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Short communication

Analysis of complex formation between crown ethers and potassium ion by determining retention factors in reversed-phase high-performance liquid chromatography

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Abstract

Retention factors in reversed-phase high-performance liquid chromatography (RP-HPLC) were utilized for the analysis of complex formation reaction of four crown ethers with potassium ion in water–methanol media. The crown ethers were injected as analytes, potassium ion was present in the eluent, and three types of reversed-phase columns were used. The retention factor of the crown ethers decreased with increasing concentrations of potassium ion in the eluent, which is attributed to the change in the species of the crown ether from a neutral ligand to a positively charged complex. The complex formation constants were determined by analyzing the changes in the retention factor with a non-linear least-squares method. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Complex formation; Retention factors; Crown ethers; Potassium

1. Introduction

Reversed-phase high-performance liquid chromatography (RP-HPLC) has been utilized for the separation and the determination of various types of analytes. The principle of the separation is based on the partition of analytes from a mobile phase to a hydrophobic stationary phase. Studies concerning retention mechanism have been performed and are reviewed elsewhere [1–3]. To control the retention factors, interacting reagents have often been used in the eluent; changes in the retention factors are attributed to an equilibrium reaction between the analytes of interest and the interacting reagent in the

eluent. Acid–base reactions have been investigated by using the changes in retention factors [4,5], and also ion interaction was investigated [6,7].

Crown ethers are one of the most famous macrocyclic compounds, and can form a host–guest complex [8], and were utilized for RP-HPLC separation of amino compounds [9,10]. Recently, Okada and co-workers investigated the quantitative analysis of complex formation reactions between alkali metal ions and some polyethers including crown ethers in cation-exchange resin phase [11–13] and in solution [14] by using ion chromatography. It is noteworthy that the complexation occurs in the stationary resin phase. However, the analysis of the complexation in the solution (mobile phase) is complicated by the ion chromatographic method [14]. In RP-HPLC, alkali metal ions are less adsorptive to the stationary phase,

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and therefore it would be very easy to analyze the complex formation reaction in the mobile phase. In this study, the change in the retention factor of crown ethers in RP-HPLC was utilized for the equilibrium analysis of the complex formation with potassium ion.

2. Experiments

2.1. Apparatus

A Yokogawa (Tokyo, Japan) LC-100 system was used for HPLC, and a Hitachi (Tokyo, Japan) D-2500 Chromato-Integrator was used to record the chromatograms. Separation columns of Devesil C8-UG-5 (50 mm×4.6 mm I.D.; Nomura, Tokyo, Japan), TSK-GEL ODS-80TM CTR (100 mm×4.6 mm I.D.; Tosoh, Tokyo, Japan), and Devesil C30-UG-5 (150 mm×4.6 mm I.D.; Nomura) were examined by attaching them to the HPLC system.

2.2. Reagent

Dibenzo-18-crown-6 (DB18C6) was synthesized according to the literature from catechol and bis(2-chloroethyl) ether [8] and used after recrystallization from benzene. Dibenzo-21-crown-7 (DB21C7), dibenzo-24-crown-8 (DB24C8), and dibenzo-30-crown-10 (DB30C10) were purchased from Wako (Osaka, Japan), Tokyo Kasei Kogyo (Tokyo, Japan), and Aldrich (Milwaukee, WI, USA), respectively. Methanol used was HPLC grade (Wako). An acetate buffer composed of acetic acid and potassium acetate was used as an eluent in RP-HPLC. Potassium chloride, potassium nitrate or potassium perchlorate was also added to the eluent to control the total concentration of K^+ . Other reagents used were of analytical-reagent grade. Water used was deionized and distilled.

2.3. Procedure

The eluent used in RP-HPLC contained 55% (v/v) methanol, 2 mM acetate buffer (pH 4.7 in water; $[K^+]=1$ mM) and variable amounts of KCl or $KClO_4$. The eluents were propelled at a flow-rate of 0.5 ml/min. After the column was equilibrated, a

sample solution containing the crown ethers ($5 \cdot 10^{-5}$ M) was injected using a six-way rotary valve (injection volume: 20 μ l), and the crown ethers were detected photometrically at 220 nm. Throughout the experiments, the column was held in a thermostated water bath kept at 25.0°C. The retention factors of the crown ethers were obtained in a usual manner.

3. Results and discussion

3.1. Retention factors of crown ethers

In the measurement of the retention factors, the acetate buffer was chosen as a pH buffer from the viewpoint of the stability of the pH of the eluent and that of the reversed-phase column. Crown ethers used in this study are electrically neutral ones, and therefore, they can be retained well on a hydrophobic stationary phase. Typical chromatograms for the crown ethers are shown in Fig. 1, where TSK-GEL ODS-80TM CTR was used. Retention times of the crown ethers were in the order of DB18C6 < DB21C7 < DB24C8 < DB30C10; this order reflected the hydrophobic character of the crown ethers. As an interacting reagent, potassium ion was investigated in this study. By adding KCl to the eluent, the retention times of the crown ethers became shorter as is shown in Fig. 1b.

Changes in the retention factors of the crown ethers are shown in Fig. 2. The retention factors became smaller with increasing concentrations of KCl in the eluent, as is expected from the complex formation reaction. Positively charged complexes formed with K^+ were less hydrophobic than the crown ethers themselves, which resulted in the decrease of the retention time.

3.2. Determination of complex formation constants by using retention factors

In the analysis of equilibrium reactions using the retention factors obtained by RP-HPLC, the components of the eluent and the stationary phase are sometimes troublesome. Complexation reactions of crown ethers are usually less dependent on pH, and the effect of ionic strength on the equilibrium constants is small, because charged reactants form

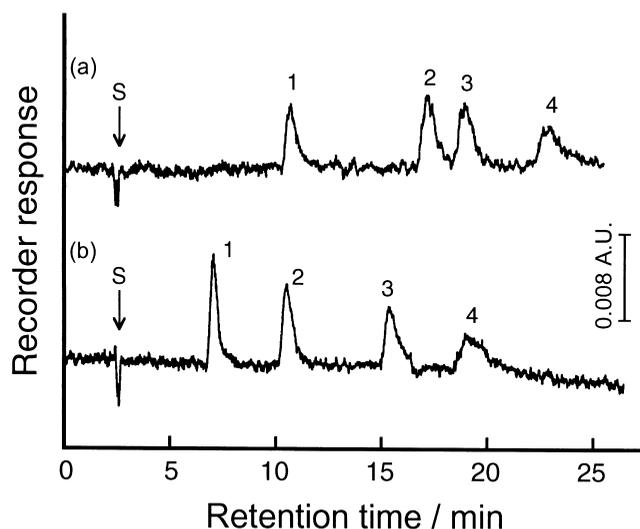


Fig. 1. Typical chromatograms for crown ethers. Sample: $5 \cdot 10^{-5}$ M crown ethers. Eluent: (a) 2 mM acetate buffer (pH 4.7) in 55% (v/v) methanol–water; (b) 2 mM acetate buffer (pH 4.7) + 10 mM KCl in 55% (v/v) methanol–water. HPLC conditions: column, TSK-GEL ODS-80TM CTR (100×4.6 mm I.D.); detection wavelength, 220 nm; column temperature, 25.0°C; sample volume, 20 μ l. Signal identifications: 1, DB18C6; 2, DB21C7; 3, DB24C8; 4, DB30C10.

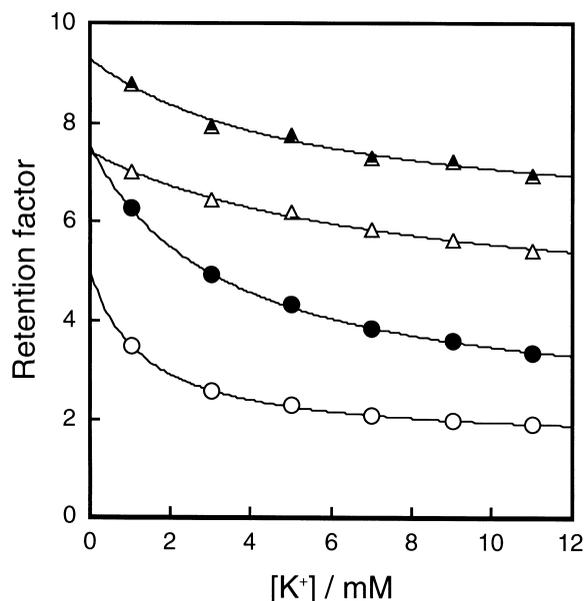


Fig. 2. Changes in retention factor of crown ethers with increasing concentration of K^+ . HPLC conditions, except for KCl concentrations, are the same as in Fig. 1. \circ , DB18C6; \bullet , DB21C7; \triangle , DB24C8; \blacktriangle , DB30C10.

equally charged products by the reactions of metal ions with electrically neutral crown ethers; in this study, the ionic strength is in the range between 0.001 and 0.011. Therefore, the reaction system adopted in this study is suitable for the equilibrium analysis.

The complex formation reactions of the crown ethers with potassium ion were analyzed by using the retention factors. When a 1:1 reaction is supposed, the reaction with its equilibrium are written in Eqs. (1) and (2):



$$K_{ML} = \frac{[KL^+]}{[K^+][L]} \quad (2)$$

where L denotes crown ether and K_{ML} is a complex formation constant. The retention factor for a certain crown ether, k , is affected by those of the free crown ether, k_L , and the complexed crown ether, k_{ML} , as is written in Eq. (3):

$$k = \frac{[L]}{[L] + [KL^+]} k_L + \frac{[KL^+]}{[L][KL^+]} k_{ML} \quad (3)$$

By the combination of the equilibrium constant

(2) and the mass balance for L with Eq. (3), the following equation can be derived:

$$k = \frac{1}{1 + K_{ML}[K^+]}k_L + \frac{K_{ML}[K^+]}{1 + K_{ML}[K^+]}k_{ML} \quad (4)$$

A series of pairs of $[K^+]$ and k were placed in Eq. (4), and a non-linear least-squares analysis provided K_{ML} value, as well as k_L and k_{ML} values. The values obtained are summarized in Table 1.

3.3. Effect of counter anions on the complex formation reaction

Reversed-phase columns sometimes retain electrically neutral ion associates even when they are hydrophilic. In the present system, the ion associates of the potassium complexes with chloride ion can be suspected. Therefore, the retention factors of the crown ethers were also measured using $KClO_4$ and KNO_3 as a source of potassium ion instead of KCl , and the complex formation constants were determined in the same manner. When KNO_3 was used, UV absorption of nitrate ion around 220 nm inter-

fered with the detection of the crown ethers, and the retention factors could not be determined. The constants of K_{ML} obtained with $KClO_4$ are also summarized in Table 1. The complex formation constants determined using $KClO_4$, as well as the retention factors of k_L and k_{ML} , are very close to those obtained using KCl . These results suggest that the side reaction of the ion association between the potassium complexes and the counter ions is sufficiently negligible.

3.4. Comparison of separation columns

Retention factors of the crown ethers using different types of the separation column were measured, and the K_{ML} values were obtained by analyzing the retention factors. Although the size and the packing materials of each column examined in this study are different, and the retention factors can be varied depending on the size and the packing material, the complex formation constants are essentially identical as is summarized in Table 1. These results indicate that the equilibrium reactions in mobile phase are not

Table 1
Complex formation constants of crown ethers with K^+ by the RP-HPLC method in 55% (v/v) methanol–water medium

Crown ether	Counter ion	Column ^a	Log K_{ML} ^b	k_L	k_{ML}
DB18C6	Cl^-	C_8	2.69 ± 0.09	4.20	0.97
DB18C6	ClO_4^-	C_8	2.65 ± 0.07	3.25	1.11
DB18C6	Cl^-	C_{18}	2.87 ± 0.03	4.97	1.56
DB18C6	Cl^-	C_{30}	2.78 ± 0.07	8.40	2.68
DB21C7	Cl^-	C_8	2.36 ± 0.07	7.87	1.38
DB21C7	ClO_4^-	C_8	2.39 ± 0.06	6.64	1.64
DB21C7	Cl^-	C_{18}	2.47 ± 0.03	7.52	2.14
DB21C7	Cl^-	C_{30}	2.41 ± 0.07	18.30	3.64
DB24C8	Cl^-	C_8	1.94 ± 0.18	8.16	3.16
DB24C8	ClO_4^-	C_8	1.91 ± 0.18	6.99	3.33
DB24C8	Cl^-	C_{18}	2.11 ± 0.07	7.43	4.14
DB24C8	Cl^-	C_{30}	2.00 ± 0.22	19.92	10.13
DB30C10	Cl^-	C_8	1.99 ± 0.19	7.87	1.38
DB30C10	ClO_4^-	C_8	1.97 ± 0.23	7.71	5.22
DB30C10	Cl^-	C_{18}	2.26 ± 0.13	9.28	5.90
DB30C10	Cl^-	C_{30}	2.25 ± 0.14	24.08	11.41

^a Type of the reversed-phase column. C_8 : Develosil C8-UG-5; C_{18} : TSK-GEL ODS-80TM CTR; C_{30} : Develosil C30-UG-5.

^b Error: 3σ .

affected by the type of the column. Among the columns examined, Develosil C30-UG-5 showed the largest retention factors for neutral crown ethers, as well as the charged complexes; the results reflected the larger hydrophobicity of the column.

3.5. Effect of methanol concentration on the complex formation reaction

Retention factors of the crown ethers were measured by varying methanol percentage in the eluent in the range of 55–70% (v/v). Peaks of the crown ethers became very broad at a methanol percentage less than 50% (v/v), and a series of the experiment with changing concentrations of KCl could not be carried out. Therefore in the RP-HPLC method, water medium cannot be employed for the equilibrium analysis of crown ethers. Methanol medium also highlights difficulties in the equilibrium analysis; the crown ethers were less retained and the analysis was quite difficult. In any case, the retention factors were smaller with the larger percentage of methanol examined. Non-linear least-squares analysis provided the K_{ML} values at each methanol percentage; they are summarized in Table 2. The K_{ML} values obtained were large when the methanol percentage was high. The results can be explained from the electrostatic interactions, which can be increased in the solvent of lower dielectric constant, and the interaction between the cation and crown ether becomes stronger with the methanol-rich solvent. Solvation of potassium ion is also supposed to be another reason. However, the effect should be supplemental, because potassium ion is less hydrated in water medium.

4. Conclusions

Complex formation constants between potassium ion and electrically neutral crown ethers are analyzed by using retention factors in RP-HPLC. The RP-HPLC method provided the equilibrium analysis of crown ethers in solutions, as well as other methods [15] including calorimetry, conductivity, ion selective electrode, solubility, spectrophotometry, and nuclear magnetic resonance spectroscopy.

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Table 2

Complex formation constants of crown ethers with K^+ by the RP-HPLC method with different methanol percentage

Methanol percentage	Column ^a	Log K_{ML} ^b			
		DB18C6	DB21C7	DB24C8	DB30C10
55	C ₁₈	2.87±0.03	2.47±0.03	2.11±0.07	2.26±0.13
65	C ₁₈	2.98±0.03	2.58±0.04	2.15±0.10	2.57±0.10
70	C ₃₀	3.29±0.11	2.83±0.08	2.38±0.15	3.13±0.18

^a Type of the reversed-phase column. C₁₈: TSK-GEL ODS-80TM CTR; C₃₀: Develosil C30-UG-5.

^b Error: 3 σ .

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