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Short communication

Enantiomer separation by counter-current chromatography Optimisation and drawbacks in the use of L-proline derivatives as chiral selectors

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Abstract

Several L-proline and (4*R*)-hydroxy-L-proline derivatives were evaluated as chiral selectors (CSs) in the separation of enantiomers by counter-current chromatography (CCC). A variety of biphasic solvent systems, all of organic/aqueous nature, were tested in order to determine the appropriate distribution for CSs and racemates (*N*-(3,5-dinitrobenzoyl)-(±)-leucine and (±)-ketoprofen). Successful separations of DNB-(±)-leucine in analogous experimental conditions allow the comparative study of the enantioselectivity displayed by the considered CSs. The low solubility of certain CSs limits their applicability for preparative purposes even for improved enantioselectivity. The effect that the nature and pH of the buffer solutions used as a component of the solvent system have on the separation was also studied.

Keywords: Counter-current chromatography (CCC); Centrifugal partition chromatography (CPC); Enantiomer separation; L-Proline derivatives; DNB-leucine

1. Introduction

During the development of chiral new active ingredients (NAI), the high stereoselectivity of the biological processes involved in their pharmacological activity must be considered. The distribution, metabolism and even the interaction with the target molecule might differ for each enantiomer of a chiral compound [1]. Therefore, there is an increasing interest in producing enantiomers separately [2,3]. HPLC, applying simulated moving bed (SMB) technology, is at present one of the first choices to perform large-scale chiral separations, in spite of costly chiral stationary phases (CSPs) and the high consumption of solvents [4,5]. However, the investment in equipment is substantial. In this regard, counter-current chromatography (CCC) [6] and its modalities, centrifugal partition chromatography (CPC) [7] among them, which are especially adapted to preparative purposes [8], can be a competitive alternative for preparative enantioseparations.

The resolution of enantiomers by CCC involves the addition of a chiral selector (CS) to the stationary liquid phase. The mixture of enantiomers comes into contact with this liquid CSP, and enantiodiscrimination may be achieved. The CSs used up to now in CCC for chiral separations come from other separation techniques, mainly HPLC, and have been recently reviewed [9]. Among the low molecular weight CSs used in multiple interaction CSPs ("Brush type" CSPs) for HPLC, L-proline derivatives exhibit particularly remarkable enantioselectivity for certain analytes [10–12]. On this basis, we previously proposed *N*-dodecanoyl-L-proline-3,5-dimethylanilide (**1**, Fig. 1) as a π-donor CS applicable to CCC in the separation of *N*-(3,5-dinitrobenzoyl)-(±)-leucine and valine derivatives [13].

In an attempt to improve selector **1**, several L-proline derivatives have been synthesised in our laboratory (Fig. 1). In this paper, we report their comparative study. The search for an adequate organic/aqueous biphasic solvent system for CPC and the effect of the nature and pH of the buffer solutions used in their application to the separation of DNB-(±)-leucine are studied. The optimisation of conditions

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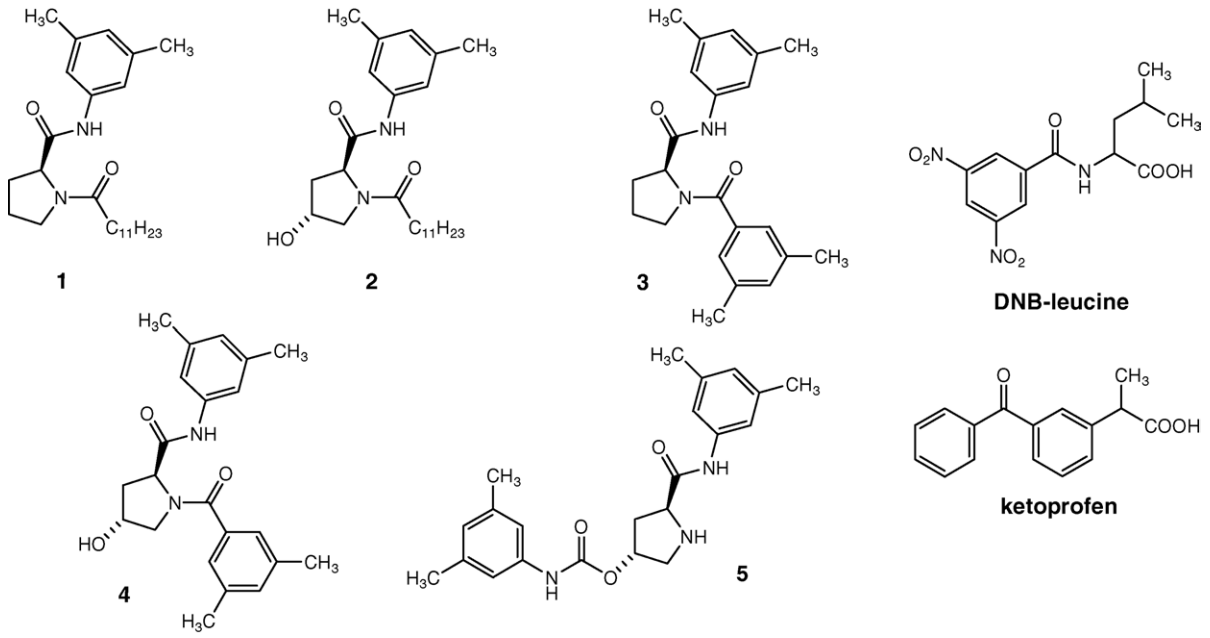

Fig. 1. Chemical structure of the chiral selectors and the racemates used in the study.

and the drawbacks encountered during the development are discussed.

2. Experimental

2.1. Reagents

CSs **1**–**5** were synthesised and characterised as described previously [14]. *N*-(3,5-Dinitrobenzoyl)-(±)-leucine was a product from Aldrich (Steinheim, Germany) and (±)-ketoprofen was supplied by Sigma (St. Louis, MO, USA).

HPLC-grade solvents were used in the preparation of liquid phases for CPC and HPLC. The buffer solutions used were prepared from analytical reagent-grade ammonium acetate, disodium monohydrogen phosphate and sodium dihydrogen phosphate and MilliQ water.

2.2. Apparatus

The CCC experiments were performed in a HPCPC model LLB-M (EverSeiko, Tokyo, Japan). This is a bench centrifuge (30 cm × 45 cm × 45 cm, 190 mL of experimentally determined internal volume) with a stacked circular partition disk rotor. This instrument was connected to a conventional HPLC system (pump, autosampler, UV detector, and chromatography data station software) model HP 1100 (Agilent Technologies, Palo Alto, CA, USA). A manual Rheodyne injector equipped with a 2.4 mL loop was used. The analytical control of the fractions collected in CPC was performed on the same HPLC system, changing the CPC device for the appropriate HPLC chiral column. Temperature was maintained at 25 °C during the CPC and HPLC runs.

2.3. Preparation of solvent systems

The binary solvent systems were prepared by mixing methyl *tert*-butyl ether (MTBE) or methyl isobutyl ketone (MIBK) with the convenient aqueous buffer solution: sodium phosphate buffer solution 0.1 M (pH 6.0, 6.7 or 8.0) or ammonium acetate buffer solution 0.1 M (pH 4.5, 6.0 or 6.7). The ternary solvent systems were prepared by mixing MTBE/ACN/10 mM hydrochloric acid solution, MIBK/ACN/10 mM HCl, and ethyl acetate/ACN/10 mM HCl or hexane/acetone/0.2 M ammonium acetate buffer pH 4.5 following the ratio 2:1:2. The quaternary solvent systems heptane/ethyl acetate/methanol/10 mM HCl were prepared by mixing their components following the ratios (4:6:5:5) and (5:5:4:6). All mixtures were shaken in a separatory funnel and allowed to equilibrate for 16 h. Each phase was then filtered and degassed separately before use.

2.4. CPC operating conditions

Previous extraction experiments were performed as described [15] to qualitatively determine the distribution of selectors and racemates in each of the biphasic solvent systems considered. The CSs were dissolved in the organic upper phase of the chosen solvent systems, which acted as stationary phase. The flow rate of the aqueous mobile phase was set at 3 mL/min and the rotation speed of the centrifuge was 1100 rpm, unless otherwise indicated. The amount of CS involved in the separations was calculated from the V_{st} value. Elution was monitored by UV-detection at 254 nm. Nevertheless, the eluate was collected in fractions and the enantiomeric content of these was determined. Elution profiles for the two enantiomers were constructed

with the results obtained. All runs were performed at least twice.

2.5. Analysis of CPC fractions

The fractions of 3 mL collected during elution were individually analysed to determine their enantiomeric content. A liquid–liquid extraction treatment, previous to the HPLC analysis, was required to process the aqueous fractions of eluate. Fractions containing DNB-leucine were analysed on a column containing *N*-(3,5-dimethylphenyl)-L-phenylalanine as CS (column CSP6c in [16]) using a mixture heptane/2-propanol/TFA (95:5:0.5) as mobile phase (α, 2.78; R_s, 6.20). Fractions containing ketoprofen were analysed on a column containing a 3,5-dichlorobenzoate of cellulose bonded to allylsilica gel as CS (CSP5 in [17]). Heptane/2-propanol/TFA (99:1:0.1) was used as mobile phase (α, 1.44; R_s, 2.37). The flow rate was 1 mL/min and the UV detection was set at 254 nm.

Elution order in the CPC experiments was established on the basis of the elution order in HPLC, determined with the use of samples enriched with one of the enantiomers of known absolute configuration.

3. Results and discussion

The election of a solvent system is crucial for the success of the separation by CCC/CPC and even more for the separation of enantiomers using this technique. Thus, the CS must be soluble in one of the phases, which will be used as the stationary phase. Simultaneously, the analyte must be conveniently partitioned into the two phases. In this scenario, the separation of the enantiomers is the result of differences in the association equilibria CS/enantiomers that occur in the stationary phase where the CS must be confined.

The partition equilibrium of the solute between the two phases, which is a function of the solvent system chosen, affects the retention of the compound and also the effectiveness of the association. Thus, a product with a high affinity for the stationary phase will be over-retained, while another with a low affinity will hardly reach the CS and the probability to be resolved will be low. Moreover, for preparative purposes the solubility of the CS in the stationary phase must be as high as possible, as column loadability is a function of the amount of CS involved in the separation [15]. Therefore, a substantial polarity is needed for this phase to reach the CS solubility required. However, such a stationary phase saturated with the other phase of the solvent system, often a water-containing mixture or solution, solvates the CS, thereby preventing its association with the enantiomers. The analyte enters in competition with this solvatation and enantioselectivity may be affected. Moreover, when polarity is similar for the two phases, the selector may be extracted from one to the other, producing leaks of the CS to the mobile phase. This phenomenon should be avoided to ensure the maximum stability for the stationary phase and the reproducibility of results. Therefore, the solvent system must reach a compromise situation between these requirements and must be adapted to the CS and the analyte.

3.1. Theoretical considerations

The selectivity factor in CCC, α, is defined as the ratio of partition coefficients, as differences in partition are on the basis of the separation of analytes by this technique [18]. In enantioselective CCC two processes, partition and association with the CS, must be considered. Assuming that the CS and its complexes with the enantiomers do not undergo partitioning to the mobile phase and considering *S* the most retained enantiomer, α_{CCC} can be expressed as [19]:

$$\alpha_{ccc} = \frac{1 + [CS]Ka_S}{1 + [CS]Ka_R}$$

[CS] being the concentration of CS that remains free in the stationary phase even in the presence of the enantiomers. That is, α_{CCC} is dependent not only on the ratio between association constants, but also on the magnitude of these association constants. The latter will determine the concentration of the CS that remains free in the stationary phase. The highest α_{CCC} value attainable in given chromatographic conditions is the ratio of association constants CS/enantiomers [20]. When CSs show low to moderate enantioselectivity when faced with a given racemate (i.e., $1.1 < Ka\ ratio < 4.0$), which is likely to be the most frequent case considering the parallelism with enantioselective HPLC, and values between 5 and 400 mM^{-1} are given to Ka constants, it can be easily shown that the effect of the free CS concentration on selectivity will be more significant for high enantioselectivity values ($Ka_{S/R}$ ratio) and low association constants. It can be assumed that, for CSs showing low association constants ($Ka < 1–2\ M^{-1}$) and enantioselectivity values in the more usual range, a 10 mM concentration of the free CS will produce an α_{CCC} value in which the difference from the maximum value attainable in the conditions considered will be negligible. Nevertheless, it should be taken into account that the concentration of free CS in the system will decrease when high amounts of analyte are injected in preparative applications, even for CSs exhibiting low Ka values.

Therefore, the relevance of the search for an adequate solvent system to perform the separation lies in the effect that this solvent may have not only on the amount of CS that is involved in a given separation, but also on the association constants CS/enantiomers. These will condition the loadability of the resulting chromatographic system and the separation of peaks.

3.2. Search for an adequate solvent system

A usual starting point in the search for an appropriate solvent system is to choose a good solvent for the CS and an immiscible solvent of whose polarity differs greatly. The system

constituted by heptane/ethyl acetate/methanol/10 mM HCl, similar to the previous applications of CS **1** in CPC [13], was first considered. The acidic solution prevents the ionisation of the racemates. Nevertheless, the application of these conditions to CSs **1** and **3** originated excessive retention of the analytes for practical purposes (more than 8 h). The distribution of CSs **1**–**4** and that of the racemates was then examined using more polar ternary solvent systems constituted by MTBE, MIBK and ethyl acetate with ACN and hexane, toluene and MIBK with acetone and 10 mM HCl. Only the less polar selector CS **1** was completely retained in the lipophilic phase of a hexane/acetone/10 mM HCl system. However, ketoprofen was still excessively retained when these conditions were used in CPC.

At this point aqueous buffer solutions were used to control the distribution of racemates. Hexane/acetone/0.2 M ammonium acetate buffer solution pH 4.5 (2:1:2) produced the complete resolution of the DNB-leucine enantiomers (t_0 = 18 min; t_1 = 38 min (*R*); t_2 = 51 min (*S*); α 1.65) as well as a partial separation for ketoprofen (*S*-enantiomer more retained than the *R*-enantiomer). Nevertheless, the solubility of the CSs and that of the racemates was rather low in this system, which constitutes a drawback for preparative applications.

More polar binary solvent systems were then tested. A comparable 10 mM-concentration of CS in the organic phase of the MIBK/0.2 M sodium phosphate buffer pH 8.0 system was attained for all four CSs. The amount of racemate to be injected was set at 150 mg (0.45 mmol, molar ratio CS/rac: 3.15), still far from the theoretical loadability limit of the stationary phase (molar ratio CS/rac: 1) when using a highly enantioselective CS [15]. CSs **1**–**4** showed enantioselectivity towards DNB-leucine in the CPC runs performed under these conditions (Table 1, Fig. 2).

The enantioselective analysis of the eluate fractions allowed us to follow the elution of enantiomers separately. Unfortunately, the peaks obtained in all four experiments did not correspond to essentially pure enantiomers. A deviation from the expected Gaussian shape was observed for the eluting profiles corresponding to the chromatographic peaks. Assuming that partition and association, the two processes involved in the separation, are fast, the lack of complete resolution of peaks may be the result of the combination of several factors. On the one hand, the low affinity between CSs and enantiomers (low association constants) may have led to a large fraction of free analyte in the stationary phase that is partitioned to the mobile phase. On the other hand, the low enantioselectivity of the CSs in these conditions (low ratio between association constants) may have determined the enantiomeric excess of the free analyte in the stationary phase and therefore, the enantiomeric excess at which it is eluting. Both processes result in a premature saturation of the chromatographic system. A third factor that could have contributed to the lack of resolution is the high polarity of MIBK, which may allow the ionisation of the racemate in the organic phase at the pH applied.

When all four selectors are compared in the same conditions the differences observed can be attributed only to differences in the association constants caused by the distinct structure of the CSs. In this regard, CSs **3** and **4** originated notably higher α values than CS **1**. Taking into account the structural similarity of the CSs and the identical elution order of enantiomers for all of them, it can be deduced that the introduction of an additional π-donor aromatic ring increases the enantioselectivity for DNB-leucine. However, the limited solubility of the resulting improved CSs in certain solvent systems limits their use as preparative tools.

The effect of the second structural modification, the introduction of an additional hydroxyl group on the CS (CSs **2** and **4**), on enantioselectivity is difficult to explain. The selectivity of CS **2** in comparison with CS **1** decreased while an improvement was observed in that of CS **4** compared with CS **3**. A deeper study on the role of this group either by modifying the polarity of the CS or its involvement in the recognition mechanism will require other alternative techniques.

Concerning racemic ketoprofen, in spite of the enrichment observed when CS **1** was used in a hexane/acetone/0.2 M ammonium acetate buffer (pH 4.5) solvent system, none of the CSs studied were enantioselective for this racemate in the conditions tested.

3.3. Effect of buffer nature and pH

A series of experiments was designed to assess the effect of buffer nature of the mobile phase on separations. It was expected that enantioselectivity could be better retained in a less polar environment [19]. Therefore, binary solvent systems containing the lipophilic MTBE were used to obtain increased separation of peaks. Ammonium acetate buffer and

Table 1
CPC runs in MIBK/0.2 M sodium phosphate buffer solution (pH 8.0)

CS	V_{st}	CS_{st} (mmol)	$r_{CS/rac}$	t_0 (min)	t_1 (min)	t_2 (min)	k_1[a]	k_2[a]	α_{CCC}[a]	R_s[a]	eo	ee_{max} (%) *R*/*S*
1	142	1.42	3.15	16	41	61	1.56	2.81	1.80	0.96	*R*	32/35
2	142	1.42	3.15	16	31	35	0.94	1.19	1.26	0.72	*R*	29/45
3	142	1.42	3.15	16	25	40	0.56	1.50	2.67	0.90	*R*	46/56
4	142	1.42	3.15	16	23	38	0.44	1.37	3.14	0.79	*R*	44/38

V_{st}, volume of retained stationary phase (mL); CS_{st}, mmol of CS involved in the experiment; $r_{CS/rac}$, molar ratio CS/racemate; t_0, void time; t_1 and t_2, retention time for each enantiomer; k_1 and k_2, retention factor for each enantiomer; α_{CCC}, selectivity factor; R_s, resolution; eo, elution order, configuration of the first eluted enantiomer; ee_{max}, enantiomeric excess attained at the maximum elution of each enantiomer. Conditions: Solvent system, MIBK/0.2 M sodium phosphate buffer solution pH 8.0, flow rate, 3 mL/min; ω = 1000 rpm; amount of DNB-leucine injected, 150 mg (0.45 mmol).

[a] Calculated on the elution profiles as defined in Ref. [18].

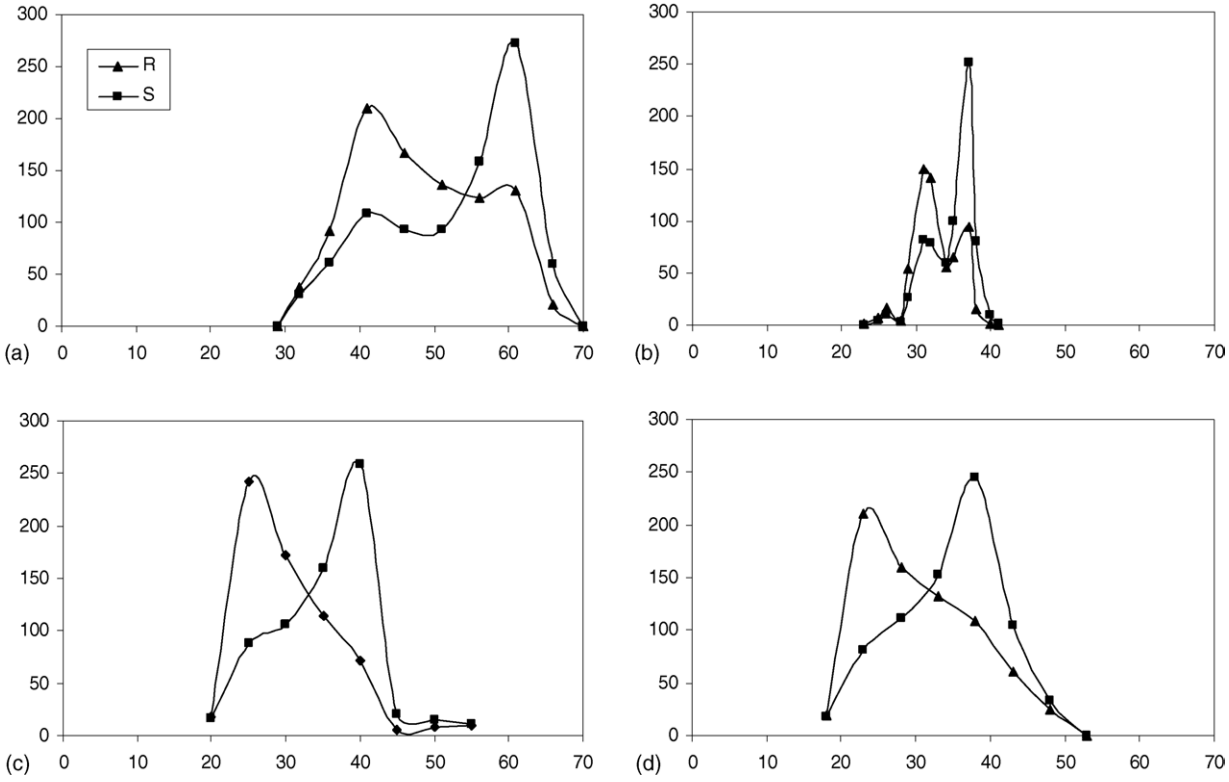

Fig. 2. Elution profiles corresponding to the separation of 150 mg (0.45 mmol) of racemic DNB-leucine using a 10 mM concentration of CS in the stationary phase (a) CS **1**, (b) CS **2**, (c) CS **3**, and (d) CS **4**. Solvent system: MIBK/0.2 M sodium phosphate buffer pH 8.0. See Table 1 for detailed chromatographic conditions. Vertical left axis, arbitrary absorbance units; horizontal axis, time (min).

sodium phosphate buffer solutions were used at the same concentration and were tested at two pH values (6.0 and 6.7). It was expected that for a considered CS, both factors would act mainly on the partition process of the racemate.

Unfortunately, the use of MTBE limited the application of certain CSs. Thus, CS **3** was used at its solubility limit (6.14 mM) while the concentration of CS **1** was set at 30 mM because of the high solubility of this CS in MTBE. At this point, CS **5** was included in the study. The ionisable amino group on CS **5** was expected to promote ion pairing formation with acidic analytes, such as DNB-leucine, within the lipophilic stationary phase, which may contribute to enhance enantioselectivity [15,21]. A concentration 13.21 mM was attained in the stationary phase for CS

Table 2
CPC runs in MTBE/buffer solution

CS	$[CS]_{st}$ (mM)	CS_{st} (mmol)	$r_{CS/rac}$	Buffer solution[a]	pH	t_1	t_2	k_1[b]	k_2[b]	α_{CCC}[b]	R_s[b]	eo
1	30.00	5.04	21.9	Phos.	6.0	90	180	11.28	23.55	2.09	1.46	*R*
				Acet.	6.0	109	269	13.87	35.70	2.57	1.63	*R*
				Phos.	6.7	56	80	6.64	9.91	1.49	0.56	*R*
				Acet.	6.7	61	111	7.32	14.14	1.93	0.89	*R*
3	6.14	1.03	4.5	Phos.	6.0	90	100	11.28	12.64	1.12	0.22	*R*
				Acet.	6.0	102	132	12.91	17.01	1.32	0.50	*R*
				Phos.	6.7	45	–	5.14	–	1.00	–	–
				Acet.	6.7	86	116	10.73	14.82	1.38	0.52	*R*
5	13.21	2.22	9.6	Phos.	6.0	108	–	13.73	–	∼1.0	–	*S*
				Acet.	6.0	149	159	19.33	20.69	1.07	–	*S*
				Phos.	6.7	58	–	6.91	–	1.0	–	–
				Acet.	6.7	128	–	16.46	–	∼1.0	–	*S*

$[CS]_{st}$, concentration of CS in the stationary phase; CS_{st}, mmol of CS involved in the experiment (V_{st} 168 mL); $r_{CS/rac}$, molar ratio CS/racemate; t_1 and t_2, retention time for each enantiomer; k_1 and k_2, retention factor for each enantiomer; α_{CCC}, selectivity factor; R_s, resolution; eo, elution order, configuration of the first eluted enantiomer; Conditions: Flow rate, 3 mL/min; ω = 1100 rpm; amount of DNB-leucine injected, 75 mg (0.23 mmol).

[a] Phos., 0.1 M sodium phosphate buffer; Acet., 0.1 M ammonium acetate buffer.

[b] Calculated on the elution profiles as defined in Ref. [18].

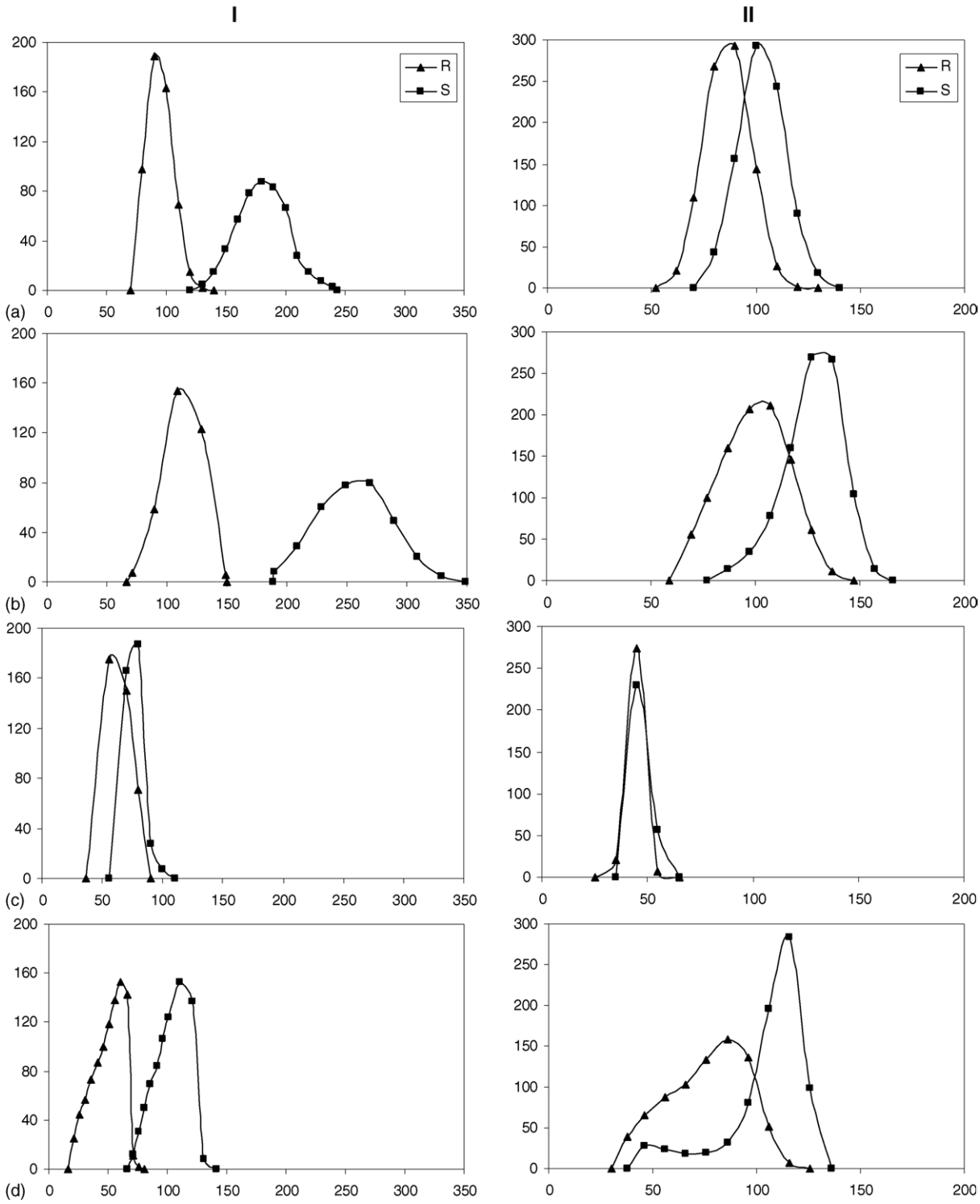

Fig. 3. Elution profiles and CPC chromatograms obtained in the separation of 75 mg (0.23 mmol) of racemic DNB-leucine using (I) CS **1** (30 mM); (II) CS **3** (6.14 mM). Solvent system: (a) MTBE/0.1 M sodium phosphate buffer solution pH 6.0; (b) MTBE/0.1 M ammonium acetate buffer solution pH 6.0; (c) MTBE/0.1 M sodium phosphate buffer solution pH 6.7; (d) MTBE/0.1 M ammonium acetate buffer solution pH 6.7. Flow rate 3 mL/min, ω = 1100 rpm. Vertical axis, arbitrary absorbance units; horizontal axis, time (min).

5. The same amount of DNB-leucine was injected in all experiments (75 mg, 0.23 mmol). This amount provides molar ratios CS/enantiomers high enough to prevent the possible saturation of the stationary phase. The results obtained are shown in Table 2 and Fig. 3.

As can be observed, most peaks show a Gaussian shape, indicating the absence of saturation even for co-eluting peaks. When analogous experiments are compared, the lower pH produces a higher retention because of the decrease in the ionisation of the analyte and, therefore, the increase in the affinity for the organic stationary phase.

When the results obtained using CSs **1** and **3** are considered, only the use of ammonium acetate buffer at pH 6.0 with CS **1** resulted in a clear improvement of the α value regarding that obtained when using the MIBK/buffer system. It is also worth noting the low α values obtained for CS **3** in comparison with the one obtained with the MIBK/buffer system. Therefore, enantioselectivity is not only a function of the polarity of the solvent system. The nature of the solvent that constitutes the stationary phase may be a major issue in this context.

Regarding the nature of the buffer solution and considering the same pH value, ammonium acetate buffer originated higher retention than sodium phosphate. However, this increased retention affected mainly the most retained enantiomer, thereby resulting in an increase in the α value. That is to say, the nature of the buffer used also affects the values corresponding to association constants CS/enantiomers. This effect may be explained by the possibility of the more lipophilic acetate/acetic acid pair to undergo partition to the stationary phase, thus modifying the environment in which the association CS/enantiomers occurs.

Concerning CS **5**, although only the solvent system containing ammonium acetate at pH 6.0 afforded a noticeable separation of DNB-leucine, the elution order of enantiomers was determined for three of the conditions used. This order was the opposite to that obtained with CSs **1**–**4**, which indicates that CS **5** has a distinct recognition mechanism. Nevertheless, the presence of an ion-pairing effect could not be confirmed. Even if the retention originated by this selector is higher than that shown by CS **3**, the higher molar ratio of CS **5** may account for this increase.

4. Conclusions

The comparative study of the chiral discrimination capacity of *N*-dodecanoyl-L-proline-3,5-dimethylanilide (**1**) and the modified CSs **2**–**4** shows the positive effect of the introduction of a second π-donor group on the structure of the CS on enantioselectivity. However, the modification reduces the solubility of the CSs in highly lipophilic solvents, which limits their applicability for preparative purposes in these conditions.

The strong effects of buffer nature and pH on the separation highlight the need to also consider these two factors when optimising the separation conditions by this technique. In the case of the CSs used in this study, ammonium acetate buffer produced higher retention times than phosphate buffer, thereby resulting in enhanced enantioselectivity. The acidification of the solvent system increased the partition of DNB-leucine towards the organic stationary phase and, consequently, increased analyte retention and separation.

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References

[1] H.Y. Aboul-Enein, I.W. Wainer (Eds.), The Impact of Stereochemistry on Drug Development and Use, Chemical Analysis, vol. 142, Wiley, New York, 1997.
[2] H. Caner, E. Groner, L. Levy, I. Agranat, Drug Discov. Today 9 (2004) 105.
[3] A.M. Rouhi, Chem. Eng. News 82 (2004) 47.
[4] E.R. Francotte, J. Chromatogr. A 906 (2001) 379.
[5] S.R. Perrin, R.M. Nicoud, in: G. Subramanian (Ed.), Chiral Separation Techniques—A Practical Approach, Wiley-VCH, Weinheim, 2001 (Chapter 10).
[6] W.C. Conway, Countercurrent Chromatography: Apparatus, Theory and Applications, VCH, New York, 1990.
[7] A.P. Foucault (Ed.), Centrifugal Partition Chromatography, Chromatographic Science Series, vol. 68, Marcel Dekker, New York, 1995.
[8] A. Berthod (Ed.), Countercurrent Chromatography the Support-Free Liquid Stationary Phase, Comprehensive Analytical Chemistry, vol. 38, Elsevier, Amsterdam, 2002.
[9] L. Oliveros, C. Minguillón, P. Franco, A.P. Foucault, in: A. Berthod (Ed.), Countercurrent Chromatography the Support-Free Liquid Stationary Phase, Comprehensive Analytical Chemistry, vol. 38, Elsevier, Amsterdam, 2002 (Chapter 11).
[10] W.H. Pirkle, P.G. Murray, J. Chromatogr. 641 (1993) 11.
[11] W.H. Pirkle, M.E. Koscho, J. Chromatogr. A 840 (1999) 151.
[12] P. Murer, K. Lewandowski, F. Svec, J.M.J. Frechet, Anal. Chem. 71 (1999) 1278.
[13] L. Oliveros, P. Franco, C. Minguillón, E. Camacho-Frias, A. Foucault, F. Le Goffic, J. Liq. Chromatogr. 17 (1994) 2301.
[14] T. González, O. Abad, M.C. Santano, C. Minguillón, Synthesis (2004) 1171.
[15] P. Franco, J. Blanc, W.R. Oberleitner, N.M. Maier, W. Lindner, C. Minguillón, Anal. Chem. 74 (2002) 4175.
[16] L. Oliveros, C. Minguillón, B. Desmazières, P.L. Desbène, J. Chromatogr. 606 (1992) 9.
[17] L. Oliveros, A. Senso, C. Minguillón, Chirality 9 (1997) 145.
[18] A. Berthod, Fundamentals of countercurrent chromatography, in: A. Berthod (Ed.), Countercurrent Chromatography the Support-Free Liquid Stationary Phase, Comprehensive Analytical Chemistry, vol. XXXVIII, Elsevier, Amsterdam, 2002 (Chapter 1).
[19] Y. Ma, Y. Ito, Anal. Chem. 67 (1995) 3069.
[20] A.P. Foucault, J. Chromatogr. A 906 (2001) 365.
[21] E. Gavioli, N.M. Maier, C. Minguillón, W. Lindner, Anal. Chem. 76 (2004) 5837.