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Optimisation methodology in the chiral and achiral separation in electrokinetic chromatography in the case of a multicomponent sample of dansyl amino acids

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ABSTRACT

Two different chiral selectors synthesised in our laboratory were used to test the possibility of separation for a sample consisting of ten different enantiomeric pairs of dansyl-derivatives of α-amino acids in electrokinetic chromatography. It was possible to observe all the peaks, though only partly resolved, due to the twenty analytes through an accurate strategy of choice of the experimental conditions. As a part of this strategy, a procedure of identification of the single peaks in the electropherograms called LACI (lastly added component identification) has been developed.

1. Introduction

Cyclodextrins are natural cyclic oligosaccharides which, owing to their molecular recognition ability and to their chirality, represent one of the most important class of chiral selectors [1,2]. Their peculiar properties have been much exploited in separation science [3,4], and particularly in capillary electrophoresis [5–11]. However, in this technique, an important limit is represented by their electrical neutrality, which excludes their use for the separation of neutral analytes. Also, as regards charged analytes, there is no doubt that the presence of a charge in the selector molecule makes the separation easier, particularly between enantiomers, by increasing the difference of electrophoretic mobility between the free and the complexed analyte [12–14].

In a series of papers [15–17], the application of cyclodextrin derivatives of different kinds, synthesised in our laboratory, to chiral separations in capillary electrophoresis (CE) has been described. All of them include a moiety with acid–base properties, which can thus become charged as a function of the pH value.

Along with other kinds of derivatives obtained by fluorescein isothiocyanate derivatisation, recently investigated by some of us [17,19], special attention has been paid to the dansyl derivatives of amino acids (DNS-AAs) [20–22], a class of chiral analytes which has been deeply studied in literature [23–26].

The chiral selectors that were used represent different kinds of cyclodextrin derivatives. Together with a γ-cyclodextrin derivatised on a secondary position, the 3-deoxy-3-amino-2(S),3(R)-γ-cyclodextrin (GCD3AM) [27], three selectors are hemispherodextrins [21,22], a class of cyclodextrin derivatives specifically designed in our laboratory, and characterised by the presence of a bridge between two opposite positions of the primary rim, in addition to the well known cyclodextrin cavity.

Given the resolution values obtained in the separation between the members of enantiomeric pairs of DNS-AAs, also in the presence of a very low concentration of the chiral selector (in some cases nearly equal to the concentration of the analytes), it can be seen how effective these molecules are for the chiral separation of this class of analytes.

As a further step in this project, tackling the problem of the contemporary separation of the investigated enantiomeric pairs was considered.

The task of separating twenty different analytes in one run appears hard, and this was also confirmed by some preliminary experiments, thus requiring an accurate strategy. Three parameters, in our opinion, appear to be the most important, namely the pH value of the BGE (background electrolyte), the analytical concentrations and the electrical field.

Apart from this last parameter, which is clearly strictly related to the electrophoretic experiment, the other two parameters are so important because they influence the equilibria occurring in solution during the run.

The pH value is strictly related to the protonation constants of both the analytes and selector, determining what the main species

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present will be, besides obviously influencing the EOF (electroosmotic flow).

This paper specifically tries to address the task of optimising the complexation equilibria just by making an accurate choice of BGE pH and analytical concentration of the selector instead of a purely empirical variation of them, as usually done in literature.

Thus, it has been decided to proceed by developing a specific methodology, and here are included the results obtained in the separation of ten enantiomeric pairs of DNS-AAs out of the eleven shown in Fig. 1 using as chiral selectors both GCD3AM [20] (without the serine pair) and the 6A,6D-dideoxy-6A,6D-N-[6,6′-di-(β-alanylamido)]-6,6′-dideoxy-α,α′-trehalose]-β-CD (THALAH) [22] (without the threonine pair), also shown in Fig. 1.

2. Experimental

2.1. Materials

The syntheses of GCD3AM [27] and of THALAH [22] have been previously reported.

Dns-amino acids were obtained from Fluka, Sigma–Aldrich, Buchs, Switzerland, NaOH and CH_3COONH_4 were purchased from Merck, Darmstadt, Germany.

2.2. Measurements

CE measurements were carried out on a Beckman P/ACE MDQ (Beckman-Coulter, Fullerton, CA, USA) equipped with a diode array detector.

An uncoated fused-silica capillary (Beckman, 61.0 cm total length, 51.0 cm effective length, 75 μm i.d.) was held at a constant temperature of 25 °C.

The system operated at a variable voltage varying in the 20–30 kV range.

BGEs for the separation experiments were prepared by dissolving CD derivatives (1.5–3 mM) in 20 mM of ammonium acetate at pH = 6.8.

The sample solutions (0.03 mM in each racemate) were obtained by dissolving the analytes in Milli-Q water.

The samples were hydrodynamically injected at 0.6 psi for 8 s.

Before each experiment, the capillary was flushed with 100 mM of NaOH (pressure of 20 psi for 5 min), 20 mM of CH_3COONH_4 (pressure of 20 psi for 8 min) and the BGE used in separation (pressure of 20 psi for 1 min).

The reported electropherograms were obtained at λ = 218 nm. A solution of acetone (3%) was used, as neutral marker, to evaluate the EOF mobility.

Values of the mobilities, corrected for the EOF (μ_{corr}), of selectivity factor S [28] and resolution R_s were calculated, as usually, by the following formula:

$$\mu(\text{anal}) = \frac{L \times l}{\Delta V \times t \times 60}; \quad \mu(\text{EOF}) = \frac{L \times l}{\Delta V \times t_{(\text{EOF})} \times 60};$$

$$S = 2\frac{\mu_2 - \mu_1}{\mu_1 + \mu_2}; \quad R_s = 2\frac{t_2 - t_1}{w_1 - w_2} \quad \mu_{corr} = \mu_{(\text{anal})} - \mu_{(\text{EOF})}$$

where, L = capillary total length; l = capillary effective length; ΔV = applied voltage; t = observed time of the analyte (minutes); t(EOF) = observed time of the EOF marker (minutes); w = peak width (minutes).

3. Results and discussion

What will be discussed is the influence of three different parameters on the separations of the enantiomeric pairs of DNS-AAs reported in Fig. 1, using as selector THALAH, and then the method for identifying the single analytes which have been developed will be shown, by using the system with the selector GCDAM3 as an example.

Table 1
Mobility values (10^{-9} m^2 V^{-1} s^{-1}) corrected for EOF (μ_{corr}), at two different pH values for the free analytes.

DNS-AAs	μ_{corr}	
	pH = 4.7	pH = 9.3
dns-asp	−17.53	−27.44
dns-met	−12.92	−14.55
dns-glu	−16.23	−26.80
dns-ser	−13.04	−16.02
dns-aba	−12.30	−15.58
dns-val	−11.58	14.94
dns-leu	−12.00	−14.46
dns-nrv	−12.20	−15.00
dns-nrl	−12.24	−14.73
dns-phe	−11.59	−14.87

3.1. Varying the pH value of the BGE

First of all, it is worth taking into account the acid-base properties both of the selector and of the analytes. All the data were directly determined by following the variation of the electrophoretic mobility in the function of the BGE pH. The selector, in absence of metal ions, behaves as a diamine, thus undergoing a double protonation ($\log K_1 = 7.7$; $\log K_2 = 5.2$, values determined elsewhere [22]).

After dansylation, the amino acid has a carboxylic group, while the primary amino group is substituted by the tertiary amino group of the naphthyl ring, whose basicity is very low ($\log K < 4.5$). The protonation values of the carboxylate are quite higher than in the parent amino acid ($\log K < 3.5$). It should be remembered that the derivatives of glutamic and aspartic acid are exceptions, due to the presence of a second carboxylate group ($\log K_1 > 5.5$).

As a test of the utility of the electrophoretic data in order to follow the protonation of the components of the investigated system, the electrophoretic mobilities of our analytes at two different pH values, reported in Table 1, should be considered. It is readily apparent that at alkaline pH the mobilities for aspartate and glutamate derivatives are far higher than for all the other analytes, due to their double negative charge, compared with one negative charge for all the other analytes. Going to pH = 4.7, while the two dianionic species undergo a partial protonation, becoming monoanionic, the other analytes slightly decrease the absolute value of their mobility, thus showing that the protonation in their cases is only started, and the anion is still the main species.

These protonation data are important for two different reasons.

One reason is that the electrostatic interaction between analyte and selector makes a significant contribution to the effectiveness of the separation, as easily shown in the separation of the DNS-AAs with GCD3AM [20], carried out at three different pH values. The influence of the selector on the increase of the mobilities of the analytes was maximum in the case of pH = 6.8, where almost all the selector is cationic and the analytes are almost completely deprotonated (though, obviously, in the cases of aspartate and glutamate the species will be dianionic).

The second reason is that at pH values near to the $\log K$ values, the influence of the pH on both the formation degrees of the species and consequently on the observed mobility values of the analytes is very strong.

Upon investigating the systems with the contemporary presence of an enantiomeric pair of DNS-AA and THALAH, it was readily seen that, in order to optimise the separation process, due to the overlapping between the pH ranges of protonation of analyte and