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Evaluation of silica gel-based brush type chiral cation exchangers with (*S*)-N-(3,5-dinitrobenzoyl)tyrosine as chiral selector: attempt to interpret the discouraging results

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Abstract

The synthesis and evaluation of new chiral stationary phases (CSPs) immobilized on silica gel and based on N-3,5-dinitrobenzoyl-(*S*)-tyrosine [DNB-(*S*)-TyrOH] as chiral selector and to be used in HPLC with buffered aqueous mobile phases are described. The CSPs differ in the linkage of DNB-(*S*)-TyrOH to the silica gel and their end-capping treatment. The applicability of the acidic and π-acidic CSPs to resolve partially enantiomers of basic drugs such as bupivacaine involving a chiral cation-exchange mechanism is demonstrated. Possible limitations of this concept are discussed.

1. Introduction

Ion-exchange chromatography is a well established chromatographic method for the separation of ionic compounds according to their pK_a values and to isolate ionizable from non-ionizable molecules. All applications have in common the use of aqueous and buffered mobile phases, thus regulating the retention of the analytes. Many applications have been described, for example nucleotide and amino acid analysis and the determination of organic acids in wine and fruit juices and of alkaloids in plant extracts [1,2]. A special analytical challenge remains the separation of enantiomers of ionic species, although to date numerous papers have been published in the field of liquid chromatographic enantioseparation (Refs. [3 and 4] and references cited therein). The enantioseparation of biologically active agents is important as it is known that stereoisomers sometimes have different pharmacological profiles, side-effects, efficacy, toxicology, metabolism and excretion rates. Despite the fact that there is a huge number of polar, basic and acidic chiral compounds, only a few papers have dealt with chiral ion-exchange chromatography. Particularly alkaloids such as quinine and brucine or amino acid derivatives have been used as chiral and ionic selectors (SOs) immobilized on different support materials as chiral stationary phases (CSPs) [5–11].

Recently, protein-type CSPs, e.g., α_1-AGP or OVM, have also become popular with respect to enantioseparation, mainly owing to their broad applicability for ionic and also for non-ionic and/or polar analytes [12–14]. The pH of the aqueous mobile phase influences significantly the overall retention of the analytes and the enantioselectivity mainly by changing the tertiary structure of the protein-type selector, but also its and

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the analytes' degree of ionization. Hence it becomes evident that electrostatic interactions between the SO and charged analytes (selectands, SAs) can be one of the main SO–SA binding principles. The above-mentioned glycosylated proteins α_1-AGP and OVM have acidic isoelectric points, and therefore they are particularly attractive to basic components, with a similar behaviour to cation exchangers. To generalize chiral recognition in such chromatographic systems one have to consider additional intermolecular interactions coming into action; their description by simple models is complicated because of the great variety of binding sites within the protein molecule and its tertiary structure, leading to diverse "chiral cavities, niches, bays or clefts" formed by the unique sequence of (glycosylated) polypeptide chains and their conformations.

However, for simple-structured but ionizable selector molecules it should be easier to elucidate the chiral recognition mechanism more rationally. Recently, Lindner and co-workers [15,16] developed CSPs based on dihydroquinine and dihydroquinidine carbamates covalently immobilized on a silica gel surface and to be used under aqueous mobile phase conditions; these CSPs and selector molecules separate chiral acidic solutes (e.g., 3,5-dinitrobenzoylated and FMOC amino acids) via an anion exchange mechanism in conjunction with additional intermolecular SO–SA interactions.

The aim of this work was to develop new silica-type CSPs based on small amino acid derivatives, in particular DNB-(*S*)-TyrOH, and combining the binding principles of so-called "Pirkle-type" CSPs (involving charge-transfer interactions, hydrogen bonding, dipole–dipole stacking and steric interactions) with cation-exchange mechanisms (similar to those with protein-type CSPs). Thus, the new CSPs (see Fig. 1) should contain free carboxylic acid functionalities and should be operated under buffered aqueous mobile phase conditions. However, similar structured CSPs were presented in 1989 by Moriguchi and Pirkle [17] and were reviewed recently [18]. Despite the poor enantioselectivity of these CSPs, judging the retention and resolution properties of such "simple"-structured CSPs and comparing the results with previous data evaluated for the above-mentioned anion-exchange type CSPs might be a possibility to obtain a clearer insight into the retention and chiral recognition processes taking place.

2. Experimental

2.1. Apparatus

Liquid chromatography was performed with a modular liquid chromatograph (Merck–Hitachi, Darmstadt, Germany), equipped with an L-6200 intelligent pump, a Model AS-2000A autosampler with a 100-μl loop, an L-4250 UV–Vis detector, controlled via D-6000 chromatography data station software, HPLC Manager Vers. 2.09. Temperature was controlled with a column thermostat (B.O. Electronics, Austria). Standard operating conditions were detection at 254 nm and a column temperature of 20°C. A guard column (LiChroCART 4-4, LiChrospher 100, RP-18, 5 μm, from Merck was used in all instances.

Infrared spectra in the diffuse reflection mode were recorded on a Perkin-Elmer (Beaconsfield, UK) 1720-x Fourier transform infrared spectrometer.

2.2. Chemicals

Acetonitrile was of gradient grade. Other chemicals were of analytical-reagent quality, purchased from Merck. Water used for HPLC was obtained from a Milli-Q system (Millipore). pH was adjusted with either glacial acetic acid or aqueous ammonia. Mobile phases were filtered and degassed in an ultrasonic bath before use.

Racemic and optically pure drugs were obtained from different pharmaceutical companies.

2.3. Chiral stationary phases

CSP-III

CSP III (see Fig. 1) was prepared from the corresponding ester phase CSP-II. The reaction

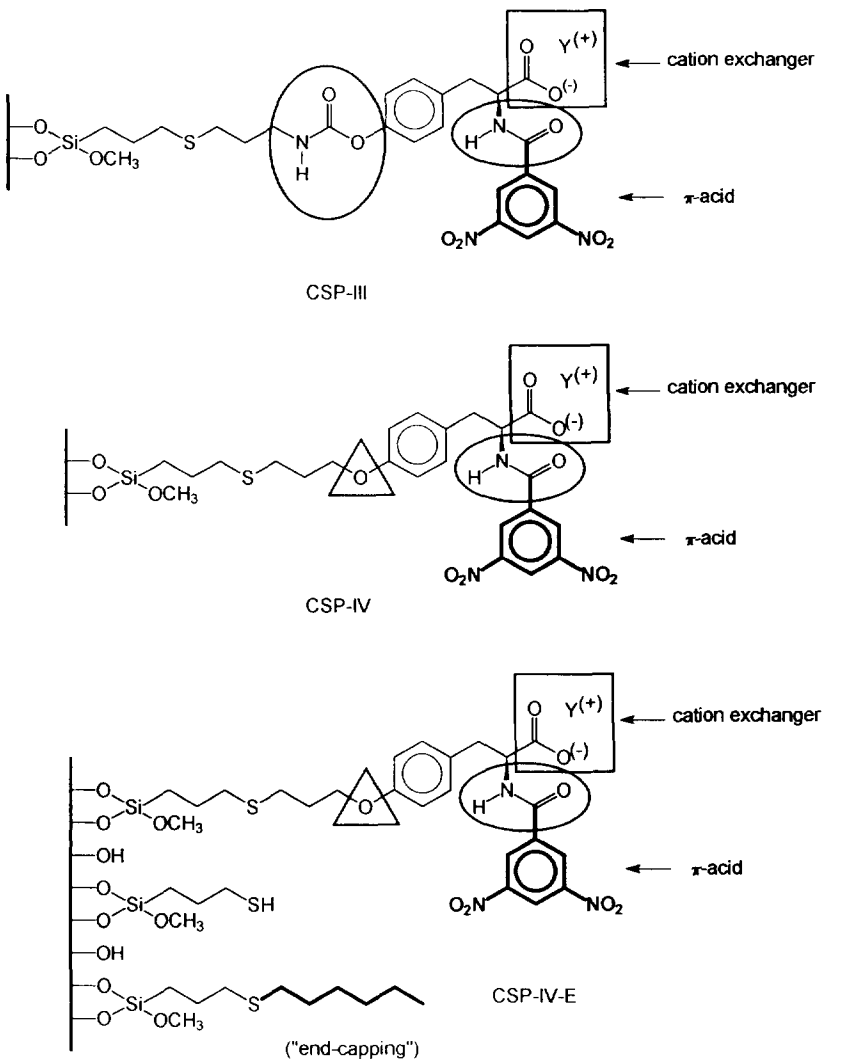

Fig. 1. Structures of CSP-III, CSP-IV and CSP-IV-E. To focus on potential binding sites, the charge-transfer interaction possibilities by the π-acid DNB residue are drawn with bold lines, the cation-exchange functionality is indicated by the square box and the amido groups, offering hydrogen bonding and dipole–dipole stacking properties, are emphasized by the ellipses. The triangle indicates the structural difference between CSP-IV and CSP-IV-E.

scheme for CSP-II, as described previously [19], is based on the following steps: (*S*)-TyrOMe and 3,5-dinitrobenzoylchloride (1.1 equiv.) were reacted in propene oxide (6 equiv.) and dioxane for 2.5 h at 40°C. The N-3,5-dinitrobenzoyl-(*S*)-tyrosine methyl ester [DNB-(*S*)-TyrOMe] formed was refluxed with allyl isocyanate (1.1 equiv.) and dibutyltin(IV) acid dilaurate (catalytic amount) in dioxane for 2 h. A 1.5-g amount of the resulting chiral selector and 2 g of 3-mercaptopropylsilica gel were refluxed with 100 mg of azobisisobutyronitrile (AIBN) in azeotropically dried chloroform for 40 h, resulting in a modified silica with a calculated coverage of the dry material of 216 μmol chiral SO/g silica gel based on C analysis. The final CSP material was obtained by stirring 6 g of modified silica gel CSP-II in 200 ml of methanol with aqueous $KHCO_3$–K_2CO_3 (10% w/v, pH 9) for 24 h at room temperature, followed by adjusting the pH

to 2.5 with hydrochloric acid (1 *M*). This modified silica gel (CSP) was sedimented and washed with methanol, methanol–water, methanol, acetone and finally light petroleum. After drying in air at 40°C, the slightly yellow silica was sieved (0.0315 mm) and slurry packed into a stainless-steel column (150 × 4.6 mm I.D.).

CSP-IV and CSP-IV-E

CSP-IV and CSP-IV-E (see Fig. 1) were similarly prepared from the previously described [19] corresponding ester phases CSP-Ia and CSP-IaE. In brief, CSP-Ia was synthesized by refluxing 6 g of 3-mercaptopropylsilica gel with 3,15 mmol (1.35 g) of N-(3,5-dinitrobenzoyl)-(*S*)-tyrosine-O-(2-propen-1-yl) methyl ester and 100 mg of AIBN in 200 ml of azeotropically dried chloroform for 40 h, resulting in a calculated coverage of the final dry material of 194 μmol chiral SO/g silica gel based on C analysis. Similarly, CSP-IaE (the "end-capped" version of CSP-Ia) was prepared by refluxing 4 g of CSP-Ia with 20 mmol (1.96 g) of 1-hexene and 0.66 g of AIBN in azeotropically dried chloroform for 35 h, resulting in a calculated coverage of 246 μmol hexyl residues/g silica gel based on C analysis. The methyl ester cleavage of the CSPs was performed by flushing the packed "ester" columns with 0.1 *M* $KHCO_3$–K_2CO_3 aqueous solution (pH 8.9) for 12 h at 20°C and at a flow-rate of 0.5 ml (see also Fig. 2).

The cleavage of the ester bond was monitored off-line by DRIFT spectroscopy. Further proof of the successful cleavage was the chromatographic behaviour and the strong dependence of the k' values on the ionic strength of the mobile

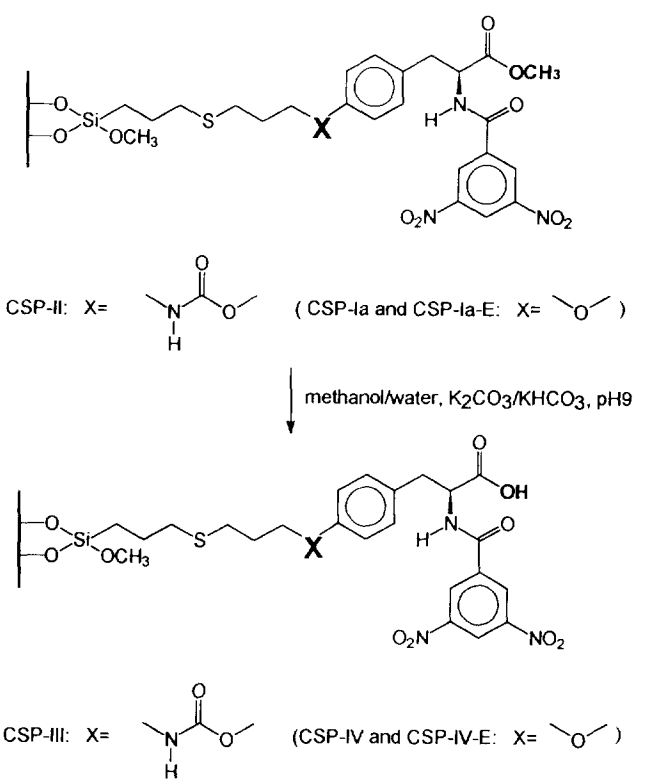

Fig. 2. Reaction scheme for preparing CSP-III, CSP-IV and CSP-IV-E from previously described CSP-II, CSP-I-a and CSP-I-aE [19].

phase, indicating an ion-exchange mechanism; the existence of free carboxylate functions on the final CSP material could safely be postulated.

3. Results and discussion

As mentioned above, the concept of chiral anion exchangers based on immobilized dihydroquinine and dihydroquinidine carbamate derivatives (having a tertiary amine group), developed by Lindner and co-workers [15,16], proved its usefulness in resolving very efficiently, for instance, the enantiomers of 3,5-dinitrobenzoylamino acids, with α-values between 1.5 and 5 and R_s values between 1.5 and 15. As indicated in Fig. 3, the chiral recognition mechanism of these cinchona alkaloid-type CSPs seems to be based mainly on interactions of charge-transfer character (the π-basic group, the quinoline ring, is indicated by bold lines, similar to the π-acid group, the DNB residue in the selector molecule), dipole–dipole binding and/or hydrogen bonding (indicated by ellipses) and ionic interaction of the basic centre of the SO (tertiary or quaternary amine, indicated by the arrow) with the acid function of the SA (carboxylic acid, indicated by the arrow) [15]. The mobile phase conditions were aqueous and pH controlled.

Adopting the principle of reciprocity and thus using chiral DNB-amino acid derivatives as CSPs, they should now express some stereoselectivity for basic analytes (amines) containing π-basic or electron-rich ring systems, and additional amide structure elements. Many drugs such as antidepressants, antihistaminics, anaesthetics and analgesics should, to a certain extent, meet such criteria. Pirkle et al. [20] introduced the concept of reciprocity first by immobilizing DNB-amino acids as chiral SOs on silica gel to resolve electron-rich compounds, but under normal-phase conditions and without involving ion-exchange interactions. To continue our work on the development of cation-exchange type CSPs, we immobilized 3,5-DNB-(*S*)-TyrOH having a free carboxylic function (see Fig. 1) to be access-

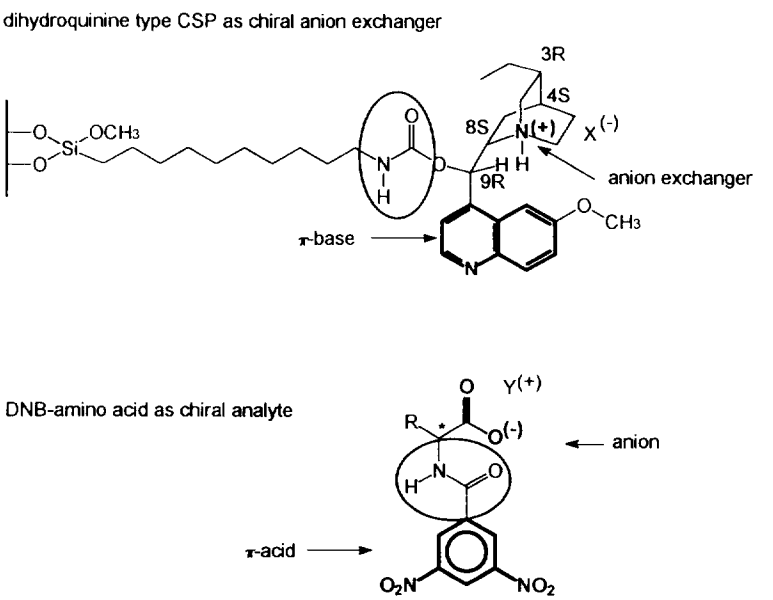

Fig. 3. Chiral anion exchanger based on dihydroquinine derivative [15,16]. The π-basic and basic centres of the stationary phase are emphasized with bold lines and arrows, whereas the amido group, offering hydrogen bonding and dipole–dipole stacking properties, is emphasized by the ellipse. DNB-α-amino acids, containing an π-acid, acidic and amido functional groups (indicated by bold lines, arrows and ellipses, respectively), were very well resolved on such stationary phases.

ible for electrostatic interactions with amines under aqueous buffered mobile phase conditions. A representative set of chiral analytes containing an amino function and π-basic aromatic ring systems was chosen (for structures see Fig. 4).

As indicated in Fig. 1, the chiral selectors of CSP-III, CSP-IV and CSP-IV-E consist of one or two amido groups (ellipses), accessible for hydrogen bonding and/or dipole–dipole interaction, a π-acid group and a free carboxylate function with ionic interaction possibilities. As deduced from previous experiments [15], for these SOs ideal reciprocal selectands (SAs) should be

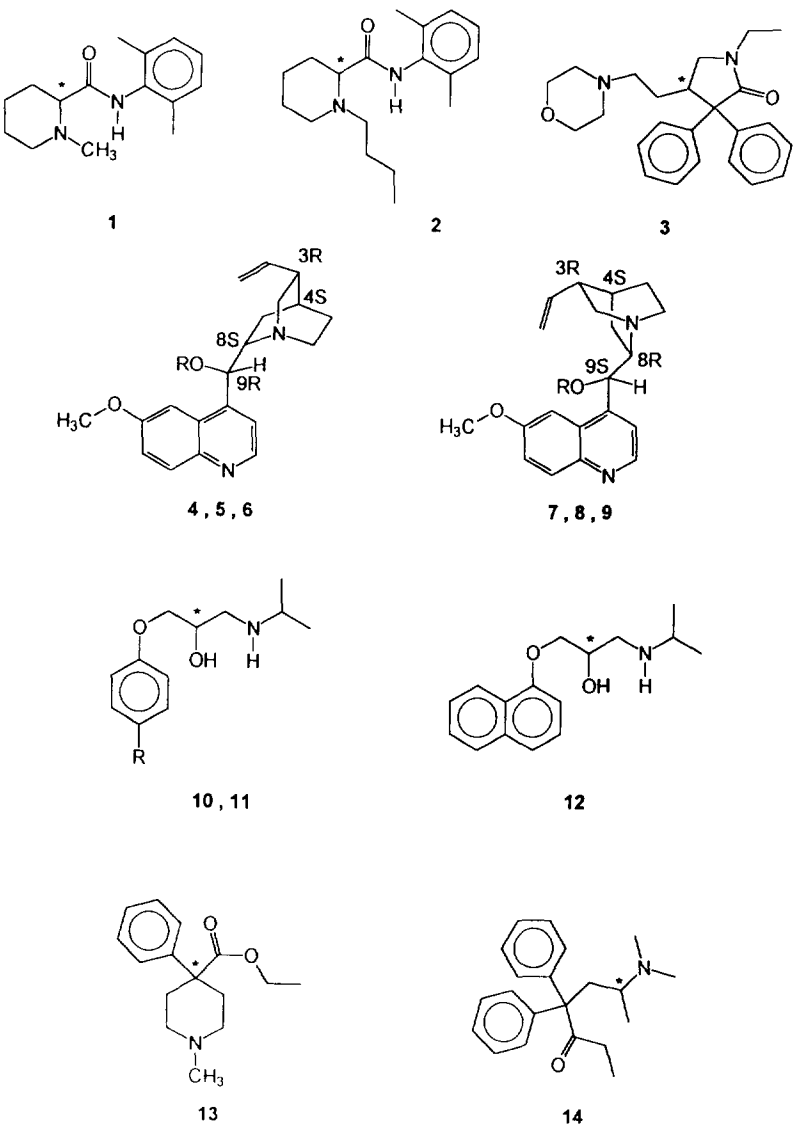

Fig. 4. Structures of some chiral drugs tested for evaluating the retention and enantioselectivity of CSP-III, CSP-IV and CSP-IV-E. **1** = Mepivacaine; **2** = bupivacaine; **3** = doxapram; **4** = quinine; **5** = 10,11-dihydroquinine; **6** = dihydroquinine allylcarbamate (R = CH_2=CH–); **7** = quinidine; **8** = 10,11-dihydroquinidine; **9** = dihydroquinidine allylcarbamate (R = CH_2=CH–); **10** = atenolol (R = H_2NCOCH_2–); **11** = metoprolol (R = $H_3COCH_2CH_2$–); **12** = propranolol; **13** = pethidine; **14** = methadone.

quinine or quinidine but as carbamate derivatives (e.g., allyl carbamates as listed in Table 2, similar to those depicted in Fig. 3).

The retention and ion-exchange properties of CSP-III were evaluated with different aqueous mobile phases. Because of the strong retention of the analytes when using methanol as organic modifier in the mobile phase (data not shown), throughout this work only acetonitrile was used as organic solvent owing to its stronger elution power; acetonitrile decreased considerably the overall retention, which is an indicator not only for a strong hydrophobic but also for strong $\pi-\pi$ type SO–SA interactions. With a mobile phase consisting of acetonitrile–40 m*M* ammonium acetate (60:40, v/v) and a pH of 7, the k' values for the test compounds were between 1.4 and 5 (see data summarized in Table 1). Under these conditions only a few analytes (quinine/quinidine and their dihydro derivatives, chloroquine and thioridazine) still showed very high capacity factors.

3.1. *Influence of buffer concentration*

As is well known in ion-exchange chromatography, the retention times of the solutes are strongly influenced by the buffer concentration and ionic strength and the pH of the mobile phase. This behaviour was observed also with CSP-III and CSP-IV, indicating that a real cation-exchange mechanism may take place as depicted in Fig. 5 for bupivacaine as a test solute (for chromatographic data see also Table 1). As can be seen from Fig. 6, the capacity factors were enhanced to about 140% on decreasing the buffer concentration from 80 to 40 m*M* whilst maintaining the acetonitrile content (60%, v/v) and the pH of the mobile phase (7) constant. A further decrease in the ionic strength to 8 m*M* (10% of the starting ionic strength) had an even greater effect on the k' values.

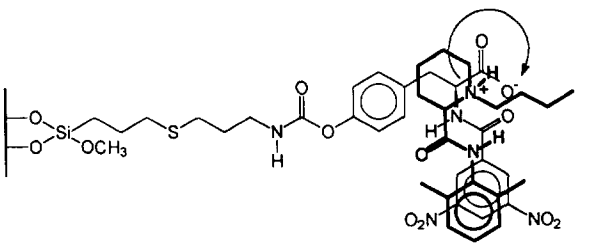

Fig. 5. Theoretical model of chiral recognition mechanism of bupivacaine on CSP-III. As can be seen from the superimposed SO and SA, a simultaneous interaction of the SO carboxylate and the SA ammonium ions (Coulombic attraction), the two amido groups (hydrogen bonding and/or dipole–dipole stacking) and the SO π-acid DNB residue and the SA π-basic dimethylphenyl residue (charge transfer) may take place, thus forming diastereomeric molecular associates with different free energy values.

Table 1
Capacity factors (k'_1) of representative chiral analytes on CSP-III

Analyte	Mobile phase A			Mobile phase B		
	80 m*M*	40 m*M*	8 m*M*	pH 6	pH 7	pH 7.9
(*R*,*S*)-Mepivacaine (**1**)	1.66	2.81	–	5.90	5.40	2.62
(*R*,*S*)-Bupivacaine (**2**)	2.27	3.01	10.16	6.30	5.99	2.82
(*R*,*S*)-Doxapram (**3**)	–	1.60	4.01	5.07	2.82	1.22
Quinine (**4**)/quinidine (**7**)	–	13.22	–	12.88	30.10	–
Dihydroquinine (**5**)/dihydroquinidine (**8**)	–	14.59	–	13.93	26.38	–
(*R*,*S*)-Atenolol (**10**)	0.82	1.39	5.18	4.13	5.87	6.31
(*R*,*S*)-Metoprolol (**11**)	1.46	2.24	8.47	4.92	7.21	8.33
(*R*,*S*)-Propranolol (**12**)	3.84	5.57	22.61	–	–	–
(*R*,*S*)-Pethidine (**13**)	2.20	3.15	11.21	6.18	8.64	7.70
(*R*,*S*)-Methadone (**14**)	3.44	4.45	17.23	7.13	10.14	10.57

Mobile phase: A = acetonitrile–ammonium acetate buffer (60:40, v/v), pH 7; B = acetonitrile–ammonium acetate buffer (75:25, v/v), 8 m*M*; flow-rate, 0.9 ml/min; temperature, 30°C; detection, UV at 230 nm. Capacity factor $k' = (t_{r_1} - t_0)/t_0$.

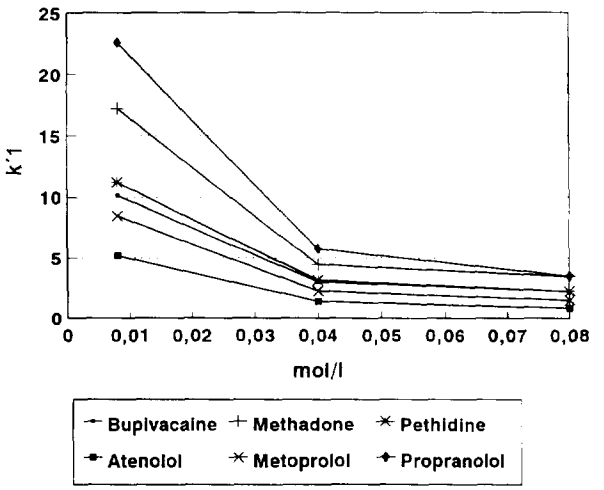

Fig. 6. k'_1-Values as a function of ionic strength. Stationary phase, CSP-III; mobile phase, acetonitrile–ammonium acetate (60:40, v/v), pH 7; flow-rate, 0.9 ml/min; detection, UV at 230 nm; temperature, 30°C.

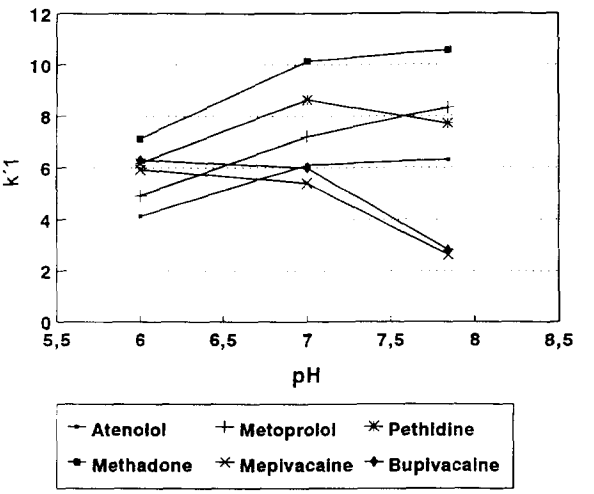

Fig. 7. k'_1-Values as function of pH. Stationary phase CSP-III; mobile phase, acetonitrile–ammonium acetate (75:25, v/v), 8 m*M*; flow-rate, 0.9 ml/min; detection, UV at 230 nm; temperature, 30°C.

3.2. *Influence of pH*

The variation of the mobile phase pH will influence the dissociation of the immobilized acidic chiral SO and the basic analytes according to their dissociation constants. The pK_a value of the carboxyl group of the chiral SO DNB-(*S*)-TyrOH was measured titrimetrically and found to be surprisingly high, namely between 6.5 and 7, depending on the composition of the aqueous–organic (methanol) titration medium (similar to mobile phase medium); therefore, these CSPs should be weak ion exchangers which are not fully dissociated within the pH range 5.5–7.5. To enhance the ionic interactions of the acidic SO with the basic SAs, a fairly high mobile phase pH should be appropriate, but at this point one must also consider that relatively weak basic solutes will not be fully protonated at high pH values. To summarize , the ionic interactions expressed as retention data are the result of the degrees of dissociation of the SO and the SAs and the applicable pH window of the mobile phase is rather limited in the present instance. Additional SO–SA interactions may become dominant. Subsequently, the influence of mobile phase pH on the capacity factors of the solutes was found to be inconsistent, as is shown in Fig. 7. For the enantiomers of mepivacaine and bupivacaine, possessing pK_a values of 7.7 and 8.1, respectively, the k'-value decreased on increasing the pH from 6 to 7.8; for pethidine ($pK_a = 8.7$) the k' value showed a maximum at pH 7 and for atenolol ($pK_a = 9.6$), metoprolol ($pK_a = 9.7$) and methadone ($pK_a = 8.3$) the k' values increased considerably. Comparing the dissociation constants of the analytes and their retention behaviour it becomes obvious that on increasing the pH the dissociation of mepivacaine and bupivacaine was decreased, resulting in weaker ionic interactions with the CSP and subsequently reduced retention times. Relatively strong bases such as atenolol and metoprolol remain protonated at higher pH values, and therefore they showed higher capacity factors owing to enhanced ionic interactions with the carboxylate function of the CSP. However, the retention behaviour of methadone (also a strong base) cannot be fully explained.

3.3. *Chiral recognition*

The overall enantioselectivity of the DNB-(*S*)-TyrOH-based CSP-III, CSP-IV and CSP-IV-E

Table 2
Enantioselectivity of CSP-III, CSP-IV and CSP-IV-E

Solute	CSP-III		CSP-IV		CSP-IV-E	
	k'_1	α	k'_1	α	k'_1	α
Mepivacaine (**1**)	5.93	1.01	6.07[+]	1.00[+]	4.02[+]	1.00[+]
Bupivacaine (**2**)	6.30	1.03	5.40	1.03	3.29	1.00
Doxapram (**3**)	5.07	1.04	3.38	1.04	2.27	1.01
Dihydroquinine (**5**)/dihydroquinidine (**8**)	11.50[a]	1.12[a]	20.07[b]	1.03[b]	10.38	1.02
5/**8** as their allylcarbamates (**6**/**9**)			5.99[b]	1.26[b]		

Mobile phase: acetonitrile–ammonium acetate (75:2, v/v), 8 m*M*, pH 6; flow-rate, 0.9 ml/min; temperature, 30°C; detection, UV at 230 nm. Capacity factor $k' = (t_{r_1} - t_0)/t_0$; selectivity $\alpha = k'_2/k'_1$.
[a] Mobile phase: acetonitrile–ammonium acetate (40:60, v/v), 120 m*M*, pH 7.
[b] Mobile phase: acetonitrile–ammonium acetate (75:25, v/v), 4 m*M*, pH 6.

was small (see Table 2). Of the various solutes tested, only the pseudo-enantiomeric analytes such as quinine/quinidine and dihydroquinine/dihydroquinidine, in addition to bupivacaine, mepivacaine and doxapram, could be partially resolved into their enantiomers. This is in great contrast to the dihydroquinine and dihydroquinidine carbamate-based CSPs, offering α-values up to 5 for DNB-α-amino acids. The quinine/quinidine and dihydroquinine/dihydroquinidine pseudo-enantiomers were resolved on the DNB-(*S*)-TyrOH-derived CSPs with α-values of only 1.03, whereas the respective carbamates, offering an additional amide function for intermolecular interaction, showed higher enantioselectivity ($\alpha = 1.26$, $R_s = 2.56$; see also Table 2). This observation is in accordance with previous statements [15,16].

Mepivacaine (**1**), bupivacaine (**2**) and doxapram (**3**) possess similar structural elements, as can be seen in Fig. 4. The basic nitrogen and the chiral centre are sterically fixed in a ring system, and additionally a carbonyl function of an amide is in close vicinity to the stereogenic centre. However, the analgesic drug pethidine, with a similar structure, was not resolvable. By comparing bupivacaine and mepivacaine, the influence of the substituent of the basic nitrogen can be clearly pointed out: the butyl residue caused higher enantioselectivity than the methyl group. Whether this behaviour is based on the steric hindrance of the residue or the enhanced basicity of the nitrogen due to the stronger inductive effect of the butyl residue is not clear.

For bupivacaine, a simplified chiral recognition model based on Coulombic attraction in combination with hydrogen bonding and/or dipole–dipole stacking and/or π–π interactions is proposed (see Fig. 5). However, the chromatographic experiments showed poor enantioseparations and therefore this chiral recognition mechanism may not be the dominant one. Charge-transfer interactions seem mainly responsible for retention of the solutes (high capacity factors for solutes containing π-basic ring systems), but concerning the chiral recognition they seem to be too dominant in comparison with the additional interactions necessary for pronounced enantioselectivity. This could also be supported by the poor resolution of quinine and quinidine lacking the dipole–dipole-type interaction possibilities, as is the case for the respective allyl carbamates.

Two types of chemical linking of the chiral SO to the silica surface, thus introducing sensitive or non-sensitive groups with respect to the overall enantioselectivity and retention characteristics, were studied (see also Fig. 1). In previous work with the corresponding methyl ester phases CSP-II and CSP-Ia applying normal-phase conditions [19], only a very small effect of the amido versus the ether linkage was noticed. Under aqueous mobile phase conditions CSP-III and CSP-IV, as presented in this paper, showed similar behaviour, despite the greater differences in the over-

all capacity factors. Assuming comparable surface coverages of CSP-III and CSP-IV, the stronger retention characteristic of CSP-III is probably based on the additional "strong" interactions of the amido group (see Fig. 1) with the solutes containing several functional groups. However, these additional dipole–dipole and/or hydrogen-bonding interactions are unspecific in terms of chiral recognition, mainly owing to the large distance to the centre of chirality.

In contrast to the earlier described DNB-(*S*)-TyrOMe phases [19], the end-capping procedure did not influence the applicability of the DNB-(*S*)-TyrOH phases in a positive way. Similarly to the other phases, end-capping reduces the overall retention of the analytes on the stationary phase, but also the enantioselectivity (see Table 2). The remaining acidic silanol groups of the support material seem to have an attractive and orientating influence for the chiral amine-type solutes (SAs) and participate in the retention and chiral recognition mechanism of basic analytes, resulting in enhanced enantioselectivity. Because of the weak acidic group of the chiral SO, the acidic silanols may enhance the overall enantioselectivity in this special case, but in general a stronger acidic chiral selector and reduced interactions of the solutes with the remaining acidic groups of the silica gel should give better results and should be easier to interpret. The close pK_a values of silanols and the chiral SO result in competing attractive forces for basic guest molecules and therefore hardly explainable chromatographic data may result.

Based on the experimental data, the following assumptions concerning retention and enantioselectivity may be drawn:

(i) The retention of the basic analytes was strongly influenced by the mobile phase pH and ionic strength, and therefore ion-exchange mechanisms are clearly involved. Owing to the unexpected high pK_a value of the acidic DNB-amino acid-type SO, ionic interactions with weak bases are relatively low.

(ii) Competing attractive forces of the acidic silanols and the carboxylate function of the SO to the amine-type SA may on the one hand enhance the resolution of enantiomers, but on the other hand may also destroy significantly the spatial arrangement of the SO and SAs. Unspecific silanol interactions generally lead to broad peaks of basic compounds at higher pH of the mobile phase.

(iii) In contrast to the earlier mentioned chiral anion exchangers based on dihydroquinine and dihydroquinidine carbamate-type CSPs, of which the SOs consist of several chiral centres close to the binding domain, including the chiral nitrogen atom, and which are responsible for a relatively rigid conformation of the SO moiety, the new, simply structured DNB-(*S*)-TyrOH-type cation exchanger seems conformationally less rigid and offers less binding features. The SO and SA interactions are not as stereoselective as expected and the reciprocity concept failed to some extent.

(iv) $\pi-\pi$ Interactions seem mainly responsible for retention, but their portion in spatial arrangements seems to be poor.

4. Conclusions

Two new chiral cation-exchange stationary phases based on DNB-(*S*)-TyrOH have been synthesized and their applicability using buffered aqueous mobile phases and chiral amines as analytes was investigated. Based on the strong influence of the mobile phase pH and ionic strength, a cation-exchange mechanism could be established but competing non-stereoselective silanol effects seem to play a significant role. The CSPs described showed some enantioselectivity towards chiral drugs with a basic nitrogen atom, an amido structural element and additional π-basic ring systems. The applicability of these new chiral cation-exchange phases was also limited, however, owing to an unexpected high dissociation constant of the chiral selector DNB-(*S*)-TyrOH and problems with the inherent silanol amine binding. It could therefore be concluded that better designed chiral selector molecules combined with a more effective shielding and end-capping of silanol groups may have some

potential also in the field of cation-exchange type CSPs based on modified silica gel.

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