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Enantiomer separation of dihydropyridine calcium antagonists with cyclodextrins as chiral selectors: structural correlation

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Abstract

Native and substituted cyclodextrins (CDs) were used as chiral selectors both in high-performance liquid chromatography and capillary electromigration separations (HPCE and MEKC). Chromatographic data of five dihydropyridine calcium antagonists obtained on three β-CD chiral stationary phases in reversed-phase mode were compared with those of capillary electrophoresis using β-CDs in the presence and absence of sodium dodecyl sulfate (SDS). Competition of separated compounds with SDS molecules for penetration into the CD cavity can limit their necessary interaction with the chiral selector and consequently even preclude enantiomer separation. Some insight into this problem can be brought about by comparing the experimental data with computer-aided energy minimization of CD–solute and CD–SDS inclusion complexes.

Keywords: Enantiomer separation; Dihydropyridine calcium antagonists; Amlodipine; Nitredipine; Nimodipine; Isradipine; Nisoldipine

1. Introduction

Cyclodextrins (CDs) – both native and derivatized – are widely used chiral selectors for enantiomer separations in high-performance liquid chromatography (HPLC) and high performance capillary electrophoresis (HPCE). During the separation process, CDs form intermediate diastereomeric complexes with chiral solutes; differences in the stability of these complexes determine the enantioselectivity of separation. It is well known that inclusion of the apolar part of the solute into the hydrophobic cavity of the CD represents an important factor in the reversed-phase mode. Additional necessary interactions are hydrogen bonding between hydroxyl groups on the rims of the CD cavity and respective functional groups in the neighbourhood of the asymmetric centre of the analyte. In the case of the substitution of secondary hydroxyl groups of the CD (in derivatized CDs) these hydrogen bonds are partially precluded, but they can be replaced by new interactions, e.g. π-donor–π-acceptor interactions, new hydrogen bonds, steric hindrances, etc., which can either improve or worsen the enantiomer separation [1].

In order to elucidate the mechanism of chiral separation, a number of both chromatographic [2,3] and electrophoretic measurements were performed [4–6], along with the application of NMR results [3] and empirical methods of molecular modelling [7–

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11]. Nevertheless, it is not yet possible to predict the success of an enantiomer separation on the basis of the chemical structure of an analyte and a selector.

As a model category of the compounds for the study of enantiomer separation by means of CD selectors, five structurally related dihydropyridine calcium antagonists (CAs) were selected. These compounds belong to the family of chiral drugs, the enantiomers of which have been proven to exert different effects on humans [12]. Therefore, attention has been paid to their enantioselective separations.

Some CAs were chirally separated by making use of polysaccharide and protein bonded phases [13–15]. Nisoldipine and nimodipine have been partially resolved on two 250 × 4.6 mm I.D. β-CD columns connected in series [16] or on an S-hydroxypropyl β-CD stationary phase [17]. In our previous paper, we reported the separation of nimodipine and nisoldipine enantiomers on β-CD and/or substituted β-CD chiral stationary phases (CSP) using only a single column in reversed-phase HPLC [18].

For electrophoretic enantiomer separation of neutral (uncharged) substances like CAs, charged CDs or admixtures of ionic surfactants with neutral CDs (as chiral selectors) must be employed. Different types of CDs have been frequently used for chiral separation of drugs in electrophoresis [19–24]. Nevertheless, an enantiomer separation of dihydropyridines by HPCE has been rarely reported [25]. A systematic study of chiral separations of dihydropyridines with β-CDs as chiral selectors in liquid chromatography and capillary electrophoresis has not been reported yet.

The aim of this work is to shed additional light on the interaction mechanism of studied compounds with CD chiral selectors. Results of HPLC and HPCE, using a set of CD selectors, were compared. The chromatographic data were obtained on three CD stationary phases: with native β-CD and *S*- or *R*-naphthylethyl carbamoyl β-CD (*S*N- or *R*N-β-CD) derivatives in both reversed-phase and polar organic modes. In electrophoretic measurements, native β-CD was used for micellar electrokinetic chromatography (MEKC) with sodium dodecyl sulfate (SDS) and 2-O-carboxymethyl-β-CD (CM-β-CD) was used in a non-micellar system of capillary zone electrophoresis (CZE). Also, the influence of SDS on chiral separation in MEKC and HPLC was studied. Some insight into this problem was brought about by molecular modelling and energy minimization of the CD–SDS inclusion complexes.

2. Experimental

2.1. Materials

Dihydropyridine CAs were extracted from the following commercially available drugs: amlodipine (Norvasc, Pfizer, Brussels, Belgium), nitredipine (Baypress, Bayer, Leverkusen, Germany), isradipine (Lomir, Sandoz, Basle, Switzerland), nimodipine (Nimotop, Bayer) and nisoldipine (Baymycard, Bayer) (Fig. 1).

Other chemicals used included triethylamine (Sigma, St. Louis, MO, USA), sodium dodecyl sulfate, with purity ≥99% (Fluka, Buchs, Switzerland), sodium dihydrogen phosphate, sodium tetraborate, sodium hydroxide, acetic acid (all of p.a. purity, Lachema, Brno, Czech Republic), urea (Merck, Darmstadt, Germany), acetonitrile for chro-

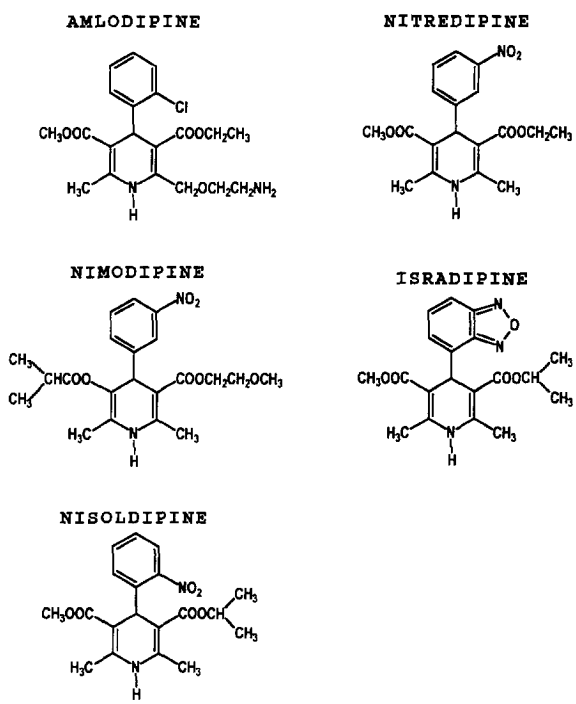


Fig. 1. Structures of the dihydropyridine calcium antagonists used in this study.

matography (Merck), methanol p.a. (Penta, Chrudim, Czech Republic).

All solutions were prepared in deionized water and filtered through a 0.45-μm filter before use.

2.2. Methods

2.2.1. High-performance liquid chromatography

All measurements were carried out with a Gilson chromatograph (Gilson, Middleton, WI, USA), consisting of an ASTED autosampler, a 115 UV Gilson detector, an 805 manometric Gilson module, 305 and 306 Gilson piston pumps and an 811C Gilson dynamic mobile phase mixer. Commercially available steel columns of either 250×4 mm I.D. packed with native β-CD covalently bonded to 5 μm silica (Chiradex, Merck) or 250×4.6 mm I.D. packed with either *S*N- or *R*N-β-CD covalently bonded to 5 μm silica (Cyclobond I 2000 SN and Cyclobond I 2000 RN, respectively) (ASTEC, Whippany, N.J., USA) were utilized. For structures of the CSP, see Fig. 2. Detection was performed at 239 nm.

Mobile phases consisted of appropriate amounts of either methanol or acetonitrile as organic modifiers added to 0.1% triethylamine (TEA) solution, the pH of which was adjusted to 5.0 with acetic acid.

The polar organic mobile phases were composed of acetonitrile and 1 to 10% methanol with 0.2% TEA and 0.3% acetic acid or with TEA and acetic acid in the opposite ratio. Flow-rate of the mobile phases used was 0.8 ml/min in all cases.

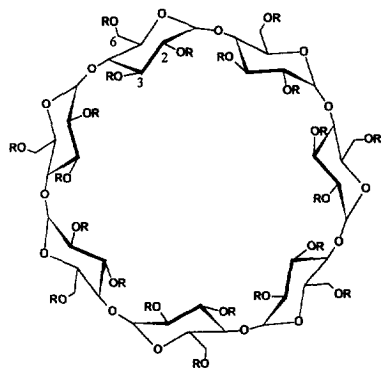
Samples to be injected were prepared from an appropriate amount of a stock solution of dihydropyridine CAs, taken to dryness and dissolved in the mobile phase.

Chromatograms were evaluated using the APEX Extra version 3.1 integration software (APEX, Prague, Czech Republic).

2.2.2. HPCE

All electrophoretic separations were carried out using a SpectraPhoresis 500 apparatus (Thermo Separation Products, Riviera Beach, FL, USA), controlled by a PC 1000 (supported on OS2 2.1, Version 2.6), equipped with an in-line variable-wavelength detector set to 240 nm.

Electrophoresis was performed at 25°C in an



R = -H	β-CYCLODEXTRIN (β-CD)
R(C2 position) = $-CH_2COOH$	carboxymethyl-β-CD
R = -(S)-OCN-CH(CH3)(naphthyl)	(S)-naphthylethyl-carbamoyl-β-CD
R = -(R)-OCN-CH(CH3)(naphthyl)	(R)-naphthylethyl-carbamoyl-β-CD

Fig. 2. Structures of cyclodextrin selectors used in HPLC or HPCE.

untreated fused-silica capillary (70 cm×75 μm I.D.) by applying a potential of 20 kV.

An uncoated silica capillary was conditioned sequentially using 0.1 *M* sodium hydroxide, water and finally the buffer to be used for the subsequent separation (5 min each). This procedure was also applied to recondition the capillary after each run.

The background electrolyte (BGE) contained 25 m*M* and 20 m*M* phosphate–borate buffer (pH 9.0–9.5) with 1% urea. Concentrations of other buffer additives used as chiral separators, i.e. β-CDs and SDS are noted in table or figure captions.

The sample was injected hydrodynamically for 1 s.

2.3. Molecular modelling

A molecule of dodecyl sulfate was constructed in an ionized form as an anion using INSIGHTII model builder [26]. CD coordinates were obtained from Cambridge crystallographic database [27]. Geometry optimizations of isolated subsystems and the respective clusters were done using CVFF (consistent

valence force-field) empirical force field implemented in DISCOVER code [28] and the dielectric constant was set to 1.0. Standard CVFF atomic charges were considered. Docking of SDS to the cyclodextrin cavity was done in a similar way as described previously for CAs [29]. In order to simplify the system only inclusion of the hydrophobic part of the SDS anion into the CD cavity from its wider side, which is more probable, has been considered.

The formation energy of the inclusion complex (ΔE) was obtained as the difference between the energy of the inclusion complex (E_{IC}) and the sum of energies of the dodecyl sulfate anion (E_S) and cyclodextrin (E_{CD}):

$$\Delta E = E_{IC} - (nE_S + E_{CD}) \quad (1)$$

where n is the number of included dodecyl sulfate anions.

Solvent molecules cannot be simply incorporated into a molecular modelling experiment; the solvation effect was not taken into account. For the calculation only the most energetically favourable conformations of SDS, CD and CAs [29] have been used; due to this fact entropic part of the Gibbs' equation ($T\,\Delta S$) has been eliminated. The inclusion complex formation energy (ΔE) characterizes only the enthalpic part (ΔH) of the equation. ΔE has been used for the comparison of CD–solute and CD–SDS inclusion complex stabilities.

3. Results and discussion

3.1. Chromatography

Interaction of dihydropyridine derivatives with CD selectors was followed by HPLC using three chiral stationary phases, namely β-CD, *S*N- and *R*N-β-CD in the reversed mode. As has been reported, enantiomer separation is favoured in systems containing a low proportion of the organic modifier, such as methanol or acetonitrile [18]. Acetonitrile acted as a stronger eluent. Both organic modifiers exert different influences on enantioselectivity of the separation and it is not possible to generally favour a particular one.

Table 1 presents a survey of the capacity ratios, separation factors and resolution. Only nisoldipine and nimodipine were chirally separated. In a mobile phase containing methanol, an increase in the capaci-

Table 1
Retention data of CAs on three different cyclodextrin stationary phases in HPLC

Compound		Mobile phase composition											
		30% MeOH			20% MeOH			20% ACN			10% ACN		
		β-CD[a]	*S*N[a]	*R*N[a]	β-CD	*S*N	*R*N	β-CD	*S*N	*R*N	β-CD	*S*N	*R*N
Nisoldipine	α		1.05	1.02	1.03	1.06	1.02				1.08	1.02	
	k	10.34	21.08	15.30	28.46	51.21	25.90	3.68	10.25	6.44	22.95	35.92	22.73
	R		0.42	<0.1	0.28	1.04	0.15				0.60	0.10	
Nimodipine	α	1.09			1.13						1.09		
	k	4.23	11.57	10.72	10.44	38.93	22.52	2.80	6.67	7.20	8.53	19.33	30.02
	R	0.71			1.15						0.60		
Nitredipine	α												
	k	2.24	9.42	9.02	3.52	32.47	–	1.64	5.50	16.73	3.33	–	–
	R												
Isradipine	α												
	k	3.23	9.08	8.93	6.65	–	–	1.96	5.58	–	5.71	–	–
	R												
Amlodipine	α												
	k	0.71	1.07	0.98	0.91	–	–	0.60	0.63	–	0.89	2.50	–
	R												

[a] Type of cyclodextrin stationary phase used.

ty ratios was observed, in agreement with the increasing hydrophobicity of the solutes [30], no matter which CD phase was used. The k values of individual derivatives increase for different CSP in the following sequence: native β-CD, *R*N-β-CD and *S*N-β-CD. It is feasible to explain this sequence in such a way that in naphthyl substituted CSPs individual solutes can in addition to inclusion interact also with the π-electrons [1]. The interpretation of results obtained with derivatized CD phases is complicated by non-univocal CD substitution; reportedly three to eight substituents can be found per single CD molecule [4]. The lower retention of CAs on *R*N-β-CD in comparison with *S*N-β-CD results from more pronounced steric hindrances of the *R*-naphthyl derivative at the cavity entrance [3].

When acetonitrile was used in the mobile phase, inversed retention sequence on *R*N-β-CD CSP was observed. This suggests the existence of a different retention mechanism on this stationary phase in comparison with the other two in which inclusion predominates.

The application of the polar organic mode, which frequently is effective for chiral separations where reversed mode fails, was also tested. With the exception of amlodipine, all other CAs exhibited exceedingly short retention times and were not chirally separated. The retention of amlodipine, however, was considerable but no enantiomer separation occurred (data not shown.)

3.2. Electrophoresis

Two modes of electrophoretic enantiomer separation – MEKC (SDS surfactant with β-CD) and HPCE (with anionic CM-β-CD) – were tested with the studied set of compounds.

In the MEKC mode of electromigration, the impact of SDS concentration in the presence of 2–5 m*M* β-CD in BGE and of SDS–β-CD ratio (Fig. 3) on migration time and resolution of enantiomers of nisoldipine, the only ones that have been separated in this system, was studied.

Fig. 3 demonstrates that as the SDS concentration (SDS–β-CD ratio) is increased, the migration time increases but resolution becomes worse. Chiral recognition of nisoldipine was optimal at a SDS–β-CD ratio of 4:1. It appears that for meaningful gain in chiral resolution the SDS concentration rather than

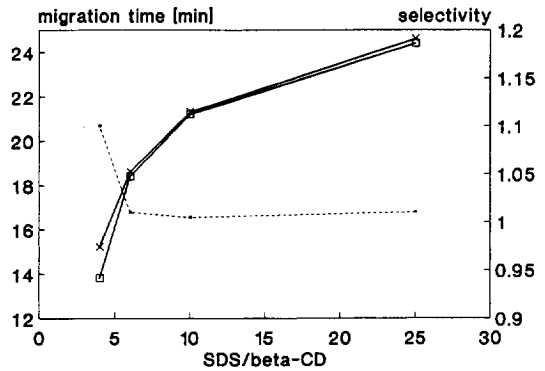


Fig. 3. Influence of the SDS–β-CD ratio on the migration time of nisoldipine enantiomers (crosses, squares) and the selectivity of chiral separation (dotted line).

the SDS–β-CD ratio is crucial. With increasing concentration of SDS the equilibrium in the solution is shifted in favour of the incorporation of uncharged CAs into the micelles, moreover, the CA molecules compete for inclusion into the CD cavity with the hydrophobic chain of free SDS molecules which are in equilibrium with the portion of the surfactant forming micelles. Of course, increasing the concentration of SDS results in a decrease of endo-osmotic flow (EOF), also increasing the migration time.

From the point of view of the existing equilibria, a simpler system is represented by capillary electrophoresis using chargeable CDs. In the present communication we have used CM–β-CD. The results are summarized in Table 2 and an electrophoregram of the chiral separation of three derivatives is shown in Fig. 4. As demonstrated in this system, baseline separation of amlodipine, nitredipine and nimodipine enantiomers was achieved.

The migration of dihydropyridine derivatives in a carboxymethylated β-CD buffer is a vector sum of the oppositely oriented electromigration of negatively charged CD–CA complexes and of EOF. Amlodipine first comes across the detector window due to possible interaction of the carboxyl group of the CD with the amino group of amlodipine. The resulting complex will exhibit a decreased charge–mass ratio, and therefore its anodic drift will be slowed down.

In the case of isradipine and nisoldipine complexes with CM–β-CD, anodic migration appears to be

Table 2
HPCE data for CAs with CM–β-CD in BGE

Compound	Electrolyte buffer											
	20 m*M* phosphate–borate buffer, pH 4.6, 1% urea 10 m*M* CM–β-CD			20 m*M* phosphate–borate buffer, pH 7.0, 1% urea 10 m*M* CM–β-CD			10 m*M* phosphate –borate buffer, pH 6.7, 0.5% urea 5 m*M* CM–β-CD			10 m*M* phosphate–borate, pH 6.7, 0.5% urea 20 m*M* SDS 5 m*M* CM–β-CD		
	tm1/tm2	α	*R*	tm1/tm2	α	*R*	tm1/tm2	α	*R*	tm1/tm2	α	*R*
Nisoldipine	n	n	n	n	n	n	n	n	n	17.15	1.00	0.00
Nimodipine	31.00/32.30	1.04	3.70	–	–	–	–	–	–	–	–	–
Nitredipine	17.90/18.40	1.03	5.70	7.26/7.33	1.01	0.45	–	–	–	–	–	–
Isradipine	n	n	n	n	n	n	n	n	n	18.41	1.00	0.00
Amlodipine	15.60/16.60	1.06	8.00	7.78/8.05	1.03	4.50	–	–	–	22.07	1.00	0.00

n: Migration time >60 min.
–: Not measured.

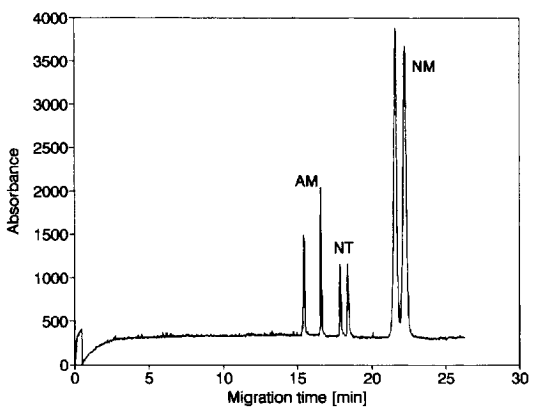


Fig. 4. Enantioseparation of three CAs in HPCE with CM–β-CD selector. 20 m*M* phosphate–borate buffer, pH 9.4, 1% urea, 10 m*M* CM–β-CD; for other conditions see Section 2. AM = amlodipine, NT = nitredipine, NM = nimodipine.

distinctly faster (within pH values 4.6–9.4) and therefore the respective complexes do not pass the detector window within the 60 min run time. There is also a possibility that nisoldipine and isradipine form complexes with two derivatized CD molecules, by which interaction the total negative charge of the complexes and their anodic mobilities are further increased. The similar behaviour of these two derivatives can be related to the values of inclusion complex energies (ΔE) [29]. The values in the preferable inclusion of the aromatic ring into the CD cavity are the highest for nisoldipine and isradipine and an analogous part of their molecules protrude from the cavity. After the addition of SDS, both nisoldipine and isradipine were eluted, but no enantiomer separation for any of the five CAs was achieved.

The results obtained cannot be compared with those obtained from HPLC on a CM–β-CD chiral stationary phase because it is not commercially available. It appears possible to use an HPLC system with a chiral selector in the mobile phase, but this approach has not been tried during this experimental work.

Table 3
Formation energies (ΔE) of inclusion complexes of β-CD: SDS calculated by molecular mechanics

Inclusion complex	Stoichiometry CD–SDS	Formation energy ΔE (kJ/mol)
1	1:1	−100.32
2 (parallel)	1:2	−132.92
3 (non-parallel)	1:2	−180.99

Note: Complex 3 was obtained by addition of another SDS molecule to optimized complex 1 (for structure see Fig. 5).

3.3. Molecular modelling

Modelling was carried out with underivatized CD because this is the only clearly defined molecule (see Section 3.1).

Because the separations obtained on β-CD CSP and in the MEKC system with an identical chiral selector (β-CD) are different, the role of SDS molecules was modelled. The results of inclusion complex energies obtained with docking one and two SDS molecules into the CD cavity are summarized in

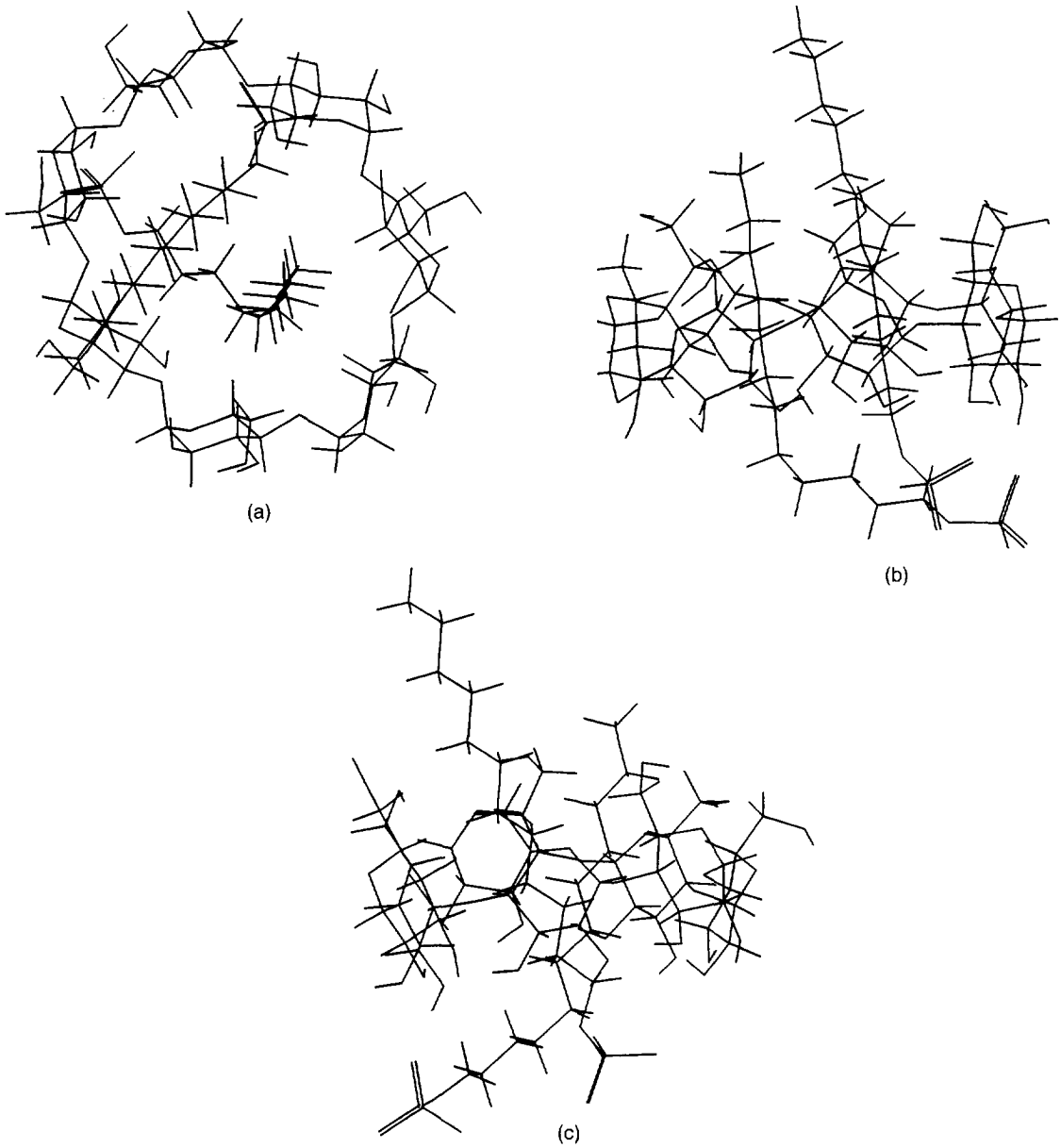


Fig. 5. Structures of the inclusion complex SDS–CD visualized by molecular modelling. Note: The stoichiometry is 2:1 (two nonparallel SDS chains inside the β-CD cavity). a = front view, b = side view I and c = side view II (as side view I turned by 90° around the x-axis). A second SDS molecule is added to the optimized complex exhibiting 1:1 stoichiometry (Table 3). In the optimized version, the first SDS molecule is placed away from the centre. From the five possible starting orientations the one with the lowest energy was considered to be most probable.

Table 3. These values are comparable to the previously published inclusion complex energies for CA–CD complexes (ranging from −127.4 to −196.5 kJ/mol) [29]. It is possible to consider SDS inclusion into the β-CD cavity as very probable and it is likely that the ratio of SDS–β-CD is 2:1 (Fig. 5). These results support the explanation of the formerly mentioned competition of SDS molecules and separated solutes for incorporation into the CD cavity in the MEKC system.

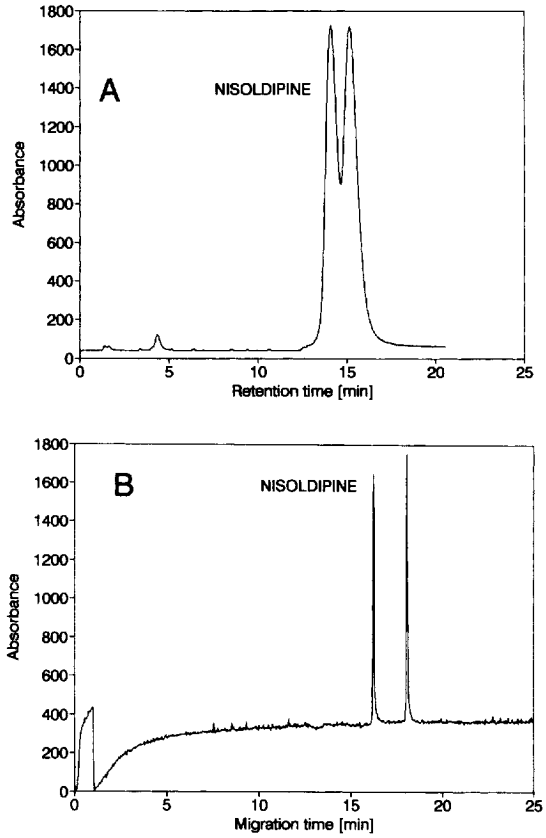


Fig. 6. Enantiomer separation of nisoldipine with β-CD selector in HPLC (A) and MEKC (B). A: HPLC with 15% acetonitrile in 0.1% triethylamine buffer, pH 5.0. B: MEKC with 25 m*M* phosphate–borate buffer, pH 9.4, with 20 m*M* SDS, 1% urea, 5 m*M* β-CD. For other conditions see Section 2.

3.4. Chiral recognition

The enantiomer separation of CAs occurred in HPLC only with derivatives exhibiting the largest retention, i.e. nimodipine and nisoldipine.

The separation of nisoldipine enantiomers was achieved, at least partially, with all CSPs in HPLC and in MEKC. A comparison of the enantiomer separation of nisoldipine in optimized HPLC and MEKC systems is presented in Fig. 6A,B, where the higher separation efficiency of the electrophoretic system at a similar selectivity of separation is documented. Nisoldipine appears to have the highest affinity for the inclusion of the benzene ring into CD. This is in concert with the highest value of the inclusion complex formation energy ($\Delta E = -187.9$ kJ/mol), which allows it to overcome a higher energetical barrier of inclusion into the cavity in the presence of SDS in the MEKC system.

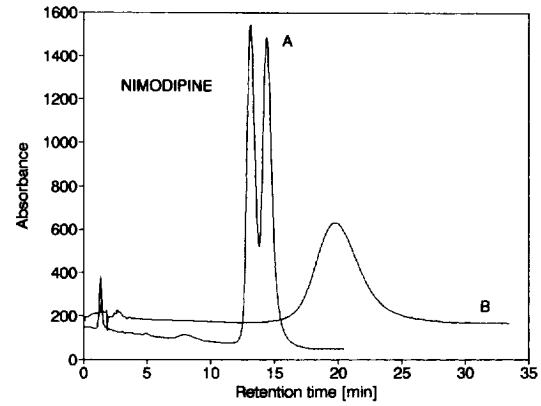


Fig. 7. Influence of SDS on the enantiomer separation of nimodipine in HPLC. A: β-CD CSP, 20% methanol in 0.1% triethylamine buffer, pH 5.0 B: β-CD CSP, 20% methanol in 0.1% triethylamine buffer, pH 5.0, with 5 m*M* SDS. For other conditions see Section 2.

Comparison of the enantiomer separation of nimodipine with the native β-CD selector in HPLC and MEKC is quite interesting. In spite of the fact that the selector is the same, good separation was obtained with CSP only. MEKC with SDS in the run buffer was unable to separate nimodipine enantiomers. The tendency toward inclusion of the aromatic ring into the CD cavity is less strong with nimodipine ($\Delta E = -145.8$ kJ/mol) than with nisoldipine. The necessary stereoselective interaction of nimodipine in MEKC is more limited by SDS and appears insufficient for enantiomer separation. This assumption was confirmed by the results of both mathematical modelling and a liquid chromatography experiment; by adding SDS to the mobile phase, the nimodipine enantiomer separation on β-CD CSP ceases to exist (Fig. 7).

In most cases SDS-containing medium limits or even precludes enantiomer separation on CD chiral selectors.

4. Conclusion

CD chiral selectors prove to be applicable to the separation of dihydropyridine CAs both on some

CSPs and in electrophoresis. Among the five derivatives followed, only isradipine resisted separation in any separation system used. CM-β-CD in the HPCE mode appeared to be the most suitable selector. This selector exhibits a higher enantioselectivity towards three solutes of the studied group. The complexity of the separation mechanism involved, even with the structurally related compounds investigated in this study, prevents formulation of a generally applicable approach.

By molecular modelling, HPLC, MEKC and non-micellar HPCE, the hypothesis about competitive interaction of CAs and SDS molecules with the CD cavity was proposed.

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