CHROM. 22 305

Influence of dissociation equilibria on the elution behaviour of the sample anion in anion chromatography

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SUMMARY

In anion chromatography, the influence of the dissociation equilibria on the measured capacity factor of a sample is controlled by the capacity factors of species in the sample, the dissociation constants and the pH of eluent. A method has been developed for interpreting the elution behaviour by using the ratio of the capacity factor of the sample to that of a standard anion with a fixed charge. The dissociation constants and the ratios of the capacity factors of various species to that of the standard were calculated. As a result, the elution behaviour of the sample at any eluent concentration was numerically estimated and the estimated values agreed with the measured values.

INTRODUCTION

Ion chromatography[1] is an effective method for the separation and determination of many sample cations and anions. Although the eluent is limited in suppressed (dual-column) ion chromatography[1], many eluents have been used in developing of non-suppressed (single-column) ion chromatography[2]. We have studied the effect of the eluent on the elution mechanism of samples.

The elution behaviour of a sample ion is controlled by four factors: (1) concentration of the eluent ion; (2) charge on the eluent ion; (3) charge on the sample ion; and (4) pH of the eluent. Whereas factors 1–3 have already been investigated[3], factor 4 has not yet been completely studied.

Owing to changes in the eluent pH, the sample ion or eluent ion may be present in several states having different charges, which complicates the analysis of elution behaviour. Jenke and Pagenkopf[4–8] studied the elution mechanism accompanying changes in eluent charge, and Hoover[9] studied variations in both eluent and sample charges.

The eluent pH also changes the physical state of an ion-exchange resin and changes the retention behaviour of the sample even if the charges of the sample and eluent ions are fixed.

In this work, we studied the influence of dissociation equilibria on the elution behaviour of samples in anion chromatography by investigating the effect of the state of the resin. It was found that this effect could be eliminated by using the ratio of the capacity factor of the sample anion to that of a standard anion having a fixed charge. As a result, we succeeded in interpreting the elution behaviour. Moreover, the behaviour at any eluent concentration could be numerically estimated by using the data obtained at a fixed eluent concentration.

THEORY

Elution behaviour of the sample anion
    In anion chromatography, the measured capacity factor (k') of a sample A is expressed by

$$
\begin{aligned}
k' &= D_A w / V_0 \\
   &= ([A]_R / [A]) w / V_0
\end{aligned}
\tag{1}
$$

where D_A is the distribution coefficient of A, w is the weight of the resin, V_0 is the dead volume of the column, $[A]_R$ is the concentration of A on the resin surface (ion-exchange phase) and $[A]$ is the concentration of A in the liquid phase. The k' value is experimentally determined as follows:

$$
k' = (V_R - V_0) / V_0
\tag{2}
$$

where V_R is the retention volume of A.

Influence of dissociation equilibria on the elution behaviour of the sample anion
    In general, a sample anion is involved in the following dissociation equilibria:

$$
A^{(i-1)-} \overset{K_{ai}}{\rightleftharpoons} A^{i-} + H^+
\tag{3}
$$

$$
K_{ai} = [H^+][A^{i-}] / [A^{(i-1)-}]
\tag{4}
$$

where K_{ai} denotes dissociation constant of $A^{(i-1)-}$. $[A]_R$ and $[A]$ in eqn. 1 can be represented as follows:

$$
[A]_R = \sum_i [A^{i-}]_R
\tag{5}
$$

$$
[A] = \sum_i [A^{i-}]
\tag{6}
$$

The ratios $[A^{i-}]/[A]$ and $[A^{i-}]_R/[A]_R$ are constant at a fixed eluent pH and the k' value is obtained by substituting eqns. 5 and 6 for eqn. 1 as follows[9]:

$$
k' = \left(\sum_i [A^{i-}]_R / \sum_i [A^{i-}] \right) w / V_0
$$

$$= \sum_i (P_i [A^{i-}]_R / [A^{i-}]) w / V_0$$

$$= \sum_i (P_i D_{Ai}) w / V_0$$

$$= \sum_i (P_i k_i') \tag{7}$$

where

$$k_i' = D_{Ai} w / V_0$$
$$= ([A^{i-}]_R / [A^{i-}]) w / V_0 \tag{8}$$

and

$$P_i = [A^{i-}] / [A] \tag{9}$$

$$\left(\sum_i P_i = 1 \right)$$

When the states $i = 0$ to $i = q$ are equilibrated, the P_i are determined by using eqns. 4, 6 and 9 as follows:

$$P_i = \left\{ \left(\prod_{y=0}^{i} K_{ay} \right) \Big/ [H^+]^i \right\} \Big/ \sum_{z=0}^{q} \left\{ \left(\prod_{y=0}^{z} K_{ay} \right) \Big/ [H^+]^z \right\} \tag{10}$$

provided $K_{a0} = 1$, where the P_i are functions of $K_{a1}, K_{a2}, \ldots, K_{aq}$ and $[H^+]$. Therefore, k' is determined only by k_i', K_{ai} and $[H^+]$, by substituting eqn. 10 for eqn. 7 as follows:

$$k' = \sum_i \left(\left\{ \left(\prod_{y=0}^{i} K_{ay} \right) \Big/ [H^+]^i \right\} \Big/ \sum_{z=0}^{q} \left\{ \left(\prod_{y=0}^{z} K_{ay} \right) \Big/ [H^+]^z \right\} k_i' \right) \tag{11}$$

As a result, k' at any eluent pH can be calculated if the k_i' values are known.

Elimination of effect of eluent pH on resin

However, even if a fixed-charge eluent anion (E^{n-}, *e.g.*, Cl^-, Br^-) is used, the k_i' value changes depending on the eluent pH because the physical state of resin is changed, accompanied by swelling.

The factor most influenced by pH is the column dead volume (V_0), as follows[10,11]:

$$V_0 = V_{all} - V_{resin,w}$$
$$= V_{all} - (V_{resin,d} + a + b\pi) \tag{12}$$

where V_{all}, $V_{resin,w}$ and $V_{resin,d}$ are the whole volume of the column, the wet resin volume and the dry resin volume, respectively, and a and b are constants determined by

the resin system; π is thermodynamic osmotic (swelling) pressure, which is determined by the equivalent fractions and partial equivalent volumes of species in the eluent[10]. When the eluent pH changes, the π value is also changed by an amount that is too large to neglect.

To eliminate the effect of V_0 on k_i', the ratio of k_i' to k_B' (capacity factor of a fixed-charge anion, B^{m-}, e.g., NO_3^-) is introduced as follows:

$$k_i'/k_B' = (D_{Ai}w/V_0)/(D_Bw/V_0)$$
$$= D_{Ai}/D_B \tag{13}$$

In other words, k_i'/k_B' is constant at a fixed eluent anion concentration, regardless of the eluent pH. Therefore, k'/k_B' is a function only of P_i, as follows:

$$k'/k_B' = \sum_i (P_i k_i')/k_B'$$
$$= \sum_i (P_i k_i'/k_B') \tag{14}$$

Determination of k_i'/k_B' values using k'/k_B' values at fixed eluent concentration

From eqns. 9 and 10, P_i is a function of pH and pK_{ai}. Therefore, the k_i'/k_B' and pK_{ai} values of sample A are determined by mathematical regression of eqn. 14 as follows:

(1) Measure k'/k_B' (equal to the ratios of retention times) at several eluent pH values and a fixed eluent concentration.

(2) Assume tentative pK_{ai} values successively at intervals of 0.01 within the range of measured pH.

(3) Calculate tentative P_i values at measured pH by using tentative pK_{ai} values obtained by process 2.

(4) Determine the tentative k_i'/k_B' values by the linear regression of k'/k_B' vs. tentative P_i with using the least-squares method corresponding to tentative pK_{ai} values.

Finally, only one set of pK_{ai} values, in which the correlation coefficient (r) of the regression is the nearest to unity, is selected, and the values of k_i'/k_B' are determined by these pK_{ai} values.

Prediction of k'/k_B' values at different eluent concentrations

There is the following correlation between k_i' or k_B' and the concentration of an eluent anion E^{n-} (ref. 3):

$$k_i' = [E^{n-}]^{-i/n} \cdot C_i \tag{15}$$
$$k_B' = [E^{n-}]^{-m/n} \cdot C_B \tag{16}$$

where C_i and C_B are constants. k_0' (k_i' with $i = 0$) is regarded as independent of $[E^{n-}]$.

By dividing eqn. 15 by eqn. 16, the relationship between k_i'/k_B' and $[E^{n-}]$ is as follows:

$$k_i'/k_B' = [E^{n-}]^{(m-i)/n} \cdot C_i/C_B \tag{17}$$

In order to eliminate C_i/C_B, by dividing eqn. 17 by $(k_i'/k_B')_0$ at a fixed concentration $([E^{n-}]_0)$, the following equation is obtained:

$$k_i'/k_B' = (k_i'/k_B')_0([E^{n-}]/[E^{n-}]_0)^{(m-i)/n} \tag{18}$$

From eqns. 7 and 18,

$$k'/k_B' = \sum_i \{P_i(k_i'/k_B')_0([E^{n-}]/[E^{n-}]_0)^{(m-i)/n}\} \tag{19}$$

EXPERIMENTAL

Analytical-reagent grade chemicals were used throughout.

Standard sample solutions

Stock standard solutions of 1000 μg/ml (as anion) of sodium chloride, bromide, iodate, sulphate, nitrate, nitrite, benzoate, acetate, propionate and hydrogenphosphate, potassium arsenate, succinic acid and fumaric acid were prepared by dissolving the chemicals in distilled water. Working standards solutions were obtained by diluting the stock solutions to 20 μg/ml (as anion) with distilled water.

Eluents

Chloride, bromide, sulphate (pK_{a2} = 1.99)[12] and salicylate (pK_{a1} = 2.98, pK_{a2} = 12.38)[12] anions were used as the eluent anion. Stock solutions (1 M) of chloride, bromide and sulphate were prepared by diluting concentrated hydrochloric acid, hydrobromic acid and sulphuric acid, respectively. A stock solution (100 mM) of salicylate was prepared by dissolving salicylic acid in dilute ammonia solution.

The reagents used for controlling the eluent pH were sodium hydroxide solution (1 M) and ammonia solution (100 mM). These solutions were prepared by dissolving sodium hydroxide in water or diluting concentrated ammonia solution.

The eluents were prepared by mixing and diluting the acidic solutions and the basic solutions, and then these eluents were deaerated.

Apparatus

A Tosoh Model HLC-601 non-suppressed ion chromatograph equipped with an anion-exchange column (50 mm × 4.6 mm I.D.) packed with TSKgel IC-Anion-PW (particle size 10 ± 0.005 μm, capacity 0.03 ± 0.003 mequiv./g) or TSKgel IC-Anion-SW (particle size 5 ± 0.005 μm, capacity 0.4 ± 0.1 mequiv./g) and a Tosoh Model UV-8 II ultraviolet detector were used. HLC-601 instrument consisted of a computer-controlled pump, conductivity detector, sample injector (100 μl) and oven. The flow-rate was maintained at 1.0 ml/min under a pressure of 20–40 kg/cm^2. The separation column and a conductivity detector were placed in an oven regulated at 30°C. The data were recorded by a Shimadzu Chromatopac C-R1A recorder. An NEC Model PC-9801VM personal computer was used to calculate the above-mentioned values.

RESULTS AND DISCUSSION

k'_i/k'_B values

In order to prove the constancy of k'_i/k'_B, the relationship between k'/k'_{NO_3} ($B^{m-} = NO_3^-$, equal to k'_1/k'_{NO_3} or k'_2/k'_{NO_3}) and eluent pH was investigated by using k' values for several anions with a fixed charge under various conditions. As shown in Fig. 1, the deviation of these k'/k'_{NO_3} values was less than 5% and it was concluded that this theoretical approach is suitable.

Determination of pK_{ai} and k'_i/k'_B values

In anion chromatography, a low concentration of the sample anion exists with relatively high concentrations of eluent anion and counter cation and flows continuously. Hence the pK_{ai} values in the literature, which were obtained statically, are not suitable. Therefore, it is necessary to determine the pK_{ai} values by using the method outlined under Theory. Moreover, this method is very useful for determining k'_i/k'_B values, particularly when the difference between the pK_{ai} and $pK_{a(i+1)}$ values is small.

Fig. 2 shows the k'/k'_{NO_3} values for phosphate and arsenate anions obtained by using 3 mM salicylate anion (charge -1) as the eluent. In Fig. 2a, the abscissa is the eluent pH and in Fig. 2b it is P_2 under the condition of maximum (nearest to 1) r value, where the pK_{a2} values are 7.36 for phosphate and 7.27 for arsenate. The lines in Fig. 2b show the results of regression under the conditions mentioned above and expressed by the following equations:

phospate:

$$k'/k'_{NO_3} = 0.348 + 2.480 \ P_2$$
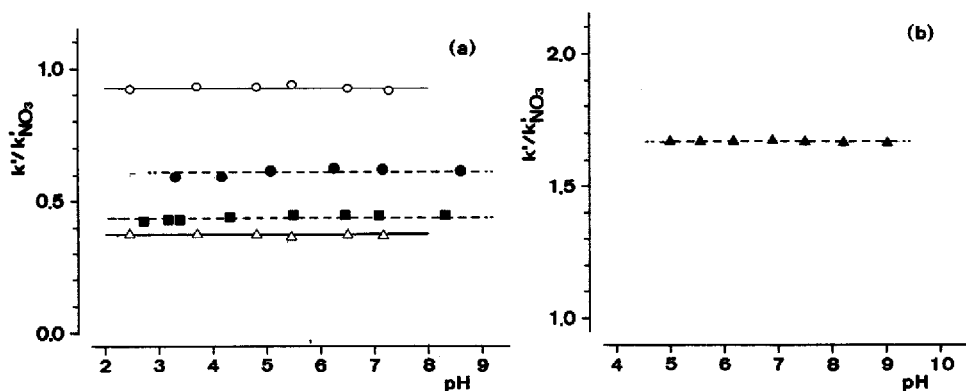$$= 0.348 \ P_1 + 2.828 \ P_2 \tag{20}$$

Fig. 1. Relationship between k'/k'_{NO_3} for solutes with a constant charge of (a) -1 or (b) -2 and eluent pH. Column: TSKgel IC-Anion-SW (solid line); TSKgel IC-Anion-PW (broken line). $\bigcirc$ = Sample bromide, eluent 20 mM chloride; $\bullet$ = bromide, 10 mM sulphate; $\blacksquare$ = chloride, 10 mM bromide; $\triangle$ = iodate, 20 mM chloride; $\blacktriangle$ = sulphate, 6 mM salicylate.
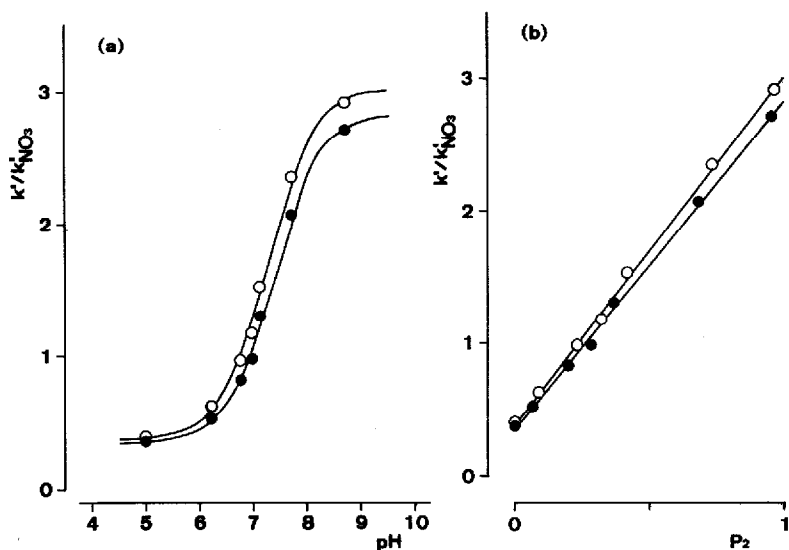
Fig. 2. Relationship between k'/k'_{NO_3} for solutes with change in charge from -1 to -2 and (a) eluent pH or (b) P_2 value obtained by using calculated pK_{a2} value. Sample: ● = phosphate; ○ = arsenate. Eluent: 3 mM salicylate. Column: TSKgel IC-Anion-PW.

arsenate:

$$k'/k'_{NO_3} = 0.375 + 2.659\ P_2$$
$$= 0.375\ P_1 + 3.034\ P_2 \tag{21}$$

In both instances, $P_1 + P_2 = 1$. The correlation coefficients were > 0.999. The values of k'_1/k'_{NO_3} and k'_2/k'_{NO_3} for phosphate were 0.348 and 2.828 and those of arsenate were 0.375 and 3.034. The curves in Fig. 2a were obtained by converting the abscissa in Fig. 2b to eluent pH.

Table I gives the pK_{ai} and k'_i/k'_B values for several anions calculated by using this method. All the calculated correlation coefficients were > 0.999.

Comparison between numerically calculated and observed k'/k'_B values at different eluent concentrations

By using eqn. 19, it is possible to establish the elution behaviour of sample anions, provided that the following two assumptions are satisfied: (1) the pK_{ai} values of the sample anion do not change with variation in the concentration of the eluent anion, and (2) the k'_0 value of the sample (k'_i with $i = 0$) does not depend on the concentration of the eluent anion.

Fig. 3 shows the results of numerically calculated and observed k'/k'_{NO_3} values. In Fig. 3a the sample is benzoate and the eluent is bromide. In 20 mM bromide eluent, pK_{a1} was 4.21 and the k'/k'_{NO_3} value was expressed as follows:

$$k'/k'_{NO_3} = 2.777\ P_0 + 1.383\ P_1 \tag{22}$$

TABLE I

CALCULATED k_i'/k_B' AND pK_{ai} VALUES

Sa$^-$ = Salicylate anion.

Sample	Eluent	Column[a]	B^{m-}	k_0'/k_B'	k_1'/k_B'	k_2'/k_B'	pK_{a1}[b]	pK_{a2}[b]
Nitrite	10 mM Br$^-$	SW	NO$_3^-$	0.042	0.797		2.98 (3.14)	
	20 mM Cl$^-$	SW	NO$_3^-$	0.006	0.829		2.92	
	20 mM Br$^-$	PW	NO$_3^-$	0.321	0.626		3.22	
Benzoate	20 mM Cl$^-$	SW	NO$_3^-$	0.149	1.747		3.82 (4.20)	
	20 mM Br$^-$	PW	NO$_3^-$	2.777	1.383		4.21	
Acetate	4 mM Sa$^-$	SW	Cl$^-$	0.028	0.782		4.56 (4.76)	
Propionate	4 mM Sa$^-$	SW	Cl$^-$	0.059	0.796		4.69 (4.87)	
Phosphate	3 mM Sa$^-$	PW	NO$_3^-$		0.348	2.828		7.36 (7.20)
Arsenate	3 mM Sa$^-$	PW	NO$_3^-$		0.375	3.034		7.27 (6.98)
Succinate	50 mM Cl$^-$	PW	NO$_3^-$	0.094	0.249	0.611	3.93 (4.21)	5.43 (5.64)
Fumarate	40 mM Cl$^-$	PW	NO$_3^-$	0.242	0.417	1.386	2.35 (3.10)	4.20 (4.60)

[a] PW = TSKgel IC-Anion-PW; SW = TSKgel IC-Anion-SW.
[b] Literature values[12] in parentheses.

The k'/k_{NO_3}' value at an arbitrary concentration of bromide was numerically calculated by using eqn. 19 as follows:

$$k'/k_{NO_3}' = 2.777\ P_0\ \{[Br^-]/(20\ mM)\} + 1.383\ P_1 \tag{23}$$

and the observed k'/k_{NO_3}' value agreed very well with the calculated value.
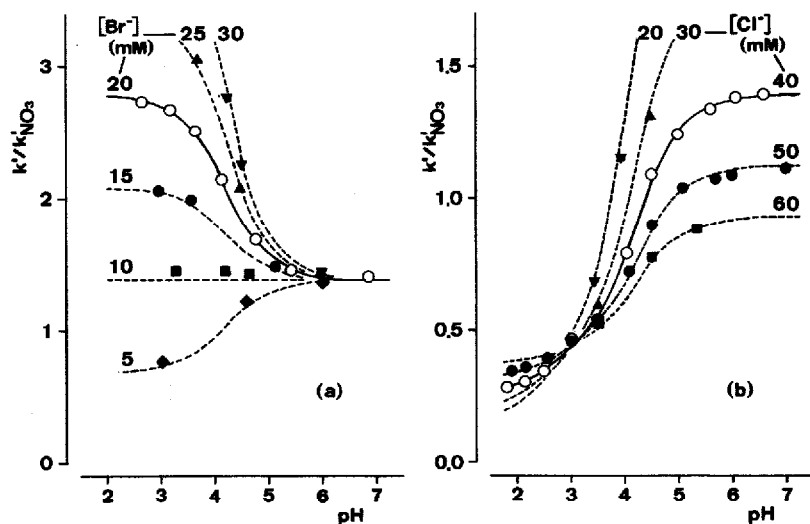

Fig. 3. Comparison between calculated and observed k'/k_{NO_3}' values. Broken curves: calculated from solid curve. (a) Sample, benzoate; eluent, bromide [$\blacklozenge$ = 5; $\blacksquare$ = 10; $\bullet$ = 15; $\bigcirc$ = 20; $\blacktriangle$ = 25; $\blacktriangledown$ = 30 mM]; column, TSKgel IC-Anion-PW. (b) Sample, fumarate; eluent, chloride [$\blacktriangledown$ = 20; $\blacktriangle$ = 30; $\bigcirc$ = 40; $\bullet$ = 50; $\blacksquare$ = 60 mM]; column, TSKgel IC-Anion-PW.
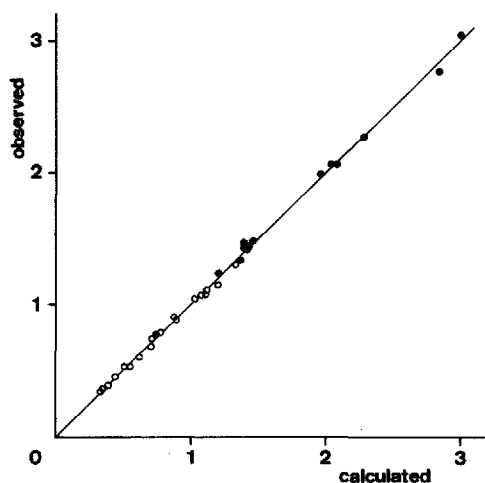
Fig. 4. Correlation between calculated and observed k'/k'_{NO_3} values. Data: ● = solid points shown in Fig. 3a; ○ = solid points shown in Fig. 3b.

In Fig. 3b, the sample is fumarate and the eluent is chloride. In 40 mM chloride eluent, pK_{a1} and pK_{a2} were 2.35 and 4.20, respectively, and the k'/k'_{NO_3} value was expressed as follows:

$$k'/k'_{NO_3} = 0.242\ P_0 + 0.417\ P_1 + 1.386\ P_2 \tag{24}$$

The k'/k'_{NO_3} value at an arbitrary concentration of chloride was calculated as follows:

$$k'/k'_{NO_3} = 0.242\ P_0\ \{[Cl^-]/(40\,mM)\} + 0.417\ P_1 + 1.386\ P_2\ \{(40\,mM)/[Cl^-]\} \tag{25}$$

and the observed k'/k'_{NO_3} value agreed very well with the calculated value.

Fig. 4 shows the relationship between the calculated and observed k'/k'_{NO_3} values shown in Fig. 3a and b. The correlation coefficients of the observed to calculated k'/k'_{NO_3} are 0.999 in both instances. As mentioned above, it is concluded that this numerical method is very accurate and precise.

CONCLUSION

A method for analysing the influence of dissociation equilibria on the elution behaviour of sample anions in anion chromatography has been presented. In this method, the ratio of the capacity factor (k') of the sample anion to that (k'_B) of an anion with a fixed charge is introduced in order to eliminate the change in physical state of the resin resulting from the change in eluent pH. The method has been demonstrated to be very useful for the analysis and numerical determination of sample anions involved in dissociation equilibria. Further, the method is useful even when using a mixed solution of water and an organic solvent as the eluent.

SYMBOLS

A	sample anion
A^{i-}	species A with charge $-i$
B^{m-}	fixed-charge anion used for the calculation of the ratio of capacity factors
D_A	distribution coefficient of A
D_{Ai}	distribution coefficient of A^{i-}
D_B	distribution coefficient of B^{m-}
E^{n-}	eluent anion
$[E^{n-}]_0$	fixed concentration of E^{n-}
i	charge number of A^{i-}
K_{ai}	dissociation constant of $A^{(i-1)-}$
k'	(measured) capacity factor of A
k'_i	capacity factor of A^{i-}
k'_B	capacity factor of B^{m-}
$(k'_i/k'_B)_0$	k'_i/k'_B at a fixed concentration of E^{n-}
m	fixed charge of B
n	fixed charge of E
P_i	ratio of $[A^{i-}]$ to total $[A]$
V_0	dead volume of the column
V_{all}	whole volume of the column
$V_{resin,d}$	dry resin volume
$V_{resin,w}$	wet resin volume
w	weight of the resin
π	osmotic (swelling) pressure

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