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Non-aqueous capillary electrophoretic separation of polyethers and evaluation of weak complex formation

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Abstract

The use of pure methanol as a separation medium for capillary electrophoresis (CE) has made possible the separation of non-ionic polyethers and the determination of their complexation constants with an electrolyte cation. The developed technique has proved to be efficient for the evaluation and detection of weak interactions (e.g., the interaction of benzo-12-crown-4 with various cations can be detected). This is a good example showing that the use of non-aqueous solvents in CE has allowed the utilization of interactions that hardly take place in water.

1. Introduction

Capillary electrophoresis (CE) has proved to be effective for the separation of compounds with various chemical properties [1,2]. This method basically differentiates ionic compounds migrating towards the oppositely charged electrode according to their mobilities in a medium. However, the separation of non-ionic compounds is also feasible if appropriate modifications are made to the electrolyte system, e.g., the use of ionic micelles has permitted the separation of a variety of electrically neutral compounds [3,4]. CE and related techniques are mostly recognized as useful for practical purposes because of their excellent separation ability, and a number of papers describing various applications have been published. However, the author believes that CE is also useful for fundamental purposes and can be used for the detection and evaluation of some physico-chemical properties in solution.

Separation methods have an essential advantage over other spectrometric or electrochemical methods in studying solution chemistry, e.g., as the evaluation method itself involves separation and purification processes, impurities contained in samples have little influences on the results obtained. From such a viewpoint, the author reported the chromatographic evaluation of the solution behaviour of ions [5,6] and polyethers [7–10]. The complexation of monodisperse polyoxyethylene (POE) with a variety of chain lengths was, for example, quantitatively evaluated by the method developed [7], and the thermodynamic origin of the selectivity in the complexation of POE with alkali metal ions was elucidated as a result [9]. However, chromatography involves several drawbacks: (1) the retention equilibria must not be affected by solution equilibria; and (2) the mobile phase conditions are restricted, and must not cause damage to the stationary phase. The fact that CE basically involves no stationary phase promises

its preference and versatility as an approach to solution chemistry.

Addition of organic solvents to an aqueous solution in CE has been extensively attempted [1,11–15]. However, in most studies, it was intended to enhance the selectivity or to modify the electroosmotic flow (EOF) by addition of organic solvents. In contrast, the use of an entirely non-aqueous solvent has rarely been attempted in CE. This might be because the high separation ability of CE did not require the use of a non-aqueous medium in a practical sense. Kenndler and Gassner [16], for example, reported non-aqueous CE (in their study 99.5% methanol), in which methanol was used to vary the selectivity of the separation of anions. Recently, Sahota and Khaledi [17] reported that CE in formamide promises higher efficiency and shorter analysis times because of the high viscosity and the high dielectric constant of the solvent.

The primary aim of this paper is to show that the use of non-aqueous solvents permits the utilization of reactions and interactions, that do not take place in water but do so in non-aqueous media in CE separations. Subsequently, information on the solution chemistry can in some instances be extracted from the retention data in the usual manner.

2. Experimental

The capillary electrophoretic system was composed of a Matsusada high-voltage power supply (HCZE-30P No. 25; the maximum voltage was 30 kV and the maximum current 250 μA), a JASCO UV-visible detector (870-CE) and a fused-silica capillary (50 μm I.D., 375 μm O.D., 44.6 cm long, 29.6 cm effective length, with a detection window located 15 cm from the negative end). Unless stated otherwise, currents typically ranged from 35 to 45 μA under a 20-kV applied voltage. Samples were introduced into the capillary by siphoning at the positive end. Conductivity measurements were carried out at 25°C with a TOA CM20S conductimeter.

Reagents were of analytical-reagent grade. If necessary, salts were dried at an appropriate

temperature under vacuum. Methanol was distilled twice. Tetraethylammonium chloride was added to adjust the ionic strength of the solution.

Crown ethers were synthesized according to the literature [18] and recrystallized from heptane. The crown ethers synthesized were benzo-12-crown-4 (B12C4), benzo-15-crown-5 (B15C5), benzo-18-crown-6 (B18C6), dibenzo-18-crown-6 (DB18C6), dibenzo-21-crown-7 (DB21C7), dibenzo-24-crown-8 (DB24C8) and dibenzo-30-crown-10 (DB30C10). Dinitrobenzoyl-POE (DNB-POE) was prepared by the reaction of POE with 3,5-dinitrobenzoyl chloride with magnesium as a catalyst in dry benzene.

3. Results and discussion

3.1. Migration of non-ionic polyethers by on-capillary complexation

Polyether complexation takes place unfavourably in solvents having a strong solvation ability for hard cations or having too low dielectric constants; in the former instance (e.g., water), polyether cannot replace solvent molecules existing in the solvation shell, and in the latter (e.g., dioxane), ion-pair formation interferes with the complexation. In this study, methanol was selected as a solvent from such a viewpoint, i.e., methanol has a moderate dielectric constant ($\epsilon = 32.6$) and is a weaker solvent than water. In addition, as this solvent has been extensively used as a solvent for the study on polyether complexation, comparison of results is also easy [19,20].

The apparent electrophoretic mobility of an electroneutral polyether is determined by both the complexation ability with an electrolyte cation and the mobility of the resulting complex. The overall mobility of a polyether (μ_{app}) can be described by

$$\mu_{\text{app}} = \mu_{\text{eo}} + \mu_{\text{ep}} \quad (1)$$

where μ_{eo} and μ_{ep} denote the electroosmotic and the electrophoretic mobility, respectively. If only

1:1 complexation takes place in the capillary, Eqn. 1 can be written as

$$\begin{aligned}\mu_{\text{app}} &= \mu_{\text{eo}} + \beta\mu_{\text{max}} \\ &= \mu_{\text{eo}} + K_1[M]\mu_{\text{max}}/(1 + K_1[M])\end{aligned}\quad (2)$$

where β represents the degree of the complexation, μ_{max} is the intrinsic electrophoretic mobility of the complex, K_1 is the 1:1 complex formation constant and $[M]$ refers to the equilibrium concentration of the uncomplexed cation in the analyte segment. As $[M]$ can be calculated from the analytical concentration of the metal ion (C_M), non-linear regression gives both K_1 and μ_{max} . However, when evaluating weak interactions, we can regard $[M]$ as C_M . The difference between C_M and $[M]$ is at most 2% over the usual concentration ranges ($C_M \geq 0.01 M$, the concentration of the polyether being assumed to be 0.5 mM), when K_1 is smaller than 68. In such a case, we can determine K_1 from the linear relationship between $1/(\mu_{\text{app}} - \mu_{\text{eo}})$ and $1/C_M$.

3.2. Separation of polyethers

Fig. 1 shows electropherograms of DNB-POE(n)D (D denotes dodecyl ether and n refers to the number of repeating oxyethylene units), where NH_4^+ is used as an electrolyte cation. In order to keep the EOF almost constant (or to keep the pH constant), triethylamine (Et_3N) was added to the solution; as triethylammonium ion (Et_3NH^+) did not form complexes with POE, the addition of Et_3N had no significant effects on the electrophoretic mobility of POE. Peaks were identified by the addition of standard monodisperse DNB-POE($n=8$)D to a sample. The peaks of POE($n > 4$)D are resolved from the peak migrating together with the EOF. As shown in Fig. 1B, POE($n=3$)D also migrates faster than the EOF when K^+ is incorporated with NH_4^+ . Similar results were obtained for Na^+ , Rb^+ and Cs^+ . These results imply that POE($n=3$) forms a complex with K^+ and POE($n=4$) does so with NH_4^+ . It was found on the same basis that 5–6 oxyethylene units are required to form the complexes with monoalkylammonium ions.

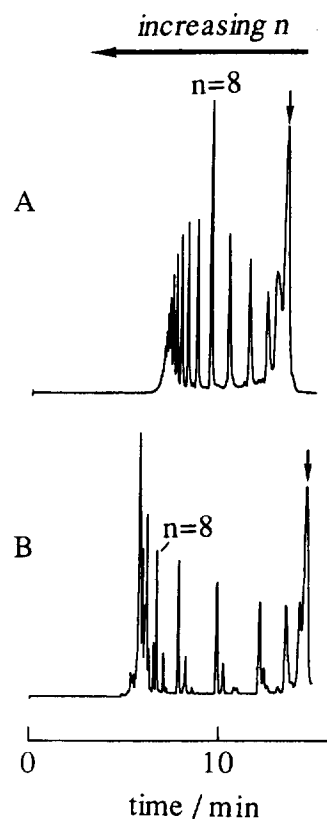


Fig. 1. Electropherograms of DNB-POE(n)D. Solution: (A) 50 mM NH_4Cl + 50 mM Et_3N ; (B) 5 mM KCl + 45 mM NH_4Cl + 45 mM Et_3N . Detection at 250 nm. Applied voltage, 20 kV. The peak of the EOF marker, which is a DNB derivative not having complexation ability, is indicated with an arrow.

In previous studies, we speculated that at least 5–6 oxyethylene units would be required to form stable complexes with heavy alkali metal ions using chromatography [7]. NMR [21] and conductimetry [22] also indicated that the minimum numbers required for K^+ complexation are six and four, respectively. However, these methods are incapable of detecting very weak complexation, e.g., a chromatographic method is suitable for the determination of complexation constants of 10^3 although it is varied by the ion-exchange capacity of the resin used as the stationary phase [23], and similarly for conductimetry [24]. These methods therefore cannot detect weak complexation such as when $K_1 < 10$.

NH_4^+ is not favourably complexed by polyethers, whereas K^+ in general forms much more stable complexes [7,19,20]. Therefore, it is predictable that the incorporation of K^+ with NH_4^+ will bring about drastic changes in separation. Fig. 1B shows an electropherogram obtained with a solution containing 5 mM K^+ . The addition of K^+ enlarges the separation window for relatively short POE chains, while the resolution becomes worse as n increases. This point will be discussed quantitatively later. In Fig. 1B, there are some small peaks other than the main series of DNB-POE(n)D. It was verified by comparison of electrophoretic mobilities that these are peaks of DNB-POEs bearing a tetradecyl, a hexadecyl or an octadecyl group.

The selectivity of POE separation was not altered by varying the electrolyte cations, i.e., the longer the POE chain length the faster is the mobility. This clearly indicates that the longer POE molecule always forms the more stable complex with any cation. In contrast, the migration of crown ethers is influenced considerably by an electrolyte cation in solution. Fig. 2 shows the variation of the separation of crown ethers with cations. The migration orders are $\text{B18C6} > \text{DB18C6} \cong \text{DB21C7} > \text{DB30C10} > \text{DB24C8} > \text{B15C5} > \text{B12C4}$ for NH_4^+ , $\text{B18C6} > \text{DB18C6} > \text{DB21C7} > \text{B15C5} > \text{DB24C8} > \text{DB30C10} > \text{B12C4}$ for monoalkylammonium ion and $\text{B18C6} > \text{DB18C6} > \text{DB24C8} > \text{DB21C7} > \text{B15C5} > \text{DB30C10} > \text{B12C4}$ for dimethylammonium ion. DB30C10, for example, forms a cage-like three-dimensional complex with K^+ [25]. If the same structure is maintained in the complexes with ammonium ions, the above changes in the migration of DB30C10 are understandable; alkyl groups interfere with the formation of the cage-like structure of this ligand, and retard the elution of DB30C10 as a result.

It should be noted that the interactions of B12C4 with all the cations listed above are detected by the present method. Although the complexation of B12C4 with Li^+ has been reported ($K_1 = 22$ in methanol [20]), its complexation with other cations has not.

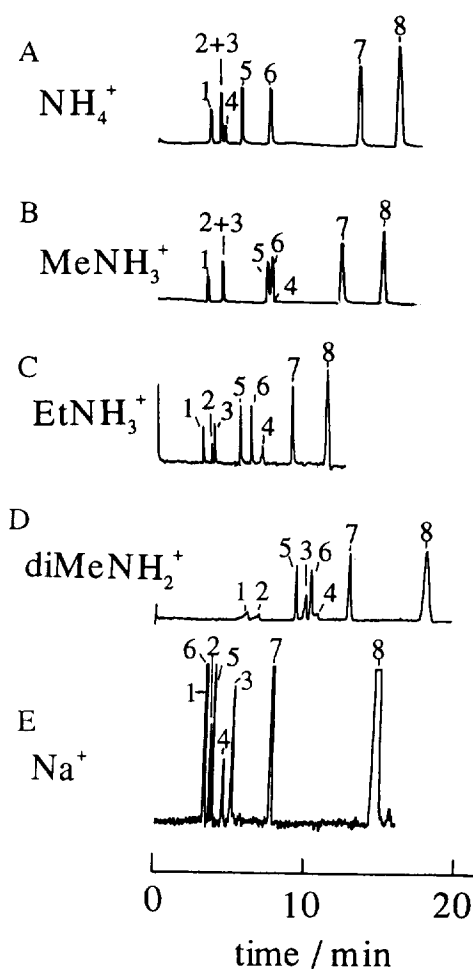


Fig. 2. Separation of crown ethers. Solution: (A) 25 mM NH_4Cl + 25 mM Et_3N + 25 mM Et_4NCl , (B) 15 mM MeNH_3Cl + 15 mM Et_3N + 35 mM Et_4NCl , (C) 40 mM EtNH_3Cl + 40 mM Et_3N + 10 mM Et_4NCl , (D) 40 mM diMeNH_2Cl + 40 mM Et_3N + 10 mM Et_4NCl , (E) 25 mM NaCl + 25 mM Et_3NHCl + 25 mM Et_3N . Peaks: 1 = B18C6; 2 = DB18C6; 3 = DB21C7; 4 = DB30C10; 5 = DB24C8; 6 = B15C5; 7 = B12C4; 8 = acetone. Detection at 280 nm. Applied voltage, 20 kV.

3.3. Determination of complexation constants

Fig. 3 shows examples of plots based on Eq. 2 obtained for NH_4^+ and DNB-POE(n)D. As predicted, linear relationships are obtained, which allow us to determine both the K_1 and μ_{max} values.

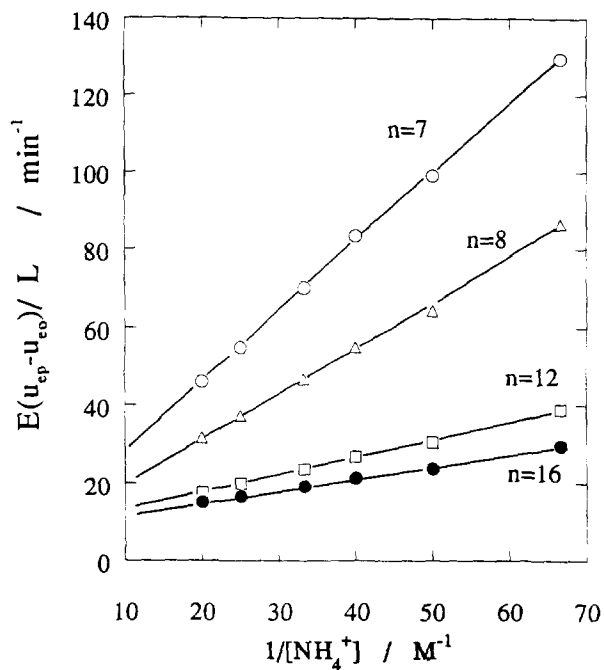


Fig. 3. Example plots based on Eq. 2 for DNB-POE(*n*)D and NH_4^+ .

Table 1 summarizes K_1 and μ_{max} for 1:1 complexes of several cations with DNB-POE(*n*)D and B12C4. The dissociation of cations except for Na^+ was calculated from conductimetric measurements according to the following equation:

$$\alpha = (\Lambda - \Lambda_2) / (\Lambda_1 - \Lambda_2)$$

where Λ_1 , Λ_2 and Λ are molar conductances, measured at the identical concentration, of the chloride salt of a given ammonium ion, Et_3NHCl , that is, the chloride salt of the conjugate acid of Et_3N added to the solution for pH adjustment, and an equimolar mixture of these, respectively. For a 0.05 M solution, the α values were 0.49, 0.73, 0.62 and 0.69 for NH_4^+ , $MeNH_3^+$, $diMeNH_3^+$ and $EtNH_3^+$, respectively. It was difficult to determine α of $PrNH_3^+$ according to the above equation because of a small difference between the Λ_1 and Λ_2 values. $PrNH_2$ was therefore used instead of Et_3N ; in this case, $\alpha = 1$.

Taking POE(*n* = 10) as an example, the K_1 values decrease in the order NH_4^+ (26.7) > $MeNH_3^+$ (15.8) > $EtNH_3^+$ (13.2) > $PrNH_3^+$ (7.8); this order correlates with the order of the size of the alkyl group. The complexation of POE with $diMeNH_3^+$ was also detected, but was so weak that we could not evaluate it quantitatively. This also implies a correlation between the complexation strength and the number of methyl groups. POE traps a cation in a helix structure [26], and usually forms a spherical coordination shell around the cation. An ammonium ion-POE complex will have a similar structure, although the details have not been elucidated. Such a spherical coordination shell is unfavourably formed around an ammonium ion having a large alkyl group or more alkyl groups. Hence the above results are simply explained by steric hindrance.

The mobilities listed in Table 1 are smaller than expected from the conductivity measurements. Taking NH_4^+ as an example, the molar conductance of NH_4Cl was $67.68 \text{ S cm}^2 \text{ mol}^{-1}$ in 0.05 M methanolic solution. Although we did not measure the transference number, it will be reasonable to assume identical ionic conductances for NH_4^+ and Cl^- . The ionic conductance of NH_4^+ , $34 \text{ S cm}^2 \text{ mol}^{-1}$, corresponds to $\mu_{max} = 3.5 \cdot 10^{-8} \text{ m}^2 \text{ s}^{-1} \text{ V}^{-1}$, which is nearly three times larger than the values for the complexes listed in Table 1. Although it is usual for complexation to lower the mobility, this difference is too large. The reason has not been elucidated.

Fig. 4 shows the variations of K_1 and μ_{max} for NH_4^+ -DNB-POE(*n*)D complexes with *n*. As is known for POE complexation with other cations [7], K_1 increases linearly with increase in *n*. On the other hand, μ_{max} increases for *n* < 10 and decreases after the maximum at *n* = 10. The linear increase in K_1 with increase in *n* is explained by a statistical effect: the number of oxygen atoms capable of coordination to an ammonium ion increases as the POE chain length increases. The explanation of the change in μ_{max} is more complicated, however. Correlating the size of a ligand with that of the resulting complex is straightforward; a decrease in μ_{max} is

Table 1
Complexation constants and mobilities of POE and B12C4 complexes with various cations

	Na ⁺		NH ₄ ⁺		MeNH ₂ ⁺		diMeNH ₂ ⁺		EtNH ₂ ⁺		PrNH ₂ ⁺	
	K ₁	μ _{max}	K ₁	μ _{max}	K ₁	μ _{max}	K ₁	μ _{max}	K ₁	μ _{max}	K ₁	μ _{max}
POE(6)	4.3	2.89										
POE(7)	8.6	1.58	12.2	1.02	10.5	0.66			8.1	0.46	5.6	0.48
POE(8)	12.4	1.55	17.8	1.13	10.1	0.97			9.4	0.54	5.7	0.65
POE(9)	17.4	1.46	21.4	1.29	12.9	1.02			11.3	0.59	6.9	0.69
POE(10)	22.0	1.41	26.7	1.31	15.8	1.04			13.2	0.61	7.8	0.74
POE(11)	26.2	1.37	32.4	1.31	18.6	1.04			14.1	0.66	9.1	0.74
POE(12)	26.6	1.49	38.8	1.29	20.5	1.08			15.8	0.68	9.9	0.76
POE(13)	30.9	1.42	44.5	1.27	22.6	1.09					10.7	0.78
POE(14)	34.8	1.37	49.6	1.25	25.2	1.08						
POE(15)	37.7	1.37	54.5	1.24	26.7	1.09						
POE(16)	38.9	1.38	59.4	1.23	28.1	1.11						
POE(17)			63.3	1.23	28.9	1.13						
B12C4	15.9	2.10	50.2	0.38	20.7	0.87	31.8	0.54	28.1	0.50	19.0	0.55

The μ_{max} values are in 10⁻⁸ m² s⁻¹ V⁻¹. Ionic strength, 0.05 M.

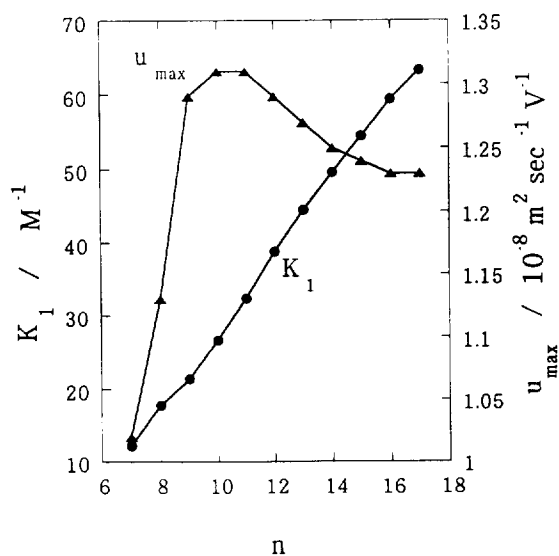


Fig. 4. Variations of K_1 and μ_{\max} with n for NH_4^+ complex.

also explained by the increasing size of the ligand. This consideration holds if a POE molecule is large enough to remove solvent molecules completely from the solvation shell of an ammonium ion. However, other factors should be taken into account in some instances. When a few solvent molecules still exist in the solvation shell of NH_4^+ , the size of the solvated complex is larger than expected from the size of the ligand, and the mobility is lowered. Such complex formation will take place when a POE chain is not large enough to replace solvent molecules completely. These phenomena are superimposed in Fig. 4.

3.4. Simulation of separation

As mentioned already, the present method is suitable for the separation of relatively short POEs. We can simulate the separation of POEs with NH_4^+ as an electrolyte cation using the values listed Table 1. K_1 and μ_{\max} values for larger POE were calculated by extrapolation of the data depicted in Fig. 4; a linear and an exponential relationship were assumed between K_1 and n and between μ_{\max} and n (≥ 10),

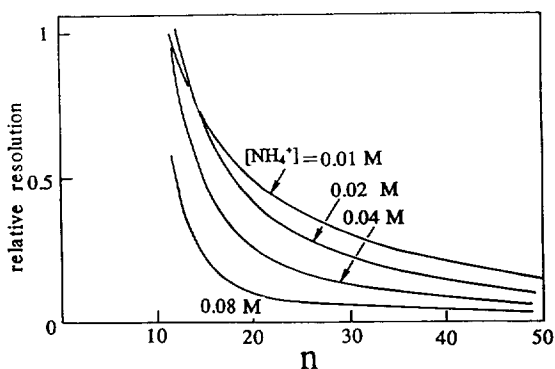


Fig. 5. Simulated decreases in relative resolution for DNB-POE(n)d separation with NH_4Cl as a carrier cation. K_1 and μ_{\max} were extrapolated on the basis of the relationships depicted in Fig. 4. Identical diffusion coefficients were assumed for all oligomers for simplicity.

respectively. The resolution (R) between adjacent peaks was calculated by [1]

$$R = 0.25 [\mu_{\text{ep}}(1) - \mu_{\text{ep}}(2)] V^{1/2} [2LD(\mu_{\text{ep}} + \mu_{\text{eo}})]^{-1/2}$$

where $\mu_{\text{ep}}(1)$, $\mu_{\text{ep}}(2)$ and μ_{ep} are electrophoretic mobilities for two solutes of interest and the average electrophoretic mobility, respectively, and V , L and D are the voltage applied, the length of the capillary and the diffusion coefficient of the solute. If identical D values can be assumed for all complexes, we can calculate the relative resolution for POE chains of $n \geq 10$. Fig. 5 shows the results. The relative resolution becomes worse as n increases, regardless of the concentration of NH_4^+ . Also, a lower C_M generally provides a better separation.

Fig. 6 shows the simulated and experimental electropherograms. Although a constant EOF was assumed for the simulation, it was difficult to keep it constant experimentally; this makes direct comparison difficult. However, the above consideration in general describes the actual separation well.

4. Conclusions

It has been shown that electrophoresis in methanol is effective for the evaluation of poly-

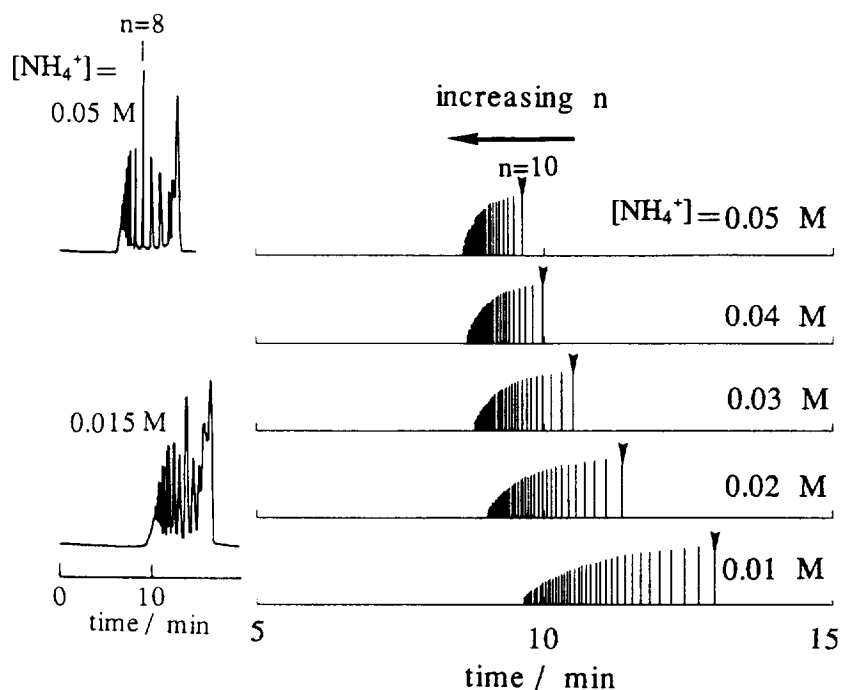


Fig. 6. Simulated and actual electropherograms for the separation of DNB-POE(n)D. Simulation was done with basically the same method as in Fig. 5. Experimental conditions: upper electropherogram, 50 mM NH_4Cl + 50 mM Et_3N ; lower electropherogram, 15 mM NH_4Cl + 15 mM Et_3N + 35 mM Et_4NCl . Detection at 250 nm.

ether complexation and the separation of polyethers. It will also be possible to use a weak interaction, which is not detected by other methods, for the separation and to evaluate it.

Interactions and equilibria taking place in non-aqueous solvents have usually been studied by, e.g., spectrometry or electrochemical methods. However, the present method is also effective for many reasons mentioned already. Although the separation in methanol was taken as an example here to show the efficiency, the present method is applicable to other non-aqueous solvents. The author believes that non-aqueous electrophoresis can be a better and more reliable approach to solution chemistry and that, in a particular case, this method can possibly elucidate interactions that have not even been detected by other methods. Novel applications of this technique will make this point clearer in the near future.

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