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Use of cyclofructan as a potential complexing agent in capillary electrophoresis

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Abstract

A novel selective complex-forming agent for metal cations in capillary electrophoresis (CE) was investigated. The complex former is cyclofructan (cyclic D-fructohexaose or cycloinulohexaose), obtained from enzymatic conversion of inulin using the enzyme, cycloinulo-oligosaccharide fructanotransferase (CFTase), obtained from a strain of *Bacillus circulans*, MCI-2554. It has a circular hexameric structure resembling that of 18-crown-6. Complex formation with a number of cations was determined using CE with indirect UV detection at 200 nm and a background electrolyte consisting of 10 m*M* 2-aminopyridine, buffered at pH 4.50 with acetic acid. Cyclofructan was added to the electrolyte at different concentrations and complexation constants with a number of cations were determined. Permethylated cyclofructan was compared with 18-crown-6 in a 50% aqueous methanol buffer.

Keywords: Complexation; Cyclofructan; Metal complexes; Metal cations

1. Introduction

Selectivity in capillary electrophoresis can be fine-tuned using a considerable number of parameters. Among these is the pH of the background electrolyte (BGE) and, to a lesser extent, ionic strength, particularly for weak monovalent and polyvalent components. Combined with the possible addition of organic modifiers, these parameters generally give ample possibilities for optimizing such separations.

For strong ions, such as metal cations, the above-mentioned parameters will generally be insufficient for selectively affecting mobilities. Crown-ethers, added to the BGE, will, in some cases, selectively complex certain metal ions by forming a 1:1 inclusion complex in dynamic equilibrium, the mobility of which will be much lower than that of the free metal ion. The complexation constants of these interactions are mainly determined by the effective ionic radius of the metal ion, which should match the size of the cavity in the crown-ether.

For chiral, organic components, some form of chirally selective interaction with a complex-forming agent is required. Cyclodextrins are the most commonly used type. The non-polar cavity generally holds the non-chiral, non-polar bulk of the molecule, whereas the polar, chiral group protrudes into the aqueous phase and interacts with the chiral -OH groups at the outer rim of the cyclodextrin molecule, the complex formation constant preferably being different for the D- and L-moieties.

Cyclofructan (cyclic D-fructohexaose or cycloinulohexaose) is a novel component obtained from enzymatic conversion of inulin [1]. The enzyme, cycloinulo-oligosaccharide fructanotransferase

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(CFTase) was obtained from a strain of *Bacillus circulans*, MCI-2554. Cyclofructan has a circular hexameric structure [2], connected by β-2,1 fructoside linkages (see Fig. 1). The fructose units are chirally oriented in the same manner. The enzymatic conversion in fact yielded a mixture of CFR-6, CFR-7 and CFR-8, which was subsequently fractionated chromatographically. In the present contribution, we only looked at CFR-6 and (for experiments in 50% methanol) its permethylated form. It is obvious that the central ring structure resembles that of 18-crown-6 (Fig. 1).

Previous publications indicate that cyclofructan can form complexes with certain metal ions [3,4]. Using proton NMR titration studies for the permethylated form of cyclofructan, *K* values of around five were found for K^+ and NH_4^+ in 70% methanol [3]. In a thin-layer chromatography (TLC) study of the parent compound, cyclofructan, considerably smaller *K* values for metal cations were found in water than in 50% methanol [4]. It appears that there is considerable similarity between cyclofructan and crown-ether interaction. Also, comparison of cycloinulohexaose, -heptaose and octaose indicates that internal ring diameter determines the selectivity, in the same manner as in the case of crown-ethers. Advantages of native cyclofructan with respect to the crown-ethers include the excellent solubility in water and non-toxic properties.

2. Experimental

2.1. Equipment

Capillary electrophoresis experiments were carried out in a P/ACE 5500-type of electrophoresis equipment from Beckman (Fullerton, CA, USA). Data acquisition was with P/ACE software version 3.0. Further data processing was achieved using DAx software (Van Mierlo Software Consultancy, Eindhoven, Netherlands). The capillary was fused-silica, 367 mm (length to the detector, 298 mm)×75 μm I.D. The temperature was set at 25°C. The separation voltage was 20 kV unless stated otherwise. The

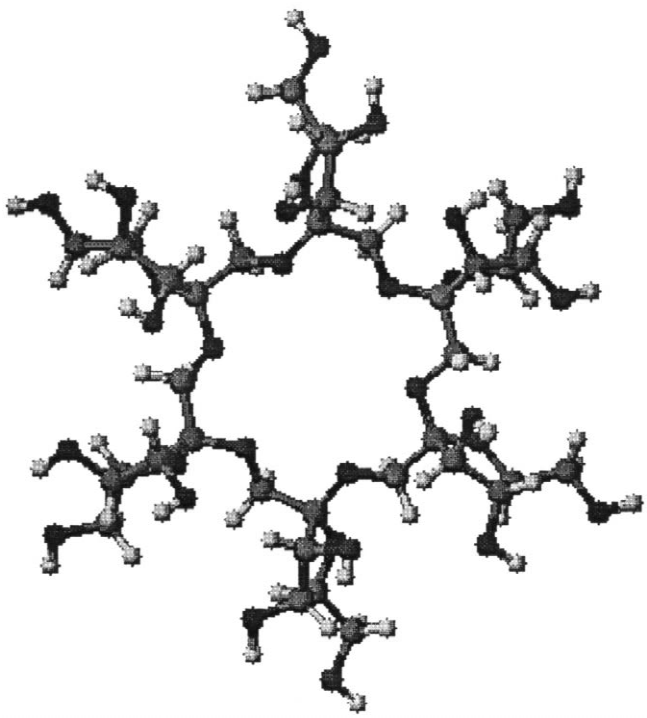

Fig. 1. Structural formula for cyclofructan. The fructose units are oriented approximately perpendicular to the central crown ether plane.

detection wavelength of indirect detection was 214 nm. A positive polarity at the inlet was used.

2.2. Procedures

After adding cyclofructan to the BGE, a filtration step appeared to be necessary to remove some slight dust-like impurities and for obtaining a smooth, spikeless baseline in the electropherogram. For this purpose, a 0.45-μm syringe-type filter (Gelman) was used. Electrophoretic experiments were all carried out in triplicate. Prior to each run, the capillary was rinsed for 1 min with BGE. Bulk viscosities of the BGEs with different concentrations of cyclofructan were measured by low-pressure displacement through the capillary, with a 1-s injection of 1% dimethyl sulfoxide (DMSO) in water as a marker. Viscosity values were determined relative to that of background electrolyte without the addition of cyclofructan. Electroosmosis was measured by observing the migration time of 1% DMSO, which was added to the sample solutions.

2.3. Chemicals

All buffer chemicals were of analytical-grade purity and obtained from the usual sources (Merck, Darmstadt, Germany). The background electrolyte consisted of 10 mmol/l 2-aminopyridine, buffered to pH 4.50 with acetic acid. The cyclofructans CFR-6 and methylated cyclofructan-6 were a gift from Mitsubishi Kagaku (Tokyo, Japan). It was produced from inulin by a strain of *Bacillus circulans*, MCI-2554. Different concentrations of cyclofructan, from 20–200 m*M*, were dissolved in the BGE. 18-Crown-6 was obtained from Sigma.

3. Results and discussion

3.1. Molecular modeling

Using the freeware molecular modeling program ACD/3D (Advanced Chemistry Development, Toronto, Canada), differences in structure between cyclofructan and 18-crown-6 were investigated. The results, indicating only small differences, are summarized in Table 1. In cyclofructan, due to the attachment of the fructose to alternating carbons in the crown-ether ring, the C–O–C bond angle is slightly larger, with a consequently larger ring inner diameter. The inner diameter given in Table 1 is given as the distance between the centers of the opposing oxygen atoms. In both molecules, the oxygen atoms, the unpaired electrons of which are responsible for metal cation complexation, are not exactly in the plane of the ring, but alternating on either side.

Table 1
Molecular modeling results using the ACD/3D program[a]

	18-crown-6		CFR-6	
	Mean	SD	Mean	SD
I.D. (nm)	0.55	0.01	0.57	0.15
Angle C–O–C	108.2	0.5	111.6	0.6
Angle O–C–C	111.8	0.2		
Angle O–C(f)–C			110.7	0.5
Angle C(f)–C–O			114.1	0.7

[a] For further explanation, see text.

3.2. Bulk viscosity measurements

The solubility of cyclofructan was excellent; 200 m*M* was easily dissolved in BGE by stirring for a few minutes at room temperature. Viscosities were determined in duplicate, as described in Section 2. Results are depicted in Fig. 2. The increase in viscosity leads one to suspect that CFR6 will decrease mobilities, possibly regardless of specific interaction taking place. This was confirmed to some extent by electrophoretic experiments. At a constant voltage, the product of the driving current through the capillary and the relative viscosity thus measured remained constant and independent of the CFR6 concentration. It must be observed that the relative bulk viscosity, as outlined in Section 2 was obtained at constant pressure drop, and not at constant shear, as would have been more appropriate for possibly non-Newtonian systems. The experimental results were fitted to the following formula:

$$RV = 1.00 + 45.989 \cdot C^2 - 156.747 \cdot C^3 \quad (1)$$

in which *RV* is the relative bulk viscosity, relative to the value of the aqueous buffer without cyclofructan and *C* is the cyclofructan concentration in mol/l. The coefficient of correlation of this fit was 0.9996.

Experiments were also carried out in 50% aqueous

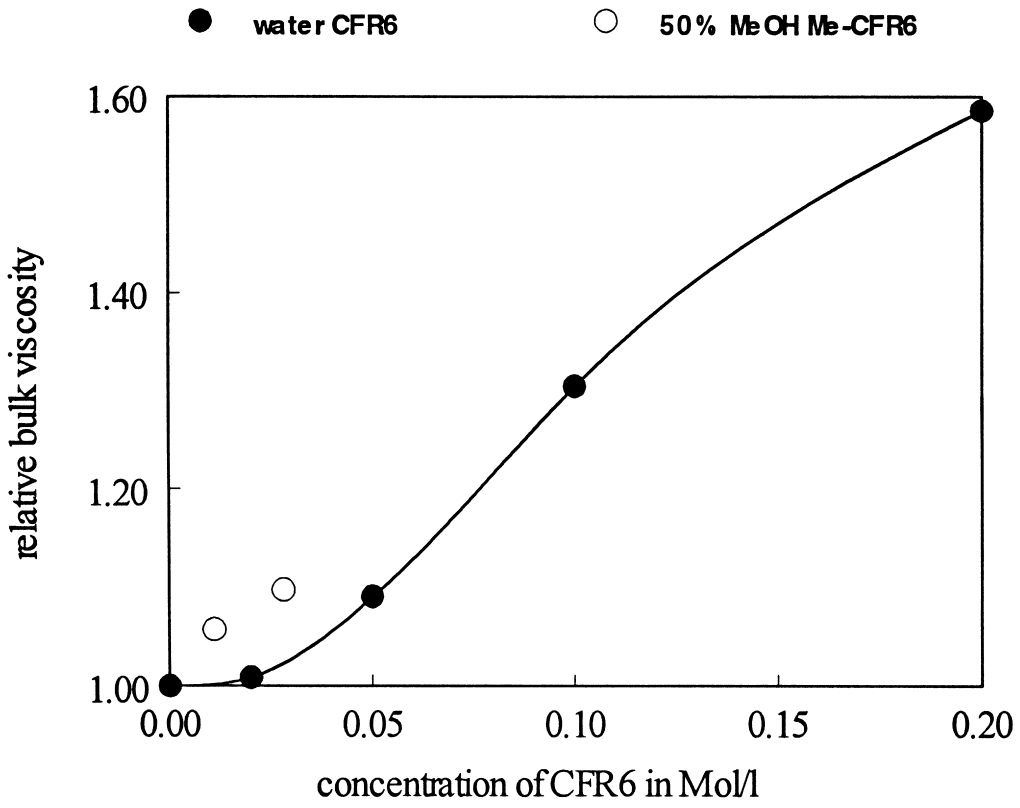

Fig. 2. Results of relative viscosity measurements of CFR-6 in water and of Me–CFR-6 in 50% methanol containing BGEs using the constant pressure capillary displacement technique at 25°C. Values are measured relative to BGE without complex former.

methanol, at lower concentrations of permethylated cyclofructan. Such a methanol–water mixture has a higher bulk viscosity itself [5], and it is also increased considerably by Me–CFR addition, the measurements being made with a buffer in 50% aqueous methanol as a reference.

3.3. Mobility measurements

Raw data were first treated in such a way as to demonstrate which were affected most by the addition of CFR6. Effective mobilities, not corrected for viscosity, were divided by their values at zero CFR6 concentration. The resulting data were fitted to the equation:

$$\mu_{eff}/\mu_0 = \exp(-K_{app}C) \quad (2)$$

in which μ_0 is the effective mobility at zero CFR6 concentration and K_{app} is considered an apparent complex formation constant. As a rough screening of raw data, the above approach is one of several that can be employed. The degree of interaction decreased in the following order: Ba>Ag>Pb>K>Rb>Cs>Ca>Sr>Na>NH_4. As an alternative, one can plot the reciprocal value of the effective mobility as a function of the concentration of the complex former. The resulting straight-line slope even equals the complex formation constant of a 1:1 complex in the case where the mobility of the complex is zero, which is not the case in the present investigation. Values of K_{app} thus determined are tabulated in Table 2, together with standard deviation and literature values for ionic diameter. The latter values refer to crystal ionic diameters rather than those of solvated ions. Best candidates were obviously Ba, Ag and Pb.

From Fig. 3, it must be observed that the decrease in the effective mobility due to complex formation approaches that due to viscosity increase, also caused

Table 2
Apparent complexation constants according to Eq. 2, together with literature values for complexation with acetic acid [6]

Ion	Diameter (nm)	K_{app}	SD	K_1 (HAc)	K_2 (HAc)
Ba	0.268	11.6	0.9	2.5	
Ag	0.252	2.8	0.9	2.3	
Pb	0.24	2.3	0.5	141.3	3162
K	0.266	1.6	0.3		
Rb	0.294	1.2	0.3		
Cs	0.334	0.9	0.4		
Ca	0.198	0.6	0.3	3.4	
Sr	0.224	0.3	0.3	2.7	
Na	0.194	0.2	0.3	0.7	
NH_4	0.286	0.2	0.4		

by the presence of the complex formation agent. This will no doubt inhibit accurate determination of complex formation constants when properly corrected for viscosity. Correction for viscosity will lead to incidental overcorrection and an enormous increase in data-scatter.

One has to realize that such an approach would inherently assume that the viscosity effect is the same for all ions, and that the effects of complexation and of viscosity are mutually independent. In our view, these assumptions are not a priori valid. We did however observe that the driving current was inversely proportional to the relative viscosity, so that the assumption is at least valid for the combined buffer ions.

The effective mobility of an ion in the presence of a neutral complex former can be written as a weighted average of the effective mobilities of the free cation μ_{free} and of the positively charged complex $\mu_{complex}$. Weighting factors are the relative concentrations of free cation and complexed cation, respectively:

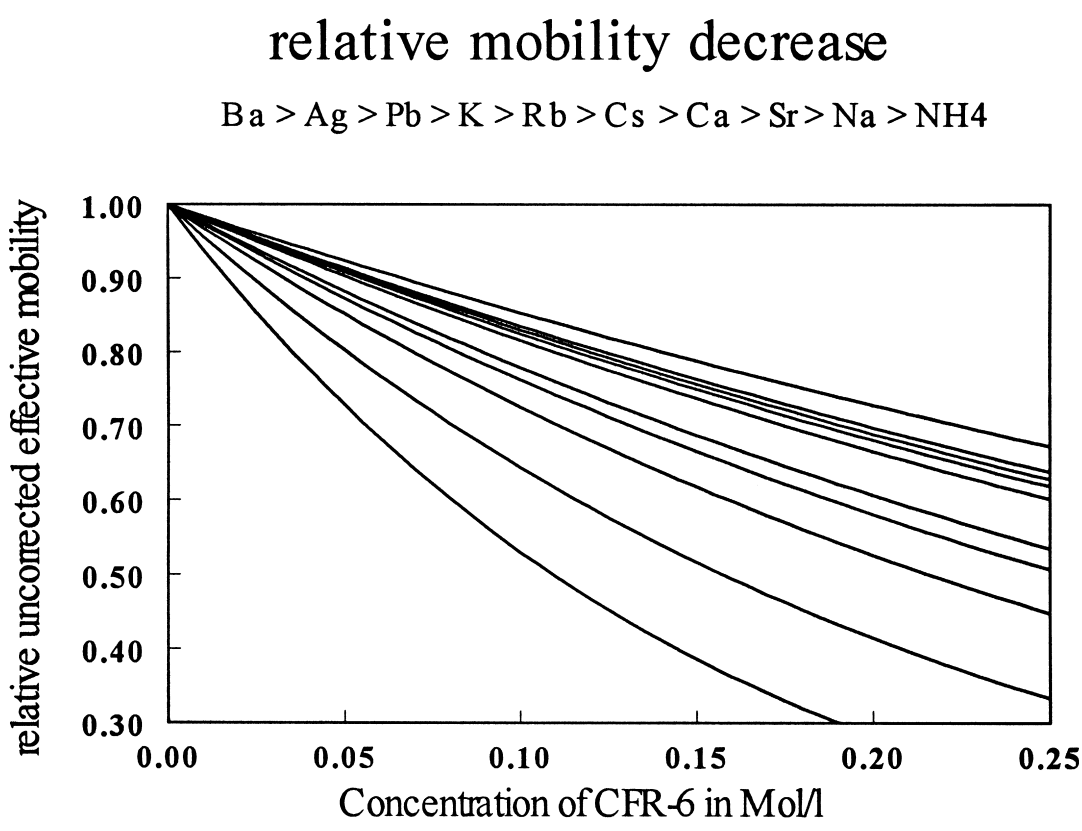

Fig. 3. Relative uncorrected effective mobilities as a function of CFR6 concentration, curves were fitted to a one-parameter exponential relation (see text).

$$\mu_{eff} = [M]/([M] + [MC])\mu_{free} + [MC]/([M] + [MC])\mu_{complex} \quad (3)$$

where [M] and [MC] are the equilibrium concentrations of free metal and complexed metal, respectively. The complex stability constant, K, is defined as:

$$K = [MC]/[M][C] \quad (4)$$

where [C] is the equilibrium concentration of the (non-complexed) complex former, C. The latter can be approximated by the analytical concentration of the complex former, as long as $[M] << C$, which is the case in the present investigation. This makes it possible to obtain the following rearrangement:

$$\mu_{eff} = (\mu_{free} + KC\mu_{complex})/(1 + KC) \quad (5)$$

The experimental data gathered in order to obtain a curve fit of the above equation were now corrected for viscosity by multiplication of μ_{eff} by the RV factor of Eq. 1, thus relating all data to the conditions at zero complex former concentration, thus:

$$(1.00 + 45.989C^2 - 156.747C^3)^*\mu_{eff} = (\mu_{free} + KC\mu_{complex})/(1 + KC) \quad (6)$$

Because the viscosity effect is at least in same order of magnitude, overcorrection and resulting scatter inhibited reasonable curve fits to Eq. (6) for all ions except three, i.e. Ba, Pb and Rb; the results are presented in Fig. 4. and tabulated in Table 3 for the first two metal ions. Standard deviations may seem reasonable, and the values of $\mu_{complex}$, obtained from Ba and Pb, are the same as they are supposed to be. The order of magnitude of $\mu_{complex}$ (considering the charge-to-size ratio) is also realistic for these bivalent complexes. The Rb data however, where two outliers were first removed, yielded unrealistic results, i.e., a complex mobility for a monovalent complex that was twice as high as in the case of the other bivalent ions. The K value for Rb is also unrealistically high, considering the K_{app} values.

When applying the above relations for curve-fitting the experimental data, there is the additional complication that the mobilities of the individual species (free metal ion and complexed metal ion),

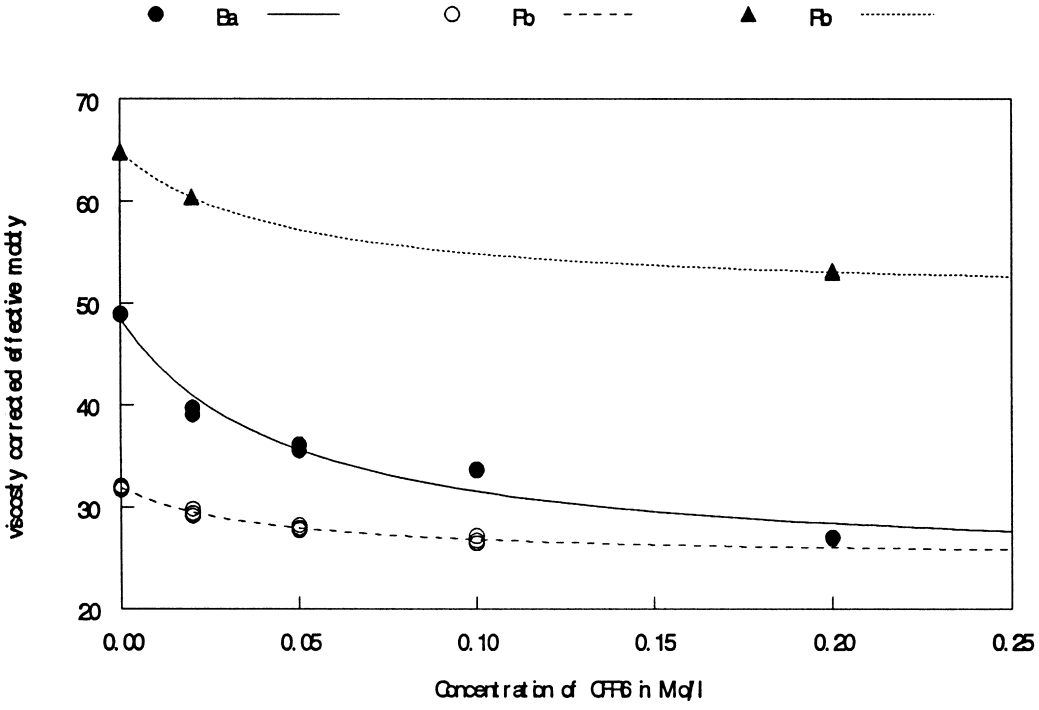

Fig. 4. Viscosity-corrected effective mobilities of Ba, Pb and Rb, as a function of CRF6 concentration.

Table 3
Complexation constants fitted from Eq. 5 (non-corrected) and Eq. 6. (viscosity-corrected) and taking into account competitive acetate complexation

	Eq. no.	Diameter (nm)	CC	*K*	Sd	$\mu_{complex}$	SD	*n*
Ba	5	0.268	0.995	11	1.1			15
	6	0.268	0.97	22	5	23.8	1.7	15
Rb	5	0.294	0.998	0.8	0.3			15
	6	0.294	0.9997	22	0.7	50.4	0.1	9
Pb	5	0.24	0.5	52	65			14
	6	0.24	0.98	26	5.2	24.8	0.5	14
	7	0.24	0.997	6.2	1.9	−29.5	15	14
	8	0.24	0.98	69.7	14	24.8	0.5	14

μ_{free} and $\mu_{complex}$ are not constant, because they also depend on the viscosity and thus on the cyclofructan concentration. In order to cope with this complication, both μ_{free} and $\mu_{complex}$ in Eq. 5. should each be multiplied by the relative viscosity factor, defined in Eq. 1, in order for these two mobilities to refer to zero complex former concentration as well.

The results processed in this manner are tabulated in Table 3 for those cases that resulted in a *K* value that deviated significantly from zero. At those relatively small *K* values, the degree of complexation is very limited; saturation will not occur even at 200 m*M* CFR-6. Consequently, estimation of $\mu_{complex}$ is hardly possible for the other species.

3.4. Competitive counter-ion complexation

In the BGE used, complexation between the sample cations and the buffer anion (acetic acid) cannot be excluded for all of the metal ions analyzed. The literature values of the acetate complexation constants [6] are listed in Table 2. That of lithium is 1.8, and of sodium is 0.7, so that one can safely assume that those of potassium, rubidium and strontium are negligible, as predicted by their sequence in the periodic system. Those of the alkaline earth metal ions and silver are of the same order of magnitude. Lead is apparently complexed with acetate with a higher complexation constant. This also explains the low mobility at zero CFR concentration. On the basis of literature values at infinite dilution and an empirical ionic strength correction [7], a value of $65 \cdot 10^{-9}$ m^2/Vs was expected without complexation. Taking into account 1:1 and 1:2 complexation with the acetate counter-ion, using the complex stability constants from literature [6], this would amount to a mobility of $15 \cdot 10^{-9}$ $m^2/V\,s$ for $PbAc^+$, which is not an unrealistic value.

Exact calculation of the complexation constant with cyclofructan therefore would require taking the acetate complexation equilibria into account. This will be illustrated with lead, where the non-corrected mobility follows from:

$$\mu_{eff} = (\mu_{free} + K_1[Ac^-]\mu_{PbAc} + KC\mu_{complex})/(1 + K_1[Ac^-] + K_2[Ac^-]^2 + KC) \quad (7)$$

Using viscosity correction, the left-hand side of Eq. 7 will read:

$$(1.00 + 45.989C^2 - 156.747C^3)\mu_{eff} = (\text{see Eq. 7}) \quad (8)$$

The values for *K* according to Eqs. 7 and 8 are tabulated in Table 3. Realistic values were obtained after viscosity correction. In spite of the considerable competition from the acetate complexation, considerable saturation in cyclofructan complexation occurs. The situation is slightly less complicated for the other metals. The number of data points is not large enough for sufficiently precise determination of all stability constants in this manner.

3.5. Comparison with crown ether

A water–methanol (50:50, v/v) mixture was used as a solvent for the comparison of methylated CFR and 18-crown-6. A mixture of potassium, ammonium, sodium and calcium was analyzed, as well as solutions of the individual ions. First, the mixture was analyzed in the aqueous system, where K and

NH_4 co-migrated as expected. In the methanolic system, selectivity increased. For an overview, see Fig. 5. This was in part due to different solvations of the ions, in part due to an increased migration time because the EOF is considerably less in the methanolic system. When adding either 18-crown-6 or CFR-6 to a concentration of 11 m*M*, resolution became excellent. Further increasing the additive concentration to 28 m*M* in both cases resulted in a reversed migration order for potassium and calcium (Fig. 6).

Lack of literature data on acetate complexation in 50% aqueous methanol prohibits calculation of cyclofructan stability constants under these competitive conditions.

3.6. Chiral interaction

The manufacturers (Mitsubishi Kagaku, Japan), in their production leaflet [2], claim that the six chirally oriented, D-fructose groups give the molecule cyclodextrin-like characteristics. From the point of view of interaction with the central crown-ether ring with organic molecules, this is unlikely. The fructose groups clearly point away from the center of the crown ether ring and are oriented perpendicular to the plane of this ring. This is very unlike cyclodextrins, where the sugar molecules form the entrance of the conical wall of a cavity. Some initial CE experiments with ephedrine revealed no interaction whatsoever, let alone chiral selectivity of a racemate.

4. Conclusion

Cyclofructan and its permethylated form appear to be non-toxic, highly soluble alternatives to 18-

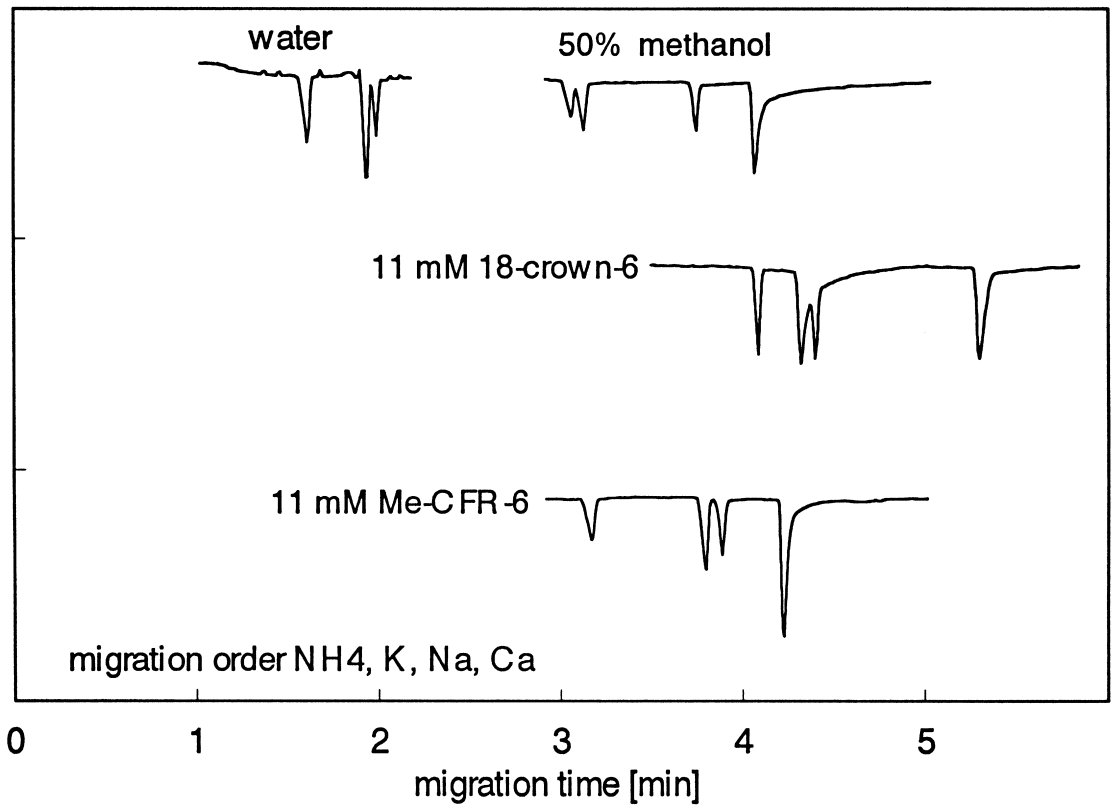

Fig. 5. Separation of ammonium, potassium, sodium and calcium in water–methanol (50:50, v/v) and with the addition of 11 m*M* 18-crown-6 or Me–CFR-6.

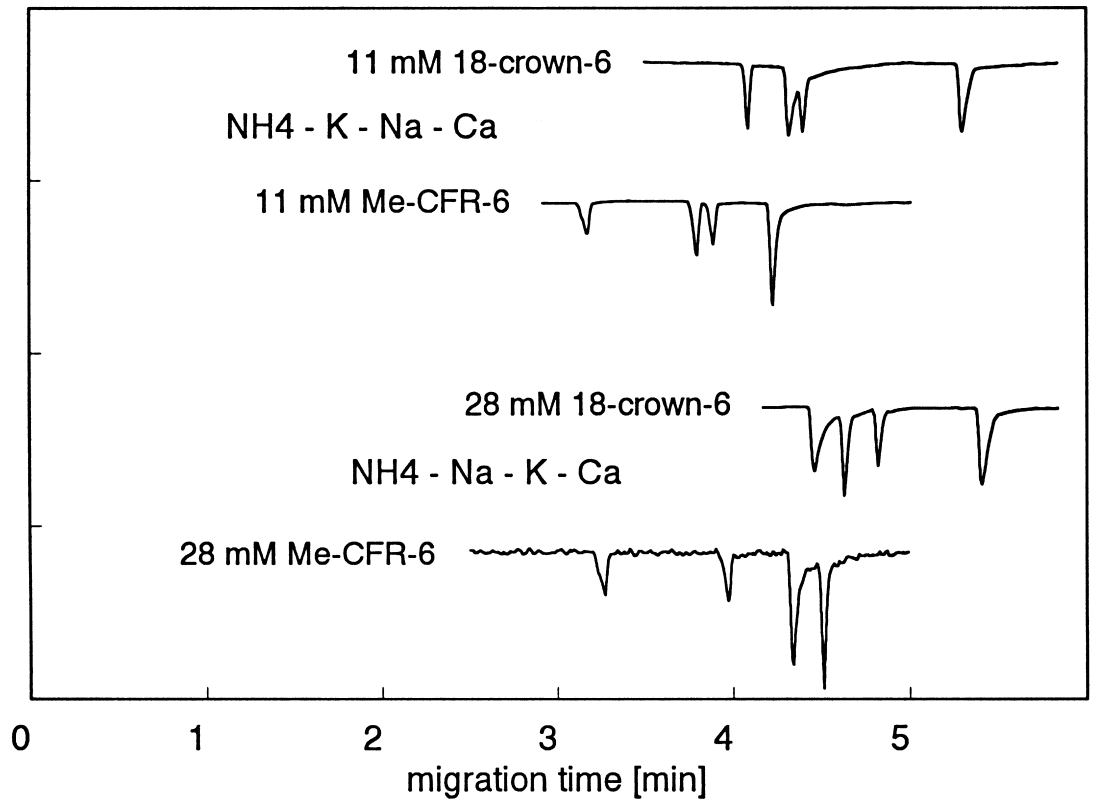

Fig. 6. Separation of ammonium, potassium, sodium and calcium in water–methanol (50:50, v/v) and with the addition of 11 and 28 m*M* 18-crown-6 or Me–CFR-6. With both complex formers, the migration order of sodium and potassium was reversed.

crown-6 for selective complexation of metal ions in aqueous or methanolic systems.

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