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Non-aqueous capillary electrophoretic enantioseparation of *N*-derivatized amino acids using cinchona alkaloids and derivatives as chiral counter-ions

Véronique Piette[a], Marianne Fillet[b], Wolfgang Lindner[c], Jacques Crommen[b,*]

[a]*Scientific Institute of Public Health – Louis Pasteur, rue Juliette Wytsman 14, B-1050 Bruxelle, Belgium*

[b]*Department of Analytical Pharmaceutical Chemistry, Institute of Pharmacy, University of Liège, CHU, B36, B-4000 Liège 1, Belgium*

[c]*Institute of Analytical Chemistry, University of Vienna, Währingerstrasse 38, A-1090 Vienna, Austria*

Abstract

A non-aqueous capillary electrophoretic method developed with quinine and *tert*.-butyl carbamoylated quinine as chiral selectors for the enantioseparation of *N*-protected amino acids was applied to the investigation of other quinine derivatives as chiral additives. The optimum composition of the background electrolyte was found to be 12.5 m*M* ammonia, 100 m*M* octanoic acid and 10 m*M* chiral selector in an ethanol–methanol (60:40, v/v) mixture. Under these conditions, a series of chiral acids, as various benzoyl, 3,5-dinitrobenzoyl and 3,5-dinitrobenzyloxycarbonyl amino acid derivatives were investigated with regards to selectand–selector relationships and enantioselectivity employing quinine, quinidine, cinchonine, cinchonidine, *tert*.-butyl carbamoylated quinine, *tert*.-butyl carbamoylated quinidine, dinitrophenyl carbamoylated quinine and cyclohexyl carbamoylated quinine as chiral selector.

Keywords: Non-aqueous capillary electrophoresis; Enantiomer separation; Chiral selectors; Buffer composition; Amino acids; Alkaloids

1. Introduction

In capillary electrophoresis (CE), enantiomeric separations are most often obtained by addition of the chiral selector (SO) to the background electrolyte [1–4]. Unfortunately, some of these chiral selectors, used successfully on stationary phases in chiral high-performance liquid chromatography (HPLC) have only limited solubility in aqueous buffers which are generally used in CE. Non-aqueous CE (NACE), employing small amounts of well-characterized chiral additives, offers an attractive alternative for screening the chiral discrimination potential of selectors with low solubility in water and studying the interactions between these selectors and various kinds of analytes [5,6].

Quinine has been used as a chiral solvating agent in nuclear magnetic resonance (NMR) studies [7], as a chiral ion-pairing agent for enantioseparation in HPLC [8,9] as well as in capillary electrochromatography (CEC) [10] and as a selector immobilized on a chiral stationary phase in HPLC [11,12]. Chiral stationary phases based on the use of carbamoylated derivatives of quinine and quinidine as selectors were found to be highly stereoselective for the direct resolution of chiral acids in HPLC using mixtures of aqueous buffers and methanol or acetonitrile as mobile phases [13–18]. This new class of chiral

*Corresponding author. Tel.: +32-4-3664-346; fax: +32-4-3664-347.

E-mail address: jcrommen@ulg.ac.be (J. Crommen)

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selectors can be classified as weak anion exchangers, due to the presence of a tertiary amino group within the quinuclidine ring which is protonated at the usual working pH of the mobile phase. The primary ionic interaction between the anionic solutes (selectands, SAs) and the cationic SO is significantly accompanied by additional intermolecular interactions as hydrogen bonding, dipole–dipole, charge transfer (π–π), hydrophobic and steric interactions. These simultaneously acting multiple interactions can be seen as a basis for the high stereoselectivity potential shown by these SOs. Moreover, the introduction of a bulky alkyl substituent at the carbamate function turned out to be advantageous for a further increase in stereoselectivity [13,14].

Recently, a NACE system using a background electrolyte composed of 12.5 m*M* of ammonium acetate in methanol was found to be useful for the investigation of the potential of quinine and *tert*.-butyl carbamoylated quinine as chiral SOs for the enantioseparation of *N*-protected amino acids [19]. The influence of the nature of the organic solvent, the combination of different solvents, the nature and the concentration of the background electrolyte, the concentration of the selector, the capillary temperature and the applied voltage was systematically studied, using DNB-Leu and DNB-Phg as model compounds. A buffer made of 12.5 m*M* ammonia, 100 m*M* octanoic acid and 10 m*M* SO in an ethanol–methanol (60:40) mixture was found to give the best compromise in terms of selectivity, resolution, efficiency, peak symmetry and analysis time [19].

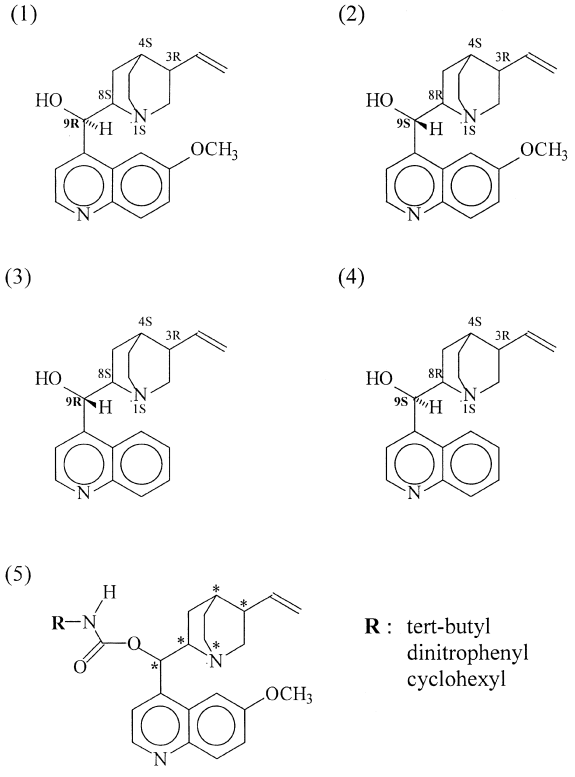

Fig. 1. Chiral selectors. (1) Quinine, (2) quinidine, (3) cinchonine, (4) cinchonidine and (5) carbamoylated quinine derivatives.

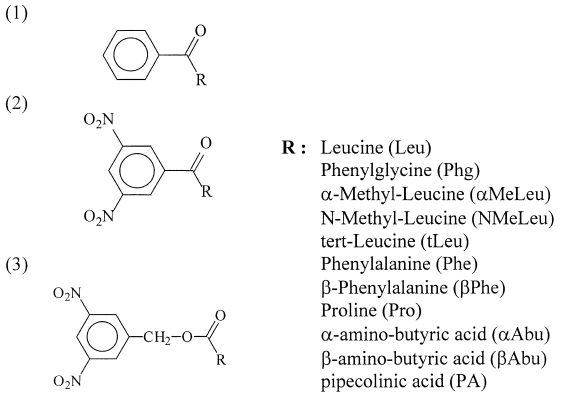

Fig. 2. *N*-Protected amino acid derivatives. (1) Bz (benzoyl), (2) DNB (3,5-dinitrobenzoyl), (3) DNZ (3,5-dinitrobenzyloxycarbonyl).

In this study, such a NACE system was applied for the rapid screening of different kinds of cinchona alkaloids and derivatives tested as SOs: quinine (QN), quinidine (QD), cinchonine (CN), cinchonidine (CD), *tert*.-butyl carbamoylated quinine (tBuCQN), *tert*.-butyl carbamoylated quinidine (tBuCQD), dinitrophenyl carbamoylated quinine (DNPCQN) and cyclohexyl carbamoylated quinine (cHexCQN) (cf. Fig. 1). A series of various *N*-protected amino acid derivatives were tested as chiral acidic selectands, including Bz- (benzoyl), DNB- (3,5-dinitrobenzoyl) and DNZ- (3,5-dinitrobenzyloxycarbonyl) amino acids (cf. Fig. 2).

2. Experimental

2.1. Apparatus

All experiments were performed on a Spec-

traphoresis 1000 CE instrument (Spectraphysics, San Jose, CA, USA) equipped with an autosampler, a UV–visible detector (190–800 nm) and a temperature control system (15–60°C). An IBM PS/2 Model 90 486 was used for instrument control and data handling. Electropherograms were printed on a HP DeskJet 500 printer. A column cartridge was obtained from Spectraphysics. The apparent pH (pH_a) of the non-aqueous electrolyte solutions was measured by means of a Model Delta 345 pH meter from Mettler (Healstead, UK).

2.2. Chemicals and reagents

QN was obtained from Sigma (St. Louis, MO, USA); QD, CN and CD were from Buchler (Brannschweig, Germany). TBuCQN, tBuCQD, DNPCQN and cHexCQN were synthesized according to a standard procedure described elsewhere [20]. The organic solvents were HPLC-grade: ethanol absolute from Merck (Darmstadt, Germany) and methanol from Fisher Scientific (Leicestershire, UK). Octanoic acid was from Sigma and ammonia solution 25% from Carlo Erba (Rodano, Italy). The amount of water in electrophoresis media was determined by Karl–Fisher titration using Hydranal Composit 1 from Riedel-de Häen (Seelze, Germany) standardized against sodium tartrate dihydrate (15.66% water, Merck). The racemic and enantiomerically pure amino acids were purchased from Sigma. Bz and DNB derivatives were synthesized according to standard derivatization procedures [17] except DNB-Leu and DNB-Phg obtained from Sigma. To synthesize the DNZ compounds, aqueous solutions of amino acid were derivatized with 3,5-dinitrobenzyl chloroformate [18]. The sample solutions were prepared by dissolving each amino acid derivative at a concentration of 50 μg/ml in methanol. Benzylic alcohol from Sigma (0.01% methanolic solution) was used as neutral marker to visualize the electroosmotic flow μ_{EOF}. Buffers and samples were filtered through a Polypure polypropylene membrane filter (0.2 μm) from Alltech (Laarne, Belgium) before use.

2.3. Electrophoretic technique

Electrophoretic separations were carried out with uncoated fused-silica capillaries, 44 cm (37 cm to the detector)×50 μm I.D., provided by Supelco (Bellefonte, PA, USA). The buffer was made of 100 m*M* octanoic acid and 12.5 m*M* ammonia in a mixture of ethanol–methanol (60:40). The amount of water determined in this electrophoretic medium was 0.25%. At the beginning of each working day, the capillary was washed with ethanol–methanol (60:40) for 5 min and with the running buffer for 10 min, while after each injection the capillary was washed with the solvent mixture for 1 min and was equilibrated with the buffer mixture for 10 min. The injections were made at the cathodic side and the applied voltage was −25 kV (reversed polarity mode). The normal polarity mode (+25 kV) was used to measure the cathodic electroosmotic flow μ_{EOF} (current: about 6.5 μA). The separations were performed with the electrolyte solution containing the selector in the reservoir at the cathodic side and with the same electrolyte solution devoid of the selector in the reservoir at the anodic side. The UV detection (at the anodic side) was performed at 214 nm. Injections were made in the hydrodynamic mode for a period of 5 s (corresponding to 13.3 nl) and the capillary was thermostated at 15°C. The resolution (R_s) and the plate number (N) were calculated according to the standard expressions based on peak width at half-height [21]. The asymmetry factor (A_s) was determined using the expression: $A_s = w(0.10)/2F(0.10)$ where $w(0.10)$ is the width of peak at 10% of height and $F(0.10)$ is the distance between the front edge and the top of the peak at 10% of peak height. The selectivity (α) was calculated according to $\alpha = \mu_{e1}/\mu_{e2}$ where $\mu_e = \mu_a - \mu_{EOF}$ (μ_e is the effective mobility, μ_a is the apparent mobility and μ_{EOF} is the electroosmotic mobility).

3. Results and discussion

3.1. Detection of the analytes in the presence of a highly UV absorbing selector

Cinchona alkaloids and their derivatives exhibit high molar absorptivities at the detection wavelength used (214 nm), so that they might give rise to a high absorbance background when they are added to the background electrolyte, making the direct UV detection of the analyte enantiomers difficult.

In order to cope with this problem, the electrolyte

solution introduced in the reservoir at the anodic (detector) side was devoid of the UV absorbing chiral selector [19].

Due to the electrophoretic migration of the positively charged selector towards the cathode (injection side) and the presence of a cathodic electroosmotic flow, the part of the capillary situated close to the detection window was progressively depleted from the chiral selector during the run and after a certain time (6–12 min after the injection, depending of the nature of the chiral selector), a rapid decrease of the background absorbance was observed, indicating that the chiral selector had left the detection window (cf. Fig. 3). Since the migration times of the analyte enantiomers were generally higher than 13 min, they could be detected with high sensitivity, in a portion of selector-free background electrolyte. The breakthrough times, corresponding to the disappearance of the selector from the detection window, were fairly reproducible for a given selector under the same operational conditions. Breakthrough times were somewhat lower, however, for cinchona alkaloids (QN: 7.7 min, QD: 7.9 min, CN: 5.6 min and CD: 6.5 min) than for the carbamoylated derivatives (tBuCQN: 7.3 min, tBuCQD: 7.4 min, DNPCQN: 11.8 min and CHexCQN: 8.6 min) under the same conditions, which might indicate that the latter (DNPCQN in particular) have a more pronounced tendency to interact with the capillary wall. This is confirmed to some extent by the corresponding μ_{EOF} values (in 10^{-5} $cm^2/V{\cdot}s$) obtained with the different selectors. (QN: 2.4, QD: 2.3, CN: 3.0, CD: 2.8, tBuCQN: 2.2, tBuCQD: 2.3, DNPCQN: 1.5 and CHexCQN: 1.8).

3.2. Selection of the experimental conditions for non-aqueous capillary electrophoresis

In previous work [19], different background electrolytes were investigated for the optimization of the NACE method, using DNB-Leu and DNB-Phg as model compounds. A long chain buffer anion which present an electrophoretic mobility similar to that of the SAs was found to improve peak symmetry and efficiency.

The addition of ethanol to methanol gave rise to higher selectivity and resolution values. This solvent with hydrogen bonding properties may provide a better environment for achieving ion-pair interactions in contrast to addition of acetonitrile. The concen-

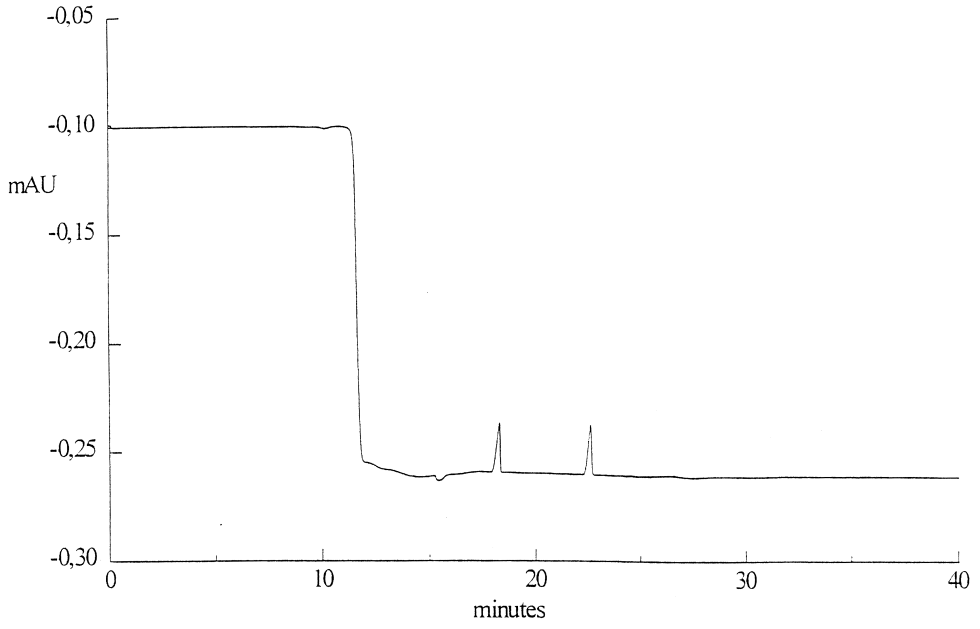

Fig. 3. Enantioseparation of DNB-Phe. Buffer: 100 m*M* octanoic acid and 12.5 m*M* ammonia in methanol–ethanol (40:60) containing 10 m*M* DNPCQN.

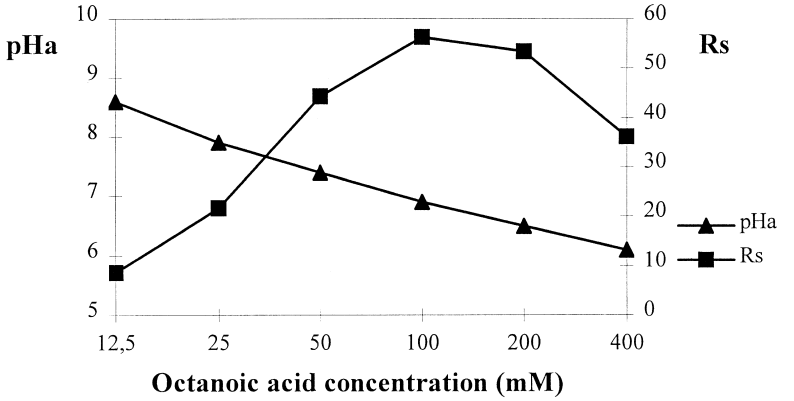

Fig. 4. Influence of the octanoic acid concentration on the pH_a and on the resolution (R_s) of the DNB-Leu enantiomers. Buffer: 12.5 m*M* ammonia and 5 m*M* tBuCQN in methanol–ethanol (40:60). Other conditions as described in Section 2.3.

Table 1
Enantioresolution of amino acid derivatives with QN, QD, CN and CD as selectors[a]

Analyte	QN				QD				CN				CD			
	t_1 (min)	t_2 (min)	α	R_s	t_1 (min)	t_2 (min)	α	R_s	t_1 (min)	t_2 (min)	α	R_s	t_1 (min)	t_2 (min)	α	R_s
DNB-Leu[b]	20.03	21.55	1.057	5.5	22.52	24.80	1.060	8.3	20.12	21.75	1.055	7.0	21.59	23.06	1.046	5.3
DNB-Phg	15.73	16.54	1.041	4.3	14.08	15.04	1.048	6.3	16.25	17.07	1.037	4.8	23.16	24.16	1.028	3.7
DNB-tLeu	19.17	19.61	1.018	2	13.90	14.26	1.019	2.3	18.96	19.49	1.020	2.6	18.59	18.97	1.014	1.8
DNB-αMeLeu	16.40	–[c]	–	–	20.14	–	–	–	15.51	–	–	–	16.51	–	–	–
DNB-NMeLeu	15.60	15.81	1.011	<0.7	21.46	21.75	1.008	<0.7	16.23	16.42	1.009	<0.7	17.78	17.94	1.006	<0.7
DNB-Phe[b]	19.90	20.55	1.025	2.6	14.93	15.54	1.028	3.6	19.08	19.82	1.027	3.1	22.34	23.07	1.022	2.7
DNB-Pro	14.40	–	–	–	15.76	–	–	–	18.83	–	–	–	21.13	–	–	–
DNB-αAbu	18.77	19.92	1.047	4.8	14.77	16.07	1.060	7.6	19.28	20.47	1.043	5.6	18.72	19.69	1.036	4.1
DNB-βAbu	35.04	38.13	1.056	5.8	24.69	27.34	1.061	7.4	44.78	49.85	1.054	6.2	40.11	43.90	1.049	6.6
DNZ-Leu	26.99	27.58	1.015	1.7	24.30	24.84	1.013	1.8	32.60	33.32	1.013	1.7	31.32	31.96	1.012	1.6
DNZ-αMeLeu	19.60	–	–	–	13.43	13.53	1.005	<0.7	24.75	–	–	–	21.84	–	–	–
DNZ-NMeLeu	30.91	31.16	1.006	<0.7	22.59	22.69	1.003	<0.7	32.31	–	–	–	28.44	–	–	–
DNZ-Phe	22.70	–	–	–	19.24	–	–	–	26.31	26.54	1.006	<0.7	24.51	24.77	1.007	0.7
DNZ-βPhe	39.41	–	–	–	27.87	–	–	–	52.04	–	–	–	49.50	–	–	–
DNZ-Pro	30.49	–	–	–	19.11	–	–	–	29.80	–	–	–	27.02	–	–	–
DNZ-αAbu	24.03	24.46	1.013	1.5	20.04	20.40	1.011	1.6	30.14	30.68	1.011	1.4	25.89	26.36	1.012	1.5
DNZ-βAbu	52.51	–	–	–	38.73	–	–	–	48.84	–	–	–	59.14	59.92	1.006	<0.7
DNZ-PA	27.96	–	–	–	22.71	–	–	–	35.08	–	–	–	30.96	–	–	–
Bz-Leu	25.37	26.07	1.020	2.3	20.90	21.55	1.019	2.6	28.41	29.30	1.019	1.7	25.82	26.52	1.017	2.1
Bz-Phe	19.84	20.05	1.008	<0.7	19.74	–	–	–	22.25	22.61	1.011	1	19.11	19.39	1.010	1.2
Bz-βPhe	31.84	–	–	–	30.41	30.66	1.005	<0.7	42.47	43.42	1.012	0.9	31.69	32.12	1.008	0.9
Bz-αAbu	18.63	19.09	1.019	1.8	16.14	16.52	1.016	2	22.94	23.50	1.016	1.4	20.60	21.04	1.014	1.5
Bz-βAbu	39.41	–	–	–	36.98	–	–	<0.7	54.85	56.15	1.011	<0.7	45.06	45.72	1.007	<0.7
Bz-PA	23.55	–	–	–	27.94	–	–	–	30.04	–	–	–	25.26	–	–	–

[a] Conditions as described in Section 2.3.
[b] Data from Ref. [22].
[c] –: No enantiomeric separation observed (R_s<0.5).

tration of octanoic acid was varied and the influence of the resulting pH_a and resolution values was studied (cf. Fig. 4). A significant increase in R_s appeared by lowering the pH_a and an optimum was found at the 100 m*M* octanoic acid concentration (R_s=56.2 for DNB-Leu). The highest enantioselectivity was found at the 200 m*M* concentration (α= 1.653 for DNB-Leu) but at this higher acid concentration μ_e and μ_{EOF} were further decreased, resulting in higher migration times, lower efficiencies and increasing tendency to peak leading. A 100 m*M* concentration of octanoic acid and a 10 m*M* concentration of chiral counter-ion were found to be suitable for all selectors and solutes investigated. Under the conditions selected, good results with respect to the repeatability of migration and resolution were obtained, as illustrated by the RSD values obtained for DNZ-Phe with tBuCQN as selector (0.56, 0.92 and 1.59% for the migration times of the first and the second migrating enantiomer and resolution, respectively).

Using these experimental conditions, the tertiary quinuclidine moiety within the chiral SO (cf. Fig. 1) is protonated and may interact with the negatively charged SAs by ionic interaction to form electrically neutral ion-pairs. Due to their opposite charge, free SO and SAs species exhibit countercurrent-like electrophoretic migration and their overall velocity is also influenced by the μ_{EOF} of the system. On the other hand, the neutral ion-pairs will move only with the μ_{EOF} (cathodic flow). Thus, the free and complexed SA species show significantly different mobilities, a fact that gives rise to high enantioselectivity. The difference between the ion-pair formation

Table 2
Enantioresolution of amino acid derivatives with tBuCQN, tBuCQD, DNPCQN and cHexCQN as selectors[a]

Analyte	tBuCQN				tBuCQD				DNPCQN				cHexCQN			
	t_1 (min)	t_2 (min)	α	R_s	t_1 (min)	t_2 (min)	α	R_s	t_1 (min)	t_2 (min)	α	R_s	t_1 (min)	t_2 (min)	α	R_s
DNB-Leu[b]	15.97	36.14	1.572	64.3	19.84	51.32	1.783	78.3	19.52	23.98	1.151	17.3	19.55	44.58	1.787	66.9
DNB-Phg	15.67	26.65	1.370	48.9	15.00	30.72	1.650	66.4	15.34	17.60	1.107	10.2	14.83	28.86	1.673	59.6
DNB-tLeu	16.97	34.14	1.473	57.3	16.72	36.06	1.673	71.5	16.61	22.72	1.247	24.3	17.17	40.71	1.871	70.8
DNB-αMeLeu	12.31	12.72	1.023	2.8	14.32	15.19	1.046	5.7	14.78	15.36	1.029	3.1	13.96	14.60	1.038	4.3
DNB-NMeLeu	12.95	13.11	1.008	<0.7	15.02	–[c]	–	<0.7	16.55	16.70	1.007	<0.7	16.39	16.54	1.007	<0.7
DNB-Phe[b]	16.94	35.66	1.504	61.1	16.28	38.03	1.759	80	18.30	22.60	1.158	13.4	16.82	40.78	1.905	56.2
DNB-Pro	13.06	–	–	–	17.77	–	–	–	24.93	–	–	–	15.51	–	–	–
DNB-αAbu	16.86	35.50	1.506	61.7	16.07	37.29	1.757	90.8	17.18	20.39	1.130	10.1	16.57	32.61	1.663	55.4
DNB-βAbu	37.64	53.02	1.147	17.1	27.76	61.43	1.544	45.5	41.07	50.76	1.113	11.9	35.52	61.55	1.392	43.5
DNZ-Leu	28.80	38.95	1.153	20.6	25.94	36.22	1.230	25.5	28.04	30.95	1.063	6.8	24.30	31.62	1.209	19
DNZ-αMeLeu	18.95	19.19	1.008	1.1	21.36	21.65	1.010	1.1	20.59	21.27	1.023	2.8	18.95	19.32	1.016	1.8
DNZ-NMeLeu	26.05	26.78	1.015	1.8	25.11	25.75	1.017	1.8	40.91	41.50	1.008	0.9	24.33	24.68	1.011	0.9
DNZ-Phe	20.98	25.40	1.114	14.5	20.47	25.56	1.165	18.2	21.47	26.94	1.162	16.3	21.01	27.19	1.212	19
DNZ-βPhe	40.01	47.21	1.070	9.8	41.58	50.92	1.111	14.7	47.58	55.23	1.074	8.6	37.46	44.56	1.117	11.8
DNZ-Pro	24.55	25.52	1.021	1.4	26.21	27.29	1.027	1.6	35.22	37.13	1.030	2.4	23.45	24.11	1.021	1.1
DNZ-αAbu	23.76	30.62	1.017	19.4	22.03	28.90	1.197	22.5	30.16	34.54	1.084	9.9	20.81	25.99	1.182	17.7
DNZ-βAbu	28.10	32.95	1.082	10.1	33.79	40.84	1.115	11.8	75.06	84.12	1.043	4.2	51.65	59.46	1.082	8.8
DNZ-PA	28.65	29.11	1.008	1.1	26.77	27.09	0.867	0.94	28.77	29.21	1.009	<0.7	27.57	–	–	–
Bz-Leu	24.56	29.11	1.094	12.4	23.69	29.57	1.156	17.1	39.93	41.83	1.025	3.3	24.91	28.45	1.102	11
Bz-Phe	20.85	23.51	1.072	10.1	16.92	19.55	1.112	13.3	16.94	17.77	1.036	3.5	18.57	20.76	1.091	10
Bz-βPhe	37.00	40.49	1.040	5.1	28.83	31.54	1.058	6.7	31.98	32.90	1.017	1.8	30.75	32.66	1.042	4.4
Bz-αAbu	18.95	21.46	1.077	9.6	18.26	20.77	1.097	11.2	18.94	19.57	1.024	1.4	17.90	19.29	1.061	5.5
Bz-βAbu	48.32	52.36	1.030	3.5	38.93	41.46	1.036	2.3	48.86	49.88	1.010	<0.7	44.86	–	–	–
Bz-PA	17.25	–	–	–	19.51	–	–	–	18.09	18.39	1.012	<0.7	22.62	–	–	–

[a] Conditions as described in Section 2.3.
[b] Data from Ref. [22].
[c] –: No enantiomeric separation observed (R_s<0.5).

constants for (*R*)- and (*S*)-enantiomers of the SAs, based on the enantioselective intermolecular interactions mentioned above, represents the other contribution to selectivity in these systems.

3.3. Cinchona alkaloids as chiral selectors

The enantioresolution of all amino acid derivatives was studied first with the four natural cinchona alkaloids under the selected operating conditions.

Using QN, QD, CN and CD, the migration times (t), the enantioselectivity (α) and the resolution (R_s) for the two SAs enantiomers are presented in Table 1.

Rather poor enantioseparations were observed for the amino acid derivatives examined using these SOs. Compared to DNZ and Bz derivatives, the DNB derivatives seem, however, to be favourable with respect to enantiodiscrimination capability of these types of SOs. Using QN and CD, the (*S*)-DNB-Leu enantiomer migrated first but with QD and CN, the (*R*)-DNB-Leu enantiomer migrated first. Higher mobility differences and selectivity values were obtained for DNB derivatives enantiomers using QD and, compared to QN, enantiomeric separations could be achieved for DNZ-αMeLeu, Bz-βPhe and Bz-βAbu. The following order in α values is observed: QD>QN>CN>CD.

3.4. Quinine and quinidine carbamoylated derivatives as chiral selectors

Using tBuCQN, tBuCQD, DNPCQN and cHexCQN, t, α and R_s are presented in Table 2.

In most cases higher resolution values were obtained with these SOs, resulting in the enantioseparation of all the derivatives excepted DNB-Pro and Bz-PA (which is partly separated using DNPCQN). The additional substituent (*tert*.-butyl, dinitrophenyl or cyclohexyl) in the SO and in particular the carbamate function, that may serve as hydrogen donor–acceptor, has obviously a favourable effect on enantioselectivity. It is also worth noting that in the presence of carbamoylated QN derivatives the migration order was reversed compared to QN: the (*R*)-DNB-Leu enantiomer migrated before the (*S*)-DNB-Leu enantiomer with tBuCQN, DNPCQN and cHexCQN. This reversal of migration order, which has also been observed in the corresponding liquid chromatographic enantioseparations with chiral

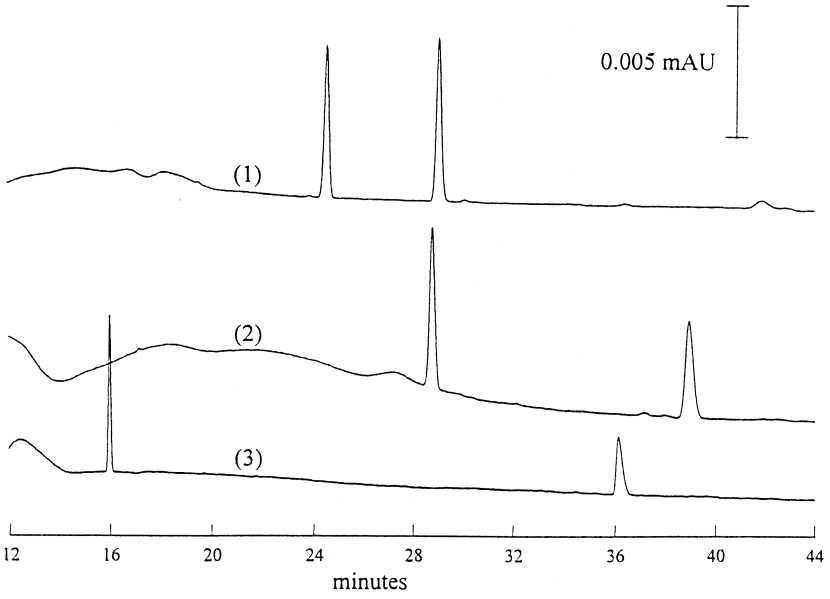

Fig. 5. Enantioseparation of (1) Bz-, (2) DNZ- and (3) DNB-Leu. Buffer: 100 m*M* octanoic acid and 12.5 m*M* ammonia in methanol–ethanol (40:60) containing 10 m*M* tBuCQN.

stationary phases based on QN and carbamoylated QN derivatives as chiral SOs, is probably due to a change of relative binding affinities of the corresponding SA enantiomers and indicates a change in the chiral recognition mechanism. Using tBuCQD, the (*S*)- migrates before the (*R*)-DNB-Leu enantiomer [R_s=78.3, α=1.783, $N(S)$=129 000, $A_s(S)$=0.96] and the enantioselectivities were higher than using tBuCQN [for DNB-Leu, R_s=64.3, α=1.572, $N(S)$=127 000, $A_s(S)$=0.93]. With DNPCQN, lower enantioseparations were observed but Bz-PA enantiomers could be separated. CHexCQN gave selectivities almost as high as tBuCQD. The following order in α values is observed: cHexCQN> tBuCQD>tBuCQN>DNPCQN.

For example, Fig. 5 presents the electropherograms obtained for Bz-Leu, DNZ-Leu and DNB-Leu with tBuCQN as SO.

4. Conclusions

A NACE system using a background electrolyte made of 100 m*M* octanoic acid and 12.5 m*M* ammonia in ethanol–methanol (60:40) was applied to the investigation of the potential of QN chiral derivatives for the enantioseparation of *N*-protected amino acids. The best enantioseparations were obtained using DNB derivatives as SAs and carbamoylated derivatives as SOs (α=1.905 for DNB-Phe using cHexCQN). In further work, the collected enantioselectivity values will be correlated with those obtained in HPLC using the same SOs immobilized onto silica as chiral stationary phase in order to apply this NACE method as a screening tool for a fast evaluation of the chiral discrimination potential of a larger set of newly developed quinine and quinidine derivatives.

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