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Dependence of retention of ionic solutes on the composition of the mobile phase electrolytes in partition chromatography

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ABSTRACT

On the basis of the model for partition chromatography of ionic solutes in the presence of background electrolytes, theoretical equations are developed to describe the retention dependence of ionic solutes on the composition of electrolytes in the mobile phase. The theoretical equations were tested by calculating the expected capacity factors of various inorganic ions in the presence of two or three background electrolytes using experimental values obtained in a single electrolyte system. The predictions from the equations agreed well with experimental retention data on hydrophilic polymer packings for both binary (acetone–water) and single solvent (water) systems.

INTRODUCTION

In any mode of liquid chromatography, it is essential to use an electrolyte solution as the mobile phase for elution of ionic solutes. In both partition chromatography and size-exclusion chromatography, an electrolyte is added to the mobile phase in order to eliminate ion exclusion and ion-exchange adsorption effects due to the low concentration of some fixed charges on the column packings.

The presence of a background of eluting electrolyte allows an analyte ion to move independently of the counter ion which is a constituent of the analyte salt injected. However, the presence of background electrolyte does not imply that the partitioning of an analyte ion is independent of its counter ion. The retention of the analyte ion depends strongly on the type of

counter ion and co-ion in the mobile phase [1–10].

Shibukawa and Ohta [9] presented a simple model for partition chromatography of ionic solutes in the presence of background electrolyte and showed that the effect of various types of electrolytes on the retention of inorganic ions on non-ionic porous polymers, when water is used as the mobile phase solvent, can well be interpreted by the proposed model. This ion partition model was also used successfully to account for the effect of type of the background ions in binary solvent systems [10].

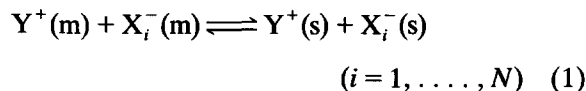
In this study, the ion partition model was used to describe the effect of the composition of uni-univalent electrolytes in the mobile phase on the retention of inorganic ions. A set of equations was derived to predict the capacity factors for cations and anions. Predictions from the theory

were compared with experimental data obtained on hydrophilic porous polymers with acetone–water or water alone and it is shown that the equations can be used to predict the retention of analyte ions.

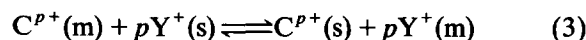
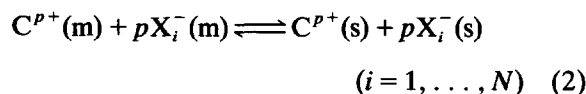
THEORETICAL

Let us consider the equilibrium distribution of an analyte cation, C^{p+} , between the mobile and stationary phases in the presence of N background univalent anions, X_1^-, \dots, X_N^- and a common cation, Y^+ , based on the ion partition model [9]. The basic assumption of the ion partition model is that the association of an analyte ion with counter ions is negligible in both the mobile and stationary phases [9].

If the number of ionic groups in the stationary phase is so small that their electrostatic effect on the retention of the analyte ion is suppressed by the background electrolyte, the distribution equilibria of Y^+ and X_i^- ($i = 1, \dots, N$) are expressed as



where m and s denote the mobile and stationary phase, respectively. The distribution of C^{p+} can be expressed by either a set of N equilibria given by eqn. 2 or one “ion-exchange” equilibrium represented by eqn. 3 [9]:



If the ionic activity coefficient can be regarded as being approximately constant, we may use concentrations in place of activities to write the equilibrium constants. The molar equilibrium constants for the equilibria 1, 2 and 3 are given by eqns. 4, 5 and 6, respectively:

$$K_{YX_i} = \frac{[Y^+]_s [X_i^-]_s}{[Y^+]_m [X_i^-]_m} \quad (i = 1, \dots, N) \quad (4)$$

$$K_{CX_i} = \frac{[C^{p+}]_s [X_i^-]_s^p}{[C^{p+}]_m [X_i^-]_m^p} \quad (i = 1, \dots, N) \quad (5)$$

$$K_{C/Y} = \frac{[C^{p+}]_s [Y^+]_m^p}{[C^{p+}]_m [Y^+]_s^p} \quad (6)$$

The ionic activity coefficient in dilute solution ($\leq 0.1 M$) depends only on the total ionic strength of the solution and is independent of the type and concentration of the coexisting ions [11]. It is not difficult to maintain the ionic strength of the mobile phase constant, whereas that of the stationary phase depends on the distribution constant of the background electrolyte. However, it is reasonable to assume that the ionic activity coefficient in both the mobile and stationary phases is approximately unchanged if there is no extreme difference in the distribution constant of the background electrolyte; the constancy of the ionic activity coefficient was discussed in a previous paper [4].

It should be noted that K_{YX_i} , K_{CX_i} and $K_{C/Y}$ are not independent of one another [9]. The distribution of C^{p+} can be described by using either a combination of eqns. 4 and 5 or a combination of eqns. 5 and 6.

In most cases, the analyte ion is present in both the mobile and stationary phases at concentrations much lower than those of other background ions. Hence we have the equations of charge balance for the mobile phase and the stationary phase as follows, respectively:

$$[Y^+]_m = [X_1^-]_m + [X_2^-]_m + \dots + [X_N^-]_m \quad (7)$$

$$[Y^+]_s = [X_1^-]_s + [X_2^-]_s + \dots + [X_N^-]_s \quad (8)$$

From eqns. 4, 7 and 8, we obtain

$$\frac{[Y^+]_s}{[Y^+]_m} = \left(\frac{\sum K_{YX_i} [X_i^-]_m}{\sum [X_i^-]_m} \right)^{1/2} \quad (9)$$

The capacity factor, $k'_C{}^{YX_i}$, of the analyte ion, C^{p+} , when eluted with a single electrolyte, YX_i , is given by [9]

$$k'_C{}^{YX_i} = \phi \cdot \frac{[C^{p+}]_s}{[C^{p+}]_m} = \phi K_{C/Y} K_{YX_i}^{p/2} \quad (10)$$

where ϕ is the phase ratio. Combining eqn. 10

with eqns. 6 and 9 gives the following equation for the capacity factor of C^{P+} in the mixed background ion system:

$$k'_C{}^{Y(X_1+\dots+X_N)} = \phi K_{C/Y} \frac{[Y^+]_s^p}{[Y^+]_m^p} = \left\{ \frac{\sum (k'_C{}^{YX_i})^{2/p} [X_i^-]_m}{\sum [X_i^-]_m} \right\}^{p/2} \quad (11)$$

The equation for the capacity factor of an anion, A^{P-} , is derived in a similar manner to that described for that of C^{P+} . The capacity factor, $k'_A{}^{YX_i}$ of A^{P-} is expressed as

$$k'_A{}^{YX_i} = \phi \cdot \frac{[A^{P-}]_s}{[A^{P-}]_m} = \phi \cdot \frac{K_{YA}}{K_{YX}} \quad (12)$$

where

$$K_{YA} = \frac{[Y^+]_s^p [A^{P-}]_s}{[Y^+]_m^p [A^{P-}]_m} \quad (13)$$

By combining eqns. 9, 12 and 13, we obtain the following equation, which represents the dependence of the capacity factor of A^{P-} on the composition of the background co-ions:

$$k'_A{}^{Y(X_1+\dots+X_N)} = \phi K_{YA} \cdot \frac{[Y^+]_m^p}{[Y^+]_s^p} = \left\{ \frac{\sum [X_i^-]_m}{\sum \frac{[X_i^-]_m}{(k'_A{}^{YX_i})^{2/p}}} \right\}^{p/2} \quad (14)$$

In order to calculate the capacity factor of an analyte ion in a mixed background electrolyte system, $k'_C{}^{Y(X_1+\dots+X_N)}$ or $k'_A{}^{Y(X_1+\dots+X_N)}$, according to eqn. 11 or 14, one must obtain the capacity factors in all of the individual electrolyte solutions which constitute the mixed electrolytes, namely $k'_C{}^{YX_1}, \dots, k'_C{}^{YX_N}$ or $k'_A{}^{YX_1}, \dots, k'_A{}^{YX_N}$. However, it is not necessary to determine all of them experimentally. The capacity factor of the analyte ion, C^{P+} , in the electrolyte system YX_i can be calculated from the following equation by using the capacity factors of C^{P+} , X_i^- and X_j^- determined in the electrolyte system YX_j [9]:

$$k'_C{}^{YX_i} = k'_C{}^{YX_j} \left(\frac{k'_{X_i}{}^{YX_j}}{k'_{X_j}{}^{YX_j}} \right)^{p/2} \quad (15)$$

The capacity factor of the anionic analyte, A^{P-} , is calculated as

$$k'_A{}^{YX_i} = k'_A{}^{YX_j} \left(\frac{k'_{X_i}{}^{YX_j}}{k'_{X_j}{}^{YX_j}} \right)^{p/2} \quad (16)$$

EXPERIMENTAL

Materials

All chemicals used in this study were of analytical-reagent grade unless indicated otherwise.

HPLC-grade acetone was obtained from Kanto (Tokyo, Japan). Water purified through a Millipore Milli-Q water purification system was used throughout.

The column packings used were a cross-linked dextran gel, Sephadex G-25 (10–40 μ m), purchased from Pharmacia (Uppsala, Sweden), and a cross-linked polyacrylamide gel, Bio-Gel P-2 (200–400 mesh), from Bio-Rad Labs. (Richmond, CA, USA). These hydrophilic polymer gels were washed with water, ethanol and acetone in that order and then dried at 90°C before use.

Chromatographic conditions

The pump was a Kyowa Seimitsu (Tokyo, Japan) Model KHP-010 solvent-delivery system. The eluent reservoir was a commercially available glass syringe with a 200-ml capacity [12]. Inorganic anions were detected with a Kyowa Seimitsu Model KLC-800 UV-visible variable-wavelength absorption detector or a Tosoh (Tokyo, Japan) Model CM-8 conductivity detector. A Perkin-Elmer (Norwalk, CT, USA) Model 3100 atomic absorption spectrometer was used for the detection of alkali and alkaline earth metal ions in a similar manner to that described in the literature [13].

The column packings were allowed to swell for 24 h in a large excess of the eluent solvent to be used, the solvent being decanted several times. The swollen gels were slurry packed into a 300 \times 4 mm I.D. stainless-steel column. The column

was thermostated at $25.0 \pm 0.1^\circ\text{C}$ through a column jacket, using a Yamato (Tokyo, Japan) Model BH-71 constant-temperature circulator.

Eluents were water or acetone–water (70:30, v/v) containing sodium salts. Sample solutions were prepared by dissolving salts of the analyte ions in solutions whose compositions were the same as those of the eluents used.

Portions of $10 \mu\text{l}$ of the sample solutions (1–5 mM) were injected into the column with a Kyowa Seimitsu Model KHP-UI-130A injection valve. Elutions were carried out at a constant flow-rate of ca. 0.8 ml/min. Exact values of the volumetric flow-rate were measured using a burette designed to prevent vaporization of the solvent.

The mobile phase volume was determined according to the method proposed by Shibukawa and Ohta [14].

RESULTS AND DISCUSSION

Sephadex G-25/acetone–water system

In order to test eqns. 11 and 14, Sephadex G-25 equilibrated with acetone–water (70:30, v/v) was chosen as a partition chromatographic system, since Sephadex G-25 preferentially takes up water when it is brought into contact with a mixture of water and acetone and then forms a typical normal-phase partition system [10].

Capacity factors of inorganic ions were determined by elution with acetone–water (70:30, v/v) containing NaCl, NaNO_3 or NaClO_4 or two or three of them. The ionic strength of the eluents was kept constant at 0.01 M; ion exclusion and ion-exchange effects of fixed ionic groups of the matrix of Sephadex G-25 are almost negligible in these media [15].

Fig. 1 shows the capacity factor vs. background electrolyte composition data for the NaNO_3 – NaClO_4 system. The solid lines represent the k' values calculated from eqn. 11 (for cations) or eqn. 14 (for anions) using only the experimental k' data obtained in NaNO_3 solution. The k' values of the analyte ions in the NaClO_4 system were calculated from eqn. 15 or 16 using the k' values of the analyte ions, NO_3^- and ClO_4^- , determined in the NaNO_3 system. For example, the capacity factor of K^+ in the

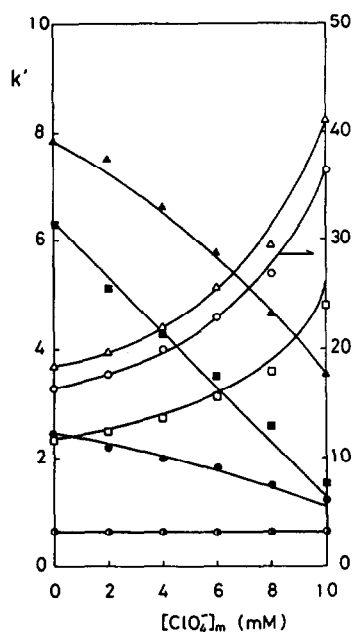


Fig. 1. Capacity factors of inorganic ions on Sephadex G-25 as a function of concentration of ClO_4^- in acetone–water (70:30, v/v) containing NaNO_3 and NaClO_4 . The solid lines were calculated for the cations and anions according to eqns. 11 and 14, respectively. $[\text{Na}^+]_m = [\text{NO}_3^-]_m + [\text{ClO}_4^-]_m = 10 \text{ mM}$. $\bullet = \text{Li}^+$; $\blacktriangle = \text{K}^+$; $\blacksquare = \text{Mg}^{2+}$; $\circ = \text{Cl}^-$; $\triangle = \text{I}^-$; $\square = \text{SCN}^-$; $\blacklozenge = \text{nitrobenzene}$.

NaClO_4 system, $k'_K{}^{\text{NaClO}_4}$, is obtained by substituting $k'_K{}^{\text{NaNO}_3} = 7.87$, $k'_{\text{ClO}_4}{}^{\text{NaNO}_3} = 1.06$ and $k'_{\text{NO}_3}{}^{\text{NaNO}_3} = 5.31$ into eqn. 15 as follows:

$$k'_K{}^{\text{NaClO}_4} = 7.87 \cdot \left(\frac{1.06}{5.31} \right)^{1/2} = 3.52 \quad (17)$$

The calculated k' values in NaClO_4 and NaCl systems obtained with eqn. 15 or 16 are listed in Table I together with the experimental values.

The capacity factor of NO_3^- in the NaNO_3 system was assumed to be the same as that of the system peak observed when NaNO_3 was injected. This assumption is reasonable if ion exclusion and ion-exchange effects exerted by the fixed ionic groups of the polymer matrix are negligibly small [16]. The details of the elution behaviour of system peaks in mobile phase systems containing more than one electrolyte will be reported in a subsequent publication.

It is seen from Fig. 1 that the calculated k' values are in remarkably good agreement with

TABLE I

CALCULATED AND EXPERIMENTAL CAPACITY FACTORS OF INORGANIC IONS ON SEPHADEX G-25 WITH ACETONE–WATER (70:30, v/v)

Background electrolyte	Value	Analyte ion							
		Li ⁺	K ⁺	Mg ²⁺	ClO ₄ ⁻	SCN ⁻	I ⁻	NO ₃ ⁻	Cl ⁻
NaNO ₃	Exptl.	2.44	7.87	6.29	1.06	2.36	3.69	5.31 ^a	16.56
NaClO ₄	Exptl.	1.23	3.55	1.67	2.15 ^a	4.80	8.22	11.83	36.48
	Calcd.	1.09	3.52	1.26	2.37	5.28	8.26	11.88	37.06
NaCl	Exptl.	4.59	15.45	17.80	0.83	1.62	2.54	3.88	12.02 ^a
	Calcd.	4.31	13.89	19.61	0.60	1.34	2.09	3.01	9.38

^a System peak.

the experimental values for both cations and anions. The k' value of nitrobenzene, which is a non-ionic solute, is independent of the composition of the mobile phase anions.

In Fig. 2, the experimental and theoretically predicted k' values are compared for NaCl–NaNO₃–NaClO₄ ternary mixtures, where concentrations of Na⁺ and NO₃⁻ are kept constant at 10 and 2 mM, respectively. Again, theoretical k'

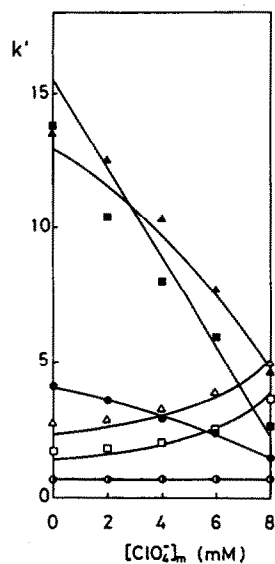


Fig. 2. Capacity factors of inorganic ions on Sephadex G-25 as a function of concentration of ClO₄⁻ in acetone–water (70:30, v/v) containing NaNO₃, NaCl and NaClO₄. [NO₃⁻]_m = 2 mM; [Na⁺]_m = [NO₃⁻]_m + [Cl⁻]_m + [ClO₄⁻]_m = 10 mM. ● = Li⁺; ▲ = K⁺; ■ = Mg²⁺; △ = I⁻; □ = SCN⁻; ○ = nitrobenzene. For other details, see Fig. 1.

values were calculated from eqn. 11 or 14 using only the experimental k' data obtained in NaNO₃ solution. Fig. 2 shows that there is fairly good agreement between the experimental and predicted k' values. The larger deviation between the predicted and experimental points for Mg²⁺ may be attributed to the possible ion association of Mg²⁺ with Cl⁻.

The results shown in Figs. 1 and 2 indicate not only the validity of the theoretical approach in this paper but also the practical usefulness of the proposed equations for calculating capacity factors of ionic solutes from a very limited amount of experimental data.

Sephadex G-25/water and Bio-Gel P-2/water systems

As a second test of the validity of eqns. 11 and 14, the retention data of inorganic ions on Sephadex G-25 and Bio-Gel P-2 obtained when eluted with aqueous solutions of NaCl and NaClO₄ were used.

It is well known that water molecules sorbed in hydrophilic polymer gels generally exhibit physical properties distinct from those of ordinary free water by the interaction with polar groups and/or the hydrophobic part of the polymer matrices [17–21]. The retention of a solute compound on a water-swollen non-ionic polymer gel can be regarded as being governed by the partitioning of the compound between the external bulk water and the water in polymer

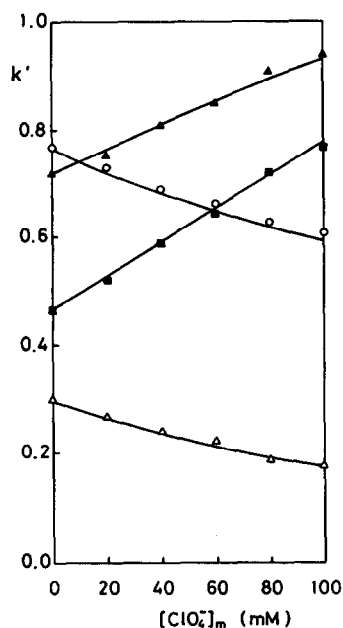


Fig. 3. Capacity factors of inorganic ions on Sephadex G-25 as a function of concentration of ClO_4^- in aqueous solution containing NaCl and NaClO_4 . $[\text{Na}^+]_m = [\text{Cl}^-]_m + [\text{ClO}_4^-]_m = 100 \text{ mM}$. $\blacktriangle = \text{K}^+$; $\blacksquare = \text{Mg}^{2+}$; $\circ = \text{NO}_3^-$; $\triangle = \text{SO}_4^{2-}$. For other details, see Fig. 1.

gels, which has properties different from those of ordinary bulk water [14,21].

Figs. 3 and 4 show the capacity factors of some inorganic ions on Sephadex G-25 and Bio-Gel P-2 as a function of the concentration of ClO_4^- in the mobile phase containing NaCl and NaClO_4 ; the total concentration of Cl^- and ClO_4^- , that is, the concentration of Na^+ , was kept constant at 0.1 M. The theoretical k' values (solid lines) were calculated from eqn. 11 or 14 using the experimental k' data obtained in the NaCl eluent system; $k'_{\text{Cl}^-}^{\text{NaCl}}$ was assumed to be the same as k' of the system peak observed when NaCl was injected into the column equilibrated with 0.1 M NaCl aqueous solution. As can be seen from Figs. 3 and 4, the calculated k' values are in good agreement with the experimental values. It should be noted that only divalent cations show the linear relationship between k' and the concentration of ClO_4^- , as expected from eqn. 11.

The results described above support the generality and practical applicability of the ion partition model to both single solvent systems

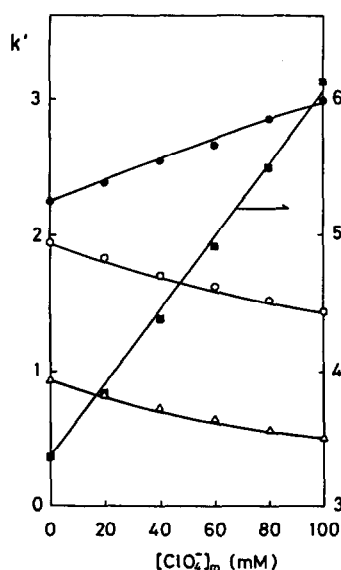


Fig. 4. Capacity factors of inorganic ions on Bio-Gel P-2 as a function of concentration of ClO_4^- in aqueous solution containing NaCl and NaClO_4 . $[\text{Na}^+]_m = [\text{Cl}^-]_m + [\text{ClO}_4^-]_m = 100 \text{ mM}$. $\bullet = \text{Li}^+$; $\blacksquare = \text{Mg}^{2+}$; $\circ = \text{NO}_3^-$; $\triangle = \text{SO}_4^{2-}$. For other details, see Fig. 1.

and binary solvent systems. Although this work was carried out only on hydrophilic polymer gels, the argument presented here can be applied to the results obtained with other non-ionic packings as the model does not depend on a specific retention mechanism.

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