz}$ , OBTAINED BY EXTRAPOLATION *VERSUS*  $I$  AT  $I = 0$  AND STANDARD DEVIATIONS OF THE EXTRAPOLATED VALUES

Species	$I \text{ (mol l}^{-1}\text{)}$			
	0.3	0.1	0.03	$\rightarrow 0$
$\text{Mg}^{2+}$	0.198	0.212	0.177	$0.189 \pm 0.022$
$\text{Na}^+$	0.077	0.094	0.090	$0.095 \pm 0.005$
MeOH	0.016	-0.030	0.007	$-0.013 \pm 0.029$
$\text{TcO}_4^-$	-0.191	-0.157	-0.099	$-0.106 \pm 0.025$
$\text{Cl}^-$	-0.140	-0.119	-0.088	$-0.091 \pm 0.013$
$\text{SO}_4^{2-}$	-0.202	-0.231	-0.225	$-0.233 \pm 0.009$
$\text{Fe}(\text{CN})_6^{3-}$	-0.483	-0.359	-0.341	$-0.316 \pm 0.014$
$\text{Fe}(\text{CN})_6^{4-}$	-0.592	-0.480	-0.457	$-0.436 \pm 0.009$
$\text{Mo}(\text{CN})_8^{4-}$	-0.617	-0.541	-0.447	$-0.453 \pm 0.036$

the real charge found for some ions by the aqueous polymer two-phase partition method<sup>1</sup> and by ion-exchange chromatography<sup>1,7</sup> are also given in Table IV.

We conclude from the data in Table IV that the precision of this gel permeation chromatographic method is almost identical with that of the method based on partition in an aqueous polymer two-phase system and is much better than that attainable by ion-exchange procedures. The gel permeation chromatographic method is the method of choice in cases where the charge of individual components of an ionic

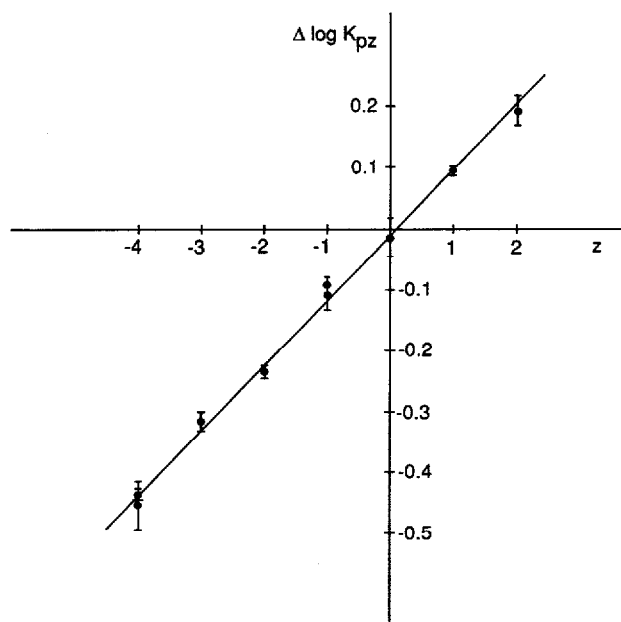


Fig. 1.  $\Delta \log K_{pz}$  versus  $z$ . Data from Table III. Regression line:  $\Delta \log K_{pz} = 0.106 z - 0.010$ .

TABLE IV

DEVIATIONS FROM THE REAL CHARGE FOR SOME IONS FOUND BY GEL PERMEATION CHROMATOGRAPHY (THIS WORK), BY AQUEOUS POLYMER TWO-PHASE PARTITION<sup>1</sup> AND BY ION-EXCHANGE CHROMATOGRAPHY<sup>1,7</sup>

Ion	Gel permeation chromatography	Aqueous polymer two-phase partition	Ion-exchange chromatography	
			DEAE-Trisacryl	Aminex 28
Mg <sup>2+</sup>	-0.12	0.00		
Na <sup>+</sup>	-0.01	+0.11		
TcO <sub>4</sub> <sup>-</sup>	+0.09	-0.08		
Cl <sup>-</sup>	+0.24		+0.37	
SO <sub>4</sub> <sup>2-</sup>	-0.10	-0.11	+0.60	-0.20
Fe(CN) <sub>6</sub> <sup>3-</sup>	+0.11	-0.12	+1.80	
Fe(CN) <sub>6</sub> <sup>4-</sup>	-0.02	+0.18		
Mo(CN) <sub>8</sub> <sup>4-</sup>	-0.18			

mixture has to be determined. In fact, by this method the charge of each separable component can be determined, whereas only the mean charge is accessible by the aqueous polymer two-phase partition method.

From the standard deviation of the experimental points to the calibration line,  $s(\Delta \log K_{P^z}) = 0.0148$  and the values of the slope  $b$  and its standard deviation  $s_b$  ( $b = 0.106 \pm 0.002$ ), the standard deviation  $s_z$  of a charge  $z$ , to be determined by the proposed method, can be estimated using<sup>8</sup>

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

where  $n$  is the number of experimental data points and  $p$  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  $n = 9$ ,  $p = 2$  and  $\bar{z} = -1.33$ ) are given in Table V.

TABLE V

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

$z$	-4	-3	-2	-1	0	+1	+2
$s_z$	0.18	0.17	0.17	0.17	0.17	0.18	0.18

## APPENDIX

The activity coefficient  $y_{P^z}$  of an ion  $P^z$ , present in a trace amount in an electrolyte solution of ionic strength  $I$  is given by the extended Debye-Hückel equation<sup>5</sup> (see also ref. 1)

$$-\log y_{P^z} = \frac{z^2 A \sqrt{I}}{1 + \beta a_i \sqrt{I}} + \log \left[ \frac{d + 10^{-3} c(vM - M_{\text{soln.}})}{d^0} \right] + CI \quad (\text{A1})$$

where  $A$ ,  $\beta$  and  $C$  are constants,  $\hat{a}_i$  is the distance of closest approach of the electrolyte ions to the ion  $P^z$ ,  $d$  and  $d^0$  are the densities of the solution and the solvent, respectively,  $c$  is the concentration of the electrolyte,  $\nu$  is the number of ions into which one molecule of electrolyte dissociates and  $M$  and  $M_{\text{solv.}}$  are the molecular weights of the electrolyte and the solvent, respectively. The activity coefficient term, occurring in eqn. 2, can then be written as

$$\log \left[ \frac{y_s(\text{NX})}{y_s(\text{N}_2\text{Y})} \right] = -z^2 A \left[ \frac{\sqrt{I_s(\text{NX})}}{1 + \beta \hat{a}_i \sqrt{I_s(\text{NX})}} - \frac{\sqrt{I_s(\text{N}_2\text{Y})}}{1 + \beta \hat{a}_i \sqrt{I_s(\text{N}_2\text{Y})}} \right] - \log \left( \frac{[d + 10^{-3} c_s(\nu M - M_{\text{solv.}})]_{\text{NX}}}{[d + 10^{-3} c_s(\nu M - M_{\text{solv.}})]_{\text{N}_2\text{Y}}} \right) - C_s(\text{NX}) I_s(\text{NX}) + C_s(\text{N}_2\text{Y}) I_s(\text{N}_2\text{Y}) \quad (\text{A2})$$

The ionic strength of the stationary phase,  $I_s$ , and the concentration of the electrolyte in the stationary phase,  $c_s$ , are related to those in the mobile phase,  $I$  and  $c$ , by

$$\frac{I_s(\text{NX})}{I} = \frac{c_s(\text{NX})}{c} = K'_{\text{NX}} \quad (\text{A3})$$

$$\frac{I_s(\text{N}_2\text{Y})}{I} = \frac{c_s(\text{N}_2\text{Y})}{c} = K'_{\text{N}_2\text{Y}}$$

Substitution in eqn. A2 gives an expression that can be written in the following simplified form

$$\log \left[ \frac{y_s(\text{NX})}{y_s(\text{N}_2\text{Y})} \right] = -z^2 A \sqrt{I} f(I, \hat{a}_i) - f'(c) + C'I \quad (\text{A4})$$

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

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