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# The effect of achiral calixarenes on chiral separation of propranolol-HCl and brompheniramine maleate in capillary electrophoresis using cyclodextrin as chiral selector

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In this study we have examined the effect of achiral water soluble p-sulfonatocalixarenes ($SCX_{[n]}$) on chiral separation propranolol-HCl and brompheniramine maleate. Several cyclodextrins (CDs) and cyclodextrin derivatives were examined as chiral selectors applying complete filling technique (CFT) accompanied with the partial filling technique PFT of ($SCX_{[n]}$) as achiral modifier. Only with hydroxypropyl-β-cyclodextrin (HP-β-CD) chiral separation could be achieved. The effect of the organic modifier on these chiral separations was examined. The results indicate that at pH 4.65, the use of HP-β-CD (CFT) alone could not initiate chiral separations of both analytes and these chiral separations could be induced using HP-β-CD (CFT) followed by $SCX_{[n]}$/HP-β-CD (PFT).

## 1. Introduction

Macrocyclic compounds, such as CDs, crown ethers and calixarenes, have been widely used as buffer additives to improve the selectivity in CE (Fillet et al. 1998; Verleysen et al. 1998). The enantioselectivity of the CDs stems from a combination of inclusion complexation with the hydrophobic cavity of the CDs and the interaction with the chiral environment at the entrance of the cavity (Szejtli 1988; Krustulovic 1989). A wide variety of chiral selectors, including CDs and their derivatives have been used in capillary zone electrophoresis (CZE) for resolution of various racemic compounds (Fanali 1989, 1991; Snopek et al. 1991). Calixarenes, which are counted as the third generation of supramolecules, are cavity-shaped cyclic molecules made up of phenol units linked via alkylidene groups. They have shown unique recognition ability to a variety of guest molecules, such as metals ions, uncharged solutes, chiral solutes and isomers (Shinkai 1993; Ludwig 2000). Therefore calixarenes have attracted much attention as powerful additives in CE. Whereas the CDs are quite rigid molecules, the parent calixarenes are highly flexible molecules, capable of minor flexing and possessing the ability to undergo complete ring inversions (Gutsche 1989). Arimura (1989) stated that the calix$_{[6]}$arene cavity is more hydrophobic than that of β-CD. $SCX_{[n]}$, which are used in this study, provide not only a hydrophobic environment (benzene rings), but also hydrophilic heads ($SO_3^-$), that is, they possess properties of both CDs and micelles (Schuette et al. 1992). The $pK_{a1}$ of $SCX_{[4]} = 3.26$ and $pK_{a2} = 11.8$ (Yoshida et al. 1992). The $pK_{a1}$ for $SCX_{[6]}$ is $<1$, $pK_{a2} = 3$, $pK_{a3} = 4$ and $pK_{a4} > 11$ (Shinkai 1986). The acidities of $SCX_{[n]}$ phenolic hydroxyl groups are determined by the system of intramolecular hydrogen bonds. The $pK_{a1}$ value of $SCX_{[4]}$ is considerably reduced in comparison with the $pK_a$ values of the corresponding linear phenol, and the reduction of $pK_{a1}$ is less for $SCX_{[6]}$ and $SCX_{[8]}$ and their $pK_a$ values are comparable with the linear oligomers (Böhmer 1995).

The partial filling technique (PFT) was introduced in 1993 (Valtcheva et al. 1993) then modified in 1995 (Tanaka and Terabe 1995), and after that applied by Amini and Paulsen-Sörman (1997) and by Amini et al. (1999a). In PFT, the capillary is filled with the selector solution up to the detection window, the sample is then injected, and finally the separation process is pursued by applying voltage while the capillary ends are dipped into the background electrolyte (BGE) containing no selector. The PFT was originally developed to avoid detection interferences by a UV active selector as calixarenes by choosing separation conditions that held the selector stationary or moving away from the detection window (Ward et al. 1996; Amini et al. 1997). When the selector has no UV interference or low UV absorptivity as CDs, the capillary can be entirely filled with the selector, while the capillary ends are dipped into the background electrolyte containing no selector and this technique is called Complete filling technique (CFT). When the selector is expensive, the use of PFT and CFT is of a great importance because these techniques decrease the consumption of the selector. PFT has been applied in separation, enantioseparation and determination of binding constants (Amini and Paulsen-Sörman 1997; Ward et al. 1996; Amini and

Westerlund 1998; Bazzanella 1999; Heintz et al. 1999; Zhang and Gomez 2000). Al-Nouti and Bartlett (2002) employed CFT for CD in CE to study the non-covalent complex between local anaesthetic and CD. Martinez-Pla et al. (2004) applied the CFT to separate the enantiomers of propranolol in less than 5 min using human serum albumin as chiral selector. Amini et al. (1999a) applied the CFT of methyl-β-cyclodextrin for quantitative enantiomeric separation of ropivacaine by CE. The same group examined also the dependence of the chiral separation on the amount of CD as selectors employing the PFT in CE. It was found that during the employing of PFT the enantioseparation can be regulated by the effective amount of the chiral selector at a given concentration (by regulation of the length of the applied zone) (Amini et al. 2000). The effect of partially filled $SCX_{[4]}$ as selector was examined on the CE separation of phenolic isomers (Xu et al. 2003). PFT was applied by our group (Sokoließ et al. 2003) using calixarene as selector to separate *cis/trans*-isomers of doxepin and some thioxanthenes. Yang and De Villiers (2004) found that the increasing of the solubility of furosemide using $SCX_{[n]}$ is in the following order $SCX_{[6]} > SCX_{[4]} > SCX_{[8]}$. The increase in furosemide solubility afforded by $SCX_{[n]}$ was most probably the result of the incorporation of the non-polar portions of the furosemide molecule into the non-polar cavity of $SCX_{[n]}$. Xu et al. (2004) achieved enantiomeric separation of three basic drugs (propranolol, chlorpheniramine and mexiletine) with partially filled serum albumin as chiral selector in CE.

Some achiral modifiers were employed to improve the enantioseparation of racemic mixtures in the presence of chiral selectors (Billiot et al. 1997; Huang et al. 1997; Armstrong et al. 1998; Jira et al. 1998; Karbaum 2000; Servais et al. 2003). Huang et al. (1997) found that the combination of achiral crown ether plus β-CD could produce enantioseparations of racemic amines that could not be resolved using only CD. Karbaum (2000) used achiral crown ether with CD for chiral separation of racemates, which contained no any primary amine groups. The result showed that although there was no any primary amine, the enantioseparations were enhanced upon the addition of achiral crown ether. Jira et al. (1998) and Servais et al. (2003) used chiral and achiral ion-pairing reagents in combination with CD in CE for chiral separations. They assumed that the more hydrophobic alkanoic acids with longest alkyl chains are better included in the CD cavity, resulting in a stronger displacement of the analyte from the CD cavity. The altered position of the guest molecules in CD led to a change of chiral recognition.

Propranolol is belonging to beta-adrenoreceptor blocking drugs, which can be used in the treatment of hypertension, angina pectoris, arrhythmia and congestive heart failure (Chavey 2000). It is known that (*S*)-propranolol is 100 times more potent as beta-blocking agent than the (*R*)-enantiomer (Walle et al. 1998). Brompheniramine maleate is antihistaminic and its antihistaminic activity reportedly exists predominantly in the dextro-isomer (Delgads and Remers 1991; Gennaro 1995).

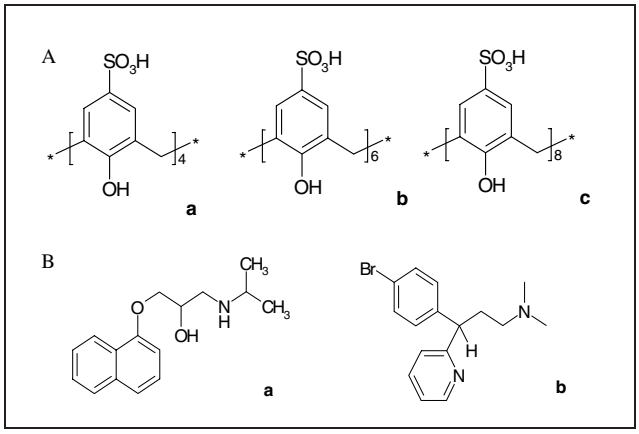


Fig. 1: Structure of the used p-sulfonatocalixarenes and analytes
A: Structure of p-sulfonatocalixarenes
a – sulfonatocalix$_{[4]}$arene ($SCX_{[4]}$)
b – sulfonatocalix$_{[6]}$arene ($SCX_{[6]}$)
c – sulfonatocalix$_{[8]}$arene ($SCX_{[8]}$)
B: Structure of the analytes
a – propranolol
b – brompheniramine

The main aim of this study was to examine if the achiral calixarenes have an effect on the chiral separations with CDs applying combination between PFT and CFT (Fig. 1). As far as we know, no other investigations in this direction exist up to now.

## 2. Investigations, results and discussion

### *2.1. Effect of the application time of calixarenes*

Because of the strong UV-absorbance the addition of the calixarenes to the whole electrolyte was unfavorable. Thus, PFT seemed to be advantageous. Table 1 shows the effect of application time of partially filled $SCX_{[4]}$ on the chiral separation of the analytes in presence of HP-β-CD. The results indicate that in absence of $SCX_{[4]}$ at pH 2.5, the stereoselectivity for propranolol-HCl and brompheniramine maleate isomers were 1.012 and 1.018, respectively. The examination was carried out by application of 1 mM $SCX_{[4]}$/HP-β-CD/BGE solution with PFT for 0.5, 1.0, 2.0, 4.0 and 10.0 min. For propranolol-HCl, when $SCX_{[4]}$/BGE solution was injected for 1.0 min, the best α value was obtained, while the other application times gave lower values than that in case of $SCX_{[4]}$ absence. The results indicate also that with increasing application time, the migration times ($t_m$) of both isomers decreased for the application time of 0.5 min, then increased again with increasing appli-

**Table 1: Effect of the application time of $SCX_{[4]}$**

| Time of PFT (min) | | 0.0 | 0.5 | 1.0 | 2.0 | 4.0 | 10.0 |
|---|---|---|---|---|---|---|---|
| Propranolol-HCl | peak 1 | 12.82 | 11.99 | 12.46 | 12.81 | 12.84 | 14.87 |
| | peak 2 | 12.97 | 12.12 | 12.69 | 12.94 | 12.94 | 14.98 |
| | α | 1.012 | 1.011 | 1.019 | 1.010 | 1.008 | 1.007 |
| Brompheniramine maleate | peak 1 | 9.53 | 8.05 | 8.60 | 9.29 | 13.78 | 14.91 |
| | peak 2 | 9.70 | 8.17 | 8.73 | 9.41 | 13.88 | 15.03 |
| | α | 1.018 | 1.015 | 1.015 | 1.013 | 1.007 | 1.008 |

Electrophoretic conditions:
PFT: 1mM $SCX_{[4]}$/15 mM HP-β-CD/50 mM $NaH_2PO_4$ pH 2.5
BGE: 15 mM HP-β-CD in 50 mM $NaH_2PO_4$ pH 2.5, 20 KV, 27 °C, 50/57 cm capillary.

cation time. But although $t_m$ values increased, α values decreased under the longer application time. For brompheniramine maleate, the $t_m$ values decreased under the application of $SCX_{[4]}$ for 0.5 min then increased with further increased application time. But until 2.0 min, $t_m$ values were lower than those in case of $SCX_{[4]}$ absence. The α values between brompheniramine maleate isomers in case of HP-β-CD alone was better than in all cases of combination with $SCX_{[4]}$. Under the increase in application time, α values decreased. So it is clear that $SCX_{[4]}$ decreased the difference between the stabilities of the complexes of both isomers of brompheniramine maleate with HP-β-CD under all application times leading to decreased stereoselectivity. In case of propranolol-HCl the application of $SCX_{[4]}$ for 1.0 min led to the best stereoselectivity and this can be attributed to a greater difference between the stabilities of the complexes of both isomers with HP-β-CD. The low concentration of $SCX_{[4]}$ (shorter application time (0.5 min)) led to lower stability of the complexes between the propranolol-HCl isomers and HP-β-CD resulting in reduction of $t_m$ values. This decrease of the stability may be due to certain interactions between the two additives resulting in change of the character of the complex between HP-β-CD and the propranolol-HCl isomers. While the increase of $t_m$ values under the increase of $SCX_{[4]}$ concentration (longer application time) can be attributed to the change in the viscosity and/or the stronger migration of $SCX_{[4]}$ in opposite direction to the EOF (electroosmotic flow) due to the higher amount of negative charges.

### 2.2. *Effect of the calixarene ring size*

From Fig. 2 and Table 2, it can be concluded that the ring size of $SCX_{[n]}$ played an important role. Under two pH conditions (2.50 and 4.65) and the combination between PFT of $SCX_{[n]}$ and CFT of HP-β-CD, the effect of the ring size was examined. At pH 2.50, the increase of the ring size led to decreased $t_m$ values of both isomers of propranolol-HCl, while α values increased. Different results were obtained in case of brompheniramine maleate; as $SCX_{[4]}$ and $SCX_{[8]}$ gave nearly the same $t_m$ and α values, which were higher than those in case of $SCX_{[6]}$. This difference of behavior for both analytes reflected that the characters of the analyte (its hydrophobicity, chemical structure and charge/mass) played also an important role beside the $SCX_{[n]}$ ring size. In case of pH 4.65, the behavior was – as expected – different than that in case of pH 2.50, the increase of the $SCX_{[n]}$ ring size led to increase of $t_m$ values for both isomers of each analyte. Under the condition of pH 4.65, the α values for propranolol-HCl and brompheniramine maleate isomers were in the following order

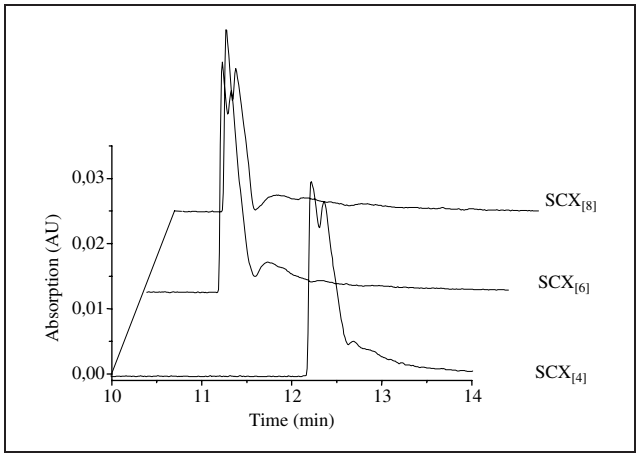


Fig. 2: Effect the ring size of calixarene on chiral separation of propranolol-HCl
Electrophoretic conditions:
CFT: 15 mM HP-beta-CD/50 mM $NaH_2PO_4$ pH 2.5
PFT: 2 mM $SCX_{[n]}$/15 mM HP-beta-CD/$NaH_2PO_4$

$SCX_{[6]} < SCX_{[4]} < SCX_{[8]}$ and $SCX_{[6]} < SCX_{[8]} < SCX_{[4]}$, respectively. These differences ensured the important effect of the analyte characters.

The different behavior under the two pH values can be attributed to the change of the ionization of $SCX_{[n]}$ under these two pH conditions. At pH 4.65, the ionization of acidic phenolic hydroxyl groups of $SCX_{[n]}$ is higher than that in case of pH 2.50. And at pH 4.65 the ratio charge/mass will be in the following order $SCX_{[4]} < SCX_{[6]} < SCX_{[8]}$. Thus the migration in the opposite direction is the highest for $SCX_{[8]}$ and the lowest for $SCX_{[4]}$ and this is maybe one of the reasons, why at pH 4.65 the increase of the ring size led to increased $t_m$ values. The effect upon the α values (their increase or decrease) can be attributed to the change of the difference between the stabilities of the complexes of both isomers of each analyte with HP-β-CD, which resulted from the different interactions of HP-β-CD with each of the three $SCX_{[n]}$.

### 2.3. *Effect of the organic modifier*

Methanol and acetonitrile were examined as organic modifiers (Table 3). MeOH and ACN possess vastly different physicochemical properties. The viscosity η and dielectric constant ε of the solvent have a major effect on the electrophoretic and electroosmotic mobility; there is proportionality to the ratio ε/η (Karbaum and Jira 1999; Riekkola et al. 2000). Under the addition of organic modifier, a

**Table 2: Effect of the ring size of calixarene**

| | | pH 2.50 | | | pH 4.65 | | |
|---|---|---|---|---|---|---|---|
| | | $SCX_{[4]}$ | $SCX_{[6]}$ | $SCX_{[8]}$ | $SCX_{[4]}$ | $SCX_{[6]}$ | $SCX_{[8]}$ |
| Propranolol-HCl | peak 1 | 12.31 | 10.82 | 10.48 | 2.36 | 2.40 | 2.60 |
| | peak 2 | 12.36 | 10.92 | 10.58 | 2.70 | 2.69 | 2.98 |
| | α | 1.004 | 1.009 | 1.010 | 1.144 | 1.121 | 1.146 |
| Brompheniramine maleate | peak 1 | 8.32 | 6.60 | 8.41 | 2.26 | 2.55 | 2.54 |
| | peak 2 | 8.45 | 6.68 | 8.55 | 2.82 | 2.95 | 3.06 |
| | α | 1.016 | 1.012 | 1.017 | 1.248 | 1.157 | 1.205 |

Electrophoretic conditions:
CFT: 15 mM HP-β-CD /BGE
PFT: 2 mM $SCX_{[n]}$/15 mM HP-β-CD/BGE for 0.5 min
BGE: 50 mM $NaH_2PO_4$, 20 KV, 27 °C, in case of pH 2.5 (50/57 cm capillary) and in case of pH 4.65 (40/47 cm capillary).

**Table 3: Effect of the organic modifier**

| | | without | 5% MeOH | 5% ACN | 10%ACN |
|---|---|---|---|---|---|
| Propranolol-HCl | peak 1 | 10.48 | 10.59 | 12.13 | 12.28 |
| | peak 2 | 10.58 | 10.69 | 12.27 | 12.41 |
| | α | 1.010 | 1.009 | 1.012 | 1.011 |
| Brompheniramine maleate | peak 1 | 8.41 | 8.93 | 7.97 | 7.77 |
| | peak 2 | 8.55 | 9.05 | 8.34 | – |
| | α | 1.017 | 1.013 | 1.046 | 1.000 |

Electrophoretic conditions:
CFT: 15 mM HP-β-CD/50 mM $NaH_2PO_4$ (or/$NaH_2PO_4$ + organic modifier) pH 2.5
PFT: 2 mM $SCX_{[8]}$/15 mM HP-β-CD/50 mM $NaH_2PO_4$ (or/$NaH_2PO_4$ + organic modifier) pH 2.5 for 0.5 min, 20 KV, 27 °C, 50/57 cm capillary

strong influence on $pK_a$ values of analytes and selectors can be expected.

The results indicate that 5% MeOH led to slightly increased $t_m$ values of isomers of both analytes, while in case of 5% ACN, $t_m$ values of propanolol-HCl increased and those of brompheniramine maleate decreased and this difference in behavior of both analytes under ACN condition ensures the important effect of analyte characters. The stereoselectivities of both analytes decreased in case of MeOH and increased in case of ACN. Under increasing ACN content from 5% to 10%, α values for propranolol-HCl became lower and there was total loss of the stereoselectivity in case of brompheniramine maleate. So it is clear that the presence of an organic modifier, its type and content must be considered in such studies.

### *2.4. Effect of the combination between PFT and CFT*

By comparison the results under conditions F and G (Table 4), we can say that the presence of calixarene increased the stability of both isomer complexes of each analyte with HP-β-CD at pH 4.65; as $t_m$ values increased. From Table 2 and Table 4 condition A and B, it can be seen that the presence of $SCX_{[6]}$ at pH 2.5 led to a decrease of the α values, while in case of pH 4.65 it led to enantioseparation, which could not be initiated without it. So the effect of combination between the two techniques was examined under the condition of pH 4.65. In Fig. 3 and Table 4, the effectiveness of the combination between PFT and CFT is shown. The 1st condition (F in Table 4) represented complete filling of HP-β-CD/BGE. The 2nd condition (G in Table 4) is the same as the 1st but followed with partial filling of 2 mM $SCX_{[6]}$/HP-β-CD/BGE for 0.5 min. In the 3rd condition (E in Table 4), 2 mM $SCX_{[6]}$/HP-β-CD/BGE were partially filled in the capillary for 0.5 min. In the 4th condition (D in Table 4), the capillary was partially filled with HP-β-CD/ BGE for 0.5 min. In all these four cases, during the analysis the BGE contained not any selector. The 2nd condition differs from the double plug technique (Amini et al. 1999b), in that CD filled the entire capillary (CD existed in the achiral selector zone also). While in the two plug technique, there are two adjacent separation zones containing selectors of different characters and in the zone of achiral selector there is no CD. Amini et al. (1999b) applied the double plug technique to avoid interaction between the chiral selector and surfactant.

The results indicate that under all conditions for the two analytes, there was no stereoselectivity except under the 2nd condition, which represented the combination between PFT and CFT. The absence of stereoselectivity under the 1st ,3rd and 4th conditions can be attributed to the fact that the amount of HP-β-CD was not enough to make recognition between the two isomers. Although the same amount of CD in 1st condition was used in the 2nd condition, there was a chiral separation in the 2nd condition only. But the main difference was the presence of $SCX_{[6]}$ in the 2nd condition. So it is clear that the presence of $SCX_{[6]}$ decreased the minimum concentration of HP-β-CD required to initiate chiral separation. It seemed also that the interaction between $SCX_{[6]}$ and HP-β-CD and between $SCX_{[6]}$ and the analyte, which led to the change of characters of the formed complex between HP-β-CD and the analytes, made the chiral recognition more easier.

According to other studies (Huang et al. 1997; Billiot et al. 1997; Armstrong et al. 1998; Karbaum 2000; Buschmann et al. 2001; Liu et al. 2001; Yang and de Villiers 2005) we cannot speak only about a single complex between $SCX_{[6]}$ and the analyte and another between HP-β-CD and the analyte but also an interaction between the two selectors

**Table 4: Effect of the different application techniques**

| | | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|---|
| Propranolol-HCl | peak 1 | 12.82 | 4.84 | 5.47 | 4.62 | 4.57 | 4.48 | 4.57 |
| | peak 2 | 12.97 | – | 5.83 | – | – | – | 4.95 |
| | α | 1.012 | 1.000 | 1.066 | 1.000 | 1.000 | 1.000 | 1.083 |
| Brompheniramine maleate | peak 1 | 9.53 | 4.88 | 5.69 | 4.36 | 4.39 | 4.38 | 4.74 |
| | peak 2 | 9.70 | – | 5.90 | – | – | – | 5.19 |
| | α | 1.018 | 1.000 | 1.037 | 1.000 | 1.000 | 1.000 | 1.095 |

Electrophoretic conditions:
50/57 cm capillary, 20 KV, 27 °C
A: BGE: 15 mM HP-β-CD in 50 mM $NaH_2PO_4$ pH 2.5
B: BGE: 15 mM HP-β-CD in 50 mM $NaH_2PO_4$ pH 4.65
C: BGE: 15 mM HP-β-CD + 10 mM SDS in 50 mM $NaH_2PO_4$ pH 4.65
D: PFT with 15 mM HP-β-CD in 50 mM $NaH_2PO_4$ pH 4.65 for 0.5 min
E: PFT with 2 mM $SCX_{[6]}$ in 15 mM HP-β-CD/50 mM $NaH_2PO_4$ pH 4.65 for 0.5 min
F: CFT with 15 mM HP-β-CD in 50 mM $NaH_2PO_4$ pH 4.65
G: CFT with 15 mM HP-β-CD in 50 mM $NaH_2PO_4$ pH 4.65 followed by PFT with 2 mM $SCX_{[6]}$ in 15 mM HP-β-CD/50 mM $NaH_2PO_4$ pH 4.65 for 0.5 min
BGE in D, E, F and G is: 50 mM $NaH_2PO_4$ pH 4.65

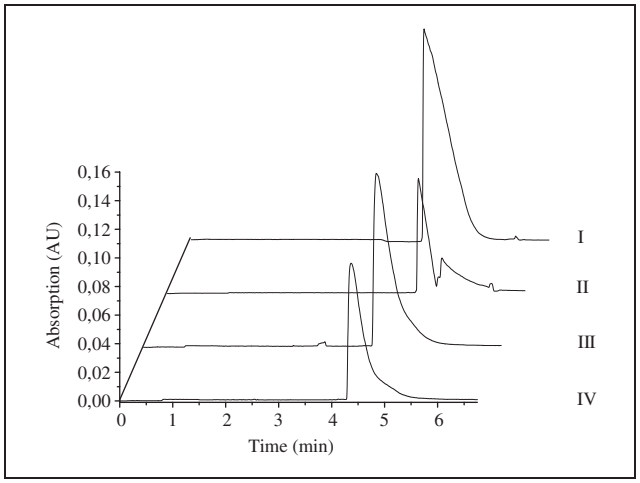


Fig. 3: Effect of the different application techniques on chiral separation of brompheniramine maleate
Electrophoretic conditions:
50/57 cm capillary, 20 KV, 27 °C,
BGE: 50 mM $NaH_2PO_4$ pH 4.65

and the analyte. Billiot et al. (1997) used four achiral modifiers, to see their effect on the chiral separation of propranolol with β-CD. They found that the hydrogen bonding of the heteroatoms between the co-modifier and propranolol probably leads to a more rigid ternary complex with increased stereoselectivity, as compared to the binary complex of β-CD and propranolol, leading to an increase in chiral recognition. Armstrong et al. (1998) used achiral crown ether plus CD to achieve enantioseparation in CE for organic racemates containing a primary amine functional group. They found that a specific three-body complex involving simultaneous, dual inclusion complex formation can be used to explain both the enhanced and diminished enantioselectivities observed when achiral crown ether was added to the buffer. This three-body complex consisted of an inclusion complex of the aromatic portion of the analyte with the CD, and a simultaneous complex between the crown ether and the analyte amine moiety, which was projected away from the mouth of the CD cavity. Buschmann et al. (2001) have found that the solubility of some supramolecules, including p-tert-butylcalix$_{[4]}$arene, p-tert-butyl-calix$_{[6]}$arene and crown ether, increased in the presence of CD due to complex formation. It was also found that the sulfonate groups in calixarene can interact with the secondary hydroxyl groups of CD through the electrostatic and/or hydrogen-bridges interactions (Liu et al. 2001). Yang and de Villiers (2005) found that the increased solubility of niclosamide in the presence of combination of $SCX_{[6]}$ and CDs indicated that these complexing agents may form bimacromolecular complexes with niclosamide and the linear increase in solubility seen in these phase-solubility profiles could be attributed to one or more molecular interactions among the niclosamide and $SCX_{[6]}$ and CD to form distinct chemical species, which may be called soluble niclosamide : $SCX_{[6]}$ : CD complexes. According to the authors, because $SCX_{[6]}$ and CD contain free hydroxyl groups, they will also interact with each other by hydrogen bonding in the solution.

This assumption that there is a complex between calixarene and CD can be used here in our study to explain what happened. According to it, the interaction between the two selectors led to change the position of the isomers in CD resulting in change of the stability of the original complex between HP-β-CD and the isomers and hence change the stereoselectivity.

Schuette et al. (1992) mentioned that calixarenes possess properties of both CDs (hydrophobic environment) and micelles (hydrophilic heads). In CE, micelles have been added to the BGE containing CD in order to improve enantioseparation (Terabe 1989, 1990; Nishi et al. 1991). Comparing the results under conditions B and C (Table 4) it is clear that SDS had positive effect on chiral recognition. In absence of SDS, there was no chiral recognition and in its presence, chiral recognition was achieved. And this improvement can be attributed to the partitioning of the analyte isomers between the CD and the negatively charged SDS micelle, which migrates in the opposite direction to the EOF.

The migration of $SCX_{[6]}$ in the opposite direction to EOF and CD – in a similar manner to SDS – can also explain the improvement of the chiral separation in the presence of $SCX_{[6]}$; as there will be a partitioning of the analyte between the two selectors and the relative distribution of the analyte between the two selectors is a function of its charge, hydrophobicity and its ability to make a hydrogen bond.

In this study, it was found that the application of HP-β-CD (CFT) followed by p-$SCX_{[n]}$/HP-β-CD (PFT) at pH 4.65 gave better enantioseparation for propranolol-HCl and brompheniramine maleate than in the case of HP-β-CD (CFT) alone, although the peak of the second isomer had always strong tailing, which can be explained with a stronger interaction between the second isomer and the selectors. The first peak is higher than the second one, but the peak areas were nearly the same and the injection of single isomers indicated that the second one is the *R*-isomer. There are several assumptions to explain this positive effect of calixarenes; including the formation of three body complexes, the change of the analyte position inside the cavity of CD through the complexation between the two selectors and partitioning of the analyte between the two selectors due to migration of calixarenes in an opposite direction to the EOF and CD. But further investigations and examinations with other techniques, for example NMR, are required to determine the nature of the interaction between the two selectors and the isomers. Also an evaluation including other drugs is necessary to justify the use of that combination of the two techniques and the two selectors. We will examine in another study the effect of p-$SCX_{[n]}$ on chiral separations in presence of other types of chiral selectors as chiral crown ethers and chiral ion-pairing agents using drugs with different characters (acidic, basic and neutral) and comparing the two plugs technique with the used technique in this study.

## 3. Experimental

### *3.1. Chemicals*

HPLC grade methanol (MeOH) and acetonitrile (ACN) were purchased from Applichem (Darmstadt, Germany). Water was deionised and doubly distilled. Phosphoric acid, sodium hydroxide and sodium dihydrogen phosphate were purchased from Merck KgaA (Darmstadt, Germany). HP-β-CD was obtained from Wacker AG (Munich, Germany). Sodium dodecyl sulfate (SDS) was supplied by Acros (Heidelberg, Germany). p-$SCX_{[n]}$ were obtained from PD Dr. Ludwig, FU Berlin. Propranolol-HCl was purchased from Sigma (St. Louis, MO, USA). Brompheniramine maleate was obtained from Kreussler Pharma (Wiesbaden, Germany).

### *3.2. Apparatus and separation conditions*

CE was performed using a P/ACE 2100 capillary electrophoresis instrument (Beckman, Fullerton USA) equipped with an on-column UV-detector. GOLD software (Beckman) was used for data acquisition.

pH values were always measured with a pH-meter (Knick Elektronische Messgeräte GmbH & Co., Berlin, Germany) after addition of CD and other additives and adjusted using phosphoric acid or sodium hydroxide.

Analyses were performed at a detector wavelength of 214 nm with an applied voltage of 20 KV in fused silica capillaries (47/40 or 57/50 cm × 50 µm I.D.; CS-Chromato-graphie Service GmbH, Langerwehe, Germany) thermostated at 27 °C. Each analyte was dissolved in bidistilled water (1 mg/ml) and before the injection the solution was mixed with electrophoresis medium (1 : 1) to prevent the breakdown of the current flow. Samples were injected hydrodynamically for 4 s under low pressure (0.5 psi). All the used solutions were filtered before use through a 0.45 µm filter.

Before the first use, the capillary was rinsed with 1 M HCl, bidistilled water, 0.1 M NaOH then finally with bidistilled water each of them for 10 min. At the beginning of the analysis each day, the capillary was rinsed with MeOH, 0.1 M NaOH then with bidistilled water each of them for 5 min. Between the runs, a rinsing was performed with MeOH for 2 min, 0.1 M NaOH for 2 min then with BGE for 4 min and in case of CFT the capillary was rinsed as mentioned before with MeOH for 2 min and 0.1 M NaOH for 2 min then for 4 min using HP-β-CD/BGE instead of BGE alone and during the analysis BGE containing no any HP-β-CD was applied. After the last run each day the capillary was rinsed with MeOH, 0.1 M NaOH, bidistilled water then with air each of them for 2 min. All rinsing steps were applied under high pressure of 5 psi. In case of PFT, p-$SCX_{[n]}$/HP-β-CD/BGE was injected under the low pressure (0.5 psi) for the required time direct before injection of the sample. For each run three injections were performed.

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