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## Review

# Polyethers in inorganic capillary electrophoresis

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### Abstract

Various additives are employed in running solutions in capillary electrophoresis (CE) to enhance separation performance and selectivity. Complexing agents are successfully employed (in most cases indispensable) in inorganic CE, especially for the separation of metal ions. Studies in inorganic analytical chemistry have been mostly directed to the methodological developments of the analyses of transition metal ions, and extensive efforts have permitted development of a number of effective reagents for their determination. Thus, there are numerous reagents forming complexes with transition metal ions. In contrast, ligands effectively complexing main group metal cations are very few. Polyethers are rather unique examples of such ligands capable of effectively interacting with hard cations. Thus, this naturally leads to designs of separation where polyethers are incorporated in running solution of CE to achieve better separations of hard cations. Polyethers have another interesting feature which is also potentially useful in CE separation; polyether–water mixtures provide unique electrophoretic separation media, which allow one to modify electrophoretic separation selectivity much more effectively than usual organic solvents. In this review, the author discusses roles of polyethers in inorganic CE from two different viewpoints, (1) complex formation and (2) separation medium modifiers, and to provide the perspectives of these useful compounds in inorganic CE. Some new data are also presented to show the ability of polyethers as medium modifiers. © 1999 Elsevier Science B.V. All rights reserved.

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## 1. Introduction

Ion-exchange chromatography and its related methods, such as ion-exclusion and ion-pair (or interaction) chromatography, have been extensively used for the separation and the determination of inorganic ionic species for both fundamental and practical purposes; these chromatographic modes are often combined under the single term ion chromatography [1,2]. These chromatographic methods have allowed us to analyze simple ions of various charges, contained in a wide variety of samples. However, chromatographic methods have the common limitation concerning the nature of stationary phases; for example, pH values, organic solvent concentrations, and the viscosity of mobile phases are restricted by the properties and the chemical or mechanical durability of the stationary phases. Capillary electrophoresis (CE), which has been rapidly developed for the past decade, can be an alternative choice in ion analyses. CE has some advantages over chromatographic methods: (1) separation performance (e.g., theoretical plate number) is higher; (2) CE generally contains no stationary phases, and thus we may use any separation media; (3) varying separation media is much easier; (4) separation mechanisms of CE are much clearer than those in chromatography, and therefore the prediction and the modification of separation are also straightforward.

As discussed below, the intrinsic electrophoretic mobility of an ion is determined by the charge and the size of the ion in solution, and can be calculated from ionic molar conductivity data. One may indicate that ionic conductivity is closely related to the ion-exchange selectivity as well. However, the ion-exchange selectivity is determined by a number of factors, such as the electrostatic interaction between an ion and an active group in a resin, the specific adsorption of the ion on the resin, a change in the solvation of the ion etc., and thus should be explained on the basis of much more complex mechanisms [3–5]. The intrinsically simple mechanisms of CE facilitate the prediction and optimization of separation, but possibly result in worse separation when some solute ions have similar ionic conductivity. In such cases, appropriate separation modifiers should be incorporated in running solutions to

achieve required separation. There are two simple ways to modify apparent conductivity of ions; one is changing solvent properties, and the other is the addition of reagents selectively interacting with solutes. If an appropriate cosolvent is added to the primary solvent (usually water), the solvent properties can be successively modified. However, it is of general difficulty to predict the nature of mixed solvents from physicochemical properties of individual components, and thus a use of mixed solvents somewhat diminishes an advantage of simple mechanisms in CE. In contrast, effects of interacting agents on CE separation are predictable from usual equilibrium rules. We can, for example, utilize complexing agents to modify the microenvironments around the cations; this allows to change the effective charges, the sizes and the mobility of cations. In addition, compiled equilibrium data for complexation of various reagents facilitate the prediction of CE behaviors of solutes under given conditions. Thus, the addition of appropriate ligands in running buffers is a basic and mostly effective strategy to enhance CE separation selectivity ([6], and references cited therein).

Most of complexing reagents utilized to the chromatographic separation of inorganic cations are directly applicable to CE separation of cations. CE promises further versatility in the applicability of reagents because careful attention to the nature of media is not necessary; we can use very concentrated, highly acidic or alkaline, and viscous solutions, which cannot be applied to chromatography. In addition, high separation performance of CE allows to utilize even weak interactions, and provide us with useful information of the chemistry taking place in the media. In this review, the author focuses his discussions on polyethers in inorganic CE. Polyethers and their complexation have received much attention in various branches of chemistry for the past two decades. These studies have indicated that water is not a suitable solvent for polyether complexation because of its rather strong solvation ability. Although polyethers can strongly interact with cations in some other solvents, the methodological developments of CE in water or water-rich media are preferable for its practical utilization. Water and hydro-organic mixtures can therefore be assumed to be main CE separation media. Roles of

polyethers in CE are discussed from two different viewpoints, i.e., complexing agents and medium modifiers.

## 2. Complexation of polyethers

### 2.1. Relation between complexation and electrophoretic mobility

As is well known, the electrophoretic mobility of an ion is related to its molar conductivity ( $\lambda$ ), which is represented by the following relation [7].

$$\lambda = \kappa/c = z^2 F^2 v \quad (1)$$

where  $\kappa$ ,  $c$ ,  $z$ ,  $v$  and  $F$  are the conductivity, the concentration, the charge, the velocity under unit

electrochemical potential slope of an ion of interest and the Faraday constant, respectively. The molar conductivity can then be related to the ionic mobility ( $u$ ) of the ion

$$u = \lambda/(|z|F) \quad (2)$$

Table 1 lists the limiting molar conductivity data for selected ions [8–13]. Some ions, for example  $K^+$  and  $NH_4^+$ , and  $Mg^{2+}$ ,  $Mn^{2+}$  and  $Ni^{2+}$ , have almost identical  $\lambda$  values; this predicts identical mobility and in turn difficulty in CE separation of these ions. The relation between ionic mobility and the size of the ion is known as Stokes' law.

$$u = \frac{|z|e}{6\pi r\eta} \quad (3)$$

The Stokes radii ( $r$ ), estimated from Eq. (3) are

Table 1  
Molar conductivity ( $\lambda$ ) of cation-crown ether complexes at 25°C

Crown ether	Cation or salt	Concentration of salt (mM)	Solvent	$\lambda$ (S cm <sup>2</sup> mol <sup>-1</sup> )	Ref.
18-Crown-6	$K^+$	0	Water	25.3	[8]
	$K^+$	0	MeOH <sup>a</sup>	39.9	[8]
	$K^+$	0	MeCN <sup>b</sup>	59	[8]
	NaCl	0.856	MeOH	89.18	[10]
	CsCl	0.805	MeOH	89.39	[10]
B18C6 <sup>c</sup>	$K^+$	0	MeOH	38.4	[9]
DB18C6 <sup>d</sup>	$K^+$	0	MeOH	39.9	[9]
15-Crown-5	LiCl	0.561	MeOH	99.6 (89.3) <sup>e</sup>	[11]
	NaCl	0.515	MeOH	94.6 (94.6)	[11]
	KCl	1.089	MeOH	90.2 (98.3)	[11]
	RbCl	0.516	MeOH	105.6 (89.4)	[11]
Without	$K^+$	0	Water	73.5	[13]
		0	MeOH	52.4	[13]
		0	MeCN	83.6	[13]
	$Na^+$	0	Water	50.1	[13]
	$NH_4^+$	0	Water	73.5	[13]
	1/2 $Mg^{2+}$	0	Water	53.3	[13]
	1/2 $Mn^{2+}$	0	Water	53.5	[13]
	1/2 $Ni^{2+}$	0	Water	53.4	[13]
	$Cl^-$	0	Water	76.3	[13]
		0	MeOH	52.3	[13]
	$Br^-$	0	Water	78.1	[13]
	$I^-$	0	Water	76.8	[13]

<sup>a</sup> Methanol.

<sup>b</sup> Acetonitrile.

<sup>c</sup> Benzo-18-crown-6.

<sup>d</sup> Dibenzo-18-crown-6.

<sup>e</sup> Conductivity of an uncomplexed salt in parentheses.

sometimes inconsistent with other radius data, because the viscosity of a bulk solvent ( $\eta$ ) is different from that around the ion. Thus, conductivity (or mobility) well reflects circumstances around ions, which can be effectively modified by complexation.

Conductometry is one of the most powerful methods for the determination of the complexation constants of polyethers with ions in solution [8–12]. The conductometric determination of polyether complexation constants is based on differences in the molar conductivity between a solvated ion and a complexed ion. Fig. 1 shows changes in molar conductivity, calculated on the basis of the assumption of constant salt concentration and 1:1 complexation.

$$\Lambda = \lambda_A + (1 - \alpha)\lambda_M + \alpha\lambda_{ML} \quad (4)$$

where  $\alpha$  is the degree of complexation, and subscripts, A, M and ML are an anion, a cation, and its 1:1 complex, respectively. As shown in Table 1,  $\lambda_M$  is usually larger than  $\lambda_{ML}$ , indicating that electrophoretic mobility is reduced by complexation. In any case, the regression analysis based on Eq. (4) gives  $K$  and  $\lambda_{ML}$ .

Electrophoretic velocity ( $v_{ep}$ ) of a cation in the presence of polyethers can be given by

$$v_{ep} = \frac{E}{F} \cdot [(1 - \alpha)\lambda_M + \alpha\lambda_{MCr}] \quad (5)$$

where  $E$  is an electric field. Eq. (5) indicates that the electrophoretic mobility of a cation under a given electric field changes in a manner similar to depicted in Fig. 1. Molar conductivity is a function of the concentration of a salt or the ionic strength of a solution, and thus not a constant. This concentration dependence of molar conductivity can be described by e.g., Onsager's equation.

$$\lambda = \lambda_0 - \left\{ \frac{e^2 \lambda_0}{24 \pi \epsilon_0 \epsilon k T} (1 + \sqrt{0.5}) \right\} - \frac{F^2}{6 \pi \eta N} \kappa / (1 + \kappa a) \quad (6)$$

$$\kappa = [2N^2 e^2 / (\epsilon_0 \epsilon R T)]^{1/2} \sqrt{I} \quad (7)$$

where  $\lambda_0$ ,  $e$ ,  $\epsilon_0$ ,  $\epsilon$ ,  $k$ ,  $\eta$ ,  $N$ ,  $a$  and  $I$  are the molar conductivity at infinite dilution, the elementary electric charge, the dielectric constant of vacuum, a specific dielectric constant, the Boltzmann constant, the viscosity of a solvent, the Avogadro's number,

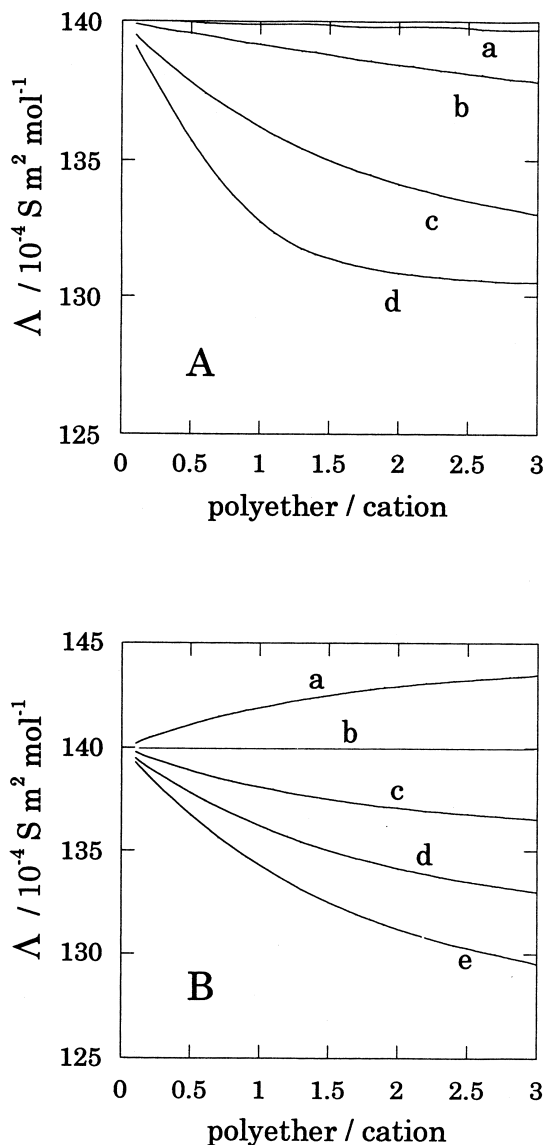


Fig. 1. Estimated changes in molar conductivity by polyether complexation. (A) An effect of complex formation constants ( $K$ ) of a polyether with a cation. (B) An effect of ionic molar conductivity of a cation–polyether complex ( $\lambda_{MCr}$ ). (A)  $\lambda_{MCr} = 60 \text{ S cm}^2 \text{ mol}^{-1}$ , (a)  $K = 10 \text{ M}^{-1}$ , (b)  $K = 10^2 \text{ M}^{-1}$ , (c)  $K = 10^3 \text{ M}^{-1}$ , (d)  $K = 10^4 \text{ M}^{-1}$ ; (B)  $K = 10^3 \text{ M}^{-1}$ , (a)  $\lambda_{MCr} = 75 \text{ S cm}^2 \text{ mol}^{-1}$ ,  $\lambda_{MCr} = 70 \text{ S cm}^2 \text{ mol}^{-1}$ ,  $\lambda_{MCr} = 65 \text{ S cm}^2 \text{ mol}^{-1}$ ,  $\lambda_{MCr} = 60 \text{ S cm}^2 \text{ mol}^{-1}$ ,  $\lambda_{MCr} = 55 \text{ S cm}^2 \text{ mol}^{-1}$ ;  $\lambda_M = \lambda_A = 70 \text{ S cm}^2 \text{ mol}^{-1}$  for both A and B.

the distance between ions, and molar ionic strength. If no ion association occurs, this equation allows the concentration corrections for molar conductivity. Thus, we can predict the electrophoretic mobility under a given condition from complexation constants and molar conductivity data. Since the complexation of polyethers is generally weak in water, conductivity studies of polyether complexation in water are very few. The  $\lambda_{ML}$  data listed in Table 1 are mostly obtained in methanol not in water, but imply the general tendency of mobility changes by polyether complexation. The  $\lambda_{ML}$  value is generally smaller than  $\lambda_M$ , indicating that the complexation with polyethers makes an ion bulkier than its solvated counterpart. In a few cases, the conductivity of ions becomes larger ( $\text{Li}^+$  with 15-crown-5 in Table 1) by complex formation. Such unusual behaviors can be seen for weak complexation, implying that there remains strong solvation even after the complexation because crown ethers cannot completely wrap the ion. Thus, the electrophoretic mobility of an ion is usually reduced by the complexation of polyethers.

## 2.2. Choice of polyether ligands

Polyethers employed in CE running buffers as complexing agents should reasonably have the following properties to enhance the separation:

1. The solubility should be high
2. The complexation ability should be preferably high
3. The mobility of a resulting complex should be different from that of a solvated cation
4. The complexation should be selective to allow electrophoretic separation of target ions
5. If spectrometric detection is applied, a polyether should be transparent (or has low absorptivity) at a detection wavelength

Table 2 lists the selected complex formation constants of polyethers in water ([14], and references cited therein). Although water and hydro-organic mixed solvents are most generally used in CE, the polyether complexation data in these media are much fewer than those in organic solvents. Thus, the complete prediction of CE behaviors of cations in the presence of polyethers is sometimes difficult because of the lack of complexation and molar conductivity data. From the above criteria, only a

Table 2  
Complexation constants of polyethers in water at 25°C.

Polyether	Cation	log <i>K</i>
15-Crown-5	$\text{Na}^+$	0.7
	$\text{K}^+$	0.74
	$\text{Rb}^+$	0.62
	$\text{NH}_4^+$	1.71
	$\text{Pb(II)}$	1.85
	$\text{Tl(I)}$	1.23
	$\text{Ag(I)}$	0.94
18-Crown-6	$\text{Li}^+$	~0.80
	$\text{Na}^+$	0.80
	$\text{K}^+$	2.1, 2.03
	$\text{Rb}^+$	1.56
	$\text{Cs}^+$	0.99
	$\text{NH}_4^+$	1.23
	$\text{Ca}^{2+}$	0.5
	$\text{Sr}^{2+}$	2.72
	$\text{Ba}^{2+}$	3.87
	$\text{Ag(I)}$	1.50
	$\text{Cd(II)}$	5.31
	$\text{Pb(II)}$	4.27
	$\text{Tl(I)}$	2.27

From Ref. [14].

few polyethers can be employed to modify CE separation by complexation. Benzo- and dibenzo-crown ethers are, for example, effective ligands, and widely applied to various analytical means. However, dibenzo-crown ethers are quite insoluble in water, and, in addition, have rather strong absorption bands at wavelengths lower than 290 nm. Benzo-crown ethers are also UV-absorbing. Thus, these crown ethers are not suitable ligands according to the above criteria (1) and (5). Table 3 summarizes polyethers used in CE of inorganic cations [15–31], and clearly indicates that 18-crown-6 is the most common ligand [15–28]; this compound is not only freely soluble in water but UV-transparent, and shows rather strong complexation ability even in water as shown in Table 2. Although other crown ethers, such as 15-crown-5 and 12-crown-4, are also used in a few instances [15,16], the enhancement of separation selectivity is very small because of their rather weak complexation ability and poor selectivity.

Table 3 indicates that poly(ethylene glycol) (PEG) is another possible choice [30,31]. Though this compound is also UV-transparent and soluble in water, its complexation ability, depending on the

Table 3  
Use of polyethers to enhance CE separation selectivity

Polyether	Concentration	Media	Cations separated	Enhanced separation	Ref.	
18-Crown-6	<20 mM	Water	A, B, E	K <sup>+</sup> –NH <sub>4</sub> <sup>+</sup>	[15]	
	<3 mM	8% MeOH	A, B, D, E	K <sup>+</sup> –NH <sub>4</sub> <sup>+</sup>	[16]	
		Lactic acid, pH 4.3			Pb(II)–Ni(II) and Co(II)?	[16]
	<300 mM	32% MeOH			Na <sup>+</sup> –alkaline earth	[16]
		Water	A, B, E	K <sup>+</sup> –NH <sub>4</sub> <sup>+</sup>	[17]	
	2 mM	Imidazole, pH 4.5				
		2% MeOH	A, B, D, E	K <sup>+</sup> –NH <sub>4</sub> <sup>+</sup> , Sr(II)–Ba(II)	[18]	
	40 mM	Glycine, pH 6.5		Alkylammonium ions		
		Water	A, B, E	K <sup>+</sup> –NH <sub>4</sub> <sup>+</sup> –Rb <sup>+</sup> –Cs <sup>+</sup>	[19]	
	<10 mM	Tart, pH 5.2			Ba(II)–Mg(II), Li <sup>+</sup> –Sr(II)	
		Water	A, B, D, E	K <sup>+</sup> –NH <sub>4</sub> <sup>+</sup> , Na <sup>+</sup> –Pb(II)	[20]	
	<2 mM	Lactic acid, pH 4.5				
		Water	A, B, E	K <sup>+</sup> –NH <sub>4</sub> <sup>+</sup>	[21]	
	<2.7 mM	Tart, pH 4.05				
		20% MeOH	A, B, D, E	K <sup>+</sup> –NH <sub>4</sub> <sup>+</sup>	[22]	
2.5 mM	(6.5 mM HIBA, pH 4.5)					
	Water	A, B, E	K <sup>+</sup> –NH <sub>4</sub> <sup>+</sup> , Ca(II)–Sr(II)	[23]		
<5 mM	Ce(III)					
	Water	A, B, E	K <sup>+</sup> –NH <sub>4</sub> <sup>+</sup>	[24]		
<10 mM	Water	A, E	K <sup>+</sup> –NH <sub>4</sub> <sup>+</sup>	[25]		
	Water	A, B, E	K <sup>+</sup> –NH <sub>4</sub> <sup>+</sup> , Ca(II)–Sr(II)	[23]		
DicyHex-18-C-6	– <sup>a</sup>	Water	A, B, E	K <sup>+</sup> –NH <sub>4</sub> <sup>+</sup>	[15]	
	<100 mM	Water	A, B, E	None	[16]	
15-Crown-5	– <sup>a</sup>	Water	– <sup>b</sup>	None	[15]	
	<80 mM	Water	A, B, E	None	[15]	
12-Crown-4	– <sup>a</sup>	Water	– <sup>b</sup>	None	[16]	
	<80 mM	Water	A, B, E	None	[16]	
Cryptand-22	0.1 mM	Water–MeOH	A, E	Na <sup>+</sup> –NH <sub>4</sub> <sup>+</sup>	[29]	
PEG	<10%	Water	A, B, C, D, E	Zn(II)–Mn(II)	[30]	
(200–2·10 <sup>7</sup> )						
200	<60%	Water, tartrate	A, B, E	K <sup>+</sup> –NH <sub>4</sub> <sup>+</sup> , Rb <sup>+</sup> –Cs <sup>+</sup>	[31]	

A, Alkali metal ions; B, alkaline earth metal ions; C, lanthanide; D, d-block transition metal ions; E, ammonium ion.

<sup>a</sup> Not reported.

<sup>b</sup> Not specified.

chain length, is much lower than 18-crown-6, and not very effective ligands. However, much lower costs of PEG have allowed researchers to employ this compound at very high concentrations. Effects of polyethers on CE separation are discussed in more detail in the following section.

### 2.3. Separation enhancements by polyether complexation

As stated above, CE separation often suffers from the identical electrophoretic mobility (or conductivity) of more than two ions. Since the electrophoretic mobility of ions is determined by the charges and the sizes of the ions, changing solvents

and adding appropriate ligands are usually effective to enhance electrophoretic separation selectivity of cations. For the CE separation of transition metal ions, various ligands are added in running buffers, where two cases can be distinguished, i.e., pre-column (or off-line) complexation and on-column complexation. Porphine-derivative [32] and  $\beta$ -diketonato complexes [33] have been separated by CE after off-line complexation, and tartrate (tart), lactate,  $\alpha$ -hydroxyisobutyrate (HIBA), EDTA derivatives etc., [16,18–22,34–39] have been extensively used for on-line complexation. Polyether complexation is kinetically very fast ([14], and references cited therein), and thus applicable only to on-line procedure (simple addition of a polyether in running

buffers). Since polyethers form complexes with hard cations, including alkali, alkaline earth and lanthanide ions, the selectivity enhancements in the separation of these cations are expected.

Fig. 2 shows effects of the addition of 12-crown-4, 15-crown-5 and 18-crown-6 on the relative electrophoretic mobility of alkali and alkaline earth cations [16]. The effects of 18-crown-6 are remarkable, while only very small effects can be seen for the others even with rather high concentrations. As shown in Table 2, 18-crown-6 complexation ability is highest for  $K^+$  within a series of alkali metal cations, and for  $Ba^{2+}$  within that of alkaline earth cations. Thus, the electrophoretic mobility of  $K^+$  and  $Ba^{2+}$  is more markedly reduced by 18-crown-6 complexation than other alkali or alkaline earth cations. The complexation selectivity of crown ethers has been often explained by the size-fit theory, where fits of the cavity sizes of crown ethers with the radii of cations are thought to be a predominant factor governing complexation selectivity. Indeed, the cavity of 18-crown-6 fits well with naked  $K^+$  and  $Ba^{2+}$  ([14], and references cited therein). However, crown ethers having larger cavity sizes, such as dibenzo-30-crown-10, still prefer  $K^+$  to other alkali cations; in the complexes of such large cyclic ligands, it is known that they can completely wrap the ion and stabilize complexes. This indicates that the cavity sizes determined by assuming rigid structures of crown ethers have limited significance in the discussion of the complexation selectivity and ability of crown ethers. In addition, though the cavity size of 15-crown-5 (ca. 0.9 Å) is almost equal to the size of  $Na^+$  (1.02 Å) rather than that of  $K^+$  (1.38 Å), this crown ether forms a stable complex with  $K^+$ . Thus, the size-fit theory is not necessarily valid. In the complexation of smaller cations, such as  $Na^+$ ,  $Li^+$  and  $Mg^{2+}$ , complexation energy cannot compensate for the very large energy loss in the desolvation process of cations.

$K^+$  and  $NH_4^+$  have almost identical sizes and hydration energies [13]. Although these data predict the similar crown-ether complexation constants for both cations, log  $K$  values are substantially different as listed in Table 2. This is due to the structural difference between  $K^+$ - and  $NH_4^+$ -complexes. The cation is located at the center of the crown ether cavity in  $K^+$ -18-crown-6 complex, while  $NH_4^+$  is

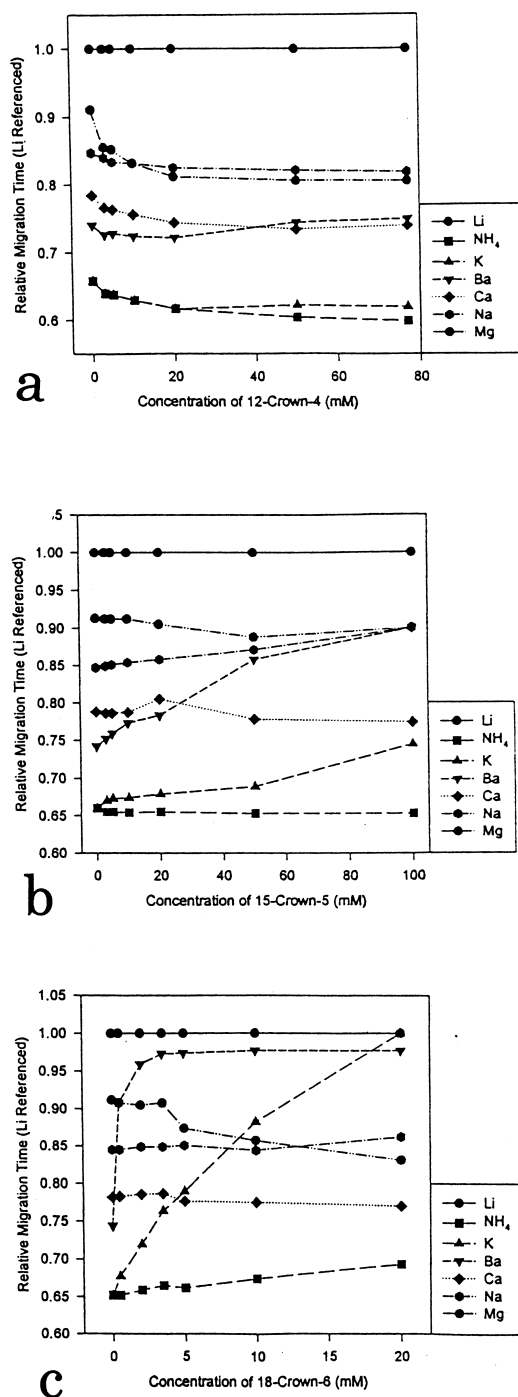


Fig. 2. Effects of added crown ethers on relative migration times. From Ref. [17].

located at a distance of ca. 0.4 Å above the mean plane of the crown ether [40]. Thus, the structural difference results in more stable complexation of 18-crown-6 with  $K^+$ . This selective complexation of 18-crown-6 enables the CE separation of  $K^+$  and  $NH_4^+$ ; Table 3 suggests that the major purpose of 18-crown-6 addition is the separation of  $K^+$  and  $NH_4^+$ . The migration order of alkali metal cations (plus ammonium ion) without ligands is  $Rb^+ \sim Cs^+ < K^+ = NH_4^+ < Na^+ < Li^+$ , whereas the order with e.g., 40 mM 18-crown-6 is  $Cs^+ < NH_4^+ < Rb^+ < Na^+ < Li^+ < K^+$ ; selectivity depends on the concentration of 18-crown-6. Also, the migration order of alkaline earth metal cations is completely reversed from  $Ba(II) < Sr(II) < Ca(II) < Mg(II)$  to  $Mg(II) < Ca(II) < Sr(II) < Ba(II)$  by the addition of this ligand. This result is also predictable from the complexation selectivity of 18-crown-6 in MeOH; complexation constants increase in the order of  $Mg(II) < Ca(II) < Sr(II) < Ba(II)$  ([14], and references cited therein).

The 18-crown-6 modifies CE separation of other relatively hard transition metal cations as well. Effects on the electrophoretic migration of  $Pb(II)$  have been reported in some instances [16,20]. It is reported that the migration time of  $Pb(II)$ , for example, is increased by 47% with a running solution containing 0.5 mM 18-crown-6 [20]; this effect depends on the nature and concentrations of incorporated complexing agents because complexing agents (lactic acid in this particular case), which are employed to enhance the separation of transition cations, compete with 18-crown-6 in the complexation with target cations. Although not the research on CE, isotachopheresis study showed that 18-crown-6 enhances the separation of  $Tl(I)$  from  $NH_4^+$  and  $Pb(II)$  [28]. Thus, 18-crown-6 must be applicable to the CE determination of  $Tl(I)$  as well.

Fig. 3 shows electrophoretic separations of some cations. In the absence of 18-crown-6 (Fig. 3a), some ions [ $K^+$  and  $NH_4^+$  and  $Na^+$  and  $Pb(II)$ ] comigrate. However, the addition of 18-crown-6 retards the migration of  $K^+$  and  $Pb(II)$  and then allows to separate these ions.

Thus, the literature clearly indicates that 18-crown-6 is the most effective water-soluble polyether ligand. However, the addition of a ligand for the improvement of particular separation may cause the overlapped migration or the worse separation for

other analytes.  $K^+$  for example migrates rather fast in the absence of 18-crown-6, but very slowly in the presence of 18-crown-6. During the retardation, the apparent mobility of  $K^+$  becomes lower than  $NH_4^+$ , but possibly close to that of other metal cations. Fig. 2c clearly shows this situation, where the plot for  $K^+$  intersects other plots as the concentration of 18-crown-6 increases. Therefore, the concentration of 18-crown-6 and other conditions should simultaneously be optimized for given samples on the basis of appropriate criteria. François et al. [17] reported that the best resolution in total separation was obtained with 2.5 mM 18-crown-6 for a sample composed of alkali, alkaline earth metal and ammonium cations. In their work,  $K^+$  and  $NH_4^+$  were not well resolved with lower concentration of 18-crown-6 as usual, while the separation between  $K^+$  and  $Ca^+$  and between  $Na^+$  and  $Mg^+$  became worse with increasing 18-crown-6 concentration. Thus, the optimization of 18-crown-6 concentration is necessary when this method is applied to the separation of numerous ions. The complexation of 18-crown-6 was applied to the further methodological developments of inorganic CE. Shi and Fritz [16] showed that 18-crown-6 effectively retards the migration of alkaline earth metal cations while  $Na^+$  is hardly affected, and thus permits to determine trace alkaline earth metal cations in the presence of large amount of  $Na^+$  in conjunction with the addition of methanol. Again, these results are basically predictable from the complexation constant data.

As mentioned above, changing solvents is another effective option to modify CE separation selectivity. Indeed, CE selectivity enhancements by changing solvent compositions have been reported [16,18,22,27,29,41–43]. If mixed solvents are used in conjunction with polyethers, additional effects are expectable. The solvation of cations usually becomes weaker, when poorer donor solvents, such as methanol, acetonitrile etc., are added to aqueous solutions. The cation solvation weakened in mixed solvents facilitates the complexation with polyethers; in most instances, the desolvation process from a cation occupies a large part of total energy expense in cation complexation with polyethers. Thus, polyether complexation is rather weak in purely aqueous solutions, but possibly becomes stronger by the addition of appropriate organic solvents. Unfortuna-



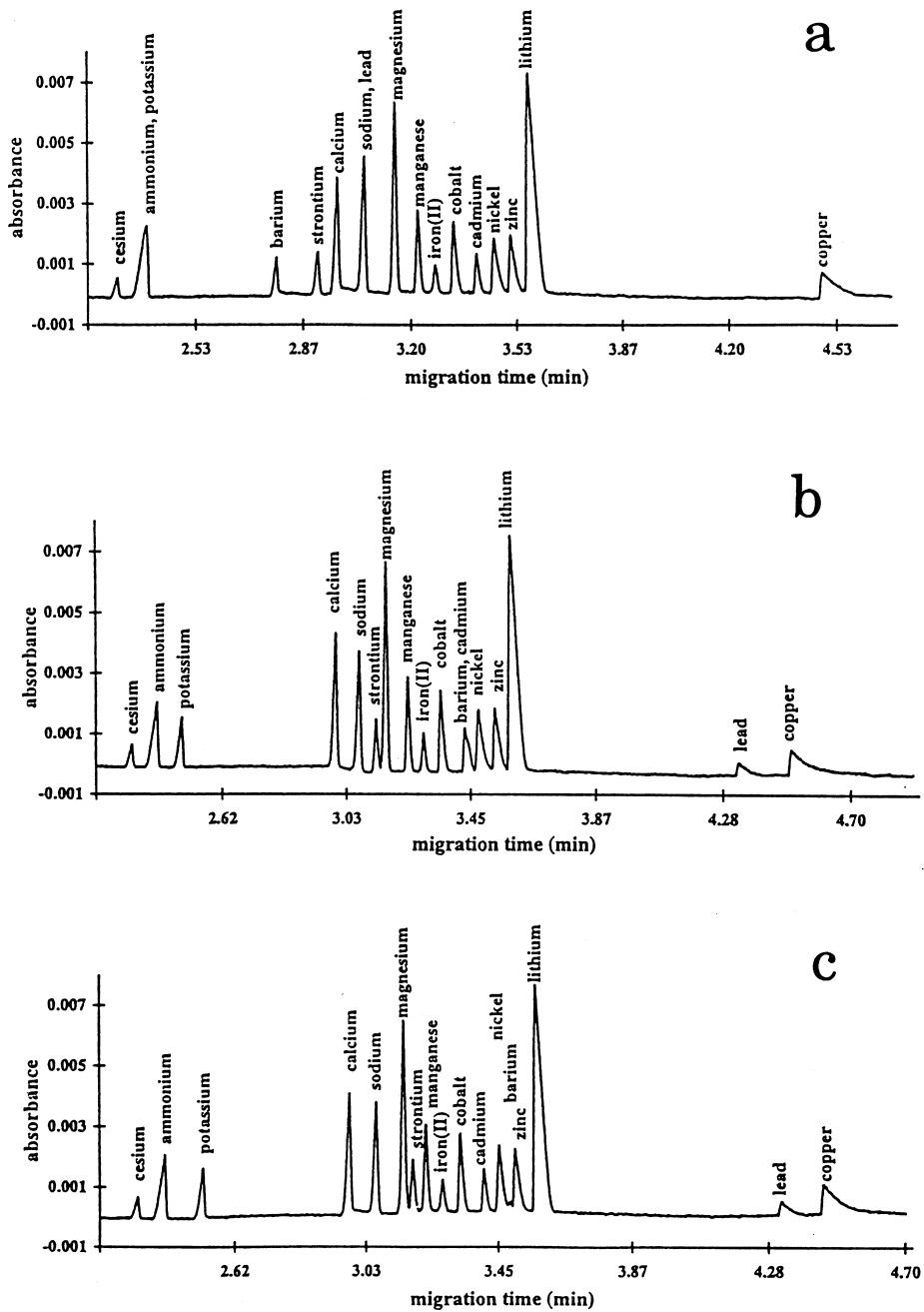


Fig. 3. Effects of 18-crown-6 on electrophoretic separation of cations. Electrolytes; 10 mM imidazole + 5 mM lactic acid (pH 4.5); (a) no 18-crown-6, (b) 1 mM 18-crown-6, (c) 1.5 mM 18-crown-6. Capillary, 57 cm  $\times$  75  $\mu$ m I.D. 20 kV applied. Indirect detection at 214 nm. From Ref. [17].

tely, there have been only a few systematic researches concerning cooperative effects of polyether complexation and solvation changes in CE. Yang et al. [27] studied the cooperative effects of 18-crown-6 and methanol in the separation of alkali, alkaline earth and some transition metal cations. After simple modeling of CE mobility, they illustrated contour

plots shown in Fig. 4, where a difference in electrophoretic mobility between ions migrating most closely ( $\Delta u$ ) was taken as criteria. Better conditions (higher  $\Delta u$  values) are arranged parallel to the 18-crown-6 axis in the lower contour plot, suggesting that the concentration of methanol is more critical than that of 18-crown-6. This was caused by the fact

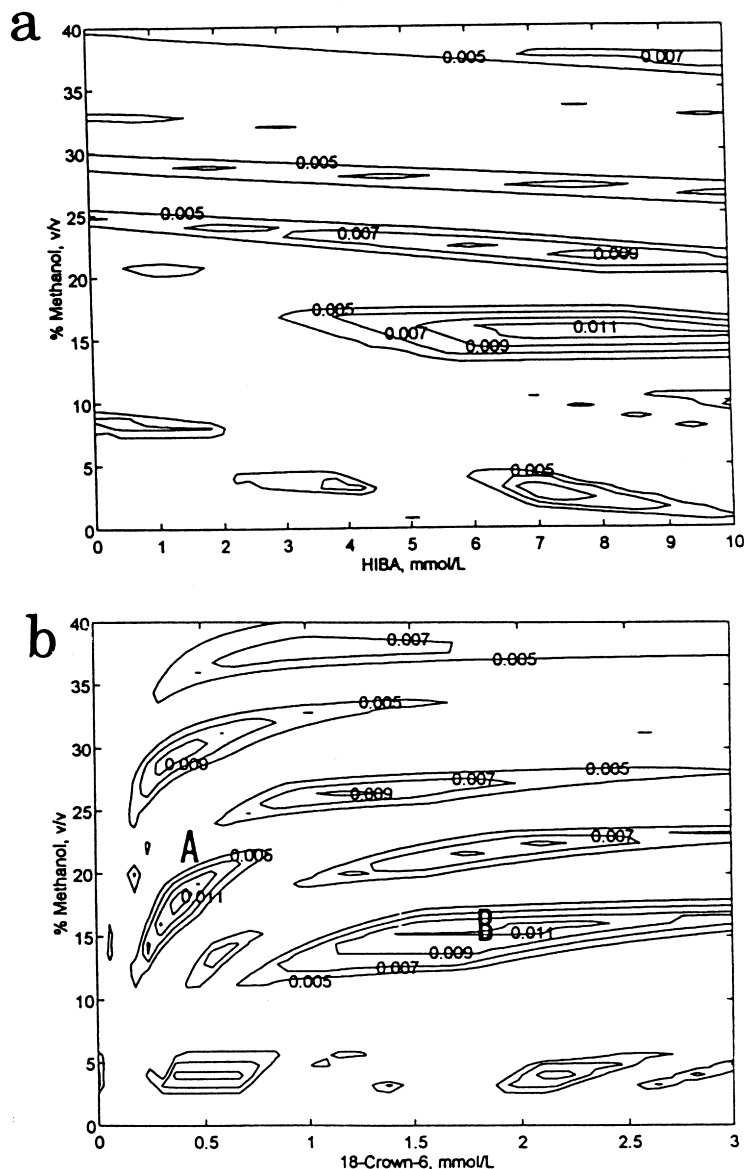


Fig. 4. Contour plots of minimum differences ( $\Delta u$ ) in electrophoretic mobility between closely migrating ions on the two-dimensional surfaces, (a) methanol–HIBA concentrations, (b) methanol–18-crown-6 concentrations. The separations of ten cations including  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cu}^{2+}$  were studied. From Ref. [27].

that increasing methanol concentration retarded the migration of many cations,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn(II)}$ ,  $\text{Li}^+$ ,  $\text{Ni(II)}$  and  $\text{Zn(II)}$ , while 18-crown-6 affects the migration of  $\text{Sr}^{2+}$ ,  $\text{Ba}^{2+}$  and  $\text{K}^+$ . This strongly implies two possibilities; (1) the addition of methanol, at least up to 40%, changes solvation of cations, but does not enhance 18-crown-6 complexation; (2) the complexation of  $\text{Sr}^{2+}$ ,  $\text{Ba}^{2+}$  and  $\text{K}^+$  with 18-crown-6 is almost completed even in water, and the complexation of other cations is enhanced by the addition of methanol. Thus, though the details are somewhat unclear, studies on the cooperative use of a polyether and an organic solvent is expected to provide interesting insights into polyether complexation chemistry in mixed solvents and to open a novel possibility in CE separation enhancements.

The data shown in Table 3 and Fig. 2a and b indicate that 15-crown-5 and 12-crown-4 have not provided positive results [15,16]. In contrast, some researches suggest that PEG be an efficient alternative [30,31]. Ito and Hirokawa [31] showed that the addition of PEG 200 (up to 60%) improves the separations of  $\text{K}^+ - \text{NH}_4^+$  and  $\text{Rb}^+ - \text{Cs}^+$ , and total separation of alkali and alkaline earth metal cations becomes much better. Selected electropherograms are shown in Fig. 5. With 35% PEG, all solute ions are separated. Further addition causes another overlap of peaks. This comigration was avoidable by adding tartaric acid, allowing the class separation between alkali and alkaline earth metal cations (Fig. 5e and f). It was inferred that PEG complexation is responsible for the migration changes because effects of PEG are similar to those of 18-crown-6. Selected complexation data for PEG and related compounds are listed in Table 4 [44,45]. It is known that PEG complexation constants also show maxima at  $\text{K}^+$  and  $\text{Ba}^{2+}$  in alkali and alkaline earth series. It was confirmed that smaller entropic losses are responsible for the preferable  $\text{K}^+$  complexation in the complexation of heavy alkali metal cations [46]. Stathakis and Cassidy [30] also pointed out the efficiency of PEG. They used PEG with various average molecular masses ranging 200–20 000 000. They focused their attention on the migration of some transition metal ions as well as alkali and alkaline earth metal cations in the presence of PEG. The migration of lanthanide cations are retarded very much, and could not be confirmed within reasonable

analysis times in some cases. However, this can be applied to the determination of trace cations in the presence of large excess of lanthanide ions. In addition, PEG enabled the separation of  $\text{Zn(II)}$  and  $\text{Mn(II)}$ , whose intrinsic mobility differs by only 2%, without assistance of other complexing agents. As well as Ito and Hirokawa [31], Stathakis and Cassidy [30] concluded that enhanced separation is ascribed to the complexation of metal cations with PEG. Since PEG is usually a mixture and the complexation ability is enhanced by increasing chain length, it must be kept in mind that the discussion on the complexation based only on average chain length (or molecular mass) possibly leads to misunderstandings of the chemistry taking place [47]. Although the complexation with PEG must be a major factor governing electrophoretic separation selectivity, changes in the nature of separation media caused by the addition of PEG may also be responsible for resulting selectivity. Medium effects will be discussed in the following section.

### 3. Polyethers as separation media

#### 3.1. Solution behaviors of polyoxyethylene in water

Another important feature of polyethers is its characteristic solution behaviors. When a long hydrocarbon chain is introduced in a polyether molecules, resulting compounds behave as surfactants. Triton X-100, Brij 35 and Tween 20 are the trademarks of typical polyether surfactants, bearing polyoxyethylene groups [48]. Micellar formation is a common but important nature of amphiphilic compounds. In addition to micellar formation, polyoxyethylene–water mixtures show interesting solution behaviors; i.e., a macroscopically homogeneous mixture is separated into two discrete phases at the particular temperature dependent on solution compositions [49–53]. This physicochemically well-defined temperature is known as a cloud point. The phase separation of homogeneous solution is thermodynamically described as

$$\left( \frac{\partial^2 \Delta G_{\text{mix}}}{\partial n_1^2} \right)_{n_2, T, P} > 0 \quad (8)$$

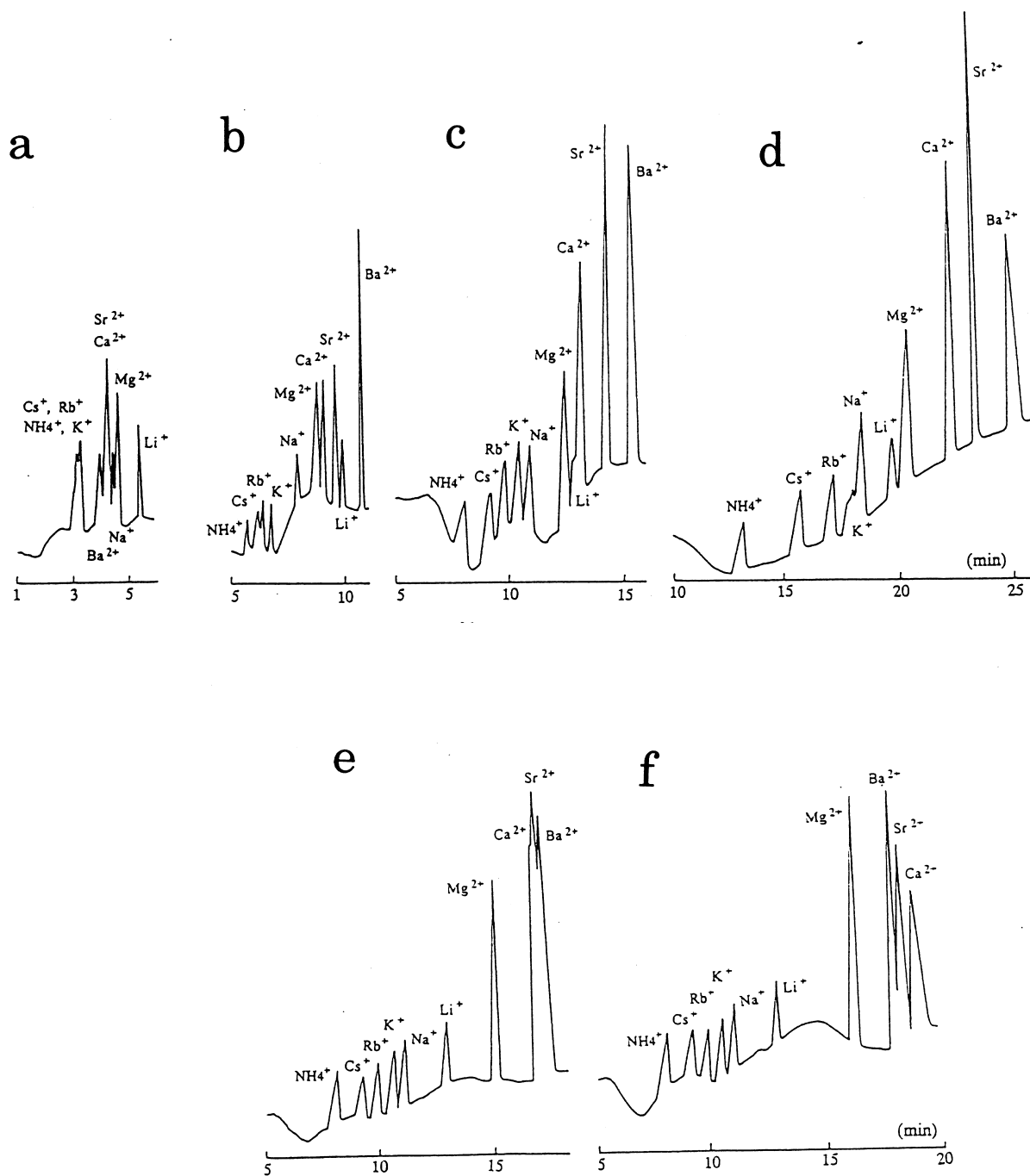


Fig. 5. Electrophoretic separations of alkali, alkaline earth and ammonium cations with running solutions containing PEG and tartaric acid. Electrolytes (pH 4.5), (a)–(d) 30 mM creatinine+15 mM HCl; (e) 30 mM creatinine+13 mM HCl+1 mM tartaric acid; (f) 30 mM creatinine+12 mM HCl+1.5 mM tartaric acid. (a) No PEG 200, (b) 35% PEG 200, (d) 60% PEG 200, and (c), (e) and (f) 50% PEG 200. Capillary, 50 cm×75  $\mu$ m I.D. 20 kV applied. Indirect detection at 254 nm. From Ref. [27].

Table 4  
Complexation constants for PEG oligomers in methanol at 25°C

PEG $n^a$	Log $K$			
	Na <sup>+</sup> <sup>b</sup>	K <sup>+</sup> <sup>c</sup>	Cs <sup>+</sup> <sup>c</sup>	NH <sub>4</sub> <sup>+</sup> <sup>b</sup>
8	1.09	2.76	2.52	1.25
9	1.24	2.95	2.67	1.33
10	1.34	3.02	2.77	1.43
11	1.42	3.08	2.88	1.51
12	1.42	3.20	2.96	1.59
13	1.49	3.29	3.03	1.65

<sup>a</sup> The number of repeating oxyethylene units.

<sup>b</sup> PEG derivatives, determined by CE from Ref. [45].

<sup>c</sup> Determined by chromatography from Ref. [44].

where  $n_1$  and  $n_2$  are the moles of water and polymers, and  $\Delta G_{\text{mix}}$  is the Gibbs free energy of mixing [53]. It has been reported that the conformation of polyoxyethylene is related to the phase separation, which can be explained e.g., according to the Florry–Hüggins theory [49–52]. Although the mechanisms of this phenomenon are still debated, it can be reasonably considered that this phenomenon is caused by the attractive interaction between polyoxyethylene chains. In addition, it has been reported that PEG forms clusters in aqueous solutions [54]. These researches indicate that PEG (or polyether surfactants)–water mixtures are microscopically heterogeneous though macroscopically homogeneous. It is expectable that the behaviors of ions in such microscopically heterogeneous media are different from those in usual solutions. If PEG aqueous solutions involve PEG-rich clusters, the partition of ions to the clusters can be an additional separation parameter. Even if not the case, water structures will be changed by the interaction with polymer domains of PEG or polyoxyethylene chains and in turn the solvation of ions are also influenced. Thus, polyether–water mixtures can be unique separation media.

### 3.2. Application to electrophoresis

In inorganic CE, the utilization of micelles has been much less common than in organic CE. Even in organic CE, nonionic surfactants are not very common separation medium-modifiers in comparison with ionic surfactants. We found only a few examples in the literature, where nonionic surfactants are

used for inorganic CE separation. Triton X-100 was, for example, employed for the separation of metal species together with HIBA [55]. However, the function of Triton X-100 was not clearly mentioned in this study. There have been no systematic studies on the use of nonionic surfactants in CE separations of inorganic species.

PEGs have proved to be effective (yet inexpensive) separation media in organic CE, and allowed researchers to enhance the separation of simple organic compounds as well as biopolymers [56,57]. In these studies, it was inferred that PEG molecules act as polymer network matrices or hydrogen bond donors. I believe that medium modifier effects of PEG should be involved in these separations as well. No work has been, so far, done from the viewpoint of the CE medium-modification by polyethers. In the preceding sections, the author mentioned the CE separation of cations with PEG solutions. In some cases, the concentration of PEG was higher than 50%. Although selectivity was discussed only on the basis of complexation mechanisms, the medium-modification effects may be superimposed with the complexation mechanisms because the water structure should be changed in the presence of large amount of PEG. However, both the complexation and medium-modification mechanisms similarly affect CE behaviors of cations. Both mechanisms involve the dehydration of ions, which plays a decisive role in the determination of overall energy. Thus, it is difficult to distinguish these two effects in the separation of cations.

Since anions are basically solvated through hydrogen bonds [13], direct interactions of PEG with anions should be weaker than those with cations. Thus, the complexation mechanism is negligible for the behaviors of anions. Therefore, CE separation of anions allows to distinguish the medium-modification mechanism from the complexation mechanism. Separation enhancements by the addition of PEG are illustrated in Fig. 6. Almost identical intrinsic mobility of Br<sup>−</sup> and I<sup>−</sup> makes their separation difficult in the absence of PEG, whereas their separation is obviously enhanced by the addition of PEG. The electrophoretic migration times of ions were proportional to the concentration of PEG. Table 5 summarizes the slopes of the linear plots of electrophoretic migration times against PEG con-

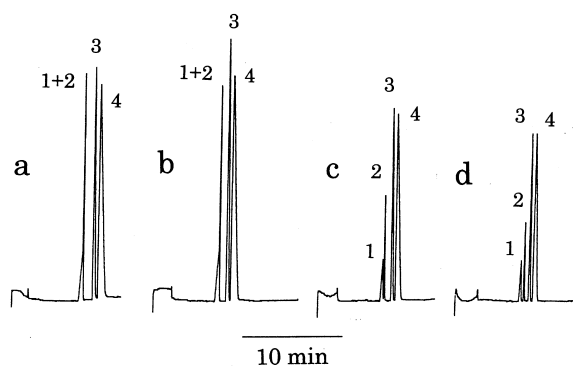


Fig. 6. Electrophoretic separations of inorganic anions, (1)  $\text{Br}^-$ , (2)  $\text{I}^-$ , (3)  $\text{NO}_2^-$  and (4)  $\text{NO}_3^-$ . (a) No PEG, (b) 1% PEG 1000, (c) 2% PEG 1000 and (d) 4% PEG 1000. Electrolyte, 25 mM phosphate buffer (pH 7.0). Capillary, 50 cm  $\times$  50  $\mu\text{m}$  I.D. 10 kV applied. UV detection at 215 nm.

centrations. In all cases, the slopes for  $\text{I}^-$  are larger than those for other ions. The PEG effects are enhanced in the order of  $\text{I}^- > \text{NO}_3^- > \text{NO}_2^- > \text{Br}^-$ , which is closely related to their hydration energies,  $\text{I}^- > \text{NO}_3^- > \text{Br}^- > \text{NO}_2^-$  [13]. PEG effects are maximized with PEG 1000 for all anions. There are some possible explanations for these phenomena; (1) PEG plays a role similar to usual organic solvents; direct solvation toward anions occurs or the solvation by water is affected by PEG; (2) PEG forms clusters, and the partition of solutes into the cluster phases causes the above phenomenon. If PEG behaves as an organic solvent, the direct solvation of PEG molecules to anions should occur through hydrogen atoms of terminal hydroxyl groups. In this case, the PEG effects should simply become smaller with increasing molecular masses. The direct solvation of PEG toward anions can thus be negligible. However, the addition of PEG should change the structures of water, and in turn the hydration of anions.  $\text{I}^-$  is the

Table 5

Slopes of linear plots of electrophoretic migration times of anions against PEG concentrations

Anion	Slope (min/%)			
	PEG 400	PEG 1000	PEG 2000	PEG 7500
$\text{Br}^-$	0.0813	0.117	0.0997	0.0706
$\text{I}^-$	0.102	0.143	0.127	0.0889
$\text{NO}_2^-$	0.0897	0.126	0.105	0.0710
$\text{NO}_3^-$	0.0927	0.131	0.111	0.0686

most weakly hydrated anion of anions tested, and is most sensitive to changes in water activity and its structure. Thus, the above selectivity enhancements can be interpreted by medium modification mechanism induced by PEG. However, the partition mechanism can also explain the present results. When anions are partitioned to PEG clusters, the desolvation from anions should take place. This process is most favorable for  $\text{I}^-$ . Although data shown here do not necessarily support either the partition or the changed water structure mechanism, it can be reasonably concluded that the addition of PEG more effectively changes the solvation of anions than the addition of usual organic solvents.

#### 4. Conclusions

Selectivity enhancements using polyethers in CE running solution are reviewed from viewpoints of the complexation and medium-modification, mainly from the former. It appears that the methods based on 18-crown-6 complexation in aqueous solutions have already been matured. However, studies on the combination of 18-crown-6 with organic solvents have been insufficient. Rather strong complexation ability, moderate selectivity and simple synthesis (in other words, low cost) of 18-crown-6 have prevented us from making further efforts to study separation conditions and to seek more efficient polyethers than 18-crown-6. CE is not only an effective tool for practical separation and analyses but also an efficient method for the screening of novel polyethers. For the latter purposes, it is an advantage of CE to demand only very small volume of samples or running solutions. Further studies should also be done from this viewpoint.

Compared to rather active investigations on CE utilizing polyether complexation, studies on the medium modification by polyethers are very few. One of the reasons must be unclear mechanisms of the medium modification. However, this is worth attempting. As stated above, nonionic micelles, involving polyethers as a part of the structures, show very interesting phase-behaviors, which are possibly applicable to inorganic separation. Even in the absence of molecular aggregation polyether provides potentially useful separation media in CE. Studying

such features of polyether solutions is expected not only to allow the developments of novel methodology but also to facilitate the elucidation of physico-chemical aspects of polyether–water mixtures. Thus, the author believes that more extensive utilization of polyethers in inorganic CE gives us an important perspective of this separation method as well as insights into polyether chemistry in solution.

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