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Retention model of multiple eluent ion chromatography

A priori estimations of analyte capacity factor and peak intensity

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ABSTRACT

In multiple eluent ion chromatography, the analyte elution behaviour still remains unclear. In this work, the inter-eluent selectivity coefficient was accurately determined by re-interpreting Hoover's model. Obtaining the inter-eluent selectivity coefficient was greatly simplified by introducing the concept of an "inter-eluent separation factor". From the coefficients obtained, analyte capacity factors in polyprotic eluent systems can be estimated *a priori*. Moreover, this new factor permits the calculation of eluent species with different charges and, through stoichiometric simulation of the chromatogram, makes possible the *a priori* estimation of analyte peak intensities.

INTRODUCTION

In ion chromatography (IC), the retention time and the peak intensity of an analyte have been dominated by the eluent composition and its concentration. Usually, optimum conditions for analyte separation and detection are determined experimentally each time the elution conditions change. If the retention time and the peak intensity of target analyte can be calculated *a priori*, then analysis can be greatly simplified and estimation of the composition of unknown peaks made possible.

The selectivity coefficient between an analyte and the eluent can be defined theoretically for a given ion-exchange equilibrium [1]. When the analyte and the eluent ion are both composed of a single species under fixed conditions, not only can the analyte capacity factor be estimated *a priori* [2,3], but also the peak intensity after simple stoichiometric treat-

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ment [4,5]. However, multi-component eluents are generally used in IC, *e.g.*, carbonate–hydrogencarbonate and phthalate–hydrogenphthalate eluents for suppressed and non-suppressed IC, respectively, and hence the analyte behaviour becomes complicated.

Hoover [6] proposed the use of an “inter-eluent selectivity coefficient”, in which there is a constant selectivity coefficient between every pair of active eluent species, in order to solve this problem. Jenke and Pagenkopf [7] applied this concept to non-suppressed IC using a low-capacity ion-exchange column, but they could not correctly obtain the inter-eluent selectivity coefficient. Kuwamoto and coworkers [8,9] tried to omit this coefficient from their equations, thus avoiding the difficulties involved in calculating it. However, the elution mechanism in multi-eluent systems cannot be calculated without the inter-eluent selectivity coefficient.

In this paper, we define a new “inter-eluent separation factor” as a method for easily calculating the inter-eluent selectivity coefficient. The use of stoichiometric analysis together with the coefficients obtained permitted the elution and detection behaviour of an analyte in a multi-species eluent to be determined.

THEORY

Experimental observations were made using a diprotic organic acid as the eluent. We have already reported that the equilibrium in Fig. 1 holds, in an anion-exchange column, for an eluent acid, EH_2, that dissociates into EH^- and E^{2-}, with K_{e1} and K_{e2} as its first and second acid dissociation constants, respectively [4]. From Hoover’s model, all of the exchange sites are occupied by eluent anions.

$$[Cap] = 2[E^{2-}]_s + [EH^-]_s \tag{1}$$

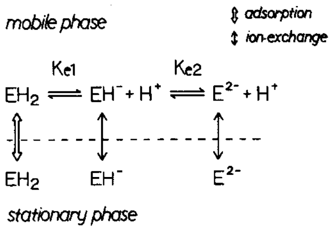

Fig. 1. Equilibrium diagram of eluent acid in the column.

where [Cap] is the anion-exchange capacity and the subscripts m and s represent existence in the mobile and stationary phase, respectively. If we assume that these eluent ions establish the equilibrium shown in eqn. 2, the inter-eluent selectivity coefficient, X_{21}, for this reaction is defined as in eqn. 3:

$$[E^{2-}]_m + 2[EH^-]_s \rightleftharpoons 2[EH^-]_m + [E^{2-}]_s \tag{2}$$

$$X_{21} = [E^{2-}]_s[EH^-]_m^2/[E^{2-}]_m[EH^-]_s^2 \tag{3}$$

This coefficient is considered to be a constant on condition that the eluent ionic strength is very low, because the contributions of the activity of ionic species to the calculation are negligible.

We can define the inter-eluent separation factor, R_E, as

$$R_E = [E^{2-}]_s[EH^-]_m/[E^{2-}]_m[EH^-]_s \tag{4}$$

Unlike the inter-eluent selectivity coefficient, this factor varies with change in eluent concentration and/or pH. However, it can be regarded as a constant when the eluent conditions are fixed, and substitution of eqn. 3 into eqn. 4 gives

$$R_E = X_{21}[EH^-]_s/[EH^-]_m \tag{5}$$

Solution of eqns. 1 and 3 for $[EH^-]_s$ and substitution into eqn. 5 gives

$$R_E = [H^+]\{(1 + 8X_{21}K_{e2}[Cap]/[H^+][EH^-]_m)^{1/2} - 1\}/4K_{e2} \tag{6}$$

When the analyte anion, S^-, is a monoprotic acid, the selectivity coefficients between this anion and differently charged eluent ions are K_{11} and K_{12}, respectively:

$$K_{11} = [S^-]_s[EH^-]_m/[S^-]_m[EH^-]_s \tag{7}$$

$$K_{12} = [S^-]_s^2[E^{2-}]_m/[S^-]_m^2[E^{2-}]_s \tag{8}$$

If the undissociated analyte, SH, and the column packing material do not interact at all, then the following ralationship between the experimental capacity factor, k'_{ex}, and the capacity factor of dissociated analyte, k'_{s^-}, holds:

$$k'_{ex} = [S^-]_s\phi/([SH]_m + [S^-]_m) = K_{s1}k'_{s^-}/([H^+] + K_{s1}) \tag{9}$$

where K_{s1} is the acid dissociation constant of the analyte and ϕ is the phase ratio of the column. The ratio of eluent species in the stationary phase becomes, according to eqn. 4,

$$[E^{2-}]_s/[EH^-]_s = R_E K_{e2}/[H^+] \qquad (10)$$

Solution of eqns. 1 and 10 for $[EH^-]_s$ and substitution into eqn. 7 gives

$$K_{11}\phi = k'_{s^-}[EH^-]_m(2R_E K_{e2} + [H^+])/[Cap][H^+] \qquad (11)$$

Since eqn. 11 is first order for $K_{11}\phi$ and R_E, they can be easily calculated using the value of k'_{s^-} obtained from eqn. 9. From eqns. 3, 7 and 8, the following relationship holds between K_{11} and K_{12}:

$$X_{21} = K_{11}^2/K_{12} \qquad (12)$$

Consequently, the selectivity coefficients determined in the above way include the phase ratio. The introduction of R_E obtained enables X_{21} to be calculated according to eqn. 6. As R_E is a function of $[EH^-]_m$, eqn. 11 implies that there is not a linear relationship between log k' and the logarithm of the eluent concentration in a multiple eluent IC system even if [Cap] is constant.

Next we deal in an analogous manner with polyprotic acid analytes. When differently charged analyte ions are treated as different ionic species, similarly in the case of the eluent, there are two by i kinds of selectivity coefficients, K_{i1} and K_{i2}, made between the analyte (i dissociation steps) and eluent (two dissociation steps). The capacity factor of each charged analyte, k'_{si-}, are determined by the following equations.

$$k'_{ex} = \left[\sum_{n=1}^{i} k'_{sn-}[H^+]^{i-n}\left(\prod_{l=1}^{n} K_{sl}\right)\right] \Bigg/ \left[[H^+]^i + \sum_{n=1}^{i} [H^+]^{i-n}\left(\prod_{l=1}^{n} K_{sl}\right)\right] \qquad (13)$$

$$k'_{si-} = R_E^{i-2} K_{i1} k'_{s2-}/X_{21}^{i-1} K_{22} \qquad (14)$$

where K_{sl} is the lth acid dissociation constant of the analyte. By using the value of X_{21} obtained experimentally for the monoprotic analyte, the values of these inter-eluent selectivity coefficients can be calculated by eqns. 13 and 14.

All of the selectivity coefficients can be calculated in the same manner as described above. By using these coefficients, the elution behaviour of analyte can be calculated in a diprotic eluent system, *i.e.*, the changes of each eluent species fraction in the stationary phase, according as the analyte fraction changes, can be calculated. Dividing eqn. 7 by eqn. 8 gives

$$\Delta[E^{2-}]_s/\Delta[EH^-]_s = K_{11}\phi k'_{s^-}\Delta[E^{2-}]_m/K_{12}\phi^2\Delta[EH^-]_m$$
$$= \Delta[E^{2-}]_m R_E/\Delta[EH^-]_m \qquad (15)$$

where Δ means the change of the bracketed species fraction. As the amount of analyte relative to that of the eluent is negligible in the overall stoichiometry of the stationary phase, the following equation applies:

$$\sum_{n=1}^{i} n\Delta[S^{n-}]_s = \Delta[EH^-]_s + 2\Delta[E^{2-}]_s \qquad (16)$$

Using eqns. 15 and 16, the simulation of chromatogram using the diprotic eluent system can be performed stoichiometrically.

In the case of a triprotic eluent system, the calculation of these coefficients is very complicated. All the selectivity coefficients, K_{ij}, of the triprotic analyte to be used as an eluent must first be calculated in mono- and/or diprotic eluent systems, where j represents the charge of the eluent. The inter-eluent selectivity coefficients of this triprotic acid eluent, X_{ij}, are determined by the following equation:

$$X_{ij} = K_{ij}/K_{jj}^i \qquad (17)$$

EXPERIMENTAL

The IC system consisted of a Shimadzu (Kyoto, Japan) LC-5A pump, a Rheodyne (Cotati, CA, USA) Model 7125 injector, a Shimadzu SPD-6AV UV–visible detector and a Shimadzu Chromatopac C-R4A integrator.

Chromatographic separation of anions was performed on a 5 cm × 4.6 mm I.D. column packed with low-capacity anion exchangers (Tosoh, Tokyo, Japan, TSK gel IC-Anion-PW, 0.03 mequiv./ml) maintained at 25°C. Phthalic acid eluents of various concentrations and pH values were delivered at 0.8 ml/min. Helium purging was performed to prevent atmospheric carbon dioxide from dissolving in the eluent at higher pH values. The detection wavelength was adjusted so that the absorbance of the eluent was approximately unity. The column void volume was determined from the front peak by injecting sodium hydroxide solution.

Chromatographic simulations were run on an

NEC (Tokyo, Japan) PC-9801 RA personal computer as described previously [4].

RESULTS AND DISCUSSION

The capacity factors of the four analytes at various phthalate concentrations and pH values are summarized in Table I. The equations in the theoretical section hold, as the ion-exchange capacity is constant. The IC-Anion-PW column used in this work was packed with a strongly basic anion-exchange resin of the quaternary ammonium salt type and its capacity is negligibly affected by altering the pH value of the eluent. In practice, there was not a significant change in the capacity for eluents of pH 4–6. Consequently, the value of the inter-eluent selectivity coefficient was calculated from the elution data for chloride anion, completely dissociated species, in the pH range mentioned above. Other coefficients were determined by using this coefficient. Four diprotic analyte selectivity coefficients were calculated from eqns. 13 and 14. Introduction of the concept of an "inter-eluent separation factor" enabled these values to be calculated accurately and readily. The calculated coefficients are shown in Table II.

TABLE I

CAPACITY FACTORS OF ANALYTES

Phthalate concentration (10^{-3} *M*)	pH	Capacity factor			
		Lactate	Chloride	Phosphate	Malate
0.5	3.95	8.45	24.8	–	45.6
	4.50	7.06	15.6	7.58	56.9
	5.9[a]	3.50	7.39	6.35	38.7
	6.4[a]	2.92	6.26	9.10	31.5
	7.1[a]	3.00	6.40	16.2	33.2
0.7	3.95	6.89	19.5	–	32.8
	4.49	5.73	12.7	6.15	39.0
	5.18	3.79	7.96	4.00	33.8
	6.4[a]	2.74	5.83	7.10	25.0
	7.1[a]	2.68	5.66	13.2	24.5
1.0	3.41	4.48	26.0	12.4	16.3
	3.95	5.47	15.1	7.71	22.7
	4.49	4.84	10.5	5.12	27.9
	5.19	3.15	6.48	3.27	22.8
	5.9[a]	2.47	5.21	3.95	19.3
	6.4[a]	2.26	4.81	5.04	17.4
	7.1[a]	2.26	4.81	8.91	17.2
1.5	3.41	3.54	18.0	9.17	10.7
	3.98	4.24	10.8	5.82	14.2
	4.51	3.79	8.00	3.94	18.0
	5.18	2.58	5.21	2.63	14.7
	5.9[a]	2.05	4.28	2.94	13.0
	6.5[a]	1.85	3.91	3.57	11.3
	7.0[a]	1.80	3.83	5.63	11.3
2.0	3.41	2.92	13.9	7.19	8.06
	3.98	3.60	8.89	–	10.8
	4.49	3.21	6.54	3.25	13.1
	5.19	2.23	4.58	2.27	11.3
	5.9[a]	1.78	3.74	2.45	9.82
	6.5[a]	1.53	3.38	3.02	8.38
	7.1[a]	1.54	3.29	4.28	8.22

[a] Helium purging.

TABLE II

SELECTIVITY COEFFICIENTS AND THEIR STANDARD DEVIATIONS FOR HOOVER'S MODEL

Analyte	$K_{11}\phi$	$K_{12}\phi^2$	$K_{21}\phi^a$	$K_{22}\phi$	X_{21}
Chloride	1.29 ± 0.07	1.49	–	–	1.12 ± 0.18
Lactate	0.68 ± 0.04	0.41	–	–	
Phosphate	0.61	0.33	1.68	1.50 ± 0.43	
Malate	0.69	0.43	1.48	1.32 ± 0.09	

[a] $K_{21} = K_{22}X_{21}$.

The relationhips between calculated (using the coefficients obtained) and observed analyte capacity factors show good linearity. The correlation coefficients and relative standard deviations (in parentheses) for lactate, chloride, phosphate and malate were 0.996 (6.5%), 0.994 (5.3%), 0.998 (2.8%) and 0.990 (6.1%), respectively. An example with a 1 m*M* phthalate eluent is shown in Fig. 2. The solid lines represent the calculated capacity factors and the symbols the observed values. The small discrepancies for lactate in the low-pH region can be attributed to the adsorption of its undissociated form and those for diprotic analytes in the high-pH region to the decrease in the ion-exchange capacity of the column. However, it should be noted that the *a priori* calculation of a polyprotic analyte capacity factor is possible even if both analyte and phthalate eluent are dissociated in the form of mixed charges.

As there was good agreement between the calculated and observed values, chromatographic simulation was carried out based on eqns. 15 and 16. The observed and simulated peak areas as a function of pH in a 1 m*M* phthalate eluent for the diprotic analytes malate and phosphate show good agreement, as can be seen in Fig. 3. The above findings show that the *a priori* estimation of analyte capacity factors and peak intensities is possible over a wide range of eluent pH. This calculation is considered to be practicable in polyprotic analyte and eluent systems. Consequently, these coefficients are useful in cases where the optimum conditions are to be established for multiple eluent IC.

If $\Delta[H^+]$ in the analyte zone is negligibly small, eqn. 15 can be rewritten as follows:

$$\Delta[E^{2-}]_s/\Delta[EH^-]_s = 2K_{e2}^2 R_E/([H^+]^2 + 4K_{e2}[H^+]) \quad (18)$$

Eqn. 18 implies that the ratio of the changes of eluent species fraction in the stationary phase is constant regardless of the analyte species. The value of $\Delta[E^{2-}]_s/\Delta[EH^-]_s$ in eqn. 18 is smaller than that of

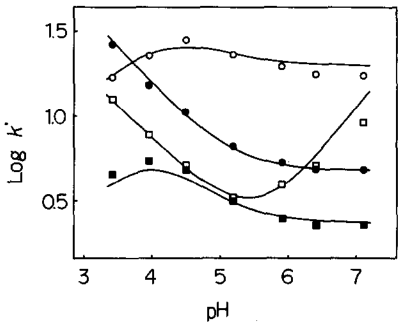

Fig. 2. Comparison of calculated capacity factors (solid lines) with observed values for malate (○), chloride (●), phosphate (□) and lactate (■).

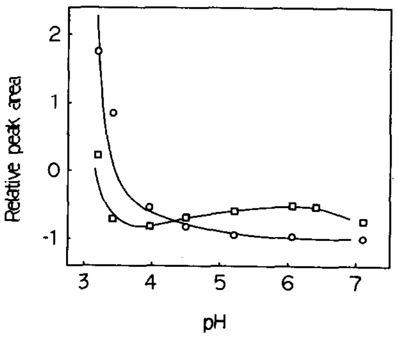

Fig. 3. Comparison of simulated relative peak areas (solid lines) with observed values for malate (○) and phosphate (□).

$[E^{2-}]_s/[EH^-]_s$ in eqn. 10. As far as the phthalate eluent is concerned, the results of the calculation show that this value is close to that of $[E^{2-}]_m/[EH^-]_m$, that is, there is no great difference between the ratio of eluent species participating in the elution of the analyte and that present in the mobile phase. This means that there is not a large difference in elution power between the two eluent species.

Overall, it can be concluded that the selectivity coefficients, based on the modified Hoover model, can be obtained accurately and readily by introducing the concept of an "inter-eluent separation factor". All equations are simplified when this new factor is used. Moreover, the use of the coefficients obtained reveals the behaviour of multiple eluents in the stationary phase and makes the *a priori* estimation of analyte peak intensity possible through stoichiometric simulation of the chromatogram.

REFERENCES

1 R. W. Grimshaw and C. E. Harland, *Ion-Exchange: Introduction to Theory and Practice*, Chemical Society, London, 1975.
2 P. Jandera and J. Churacek, *J. Chromatogr.*, 91 (1974) 207.
3 D. T. Gjerde, G. Schmuckler and J. S. Fritz, *J. Chromatogr.*, 187 (1980) 35.
4 A. Yamamoto, A. Matsunaga, M. Ohto, E. Mizukami, K. Hayakawa and M. Miyazaki, *J. Chromatogr.*, 482 (1989) 145.
5 H. Sato, *Anal. Chem.*, 62 (1990) 1576.
6 T. B. Hoover, *Sep. Sci. Technol.*, 17 (1982) 295.
7 D. R. Jenke and G. K. Pagenkopf, *Anal. Chem.*, 56 (1984) 88.
8 M. Maruo, N. Hirayama and T. Kuwamoto, *J. Chromatogr.*, 481 (1989) 315.
9 N. Hirayama and T. Kuwamoto, *J. Chromatogr.*, 508 (1990) 51.