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Journal of Chromatography A, 804 (1998) 17–28

JOURNAL OF CHROMATOGRAPHY A

Review

Nonaqueous ion-exchange chromatography and electrophoresis Approaches to nonaqueous solution chemistry and design of novel separation

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Abstract

Compiling ion-exchange chromatographic data, which has been allowed by recent developments in chromatographic techniques, indicates that solvation plays important roles in determining selectivity. Ion-exchange chromatography in nonaqueous solvents is not a novel idea, but is expected to give insights into roles of solvation in separation mechanisms, because changing solvents is the simplest way to study solvation effects. Thus, nonaqueous media facilitate to understand the chemistry taking place in ion-exchange resins or at the interface between an ion-exchange resin and solution. In contrast, capillary electrophoresis is a remarkable method to study solution chemistry, because the migration of solutes can be directly related to the reactions occurring in solution. The application of this method to nonaqueous media is also expected to enhance the separation selectivity and understanding of nonaqueous solution chemistry. In this paper, nonaqueous ion-exchange chromatography and capillary electrophoresis are reviewed mainly from fundamental viewpoints. © 1998 Elsevier Science B.V.

Keywords: Reviews; Non-aqueous ion-exchange chromatography; Non-aqueous capillary electrophoresis; Buffer composition; Mobile phase composition; Polyethers; Polymers; Acids; Phenols; Crown ethers

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1. Introduction

Separation of ionic compounds is of fundamental and practical importance, and has been done by means of solvent (solid-phase) extraction, flow separation techniques, membrane separation, etc. Of

0021-9673/98/$19.00
PII S0021-9673(97)01234-X

these, flow separation techniques have been most frequently employed because of their high applicability and versatility. Chromatography and recently developed capillary electrophoresis are typical representatives of efficient flow separation techniques.

Chromatography is an effective technique to separate compounds of various properties. Ion-exchange chromatography has covered the separation of almost all ionic compounds differing in molecular sizes and molecular nature. Despite extensive use of this separation technique, the proper separation mechanism has not completely been elucidated partly because principal efforts in this discipline have been directed to technical and practical developments. For these reasons, ion-exchange chromatography has been mostly done in aqueous or hydro-organic media, in which most of the ionic compounds are dissolved better than in other solvents and thus, in water, the application to various samples is much easier [1–6] than in nonaqueous media. Although results in aqueous ion-exchange chromatography should involve important information concerning the separation mechanisms, varying the nature of media is expected to provide more essential clues to elucidate the origin of separation mechanisms and selectivity.

It is one of serious disadvantages of ion exchange that this method provides no direct information on events occurring at the surface of the stationary phase, because the ion-exchange equilibrium is always determined by the balance between the solute interaction and the eluent interaction with active sites of a resin. However, the separation selectivity obtained with a particular eluent should reflect the order of the retention interactions of solutes. A number of data have been compiled for aqueous ion exchange, especially since the introduction of ion chromatography, and have allowed us to infer mechanisms of this separation method. One of important results reported so far is that the selectivity can be varied by the chemical structures of ion-exchange sites [7–10]. This suggests that chemical interaction such as ion-pair formation between a solute ion and an ion-exchange site be an essential factor to determine the selectivity. In solution, ion-pair formation equilibria are usually athermic or endothermic, indicating that the desolvation entropy is a driving force of this interaction [11]. If ion-pair formation is closely related to ion-exchange equilibria, the information on the solvation of solute ions must be useful in considering the origin of ion-exchange selectivity. Since both solutes and ion-exchange sites are solvated in different ways in different solvents, changing the solvation states of ions must result in different ion-exchange selectivity. Thus, uses of nonaqueous mobile phases in ion-exchange chromatography should give unique selectivity and insights into ion-exchange mechanisms.

As mentioned above, it is difficult to obtain direct information on the basic interaction involved in ion-exchange equilibria. In contrast, capillary electrophoresis (CE) possibly provides direct information on interactions in solutions. This is an advantage of this method as an approach to solution chemistry over chromatography. Though the basic principles of CE separation are similar to those of conductometric measurements, CE is superior to conductometry in that differences in ionic mobility or in reactivity are, in CE, shown in a very comprehensible manner, i.e. peak separation [12–14]. For the same reason as in ion exchange, nonaqueous CE is much less common than aqueous CE so far. However, nonaqueous CE can be a remarkable approach to nonaqueous solution chemistry, as shown later, and the excellent applicability of this method has recently been accepted [15–17].

In this review, the author discusses the usefulness of ion-exchange chromatography and CE as fundamental tools to study nonaqueous solution chemistry, as well as the unique separation that is impossible or very difficult to achieve in aqueous media.

2. Nonaqueous ion-exchange chromatography

2.1. Separation mechanism in ion-exchange chromatography

No one doubts that the primary separation mechanism in ion-exchange chromatography is the electrostatic interaction between ion-exchange sites and counter ions. However, other interactions seen in usual solute–solute, solute–solvent, and solvent–solvent interaction in solution contribute to overall

retention or selectivity in ion-exchange. Ion-dipole and dispersion interaction, for example, should be included as important mechanisms [18]. In addition, in polar solvents such as water, the entropic contribution originating from solvent structures around ion-exchange sites should also be taken into consideration [19,20]. These phenomena together are called 'ion-exchange interaction', and are often represented by a single equilibrium such as

$$z_Y\overline{X}^{z_X} + z_X Y^{z_Y} = z_Y X^{z_X} + z_X\overline{Y}^{z_Y} \quad (1)$$

where X and Y with a bar are species in an ion-exchange resin phase, and z_X and z_Y denote the charges of ions X and Y. The thermodynamic equilibrium constant for Eq. (1) well describes various phenomena, if activity coefficients are correctly taken into account. However, equilibrium constants represented by concentrations, called selectivity coefficients, are often used instead of thermodynamic equilibrium constants. It has been pointed out that treating the selectivity coefficients as thermodynamic values possibly draws a wrong conclusion [21–23].

There are some criticisms against the above discussion based on Eq. (1). Ståhlberg indicated that the stoichiometric consideration is inapplicable to long-range mechanisms, such as electrostatic interaction, because the distribution of ions in solution is also influenced by the electrostatic potential [23–25]. On the basis of this idea, Ståhlberg derived a useful equation capable of evaluating contributions from the accumulation of a solute ion in the diffuse layer [23]. Similar, but slightly different, approaches have been presented by Cantwell and co-workers [26–28] and Höll and co-workers [29–32]. In order to explain ion-exchange selectivity, various chemical interactions are taken into account in their models; surface adsorption [23,26–28], surface complexation [29–32], ion exclusion [26–28], etc. The author considered that three possible mechanisms are involved in ion-exchange equilibria; (1) ion-pair formation between an analyte and an ion-exchange site; (2) the specific adsorption of ions on the resin matrices; and (3) the nonselective accumulation of analytes in the diffuse layer [33]. In nonaqueous media, surface adsorption must be suppressed because the polarizability of such molecules is usually larger or much larger than that of water, but the ion-pair formation should basically be enhanced because of the low solvation ability of nonaqueous solvent molecules toward ions. The assumption of the surface ion-pair formation must be justified by the discussions on ion-exchange selectivity in nonaqueous solvents (see Section 2.2).

Let us consider anion separation as an example. In aqueous anion exchange, the basic order of the affinity to a resin bearing tertiary ammonium ions as active groups is, e.g. $F^- < Cl^- < Br^- < I^- < ClO_4^-$ (hereinafter denoted as usual selectivity). This order agrees well with the order of decreasing radii of solvated ions, that of increasing crystal radii, and that of decreasing numerical hydration energy [18,34]. The surface adsorption of ions should be related to the dispersion forces, which are usually enhanced with increasing molecular size; the above anion-exchange selectivity is understandable if the surface adsorption is a main factor governing usual selectivity. However, ion-pair formation also explains the above selectivity because larger ions favorably undergo this reaction in water because of smaller desolvation energy expense. Thus, these two mechanisms, surface adsorption and ion-pair formation, result in the same selectivity in aqueous ion-exchange even if either is a major mechanism.

Though it is difficult to find direct evidence for the ion-pair formation, this interaction can account for the dependence of ion-exchange selectivity on the structures of ion-exchange. The anion separation selectivity obtained with primary ammonium ions as anion-exchange sites can be evidence for ion-pair formation; unusual selectivity not obeying the above hydration consideration can be seen [9,35]. In this case, the hydrogen bond formation between anions and ammonium ions must be dominant; smaller ions are better hydrogen bond acceptors because of their high charge density.

A novel model has been recently proposed, which includes surface adsorption, ion-pair formation, and diffuse layer accumulation as possible mechanisms [33]. The model, developed on the basis of linearized Poisson–Boltzmann and Gouy–Chapman–Stern theory, indicated that the accumulation of analyte ions in the diffuse layer is important when an eluent ion forms a strong ion-pair with an ion-exchange site, but in general plays a minor role. The surface

adsorption, which depends on the properties of resin matrices, and the ion-pair formation, are basically responsible for the determination of the overall selectivity. In nonaqueous solvents, the surface adsorption should be suppressed in comparison with in water. Thus, the ion-exchange selectivity in nonaqueous solutions should well reflect the relative ion-pair formation abilities of solute ions. In the following section, unless otherwise stated, the ion-exchange selectivity obtained in nonaqueous mobile phases is discussed from a viewpoint of ion-pair formation.

2.2. Changes in the anion-separation selectivity by varying the solvent properties and/or the structures of anion-exchange sites

As mentioned above, the desolvation process plays very important roles in ion-pair formation in solution. If ion-exchange equilibria include ion-pair formation, its separation selectivity must be changed by varying solvation states of ions. Such modification of the solvation is most simply done by changing solvents [34,36–39]. Figs. 1 and 2 show chromatograms of anions obtained with two different stationary phases ($-NH_3^+$ and $-NEt_3^+$ polymer matrix resin) with four different solvents. Water, methanol (MeOH), acetonitrile (MeCN), and *N*,*N*-dimethylformamide (DMF) were selected as solvents. Though examples obtained with pure solvents are shown when possible, chromatograms obtained with mixed solvents, containing the solvent of interest as much as possible, are shown in some cases because the extremely strong affinity of some ions to the resin prevented the confirmation of peak appearance (e.g. Cl^- with the $-NH_3^+$ stationary phase with DMF or MeCN or ClO_4^- with the $-NEt_3^+$ stationary phase in water). Selectivity varys with solvents: $Cl^- < Br^- < I^- < SCN^- < ClO_4^-$ (usual selectivity) with MeOH and water, $ClO_4^- < I^- (SCN^-) < SCN^- (I^-) < Br^- < Cl^-$ (denoted as unusual selectivity) with MeCN and DMF (in parentheses) irrespective of the structures of ion-exchange functional groups. It should be noted that the separation window becomes narrow on going from in water to in MeOH, indicating that usual selectivity tends to diminish in MeOH.

Similar selectivity was obtained with silica-based anion-exchange resins (denoted by $Si–NH_3^+$ and $Si–N(Et)_2Me^+$ according to the structure of active sites). The specific adsorption of anionic species is thought to be weaker on silica gel than on polymer

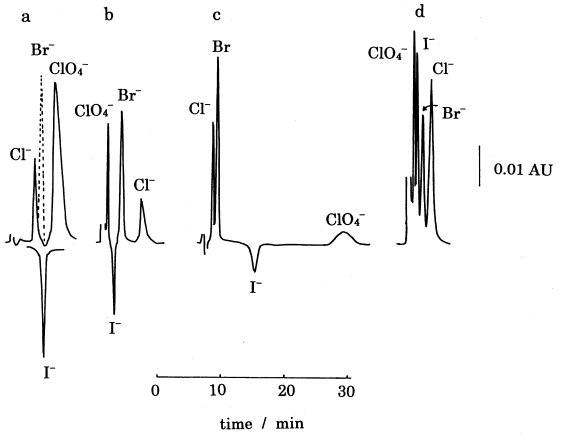

Fig. 1. Anion-exchange chromatograms with the $-NH_3^+$ resin. Mobile phase: (a) 0.02 *M* $LiNO_3$ in MeOH, (b) 0.02 *M* Et_4NNO_3 in MeCN–MeOH (80:20, v/v), (c) 0.02 *M* $LiNO_3$ in water, and (d) 0.01 *M* Et_4NpNB (tetraethylammonium *p*-nitrobenzoate) in DMF–MeOH (75:25, v/v). Detection, at 246 nm for NO_3^- eluent and at 390 nm for pNB^- eluent. Flow-rate, 1 ml min^{-1} for (a–c) and 0.6 ml min^{-1} for (d). From Ref. [34].

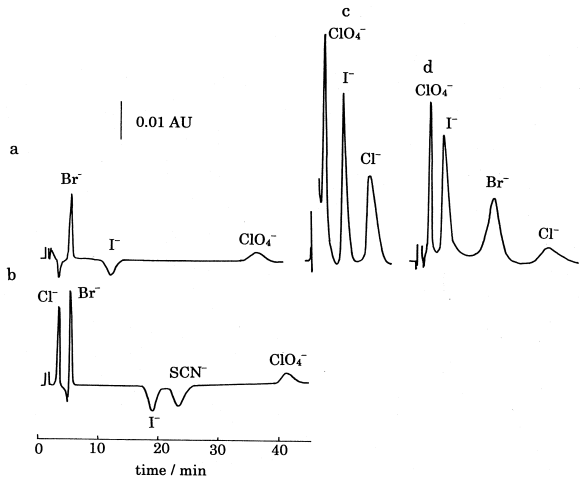

Fig. 2. Anion-exchange chromatograms with the $-NEt_3^+$ resin. Mobile phase: (a) 0.02 *M* $LiNO_3$ in MeOH, (b) 0.02 *M* $LiNO_3$ in MeCN–water (60:40, v/v), (c) 0.01 *M* Et_4NpNB in MeCN, and (d) 0.01 *M* Et_4NpNB in DMF. Detection, at 246 nm for NO_3^- eluent and at 395 nm for pNB^- eluent. Flow-rate, 1 ml min^{-1} for (a–c) and 0.7 ml min^{-1} for (d). From Ref. [34].

gel. Anion-exchange selectivity is similar, except that partially unusual selectivity appears for the Si–NH_3^+ even with methanolic mobile phases; $I^- < ClO_4^- \sim Br^- < Cl^-$. These results can be understood as follows: the specific adsorption on the polymer gel still acts as a part of the retention mechanisms even in MeOH, but is much reduced in comparison with in water; the weak specific adsorption on the polymer gel results in usual selectivity in MeOH; this effect is almost suppressed on the silica gel, and eventually partly unusual selectivity appears; thus, partly unusually selectivity obtained with the Si–NH_3^+ resin in MeOH should reflect actual interaction between solute ions and ion-exchange sites in this solvent. Thus, the structures of ion-exchange sites more strongly affect separation selectivity than the matrices of the resin; in other words, the interaction between solutes and active sites dominantly determines overall selectivity especially in nonaqueous ion-exchange.

The $-NH_3^+$ and $-NEt_3^+$ sites have different ion-exchange features. The latter site is principally a poor electron acceptor due to the low charge density and a poor hydrogen-bond donor, but undergoes strong ion-induced dipole interaction and London dispersion interaction. From these, we can infer that the $-NEt_3^+$ resin prefers larger ions, while the $-NH_3^+$ resin prefers smaller ions. This inference agrees well with experimental results in anion-exchange chromatography with wide varieties of anion-exchange groups; the relative affinity to large ions is enhanced by increasing molecular sizes of anion-exchange sites in water [7].

If the complete desolvation occurs for both ion-exchange sites and counter ions upon the ion-pair formation, not only the solvation states of the latter but those of the former possibly influence separation selectivity. However, almost the same results were obtained with MeCN and DMF despite their much different donor abilities. It is understandable that no difference between results obtained with these two solvents was found for the $-NEt_3^+$ resin, because the free energies of transfer from DMF to MeCN for tetraalkylammonium ions are almost zero [34]. However, though no quantitative data are available for the solvation of monoalkylammonium ions, their solvation energy in DMF should be different from that in MeCN; these ions must be more stable in DMF. The fact that identical selectivity was obtained for the $-NH_3^+$ resin in these two solvents implies that the relative extents of the desolvation from the $-NH_3^+$ sites are independent of the nature of solvents; the absolute extents of desolvation must depend on the nature of solvents. Thus, in anion exchange, the solvation states of anions is responsible for the determination of separation selectivity, while those of ion-exchange sites formally have no effects. This agrees well with results of solvent swelling measurements. According to the results of swelling measurements for tetraalkylammonium-type resins, Cl^--form anion-exchange resin swells in water almost five times better than a ClO_4^--form resin of the same cross-linking when compared on the basis of moles of water sorbed in the equivalent resin, whereas the latter swells in DMF almost 10 times better than the former [40]. In water, the ion-pair formation between a counter anion and an anion-exchange site is not very strong and the desolvation is not facilitated very much. Though Cl^- is still solvated better than ClO_4^- even in DMF solution, the strong ion-pair formation of Cl^- in anion-exchange resins, which is accompanied by the desolvation from interacting ions, results in poor swelling in this medium.

The results can be summarized as follows: (1) the $-NH_3^+$ resins bind smaller anions more strongly than the $-NR_3^+$ (R = alkyl groups) resins do; (2) in water unusual selectivity does not appear even with $-NH_3^+$ resins because of stronger acceptor ability of water and surface adsorption; (3) unusual selectivity can be seen in solvents of poor acceptor ability irrespective of the structure of anion-exchange sites; and (4) the donor ability of a solvent plays minor roles in the determination of anion-exchange selectivity in comparison with its acceptor ability (reversed situation must be true for cation exchange).

Thus, ion-exchange selectivity, in most cases, can be discussed on the basis of the extent of ion-pair formation and the solvation states of solute ions. Since other possible mechanisms, such as specific adsorption, are suppressed in nonaqueous solvents, it can be concluded that ion-pair formation is the most important mechanism in nonaqueous ion exchange; in other words, we can understand nonaqueous ion-exchange selectivity by ion-pair formation, and infer ion-pair formation taking place on the ion-exchange resin surface from ion-exchange data.

2.3. Modification of anion-exchange selectivity by crown ether complexation

It was shown in the preceding section that changing solvents and/or the structures of ion-exchange sites results in different separation selectivity. Changing solvents in a chromatographic run is routinely done in, e.g. gradient elution, while changing the structures of functional groups is much laborious and should usually be carried out by off-line procedures; e.g. the on-line reversible conversion between tertiary alkylammonium ion and primary ammonium ion must be impossible. However, use of nonaqueous solvents allows reversible on-line modification of ion-exchange resins.

It is known that the $-NH_3^+$-type resin retains polyethers by the complex formation, where counter anions play important roles, because this reaction involves the dissociation of ion-pairs between the $-NH_3^+$ and a counteranion as well as the ion-pair formation between the $-NH_3^+$ complexed by a polyether and a counter anion [41–43]. As shown above, the $-NH_3^+$ chemically bonded on silica gel showed a selectivity different from the $-NEt_3^+$-type anion-exchange resin. The difference in the selectivity is marked in MeOH. This difference in ion-exchange selectivity is caused by lower charge density and lower hydrogen bond formation ability of the latter. The complex formation of the $-NH_3^+$ groups with polyethers, which preferably occurs in MeOH, causes the dispersion of effective charges of the ammonium ion, and lowers hydrogen bond formation ability. Thus, the $-NH_3^+$ site complexed by a polyether is expected to behave like a tertiary ammonium group [44].

Fig. 3 shows changes of corrected retention times for selected anions with the concentration of 15-crown-5 (15C5) in MeOH mobile phases. It can be seen that partly unusual selectivity changes into entirely usual one with increasing 15C5 concentration (see retention, e.g. at zero and at 0.05 *M* 15C5). Anions are separated into two classes according to their retention behaviors; (1) anions whose retention decreases with increasing 15C5 concentration; and (2) anions showing reversed tendency. Group (1) includes smaller anions, Cl^- and Br^-, while group (2) includes larger anions. Ion-exchange retention is determined not by the absolute affinity of solute ions but by the affinity relative to that of an eluent ion (NO_3^- in this case). The ion-exchange affinity of NO_3^- is intermediate between those of groups (1) and (2). The $-NH_3^+$ groups binds group (1) ions better than NO_3^- before polyether complexation; this causes partly unusual selectivity. However, after polyether complexation, the relative affinity of group (1) is lowered while that of group (2) is enhanced; this allows the appearance of usual selectivity. These results are consistent with the above consideration about the $-NH_3^+$ and the $-NEt_3^+$ resins; the former prefer smaller ions as counteranions, but the latter prefers larger ions. Thus, crown ether complexation allows on-line conversion of the structures of anion-exchange sites, and drastically changes separation selectivity.

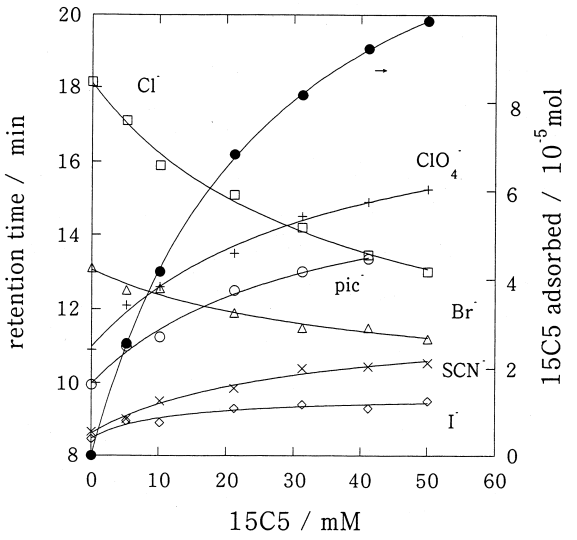

Fig. 3. Changes in retention times of anions with the addition of 15C5 to 0.01 *M* $LiNO_3$ methanolic solution. Solid circles represent amounts of 15C5 adsorbed on Si–NH_3^+ resin. From Ref. [41].

3. Nonaqueous CE: an approach to the evaluation of some equilibria occurring in nonaqueous solution

Advantages of nonaqueous solvents in CE have been discussed from various view points [15–17,45–50]. Nonaqueous solvents can solubilize organic large compounds that are insoluble in water, and thus give the possibility to separate such compounds by

CE. In addition, according to the Helmholtz–Smoluchovky equation, both electrophoretic mobility and electroosmosis are represented as a function of ϵ/η, where ϵ is the permittivity and η is the viscosity of a solvent. Some solvents, such as *N*-methylformamide and acetonitrile, provide larger ϵ/η ratios than water, indicating that larger electrophoretic mobility and electroosmotic flow-rates are possibly obtained, albeit the dissociation of solutes must be suppressed in some media. Changing solvents results in the different solvation states of solutes, different total mobility, and eventually different separation selectivity. Thus, use of nonaqueous solvents possibly brings about unusual selectivity even for the separation of usual ions.

As mentioned already, in our work, CE has been used as an approach to nonaqueous solution chemistry. Though other approaches, such as electrochemical and spectroscopic methods, have been usually employed to probe solution chemistry, CE has some advantages over these conventional methods; e.g. impure or even mixed samples can be used for physicochemical measurements, changes in the spectrometric or electrochemical properties of compounds are not necessary, net changes of solute charges are very clearly shown, etc. If the reactions, not occurring in aqueous media, are appropriately combined with CE separation, we can design novel separation, and in some cases quantitatively evaluate reactions involved in the separation. Two examples are shown below.

3.1. Separation of polyethers and determination of polyether complex formation constants

Polyether chemistry has been one of the main topics in chemistry in the last three decades [51–58]. In analytical chemistry, polyethers have been used for the separation and sensing of hard Lewis acids such as alkali, alkaline earth, and lanthanoid ions [58–60]. Crown ethers have, in particular, been utilized to develop various analytical methods because of their high complexation ability and selectivity toward hard cations. Acyclic polyethers have also been studied, e.g. to understand the origin of high complexation selectivity of cyclic counterparts, albeit the complexation ability and selectivity are mostly lower than those of crown ethers [58]. It is essential to know the complex formation constants of various polyethers in order to understand polyether chemistry and develop novel ligands and analytical methods.

Polyethers coordinate a cation through ethereal oxygen atoms arranged on the coordination shell of the cation. Since the coordination shell should usually be occupied by solvent molecules before the complexation, coordination bond formation competes with solvation, meaning that polyether complexation is seriously affected by the nature of solvents. It is well known that water is an unfavorable solvent for polyether complexation because of its high donor ability. For this reason, polyether complexation has been investigated in solvents of lower donor ability and relatively high polarity; the latter condition is necessary to allow the sufficient dissolution and dissociation of salts. MeOH is a suitable choice to study polyether complexation, and has been most extensively used for this purpose [55,56,58].

If electrically neutral polyethers form cationic complexes, they must migrate towards a cathode under an electric field. Their mobility is determined not only by the charge and the molecular sizes of the complexes but by the degree of complexation. The apparent velocity (v_{app}) of a polyether in the presence of a complexed cation can be written as [61]

$$v_{app} = v_{eo} + v_{ep} = v_{eo} + \frac{K[M]v_{max}}{1 + K[M]} \quad (2)$$

where v_{eo} and v_{ep} are the electroosmotic and electrophoretic velocity, v_{max} is a limiting velocity of a complex, K and [M] are the complex formation constant and the equilibrium concentration of a cation of interest. Eq. (2) indicates that there are two factors governing v_{app}; i.e. v_{max} and the degree of complexation.

Fig. 4 shows the separation of oligomers of polyoxyethylene (POE) with running MeOH solution containing NH_4^+ as a complexed cation. Electropherograms indicate that the longer POE migrates faster than the shorter counterparts despite the larger molecular size of the former. Since it is known that K values of POE systematically increase with increasing chain length [59,60], the degree of complexation is a primary factor affecting the migration and the variation of v_{max} values is a minor factor in this instance.

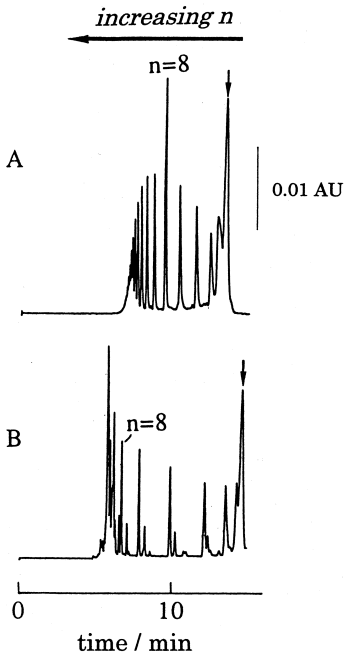

Fig. 4. Electropherograms of dinitrobenzoyl-POE-dodecylether. Solution: (A) 50 m*M* NH_4Cl+50 m*M* Et_3N, (B) 5 m*M* KCl+45 m*M* NH_4Cl+45 m*M* Et_3N. Capillary: 44.6 cm×50 μm I.D.×375 μm O.D. Detection at 250 nm. Applied voltage, 20 kV. The peak of the electroosmotic flow marker is shown by an arrow. From Ref. [58].

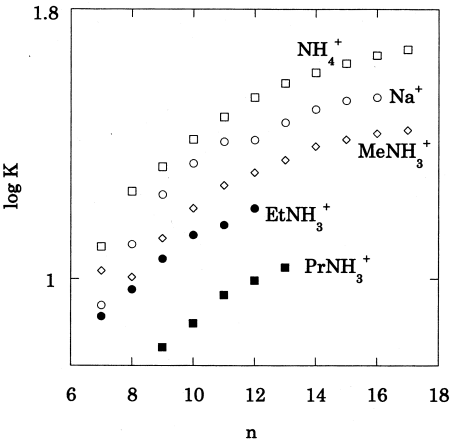

Fig. 5. Relations between *n* and log *K* determined by MeOH CE for Na^+ and some ammonium ions.

It was assumed that [M] is much larger than the concentration of sample polyethers and a difference between [M] and the analytical concentration of the cation should be negligible. According to Eq. (2), we can determine *K* from the dependence of v_{ep} on [M]. Results are illustrated in Fig. 5. The application of Eq. (2) to the determination of *K* values has some limitation that *K* values should be lower than 200 M^{-1} for reliable determination. This limitation comes from the above assumption of the equality of the equilibrium concentration with the analytical concentration of a cation. This difference is negligible when *K* is sufficiently small, but becomes critical as *K* increases. The above threshold, $K=200$ M^{-1}, was calculated by assuming differences between analytical and equilibrium concentration of a cation to be lower than 5%. This limitation must be improved by reducing sample concentration, in other word by enhancing detectability. The liquid chromatographic method has been reported as an alternative method to determine *K* values of monodispersed POE compounds contained in polydispersed samples [62–65]. This method is suitable for the determination of relatively large formation constants (>200). Thus, liquid chromatographic and CE approaches are complementary for the determination of *K* values of polyethers.

Nonaqueous CE is applicable to the separation of crown ethers as well [61]. Fig. 6 shows the separation of crown ethers obtained with some different cations. In any case, the separation of B12C4 from acetone added as the electroosmotic flow marker is seen, suggesting the weak interaction of B12C4 with cations tested. Though reliable complex formation constants cannot be determined because of the limitation as stated above, the complexation selectivity agrees well with the migration order.

3.2. Heteroconjugated anion formation

Heteroconjugated anion formation is also studied by nonaqueous CE [66,67]. It is known that both heteroconjugation and homoconjugation play important roles in acid–base chemistry in solvents with low acceptor ability, such as MeCN and DMF [68–73]. Though Kolthoff and co-workers [69–71] studied heteroconjugation of various compounds in various aprotic solvents by potentiometry and spectrometry, these methods sometimes fail to give obvious evidence of heteroconjugation. Nonaqueous

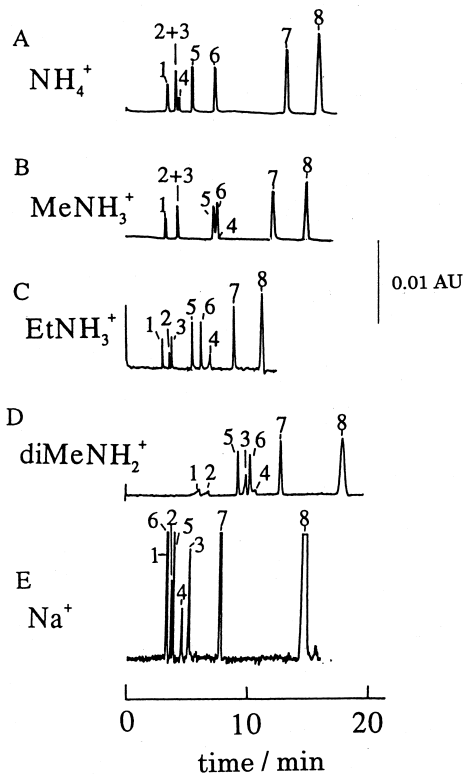

Fig. 6. Separation of crown ethers. Solution: (A) 25 m*M* NH_4Cl+25 m*M* Et_3N+25 m*M* Et_4NCl, (B) 15 m*M* $MeNH_3Cl$+15 m*M* Et_3N+35 m*M* Et_4NCl, (C) 40 m*M* $EtNH_3Cl$+40 m*M* Et_3N+10 m*M* Et_4NCl, (D) 40 m*M* $diMeNH_3Cl$+40 m*M* Et_3N+10 m*M* Et_4NCl, (E) 25 m*M* NaCl+25 m*M* Et_3N+25 m*M* Et_3NHCl. Peaks: (1) B18C6, (2) DB18C6, (3) DB21C7, (4) DB30C10, (5) DB24C8, (6) B15C5, (7) B12C4, (8) acetone. Detection at 280 nm. Other conditions are the same as for Fig. 4. From Ref. [58].

CE is capable of distinguishing neutral compounds from ionic compounds, and of detecting even very weak heteroconjugation. Thus, nonaqueous CE is a suitable choice to study such hydrogen bond complexes in solution.

Most Brønsted acids, if excluding a few exceptions, are weak acids in aprotic solvents, such as MeCN, mainly because of the low acceptor (or low hydrogen-bond formation) abilities of the solvents. Anions added in solution thus undergo very weak solvation, and interact with stronger hydrogen donor, Brønsted acids, rather than be solvated. Table 1 lists pK_a values of selected Brønsted acids in water and MeCN [34,74]. If phenols are, for example, dissolved in aprotic solvents together with inorganic

Table 1
pK_a values of some acids in water and MeCN

	pK_a[a]	
	Water	MeCN
Acids		
HCl	—	8.9
HBr	—	5.5
HNO_3	—	8.9
$HClO_4$	—	1.57
Phenols		
Phenol	9.9	27.2
o-NO_2	7.2	22.2
p-NO_2	7.1	20.7
2,6-diNO_2	3.7	16.5
Picric acid	0.3	11
Benzoic acids		
Benzoic acid	4.2	20.7
3,5-di-NO_2	2.8	16.9

[a] pK_a values taken from Ref. [74].

anions (conjugated base of moderately strong acids in the medium), heteroconjugated anion formation rather than the proton transfer from the phenols to the anions takes place. Thus, weak Brønsted acids behave as anions though they are not dissociated into their conjugated bases. If this phenomenon is applied to CE separation of weak Brønsted acids, the following equation must describe the migration of these compounds

$$v_s = v_{app} - v_f$$
$$= L(1/t_{app} - 1/t_f)$$
$$= \alpha v_{hetero}$$

Substituting $\alpha = (K_{HAX}C)/(K_{HAX}C+1)$ and $v_{hetero} = (E\lambda_{hetero})/F$ in this equation gives

$$1/(1/t_{app} - 1/t_f) = [1/(K_{HAX}C) + 1]LF/(E\lambda_{hetero})$$

where v and t denote an electrophoretic velocity and the time of migration, subscripts, s, app, f, and hetero are the abbreviations of a solute, apparent, a flow, and a heteroconjugated anion, and α, K_{HAX}, C, L, E, F, and λ_{hetero} refer to the degree of heteroconjugated anion formation, the heteroconjugated anion formation constant, the concentration of an anion in running solution, the effective length of a capillary, the strength of an electric field, Faraday constant,

and the molar ionic conductivity of a heteroconjugated anion.

This equation indicates that the apparent migration of a weak Brønsted acid is determined by the mobility (or limiting conductivity in the equation) and the extent of heteroconjugation similar to the separation of polyethers. Figs. 7 and 8 show selected electropherograms of phenols with MeCN containing ClO_4^- and Cl^- as running electrolytes, where samples were injected at the anodic end and the detection window was placed at the cathodic end. The first peak is of a flow marker (acetone). The slower migration of phenols than the flow marker implies that these compounds form anionic compounds. Even with ClO_4^-, that is usually regarded as an inert anion, peak separation due to heteroconjugation is seen. Though the above equation indicates that this scheme is applicable to the determination of K_{HAX}, the adjustment of ionic strengths, which is necessary for the constancy of λ_{hetero} values, is difficult because even ClO_4^- undergoes the heteroconjugation. Therefore, instead of the adjustment of the ionic strength, the following Onsager's equation was used

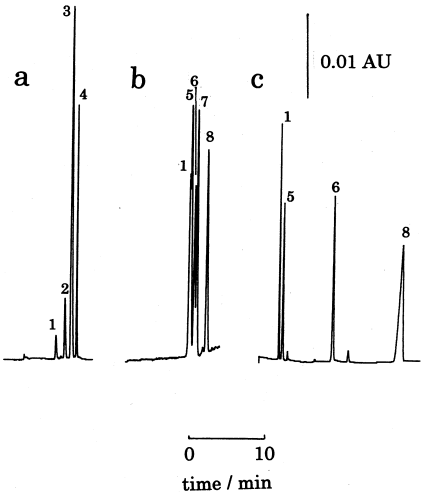

Fig. 7. Electrophoretic separation of phenols: (a,b) 0.1 *M* Et_4NClO_4 in MeCN; (c) 0.01 *M* Et_4NCl in MeCN. Peak identification: (1) acetone, (2) *m*-aminophenol, (3) phenol, (4) *p*-hydroxybenzaldehyde, (5) *o*-nitrophenol, (6) 2,4-dinitrophenol, (7) picric acid, and (8) *p*-nitrophenol. Detection at 265 nm. Capillary: 57.8 cm×50 μm I.D.×375 μm O.D. Applied voltage, 15 kV. From Ref. [63].

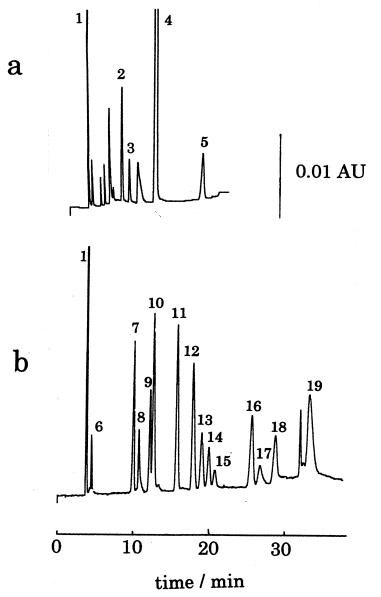

Fig. 8. Electrophoretic separation of phenols: (a) 0.01 *M* Et_4NCl in MeCN; (b) 0.02 *M* in MeCN. Peak identification: (1) acetone, (2) resorcinol, (3) *p*-chlorophenol, (4) *p*-hydroxybenzaldehyde, (5) *p*-nitrophenol, (6) *o*-nitrophenol, (7) *p*-isopropylphenol, (8) *p*-aminophenol, (9) *p*-*tert*-butylphenol, (10) *m*-aminophenol, (11) *p*-ethylphenol, (12) *o*-cresol, (13) *m*-cresol, (14) *p*-cresol, (15) *p*-hydroxybenzylalcohol, (16) phenol, (17) *o*-aminophenol, (18) 2-naphthol, and (19) 1-naphthol. Detection at 265 nm. Other conditions are the same as for Fig. 7. From Ref. [58].

for the correction of λ_{hetero} values. This equation for an X^+Y^--type electrolyte can be written as

$$\lambda = \lambda_0 - [e^2\lambda_0/\{24\pi\epsilon_0\epsilon kT(1+\sqrt{0.5})\} - F^2/(6\pi\eta N)]\kappa/(1+\kappa a)$$

$$\kappa = \{2N^2e^2/(\epsilon_0\epsilon RT)\}^{1/2}\sqrt{I}$$

where λ_0, e, ϵ_0 ϵ, k, η, 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, Avogadro's number, the distance between ions, and molar ionic strength.

Table 2 lists selected K_{HAX} and λ_{hetero} values for phenols determined by the above scheme. The K_{HAX} values for $X=ClO_4^-$ are too small for the other usual method to detect this interaction. Again, the ability to detect the weak interaction is a remarkable advantage of nonaqueous CE.

Table 2
K_{HAX} for selected phenols with Cl^-, NO_3^-, and ClO_4^- in MeCN

Phenols	Log K_{HAX}		
	Cl^- [a]	NO_3^- [a]	ClO_4^- [b]
Phenol	2.09(0.01)	1.03(0.04)	0.44(0.02)
o-Me	1.72(0.13)	0.96(0.11)	0.34(0.01)
m-Me	1.95(0.16)	0.93(0.18)	0.34(0.01)
p-Me	1.87(0.15)	0.88(0.17)	0.34(0.01)
p-Et	1.84(0.03)	0.95(0.19)	0.32(0.02)
p-tBu	1.79(0.04)	0.89(0.12)	0.25(0.01)
o-NH_2	2.14(0.13)	0.91(0.04)	
m-NH_2	1.81(0.04)	0.65(0.09)	
p-NH_2	1.72(0.04)		
o-NO_2	1.27(0.17)		
p-NO_2	2.69(0.00)		0.95(0.10)
p-CHO	2.43(0.16)		
p-Cl	2.17(0.03)	1.05(0.13)	0.54(0.03)
p-CH_2OH	1.79(0.11)	1.01(0.13)	
m-OH	2.02(0.05)	1.03(0.04)	0.79(0.05)

Standard deviations are in parentheses.
[a]From Ref. [66].
[b]From Ref. [67].

This scheme has been applied to the separation of carboxylic acids and alcohols as well.

4. Conclusion

Nonaqueous ion-exchange and CE are reviewed from a fundamental point of view. Use of such media allows to study the solvation of ions in ion-exchange resins, to provide a clue to the proper mechanism of this separation, to evaluate nonaqueous solution chemistry, and to modify or alter the separation selectivity. Thus, though uses of nonaqueous solvents in ion-exchange and CE have not been common so far, it is expected that these contribute to the development of novel separation methods and to the elucidation of reactions unknown or undetectable by other conventional methods.

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