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Designed combination of chiral selectors for adjustment of enantioseparation selectivity in capillary electrophoresis

In this study an attempt has been made to explain the reasons for changing the enantioseparation selectivity in some dual cyclodextrin (CD) systems compared to the use of single chiral selectors in capillary electrophoresis (CE). An explanation for selectivity changes is proposed based on the effect of the chiral selector on the mobility of the analyte. In order to support the proposed mechanism, several dual systems were designed on the basis of the known recognition pattern of enantiomers for individual CDs. In most cases the separation selectivity could be adjusted in a designed way. There was no experimental evidence for simultaneous binding of a given chiral analyte with both chiral selectors or of chiral recognition of an analyte complex with one CD by another CD.

1 Introduction

1.1 Multiple chiral selectors in CE

Capillary electrophoresis (CE) is established as one of the major techniques for analytical-scale enantioseparations [1, 2]. One of the advantages of CE compared to chromatographic techniques is that the utilization of multiple chiral selectors is easier [3–21]. In fact, it has been illustrated in several studies that enantioseparations which were impossible to achieve with single chiral selectors could sometimes be obtained by a combination of these selectors [3–9]. Another important point is that chiral recognition which is present but impossible to observe in certain single selector systems due to intrinsic characteristics of CE can be observed after the addition of components which do not necessarily contribute to chiral recognition [4–9]. In separation systems containing two (or more) chiral selectors, the latter can act in a cooperative way as well as counteract each other [3, 5–7, 11, 12]. Therefore, better understanding of the mechanisms contributing to enantioseparation selectivity in dual CE systems is required. Information about the chiral recognition (at least chiral resolving properties) of each chiral selector seems to be of crucial importance for their optimal use in combination.

There is at least one example in the literature [13] of CE separation in a dual chiral selector system based on the enantiomer migration order observed in systems where these selectors were used separately. Terabe *et al.* [13] noted that the enantiomer migration order of dansyl (Dns)-DL-amino acids was opposite, when either CDs [13] or a chiral surfactant, sodium taurodeoxycholate (STDC) [22], were used as chiral selectors. At the same time, in CD-modified micellar electrokinetic chromatography (CD-MEKC), these two chiral selectors were found to have an opposite effect on the migration of the Dns-amino acids: CDs accelerated the analytes and STDC decelerated them. Following the principle that the mobility difference between the enantiomers will be maximal when a chiral selector accelerates one enantiomer and the other chiral selector decelerates the opposite enantiomer, the authors used CDs and STDC in combination and observed an improvement in selectivity [13]. Later, other authors also came to the conclusion that when two chiral selectors are used in combination, an opposite chiral recognition to a given analyte is required for improvement of enantioseparation [9, 12].

The aim of the present study is to show that the requirement of opposite chiral recognition for two chiral selectors

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CE and CEC

used in combination is correct in principle but it does not cover all possible cases in chiral CE. Indeed, two chiral selectors used in combination do not always affect the mobility of an analyte in opposite mode. Besides the acceleration by one chiral selector and deceleration by the other, it is also possible that both chiral selectors accelerate or decelerate the analyte. In contrast to the above cases, the same chiral recognition pattern will be required for both chiral selectors for an enhancement of separation selectivity in the latter case [18]. In addition, note that it is impossible to explain the variation of selectivity on the basis of the effect of the chiral selector on analyte mobility in all dual systems. Certain synergistic or antagonistic effects at the level of molecular recognition are also possible [4, 19]. This means that the net effect of two chiral selectors will not necessarily be a "sum" of their independent effects on the mobility of the analyte. All dual separation systems described in this paper were designed *a priori* in such a way as to achieve increased or decreased selectivity compared to single selector systems. This was performed by taking into account the enantiomer binding pattern and migration order observed with single chiral selectors in previous studies [23–28].

1.2 Fundamentals

When two selectors are used in combination and they act independently, the overall mobility difference ($\Delta\mu_{ov}$) between the enantiomers can be expressed as the factorial:

$$\Delta\mu_{ov} = i\Delta\mu_1 + j\Delta\mu_2 \tag{1}$$

where $\Delta\mu_1$ and $\Delta\mu_2$ are the mobility difference generated by chiral selector 1 and chiral selector 2, respectively, and i and j are the statistical weights of $\Delta\mu_1$ and $\Delta\mu_2$. The statistical weights of each chiral selector are determined by their concentration and the competitive affinity of analytes to each chiral selector.

$\Delta\mu_1$ and $\Delta\mu_2$ can be calculated as follows [29]:

$$\Delta\mu_1 = \frac{C_1 (\mu_i - \mu_{c_1}) (K_{R_1} - K_{S_1})}{1 + C_1 (K_{R_1} + K_{S_1}) + K_{R_1} K_{S_1} C_2^1} \tag{2}$$

$$\Delta\mu_2 = \frac{C_2 (\mu_f - \mu_{c_2}) (K_{R_2} - K_{S_2})}{1 + C_2 (K_{R_2} + K_{S_2}) + K_{R_2} K_{S_2} C_2^2} \tag{3}$$
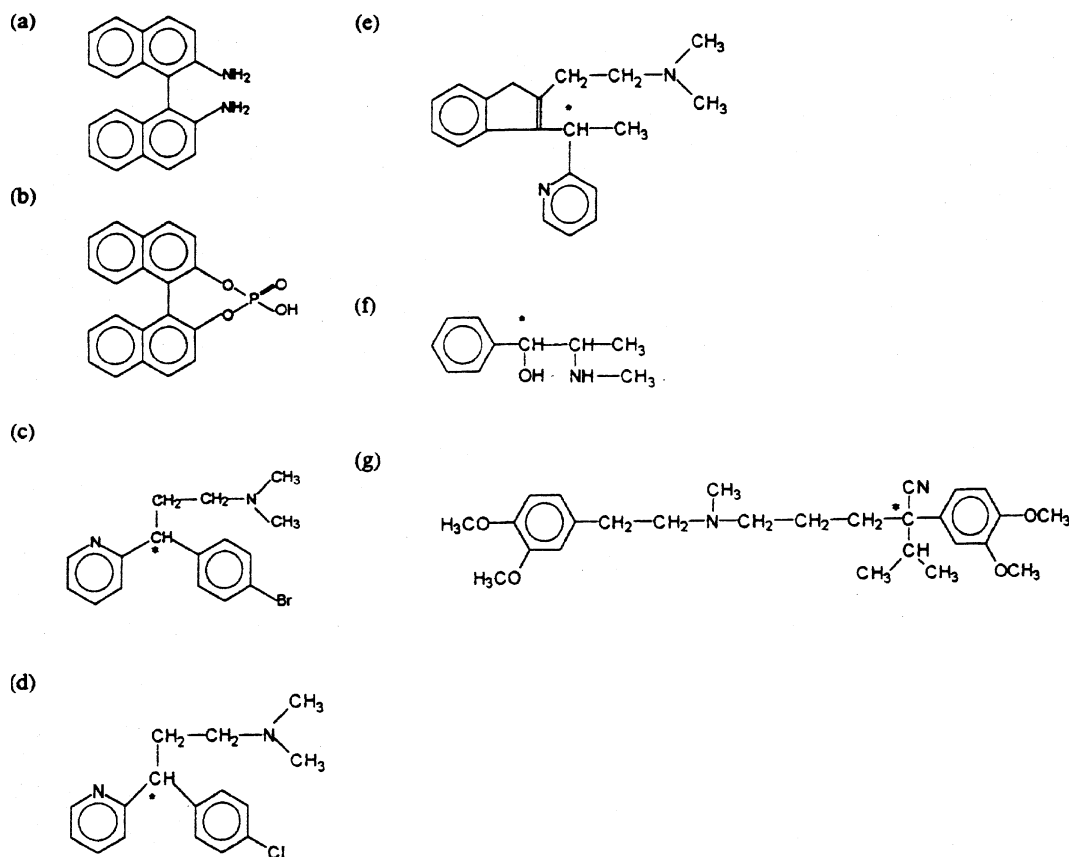
Figure 1. Structure of chiral compounds: (a) 1,1'-binaphthyl-2,2'-diamine, (b) 1,1'-binaphthyl-2, 2'-diyl hydrogen phosphate, (c) brompheniramine, (d) chlorpheniramine, (e) dimethindene, (f) ephedrine, (g) verapamil.

where μ_f is the mobility of the uncomplexed analyte, C_1 is the concentration of chiral selector 1, μ_{c1} is the mobility of the complex between the analyte and chiral selector 1, and K_{R1} and K_{S1} are the so-called competitive affinity constants of the *R* and *S* enantiomers for chiral selector 1, respectively. The same parameters in the case of chiral selector 2 are labeled with index 2.

For a quantitative evaluation of $\Delta\mu_{ov}$ it would be necessary to estimate so-called competitive binding characteristics of both enantiomers for both chiral selectors. However, a qualitative description can be performed as follows: the sign of $\Delta\mu_1$ and $\Delta\mu_2$ must be the same in order to obtain an improvement of mobility difference in a dual system. However, the signs of $\Delta\mu_1$ and $\Delta\mu_2$ not only depend on the affinity terms (K_R–K_S) but also on the mobility terms (μ_f–μ_c). In the majority of cases described in the literature, the signs of (μ_f–μ_c) terms were opposite for the two chiral selectors used in combination. Therefore, opposite signs were also required for the (K_R–K_S) terms in order to obtain $\Delta\mu_1$ and $\Delta\mu_2$ values with the same signs. However, it is obvious that if either (μ_f–μ_c) or (K_R–K_S) terms have the same sign for both chiral selectors, then the same sign will also be required for the other terms, (K_R–K_S) or (μ_f–μ_c). This is illustrated below by several examples of enantioseparations of acidic and basic compounds.

2 Materials and methods

2.1 Chemicals

The racemic compounds (Fig. 1), (±)-chlorpheniramine maleate (CHL), (±)-brompheniramine maleate (Brp), (±)-verapamil hydrochloride (VP), and (+)-chlorpheniramine maleate, (+)-brompheniramine maleate, and (+)- and (−)-ephedrine hydrochlorides were obtained from Sigma Aldrich (Deisenhofen, Germany). The racemic dimethindene (DM) was a gift from Zyma (Munich, Germany). The optically pure (+)- and (−)-enantiomers of verapamil were obtained by diastereomeric crystallization with (+)- or (−)-1,1'-binaphtyl-2,2'-diyl-hydrogen phosphate as described earlier [30]. The enantiomers of DM were prepared by diastereomeric crystallization with optically pure tartaric acid in ethanol as described previously [31]. The pure enantiomers of 1,1'-binaphthyl-2,2'-diamine (BNDA) and 1,1'-binaphthyl-2,2'-diyl hydrogen phosphoric acid (BDHP) were supplied by Aldrich (Steinheim, Germany). β-CD, carboxymethyl-β-CD (CM-β-CD) with an averaged substitution degree (D.S.) of 3.5, 2-hydroxypropyltrimethylammonium salt of β-CD (TMA-β-CD) with a D.S. of 3.5 were gifts from Wacker Chemie (Munich, Germany). Heptakis-(2,6-di-*O*-methyl)-β-CD (DM-β-CD) and heptakis-(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) were from Sigma Aldrich.
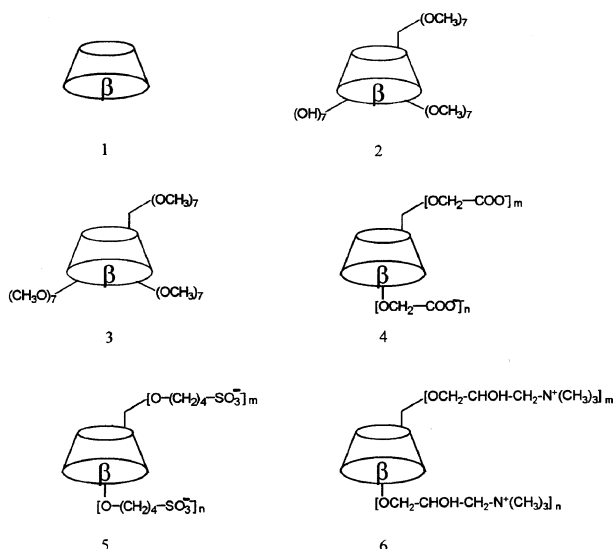
Figure 2. Structure of CDs: (1) β-CD, (2) DM-β-CD, (3) TM-β-CD, (4) CM-β-CD, (5) SBE-β-CD, (6) TMA-β-CD.

Sulfobutylether-β-CD (SBE-β-CD) with a D.S. of 4.0 was from CyDex, L. C. (Overland Park, KS, USA). TMA-β-CD with a D.S. of 1.0 was synthesized in our laboratory. The structures of chiral selectors are represented in Fig. 2. Analytical-grade KH_2PO_4, H_3PO_4, triethanolamine, NaOH, boron trifluoride ethyl ether, tris(hydroxymethyl)a-
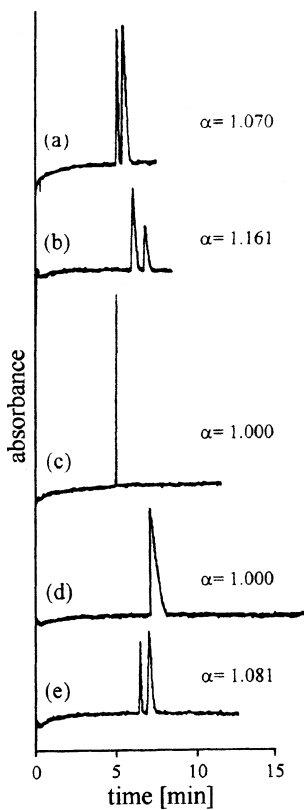
Figure 3. Electropherograms of the nonracemic mixture ((+)/(−) = 2/1) of dimethindene enantiomers in the presence of (a) 0.5 mg/mL CM-β-CD, (b) 1 mg/mL SBE-β-CD, (c) 10 mg/mL TM-β-CD, (d) a combination of 0.5 mg/mL CM-β-CD + 1 mg/mL SBE-β-CD, and (e) 0.5 mg/mL CM-β-CD +10 mg/mL TM-β-CD. Methylcellulose-coated capillary, 60 cm total length (43 cm to the detector), 50 μm ID; buffer, 100 mM phosphoric acid/triethanolamine (pH 3); injection, at the anodic side; voltage, 25 kV.

minomethane (Tris), chloroform, and acetone were purchased from Merck (Darmstadt, Germany). Methocel MC with a viscosity of 3000–5000 mPas and (3-glycidyloxypropyl)trimethoxysilane were purchased from Fluka (Deisenhofen, Germany).

2.2 Appartus

CE separations were performed using the Grom capillary electrophoresis modular system 100 (Grom, Herrenberg, Germany) equipped with a Linear Instrument UVIS 200 detector (Reno, NV, USA). The samples were introduced hydrostatically (10 cm). Fused silica and methylcellulose-coated capillaries of 50 μm ID, 60 cm total length and 43 cm effective length were used. The coated capillaries were prepared as described by Liao *et al.* [32]. The methylcellulose used as a neutral coating is a chiral polymer and, when used as a buffer additive at higher concenta-

tions, can resolve the enantiomers of some analytes used in this study [33]. However, no enantioseparations were observed in achiral buffers in these capillaries. Detection of the analytes was performed at 214 nm. Other experimental conditions are indicated in the legends to the figures.

3 Results and discussion

3.1 Combination of two chiral selectors with opposite chiral recognition but with the same effect on the analyte mobility

The separations of a nonracemic mixture of DM enantiomers are depicted in presence of CM-β-CD (in Fig. 3a, SBE-β-CD in Fig. 3b, and their combination in Fig. 3d. As can be seen, these two chiral selectors meet the requirement for opposite affinity pattern. However, their use in combination results in a decrease of separation selectivity

Table 1. CE enantioseparation of some chiral bases in dual separation systems containing different combinations of CDs

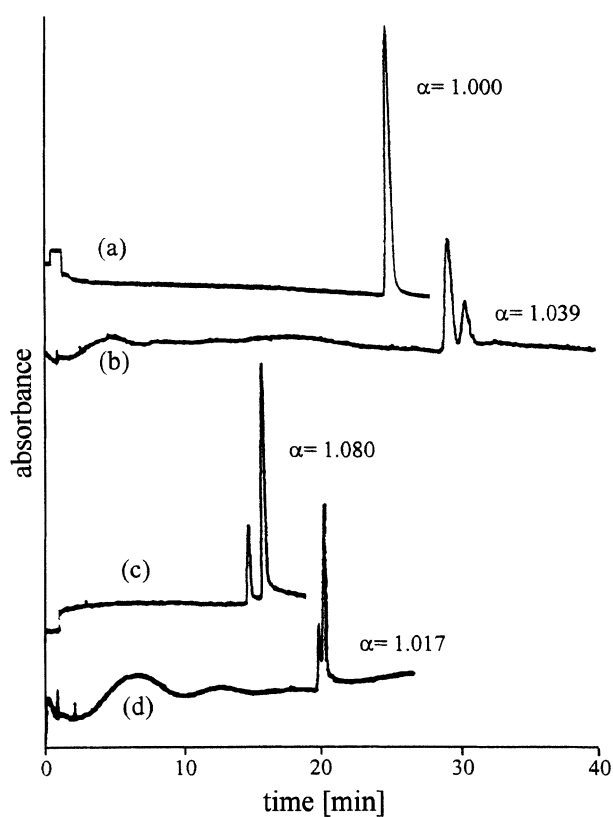| Analyte	CD	[CD] (mg/mL)	t_1 (min)	t_2 (min)	α
Brompheniramine	CM-β-CD	0.1	6.82	7.17	1.051
	TM-β-CD	10	6.16	6.16	1.000[a]
	β-CD	5	8.05	8.17	1.015
	CM-β-CD/TM-β-CD[b]	0.1/10	7.19	7.51	1.045
	CM-β-CD/β-CD[c]	0.1/5	11.72	12.43	1.061
Chlorpheniramine	CM-β-CD	0.1	6.52	6.76	1.037
	TM-β-CD	5	6.43	6.43	1.000[a]
	β-CD	5	8.57	8.75	1.021
	CM-β-CD/TM-β-CD[b]	0.1/5	7.35	7.58	1.031
	CM-β-CD/β-CD[c]	0.1/5	9.06	9.55	1.054
Dimethindene	CM-β-CD	0.5	6.67	7.14	1.070
	TM-β-CD	10	5.35	5.35	1.000[a]
	β-CD	5	6.42	6.42	1.000[a]
	CM-β-CD/β-CD[b]	0.5/5	7.61	8.02	1.054
	CM-β-CD/TM-β-CD[c]	0.5/10	6.88	7.44	1.081
Ephedrine	CM-β-CD	2	9.21	9.62	1.044
	DM-β-CD	15	10.60	10.83	1.022
	β-CD	15	11.02	11.21	1.017
	CM-β-CD/DM-β-CD[b]	2/15	11.96	12.16	1.017
	CM-β-CD/β-CD[c]	2/15	12.07	12.67	1.050
Verapamil	CM-β-CD	0.5	15.43	16.14	1.046
	TM-β-CD	10	11.96	12.22	1.022
	β-CD	5	16.62	16.76	1.008
	CM-β-CD/TM-β-CD[b]	0.5/5	18.63	19.93	1.070
	CM-β-CD/β-CD[c]	0.5/10	16.57	17.13	1.034

Conditions: Buffer, 100 mM phosphoric acid adjusted to pH 3 with triethanolamine; voltage, 25 kV (injection at the anodic side)
a) No enantioseparation was observed at this concentration. However, at higher concentration of the same chiral selectors, baseline enantioseparations were obtained for all compounds in this table and the enantiomer migration orders were as indicated.
b) Opposite recognition pattern
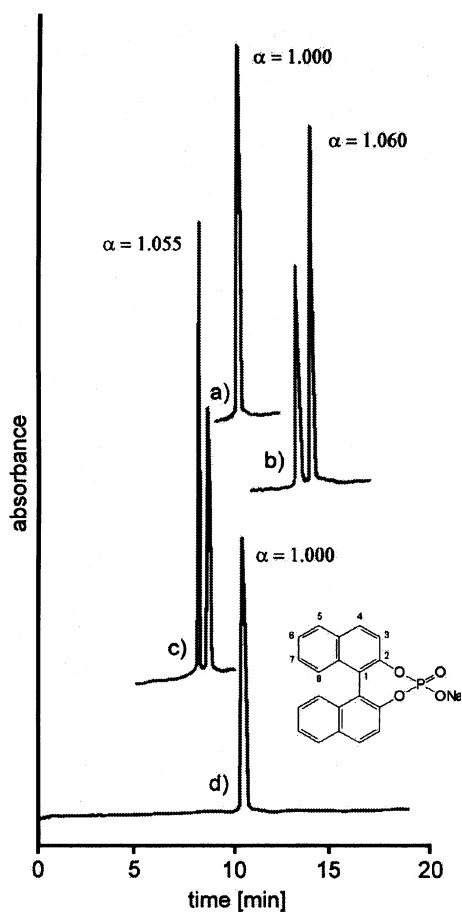c) Same recognition pattern

(total loss of resolution; Fig. 3d). The reason for this is that both chiral selectors have the same effect on the mobility of the analyte: both decelerate it. Therefore, according to the principles discussed above, two chiral selectors with the same effect on analyte mobility and the same recognition pattern should be beneficial for the enantioseparation. This is actually the case when CM-β-CD and TM-β-CD are used in combination (Fig. 3e). Additional examples of enantioseparations designed according to the same principles are summarized in Table 1. In these examples an adjustment of separation selectivity was obtained by manipulation of the sign of the $(K_R–K_S)$ term in the mobility difference Eqs. (2) and (3). In all cases, the cationic analytes were decelerated by all CDs studied and, as expected, an improvement in separation selectivity was only observed when the two CDs used in combination had the same recognition pattern. An alternative possibility of achieving similar effects is to modify the $(\mu_f–\mu_c)$ term while keeping the sign of the $(K_R–K_S)$ term unchanged.

3.2 Combination of two chiral selectors with the same chiral recognition pattern but with opposite effects on the analyte mobility

The chiral recognition pattern of a given chiral selector is an intrinsic, thermodynamic property which is not easy to adjust in a designed way. However, in contrast to chromatographic techniques, CE offers an attractive alternative for a design of the enantiomer migration order and separation selectivity by varying the mobility of a chiral selector [34]. The examples described in this section illustrate that a designed change of separation selectivity in dual selector systems is possible without changing the affinity pattern of the enantiomers for the chiral selector. In the examples described in Fig. 4, the nonracemic mixture ((+)/(−) = 2/1) of BNDA was resolved with the positively
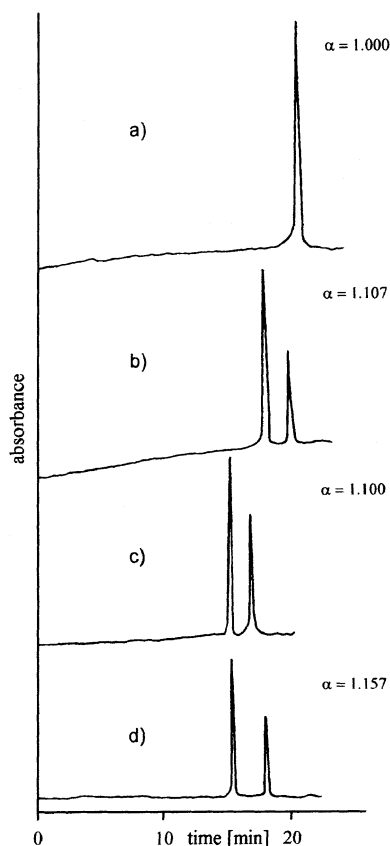
Figure 4. Electropherograms of nonracemic mixture ((+)/(−) = 2/1) of BNDA (a) in the absence of cyclodextrin, (b) with 20 mg/mL TMA-β-CD (D.S. = 1.0), (c) 20 mg/mL TMA-β-CD (D.S., 3.5) and (d) with 20 mg/mL TMA-β-CD (D.S., 1.0) + 20 mg/mL TMA-β-CD (D.S., 3.5). Voltage, 20 kV. Other conditions as in Fig. 3.

Figure 5. Electropherograms of nonracemic mixture ((+)/(−) = 2/1) of BDHP (a) in the absence and (b) in the presence of 5 mg/mL CM-β-CD, (c) 5 mg/mL SBE-β-CD and (d) combination of 5 mg/mL CM-β-CD + 5 mg/mL SBE-β-CD. Buffer, 100 mM phosphoric acid/triethanolamine (pH 2.0); injection, at the cathodic side; capillary, fused silica, 60 cm total length (43 cm to the detector), 50 μm ID. Other conditions as in Fig. 3.

Figure 6. Electropherograms of nonracemic mixture ((+)/(−) = 2/1) of BDHP (a) in the absence and (b) in the presence of 2.5 mg/mL CM-β-CD, (c) 2.5 mg/mL SBE-β-CD and (d) a combination of 2.5 mg/mL CM-β-CD + 2.5 mg/mL SBE-β-CD. Buffer, 100 mM phosphoric acid / triethanolamine (pH 5.5); injection, at the cathodic side; capillary, fused-silica capillary, 60 cm total length (43 cm to the detector), 50 μm ID.

charged TMA-β-CD. The chiral selectors used possess different average D.S. of *ca.* 1.0 (Fig. 4b) and 3.5 (Fig. 4c). As expected, the affinity pattern of the enantiomers for both chiral selectors is the same ((+)-BNDA is more tightly complexed) [27]. However, the enantiomer migration order was found to be opposite with these two chiral selectors [18]. The reason for this is that TMA-β-CD with a D.S. of 1.0 decelerates BNDA enantiomers while TMA-β-CD with D.S. 3.5 accelerates it [18]. As expected, the combination of these chiral selectors is not favorable to the enantioseparation of this compound (Fig. 4d).

The effect of the chiral selector on the mobility of the analyte can also be adjusted by modifying the CE separation conditions [28]. Both chiral selectors (CM-β-CD and SBE-β-CD) used in the enantioseparations shown in Fig. 5 bind BDHP more tightly [27]. All these separations were performed in the reversed polarity mode, *i.e.*, with injection at

the cathodic side of the capillary and detection at the anodic side. However, at pH 2.0, SBE-β-CD accelerates the analyte enantiomers (Fig. 5c) and CM-β-CD (Fig. 5b) decelerates them. Therefore, the addition of CM-β-CD (Fig. 5d) to the buffer containing SBE-β-CD causes the decrease in separation selectivity. However, at pH 5.5, while retaining the same chiral recognition pattern, CM-β-CD changes its effect on the mobility of BDHP in the same way as SBE-β-CD, now also leading to an acceleration of this compound (Fig. 6b). Therefore, a combination of CM-β-CD and SBE-β-CD, which was unfavorable to the enantioseparation at pH 2.0 (Fig. 5d), becomes favorable at pH 5.5 (Fig. 6d).

Thus, in order to develop a dual separation system it is feasible to manipulate either by affinity or mobility terms. The latter seems to be more flexible and predictable in CE. In addition, the requirements for an improvement of separation selectivity are as follows: (i) If the two chiral selectors used in combination affect the analyte mobility in opposite ways (one accelerates and the other one decelerates it), then the analyte enantiomers must possess opposite affinity patterns for these two chiral selectors; (ii) if both chiral selectors affect the analyte mobility in the same way (both accelerate or both decelerate it), then the analyte enantiomers must possess a similar affinity pattern for these two chiral selectors.

5 Concluding remarks

Mobility effects are as important as affinity properties for the design of CE separation systems containing two selectors. The advantage of the manipulation of mobilities is its predictability and easier design. Finally, this study has been devoted to relatively simple cases where both chiral selectors most likely act independently. Much more difficult (if not impossible) for a design are probably those cases in which both chiral additives participate in the elementary process of molecular recognition simultaneously.

6 References

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