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Influence of the nature of the buffer on chiral separation in capillary electrophoresis

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Abstract

The capillary electrophoretic separation of the enantiomers of a number of anionic sulfonamides was studied. Enantioselectivity of a range of native and modified cyclodextrins was tested. Also, from the experimental results, equilibrium constants and complex mobilities in different electrolyte systems were determined. In the present study it was found that the nature of the co-migrating buffer anion may significantly influence the magnitude of equilibrium constants, depending on the type of modification of a specific cyclodextrin. Consequently, this may also strongly influence the optimum cyclodextrin concentration for a particular separation. The results of the separation of sulfonamide enantiomers with cyclodextrins do not agree with the theoretical model suggested by Wren and Rowe, concerning the existence of a maximum in the mobility difference between two optical isomers.

Keywords: Enantiomer separation; Buffer composition; Sulfonamides; Cyclodextrins

1. Introduction

Capillary electrophoresis (CE) has shown to be a powerful separation technique for optical isomers [1,2]. Compared to other chromatographic techniques such as liquid chromatography (LC), in CE often shorter migration times together with higher efficiencies are obtained. Furthermore, CE has a low sample and buffer consumption. Until now, cyclodextrins (CDs) have been the most widely used chiral selectors in CE. Cyclodextrins have the ability to form enantioselective inclusion complexes with many kinds of solutes involving the secondary hydroxyl groups on the larger rim. Inclusion complexation is controlled by charge, size and shape of both the solute and the cyclodextrin [3,4]. Besides that, the composition of the buffer may also play a role in enantioselectivity. Optimization of selectivity must therefore be accomplished by screening of native (α-, β-, γ-CD) and derivatized cyclodextrins, combined with different buffer systems.

Recently, an increasing number of studies has been published concerning the retention and selectivity mechanism in chiral separation. Wren and Rowe [5,6] described the difference in mobility of two enantiomers as a function of the cyclodextrin concentration, predicting an optimum cyclodextrin concentration as a function of equilibrium constants. Rawjee et al. [7] extended this model studying the chiral selectivity as a function of pH and CD

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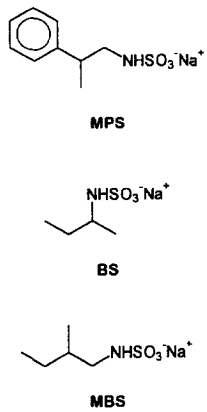

Fig. 1. Chemical structures of the sodium salts of β-methyl-phenethylsulfonamide (MPS), 2-butylsulfonamide (BS) and 2-methylbutylsulfonamide (MBS).

concentration. Other parameters influencing the resolution of enantiomers that were studied included temperature [8,9], concentration of organic modifier and buffer concentration [10].

In the present study, the chiral separation of some sulfonamide enantiomers was investigated, using native and modified cyclodextrins as chiral selectors. The aim of this study was to research both complex formation and stereoselectivity for these solutes as a function of the properties of different native and modified cyclodextrins. Another objective of this study was to investigate the influence of the co-migrating buffer anion on complex formation and enantioselectivity.

2. Experimental

Native α- and β-cyclodextrins, as well as heptakis(2,6-di-O-methyl)-β-cyclodextrin (DIMEB), heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TRIMEB), (2-hydroxy)propylated β-cyclodextrin; D.S:4.6 (HP-β-CD), and soluble α- and β-cyclodextrin polymers were purchased from Cyclolab (Budapest, Hungary). The sodium salts of the racemic sulfonamides, 2-methylbutylsulfonamide (MBS), β-methyl-phenethylsulfonamide (MPS) and 2-butylsulfonamide (BS), were gifts of DSM Research (Geleen, Netherlands). The enantiomers (*R*)- and (*S*)-BS, and (*S*)-MBS were synthesized in the Laboratory of Organic Chemistry of the Eindhoven University of Technology. Chemical structures of these substances are presented in Fig. 1. All solutions were prepared in demineralized water. The background electrolytes (BGEs) were prepared by adjusting a 100 m*M* Tris [tris(hydroxymethyl)-aminomethane, Merck, Darmstadt, Germany] solution with maleic acid, fumaric acid, hydrochloric acid or chromic acid to pH 8.2, or with benzoic acid to pH 8.5. Chromic acid was prepared by percolating a potassium chromate (Merck) solution over a strong acid cation exchanger (type I, Merck).

A P/ACE 2200 capillary electrophoresis system (Beckman, Fullerton, CA, USA) was used for all electrophoretic experiments. Polyacrylamide coated and uncoated fused-silica capillaries (50 μm I.D.) of different lengths were applied. Capillaries were coated according to the procedure of Van der Schans et al. [11]. The capillary cartridge was thermostated at 20°C. When coated capillaries were used, only the capillary was filled with BGE containing a specific cyclodextrin concentration. In case of uncoated capillaries the capillary and the inlet vial were filled with BGE containing cyclodextrin. In none of the experiments were cyclodextrins present in the outlet vial during separation. The UV detector was operated at 214 nm, except for the indirect UV experiments using Tris–benzoate buffer (230 nm) and Tris–chromate buffer (280 nm). To measure relative viscosities of cyclodextrin containing BGEs, first the capillary was filled with a BGE containing a cyclodextrin in a specific concentration, next a small amount of water was injected, after which the BGE under study was pumped through the capillary at constant low pressure ($3.4 \cdot 10^3$ Pa). The same experiment was also carried out with the corresponding BGE without cyclodextrin. The relative viscosity of BGE containing a specific cyclodextrin was calculated from the quotient of the migration time of the water dips obtained from two corresponding BGE solutions, with and without a specific cyclodextrin.

Samples were injected hydrodynamically for 10 s ($3.4 \cdot 10^3$ Pa). The concentration of injected samples was 10^{-4} *M*. The applied field strength was in the range 30 000–50 000 V/m using the constant voltage mode.

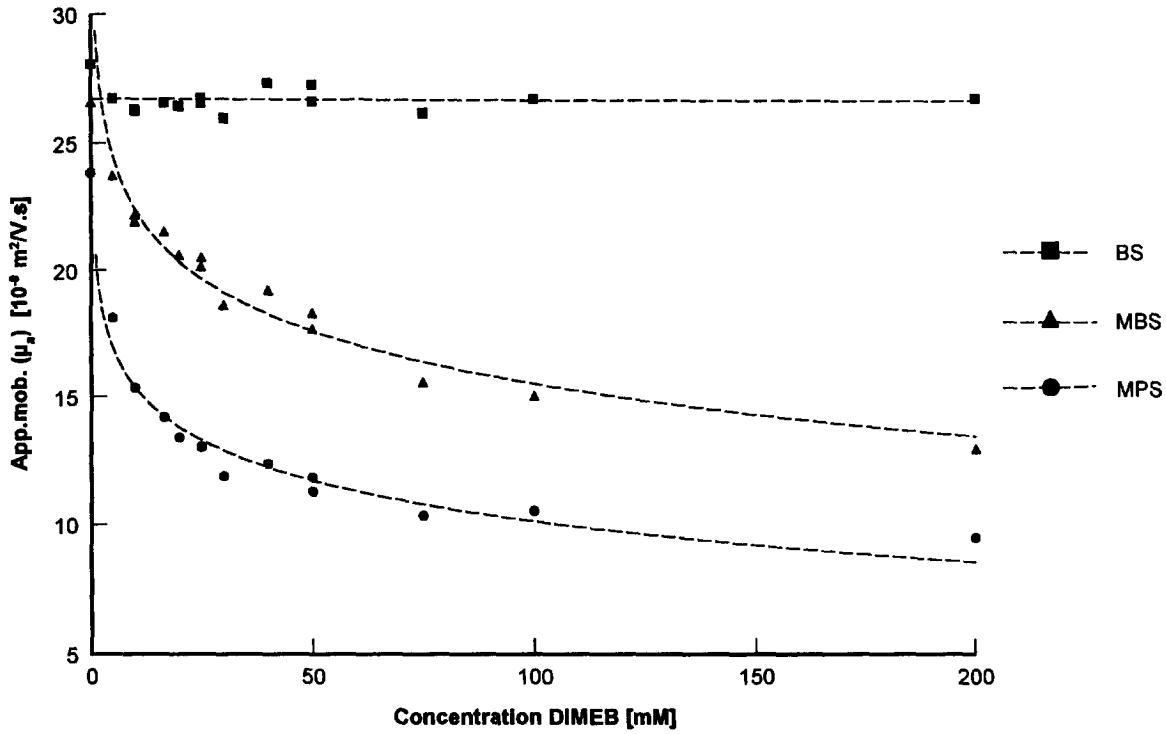

Fig. 2. Apparent mobilities (corrected for viscosity effects) of MPS, BS, and MBS versus concentration of DIMEB. BGE: 100 m*M* Tris–benzoate pH 8.5. Capillary: 468 mm (effective length 401 mm), 50 μm I.D. Separation 15 kV; T=20°C.

3. Results and discussion

Electrophoretic experiments were performed in different BGEs at pH 8.2 or 8.5. At this pH, the analytes were moving as anions, opposite to the direction of the EOF. Under these conditions, using positive polarity, both the electroosmotic mobility (water dip) and the mobility of the analytes could be determined in one run, providing an accurate calculation of effective mobilities. Native (α- and β-CD), modified (DIMEB, TRIMEB, HP-β-CD) cyclodextrins and neutral α- and β-cyclodextrin polymers were dissolved in several concentrations in the BGEs under study. One of the objectives of this research was to study the influence of the co-migrating buffer anion on the inclusion-complex formation between cyclodextrin and analytes. Therefore, electrolytes containing 100 m*M* Tris were titrated with hydrochloric acid, maleic acid, fumaric acid and chromic acid respectively to pH 8.2, and with benzoic acid to pH 8.5. Wren and Rowe [5] suggested a model for the separation of optical isomers in capillary electrophoresis describing the change in apparent mobility (corrected for the electroosmotic mobility) of enantiomers (μ_a) as a function of the equilibrium constant (K_c) for complex-formation between analytes and cyclodextrin.:

$$\mu_a = \frac{\mu_1 + \mu_2 K_c[C]}{1 + K_c[C]} \quad (1)$$

where [C]=concentration of cyclodextrin (mol/l); μ_1=effective mobility of free enantiomer (m^2/V s); μ_2=effective mobility of cyclodextrin–enantiomer complex (m^2/V s); K_c=equilibrium constant for complex formation (l/mol).

This equation can be transformed to:

$$\frac{\mu_1 - \mu_a}{[C]} = K_c(\mu_a - \mu_2) \quad (2)$$

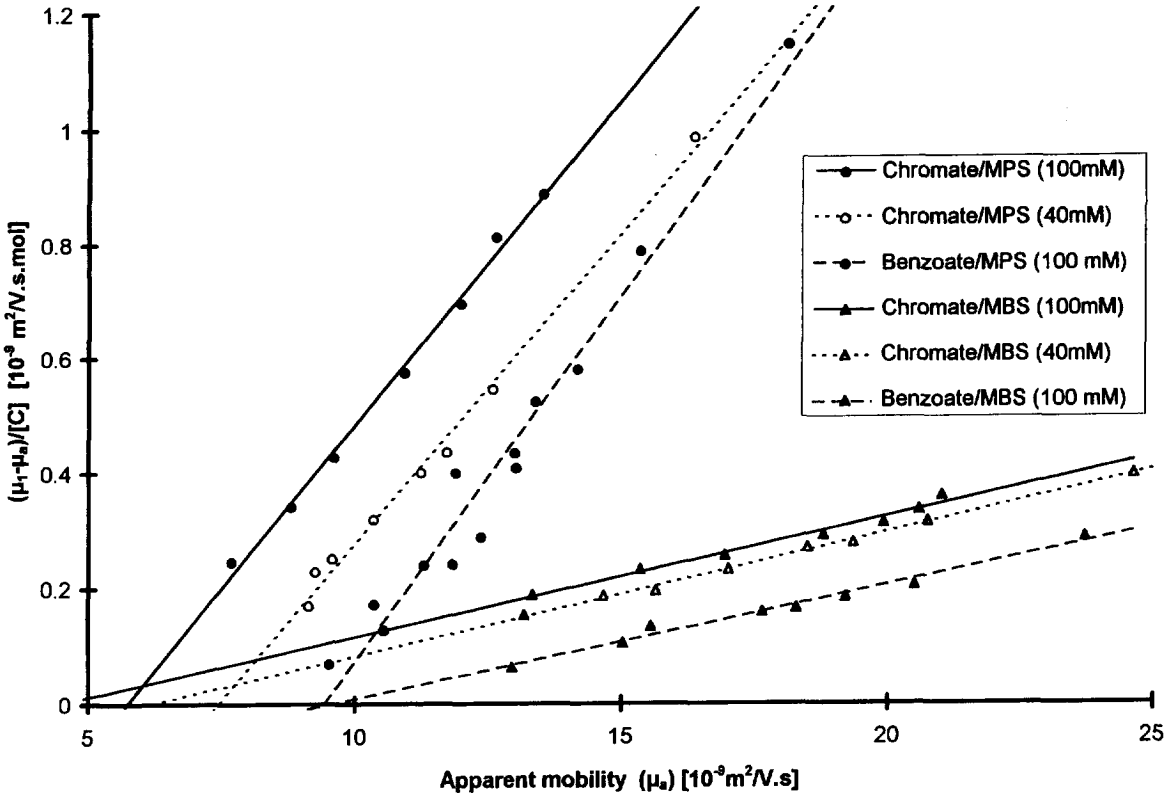

Fig. 3. Graphical determination of K_c values and mobilities of selector enantiomer complexes (μ_2) for complexes between DIMEB and MBS and MPS in different BGEs. Experimental conditions as in Fig. 2.

Consequently, according to this model, if complex formation occurs, a graphical representation of $(\mu_1 - \mu_a)/[C]$ versus μ_a must show a linear relationship, with a slope equalling K_c and the μ_a-axis being intercepted at $\mu_a = \mu_2$. In our experiments, standard errors of K_c and μ_2 calculations were smaller than 10%. Day to day reproducibilities were also within 10%. The relative viscosities of BGEs containing cyclodextrins were determined as a function of the concentration of cyclodextrins added to the BGE and

Table 1
Equilibrium constants of complex formation (K_c l/mol) for different modified cyclodextrins and different buffer systems

	DIMEB		α-CD-polymer		β-CD-polymer	HP-β-CD	TRIMEB	
	MPS	MBS	MPS	MBS	MPS	MPS	MPS	MBS
100 m*M* Tris–benzoate	120	22	30	28	80	70	–	–
100 m*M* Tris–maleate	–	25	–	48	–	–	–	–
100 m*M* Tris–fumarate	108	25	–	51	–	–	–	–
100 m*M* Tris–chloride	120	–	48	–	155	130	16	–
100 m*M* Tris–chromate	117	22	52	59	–	–	14	20

Average values are tabulated for each pair of enantiomers.

all measured mobilities were corrected for the influence of viscosity by multiplying with the relative viscosity.

3.1. DIMEB

First the influence of the co-migrating buffer ion on complex formation was studied for the modified cyclodextrins DIMEB and TRIMEB. As an example in Fig. 2 apparent mobilities of MPS, BS, and MBS enantiomers are plotted as a function of the concentration of DIMEB in the BGE. Obviously, increasing concentrations of DIMEB results in a decrease of the apparent mobilities of MPS and MBS, with MPS showing a higher affinity towards the cyclodextrin as compared to MBS. However, BS apparently shows very little interaction with the cyclodextrin, since hardly any decrease in mobility is observed.

In Fig. 3 the relationships of $(\mu_1 - \mu_a)/[C]$ versus the apparent mobility μ_a of MPS and MBS for the different BGEs are plotted. As can be seen from the slopes of the curves, the mean equilibrium constant K_c is larger for MPS than for MBS. In Table 1 all K_c values for the different chiral selectors and BGEs are summarized. We think that the larger K_c values for MPS in case of DIMEB can be attributed to the aromatic structure of MPS, which is more likely to fit in the hydrophobic cavity of the cyclodextrin than the aliphatic chain of MBS. Furthermore, for DIMEB as the chiral selector, the different magnitudes of the K_c values of MPS and MBS appeared to be independent of the applied BGE anion. Since benzoic acid is likely to enter the CD-cavity and as a consequence could decrease the interaction between

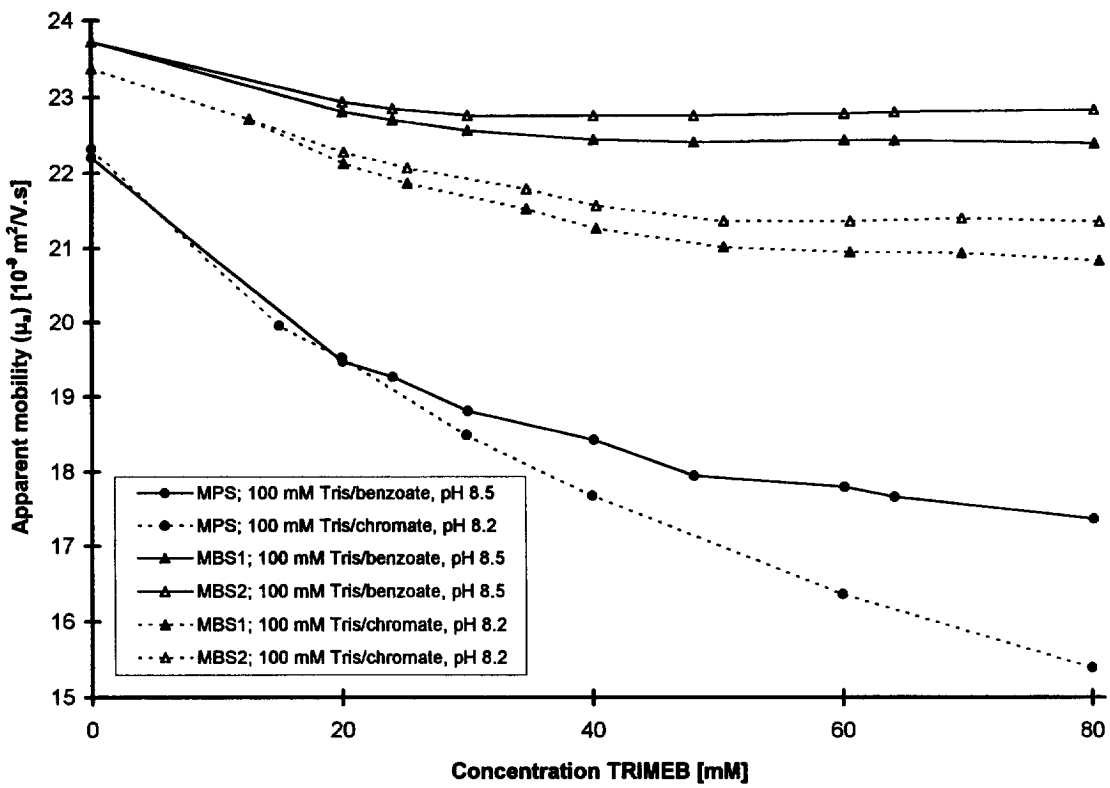

Fig. 4. Apparent mobilities for MPS and MBS enantiomers (corrected for viscosity effects) versus concentration TRIMEB. BGE: as indicated. Other experimental conditions as in Fig. 2. (MBS1 = first eluting enantiomer.)

analyte and CD it was expected that the equilibrium constant in buffers containing benzoic acid would be lower compared to BGEs not containing aromatic compounds. However, for both MPS and MBS, K_c values appeared to be independent of the buffer anion under these conditions.

It also appears from Fig. 3 that the magnitude of the complex mobility (μ_2) is approximately the same for the MPS- and the MBS-DIMEB complexes, in case the same BGE was applied. Opposite to that, μ_2 values for both MPS and MBS appeared to be dependent on the BGE nature and composition. When benzoate was used as the co-migrating anion, the μ_2 values for both MBS and MPS were about 50% larger as compared to chromate. Besides the nature of the buffer this observation might also be attributed to the higher ionic strength of the Tris–chromate buffer (I=64.5 mmol/l) compared to the Tris–benzoate BGE (I=27.6 mmol/l). In order to allow a fair comparison at equal ionic strengths, also experiments were carried out at 40 mM Tris–chromate BGE (pH 8.2, I=25.8). As illustrated in Fig. 3, μ_2 does increase upon decreasing the Tris–chromate buffer ionic strength, although the complex mobility still does not match the value obtained in the Tris–benzoate BGE. Furthermore, from the data μ_2 values for MPS and MBS were calculated and compared for 100 mM Tris–benzoate pH 8.2 (I=27.6), 100 mM Tris–Cl pH 8.2 (I=43.1 mmol/l), and 60 mM Tris–Cl pH 8.2 (I=25.9 mmol/l). The mobilities of the MPS and MBS complexes were $-9.0\cdot10^{-9}$ in Tris–benzoate, $-7.5\cdot10^{-9}$ in Tris–Cl (100 mM), and $-8.2\cdot10^{-9}$ m^2/V s in Tris–Cl (60 mM) respectively. These results suggest that μ_2 values decrease upon increasing the BGE ionic strength, as expected.

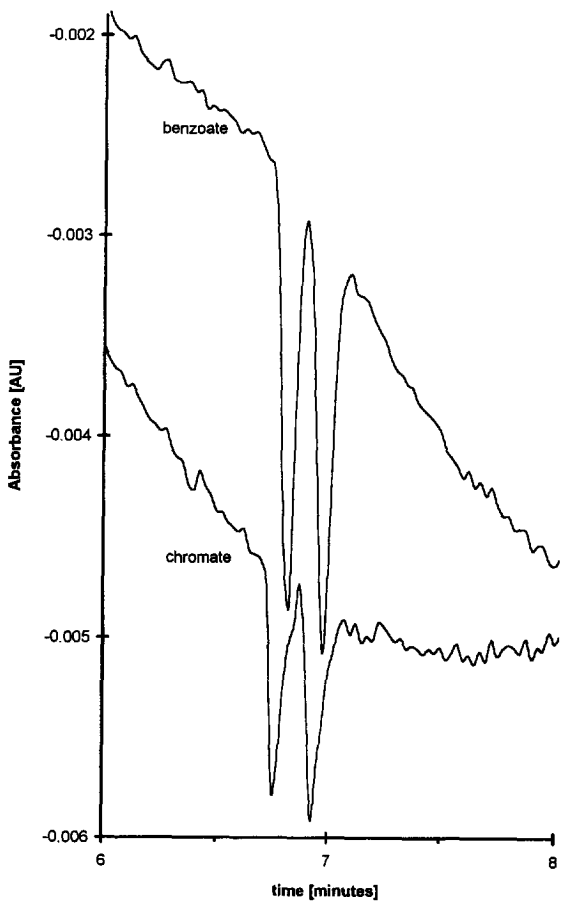

Fig. 5. Chiral separation of MBS enantiomers using a 100 mM Tris–chromate pH 8.2 and a 100 mM Tris–benzoate pH 8.5 as BGEs. Chiral selector: 80 mM TRIMEB. Other experimental conditions as in Fig. 2.

According to the model suggested by Wren and Rowe, optimum difference in apparent mobility (as defined by Eq. 1) between two optical isomers is obtained at:

$$[C] = 1/\sqrt{K_{c1}K_{c2}} \quad (3)$$

with K_{c1} and K_{c2} being the equilibrium constants of both enantiomers.

This is in good agreement with the results of our experiments, showing optimum mobility-difference between the optical isomers of MPS, at a DIMEB concentration of 10 mM (results not shown). As discussed earlier K_c values appeared to be independent on the nature of the BGE. The same might be true for the selectivity (S) which is given by the ratio of the mobility difference of the two enantiomers and the mean mobility value of both optical isomers. This was confirmed in first instance by our selectivity results. For MBS however, no resolution was obtained using Tris–benzoic acid as the BGE whereas the optical isomers were partly resolved (R_s=0.5) using either maleate, chromate or fumarate as co-migrating buffer ion. This maximum in resolution was obtained at about 50 mM DIMEB, which corresponds with the optimum concentration predicted by Eq. 3, using the results from Table 1.

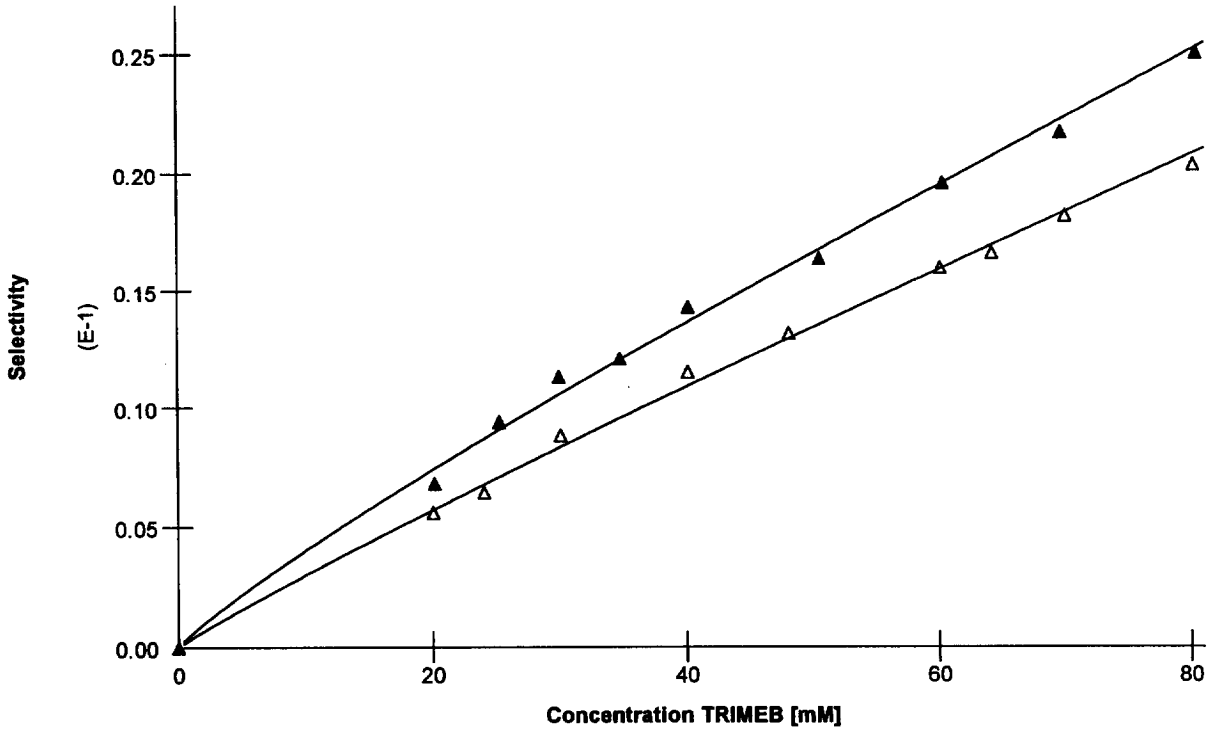

Fig. 6. Selectivity of MBS enantiomers versus concentration of TRIMEB for two different BGEs. ▲ = 100 m*M* Tris–chromate pH 8.2, △ = 100 m*M* Tris–benzoate pH 8.5. Other experimental conditions as in Fig. 2.

For the enantiomers of BS a partial resolution at 200 m*M* DIMEB was obtained, which was shown to be independent of the nature of the corresponding BGE. Evidently, interaction occurred between this analyte and DIMEB. Spiking of the racemic sample with (*S*)-BS demonstrated that the (*R*)-enantiomer had a stronger interaction with DIMEB than the (*S*)-enantiomer.

3.2. TRIMEB

In Fig. 4 the apparent mobilities of MPS and MBS enantiomers, after correction for the BGE viscosity, were plotted against the TRIMEB concentration for two different BGEs. Compared to the results for DIMEB (Fig. 2), for TRIMEB the decrease in mobilities of the MBS and MPS solutes is less steep. We think that this might be attributed to sterical hindrance by the methoxy group on the 3-position located on the wider entrance of the cyclodextrin rim, while DIMEB is only methoxy substituted on the 2- and 6-position. For TRIMEB, K_c values were experimentally determined for the BGEs Tris–chromate and Tris–chloride, while no reliable data could be obtained for Tris–benzoate. This latter result might be explained by the weaker interaction and retardation of the analytes with TRIMEB in Tris–benzoate electrolytes. Consequently in such cases, standard errors in K_c calculations become relatively high. The results are summarized in Table 1.

Due to the lower ionic strength of the Tris–benzoate BGE we might expect complex-mobilities (μ_2) to be larger than in other electrolyte systems. In Fig. 4 it is shown that under the experimental conditions MPS is more retarded than MBS. This is not in agreement with the calculated equilibrium constants (K_c) for the optical isomers of MBS (MBS1 and MBS2) of 17 respectively 22 l/mol, in a 100 m*M* Tris–chromate BGE (pH 8.2), whereas $K_c = 14$ l/mol for MPS in the same BGE. The calculated complex-mobility (μ_2) however for MBS is rather high ($-19.5 \cdot 10^{-9}$ m^2/V s) compared to MPS–TRIMEB complexes ($-9.2 \cdot 10^{-9}$ m^2/V s), thus explaining the higher retardation for MPS. A

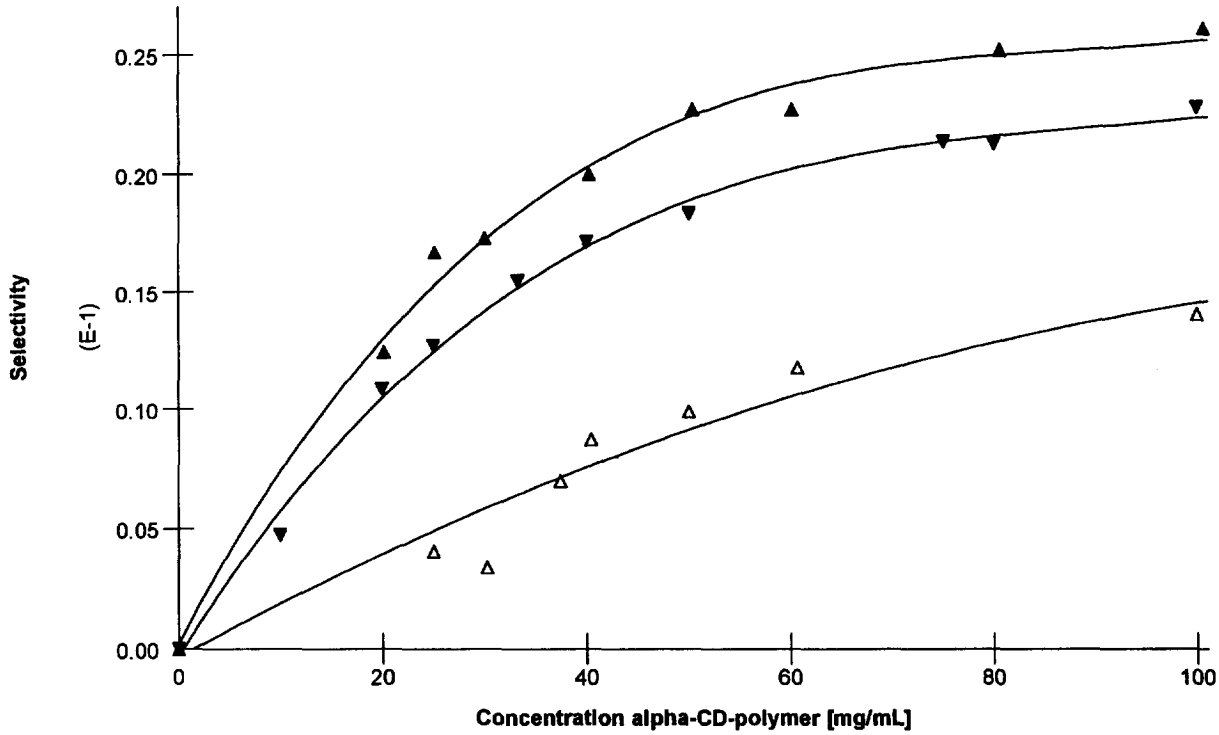

Fig. 7. Selectivity of MPS enantiomers versus concentration of the α-cyclodextrin polymer using different BGEs. ▼=100 m*M* Tris–chloride pH 8.2. Other symbols as in Fig. 6. Experimental conditions as in Fig. 2.

possible explanation of the high value of μ_2 for TRIMEB–MBS complexes could be that complex formation differs from the regular "single analyte–single CD-molecule" mechanism.

Furthermore from the data it can be seen that the analytes are more retarded in chromate containing BGEs than in BGEs containing benzoate. This can be explained either by a stronger complex formation, or by a lower complex-mobility in the Tris–chromate buffer. As an example in Fig. 5 the baseline resolution of the MBS enantiomers for TRIMEB as the chiral selector in 100 m*M* Tris–chromate and Tris–benzoate buffers (pH 8.5) is presented. Resolutions (R_s) were calculated according to Eq. 4

$$R_s = \frac{t_2 - t_1}{\frac{1}{2}(w_{b1} + w_{b2})} \quad (4)$$

with t_1 and t_2 being the migration times of the first and second enantiomers, and w_b the peak width at the baseline. Resolution of the MBS enantiomers in Tris–benzoate buffers is slightly better (1.2) compared to Tris–chromate (1.0). This might be explained by a much better mobility-matching between MBS ($m_{eff} = -23 \cdot 10^{-9}$ m^2/V s) and benzoate ($m_{eff} = -30 \cdot 10^{-9}$ m^2/V s), compared to MBS and chromate ($m_{eff} = -65 \cdot 10^{-9}$ m^2/V s) [12]. Spiking of racemic MBS with (*S*)-MBS proved that (*S*)-MBS has larger interaction with TRIMEB under these conditions.

Also selectivities of the MBS enantiomers for different BGEs were calculated according to the definition mentioned earlier. From the results in Fig. 6 it can be seen that selectivities of the MBS enantiomers are larger for chromate containing BGEs than for BGEs containing benzoate. These observations are in good agreement with the results obtained with DIMEB for the separation of MBS enantiomers. According to the data in Table 1 and to Eq. 3, a maximum mobility difference between MBS1 and MBS2 was expected at 50 m*M* TRIMEB using a 100 m*M* Tris–chromate BGE. From our results however,

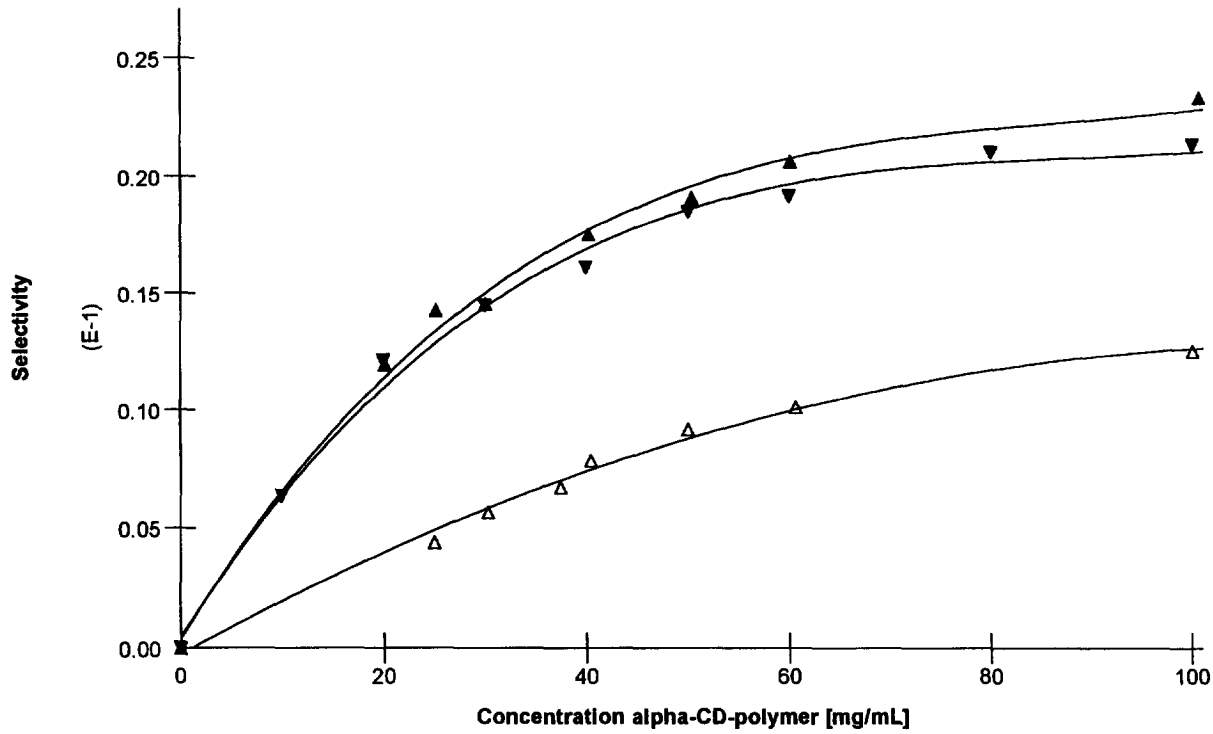

Fig. 8. Selectivity of MBS enantiomers versus concentration of the α-cyclodextrin polymer using different BGEs. ▼ = 100 m*M* Tris–fumarate pH 8.2. Other symbols as in Fig. 6. Experimental conditions as in Fig. 2.

no clear maximum in mobility differences could be distinguished. This could be explained by a different mobility (μ_2) between MBS1–TRIMEB and MBS2–TRIMEB complexes. According to our data this mobility-difference valued 7%. Wren recently modified the mathematical model [5] to deal with such differences [13], although in his case (atenolol in modified β-cyclodextrin) the relative differences were smaller.

3.3. Neutral α- and β-cyclodextrin polymer

Neutral α- and β-cyclodextrin polymers were also included in this study as chiral additives to the BGE.

β-Cyclodextrin polymer did not show any enantioselectivity towards BS and MBS. However for the MPS enantiomers good selectivity was obtained. Equilibrium constants between β-cyclodextrin polymer and MPS were determined for the Tris–benzoate and Tris–Cl BGEs. K_c values for the MPS enantiomers were 78 and 83 l/equiv. for benzoate and 151 and 160 l/equiv. for chloride as the co-migrating anion. The amount of cyclodextrin units per mg of polymer was calculated according to the specification of the manufacturer (β-CD polymer sample: β-CD content 58.2% (w/w), α-CD polymer sample: α-CD content 54%, w/w) as in reference [10]. Consequently 100 mg β-CD-polymer/ml equals 50 mequiv. β-CD/ml, while 100 mg/ml α-CD-polymer is equal to 55 mequiv. α-CD/ml. Opposite to DIMEB, for the β-cyclodextrin polymer, the K_c values for MPS were strongly influenced by the nature of the co-migrating anion. Presumably, complex formation may be suppressed by benzoate, competing with the analyte for inclusion in the cyclodextrin cavity, without however affecting selectivity. From the K_c values shown in Table 1 similar trends can be observed for the MPS and MBS racemates with the α-CD polymer. K_c values increased twofold if chromate was used instead of benzoate as the co-migrating anion. According to Eq. (3), this will influence the optimum cyclodextrin concentration in the BGE. Since the experimentally measured K_c values are nearly the same for MPS and

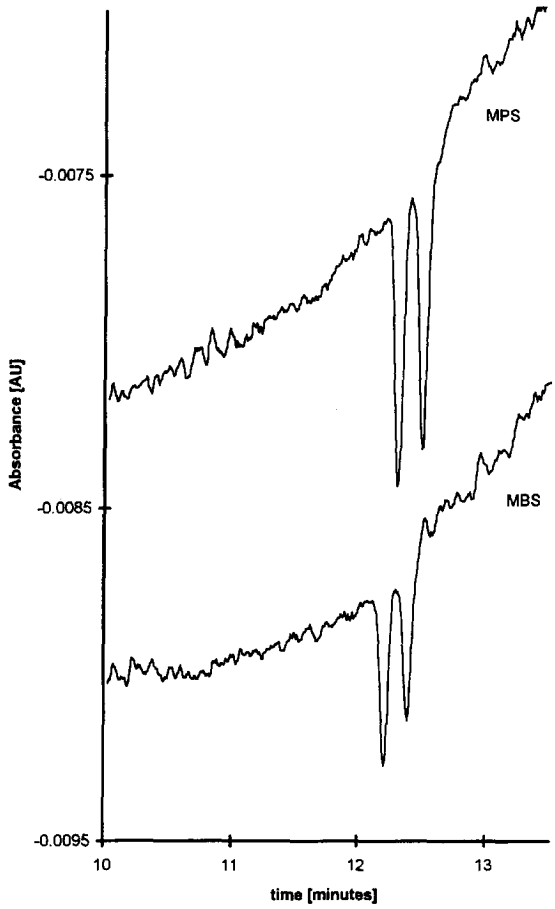

Fig. 9. Separation of MPS and MBS enantiomers. BGE: 100 m*M* Tris–chromate, pH 8.2 containing 100 mg/ml α-cyclodextrin polymer. Coated capillary: 370 mm (effective length 300 mm), 50 μm I.D.. Separation: 15 kV.

MBS, we expected $[C]_{opt}$ to be about the same for both pairs of enantiomers equalling 34 mequiv./l (63 mg/ml) for Tris–benzoate, and 18 mequiv./l (32 mg/ml) for Tris–chromate. In Figs. 7 and 8 selectivities of MPS and MBS enantiomers respectively, for different BGEs are plotted as a function of the α-CD polymer concentration.

From these results two observations can be made. First, as was also the case for TRIMEB with MBS (Fig. 6), no clear maximum in either selectivity or mobility-difference could be distinguished. As was the case with TRIMEB, this might be explained by a different mobility (μ_2) between complexes of α-CD polymer and the two optical isomers. According to our data this mobility-difference valued 4%. For MBS complexes this difference valued 3%. Also from the data it is obvious that the selectivity in Tris–benzoate is again substantially lower than for the other BGEs. For example, to obtain a selectivity for MBS enantiomers (Fig. 8) of 0.013, a concentration of 100 mg/ml α-CD-polymer is needed in a Tris–benzoate BGE, while 20 mg/ml polymer is sufficient in the other BGEs. Thus, from our data it is obvious that benzoate has a negative effect on the enantioselectivity for both these sulfonamides. In this case this effect was even more pronounced than with TRIMEB as the chiral selector (Fig. 6). As an example Fig. 9 shows a full resolution electropherogram of MPS and MBS enantiomers for α-cyclodextrin polymer as the chiral selector. Spiking of racemic MBS with (*S*)-MBS demonstrated that (*S*)-MBS has the largest interaction with the polymer under these conditions, and was the first peak to be detected, as was also the case for TRIMEB.

3.4. HP-β-CD

HP-β-CD (degree of substitution 4.5) was also included in this study for its potential as a chiral selector. The results in Table 1 show that complex formation here is influenced by the nature of the co-migrating anion, similarly as for α- and β-CD polymer. HP-β-CD showed enantioselectivity for the MPS enantiomers ($K_{c1}/K_{c2} = 1.05$) but no selectivity was observed for the optical isomers of BS and MBS.

4. Conclusions

Baseline resolution was obtained for the enantiomers of MPS and MBS for several modified cyclodextrins. Only partial resolution was obtained for the optical isomers of BS. Complex formation was very much influenced by the nature of the co-migrating buffer anion in case where HP-β-CD, α- and β-CD polymers were applied as chiral selectors, with an exception of DIMEB. MBS enantiomers were partly separated using DIMEB in maleate, fumarate, and chromate containing BGEs, but no separation was obtained in a Tris–benzoate BGE. Also for TRIMEB and the α-CD polymer it is shown that benzoate has

a negative effect on the enantioselectivity. However in these cases resolution is not much influenced by the nature of the BGE, probably since benzoate has a better mobility matching with the analytes than the other buffer anions such as chromate or chloride.

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