Theoretical interpretation of the retention of system peaks in partition chromatography with a mobile phase containing electrolytes

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Abstract

Theoretical equations were developed to describe the retention dependence of system peaks generated in partition chromatography with a mobile phase containing electrolytes on the composition of the mobile phase electrolytes, on the basis of the model regarding the partitioning of ionic solutes and Knox and Kaliszan's general equations for the retention of system peaks. The predictions from the equations agreed well with experimental results obtained on hydrophilic polymer packings for both water and acetone–water mobile phase systems containing two or three background electrolytes. This means that the retention of system peaks can be predicted even for three or more mobile phase component systems, once the dependence of the distribution of each mobile phase component between the mobile and stationary phases on the composition of the mobile phase has been elucidated.

1. Introduction

The use of multi-component mixtures as mobile phases leads to the formation of extra peaks that do not correspond to any component of the separated sample. These extraneous peaks are generally called systems peaks. System peaks can be visualized if at least one of the mobile phase components can be detected. Therefore, the system peaks can cause several chromatographic problems such as co-elution with solute peaks and incorrect assignment of peak identities [1].

Many investigators have examined various aspects of system peaks [1–25] and it is known that system peaks appear in the chromatograms due to the perturbation of the equilibrium composition of the mobile phase components caused by sample introduction. The equilibrium disturbances that occur when a sample solute is injected was extensively treated by Helfferich and Klein [26]. More recently, Knox and Kaliszan [27] developed a general theory on the generation of system peaks and presented a useful equation for interpreting the retention of the system peaks. The equations presented are expected to be used for the prediction of the capacity factors of the system peaks. However, the theory appears to have been substantiated by experiments only for two-component systems and not yet for three- or more-component systems.

In order to calculate the capacity factors of the system peaks, one would need to know all of the relevant sorption isotherms of the mobile phase

components. Although isotopically labelled mobile phase components could be used for this purpose, it is tedious, especially for systems of three or more components, to obtain the isotherms from the retention volumes of the labelled components.

Another approach is to use a system where the dependence of the retention of the mobile phase components on the mobile phase composition has been clarified. We have shown that the effects of the background electrolytes in the mobile phase on the retention of ionic solutes on non-ionic polymer gels with water [28] or aqueous acetone [29] can be well interpreted by the stoichiometric model presented regarding the partitioning of ionic solutes. This ion partition model was also used successfully to account for the retention dependence of ionic solutes on the composition of the background electrolytes in these systems [30]. It was felt that this partition chromatographic system should provide an opportunity not only to demonstrate the practical validity of Knox and Kaliszan's theory but also to clarify the effect of the composition of background electrolytes in the mobile phase on the retention of the system peaks. It will be shown in this paper that the dependence of the capacity factors of the system peaks on hydrophilic porous polymers with acetone–water or water alone for three- or more-component systems can indeed be interpreted on the basis of the theoretical equations derived from Knox and Kaliszan's equation and the ion partition model.

2. Experimental

2.1. Materials

All chemicals 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 μm), 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 polymer gels were washed with water, ethanol and acetone in that order and dried at 90°C before use.

2.2. Chromatographic conditions

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 250 × 4 mm I.D. stainless-steel column. The column was thermostated at 25.0 ± 0.1°C through a column jacket, using a Yamato (Tokyo, Japan) Model BH-71 constant-temperature circulator.

The pump was a Kyowa Seimitsu (Tokyo, Japan) KHP-010 solvent-delivery system. The eluent reservoir was a commercially available glass syringe of 200-ml capacity [31]. Mobile phases were water or acetone–water (70:30, v/v) containing sodium salts. 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 solvent. Portions of 5 μl of the sample solutions were injected into the column with a Kyowa Seimitsu KHP-UI-130A injection valve. The system peaks were monitored with a Tosoh (Tokyo, Japan) CM-8 conductivity detector and an Erma (Tokyo, Japan) ERC-7510 differential refractometer.

The mobile phase volume was determined according to the method proposed by Shibukawa and Ohta [32].

3. Results and discussion

3.1. Theoretical background

If the equilibrium composition of a mobile phase containing $N + 1$ components is perturbed by sample introduction, N system peaks will be generated. Knox and Kaliszan [27] showed that the relative velocities, R, of the system peaks for an $(N + 1)$-component mobile phase system are given by N roots of the following equation:

$$\begin{vmatrix} \left(f_{11} - \dfrac{1}{R}\right) & f_{12} & \cdots & f_{1N} \\[2ex] f_{21} & \left(f_{22} - \dfrac{1}{R}\right) & \cdots & f_{2N} \\ \vdots & \vdots & & \vdots \\ f_{N1} & f_{N2} & \cdots & \left(f_{NN} - \dfrac{1}{R}\right) \end{vmatrix} = 0$$

(1)

where

$$f_{ij} = \left(\frac{\partial y_i}{\partial x_j}\right)_{x_k(k \neq j, N+1) = \text{constant}}$$

(2)

and x and y are the volume fraction of each component in the mobile phase and that in the liquid phase within the column, respectively.

Eq. 1 was derived for the distribution between the bulk mobile phase and the space within the column bed. In this case, R is defined by

$$R = \frac{V_t}{V_R}$$

(3)

where V_t is the total volume of all mobile phase components within 'the column and V_R is the retention volume.

It is more convenient to use the capacity factor, which describes the solute distribution between the mobile phase and the stationary phase, to express the retention of the system peaks observed in partition chromatography of ionic solutes than to use R. It is then a simple matter to replace volume fraction by amount, n, and R by the capacity factor, k', to give

$$\begin{vmatrix} (g_{11} - k') & g_{12} & \cdots & g_{1N} \\ g_{21} & (g_{22} - k') & \cdots & g_{2N} \\ \vdots & \vdots & & \vdots \\ g_{N1} & g_{N2} & \cdots & (g_{NN} - k') \end{vmatrix} = 0$$

(4)

where

$$g_{ij} = \left(\frac{\partial n_{s,i}}{\partial n_{m,j}}\right)_{n_{m,k}(k \neq j, N+1) = \text{constant}}$$

(5)

and subscripts m and s denote the mobile and stationary phases, respectively.

In order to solve Eq. 4, all of the differentials in this equation, g_{11}, $g_{12}, \ldots, g_{NN}$, must be

obtained. It is possible to calculate these differentials provided that the dependence of the retention of each mobile phase component on the composition of the mobile phase has previously been clarified. The author has recently presented the equations that represent the dependence of the retention of ionic analytes on the composition of the mobile phase electrolytes in partition chromatography [30]. The basic assumption of the ion partition model used for the derivation of the equations is that the association of each ion with counter ions is negligible in both the mobile and stationary phases [28].

When the mobile phase contains N univalent anions, $X_1^-, \cdots, X_N^-$ and a common univalent cation, Y^+, the capacity factor of an analyte anion, A^{p-}, $k_A'^{Y(X_1^+ \cdots + X_N)}$, is given by [30]

$$k_A'^{Y(X_1 + \cdots + X_N)} = \frac{n_{s,A}}{n_{m,A}}$$

$$= \left\{ \frac{\sum [X_i^-]}{\sum \dfrac{[X_i^-]}{(k_A'^{YX_i})^{2/p}}} \right\}^{p/2}$$

(6)

where $k_A'^{YX_i}$ is the capacity factor of A^{p-} when eluted with a single electrolyte, YX_i ($i = 1, \cdots, N$), and $[X_i^-]$ is the concentration of X_i^- is the mobile phase.

In a similar manner, the capacity factor of an anion, which is one of the components of the mobile phase, X_j^-, can be expressed as

$$k_{X_j}'^{Y(X_1 + \cdots + X_N)} = \frac{n_{s,X_j}}{n_{m,X_j}}$$

$$= \left\{ \frac{\sum [X_i^-]}{\sum \dfrac{[X_i^-]}{(k_{X_j}'^{YX_i})^2}} \right\}^{1/2}$$

$$= \left\{ \frac{\sum n_{m,X_i}}{\sum \dfrac{n_{m,X_i}}{(k_{X_j}'^{YX_i})^2}} \right\}^{1/2}$$

(7)

We see that Eq. 7 enables us to calculate $g_{X_i X_j}$ for any combinations of the mobile phase component anions.

3.2. One solvent–two electrolyte system

Let us consider a mobile phase that consists of one solvent and two electrolytes, YX_1 and YX_2. This mobile phase is regarded as a three-component system since X_1^- and X_2^- must be accompanied by their counter cation, Y^+, in both the mobile and stationary phases. Hence we can write

$$\begin{vmatrix} (g_{X_1X_1} - k') & g_{X_1X_2} \\ g_{X_2X_1} & (g_{X_2X_2} - k') \end{vmatrix} = 0 \tag{8}$$

We obtain $g_{X_1X_1}$, $g_{X_2X_2}$, $g_{X_1X_2}$ and $g_{X_2X_1}$ from Eq. 7 as

$$g_{X_1X_1} =$$
$$\frac{k_{X_1}'^{YX_2}\{[X_1^-](k_{X_1}'^{YX_1}/k_{X_2}'^{YX_1})(2[X_1^-] + [X_2^-]) + [X_2^-](3[X_1^-] + 2[X_2^-])\}}{2([X_1^-] + [X_2^-])^{1/2}\{[X_1^-](k_{X_1}'^{YX_1}/k_{X_2}'^{YX_1}) + [X_2^-]\}^{3/2}} \tag{9}$$

$$g_{X_2X_2} =$$
$$\frac{k_{X_2}'^{YX_1}\{[X_2^-](k_{X_2}'^{YX_2}/k_{X_1}'^{YX_2})(2[X_2^-] + [X_1^-]) + [X_1^-](3[X_2^-] + 2[X_1^-])\}}{2([X_1^-] + [X_2^-])^{1/2}\{[X_2^-](k_{X_2}'^{YX_2}/k_{X_1}'^{YX_2}) + [X_1^-]\}^{3/2}} \tag{10}$$

$$g_{X_1X_2} = \frac{k_{X_1}'^{YX_2}[X_1^-]^2\{(k_{X_1}'^{YX_1}/k_{X_2}'^{YX_1}) - 1\}}{2([X_1^-] + [X_2^-])^{1/2}\{[X_1^-](k_{X_1}'^{YX_1}/k_{X_2}'^{YX_1}) + [X_2^-]\}^{3/2}} \tag{11}$$

$$g_{X_2X_1} = \frac{k_{X_2}'^{YX_1}[X_2^-]^2\{(k_{X_2}'^{YX_2}/k_{X_1}'^{YX_2}) - 1\}}{2([X_1^-] + [X_2^-])^{1/2}\{[X_2^-](k_{X_2}'^{YX_2}/k_{X_1}'^{YX_2}) + [X_1^-]\}^{3/2}} \tag{12}$$

Comparison of the theory with experiments through Eqs. 8–12 requires a knowledge of four k' values, $k_{X_1}'^{YX_1}$, $k_{X_1}'^{YX_2}$, $k_{X_2}'^{YX_1}$ and $k_{X_2}'^{YX_2}$. However, it is not necessary to determine all of the four values experimentally. $k_{X_1}'^{YX_2}$ and $k_{X_2}'^{YX_2}$ can be calculated from $k_{X_1}'^{YX_1}$ and $k_{X_2}'^{YX_1}$ as follows [28]:

$$k_{X_1}'^{YX_2} = \left[\frac{(k_{X_1}'^{YX_1})^3}{k_{X_2}'^{YX_1}}\right]^{1/2} \tag{13}$$

$$k_{X_2}'^{YX_2} = (k_{X_1}'^{YX_1}k_{X_2}'^{YX_2})^{1/2} \tag{14}$$

$k_{X_1}'^{YX_1}$ can be assumed to be the same as the capacity factor of the system peak observed when a solution of YX_1 with a concentration different from that of the mobile phase is injected into the column, provided that the mobile phase concentration of YX_1 is low enough to be within the portion of the isotherm that is linear from the origin [2]. Since an aqueous solution of YX_1 is a two-component system, only one system peak is generated, the capacity factor of which is given by

$$k' = g_{X_1X_1} = \frac{\partial n_{s,X_1}}{\partial n_{m,X_1}} \tag{15}$$

We compared the predictions of the theory with the experiments carried out on Sephadex G-25 and Bio-Gel P-2 with aqueous solutions of NaCl and NaClO$_4$. It has been shown that the effect of various types of electrolytes on the retention of inorganic ions on non-ionic hydrophilic porous polymers such as Sephadex G-25 and Bio-Gel P-2, when water is used as the mobile phase solvent can well be interpreted by the ion partition model [28]. The retention of an ion in these systems can be regarded as being governed by the partitioning of the ion between the external bulk water and the water in polymer gels, which has properties different from those of ordinary bulk water.

Figs. 1 and 2 show the capacity factors of the system peaks on Sephadex G-25 and Bio-Gel P-2 as a function 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 solid lines represent the k' values calculated from Eqs. 8–12 using experimental data obtained in the NaCl mobile phase system. $k_{Cl}'^{NaClO_4}$ and $k_{ClO_4}'^{NaClO_4}$ were calculated by substituting $k_{Cl}'^{NaCl}$ and $k_{ClO_4}'^{NaCl}$ into Eqs. 13 and 14, respectively. It is seen from Figs. 1 and 2 that the calculated k' values are in good agreement with
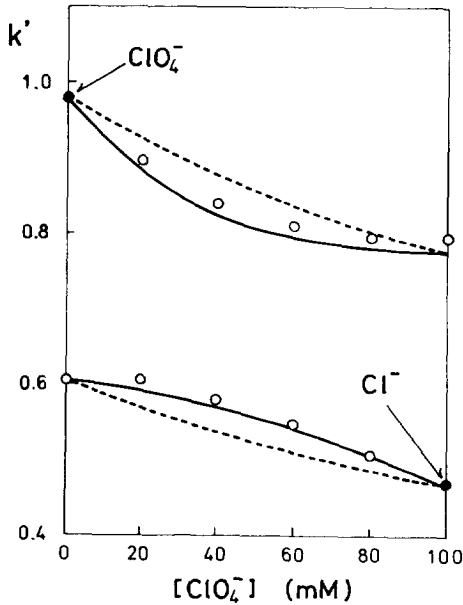
Fig. 1. Capacity factors of system peaks on Sephadex G-25 as a function of concentration of ClO_4^- in aqueous solution containing NaCl and $NaClO_4$. The solid lines were calculated from Eqs. 8–12. The dashed lines, which represent the capacity factors of Cl^- and ClO_4^- as analyte ions, were calculated according to Eq. 6. $[Na^+] = [Cl^-] + [ClO_4^-] = 100$ mM. ● = Analyte peak; ○ = system peak.
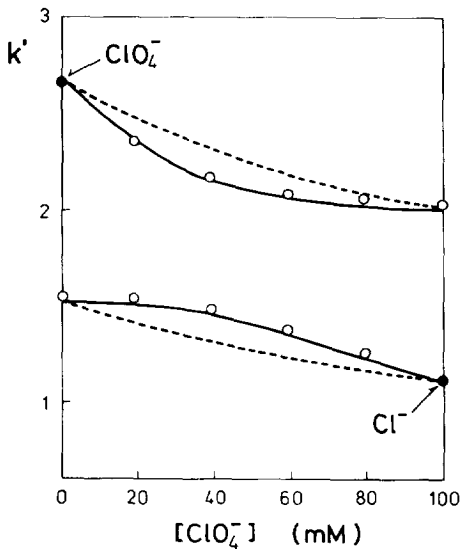

Fig. 2. Capacity factors of system peaks on Bio-Gel P-2 as a function of concentration of ClO_4^- in aqueous solution containing NaCl and $NaClO_4$. For other details, see Fig. 1.

the experimental values. It should be noted that the capacity factors of Cl^- and ClO_4^- as analyte ions injected (dashed lines), evaluated using Eq. 6, are different from those of the system peaks.

3.3. Two solvent–two electrolyte system

As the second object of this study, Sephadex G-25 equilibrated with acetone–water containing two electrolytes, YX_1 and YX_2, was chosen. 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 where the retention of inorganic ions is governed by the simple ion partition mechanism [29].

The mobile phase adopted here is a four-component system, the capacity factors of three system peaks observed in this system being given by the three roots of the equation

$$\begin{vmatrix} (g_{X_1X_1} - k') & g_{X_1X_2} & g_{X_1Ac} \\ g_{X_2X_1} & (g_{X_2X_2} - k') & g_{X_2Ac} \\ g_{AcX_1} & g_{AcX_2} & (g_{AcAc} - k') \end{vmatrix} = 0$$

(16)

where the subscript Ac represents acetone.

If the concentrations of the mobile phase ions are negligibly smaller than those of the component solvents and the concentrations of the solvents in the stationary phase can be regarded as being constant regardless of the composition of the mobile phase electrolytes, we can write

$$g_{AcX_1} = g_{AcX_2} = 0$$

(17)

Eqs. 16 and 17 then give

$$(g_{AcAc} - k') \begin{vmatrix} (g_{X_1X_1} - k') & g_{X_1X_2} \\ g_{X_2X_1} & (g_{X_2X_2} - k') \end{vmatrix} = 0$$

(18)

It is expected from Eq. 18 that the k' value of one of the three system peaks generated in this system should be constant, independent of the composition of the mobile phase electrolytes, whereas the values of the remaining two peaks are given by substituting Eqs. 9–12 into Eq. 8 in

a similar manner as described for a one solvent–two electrolyte system.

Fig. 3 shows the chromatograms of conductivity and refractive index detector responses for the injection of an acetone–water mixture with a concentration of acetone slightly less than that in the mobile phase into a column equilibrated with acetone–water (70:30, v/v) containing 8 mM NaNO$_3$ and 2 mM NaClO$_4$. The dependence of the k' values for the three system peaks in the conductivity trace on the concentration of ClO$_4^-$ is illustrated in Fig. 4, where the total con-

Fig. 4. Capacity factors of system peaks 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 from Eqs. 8–12. [Na$^+$] = [NO$_3^-$] + [ClO$_4^-$] = 10 mM. ● = Analyte peak; ○ = system peak which depends on the composition of mobile phase electrolytes; △ = system peak which does not depend on the composition of mobile phase electrolytes.
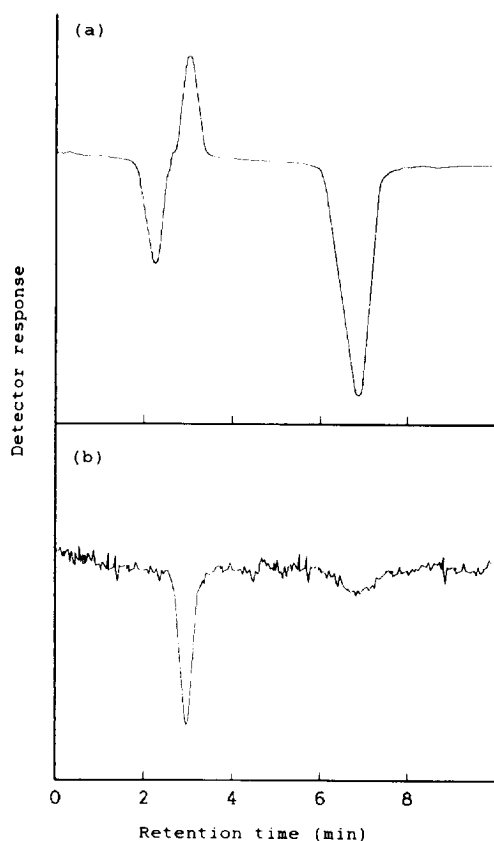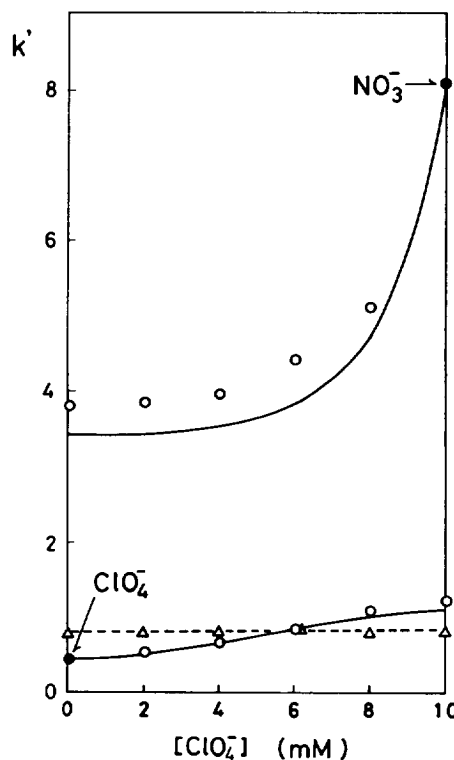

Fig. 3. Chromatograms of (a) conductivity and (b) refractive index detector responses resulting from an injection of an acetone–water mixture that is slightly enriched in water compared with the mobile phase. Column, Sephadex G-25 (250 × 4 mm I.D.); mobile phase, acetone–water (70:30, v/v) containing 8 mM NaNO$_3$ and 2 mM NaClO$_4$.

centration of NO$_3^-$ and ClO$_4^-$ was kept constant at 10 mM. As expected, one of the system peaks, the positive peak in the conductivity trace, which corresponds to the peak observed in the refractive index response, has a constant k' value of 0.81, whereas the other two peaks exhibit k' values that depend on the composition of the mobile phase electrolytes. The theoretical k' values (solid lines) were calculated from Eqs. 8–12 using experimental data obtained in the NaClO$_4$ system. $k'^{NaNO_3}_{ClO_4}$ and $k'^{NaNO_3}_{NO_3}$ were obtained by substituting $k'^{NaClO_4}_{ClO_4}$ and $k'^{NaClO_4}_{NO_3}$ into Eqs. 13 and 14, respectively. Fig. 4 shows

that there is fairly good agreement between the experimental and calculated k' values. This result indicates that the retention of the system peaks in this system can be predicted from Eq. 18 and the ion partition model.

3.4. Two solvent–three electrolyte system

We extend the theoretical approach described above to a two solvent–three electrolyte system. If acetone–water to be used as the mobile phase solvent contains three anions, X_1^-, X_2^- and X_3^-, and a common cation, Y^+, it is regarded as a five-component system. The capacity factors to be generated are given by the four roots of the following equation, provided that the distribution of acetone on Sephadex G-25 does not

depend on the composition of the mobile phase electrolytes:

$$(g_{AcAc} - k') \begin{vmatrix} (g_{X_1X_1} - k') & g_{X_1X_2} & g_{X_1X_3} \\ g_{X_2X_1} & (g_{X_2X_2} - k') & g_{X_2X_3} \\ g_{X_3X_1} & g_{X_3X_2} & (g_{X_3X_3} - k') \end{vmatrix} = 0$$

(19)

The evaluation of $g_{X_1X_1}$, $g_{X_1X_2}$, ..., $g_{X_3X_3}$ from Eq. 7 involves simple but detailed manipulations too long to reproduce here. The result for the system acetone–water (70:30, v/v) containing NaCl, NaNO$_3$ and NaClO$_4$ is shown in Fig. 5, where the solid lines represent the theoretical k' values calculated by using the experimental k' data obtained in the NaNO$_3$ system. Table 1 compares the calculated and experimental k' values for the three system peaks which depend on the mobile phase electrolyte composition. The calculated and experimental values agree well with each other, which indicates that the k' values of the system peaks in two solvent–three
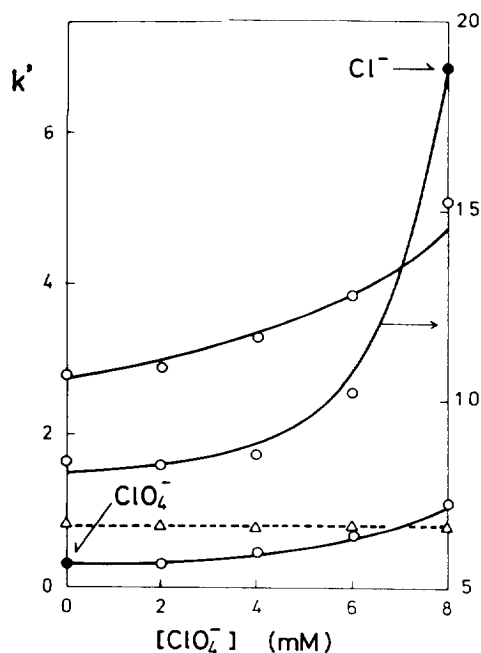

Fig. 5. Capacity factors of system peaks on Sephadex G-25 as a function of concentration of ClO$_4$ in acetone–water (70:30, v/v) containing NaNO$_3$. NaCl and NaClO$_4$. [NO$_3^-$] = 2 mM; [Na$^+$] = [NO$_3^-$] + [Cl$^-$] + [ClO$_4^-$] = 10 mM. For other details, see Fig. 4.

Table 1

Calculated and experimental capacity factors of system peaks on Sephadex G-25 with acetone–water (70:30, v/v) containing NaCl, NaNO$_3$ and NaClO$_4$

Concentration of mobile phase anions (mM)				Capacity factor of system peak		
ClO$_4^-$	NO$_3^-$	Cl$^-$		No. 1	No. 2	No. 3
2	2	6	Exptl.	0.311	2.88	8.10
			Calcd.	0.389	2.91	7.61
2	4	4	Exptl.	0.405	3.46	8.90
			Calcd.	0.423	3.25	8.19
2	6	2	Exptl.	0.443	3.89	10.18
			Calcd.	0.474	3.51	9.51
4	2	4	Exptl.	0.456	3.28	8.50
			Calcd.	0.495	3.32	8.26
4	4	2	Exptl.	0.527	3.96	10.55
			Calcd.	0.560	3.62	9.88
6	2	2	Exptl.	0.674	3.85	10.15
			Calcd.	0.671	3.88	10.20

electrolyte system can also be accurately predicted from Eqs. 7 and 19.

4. Conclusions

Theoretical equations concerning the dependence of retention of system peaks on the composition of the mobile phase electrolytes were developed on the basis of the model for partition chromatography of ionic solutes in the presence of background electrolytes and Knox and Kaliszan's theory. The capacity factors of the system peaks predicted from the equations were in good agreement with the experimental values obtained with both single (water) and binary solvent (acetone–water) systems containing two or three electrolytes. The results described above indicate that the relationship between the retention of the system peaks and the mobile phase composition can be obtained, once the dependence of the distribution of each mobile phase component between the mobile and stationary phases on the composition of the mobile phase is known, although it may sometimes be fairly complicated.

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