Correlation between Structure of Dihydropyridine Calcium Antagonists and Their Retention Behavior and Enantioseparation on the β-Cyclodextrin Stationary Phase in HPLC

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Summary

Chromatographic data for five dihydropyridine calcium antagonists (CAs) were obtained on β-cyclodextrin column in the reversed phase HPLC. Interaction energies of solute-cyclodextrin complexes formed by docking of different parts of the CAs' molecules into the cyclodextrin cavity were calculated. Results from the HPLC experiments and molecular modeling were compared. It was found that several types of inclusion complexes are energetically possible and contribute to the retention simultaneously. Only certain inclusion complexes are assumed to be responsible for enantioseparation of CAs. Nevertheless molecular modeling studies are a useful tool for explaining the enantioselectivity and predicting the elution order of enantiomers.

1 Introduction

Cyclodextrins (alpha-, beta- and gamma-) and their derivatives have been successfully employed as chiral selectors for analytical separations in gas and liquid chromatography as well as in capillary electrophoresis. During the chromatographic separation process the cyclodextrins (CD) as host molecules form intermediate diastereomeric complexes with chiral guest molecules; differences in the stability of these complexes determine the enantioselectivity of separations.

The mechanism that rules the separation in the presence of β-cyclodextrin as chiral selector has not yet been fully clarified. Inclusion of the apolar domain of the solute into the hydrophobic cavity of the selector is assumed [1,2]. However, at the moment it is not possible to predict the success of a chiral separation on the basis of the chemical structure of analyte [3].

The empirical methods of molecular modeling represent a very promising tool which is likely to help us in understanding the solute-selector interaction during the enantioseparations on cyclodextrin chiral phases [4–8,17,18]. This approach is particularly suitable for describing the chromatographic behavior in gas chromatography. In liquid chromatography the system is complicated by the composition of the mobile phase, which influence could not be simply incorporated to the process of molecular modeling.

In our previous work [9] we have studied retention behavior and enantioseparation of a series of structurally related dihydropyridine calcium antagonists (CA). These compounds belong to the family of chiral drugs the enantiomers of which have been proven to exert different effects on human organism [10–12]. Therefore attention has been paid to their enantioselective separations.

The measurements were performed on two types of cyclodextrin stationary phases: native β-cyclodextrin and a *S*-naphthylethyl carbamoyl β-cyclodextrin derivative.

In this paper we compare the experimental chromatographic data with results of molecular modeling with the goal of elucidating the type of cyclodextrin-chiral solute interactions. In this study we have limited ourselves to a rather simple situation with native β-cyclodextrin stationary phase.

2 Experimental

2.1 Chemicals

The following chemicals were used: triethylamine min. 99% (Sigma, St. Louis, Mo U.S.A.), acetic acid p.a. (Lachema, Brno, Czech Republic), acetonitrile for chromatography (Merck, Darmstadt, Germany), methanol p.a. (Penta, Chrudim, Czech Republic) and deionized water.

Calcium antagonists were extracted from the following commercially available drugs: amlodipine (Norvasc, Pfizer, Brussels Belgium), nitredipine (Baypress, Bayer, Leverkusen Germany), isradipine (Loimir, Sandoz, Basle Switzerland), nimodipine (Nimotop, Bayer, Leverkusen Germany), nisoldipine (Baymycard, Bayer, Leverkusen Germany).

For structures of the CAs studied see **Figure 1**.

2.2 High performance liquid chromatography

All measurements were carried out with the Gilson chromatograph (Gilson, Middleton, U.S.A.) consisting of ASTED autosampler, 115 UV Gilson detector, 805 manometric Gilson module, 305 and 306 Gilson piston pumps and 811C Gilson dynamic mobile phase mixer. A commercially available steel column 250 × 4 mm packed with native β-CD covalently bonded on 5 μm silica – Chiradex (Merck, Darmstadt, Germany) was used.

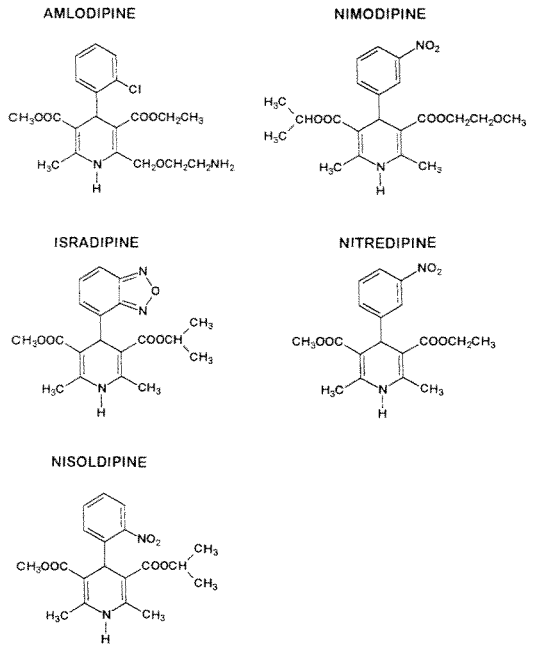

Figure 1. Chemical structures of the drugs investigated.

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 by acetic acid. The flow rate of the mobile phases used was 0.8 ml/min in all cases.

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

2.3 Molecular Modeling

R-Isomers of selected CAs were constructed using the INSIGHTII model builder [13]. Cyclodextrin coordinates were obtained from the Cambridge crystallographic database [14]. Geometric optimizations of isolated subsystems and the respective clusters were performed using CVFF empirical force field implemented in DISCOVER code [15]; the dielectric constant was set to 1.0. Standard CVFF atomic charges were considered.

Docking of CAs to the cyclodextrin cavity was done in the following way:

i) Orientation of the CA in front of the cyclodextrin cavity: four initial orientation of CAs were selected (see Figure 5).

ii) Gradient optimization of all the coordinates of CA and cyclodextrin.

iii) After the local minimum was generated, the CA was put deeper to the CD cavity; in this way the additional intermolecular hydrogen bonds (to state already existing) were generated and gradient optimization was repeated.

iv) If the energy of an obtained minimum did not change after several optimizations, the structure found was considered to be optimal.

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 a solute (E_S) and CD (E_{CD}) – Eq.(1):

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

Observation of the requirements specified in item i) results in limitation to energetically most favorable conformations of CAs; higher energy structures are not included. The flexibility of the guest molecules is thus not fully taken into account. We are further aware of the fact that the docking method used depends partially on the authors' intuition. A proper study would need to account also for the entropic effect, *i.e.* by performing random sampling optimization. This procedure will be applied in future studies.

Table 1. Capacity factors of CAs under various mobile phase compositions.

	Capacity factors k				
	Amlodipine	Nitredipine	Isradipine	Nimodipine	Nisoldipine
% of MeOH					
5	1.78	9.66			
10	1.48	6.98	15.43	29.80	82.25
20	0.91	3.52	6.65	10.44	28.46
30	0.71	2.24	3.23	4.23	10.34
40	0.58	1.69	2.01	2.26	4.16
50	0.55	1.53	1.63	1.68	2.32
80	0.54	1.36	1.37	1.37	1.39
100	0.59	1.38	1.38	1.39	1.40
% of ACN					
5	1.53	6.29	13.96	25.30	68.38
10	0.89	3.33	5.71	8.53	22.95
20	0.60	1.64	1.96	2.08	3.68
40	0.53	1.08	1.11	1.08	1.14
60	0.73	0.91	0.94	0.91	0.91
80	1.59	1.03	1.04	1.02	1.02
90	7.25	1.22	1.22	1.21	1.21
95	16.79	1.32	1.34	1.33	1.32
100		2.13	2.22	1.51	1.51

3 Results and Discussion

3.1 Retention Behavior and Chiral Separation of CAs in a Reversed HPLC System

Retention and enantioseparation of selected CAs on native β-CD in the reversed-phase mode has been investigated. The mobile phases consisted of pH 5.0 buffer (for composition see Section 2.2.) containing different proportions of methanol or acetonitrile (5–100%). The results are summarized in **Table 1**.

Dihydropyridine CAs behave on the β-CD phase similarly on the conventional C-18 reversed phase, *i.e.* their retention becomes distinctly shorter with an increasing methanol concentration.

An analogous retention data dependence was obtained with acetonitrile as the organic phase modifier. An exception was the retention of amlodipine, the retention of which starting from 60% acetonitrile in the mobile phase, increases. This behavior, besides other factors, reflects a limited solubility of amlodipine in acetonitrile.

From the enantioselectivity viewpoint only the two most retarded derivatives, nimodipine and nisoldipine, were separated into their enantiomers in the system used. The values found for the separation factors (R_S) and their dependence on the acetonitrile or methanol concentrations are presented in **Figure 2**. With decreasing content of organic modifier R_S increases; however, the retention increases beyond tolerable limits. The selectivity differs for the two modifiers used: mobile phases containing MeOH are more suited for the separation of nimodipine; enantioseparation of nisoldipine was slightly better when acetonitrile was used.

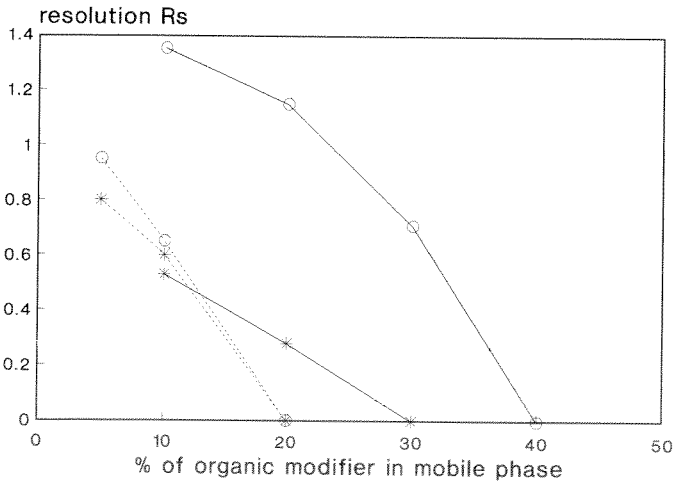

Figure 2. Resolution (R_S) of enantiomers of nimodipine and nisoldipine *versus* mobile phase composition. Asterisks – nisoldipine; circles – nimodipine; full line – methanol as organic modifier, dotted line – acetonitrile as organic modifier.

The most distinct influence on enantioselectivity is observed on carrying out the separation at a decreased temperature. At 0 °C and with the optimum mobile phase, R_S values shown in **Table 2** were obtained. A representative chromatogram showing the separation of nimodipine at decreased temperature is shown in **Figure 3**.

Table 2. Influence of decreased temperature on the chiral selectivity (α) and resolution (R_S) of nimodipine and nisoldipine. The mobile phase used consisted of appropriate amount of organic modifier and 0.1% TEA solution buffered by acetic acid to pH 5.0.

Derivative	% of org. modifier	k_1	k_2	α	R_S
Nimodipine	30 % MeOH	16.83	20.14	1.197	1.60
Nisoldipine	15 % ACN	21.95	24.62	1.122	1.06

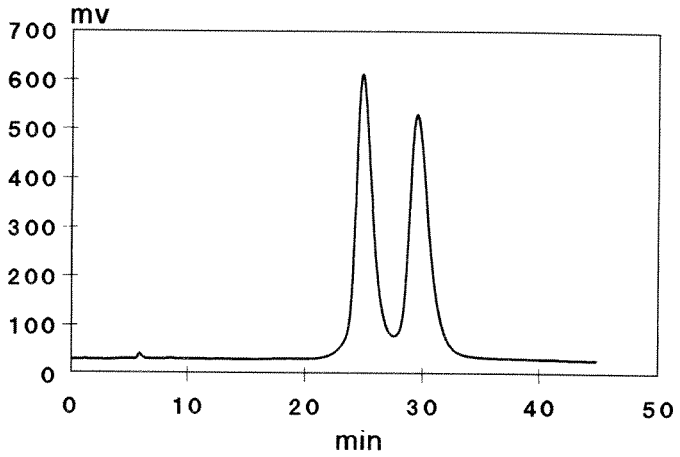

Figure 3. Separation of nimodipine enantiomers on the β-CD column. Conditions: 30 % MeOH: 70 % buffer (0.1% triethylamine, pH 5.0); flow rate 0.8 ml/min; temperature 0 °C.

3.2 Molecular Modeling

By making use of molecular modeling, energetically most favorable structures of isolated solute molecules were obtained. The spatial arrangement of the nimodipine molecule is shown in **Figure 4**. A common feature of all CAs studied is that substituted benzene and dihydropyridine rings bound to the chiral carbon exhibit an angle of approximately 109 °C (tetrahedral bond); concomitantly the planes of both rings are perpendicular.

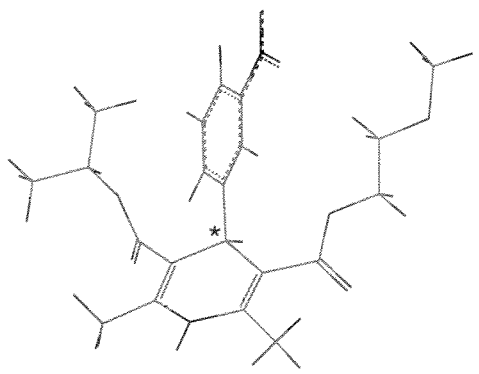

Figure 4. Molecule of nimodipine after gradient energy optimization. Asterisk marks chiral carbon.

Structures created in this way were docked into the cyclodextrin cavity. For steric reasons it is unlikely that CAs will be included completely. The following possibilities of inclusion complexes formation with the participation of different domains of the solute molecules – (1) derivatized benzene, (2) dihydropyridine ring, (3) longer carboxylic acid ester moiety, (4) shorter carboxylic acid ester moiety – were investigated. In these considerations both inclusion from the more open side and the narrower side of the cyclodextrin cone were investigated.

From the results of the energetic minimization for the nisoldipine-CD complexes it follows (**Figure 5**) that all ways of inclusion investigated are thermodynamically possible.

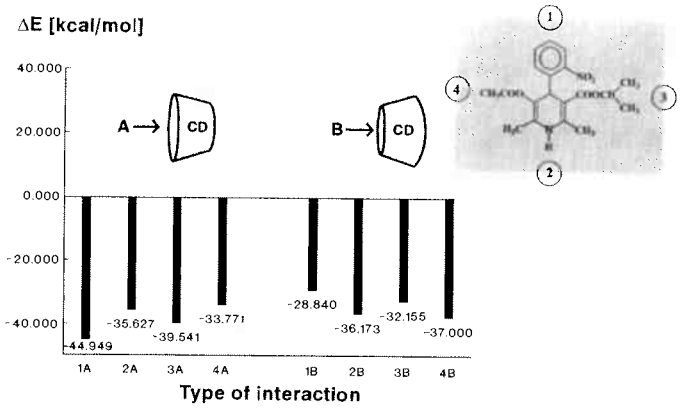

Figure 5. Comparison of formation energies of eight nisoldipine-CD inclusion complexes. A – inclusion from the more open side of CD; B – inclusion from the narrower side of CD.

Surprisingly strong complexes originated during nisoldipine inclusion from the narrower side of the CD cavity. This is caused by the ability of cyclodextrin to undergo conformational changes. That enables CD to open the narrower side of the cone and hence to accommodate even voluminous functionalities (they are not sterically excluded from inclusion from this side) and afterwards to close itself again. Also elliptic distortion of the CD ring occurs; not only in the direction of hydrophobic attractive forces between the plane of the aromatic ring and CD, but also for steric reasons.

Table 3. CA-cyclodextrin complex formation energy; four types of interaction. 1 – Benzene moiety; 2 – dihydropyridine moiety; 3 – longer carboxyester chain; 4 – shorter carboxyester chain.

Inclusion complex formation energy ΔE [kcal/mol] *				
	1	2	3	4
Nisoldipine	–44.949	–35.627	–39.541	–33.771
Nimodipine	–34.867	–34.942	–44.339	–33.731
Nitredipine	–35.775	–36.617	–46.195	–41.217
Isradipine	–37.736	–35.292	–34.928	–37.468
Amlodipine	–30.479	–38.320	–34.103	–47.017

* Inclusion from the more open side of cyclodextrin.

In order to simplify the system studied we have focused our attention only on the formation of inclusion complexes in which the solute enters the cyclodextrin cavity from the more open side of cone. In the system studied the accessibility towards the narrower cyclodextrin cavity side is limited through β-cyclodextrin bonding to the solid support. It is feasible to assume that inclusion from the broader side of the CD molecule will be favored both sterically and energetically. Last but not least, interaction from the more open side of the cyclodextrin cavity will be favored also by kinetic factors as conformational changes during the inclusion from the narrower side of the cone will require more time between inclusion and relaxation, *i.e.* releasing the solute from the cavity. The values of interaction energies of the complexes CA-cyclodextrin found by docking different parts of the molecule into the cavity are summarized in **Table 3**.

3.3 Correlation of the Results Obtained by Molecular Modeling with the Chromatographic Experiment

3.3.1 Retention Behavior of Dihydropyridine Derivatives

On comparing the chromatographic results with molecular modeling (Tables 1 and 3) no correlation of the retention behavior of CAs with the estimated interaction energies of possible inclusion complexes is apparent. For the chromatographic behavior of solutes Gibbs' energy (Eq.(2)) is of decisive importance:

$$\Delta G = \Delta H - T\Delta S \qquad (2)$$

The interaction energy ΔE (Eq. 1) characterizes only the enthalpic part (ΔH) of the equation; however, it is rather obvious that the entropic part ($T\Delta S$) will also significantly contribute to the overall ΔG value. Further, it is possible to speculate that in the present system inclusion is not the only interaction influencing retention and separation on cyclodextrin bonded phases. In contrast to gas chromatography, retention in liquid chromatography is considerably influenced by solute distribution between the stationary and mobile phase, *i.e.* by the buffer media and type of the organic modifier, mainly by its ability to exclude the solute of the CD cavity.

In accordance with the assumption in ref. [9], the interaction with the benzene ring is most favored for nisoldipine with a nitro group in the *meta* position. This is reflected by the retention of nisoldipine, which is by far the longest.

In all cases the nitro group is oriented in such a way that it does not enter the cavity, thus determining the depth of benzene penetrating into the CD cone. It is rather surprising that in the case of isradipine, in spite of its sterically voluminous substituent on the benzene ring, the whole substituent does penetrate into the cavity.

Inclusion of the benzene ring in the case of amlodipine is limited by repulsive electrostatic forces of the chlorine substituent. Therefore the inclusion is energetically the least favorable. Consequently, amlodipine is the least retained CA of all those investigated.

All interactions 1 – 4 are considered to be possible and we suppose, on the basis of the similar values of the interaction energies, that they contribute simultaneously to retention. The hypothesis about non-uniform action of chiral stationary phase (CSP) is further supported by inadequate peak spreading.

3.3.2 Enantioselectivity

From the mechanistic studies reported in the literature, retention and enantioseparation prediction on cyclodextrin phases is of only limited success even for structurally related compounds [3,16].

Application of empirical methods of molecular modeling allows description of the cyclodextrin-separated solute host-guest system not only with regard to steric contributions but also with regard to energetic contributions to the stability of the diastereomeric complex [4,6,8,17,18].

Optimized inclusions complexes have been subjected to analysis with the aim of finding out which interactions are stereoselective. CD-benzene complexes are potentially stereoselective. To what extent this interaction will be involved in enantioseparation depends upon the extent to which the benzene domain will penetrate into the cavity, or, more precisely, whether or not the chiral center will be drawn into the cone, whether the inclusion is preferred energetically, and upon the possibility of other stereoselective interactions.

The most favorable situation can be observed with nisoldipine (four hydrogen bonds) which shows clear enantioseparation (**Figure 6**). In the case of nimodipine which exhibits the most distinct enantioseparation, the benzene ring penetrates only partly into the cavity. Therefore it is proposed that this interaction has only a limited stereoselectivity.

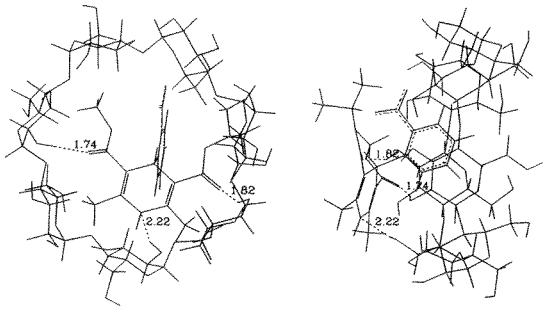

Figure 6. Optimized inclusion complex of nisoldipine with CD. A – front view, B – side view. H bonds with lengths (in Å) are indicated by dotted lines.

The type of interaction CD-pyridinium grouping, where even inclusion of both methyl groups occurs (in amlodipine even with the long chain), is not considered to be stereoselective. The chiral center does not penetrate the cavity and the three-point formal assumptions for a chiral interaction are not fulfilled.

Stereoselectivity cannot be excluded in the interaction of the two carboxy ester chains, because the chiral center lies at the mouth of the cavity and the benzene moiety can interact either hydrophobically or sterically with the more open side of CD. With nimodipine, in which the penetration into the cavity by the long side chain is preferred (energetically the most favored complex), the penetrating molecule is fixed in a position, in which the carbonyls of both chains form hydrogen bonds. The nitro group on the benzene forms an other H bond with cyclodextrin. It is proposed that this inclusion complex plays the most important role in nimodipine enantiomers separation.

Because the studied systems can display more stereoselective interactions, it can be speculated that in cases where the contributions of several stereoselective interactions are summed (all prefer a particular enantiomer), stereoselectivity increases. On the contrary if the enantioselectivities of different interactions counteract each other, the overall stereoselectivity is suppressed.

4 Conclusion

The complexity of interactions which may contribute to stereoselectivity has been demonstrated on a model system composed of native β-CD and selected CAs.

Molecular modeling confirms the importance of inclusion for chiral separation of CAs. It is unlikely that chiral recognition is possible through interaction of the solutes with the outside of cyclodextrin cavity.

In contrast to GC, in HPLC the separation mechanism is more distinctly influenced by mobile phase composition. The molecular modeling approach used involves only the interaction energy ΔE and neglects the entropic contribution as well as the kinetic factors which play a role in inclusion complex formation in real systems. For a more precise description of solute retention on CD phases it would be of an advantage to determine the total change of Gibbs' energy.

At the moment we are studying the stability of the inclusion complexes of nimodipine and nisoldipine by using molecular dynamics in order to be able to predict the elution order of both enantiomers of nimodipine and nisoldipine.

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