Journal of Chromatography A, xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Combinatorial effects of the configuration of the cationic and the anionic chiral subunits of four zwitterionic chiral stationary phases leading to reversal of elution order of cyclic β^3-amino acid enantiomers as ampholytic model compounds

Nóra Grecsó[a,b], Enikő Forró[b], Ferenc Fülöp[b], Antal Péter[a], István Ilisz[a,*], Wolfgang Lindner[c,*]

[a] Department of Inorganic and Analytical Chemistry, University of Szeged, H-6720 Szeged, Dóm tér 7, Hungary
[b] Institute of Pharmaceutical Chemistry, University of Szeged, H-6720 Szeged, Eötvös u. 6, Hungary
[c] Department of Analytical Chemistry, University of Vienna, Währingerstrasse 38, 1090 Vienna, Austria

ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form 4 May 2016
Accepted 11 May 2016
Available online xxx

Keywords:
Enantiomer separation
Zwitterionic chiral stationary phases
N_α-methyl-protected cyclic β^3-amino acids
Temperature effect

ABSTRACT

In a systematic way enantioseparations of non-methylated and the corresponding *N*-monomethylated ampholytic cyclic β^3-amino acids were carried out on four zwitterionic chiral stationary phases (CSPs; ZWIX(+)™, ZWIX(−)™, ZWIX(+A), ZWIX(-A)). CSPs were based on the combinations of quinine and quinidine as the cationic and of (*R,R*)- and (*S,S*)-aminocyclohexane sulfonic acid as the anionic sites. In polar-ionic mobile phase systems, the effects of the composition of the bulk solvents, the additives, the concentration of the co- and counter-ions, the temperature, and the structures of the ampholytic analytes were investigated. The changes in standard enthalpy, $\Delta(\Delta H^\circ)$, entropy, $\Delta(\Delta S^\circ)$, and free energy, $\Delta(\Delta G^\circ)$, were calculated from the linear van't Hoff plots derived from the ln α *vs 1/T* curves in the studied temperature range (5–40 °C). Unusual temperature behavior was observed on the ZWIX(−)™ column: decreased retention times were accompanied by increased separation factors with increasing temperature, and separation was entropically-driven. For the other three CSPs, enthalpically-driven enantioseparations were observed. *Via* the consequent determination of the elution order of the resolved enantiomers, the effects of the absolute configuration of the chiral anionic and cationic subunits of the zwitterionic CSPs could be elucidated. *N*-methylation of the amino acids led unexpectedly to a reversal of the elution sequence, which can be interpreted by a subtle shift of the hierarchical order of the sterically most important driving interaction sites from the cationic to the anionic units, and vice versa.

1. Introduction

Conceptually, this paper raises two principal questions: (*i*) What will be the effects of a systematic variation of the positively and negatively charged chiral interaction sites within zwitterionic selectors (SOs) and the related chiral stationary phases (CSPs)?; and (*ii*) What effect will the *N*-monomethylation of the amino group of cyclic β^3-amino acids (AAs) have regarding the retention and elution sequence of the resolved enantiomers?

As generally accepted, the spatial structure of a chiral compound described by the absolute configuration of the chiral centers and the conformational flexibility of the entire SO acting as chiral host determines the extent of molecular interactions and molecular recognition of the analytes (selectands, SAs). In a simplified way, this concept relates to the difference of Gibbs free binding energy $\Delta(\Delta G^\circ)$ of the [SO-(*R*)-SA] and the [SO-(*S*)-SA] associates that in chromatographic terms, leads to a stereoselectivity factor, α. In combination with the efficiency of a "chiral column," it relates to the resolution value of the enantiomers. A chiral SO moiety can in principle be divided into structural subunits, which may participate more or less strongly but simultaneously in the overall intermolecular interaction process with the chiral SAs. In the case of ionizable subunits, cationic and anionic sites in the SO can each have independent (*R*) and (*S*) configurations. For zwitterionic SOs, it is assumed that these two sites interact *via* a simultaneously occurring double ion pairing event with the chiral zwitterionic (ampholytic) SAs, whereby the spatial environment of all the ionized sites will have a

* Corresponding authors.
E-mail addresses: ilisz@chem.u-szeged.hu (I. Ilisz), wolfgang.lindner@univie.ac.at (W. Lindner).

http://dx.doi.org/10.1016/j.chroma.2016.05.041
0021-9673/

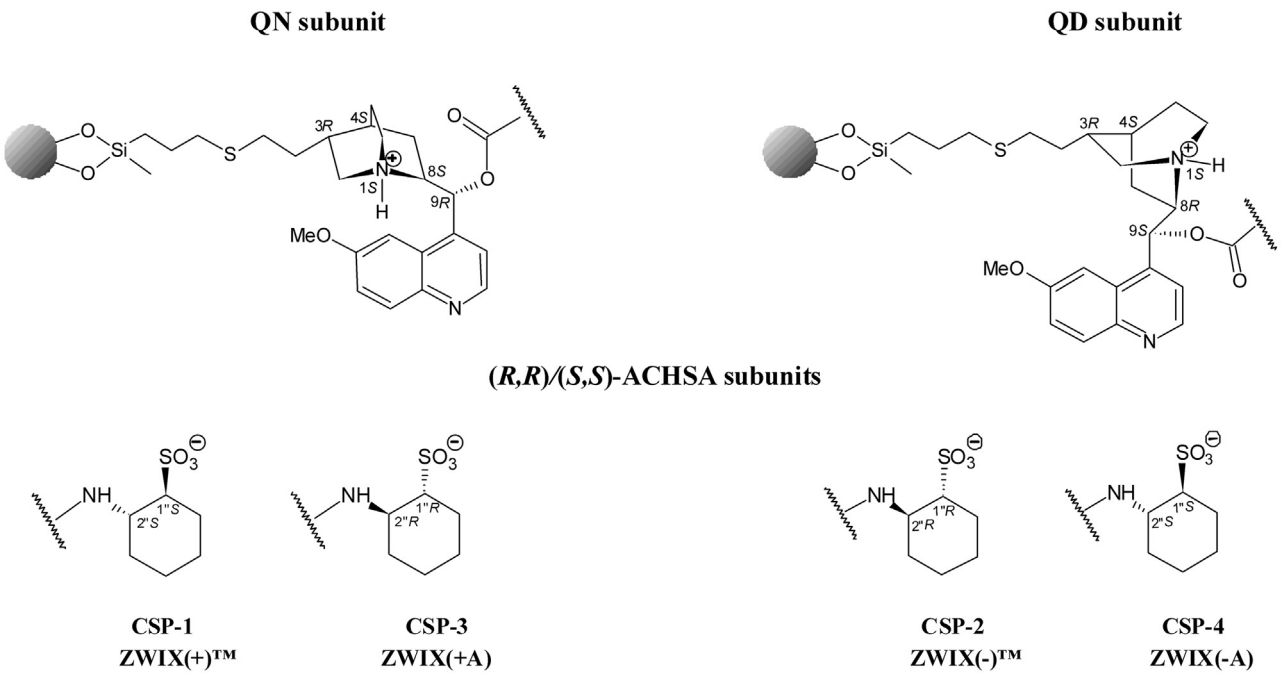

Fig. 1. Structure of the four combinatorically composed zwitterionic CSPs based on QN or QD and on (*R,R*)- and (*S,S*)-ACHSA.

directive effect, and thus determine the resulting elution sequence of the resolved enantiomers.

On the basis of previous works [1,2], we used four ZWIX type CSPs, which differ only in the chirality of the subunits (depicted in Fig. 1), for the resolution of the cyclic β^3-AA derivatives and to probe the initially raised questions.

Due to their pharmacological effects, carbocyclic β-AAs have gained great interest among synthetic and medicinal chemists in the past two decades, and they have become a "hot" topic in organic and bioorganic chemistry. A considerable number of syntheses of carbocyclic β-AA derivatives in both racemic and enantiopure form have been reported in recent years, and most of them have been reviewed [1,3]. Controlling the enantiomeric purity (*e.g.* under peptide synthesis) requires reliable and accurate analytical methods to be at hand. At an analytical level, high-performance liquid chromatography (HPLC) is undoubtedly the most important method of choice today. HPLC enantioseparations of ß-AAs have been performed by both indirect and direct methods and in the past decade new types of chiral derivatizing agents and CSPs have been applied for this purpose and were reviewed in numerous papers [4–11]. Several papers deal with the enantioseparation of *N*-acyl and *N*-aryl-protected AA derivatives; however, only few reports can be found for the enantioresolution of *N*-methylated variants. Enantioseparation of *N*-methyl-α-AAs by ligand-exchange HPLC was reported by Brückner [12]. *N*-methylleucine, *N*-methylisoleucine, *N*-methyl-phenylalanine and *N*-methylglutamate and aspartate was resolved by the application of chiral derivatizing agents by Hess et al. [13] and Tsesarskaia et al. [14]. Enantiomers of *N*-methylleucine and its 2,4-dinitrophenyl- and 3,5-dinitrobenzoyl-derivatives were separated on *Cinchona* alkaloid-based CSPs [15,16]. Piette et al. reported enantioseparation of 3,5-dinitrobenzoyl-*N*-methylleucine by non-aqueous capillary electrophoresis using 1,3-phenylene-bis(carbamoylated quinine) as a selector [17].

Chromatographic resolution can be tuned by variation of mobile phase composition, mobile phase additives, and especially in chiral chromatography, temperature. In most cases, the achiral and chiral (stereoselective) interactions are sensitive to temperature [18–21]. Therefore, during chiral separations, the column temperature often has been optimized and kept well controlled.

The temperature dependence of chromatographic retention can be expressed by the van't Hoff equation. The difference in the change in standard enthalpy $\Delta(\Delta H^\circ)$ and entropy $\Delta(\Delta S^\circ)$ for enantiomers can be expressed as [20]:

$$\ln\alpha = -\frac{\Delta(\Delta H^o)}{RT} + \frac{\Delta(\Delta S^o)}{R}$$

where R is the universal gas constant, T is temperature in Kelvin, and α is the selectivity factor. If a linear van't Hoff plot is obtained, a plot of $R \ln \alpha$ versus $1/T$ has a slope of $-\Delta(\Delta H^\circ)$ and an intercept of $\Delta(\Delta S^\circ)$. The van't Hoff plots interpreted this way yield apparent changes in standard enthalpy and entropy values, in which the respective contributions of the chiral and achiral interactions are combined. A more sophisticated approach should be based on the differentiation of stereoselective and non-selective sites [21].

In this work, we present the enantioseparation of non-methylated and *N*α-monomethylated cyclic ß^3-AAs (Fig. 2) on the quinine (QN) or quinidine (QD) and aminocyclohexane sulfonic acid [(*R,R*)- and (*S,S*)-ACHSA] based and chemically fused zwitterionic CSPs. Focus was given to the effect of *N*-methylation in comparison to the non-methylated congeners, as well as in the mobile phase composition, temperature, and the structure of the variants of chiral selectors on chromatographic performance, including enantioselectivity and elution order. The effects of the different selectors and the *N*α-methylation of the cyclic ß^3-AAs on the separation performances were studied in detail with the aim to evaluate the driving influences of the configuration of the chiral ionizable subunits of the combinatorically composed zwitterionic selector units.

Please cite this article in press as: N. Grecsó, et al., Combinatorial effects of the configuration of the cationic and the anionic chiral subunits of four zwitterionic chiral stationary phases leading to reversal of elution order of cyclic **β^3**-amino acid enantiomers as ampholytic model compounds, J. Chromatogr. A (2016), http://dx.doi.org/10.1016/j.chroma.2016.05.041

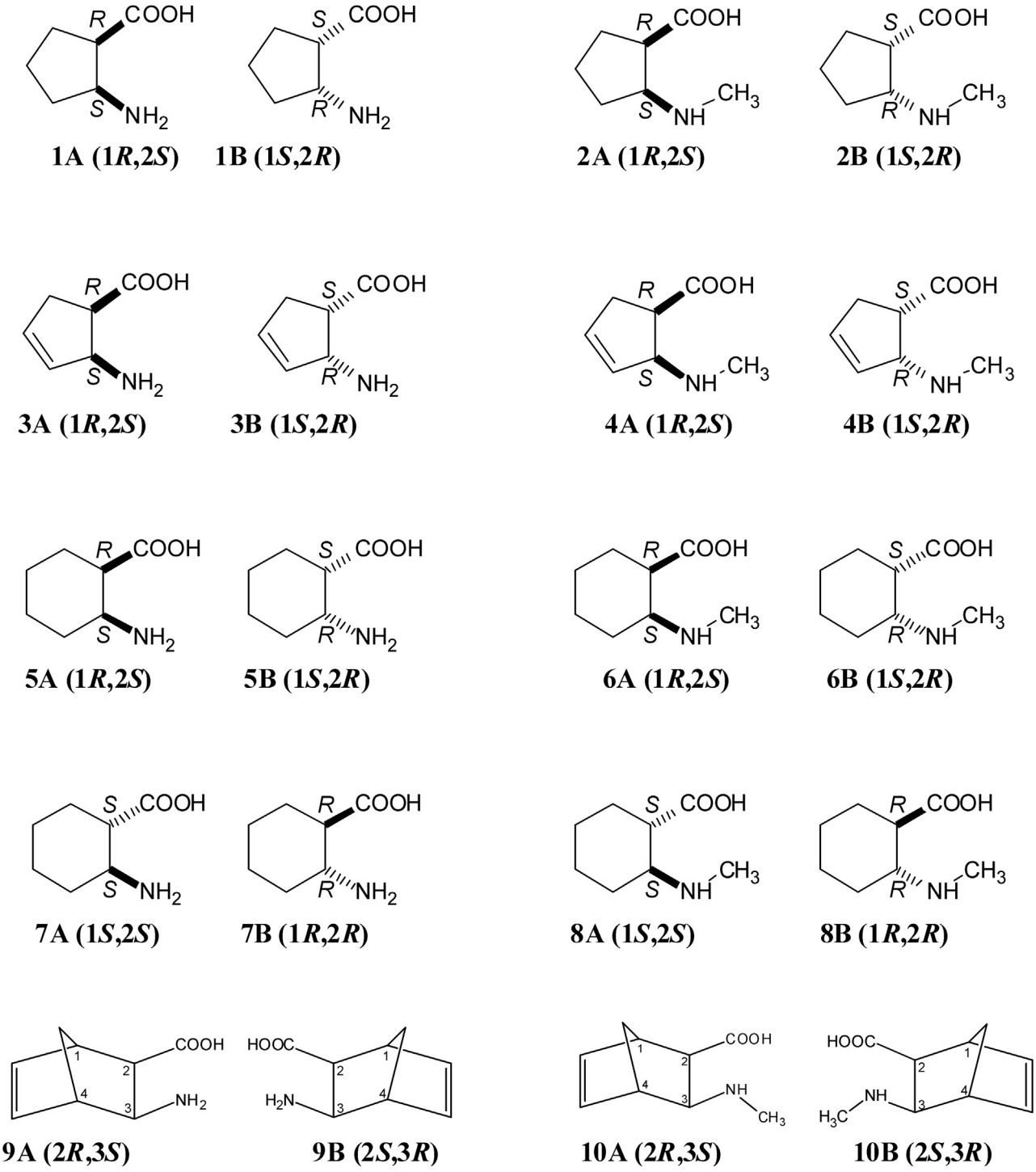

Fig. 2. Chemical structures of ampholytic cyclic β^3-amino acids.

2. Experimental

2.1. Chemicals and reagents

The *N*-methylated carbocyclic β-AAs were prepared through the general methodology described by Arvidsson et al.[22], from the corresponding Fmoc-AAs with paraformaldehyde, *via* oxazinanone intermediates, followed by triethylsilane reduction, under microwave heating.

HPLC-grade MeOH, MeCN and THF, as well as diethylamine (DEA) and acetic acid (AcOH) modifiers were obtained from VWR International (Radnor, PA, USA). Purified water was obtained from the Ultrapure Water System, Puranity TU UV/UF (VWR International).

2.2. Apparatus and chromatography

HPLC separations were performed on: A Waters Breeze system consisting of a 1525 binary pump, a 487 dual-channel absorbance detector, a 717 plus autosampler, Empower 2 data manager software (Waters Chromatography, Milford, MA, USA), and a Lauda Alpha RA8 thermostat (Lauda Dr. R. Wobser Gmbh,

Please cite this article in press as: N. Grecsó, et al., Combinatorial effects of the configuration of the cationic and the anionic chiral subunits of four zwitterionic chiral stationary phases leading to reversal of elution order of cyclic β^3-amino acid enantiomers as ampholytic model compounds, J. Chromatogr. A (2016), http://dx.doi.org/10.1016/j.chroma.2016.05.041

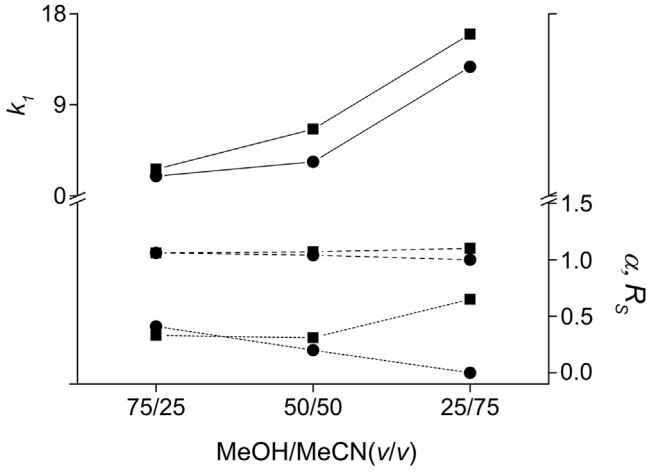

Fig. 3. Effects of the compositions of the bulk solvents on the chromatographic parameters for **1** and **3** on CSP-2.
Chromatographic conditions: column, CSP-2; mobile phase, MeOH/MeCN (75/25, 50/50 or 25/75 *v/v*) containing 25 mM DEA and 50 mM AcOH; flow rate, 0.6 ml min^{-1}; detection, 215 and 230 nm; temperature 25 °C; symbols, for k_1, α and R_S values for **1**, ■, for **3**, ●.

Lauda-Königshofen, Germany); or on a 1100 Series HPLC system from Agilent Technologies (Waldbronn, Germany), consisting of a solvent degasser, a pump, an autosampler, a column thermostat, a multiwavelength UV–vis detector and a corona-charged aerosol detector from ESA Biosciences, Inc. (Chelmsford, MA, USA). Data acquisition and analysis on the latter system were carried out with ChemStation chromatographic data software from Agilent Technologies.

The commercially available Chiralpak ZWIX(+)^{TM} (CSP-1) and ZWIX(−)^{TM} (CSP-2) (150 × 3.0 mm I.D., 3-μm particle size) were provided by Chiral Technologies Europe (Illkirch, France). The preparation of ZWIX(+A) (CSP-3) (150 × 3.0 mm I.D., 5-μm particle size) is described in [23,24], and that of ZWIX(-A) (CSP-4) (150 × 3.0 mm I.D., 3-μm particle size) in [25].

Stock solutions of AAs (1 mg ml^{-1}) were prepared by dissolving them in the mobile phase. For determination of the dead-times (t_0) of the columns, a methanolic solution of acetone was injected. The flow rate was 0.6 ml min^{-1} and the column temperature was 25 °C if not otherwise stated.

3. Results and discussion

3.1. Effect of nature and concentration of bulk solvent components and mobile phase additives

The four zwitterionic *Cinchona* alkaloid QN- or QD- and (*R,R*)- or (*S,S*)-ACHSA-based CSPs (Fig. 1) perform well in non-aqueous polar organic solvents containing MeOH as a protic bulk solvent component (which can suppress H-bonding interactions) and MeCN as an aprotic bulk solvent component (which is advantageous for ionic interactions, but disadvantageous for π-π interactions) [1]. Additionally, acid and base additives are often applied to ensure ionic properties of the mobile phase [1,24–27].

For the chromatographic experiments with the cyclic β^3- and *N*α-monomethylated cyclic β^3-AAs, variation of volume ratios of MeOH/MeCN (75/25, 50/50 and 25/75 *v/v*) as bulk solvent containing 50 mM AcOH and 25 mM DEA in most cases resulted in the separation of the enantiomers. As depicted in Fig. 3, in the case of CSP-2 for **1** and **3**, with increasing MeCN/MeOH ratio, the retention continuously increased. The observed chromatographic behavior can be explained on the basis of the characteristics of the solvents and AAs. Nonaqueous, polar organic solvents are favored for *Cinchona* alkaloid-based CSPs [1,23,24]. The increased retention observed for the applied AAs at higher MeCN content can probably be explained in terms of the decreased solvation of the ionizable compounds, *i.e.* solvation of polar compounds in a mobile phase with a higher MeCN content is less effective, with the consequence that the electrostatic interactions between the SO and the AA become stronger, resulting in higher retention. At a constant acid-to-base ratio with the change of bulk solvent composition, the acid–base equilibrium and proton activity may also change, leading to further effects on the chromatographic behavior.

In the case of **1** and **3**, the selectivity slightly increased in MeCN-rich mobile phases. This is likely due to enhanced electrostatic and H-bonding interactions. For resolution, both a slight increase and decrease could be observed.

Applying MeOH/MeCN as bulk solvent in some cases provided only partial or lack of separation (Tables 1–4). In order to obtain better separations, THF was applied instead of MeCN. In certain cases, the application of THF resulted in an improvement in enantioselectivity and resolution; however, this improvement strongly depended on the SOs and AAs involved in the experiment. In Fig. 4, for **1**, **3**, and **9** on CSP-2 and CSP-3, the MeOH/THF ratio exerted a marked effect on the retention. With MeOH/THF mobile phase systems containing 50 mM AcOH and 25 mM DEA, a moderate decrease in k_1 with increasing MeOH content was observed. The higher

Table 1
Chromatographic data, separation factor (k), selectivity factor (α), resolution (R_S) and elution sequence of free- and *N*-monomethylated cyclic β^3-amino acids on CSP-1.

Compound	Eluent	k_1	α	R_S	*Elution sequence*
1	a	3.13	1.00	0.00	–
	g	3.46	1.02	<0.3	*A < B*
2	a	2.55	1.71	7.07	*B < A*
3	a	2.82	1.00	0.00	–
	g	2.61	1.10	0.68	*A < B*
4	a	1.60	1.54	3.75	*B < A*
5	a	3.96	1.21	1.31	*A < B*
6	a	2.13	1.50	3.59	*B < A*
7	a	6.22	1.12	0.99	*B < A*
8	a	2.46	2.00	9.24	*B < A*
9	a	3.39	1.05	0.49	*A < B*
	f	0.70	1.11	0.69	*A < B*
10	a	1.47	1.38	3.37	*B < A*

Chromatographic conditions: column, CSP-1; mobile phase, a, MeOH/MeCN (50/50 *v/v*) containing 25 mM DEA and 50 mM AcOH, f, H_2O/MeCN (25/75 *v/v*) containing 25 mM DEA and 50 mM AcOH, g, MeOH/THF (80/20 *v/v*) containing 25 mM DEA and 50 mM AcOH; flow rate, 0.6 ml min^{-1}; detection, 215 and 230 nm.

Table 2
Chromatographic data, separation factor (k), selectivity factor (α), resolution (R_S) and elution sequence of free- and *N*-monomethylated cyclic β^3-amino acids on CSP-2.

Compound	Eluent	k_1	α	R_S	*Elution sequence*
1	a	6.59	1.07	0.52	*B < A*
	g	4.66	1.17	0.86	*B < A*
2	a	3.34	1.70	6.45	*A < B*
3	a	3.36	1.04	0.30	*B < A*
	g	3.48	1.20	1.21	*B < A*
4	a	1.78	1.63	3.41	*A < B*
5	a	4.33	1.35	2.17	*B < A*
6	a	2.55	1.35	2.30	*A < B*
7	a	8.42	1.41	2.83	*A < B*
8	a	2.55	2.23	8.74	*A < B*
9	a	3.69	1.16	0.95	*B < A*
	i	1.66	1.25	1.25	*B < A*
10	a	1.23	1.64	3.29	*A < B*

Chromatographic conditions: column, CSP-2; mobile phase, a, MeOH/MeCN (50/50 *v/v*) containing 25 mM DEA and 50 mM AcOH, g, MeOH/THF (80/20 *v/v*) containing 25 mM DEA and 50 mM AcOH, i, MeOH/THF (95/5 *v/v*) containing 25 mM DEA and 50 mM AcOH; flow rate 0.6 ml min^{-1}; detection, 215 and 230 nm.

Please cite this article in press as: N. Grecsó, et al., Combinatorial effects of the configuration of the cationic and the anionic chiral subunits of four zwitterionic chiral stationary phases leading to reversal of elution order of cyclic β^3-amino acid enantiomers as ampholytic model compounds, J. Chromatogr. A (2016), http://dx.doi.org/10.1016/j.chroma.2016.05.041

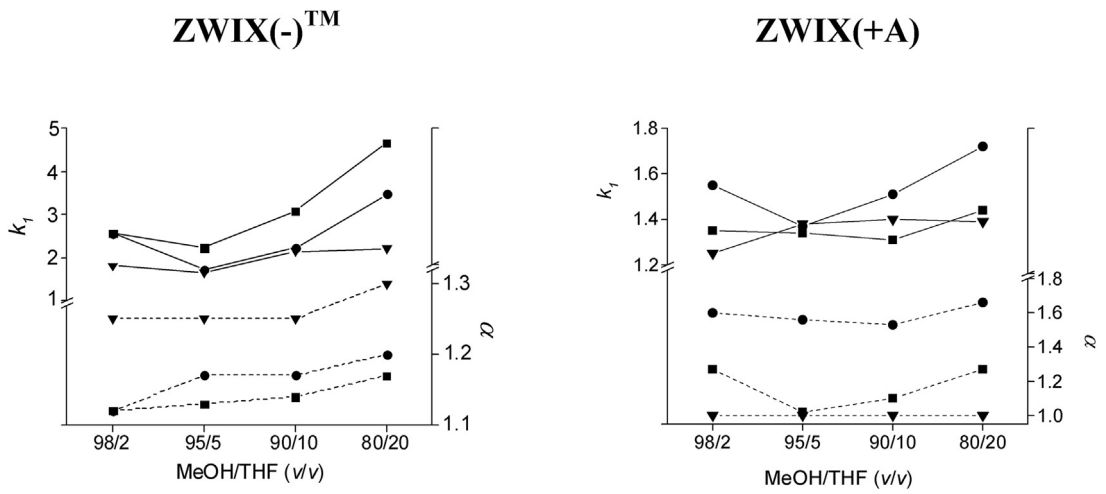

Fig. 4. Effects of the THF content in MeOH/THF mobile phase on the k_1 and α values for AAs **1, 3** and **9** on CSP-2.
Chromatographic conditions: column, CSP-2; mobile phase, MeOH/THF (98/2, 95/5, 90/10 and 80/20 *v/v*) containing 25 mM DEA and 50 mM AcOH; flow rate, 0.6 ml min^{-1}; detection, 215 and 230 nm; temperature 25 °C; symbols, for k_1 and α values for **1** ■, for **3**, ●, and for **9**, ▼.

Table 3
Chromatographic data, the separation factor (k), the selectivity factor (α), the resolution (R_S) and elution sequence of free- and *N*-monomethylated cyclic β^3-amino acids on CSP-3.

Compound	*Eluent*	k_1	α	R_S	*Elution sequence*
1	a	1.89	1.69	2.89	*B < A*
	g	1.44	1.66	2.87	*B < A*
2	a	1.41	1.00	0.00	–
	g	0.57	1.00	0.00	–
3	a	1.70	1.40	1.82	*B < A*
	g	1.27	1.39	1.40	*B < A*
4	a	1.33	1.00	0.00	–
	g	0.98	1.00	0.00	–
5	a	1.76	1.25	1.21	*B < A*
6	a	1.31	1.21	1.23	*A < B*
7	a	2.24	1.11	0.58	*B < A*
8	a	1.42	1.00	0.00	–
	g	1.20	1.00	0.00	–
9	a	2.00	1.10	0.46	*B < A*
	g	1.55	1.00	0.00	–
10	a	1.01	1.32	1.69	*A < B*

Chromatographic conditions: column, CSP-3; mobile phase, a, MeOH/MeCN (50/50 *v/v*) containing 25 mM DEA and 50 mM AcOH, g, MeOH/THF (80/20 *v/v*) containing 25 mM DEA and 50 mM AcOH; flow rate, 0.6 ml min^{-1}; detection, 215, 230 nm and corona detector.

Table 4
Chromatographic data, the separation factor (k), the selectivity factor (α), the resolution (R_S) and elution sequence of free- and *N*-monomethylated cyclic β^3-amino acids on CSP-4.

Compound	k_1	α	R_S	*Elution sequence*
1	2.80	1.80	3.73	*A < B*
2	1.50	1.46	3.31	*B < A*
3	2.47	1.51	2.21	*A < B*
4	1.56	1.07	0.65	*B < A*
5	2.38	1.24	0.90	*A < B*
6	1.85	1.11	0.75	*B < A*
7	3.00	1.44	2.53	*A < B*
8	1.50	1.32	2.09	*A < B*
9	3.36	1.14	0.68	*A < B*
10	1.35	1.19	1.46	*B < A*

Chromatographic conditions: column, CSP-4; mobile phase, a, MeOH/MeCN (50/50 *v/v*) containing 25 mM DEA and 50 mM AcOH; flow rate, 0.6 ml min^{-1}; detection, 215, 230 nm and corona detector.

content of protic MeOH progressively weakens the ionic interactions between the AA and the SO; the enhanced solvation of polar AAs results in a decrease of retention. The selectivity (and R_S, data not shown) increased slightly when the THF content was increased from 2 to 20% (*v/v*).

In a study of zwitterionic CSPs under non-aqueous and aqueous conditions, the addition of water to the MeOH-containing eluent system up to a water content of 20% (*v/v*) decreased the retention of the polar AAs [24]. Similarly, Zhang et al. found that the presence of 2.0% (*v/v*) water in the mobile phase shortened retention, but enhanced resolution of some AA enantiomer pairs on zwitterionic CSPs [26]. In our case, on CSP-1 and CSP-2 for **1**, **3**, and **9**, the addition of 2% (*v/v*) H_2O to the MeOH or MeOH/THF (95/5 *v/v*) mobile phase containing 50 mM AcOH and 25 mM DEA was also accompanied by a decrease of about 20–25% in k_1, and a slight decrease (5–10%) for α and R_S (data not shown). Thus, the presence of such a low concentration of water in the mobile phase might be advantageous because of the decreased retention obtained, without significant loss in selectivity and resolution.

3.2. Influence of the counter-ion concentration

For both anion- and cation-exchange type SOs under polar-ionic conditions, a predominant ion-exchange-driven retention mechanism has been confirmed [27], and in the ion-pairing process, the counter-ions present in the mobile phase act as competitors. Thus, retention can be adjusted by variation of the concentrations of the counter-ions. In cases like this, the retention can be described by the simple displacement model [28]; the plot of log k *vs.* log c results in a linear relationship, where the slope of the straight line is proportional to the effective charge involved in the ion-exchange. The effects of counter-ion concentration for **1**, **2**, **7**, **8**, **9**, and **10** (selected AAs exhibited *cis*- or *trans*-configurations with primary amino or *N*-monomethylated structure) were investigated in the MeOH/MeCN (50/50 *v/v*) mobile phase system containing AcOH and DEA. The concentrations of AcOH and DEA were varied from 12.5 mM up to 100 mM and from 6.25 mM up to 50 mM, respectively, while the acid to base ratio was maintained at 2:1. Under the studied conditions, linear relationships were found between log k_1 and log c_{AcOH}, with slopes varying around 0.20–0.35 (Fig. 5). The observed slopes practically were invariant with the structures of AAs. According to the simple displacement model, the slopes of these plots are determined by the ratio of the effective charges of the solute and the counter-ions. The data obtained with the four, in a combinatorial fashion, modulated ZWIX columns revealed that in general, an increasing counter-ion concentration resulted in reduced retention factors, as expected for ion-exchangers. On a cation-exchange

Please cite this article in press as: N. Grecsó, et al., Combinatorial effects of the configuration of the cationic and the anionic chiral subunits of four zwitterionic chiral stationary phases leading to reversal of elution order of cyclic β^3-amino acid enantiomers as ampholytic model compounds, J. Chromatogr. A (2016), http://dx.doi.org/10.1016/j.chroma.2016.05.041

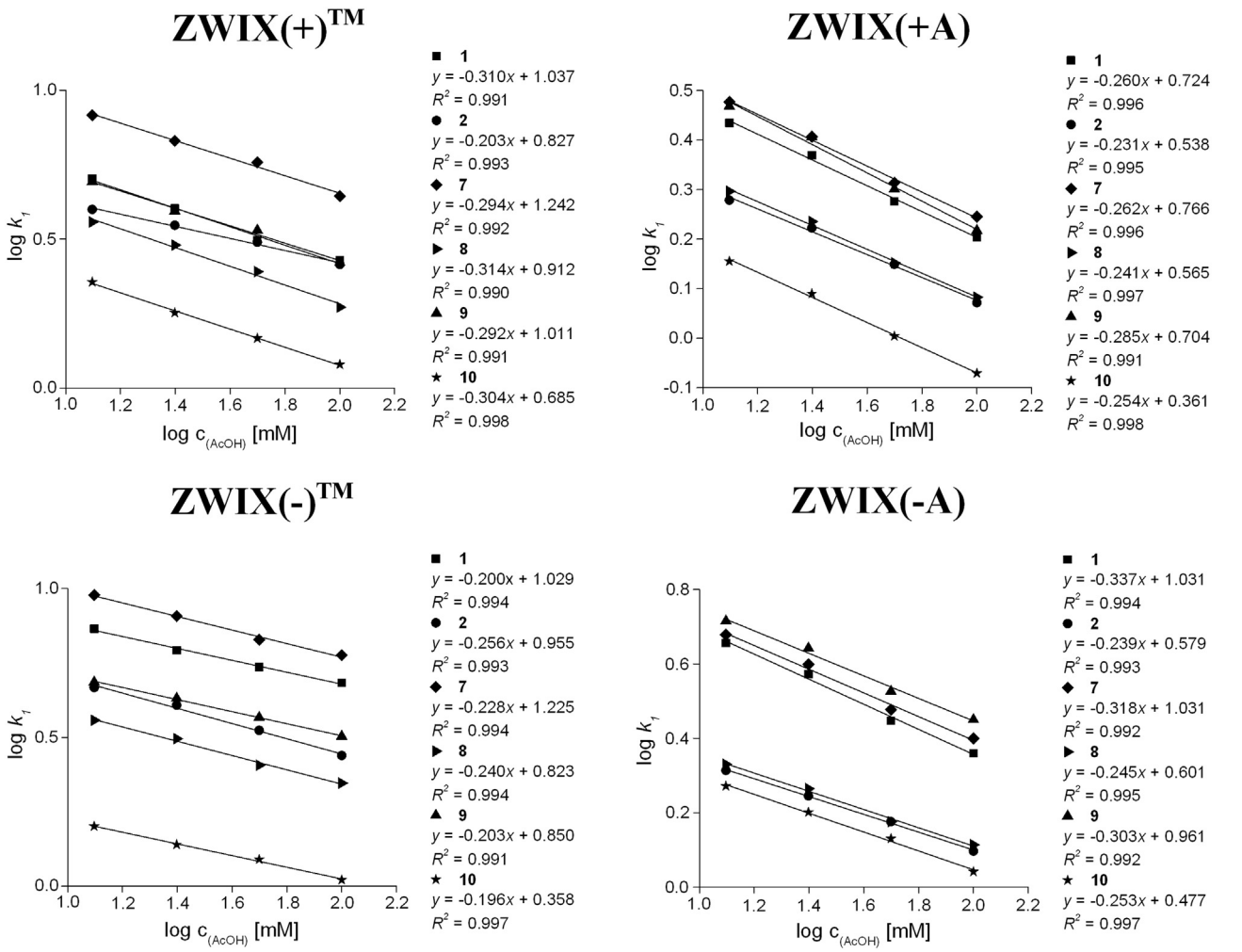

Fig. 5. Effects of counter-ion concentration on the k_1 values for **1, 2, 7, 8, 9** and **10** on the four *Cinchona* alkaloid-based CSPs.
Chromatographic conditions: column, CSP-1 – CSP-4; mobile phase, MeOH/MeCN (50/50 *v/v*) containing DEA/AcOH in concentration 6.25/12.5, 12.5/25.0, 25.0/50, and 50/100 mM/mM; flow rate, 0.6 ml min^{-1}; detection, 215 and 230 nm; temperature 25 °C; symbols, for **1** ■, for **2**, ●, for 7, ♦, for **8** ►, for **9**, ▲, and for **10**, *.

CSP, assuming a "single ionic" ion-exchange mechanism, slopes of log *k vs.* log *c* plots around 0.9 have been reported for the separation of different chiral amines [23]. However, the slopes of the log *k vs.* log *c* plots in this study were 0.20-0.35. These values indicate a marked difference between the zwitterionic and a "single ionic" ion-exchange mechanism. In the case of zwitterionic CSPs, with increased counter-ion concentration, retention can be reduced. However, almost an order of magnitude increase in the concentration of the counter-ions (from 12.5 mM to 100 mM) resulted only in 40–50% reduction in the retention factor. Under the studied conditions on all the four CSPs, practically identical slopes were obtained for each enantiomer, *i.e.* the individual enantioselectivity characteristics remained almost constant when the counter-ion concentration was varied (data not shown). This is in line with the general observation concerning the behavior of enantioselective ion-exchangers [2].

3.3. Comparison of separation performances of the four combinatorically composed zwitterionic CSPs

For the purpose of comparison, all of the CSP separations investigated were carried out at constant mobile phase composition (MeOH/MeCN (50/50 *v/v*) containing 25 mM DEA and 50 mM AcOH). The data in Tables 1–4 reveal that retention on the studied CSPs for the first eluting enantiomer followed the sequence CSP–2 > CSP–1 > CSP–4 > CSP-3 (except for **10**) with k_1 varying in the range from 1.01–8.42.

Comparing the two pairs of columns based on QN, and possessing the ACHSA subunits with opposite configuration [CSP-1 *vs.* CSP-3], or based on QD, and possessing the ACHSA subunits with opposite configuration [CSP-2 *vs.* CSP-4], it became evident that higher k_1 values were obtained on CSP-1 and CSP-2 than on the pseudoenantiomeric CSP-3 and CSP-4 (except for **10**) phases. The same was valid for values of α (exceptions were **1**, **3**, **5**, and **9** on CSP-1/CSP-3 and **1** and **3** on CSP-2/CSP-4).

The behavior of two pairs of CSPs based on QD or QN moiety and possessing ACHSA unit with the same configuration [CSP-2 *vs.* CSP-3 and CSP-1 *vs.* CSP-4] is also worth elaborating. It was observed that in the case when the ACHSA unit possesses a 1"*R*,2"*R* configuration, the QD-based CSP (CSP-2) exhibited higher k_1 values than the QN-based CSP (CSP-3), while when the ACHSA unit possesses a 1"*S*,2"*S* configuration, QN-based CSP-1 exhibited higher k_1 values than the QD-based CSP-4. The higher k_1 values generally were accompanied with higher selectivities (exceptions were SAs **1** and **3** on CSP-2/CSP-3 and SAs **1**, **3**, **5**, **7**, and **9** on CSP-1/CSP-4). The chromatographically visible effect of the structure of the QN/QD and (*R,R*)/(*S,S*)-ACHSA subunits of the SO on *k*, α, and R_S turns out to be rather complex. However, in terms of the elution order of the resolved cyclic β^3-AA enantiomers, trends can clearly be seen to depend on QD/QN and (*R,R*)/(*S,S*)-ACHSA subunits (discussed later).

Please cite this article in press as: N. Grecsó, et al., Combinatorial effects of the configuration of the cationic and the anionic chiral subunits of four zwitterionic chiral stationary phases leading to reversal of elution order of cyclic β^3-amino acid enantiomers as ampholytic model compounds, J. Chromatogr. A (2016), http://dx.doi.org/10.1016/j.chroma.2016.05.041

Table 5
Thermodynamic parameters, $\Delta(\Delta H^\circ)$, $\Delta(\Delta S^\circ)$, $T\times\Delta(\Delta S^\circ)$, $\Delta(\Delta G^\circ)$, correlation coefficients (R^2) and Q temperature of free- and *N*-monomethylated cyclic β^3-amino acids on *Cinchona* alkaloid and ACHSA based CSPs.

Compound	Column	Correlation coefficients (R^2)	$-\Delta(\Delta H^\circ)$ (kJ mol^{-1})	$-\Delta(\Delta S^\circ)$ (J mol^{-1} K^{-1})	$-T\times\Delta(\Delta S^\circ)_{298K}$ (kJ mol^{-1})	$-\Delta(\Delta G^\circ)_{298K}$ (kJ mol^{-1})	Q
1	CSP-2	0.9911	−0.7	−2.8	−0.8	0.1	0.8
	CSP-4	0.9951	5.5	13.5	4.0	1.5	1.4
	CSP-3	0.9951	6.0	15.8	4.7	1.3	1.3
2	CSP-2	0.9942	2.9	5.4	1.6	1.3	0.5
	CSP-4	0.9908	5.4	14.9	4.4	1.0	1.2
	CSP-1	0.9914	3.0	5.7	1.7	1.3	1.8
7	CSP-2	0.9923	1.3	1.5	0.5	0.8	0.2
	CSP-4	0.9909	2.4	5.0	1.5	0.9	1.6
	CSP-1	0.9941	0.7	1.6	0.5	0.2	1.6
	CSP-3	0.9990*	0.4	0.3	0.1	0.3	3.9
		0.9975**	2.1	6.2	1.8	0.3	1.1
8	CSP-2	0.9922	5.2	10.7	3.2	2.0	0.6
	CSP-4	0.9973	2.5	6.0	1.8	0.7	1.4
	CSP-1	0.9934	4.3	8.6	2.6	1.7	1.7

Chromatographic conditions: columns, CSP-1–CSP-4; mobile phase, a, MeOH/MeCN (50/50 *v/v*) containing 25 mM DEA and 50 mM AcOH; flow rate, 0.6 ml min^{-1}; detection, 215, 230 nm and corona detector; R^2, correlation coefficient of van't Hoff plot, ln α *vs* 1/*T* curves; $Q = \Delta(\Delta H^\circ)/298 \times \Delta(\Delta S^\circ)$
* Temperature range 5–20 °C.
** Temperature range 20–40 °C.

It seems that the QD-based CSP-2 and CSP-4 ensure a more pronounced sterically triggered environment for the intermolecular interactions between SO and AA. The four *Cinchona* alkaloid-based zwitterionic CSPs display a complementary character. This behavior reveals again the importance of the steric and spatial position of the ionic interaction sites within the SO moieties.

3.4. *Effects of the structure of the analytes (non-methylated versus* N*-monomethylated SAs and* cis/trans*-isomers) on chromatographic behavior and elution sequence*

It is fully consistent for all AAs and for all the four CSPs that *k* values were smaller for *N*-methylated cyclic β^3-AAs compared to the non-methylated ones. However, α and R_S values changed in different ways. With reversed elution order on CSP-1 and CSP-2, despite the lower *k* values, the enantioselectivity and the R_S were higher for *N*-methylated AAs; on CSP-3 and CSP-4 the α and R_S values changed parallel with *k* values, *i.e.* they were lower for *N*-methylated AAs (exceptions were **9** and **10**). It was also observed that the presence of a double bond in the molecule (**1** *vs.* **3** or **2** *vs.* **4**) generally resulted in lower *k*, α, and R_S values. The more polar molecules with a double bond probably were somewhat more solvated in the methanol containing mobile phase resulting in smaller *k*, α, and R_S values. A comparison of cyclohexyl skeleton-containing AAs with *cis*- *vs. trans*-configurations (**5** *vs.* **7** and **6** *vs.* **8**) revealed that AAs with *trans*-configuration (**7** and **8**) in most cases exhibited higher *k*, α, and R_S values (exceptions were **5** *vs.* **7** on CSP-1 and CSP-3 and **6** *vs.* **8** on CSP-3). **7** on all four CSPs exhibited the largest *k* values. Probably the steric arrangement in *trans*-isomer of carboxyl- and amino- (or *N*-methylamino)-groups favors the interactions with the cationic- and anionic-sites of the SO. Subsequently, it became evident that with switching the columns from CSP-1 to CSP-2 or CSP-3 to CSP-4 or from CSP-1 to CSP-3 or from CSP-2 to CSP-4, the elution sequence could be reversed (exception was **7**). This is in line with the previously obtained results, a switch from the QN based selector CSP-1 to the pseudoenantiomeric QD based CSP-2 led to a reversal of the elution order. (**1**, **3**, **5**, and **9** had an elution order *A* < *B* on CSP-1 and an elution order *B* < *A* on CSP-2.)

However, a reversal of elution order became evident when changing from the QN based CSP-1 to the also QN based CSP-3. This fact corroborates that in essence the absolute configurations of all the chiral subunits (the QN and the ACHSA subunit) determine in a concerted fashion the overall spatially oriented interaction sites of the particular ZWIX selector with the ampholytic AAs. As already outlined above, a simultaneously occurring double ion-pairing event is envisioned and a strong electrostatic interaction between the configurationally defined chiral ACHSA unit of the SO and the primary amino group of the AA seem to be responsible for the directing effect, which leads to the observed enantioselectivity. In other words, switching from an (*R*,*R*)- to a (*S*,*S*)-ACHSA subunit leads to a reversal of the elution sequence of the probed enantiomers. Exactly the same trend can be seen for the QD-based CSPs (CSP-2 and CSP-4). As a consequence of these systematic studies, it becomes evident that also the CSP-3 and CSP-4 behave pseudoenantiomerically to each other for the given cyclic β^3-AAs.

In comparison to the non-methylated cyclic β^3-AAs we observed a reversal of the elution sequence for the *N*-monomethylated analogues (see Tables 1–4). This effect was very surprising as it indicates that in the case of CSP-1 and CSP-2 the basic QN site becomes now more dominant than the ACHSA site. The same argument applies for the comparison of CSP-3 and CSP-4. In other words, there exists a subtle but hierarchically switched balance between the sterically driving subunits of the zwitterionic CSPs (SOs) in terms of the overall observed intermolecular recognition phenomena for a given set of ampholytic analytes. This determines consequently the enantioselectivity and elution order of the AAs.

Switching from the *cis*-configurated **5** and **6** to the *trans*-configurated **7** and **8** (Fig. 2 and Tables 1–4), we observed an exception of the above discussed trend. In essence, the actively involved and sterically demanding intermolecular interaction motifs of the AA and the SO sites are different. These, however, determine the elution order.

Selected chromatograms with indication of elution sequences are depicted in Fig. 6.

3.5. *Temperature dependence and thermodynamic parameters*

In order to investigate the effects of temperature on chromatographic behavior, a variable-temperature study was carried out on all four *Cinchona* alkaloid-based CSPs over the temperature range 5–40 °C. Experimental data collected for **1**, **2**, **7**, and **8** on the four columns with the mobile phase MeOH/MeCN (50/50 v/v) containing 25 mM TEA and 50 mM AcOH are listed in Supplementary material (Table S1).

The tabulated data indicate that the retention decreased in all cases with increasing temperature. Transfer of the SA from the mobile phase to the stationary phase is generally an exothermic process and consequently *k* decreases with increasing temperature. The changes observed in the selectivity and resolution with

Please cite this article in press as: N. Grecsó, et al., Combinatorial effects of the configuration of the cationic and the anionic chiral subunits of four zwitterionic chiral stationary phases leading to reversal of elution order of cyclic β^3-amino acid enantiomers as ampholytic model compounds, J. Chromatogr. A (2016), http://dx.doi.org/10.1016/j.chroma.2016.05.041

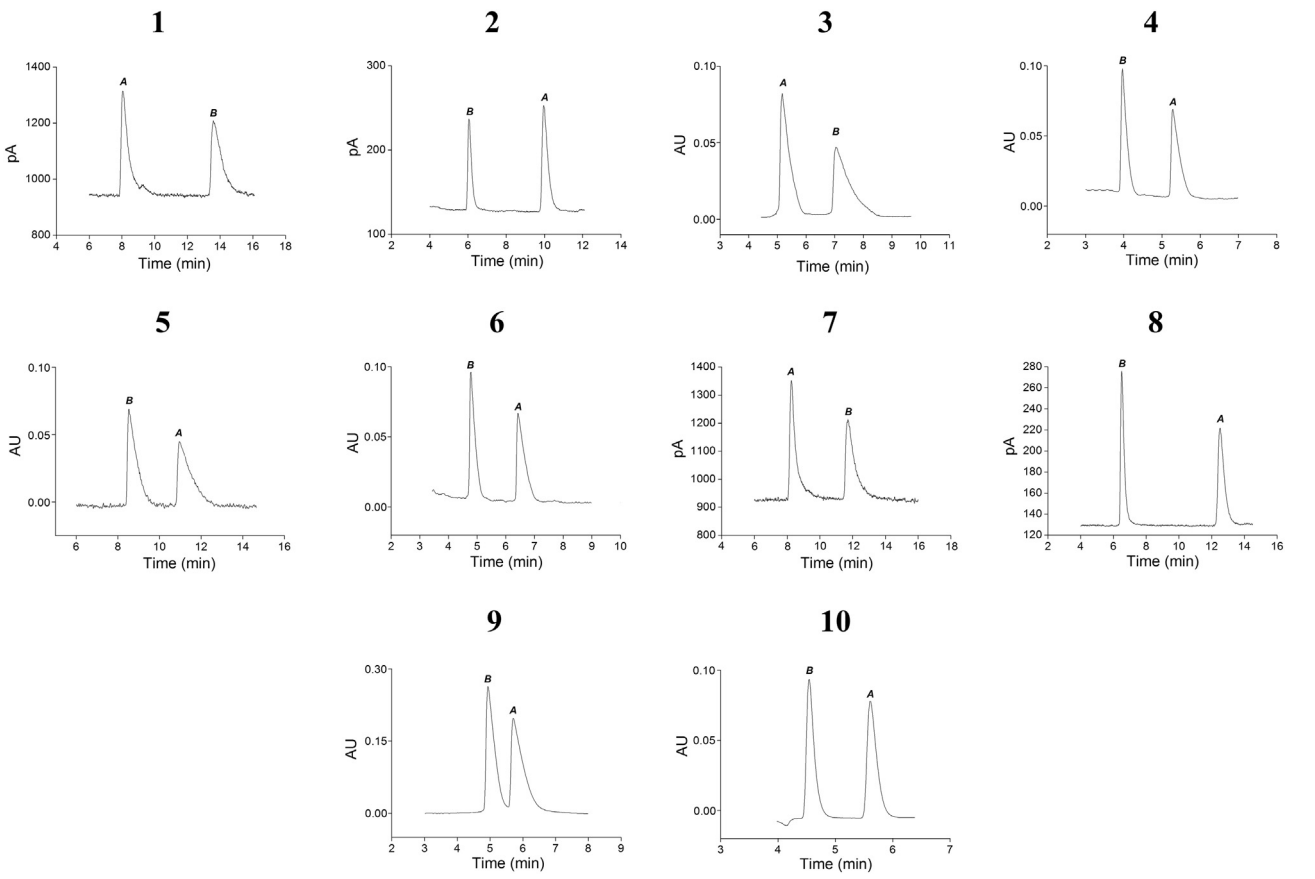

Fig. 6. Selected chromatograms for **1–10.**
Chromatographic conditions: column, for **1, 3** and **7** CSP-4, for **2, 4, 6, 8** and **10** CSP-1 and for **5** and **9** CSP-2; mobile phase, for **1** and **7** MeOH/MeCN (50/50 *v/v*) containing 6.25 mM DEA and 12.5 mM AcOH, for **2-6** and **8** MeOH/MeCN (50/50 *v/v*) containing 25 mM DEA and 50 mM AcOH, for **9** MeOH/THF (95/5 *v/v*) containing 25 mM DEA and 50 mM AcOH and for **10** MeOH/MeCN (50/50 *v/v*) containing 12.5 mM DEA and 25 mM AcOH; flow rate, 0.6 ml min^{-1}; detection, 215, 230 nm and corona detector; temperature 25 °C, except for **2** and **8** it was 5 °C

temperature were not consistent. In most cases, α and R_S decreased with increasing temperature. However, on CSP-2 for **1**, α and R_S slightly increased with increasing temperature under the applied conditions. Increasing temperature may improve the peak symmetry and efficiency, and therefore resolution may also improve. Such unusual behavior was recently observed for enantioseparation of unusual AAs on *Cinchona* alkaloid-based CSPs [29–32] and for an entirely different chiral chromatographic system by Chankvetadze et al. [33].

Since the effect of temperature on the enantiomer separation is complex, an extensive study relating to the thermodynamics was carried out. Accurate chromatographic data were collected to construct van't Hoff plots and the thermodynamic parameters were calculated from the slopes and intercepts of plots of ln α *vs. 1/T* (Table 5).

The differences in the changes in standard enthalpy $\Delta(\Delta H^\circ)$ provide information on the relative ease of transfer of AAs from the mobile to the stationary phase. A negative $\Delta(\Delta H^\circ)$ value indicates an exothermic transfer of AAs from mobile to stationary phase. The $\Delta(\Delta H^\circ)$ values ranged from −6.0 to +0.7 kJ mol^{-1}.

The differences in entropy $\Delta(\Delta S^\circ)$ is a measure of the loss of the degree of freedom or the energy balance of desolvation/solvation process when AA-SO complex is forming during the adsorption process. The trend in the change in $\Delta(\Delta S^\circ)$ is similar to that in $\Delta(\Delta H^\circ)$. Under the conditions where $\Delta(\Delta H^\circ)$ has negative values, $\Delta(\Delta S^\circ)$ was also negative [and the positive $\Delta(\Delta H^\circ)$ was accompanied by the positive $\Delta(\Delta S^\circ)$]. A negative $\Delta(\Delta S^\circ)$ indicates an increase in order and/or loss in the degrees of freedom of the adsorbed enantiomers. The $\Delta(\Delta S^\circ)$ values ranged from −15.8 to +2.8 J mol^{-1} K^{-1}. The interactions of **1** and **2** with CSP-4 and **1** or **8** with CSP-3 or CSP-2, respectively were characterized by the lowest $\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$ values reflecting a thermodynamically unfavorable process.

When the selectivity increased with increasing temperature, $\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$ were positive (*e.g.*, **1** on CSP-2). In this case, the change in the adsorption enthalpy with increasing temperature had a positive effect on the enantioselectivity. For this AA in this temperature range, enantioresolution is entropically-driven, and the selectivity increases with increasing temperature. Thermodynamically, this unusual behavior may be attributed to the positive $\Delta(\Delta S^\circ)$ values, indicating the importance of the entropy contribution to the chiral separation. On the other hand, the positive $\Delta(\Delta S^\circ)$ compensated the positive $\Delta(\Delta H^\circ)$ and resulted in a negative $\Delta(\Delta G^\circ)$.

The relative contribution of the enthalpic and entropic terms to the free energy of adsorption can be visualized by the enthalpy/entropy ratios $Q = \Delta(\Delta H^\circ)/298 \times \Delta(\Delta S^\circ)$ (Table 5). Comparison of the Q values for the SAs on different CSPs revealed that the enantioselective discrimination was in most cases enthalpically-driven ($Q > 1$) but for all investigated SAs on CSP-2 the entropic contribution to the free energy surpasses the enthalpic one ($Q < 1$). For **7** on CSP-3, the ln α *vs. 1/T* curve can be divided into two linear ranges between 5–20 °C and 20–40 °C, respectively. While the sign of the slope remained the same in both regions, the entropic contribution to the free energy in the region 20–40 °C

Please cite this article in press as: N. Grecsó, et al., Combinatorial effects of the configuration of the cationic and the anionic chiral subunits of four zwitterionic chiral stationary phases leading to reversal of elution order of cyclic β^3-amino acid enantiomers as ampholytic model compounds, J. Chromatogr. A (2016), http://dx.doi.org/10.1016/j.chroma.2016.05.041

is more expressed ($Q = 1.1$) denoting a more significant rearrangement in the solvent sphere.

Comparison of the $\Delta(\Delta G^\circ)$ values for the four columns demonstrated that in most cases lower $\Delta(\Delta G^\circ)$ were obtained on CSP-2 or CSP-4. It seems that the QD-based CSPs underwent more efficient binding with the SAs studied.

4. Conclusions

The enantiomers of non-methylated and *N*-monomethylated cyclic $ß^3$-AAs were separated on four zwitterionic CSPs, which contained the strongly acidic chiral (*R,R*)- and (*S,S*)- ACHSA subunits fused with the weakly basic chiral QN- or QD subunits. The separations could be accomplished in polar-ionic mode and the chromatographic retention behavior and resolution proved to depend on the nature and concentration of the bulk solvent and the acid and base modifiers, the temperature, and the *N*-methylation of the cyclic $ß^3$-AAs. The values of the thermodynamic parameters, such as $\Delta(\Delta H^\circ)$, $\Delta(\Delta S^\circ)$ and $\Delta(\Delta G^\circ)$, depended on the structures of the analytes and on the chiral selectors used. Most of the separations were enthalpically-driven, but entropically-driven separations were also observed. Of the studied CSPs, the QD based CSP-2 and CSP-4 appeared more suitable for the direct enantioseparation of non-methylated and *N*-monomethylated cyclic $ß^3$-AAs.

Particularly striking were the results obtained for the investigation of the molecular recognition phenomena of the systematically varied zwitterionic CSPs. In a systematic fashion, these four CSPs were screened for the resolution of five β^3-AAs and five related *N*-monomethylated β^3-AAs. Through the consequent elucidation of the elution orders of the enantiomers of this particular set of probes it became evident that:

(*i*) The pairs of CSP-1 and CSP-2, and of CSP-3 and CSP-4 behave pseudo-enantiomerically to each other, thus the elution order of the structurally similar analytes **1**, **3**, **5**, and **9** were reversed.

(*ii*) The QN based CSP-1 and CSP-3, differing in the chirality of the ACHSA subunits, led also to a reversal of the elution order of the screened probes, which indicates that both chiral subunits are simultaneously involved in the intermolecular recognition process. However, for non-methylated β^3-AAs the absolute configuration of the anionic ACHSA subunit site provided the dominant directing effect.

(*iii*) For the *N*-methylated congeners of the non-methylated β^3-AAs, an unexpected reversal of elution order was noticed, which indicates that in these cases the cationic *Cinchona* alkaloid interaction site becomes the dominant and elution order-directing interaction site.

Efforts to elucidate these diverse and surprising and subtle SO-AA interaction phenomena on a molecular level by spectroscopic, X-ray, and computer modeling studies are currently underway.

From an analytical point of view it is advantageous to elute the minor enantiomer in front of the major enantiomer, so it makes indeed sense to have a set of similar CSPs available which offer the reversal of elution order. In the case of Chiralpak ZWIX(+)™ and ZWIX(−)™ this concept is already established. In summary, from the explored examples of enantiomer separations and elution orders it becomes evident that zwitterionic chiral selectors composed of oppositely charged chiral subunits which interact simultaneously *via* electrostatic forces with *e.g.* ampholytic analytes have a great potential. Fusing chiral subunits in a combinatorial way offer the potential to optimize the overall structure of the SO to discriminate most efficiently the enantiomers of given SAs. It became also evident that such complex SOs may not always work perfectly and a loss of enantioselectivity can also occur.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

This work was supported by Hungarian National Science Foundation grant OTKA K 108847. We gratefully thank Pilar Franco (Chiral Technologies Europe, Illkirch, France) for the provision of the *Cinchona* alkaloid-based columns of the CSP-1 and CSP-2 type. CSP-4 was provided by Dr. Michal Kohout (Department of Organic Chemistry, Institute of Chemical Technology, Prague, Czech Republic). We also thank Dr. Kevin Schug (University of Arlington, Texas, USA) for proofreading the article.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2016.05.041.

References

[1] C.V. Hoffmann, R. Pell, M. Lämmerhofer, W. Lindner, Effects on enantioselectivity of zwitterionic chiral stationary phases for separations of chiral acids bases, and amino acids by HPLC, Anal. Chem. 80 (2008) 8780–8789.
[2] I. Ilisz, A. Péter, W. Lindner, State-of-the-art enantioseparations of natural and unnatural amino acids by high-performance liquid chromatography, Trends Anal. Chem. (2016), http://dx.doi.org/10.1016/j.trac.2016.01.016.
[3] L. Kiss, F. Fülöp, Synthesis of carbocyclic and heterocyclic ü-aminocarboxylic acids, Chem. Rev. 114 (2014) 1116–1169.
[4] I. Ilisz, R. Berkecz, A. Péter, Application of chiral derivatizing agents in the high-performance liquid chromatographic separation of amino acid enantiomers: a review, J. Pharm. Biomed. Anal. 47 (2008) 1–15.
[5] I. Ilisz, Z. Pataj, A. Aranyi, A. Péter, High-performance liquid chromatography of biologically important small epimeric peptides and their L,D-amino acid content, Mini Rev. Med. Chem. 10 (2010) 287–298.
[6] M. Lämmerhofer, Chiral recognition by enantioselective liquid chromatography: mechanisms and modern chiral stationary phases, J. Chromatogr. A 1217 (2010) 814–856.
[7] I. Ilisz, Z. Pataj, A. Aranyi, A. Péter, Macrocyclic antibiotic selectors in direct HPLC enantioseparations, Sep. Purif. Rev. 41 (2012) 207–249.
[8] I. Ilisz, A. Aranyi, Z. Pataj, A. Péter, Recent advances in the enantioseparation of amino acids and related compounds: a review, J. Pharm. Biomed. Anal. 69 (2012) 28–41.
[9] I. Ilisz, A. Aranyi, Z. Pataj, A. Péter, Enantiomeric separation of nonproteinogenic amino acids by high-performance liquid chromatography, J. Chromatogr. A 1269 (2012) 94–121.
[10] I. Ilisz, A. Aranyi, Z. Pataj, A. Péter, Enantioseparations by high-performance liquid chromatography using macrocyclic glycopeptide-based chiral stationary phases – an overview, in: G. Scriba (Ed.), Chiral Separations, Methods and Protocols, Humana Press, New York, 2013, pp. 137–163.
[11] M.H. Hyun, Development and application of crown ether-based HPLC chiral stationary phases, Bull. Korean Chem. Soc. 26 (2005) 1153–1163.
[12] H. Brückner, Enantiomeric resolution of *N*-methyl-α-amino acids and α-alkyl-α-amino acids by ligand-exchange chromatography, Chromatographia 24 (1987) 725–738.
[13] S. Hess, K.R. Gustafson, D.J. Milanowski, E. Alvira, M.A. Lipton, L.K. Panell, Chirality determination of unusual amino acids using precolumn derivatization and liquid chromatography-electrospray ionization mass spectrometry, J. Chromatogr. A 1035 (2004) 211–219.
[14] M. Tsesarskaia, E. Galindo, G. Szókán, G. Fisher, HPLC determination of acidic D-amino acids and their *N*-methyl derivatives in biological tissues, Biomed. Chromatogr. 23 (2009) 581–587.
[15] R. Pell, S. Sic, W. Lindner, Mechanistic investigation of *Cinchona* alkaloid-based zwitterionic chiral stationary phases, J. Chromatogr. A 1269 (2012) 287–296.
[16] A. Mandl, L. Nicoletti, M. Lämmerhofer, W. Lindner, Quinine versus carbamoylated quinine-based chiral aninon exchangers. A comparison regarding enantioselectivity for *N*-protected amino acids and other chiral acids, J. Chromatogr. A 858 (1999) 1–11.
[17] V. Piette, W. Lindner, J. Crommen, Enantiomer separation of *N*-protected amino acids by non-aqueous capillary electrophoresis with dimeric forms of quinine and quinidine derivatives serving as chiral selector, J. Chromatogr. A 948 (2002) 295–302.
[18] S. Allenmark, V. Schurig, Chromatography on chiral stationary phases, J. Mater. Sci. 7 (1977) 1955–1963.

[19] T. Fornstedt, P. Sajonz, G. Guichon, Thermodynamic study of an unusual chiral separation – propanolol enantiomers on an immobilized cellulose, J. Amer. Chem. Soc. 119 (1997) 1254–1264.
[20] T.L. Chester, J.W. Coym, Effect of phase ratio on van't Hoff analysis in reversed-phase liquid chromatography, and phase-ratio-independent estimation of transfer enthalpy, J. Chromatogr. A 1003 (2003) 101–111.
[21] G. Gotmar, T. Fornstedt, G. Guiochon, Apparent and true enantioselectivity in enantioseparations, Chirality 12 (2000) 558–564.
[22] T. Govender, P.I. Arvidsson, Facile synthesis of fmoc-*N*-methylated α- and β-amino acids, Tetrahedron Lett. 47 (2006) 1691–1694.
[23] C.V. Hoffmann, R. Reischl, N.M. Maier, M. Lämmerhofer, W. Lindner, Stationary phase-related investigations of quinine-based zwitterionic chiral stationary phases operated in anion- cation-, and zwitterion-exchange modes, J. Chromatogr. A 1216 (2009) 1147–1156.
[24] C.V. Hoffmann, R. Reischl, N.M. Maier, M. Lämmerhofer, W. Lindner, Investigations of mobile phase contributions to enantioselective anion- and zwitterion-exchange modes on quinine-based zwitterionic chiral stationary phases, J. Chromatogr. A 1216 (2009) 1157–1166.
[25] N. Grecsó, M. Kohout, A. Carotti, R. Sardella, B. Natalini, F. Fülöp, W. Lindner, A. Péter, I. Ilisz, Mechanistic considerations of enantiorecognition on novel *Cinchona* alkaloid-based zwitterionic chiral stationary phases from the aspect of the separation of trans-paroxetine enantiomers as model compounds, J. Pharm. Biomed. Anal. 124 (2016) 164–173.
[26] T. Zhang, E. Holder, P. Franco, W. Lindner, Method development and optimization on *Cinchona* and chiral sulfonic acid-based zwitterionic stationary phases for enantiomer separations of free amino acids by high-performance liquid chromatography, J. Chromatogr. A 1363 (2014) 191–199.
[27] M. Lämmerhofer, W. Lindner, Liquid chromatographic enantiomer separation and chiral recognition by *Cinchona* alkaloid-derived enantioselective separation materials, in: E. Grushka, N. Grinberg (Eds.), Advances in Chromatography, vol. 46, CRC Press/Taylor & Francis Group, Boca Raton, 2008, pp. 1–107 (F1).
[28] W. Kopaciewicz, M.A. Rounds, F. Fausnaugh, F.E. Regnier, Retention model for high-performance ion-exchange chromatography, J. Chromatogr. A 266 (1983) 3–21.
[29] I. Ilisz, N. Grecsó, A. Aranyi, P. Suchotin, D. Tymecka, B. Wilenska, A. Misicka, F. Fülöp, W. Lindner, A. Péter, Enantioseparation of β^2-amino acids on *Cinchona* alkaloid-based zwitterionic chiral stationary phases. Structural and temperature effects, J. Chromatogr. A 1334 (2014) 44–54.
[30] I. Ilisz, N. Grecsó, A. Misicka, D. Tymecka, L. László, W. Lindner, A. Péter, Comparison of separation performances of *Cinchona* alkaloid-based zwitterionic stationary phases in the enantioseparation of β^2- and β^3-amino acids, Molecules 20 (2015) 70–87.
[31] I. Ilisz, Z. Gecse, G. Lajkó, M. Nonn, F. Fülöp, W. Lindner, A. Péter, High-performance liquid chromatographic enantioseparation of unusual isoxazoline-fused 2-aminocyclopentanecarboxylic acids on *Cinchona* alkaloid-based chiral stationary phases at variable temperature conditions. Effect of mobile phase conditions and structural parameters on the chromatographic parameters, J. Chromatogr. A 1384 (2015) 67–75.
[32] I. Ilisz, Z. Gecse, G. Lajkó, E. Forró, F. Fülöp, W. Lindner, A. Péter, High-performance liquid chromatographic enantioseparation of cyclic β-amino acids on zwitterionic chiral stationary phases based on *Cinchona* alkaloids, Chirality 27 (2015) 563–570.
[33] I. Matarashvili, L. Chankvetadze, S. Fanali, T. Farkas, B. Chankvetadze, HPLC separation of enantiomers of chiral arylpropionic acid derivatives using polysaccharide-based chiral columns and normal-phase eluents with emphasis on elution order, J. Sep. Sci. 36 (2013) 140–147.

Please cite this article in press as: N. Grecsó, et al., Combinatorial effects of the configuration of the cationic and the anionic chiral subunits of four zwitterionic chiral stationary phases leading to reversal of elution order of cyclic β^3-amino acid enantiomers as ampholytic model compounds, J. Chromatogr. A (2016), http://dx.doi.org/10.1016/j.chroma.2016.05.041