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Enantiomeric separation of amino acid derivatives by non-aqueous capillary electrophoresis using quinine and related compounds as chiral additives

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INTRODUCTION

Non-aqueous capillary electrophoresis (NACE), employing small amounts of well-characterized chiral additive, offers an attractive alternative for screening potential chiral stationary phase selectors and studying the interactions between the selector and various analytes. In a previous work (Piette *et al.*, 1998), a NACE system using a background electrolyte made from 12.5 mM of ammonium acetate in methanol was found to be useful for the investigation of the potential of quinine and tert-butyl carbamoylated quinine as selectors (SOs) for the enantioseparation of various kinds of N-protected amino acids. The influence of a series of parameters on the enantioseparation of the anionic amino acid derivatives by formation of ion pairs with the cationic chiral selectors in the non-aqueous electrolyte was investigated, using uncoated fused silica capillaries: the composition and concentration of the background electrolyte, the concentration of the chiral selector used as counter-ion, the nature and proportions of organic solvents, the capillary temperature and the applied voltage. High selectivity and resolution were obtained with a background electrolyte made from a mixture of 12.5 mM ammonia and 100 mM octanoic acid, containing the chiral selector at 10 mM concentration in an ethanol–methanol mixture (60:40; v/v).

In this study, under these operating conditions, 3,5-dinitrobenzoyl (DNB) derivatives of leucine (Leu) and phenylalanine (Phe) were examined with respect to selectivity, resolution and migration order for their enantiomers. 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) were tested successively as chiral counter ions.

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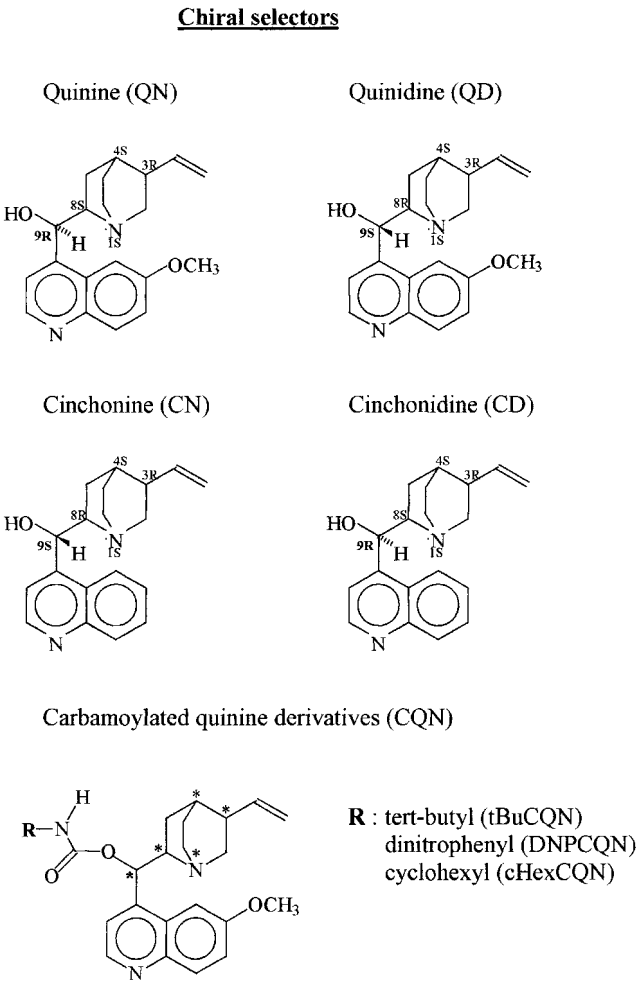

Figure 1. Chiral selectors.

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MATERIAL AND METHODS

All experiments were performed on a Spectraphoresis 1000 CE instrument (Spectraphysics, San Jose, CA, USA) equipped with an automatic injector, an autosampler, a UV/visible detector (190–800 nm) and a temperature control system (15–60°C). 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 (Lindner *et al.*, 1997). The organic solvents were HPLC grade: ethanol absolute from Merck (Darmstad, Germany) and methanol from Fisher Scientific (Leicestershire, UK). Octanoic acid was from Sigma and ammonia solution 25% from Carlo Erba (Rodano, Italy). The racemic and enantiomerically pure amino acids leucine (Leu) and phenylalanine (Phe) were purchased from Sigma. The DNB-derivative of Phe was synthesized according to a standard derivatization procedure (Kleidernigg *et al.*, 1996), while DNB-Leu was obtained from Sigma. 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.

Electrophoretic separations were carried out with uncoated fused-silica capillaries, 50 μm i.d. and 44 cm length (37 cm to the detector), provided by Supelco (Bellefonte, PA, USA). 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} ($i \approx 6.5$ μA). 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 thermostatted at 15°C. The resolution (*Rs*) was calculated according to the standard expressions based on peak width at half-height, and 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).

RESULTS AND DISCUSSION

The enantioresolution of the DNB derivatives of Phe and Leu was studied with the eight different SOs. Under the selected operating conditions, the tertiary quinuclidine moiety within the chiral SO is protonated and interacts with the negatively charged selectands (SA) to form neutral ion pairs moving with the cathodic electroosmotic flow. Thus the free and the complexed SA species have significantly different mobilities, a fact that gives rise to high enantioselectivity.

For the amino acid derivatives using the natural alkaloids as SOs, rather poor enantioseparations were observed. Higher mobility differences and selectivity values were obtained with the alkaloid derivatives (tBuCQN, tBuCQD, DNPCQN and cHexCQN). The additional substituent (tert-butyl, dinitrophenyl or cyclohexyl) in the SO and in particular the carbamate function, which may serve as hydrogen donor–acceptor, obviously has a favourable effect on enantioselectivity. For the DNB–Leu and the DNB–Phe enantiomers, the migration times (*t*), the enantioselectivity (α) and the resolution (*Rs*) are presented in Table 1.

The following order in α values was observed for DNB-Leu: cHexCQN > tBuCQD > tBuCQN > DNPCQN > QD > QN > CN > CD. The highest α (1.905) was obtained for the DNB–Phe enantiomers using cHexCQN and the highest *Rs* (80.0) for the same enantiomers with tBuCQD. In the presence of carbamoylated QN derivatives the migration order was reversed compared to QN. With QN, CD and tBuCQD, the (*S*)-DNB-Leu enantiomer was migrating first, but with QD, CN, tBuCQN, DNPCQN and cHexCQN the (*R*)-DNB-Leu enantiomer was migrating first. Using all these selectors, the enantiomers of DNB-Leu and DNB-Phe were completely separated. For example, Fig. 2

Table 1. Enantioresolution of DNB–Leu and DNB–Phe with natural and derived alkaloids as selectors[a]

	DNB-Leu				DNB-Phe			
Chiral selector	t_1 (min)	t_2 (min)	α	*Rs*	t_1 (min)	t_2 (min)	α	*Rs*
QN	20.03	21.55	1.057	5.5	19.90	20.55	1.025	2.6
QD	22.52	24.80	1.060	8.3	14.93	15.54	1.028	3.6
CN	20.12	21.75	1.055	7.0	19.08	19.82	1.027	3.1
CD	21.59	23.06	1.046	5.3	22.34	23.07	1.022	2.7
tBuCQN	15.97	36.14	1.572	64.3	16.94	35.66	1.504	61.1
tBuCQD	19.84	51.32	1.783	78.3	16.28	38.03	1.759	80.0
DNPCQN	19.52	23.98	1.151	17.3	18.30	22.60	1.158	13.4
cHexCQN	19.55	44.58	1.787	66.9	16.82	40.78	1.905	56.2

[a] Conditions as described in Material and Methods.

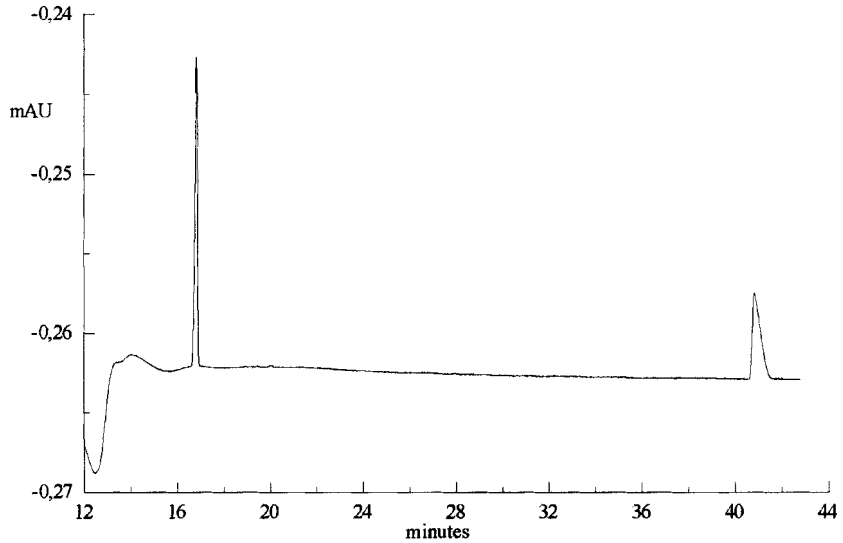

Figure 2. Enantioseparation of DNB–Phe with cHexCQN as chiral selector. Buffer: 100 mM octanoic acid and 12.5 mM ammonia in methanol–ethanol (40:60) containing 10 mM cHexCQN. Other conditions as described in Material and methods.

presents the electropherogram obtained for DNB–Phe with cHexCQN as SO.

CONCLUSIONS

A NACE system using a background electrolyte made from 100 mM octanoic acid and 12.5 mM ammonia in an ethanol–methanol mixture (60:40) was applied to the investigation of the potential of QN, other cinchona alkaloids and derivatives for the enantioseparation of N-protected amino acids. Particularly high enantiomeric resolution values (up to 80) were obtained for DNB amino acids using tert-butyl carbamoylated derivatives of quinine and quinidine as well as cyclohexyl carbamoylated quinine as chiral selectors.

In further work, the collected enantioselectivity values will be correlated with those obtained in HPLC using the same SOs imobilized onto silica as chiral stationary phase in order to apply this NACE method as a screening tool to evaluate the enantiodiscrimination potential of a larger set of newly developed chiral SOs derived from quinine and related alkaloids.

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