1 INTRODUCTION

Over the last two decades, high-performance liquid chromatography (HPLC) has become one of the most common techniques in chiral separations. HPLC has also been successfully employed for determination of the optical purity of newly synthesized organic compounds.

Asymmetric synthesis is a direct way of synthesis of enantiomers that uses suitable optically pure ligands acting as catalysts. The chiral ligands in asymmetric reactions must have stable configurations (they must be resistant towards racemization) and a high optical purity is required.

Binaphthyl derivatives have been extensively used to control many asymmetric processes and have demonstrated outstanding chiral discrimination properties, due to their unique properties derived from their rigidity and spatial arrangement. Most of 1,1´-binaphthyl molecules are C_2 symmetric with two identical naphthyl units often substituted in the 2,2´-positions. Chirality of these compounds is caused by restricted rotation of atoms or groups of atoms around the single bond on the binaphthyl skeleton (axial chirality, atropisomerism). Increased hindrance to rotation at the pivotal 1,1´-bond makes these molecules potential candidates for enantioseparation [1]. The best representatives of the binaphthyl group are 2,2´-dihydroxy-1,1´-binaphthyl (1,1´-bi-2,2´-naphthol, BINOL, OBIN) [2-5], 2,2´-diamino-1,1´-binaphthyl (BINAM) and 2,2´-bis(diphenylphosphino)-1,1´-binaphthyl (BINAP) [6,7], which have been used to develop many related chiral auxiliaries. The recent developments in the field of new chiral binaphthyl catalysts are focused to the preparation of derivatives with non-identical groups in various positions [8] and to a shifting the classical 1,1´- chiral axis to other positions [9]. Efforts in the molecular architecture of these ligands are oriented toward creating a stable chiral environment for asymmetric reactions providing a high degree of enantiopurity.

Cyclodextrins (CDs) and polysaccharides are natural oligomers and polymers, respectively, their basic constituents are glucose units. CDs and polysaccharides and their derivatives have been successfully used as chiral stationary phases (CSPs) in HPLC for enantioselective separation of a wide range of structurally different

compounds [10]. On cyclodextrin based CSPs inclusion phenomena play a dominant role in the enantioselective recognition mechanism in a reversed-phase separation system. The cavity dimension of β-cyclodextrin fits well with substituted phenyl and naphthyl (even better) moieties, so that enantioseparation of disubstituted binaphthyls can be expected. Combination of hydrogen bonding, dipole-dipole interactions, dispersion forces etc. are accented in normal or polar-organic separation modes [11,12]. Among CSPs based on polysaccharide derivatives, celulose *tris*(3,5-dimethylphenylcarbamate) ones exhibit a very good resolution capability [13,14]. The chiral recognition is based on stereogenically different fit of enantiomers into chiral grooves, the enantioselective interaction is further stabilized by other interaction types, e.g. H-bonding, π - π interactions, dipole-dipole interaction and steric effect [13].

Besides natural polymeric chiral selectors (CSs), purely synthetic polymers can provide comparable separation possibilities. Creation of well-defined chiral environment, possibility of obtaining chiral selectors with opposite absolute configurations, structures available for chemical modification are just a few features offered by the polymeric CSPs. [15]. In general, four approaches have been used to prepare the synthetic polymeric CSPs [16]. One of them is based on the creation of a chiral linear homopolymer attached to the surface of a silica gel support. Three new synthetic polymeric CSPs based on *trans*-1,2-diamino-cyclohexane (P-CAP), *trans*-1,2-diphenylethylenediamine (P-CAP-DP) and *trans*-9,10-dihydro-9,10-ethanoanthracene-(11*S*,12*S*)-11,12-dicarboxylic acid (DEABV) have been developed via radical-initiated polymerization [15,17,18]. The P-CAP CSP contains relatively rigid structure that has no aromatic moieties while the P-CAP-DP CSP has aromatic units and the conformation of the monomer is flexible. The DEABV CSP exhibits different enantioselectivity due to a number of amide linkages and aromatic groups. In general, different hydrogen bonding, dipolar and π- π interactions (with the exception of P-CAP CSP) play an important role in the chiral discrimination process and can provide complementary separations. [15,17,18]. The advantages of all these CSPs are wide range of mobile phase compositions compatible with them (multiple mobile phase modes can be used), high sample-loading capacity and therefore, possibility of semipreparative or preparative applications.

Most papers on the binaphthyl-based ligands found in the literature deal with their synthesis. Enantioselective HPLC has been used in some cases to control the enantiomeric purity or the yield of the final products. However, the separation conditions, including characterization of the separation systems, have not usually been adequately described. Only a few articles pay attention to the enantioseparation of some of these compounds. Cellulose- or amylose- based columns in the normal separation mode have mostly been used [13,19-23]. OBIN, 2,2´-diamino-1,1´-binaphthalene and 1,1´-bi-2-naphthol bis(trifluoromethane sulfonate) have been separated on newly developed synthetic polymeric chiral stationary phases – P-CAP, P-CAP-DP, DEABV and DPEVB [24].

2 OBJECTIVES OF THE THESIS

This work is aimed to study the retention and enantioseparation behavior of 2,2´-disubstituted and 2,3,2´-trisubstituted 1,1´-binaphthyls and 3,8´-disubstituted 1,2´-binaphthyls (see Fig. 1) on three different types of chiral stationary phases based on: (i) β-cyclodextrin, (ii) derivatized cellulose and (iii) synthetic polymers under various experimental conditions. The obtained results, related to different interaction and enantiodiscrimination mechanisms were critically compared.

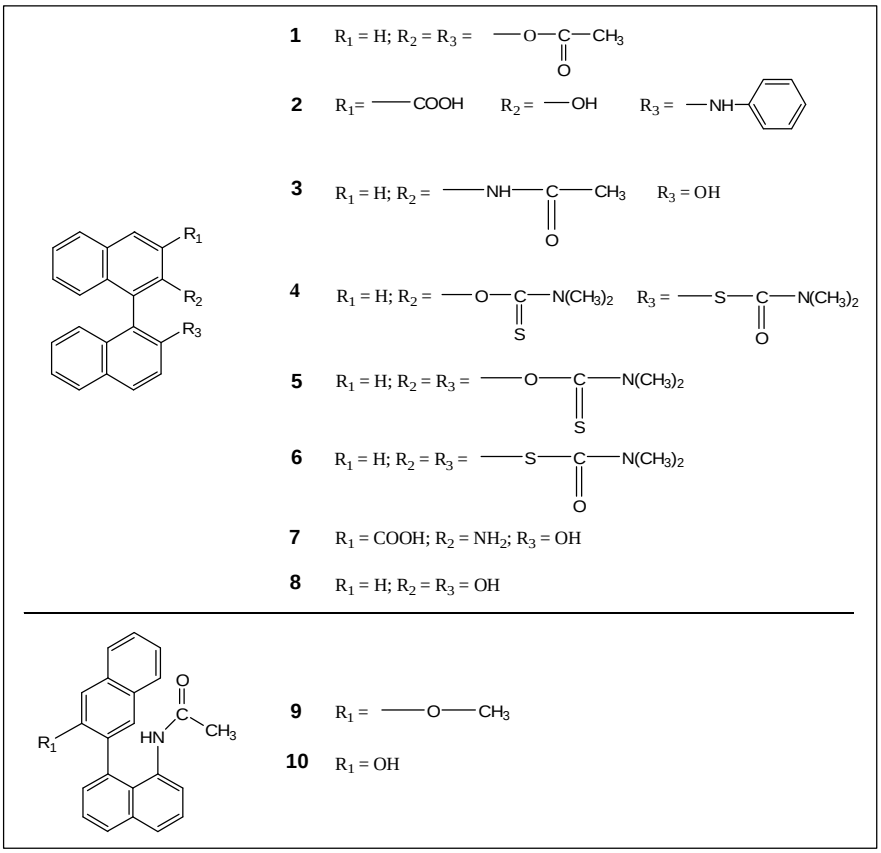

Fig. 1: Structures of the studied binaphthyl derivatives.

3 RESULTS AND DISCUSSION

3.1 Study of the influence of experimental conditions on the retention and enantioseparation of series of newly synthesized disubstituted binaphthyls

Separation on β–cyclodextrin bonded CSP

The enantioseparation was carried out in two separation modes – normal and reversed-phase. The molecules of substituted binaphthyls have a non-polar character so for that reason the normal separation mode was used as the first choice. The mobile phases (MP) consisted of *n*-hexane/propan-2-ol in various volume ratios. Retention of the analytes increased when the amount of *n*-hexane in mobile phase increased, but no enantioseparation was observed.

The reversed-phase separation mode was carried out in binary mobile phases consisted of acetonitrile (ACN) or methanol (MeOH) as the organic modifier and water. Water was later replaced by triethylamine acetate (TEAA) buffer of two different pH values – 3.0 and 6.0.

All the analytes eluted in death time when ACN was used as the organic modifier. Better results were observed in the mobile phase containing MeOH. High amount of methanol (50 - 100 vol. %) led to short retention times with no enantioseparation. As the amount of methanol decreased, the analytes were more retained and enantioseparation was observed in some cases. The appropriate mobile phase composition was proved to be MeOH/water 30/70 (*v/v*), in which all the analytes eluted with retention times ranging from 6 to 12 minutes, except of analyte **2** (Fig. 1). Just one representative of the group of 2,2´-substituted 1,1´-binaphthyls, analyte **1**, was partly separated into enantiomers (see Tab. 1). The best resolution of 8,3´-disubstituted-1,2´-binaphthyls was achieved in mobile phases consisted of MeOH/water 30/70 (*v/v*) and 20/80 (*v/v*) for **9** and **10**, respectively.

Table 1: Retention parameters of binaphthyl derivatives enantioseparated on β-cyclodextrin-based column, mobile phase MeOH/water, flow rate 0.7 mL/min and detection wavelength 254 nm.

MeOH/H_2O (*v/v*) Analyte	**20/80**			**30/70**		
	k_1	R	α	k_1	R	α
1	2.9	0.5	1.2	0.8	0.2	1.1
9	2.5	0.5	1.3	1.1	0.9	1.1
10	2.1	2.2	1.3	1.1	0.4	1.2

Replacement of water for 0.5% TEAA buffer, pH 3.0, provided considerable retention decrease, which indicates more effective reduction of silanophyl interactions between analyte and silica gel. These interactions are characteristic for this type of chiral columns. Higher pH led also to shorter retention times in comparison with the mobile phase consisted of MeOH and water. The peak symmetry, resolution and efficiency were not affected by the change in composition of the aqueous part of the mobile phase.

Separation on hydroxypropylether-β-cyclodextrin bonded CSP

As the results have shown, the chiral stationary phase based on native β-cyclodextrin is not effective for the enantioseparations of the studied compounds. Inclusion complexes are probably formed in reversed-phase separation mode in the mobile phase used but this interaction does not have any stereodiscriminative character. In further experiments hydroxypropylether-β-cyclodextrin (HP-β-CD) CSP was used. The hydroxyl group is bonded to the second carbon atom in the glucose structure and in this way the molecule of the substituted cyclodextrin is enlarged and offers other interaction possibilities for the analytes (steric repulsions, hydrogen bonds).

Normal separation mode

Firstly the normal separation mode (realized in the same way as for β-CD CSP) was tested but no enantioseparation occurred. The retention factors acquired with the β-CD CSP were lower than these obtained with HP-β-CD CSP, whereas the asymmetry values were proved to be better on the latter column.

Non-chiral separations of binaphthyl mixtures can be applied in normal separation mode for the analysis of binaphthyl derivatives that can occur during the process of asymmetric synthesis because the required derivative can be accompanied by some side products.

Reversed-phase separation mode

Based on the results obtained with the β-CD CSP binary MP consisted of MeOH/water mixtures with MeOH content between 30 - 40% on HP-β-CD CSP. Higher amount of MeOH in the mobile phase resulted in the faster elution. The retention gained on the HP-β-CD column was in most cases higher than the retention on β-CD CSP. In spite of the higher retention and better values of asymmetry, the experiments carried out on hydroxypropyl-β-cyclodextrin column did not lead to enantioseparation of all the studied analytes.

Analyte **1** as the representative of the group of disubstituted 1,1´-binaphthyls was separated in 23 minutes with resolution value R = 1.0 and partial enantioseparation of analytes 2, 4 and 8 was observed, too. Both studied 8,3´-substituted 1,2´-binaphthyls were successfully enantioseparated in the mobile phase composed of MeOH/water 30/70 (*v/v*) with the values of resolution R = 1.1 for analyte **9** and R = 1.9 for analyte **10**.

The use of TEAA buffer, pH 3.0 or 6.0, instead of water led to shorter retention, which was accompanied with a decrease of peak symmetry of all the studied analytes. Only analyte **9** gained the highest resolution value (R = 1.5) in MP containing TEAA buffer, pH 3.0, but its peak symmetry deteriorated.

Conclusions:

β–Cyclodextrin based chiral stationary phases did not provide sufficient enantioseparation ability for the studied analytes but these chiral selectors can be successfully used for separations of mixtures of these compounds produced during the synthesis process.

3.2 Cellulose *tris*(3,5-dimethylphenylcarbamate)-based chiral stationary phases as effective tools for enantioselective HPLC separation of structurally different disubstituted binaphthyls

CSPs based on cellulose *tris*(3,5-dimethylphenylcarbamate) exhibit a particularly high chiral recognition for a variety of racemic compounds. Applicability of coated versions of these CSPs to mobile phases of various polarities is limited [25]. Two cellulose *tris*(3,5-dimethylphenylcarbamate) coated CSPs have been examined - one designed for normal mode and the other for reversed-phase separations of the synthesized atropoisomers of differently substituted binaphthyls.

Normal separation mode

Based on the structure of the substituted binaphthyls [13], the normal phase separation mode was the first choice for the study of the retention and enantioseparation behavior of the selected 1,1´- and 1,2´- binaphthyl derivatives. The mobile phases were prepared by mixing *n*-hexane and propane-2-ol at various volume ratios, ranging from 5 to 80 vol. % of propane-2-ol. In general, higher propane-2-ol contents resulted in a lower retention. The enantioseparation of four analytes (**1**, **4**, **9**, **10**) in MP composed of *n*-hexane/propane-2-ol 95/5 (*v/v*) was observed, with baseline separation of three compounds. The baseline enantioseparation of analyte **4** was achieved in a very short separation time in the mobile phase with 20% vol. propan-2-ol (see Fig. 2A). In comparison with the retention of the 1,1´-binaphthyls, both the disubstituted 1,2´-binaphthyls exhibited longer retention times (particularly sample **9**) with markedly higher values of enantioresolution (see Fig. 2B) which were just slightly affected by changes in the mobile phase composition.

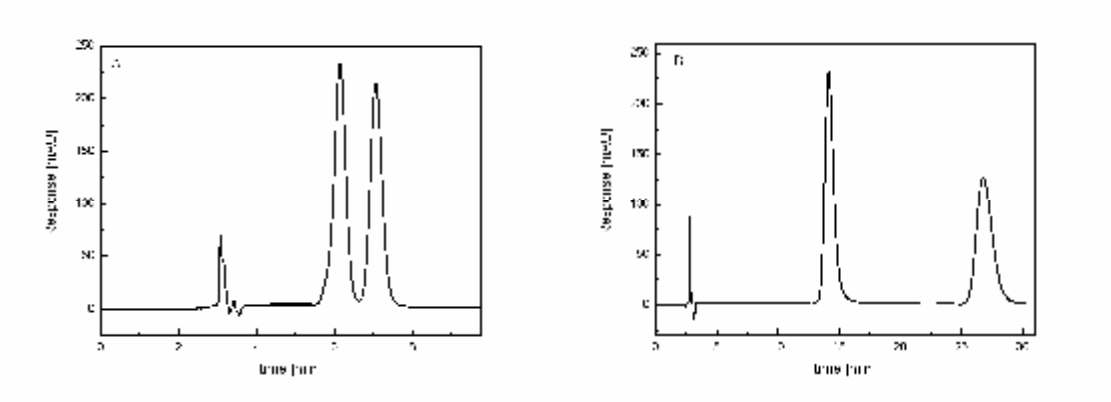

Fig. 2: Enantioseparation of analyte **4** (A) and analyte **9** (B) on CSP based on *tris*(3,5-dimethylphenylcarbamate) cellulose. Experimental conditions: Chiralcel OD-H (150 x 4.6 mm I.D.) column, mobile phase *n*-hexane/propane-2-ol, 80/20 (*v/v*); flow rate 0.7 mL/min, UV detection at 254 nm.

Reversed-phase separation mode

In the reversed-phase separation mode mobile phases consisted of ACN and water or a 20 mM phosphate buffer, pH 3.0 or 6.0. The amount of ACN in the MP ranged between 80 – 30 vol. %. Lower acetonitrile contents led to a higher retention of all the analytes, especially analytes **1**, **4**, **5**, **6**, **8** and **9** exhibited very long retention times in the MPs with 40% or 30% ACN.

In general, increased retention times result in an improved enantioresolution of most of the analytes. However, no partial enantioresolution was observed for analytes **2**, **5**, **6** and **8**. On the contrary, both the representatives of 8,3´-disubstituted 1,2´-binaphthyls (analytes **9** and **10**) provided high values of enantioresolution, even in mobile phases with high acetonitrile contents. Compound **7** showed rather high sensitivity to MP composition. Both hydrogen donor and acceptor groups, which are sensitive to ACN/water ratio, are available in analyte **7**. No other analyte studied offers such possibilities for hydrogen bonding.

The influence of the acetonitrile content in the buffered mobile phases, pH 3.0 or 6.0, on the retention is similar to that observed in the unbuffered separation systems, i.e., a decrease in the acetonitrile content leads to a higher retention with better values of enantioresolution for most of the analytes. The effect of the phosphate buffer pH is

illustrated in Fig. 3. The retention is increased and the resolution substantially improved at lower pH values (see Table 2).

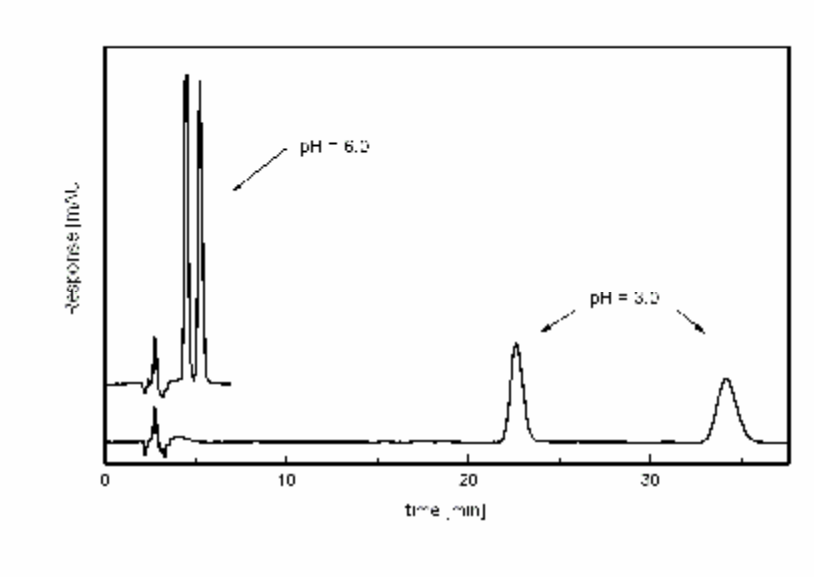

Fig. 3: A chromatogram of enantioseparation of sample **7**, Chiralcel OD-RH (150 x 4.6 mm I.D.) column, ACN/20 mM phosphate buffer, pH 3.0 or 6.0, 40/60 (*v/v*); flow rate 0.7mL/min, UV detection at 254 nm.

Table 2: Effect of the acetonitrile content and buffer pH on the chromatographic data of all the studied binaphthyl derivatives; ACN/20 mM phosphate buffer, pH 3.0, at three volume ratios, Chiralcel OD-RH column, mobile phase –flow rate 0.7 mL/min, UV detection at 254 nm.

Sample	**ACN/20 mM phosphate buffer, pH 3.0**								
	60/40			**50/50**			**40/60**		
	k_1	R	α	k_1	R	α	k_1	R	α
1	2.74	0.63	1.05	6.56	0.72	1.05	10.48	1.79	1.13
2	1.12	0.00	1.00	2.91	0.59	1.05	13.90	0.74	1.10
3	0.79	1.25	1.16	1.60	1.66	1.14	4.65	2.14	1.15
4	4.77	0.95	1.06	11.59	1.09	1.07	x	x	x
5	5.14	0.00	1.00	16.72	0.00	1.00	x	x	x
6	3.13	0.00	1.00	8.10	0.00	1.00	x	x	x
7	1.02	4.37	1.55	2.30	5.64	1.55	7.12	6.93	1.58
8	1.43	0.00	1.00	3.27	0.00	1.00	10.32	0.00	1.00
9	2.74	4.41	1.37	5.62	4.87	1.37	16.50	4.96	1.38
10	1.35	3.68	1.41	2.70	4.46	1.40	7.05	4.96	1.42

x – retention time of analyte was longer than 120 minutes

Comparing the results obtained in the unbuffered and buffered mobile phases with the same ACN contents, differences in the retention and enantioseparation can mostly be found in the mobile phases with the lowest acetonitrile content (40 vol. %) and at lower buffer pH value (3.0). These differences are mostly pronounced for samples **1**, **2** and **7**. The enantioresolution of these three compounds was improved in the buffered mobile phase (pH 3.0) while the retention factors were affected in different ways. The reduced retention of compound **1** in the mobile phase with the phosphate buffer, pH 3.0, is still accompanied by an increased resolution value. The samples **2** and **7** contain in their structures functional groups capable of creating hydrogen bonds. The H-bonding interactions are stereoselective and an increase in their strength results in a stronger retention and improved enantioseparation in the separation system with the cellulose *tris*(3,5-dimethylphenylcarbamate) chiral selector and the mobile phase buffered to pH 3.0. As the strength of the hydrogen bonding is considerably influenced by the organic modifier present in the mobile phase, the differences are more pronounced in the mobile phases with low acetonitrile contents. Comparing the separation behavior of analytes **2** and **7** in identical mobile phases demonstrates that the bulky aromatic substituent of the amino group near the hydroxyl group in analyte **2** substantially reduces the strength of the stereoselective H-bonding. This result can be documented on higher retention versus lower resolution values of analyte **2** against analyte **7** (Table 2). Unfortunately, no partial enantioresolution was observed for samples **5**, **6** and **8**. These analytes have the most symmetrical structure of all the compounds studied, which is difficult to enantioresolve.

The replacement of water with the buffer solution only slightly affects the retention and enantioresolution of 8,3´-disubstituted 1,2´-binaphthyls. The retention factors and the high values of enantioresolution remain almost unchanged. The steric arrangement of this type of derivatives probably improves their fitting to the chiral cavities of cellulose *tris*(3,5-dimethylphenylcarbamate) and a substitution, especially in position 8, provides a supplementary stereoselective interaction.

Based on the results attained in the reversed-phased separation mode, the best mobile phase composition for analytes **1**, **2** and **7** was ACN/20 mM phosphate buffer,

pH 3.0, 40/60 (*v/v*). Compounds **3**, **9** and **10** yielded better results in ACN/water 40/60 (*v/v*).

The resolution values obtained for analytes **9** and **10** in the ACN/water mobile phase enable direct use of the method developed in a semi-preparative mode. As a compromise among the demands on the solubility, retention and resolution, the mobile phase composition ACN/water 60/40 (*v/v*) was selected for analytes **9** and **10**. Mobile phase composed of ACN/0.1% acetic acid, pH 3.3, 60/40 (*v/v*) was used for analyte **7**. Overload conditions were attained by using various injection volumes (10, 50 and 100 μL) at the same concentration of analytes **7**, **9** and **10**, i.e., 5.0 mg/mL. A sufficient enantioresolution was preserved up to the injection volume, 50 μL (see Fig. 4).

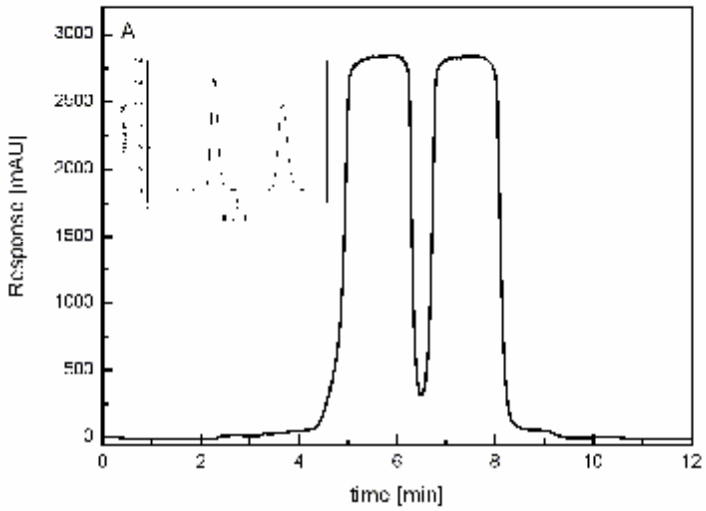

Fig. 4: Enantioseparation of sample **7** under semi-preparative conditions, Chiralcel OD-RH (150 x 4.6 mm I.D.), ACN/1%HAc, pH 3.3, 60/40 (*v/v*); flow rate 0.7mL/min, UV detection at 254 nm.

Injected sample amount, 250 μg (sample concentration, 5.0 mg/mL, sample volume, 50 μL).

Insert: The enantioseparation under analytical conditions - sample concentration 0.5 mg/mL, sample volume 10 μL.

Conclusions:

Cellulose *tris*(3,5-dimethylphenylcarbamate)-based CSPs have been found suitable for enantioseparation of most (7 from 10) of the studied binaphthyl derivatives. The separation system has also been proposed for semi-preparative application (for analytes **7**, **9** and **10**), which can be used for the preparation of these catalysts for asymmetric syntheses. The representatives of 8,3´-disubstituted 1,2´-binaphthyls (analytes **9** and **10**) have been separated with the highest values of enantioresolution in all the mobile phases tested. This result can be attributed to the proper fit of these derivatives into the helical structure of cellulose. In addition, π-π interactions of the aromatic parts of the analytes with the *tris*(3,5-dimethylphenylcarbamate) substituent on cellulose and hydrogen bonding or dipole-dipole interactions contribute to the enantiodiscrimination process. In the group of the 1,1´-binaphthyl derivatives, the best enantioseparation has been attained for analyte **7**, which offers functional groups required for H-bonding.

The comparison of the two separation modes studied (normal- and reversed-phase) shows that only analyte **4** from the family of 1,1´-binaphthyls and the two 8,3´-disubstituted 1,2´-binaphthyls can be baseline resolved in the normal phase separation mode, whereas the reversed-phase mode provides a more universal application.

3.3 Comparison of enantioseparation of substituted binaphthyls on cyclodextrin-, cellulose- and synthetic polymer-based chiral stationary phases

This work was focused on the comparison of the enantiospearation behavior of selected binaphthyls on three types of chiral stationary phases: (i) CSPs based on β-cyclodextrin and hydroxypropylether-β-cyclodextrin; (ii) CSPs based on cellulose *tris*(3,5-dimethylphenylcarbamate) and (iii) the last group of studied CSPs included three recently developed synthetic stationary phases – polymers based on *trans*-1,2-diaminocyclohexane, P-CAP, P-CAP-DP and DEABV (structures are depicted in Fig. 5) covalently bonded to silica gel support. These CSPs were prepared in the laboratory of Prof. Armstrong, The University of Texas at Arlington, USA. The measurements were carried out in normal and polar-orgnic separation modes.

The retention and separation behavior of the studied binaphthyl on the first two types of CSPs was described in the previous (chapter 3.1 and 3.2) works. Therefore, only the enantioseparation behavior of the studied compounds on polymer-based CSPs will be discussed in this part.

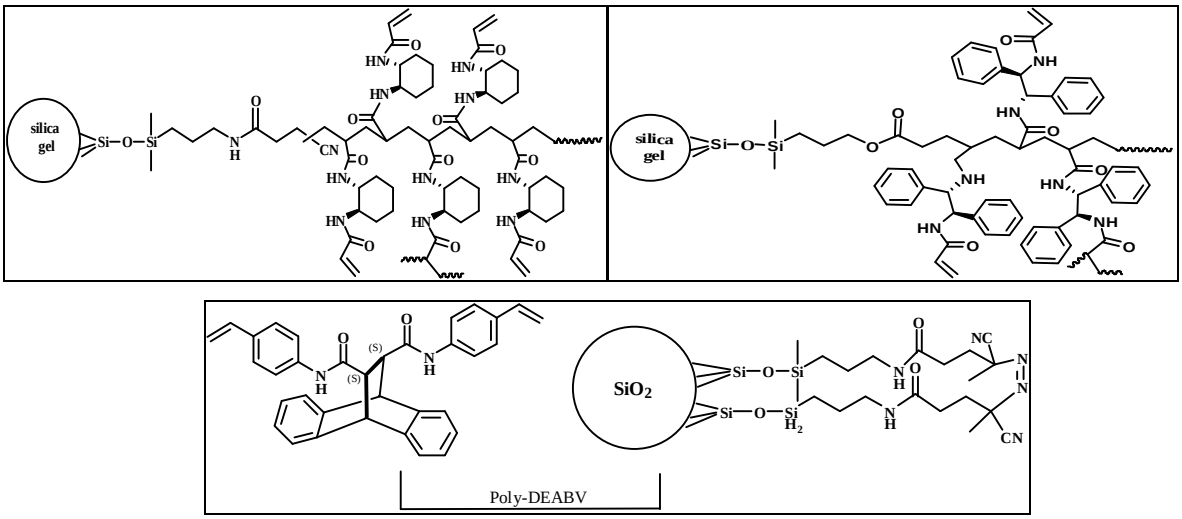

Fig. 5: Structures of polymer-based CSPs, left up – (*R,R*)-P-CAP, right up - (*R,R*)-P-CAP-DP, bottom – DEABV CSP.

N*ormal separation mode*

Mixtures of *n*-hexane or *n*-heptane and propan-2-ol or ethanol without or with addition of small amounts of acetic acid or trifluoroacetic acid were tested in NP. As could be expected, retention of the analytes increased with the increasing content of the nonpolar constituent in the mobile phase and the increase of retention was accompanied by improved enantioresolution. The most suitable *n*-hexane (*n*-heptane) to propan-2-ol (ethanol) ratio for P-CAP CSP was found 80/20 (*v/v*). Table 3 summarizes the results obtained by variation of this basic mobile phase composition. Addition of acetic acid or trifluoroacetic acid mostly resulted in drop of retention and resolution that was more pronounced with TFA.

Table 3: Retention, enantioresolution and enantioselectivity values obtained on P-CAP CSP for analytes that exhibited at least partial enantioseparation of their atropoisomers in NP.

		80/20 (*v/v*)			**80/20/0,1 (*v/v/v*)**		
Analyte		**HEX/IPA**	**HEX/EtOH**	**HEP/EtOH**	**HEP/EtOH/HAc**	**HEX/IPA/TFA**	**HEP/EtOH/TFA**
3	k_1	2.62	3.92	4.23	2.72	2.54	0.85
	R	0.91	0.85	0.75	0.64	1.06	0.00
	α	1.15	1.07	1.06	1.06	1.13	1.00
7	k_1	x	x	x	x	1.27	2.21
	R	x	x	x	x	1.62	0.69
	α	x	x	x	x	1.22	1.10
8	k_1	9.98	11.23	11.24	8.73	9.04	2.15
	R	3.43	5.02	4.22	4.52	3.66	2.93
	α	1.59	1.43	1.36	1.39	1.58	1.34
10	k_1	3.56	4.82	4.81	3.98	3.74	0.96
	R	1.97	2.28	2.28	1.93	1.96	1.00
	α	1.36	1.19	1.18	1.17	1.35	1.15

x – analyte did not elute within 120 minutes.

Separation of the derivatives with hydroxyl group in the position next to the single bond connecting the two naphthyl moieties – compounds **3**, **7**, **8**, **10** – shows the importance of the H-bonding interaction in the enantioselective recognition mechanism of the atropoisomers on P-CAP CSP. The importance of H-bonding is supported by the fact that atropoisomers of compound **9**, which has a very similar structure to compound

10 but misses the OH group, could not be, even partly, separated. The enantioseparation of analytes **7** and **8** is illustrated in Fig. 6.

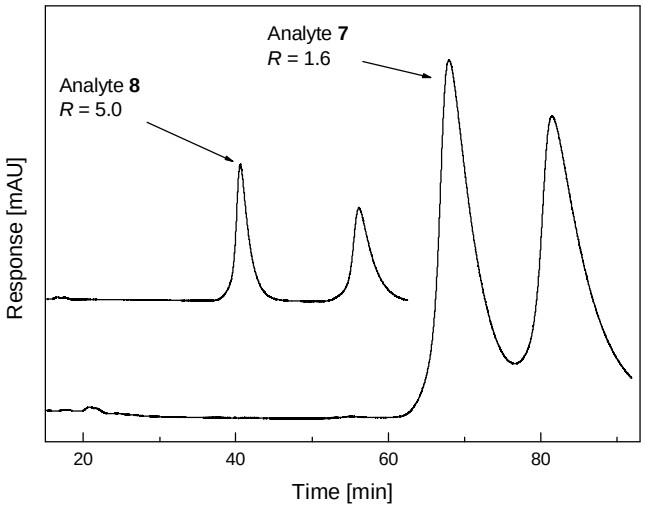

Fig. 6: Chromatograms of enantioseparation of analytes **7** and **8** on P-CAP (250 x 4.6 mm I.D.) CSP, mobile phase: for analyte **7** *n*-hexane/propane-2-ol/TFA 80/20/0.1 (*v/v/v*), for analyte **8** *n*-hexane/ethanol 80/20 (*v/v*); flow rate 0.7mL/min, UV detection at 254 nm.

On DEABV CSP higher retention than on the P-CAP column was observed in mobile phases of the same composition. Therefore, the optimized mobile phase composition (*n*-heptane/IPA 70/30 (*v/v*)) contained lower amount of *n*-heptane. The effect of addition of 0.1 % HAc to the mobile phase on chromatographic parameters of the studied compounds was minute. Partial enantioresolution of analytes **8** (R = 0.5), **9** (R = 1.3) and **10** (R = 1.0) was observed. Comparing the P-CAP and DEABV CSPs, better results – resolution, peak symmetry and separation efficiency values - were obtained on the P-CAP column.

The results obtained on column P-CAP-DP showed that this column is not suitable for enantioseparation of the binaphthyl derivatives in the normal phase mode.

Polar-organic separation mode

In polar-organic (PO) mode the mobile phases were composed of acetonitrile and small portions of methanol with HAc, TFA or triethylamine as aditives affecting dissociation/protonation of either analytes or functional groups of the chiral selectors. Retention increased with increased acetonitrile contents in the mobile phase on all these polymer-based columns. Addition of 0.1% HAc to the mobile phase resulted in decreased retention on P-CAP and P-CAP-DP CSPs while it did not much affect retention on DEABV column. Peak symmetry and separation efficiency were improved also in acidified mobile phases; this effect was less noticable on DEABV CSP. The results of some interest for enantioseparation of binaphthyls are summarized in Table 4. Five compounds were at least partly separated on P-CAP CSP. Analytes **2** and **7** (missing in this Table) were enantioresolved just in mobile phase consisted of ACN/MeOH 95/5 (*v/v*) + 10 mM HAc and TEA with the values of enantioresolution R = 1.20 for analyte **2** and R = 0.60 for analyte **7**. Three analytes were separated on P-CAP-DP and DEABV CSP. From all the tested CSPs only DEABV column provided at least partial separation of compound **5** (Fig. 7).

Table 4: Resolution and enantioselectivity values of 1,1´- or 1,2´-binaphthyls separated in polar-organic separation mode on the synthetic stationary phases P-CAP, P-CAP-DP and DEABV.

Stationary phase	**P-CAP**						**P-CAP-DP**				**DEABV**					
Analyte	**3**		**8**		**10**		**8**		**10**		**5**		**9**		**10**	
Mobile phase (*v/v*)	***R***	***α***	***R***	***α***	***R***	***α***	***R***	***α***	***R***	***α***	***R***	***α***	***R***	***α***	***R***	***α***
ACN/MeOH 95/5	0.54	1.07	3.64	1.37	1.64	1.18	0.00	1.00	1.47	1.28	0.60	1.08	1.33	1.17	1.40	1.16
ACN	0.75	1.11	3.82	1.54	1.29	1.19	0.78	1.13	2.31	1.37	0.61	1.09	1.40	1.17	1.35	1.17
ACN/MeOH/HAc 95/5/10 mM	0.57	1.07	3.86	1.37	1.88	1.19	0.49	1.06	1.53	1.27	0.58	1.08	1.27	1.16	1.37	1.16
ACN/HAc 100/10 mM	1.01	1.12	4.22	1.57	1.83	1.21	0.86	1.12	2.30	1.36	0.63	1.11	1.43	1.18	1.31	1.17

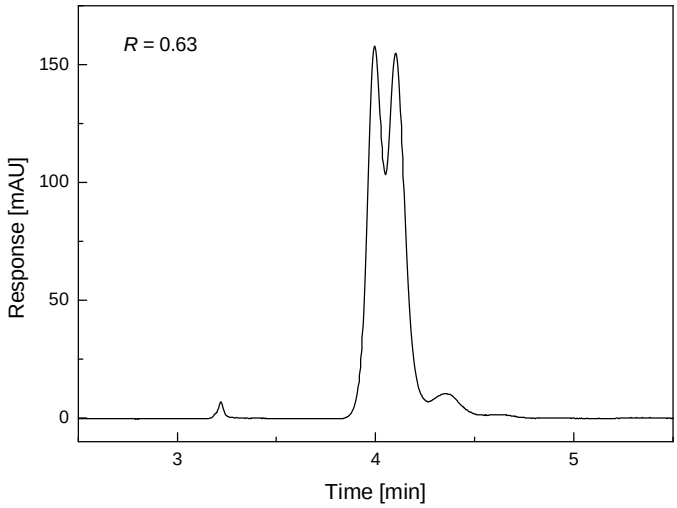

Fig. 7: Chromatogram of enantioseparation of analyte **5** in ACN/HAc 100/0.1 (*v/v*), DEABV (250 x 4.6 I.D.) column; flow rate 0.7mL/min, UV detection at 254 nm.

Conclusions:

At least partial enantioseparation of four analytes on P-CAP and three compounds on DEABV was observed in the NP mode. The best mobile phase composition was *n*-hexane/propane-2-ol/TFA 80/20/0.1 (*v/v/v*), where the highest values of enantioresolution were achieved on P-CAP CSP. All the separated analytes posses functional groups capable of H-bonding, which is important in the separation mechanism on P-CAP CSP. On DEABV CSP analytes **8**, **9** and **10** were resolved into atropisomers in MP consisted of *n*-heptane/propane-2-ol 70/30 (*v/v*). Comparing the two CSPs – P-CAP and DEABV – better results were obtained on P-CAP column.

The enantioseparation of seven compounds in PO mode was observed, five were enantioresolved on P-CAP and three compounds on P-CAP-DP and DEABV CSPs. Analyte **8** was separated with the highest enantioresolution on P-CAP CSP while the best results for analyte **10** were achieved on P-CAP-DP CSP. This derivative was as the only one separated on all three polymer-based CSPs. The enantioseparation of compounds **3** and **7** was obtained only on P-CAP CSP, analyte **2** was successfully separated on P-CAP and P-CAP-DP CSPs, and analytes **5** and **9** were enantioresolved on DEABV CSP. The complementarity of the three polymer-based stationary phases

was confirmed by the fact that on each of these CSPs enantioseparation of different binaphthyl derivatives was achieved.

4 CONCLUSION

Table 5 gives an overview of the separations of the atropoisomers of individual binaphthyl derivatives in various separation systems investigated in this work.

Table 5: Overview of the analytes separated on the CSPs studied in this work in NP, RP and PO separation modes (resolution values in parenthesis).

Separation mode **Chiral stationary phase**	**NP**	**RP**	**PO**
Cyclobond I 2000	no separation	**1** (0.5) **9** (0.9) **10** (2.2)	-
Cyclobond I 2000 RSP	no separation	**1** (0.7) **2** (1.0) **4** (0.2) **8** (0.8) **9** (1.1) **10** (1.9)	-
Derivatized cellulose	**1** (1.2) **4** (1.9) **9** (4.9) **10** (5.0)	**1** (1.8) **2** (0.7) **3** (3.2) **4** (1.2) **7** (6.9) **9** (6.3) **10** (5.9)	-
P-CAP	**3** (1.1) **7** (1.6) **8** (5.0) **10** (2.3)	-	**2** (1.1) **3** (1.0) **7** (0.6) **8** (4.2) **10** (1.9)
P-CAP-DP	-	-	**2** (0.6) **8** (0.9) **10** (2.3)
DEABV	**8** (0.6) **9** (1.3) **10** (1.0)	-	**5** (0.6) **9** (1.4) **10** (1.4)

- Enantioseparation behavior of analytes was not tested in the separation mode or the analytes did not elute within 120 minutes.

The RP separation mode has been found more advantageous for enantioresolution of the majority of analytes on cyclodextrin- and cellulose-based CSPs. Concerning the cyclodextrin-bonded CSPs better enantioseparation has been obtained on the HP-β-CD CSP with MeOH/water mobile phases containing low portion of the organic modifier. On *tris*(3,5-dimethylphenylcarbamate) cellulose-based CSP, lower content of acetonitrile (40 vol. %) in the ACN/water or ACN/phosphate buffer mobile phases provided sufficient retention of the binaphthyl derivatives to enable enantioresolution of the majority of these analytes. The best enantioseparation of 7 analytes from the total number of 10 on the cellulose-based column has been succeeded in the mobile phase composed of ACN/20 mM phosphate buffer, pH 3.0, 40/60 (*v/v*). Cellulose-based columns have been shown most suitable for enantioseparation of the studied analytes. Even semi-preparative separation mode could be employed with those CSP.

The recently introduced synthetic polymer-based CSPs have been suitable for enantioseparation of some of the binaphthyl derivatives in NP and PO separation modes. These CSPs were advantageous for enantioseparation of certain analytes that could not be successfully separated on cyclodextrin-bonded or cellulose-bonded CSPs.