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Secondary equilibrium size-exclusion chromatography of ions with polymeric mobile phase additives

Tetsuo Okada

Faculty of Liberal Arts, Shizuoka University, Shizuoka 422 (Japan)

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ABSTRACT

Polymeric ligands play an important role in the secondary equilibrium size-exclusion chromatography of small molecules. Polyethylene glycol and polybrene were employed in mobile phases to separate inorganic cations and anions, respectively. Addition of polymeric ligands to the mobile phases permits the elution order to be altered, the partition coefficients of solutes in the polymer phase to be determined and the analysis time to be reduced.

INTRODUCTION

Secondary equilibria in liquid chromatography allow one to modify the selectivity, to enhance the separation efficiency and to determine equilibrium constants in solution [1–11]. Chromatographic stationary phases are essentially developed for the separation of particular target solutes, *e.g.*, ion-exchange chromatography is for the separation of ions and size-exclusion chromatography is for polymers. However, secondary equilibria make it possible to separate analytes other than the essential solutes of the stationary phase. The author previously reported "micelle exclusion chromatography", wherein a size-exclusion chromatographic (SEC) stationary phase was employed for the separation of simple small ions in combination with micellar mobile phases [6–8]. Correspondingly, mobile phases containing polymeric additives were used for the SEC separation of simple ions in this work. It is known that stationary phases for SEC retain simple ions principally by electrostatic interaction between an ion and residual ionic groups in the stationary phase [12–14]. The selectivity obtained in the SEC separation of ions is therefore identical with that in ion-exchange chromatography in the absence of secondary equilibria, *e.g.*, the selectivity between inorganic anions is basically explained by the "Hofmeister series" [15]. Secondary equilibria, partitioning to the polymer phase or complexation with the polymer in mobile phases permit the selectivity to be altered.

Polymerized agents are excluded in terms of their effective sizes by the stationary phase, whereas small ions can penetrate into the inner part of the stationary phase, and probably interact with the stationary phase. If an ionic analyte interacts with a polymeric agent in mobile phases, the elution volume is reduced according to the partitioning to the polymer phase. A completely reversed elution order is expected if analytes interact weakly with the stationary phase.

EXPERIMENTAL

The chromatographic system consisted of a Tosoh Model CCPM or CCPD computer-controlled pump, a Tosoh CO-8000 column oven set at 25°C, a JASCO Model 875-UV UV–visible detector, a JASCO Model 830-RI refractive index (RI) detector and a Rheodyne injection valve equipped with a 100-μl sample loop. The elution of cations was monitored with RI detection and that of anions with UV detection Separation columns were Asahipak

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GS-300H and GS-320H [250 × 7.6 mm I.D. packed with poly(vinyl alcohol) gel, particle size 9 μm]. These stationary phases have different degrees of saponification and therefore show different hydrophobicities.

Methanol of analytical-reagent grade was distilled and stored over molecular sieves. Distilled methanol was redistilled daily before experiments. Distilled, deionized water was used throughout. Other reagents were of analytical-reagent grade and used as received.

Partial volumes of polymers ($\bar{v}$) were determined by measuring the density of the solution; $\bar{v}$ (PEG) and $\bar{v}$ (polybrene)

$$([-(CH_2)_6-\overset{+}{N}(CH_3)_2-(CH_2)_3-\overset{+}{N}(CH_3)_2-]_n\ [2Br^-]_n)$$

were determined as 0.8 and 0.7 ml/g, respectively.

RESULTS AND DISCUSSION

Retention model

In the present studies, the following equilibria are involved in the retention of an analyte (A):

$$A + P \rightleftharpoons AP \quad (1)$$

$$A + S \rightleftharpoons AS \quad (2)$$

where P and S refer to binding sites in a polymer and in the stationary phase, respectively. Because of difficulty in defining equilibrium constants (see below), partition coefficients for these above equilibria were used instead; K_s and K_p represent the partition coefficients of an analyte to the stationary phase and to the polymer phase, respectively. Partitioning of a polymer or a polymer–ion complex was not observed, and therefore was not taken into account in deriving the following equations.

In SEC, the retention volume of an analyte (V_r) can be described by the distribution coefficient (K_d), the volume of the external solvent (V_e) and the volume of the inner solvent, V_i:

$$V_r = V_e + K_d V_i \quad (3)$$

V_e and V_i are not constant for the stationary phase, but are changed by the effective size of the polymer employed in the mobile phase. Substitution of partition coefficients in eqn. 3 yields

$$1/K_d = V_i(1 + K_p\bar{v}C_p)/(V_i + V_sK_s) \quad (4)$$

According to eqn. 4, $1/K_d$ *vs.* C_p plots are linear, and permit the calculation of partition coefficients. This equation is analogous to that derived for micellar chromatography [5–8].

Polyethylene glycol as polymeric reagent for the separation of Ba^{2+} and alkali metal cations

Polyethylene glycol (PEG) is known to be capable of forming complexes with some hard metal ions such as alkali and alkaline earth metal ions [16–18]. The chemistry of this complexation has attracted fundamental and practical interest. The retention behaviours of alkali metal cations and Ba^{2+} were investigated here by adding PEG to methanolic mobile phases. Fig. 1 shows the changes in the retention with the concentration (C_p) of PEG 20 000 (the number following PEG represents the average molecular weight). Ba^{2+} is most strongly retained on GS-320H in the absence of PEG by electrostatic interactions; the elution order of other cations, $Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$, correlates with that in cation exchange. However, retention of cations capable of forming stable complexes with PEG decreases on addition of PEG to the mobile phase, but the retention of Na^+ and Li^+ is little changed. Although Na^+ and Li^+ are known to form PEG complexes in solution or the solid state, the complexation was too weak to be detected in the present experiments. The elution order of Ba^{2+} and Na^+ or Li^+ is thus altered by adding PEG to the mobile phase.

Fig. 2 shows the plots based on eqn. 2 for determining K_p values for Ba^{2+}. Regardless of the molecular weight of PEG, linear plots are obtained. Partition coefficients and V_e values for PEG are summarized in Table I. The K_p values vary with the molecular weight of PEG. A Ba^{2+}–PEG complex is extracted into organic solvents as an ion pair with a lipophilic anion, and precipitates its phosphomolybdate, phosphotungstate or tetraiodobismutate salt. Such studies permitted the estimation of the stoichiometry of Ba^{2+}–PEG complexes; usually 1:10–12 [Ba^{2+}: oxyethylene (EO) units] are thought to be possible molar ratios [18]. If complexation by a particular binding site consisting of ten successive EO units does not affect complexation by the adjacent binding sites, the binding constant of eqn. 1 for Ba^{2+} can be determined. However, such values calculated from results depicted in Figs. 1 and 2 are

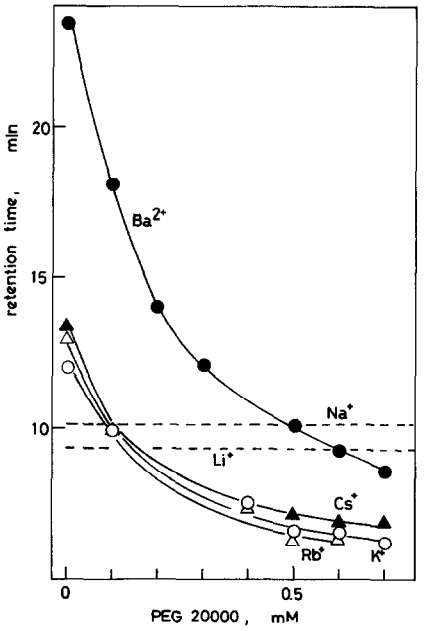

Fig. 1. Change in retention of cations with PEG 20 000 concentration in methanolic mobile phase. Mobile phase contains 0.1 *M* NH_4Cl. Stationary phase, GS-320H.

too small in comparison with literature values. The binding constant for Ba^{2+}–PEG 20 000 is, for example, estimated to be *ca.* 60 in this work, whereas that for Ba^{2+}–pentaethylene glycol was reported to be 200.

The complex formation ability of PEG is enhanced with increasing number of EO units; there is

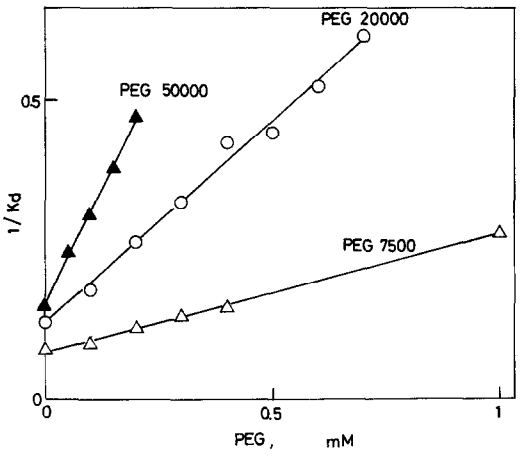

Fig. 2. $1/K_d$ *vs.* C_p plots for Ba^{2+} based on eqn. 2. Mobile phase contains 0.1 *M* NH_4Cl. Stationary phase, GS-320H.

TABLE I

PARTITION COEFFICIENTS (K_p) OF Ba^{2+} AND ALKALI METAL CATIONS TO PEG POLYMER PHASES

Polymer	Elution volume, V_e (ml)	K_p			
		Ba^{2+}	K^+	Rb^+	Cs^+
PEG 2000	6.43	980			
PEG 7500	5.27	440			
PEG 20 000	4.43	340	400	400	220
PEG 50 000	3.93	230			

a linear relationship between the molecular weight and the complex formation constant when the molecular weight is lower than 1000 [19,20]. It was reported previously that this enhancement of complex formation ability is caused by a statistical effect for relatively long PEG chains [20]. These results indicate that PEG of higher molecular weight shows a higher complex formation ability, which does not correlate with the present results. However, in the present experiments, we detected not only 1:1 complex formation but also multiple complex formation. Complex formation must affect the subsequent complexation of the adjacent binding sites. Kraus and Rogers [21] reported that the retention of PEG in SEC was changed by adding a salt to the mobile phase, and this effect was caused by the conformational change of PEG induced by the complex formation. The conformational changes and repulsion from metal cations trapped in PEG lead to unfavourable conditions for consecutive complexation. Highly multiple complex formation therefore becomes more difficult. In the present instance, the binding constant calculated from the corresponding partition coefficient is the mean value of consecutive complexation constants, and is smaller than the formation constant of a 1:1 complex.

Fig. 3 shows a chromatogram of some metal ions with PEG 20 000 as a mobile phase modifier. Although the separation efficiency is not high because of the narrow elution range, the elution order is unique. An advantage of the present method is thus not the efficiency but the unique selectivity.

Separation of anions

Despite much effort, common complexing agents for anions, comparable to crown ethers, cryptand,

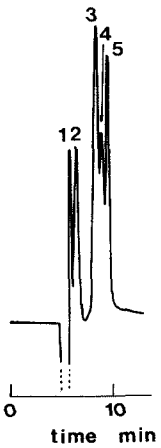

Fig. 3. Separation of alkali metal cations and Ba^{2+} with secondary equilibrium SEC with methanol containing 0.75 m*M* PEG 2000. Stationary phase, GS-320H. Detection, RI ($8 \cdot 10^{-5}$ f.s.). Peaks: 1 = K^+; 2 = Cs^+; 3 = Ba^{2+}; 4 = Li^+; 5 = Na^+.

cycrodextrins, etc., have not been found. Electrostatic interaction should therefore be employed to separate anions by secondary equilibrium SEC with a polymeric mobile phase additive. A cationic polymer (polybrene) wss selected for this purpose. As it was difficult to determine the partition coefficients precisely because of the weak retention ability of the stationary phase, the stationary phase (GS-310H) pre-equilibrated with a hexadecyltrimethylammonium (HTA) bromide solution (8 m*M* in 50% methanol) was used. Partition coefficients obtained in 0.05 *M* sodium chloride solution are listed in Table II. K_p values for NO_2^- and IO_3^- could not be determined because of their weak affinity to polybrene.

TABLE II

PARTITION COEFFICIENTS (K_p) OF INORGANIC ANIONS TO POLYBRENE POLYMER PHASE AND COMPARISON WITH PARTITION COEFFICIENTS (K_m) TO HTA CHLORIDE MICELLAR PHASE

Partition coefficients were determined in 0.05 NaCl.

Anion	K_p	K_m[a]
NO_2^-	—[b]	74.3
NO_3^-	240	189
I^-	660	850

[a] From ref. 8.
[b] Not determined.

TABLE III

EFFECTIVE REDUCTION OF RETENTION TIMES WITH POLYMER MOBILE PHASE ADDITIVES IN ION-INTERACTION CHROMATOGRAPHY

Mobile phase composition	Retention times calculated			
	IO_3^-	NO_2^-	NO_3^-	I^-
69 m*M* NaCl	7.0	8.3	10.8	20.0
0.51 g/l polybrene with 50 m*M* NaCl	7.0	8.5	11.4	20.0
0.11 *M* NaCl	6.9	6.8	9.9	15.0
2.1 g/l polybrene with 50 m*M* NaCl	7.0	8.5	10.5	15.0

Soldi *et al.* [22] reported that cationic polyelectrolytes behaved like cationic micelles with respect to counter-ion binding, counter-ion-exchange selectivity, etc., and concluded that this type of polyelectrolyte could be a micelle-mimetic system. The author previously determined partition coefficients of inorganic anions to HTA chloride micelles under various ionic strength conditions. In 0.05 *M* sodium chloride solution, the partition coefficients of NO_2^-, NO_3^- and I^- to HTA chloride micelles were determined as 74.3, 189 and 850, respectively [8]. These values in general correlate with the partition coefficients to polybrene as shown in Table II.

In anion-exchange chromatography, elution of analytes is usually accelerated or retarded only by changing the salt concentration in the mobile phase. In such cases, there is a linear relationship between log k' and the logarithm of salt concentration. This means that a high salt concentration reduces the retention time of all analytes; this often causes poor resolution between analytes that are eluted rapidly. In Table III, retention times obtained, when mobile phases capable of eluting I^- at 15 and 20 min were used are given. A high salt concentration causes co-elution of NO_2^- and IO_3^-, whereas a high polymer concentration does not result in a poor separtion between these anions.

CONCLUSIONS

Polymeric mobile phases in secondary equilibrium SEC of ions are effective for modification of the

chromatographic selectivity, reduction of the total analysis time, the separation of small analytes on a size-exclusion column even when the stationary phase is intrinsically unable to retain the analytes and the evaluation of the partition behaviours of analytes to polymer phases. Polymeric additives thus provide versatility for SEC.

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REFERENCES

1 M. T. W. Hearn, *Ion-Pair Chromatography*, Marcel Dekker, New York, 1985.
2 D. W. Armstrong, F. Nome and L. A. Spino, *J. Am. Chem. Soc.*, 108 (1986) 1418.
3 T. Nakagawa, A. Shibukawa and T. Uno, *J. Chromatogr.*, 239 (1982) 685.
4 G. J. Sevenich and J. S. Fritz, *Anal. Chem.*, 55 (1983) 12.
5 D. W. Armstrong, *Sep. Purif. Methods*, 14 (1985) 213.
6 T. Okada, *Anal. Chem.*, 62 (1988) 1511.
7 T. Okada, *Anal. Chem.*, 62 (1988) 2116.
8 T. Okada, *J. Chromatogr.*, 538 (1991) 341.
9 J. P. Foley and W. E. May, *Anal. Chem.*, 59 (1987) 102.
10 B. L. Karger and J. N. LePage, in C. Horváth (Editor), *High Performance Liquid Chromatography: Advances and Perspectives*, Vol. 1, Academic Press, New York, 1980, p. 113.
11 W. R. Melander and Cs. Horváth, in Cs. Horváth (Editor), *High Performance Liquid Chromatography: Advances and Perspectives*, Vol. 2, Academic Press, New York, 1980, p. 113.
12 M. Shibukawa and N. Ohta, in P. L. Dubin (Editor), *Aqueous Size-Exclusion Chromatography*, Elsevier, Amsterdam, 1988, Ch. 4; and references cited therein.
13 T. G. Tji, H. J. Krips, W. J. Gelsema and C. L. de Ligny, *J. Chromatogr.*, 504 (1990) 403.
14 N. V. B. Marsden, *J. Chromatogr.*, 319 (1985) 247.
15 M. Nakagaki, in *Hyomen Jotai to Colloid Jotai*, Tokyo Kagaku Dojin, Tokyo, 1968, Ch. 1.
16 B. L. Haymore, J. D. Lamb, R. M. Izatt and J. J. Christensen, *Inorg. Chem.*, 21 (1982) 1598.
17 F. Vögtle and E. Weber, *Angew. Chem., Int. Ed. Engl.*, 18 (1979) 753.
18 J. Cross, in J. Cross (Editor), *Nonionic Surfactant—Chemical Analysis*, Marcel Dekker, New York, 1987, Ch. 2; and references cited therein.
19 J. W. Gokel, D. M. Goli and R. A. Schultz, *J. Org. Chem.*, 48 (1983) 2837.
20 T. Okada, *Macromolecules*, 23 (1990) 4216.
21 S. Kraus, L. B. Rogers, *J. Chromatogr.*, 257 (1983) 237.
22 V. Soldi, N. de M. Erismann, F. H. Quina, *J. Am. Chem. Soc.*, 110 (1988) 5137.