Journal of Chromatography, 639 (1993) 375–378
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 023

Short Communication

Separation of diastereomeric derivatives of enantiomers by capillary zone electrophoresis with a polymer network: use of polyvinylpyrrolidone as buffer additive

W. Schützner[☆]

Institute for Analytical Chemistry, University of Vienna, Währingerstrasse 38, A-1090 Vienna (Austria)

S. Fanali

Istituto di Cromatografia, Consiglio Nazionale delle Ricerche, Area della Ricerca di Roma, Via Salaria km 29.300, 00016 Monterotondo Scalo, Rome (Italy)

A. Rizzi* and E. Kenndler

Institute for Analytical Chemistry, University of Vienna, Währingerstrasse 38, A-1090 Vienna (Austria)

(First received December 1st, 1992; revised manuscript received February 26th, 1993)

ABSTRACT

Diastereomeric derivatives of D- and L-tryptophan obtained by reaction with (+)-diacetyl-L-tartaric anhydride were separated using capillary zone electrophoresis with polyvinylpyrrolidone as polymeric additive to the separation buffer. This additive is able to undergo hydrophobic as well as dipolar interactions with the sample components and in this way influences the effective mobility of the analytes. As this influence is of different strength for the two diastereomers, the selectivity for these separands is enhanced. The application of such physical networks as a kind of "pseudophase" is of general significance as this method mirrors reversed-phase chromatography using aqueous mobile phases.

INTRODUCTION

The separation of enantiomeric compounds has gained widespread interest in the past decade and is still an important challenge for modern separation techniques. Gas chromatography, supercritical fluid chromatography and especially high-performance liquid chromatography (HPLC) have been the most frequently used techniques for the separation of enantiomers of compounds with a broad structural variety. Different separation strategies are applied within these chromatographic techniques. One is based on the application of chiral selectors in the chromatographic system, as a constituent of

* Corresponding author.
☆ Present address: Istituto di Cromatografia, Consiglio Nazionale delle Ricerche, Area della Ricerca di Roma, Via Salaria km 29.300, 00016 Monterotondo Scalo, Rome, Italy.

0021-9673/93/$06.00

either the stationary or the mobile phase. The alternative strategy is based on the conversion of the enantiomers into diastereomers by reacting them with an optically pure agent. These diastereomers can be separated on commonly employed non-chiral phases such as reversed-phase systems.

The concept of chiral selection has been transferred to electrophoretic methods, especially to isotachophoresis and capillary zone electrophoresis (CZE) (see, for example, refs. 1–3). In this case, it is used to try to influence the effective mobilities of the individual enantiomeric species to a different extent by utilizing interactions with chiral selectors, which are either easily soluble additives to the separation buffer or compounds that form chiral micelles. Soluble chiral selectors are successfully applied if complexes with different association constants are formed. This has been shown with chiral chelator–metal complexes [4,5] and with cyclodextrines and their derivatives [6–8], the latter forming inclusion complexes with the separated compounds. Chiral micelles [9–12], acting as a kind of "pseudophase", were introduced in analogy with chiral phases in chromatography.

This paper deals with the approach of the indirect separation of enantiomers by CZE, namely via their diastereomeric derivatives. In this case the analytes are transformed into compounds with chemically different properties. Such separations have been reported previously, *e.g.* by applying sodium dodecyl sulphate (SDS) micelles [13–15].

In the present paper a non-chiral physical network is applied instead of the charged SDS micelles as an additive to the separation buffer, namely polyvinylpyrrolidone (PVP). It is used as a polymer that is able to offer hydrophilic and hydrophobic interactions with the analytes. This paper illustrates the particular example, using diastereomeric tryptophan derivatives, of the amplification of the differences in the effective mobilities of the diastereomers. However, it is meant to be typical of the general approach of using interactions between the chains of physical networks and analytes to obtain separation.

EXPERIMENTAL

Chemicals and reagents

The chemicals used had the following specification: DL-, D- and L-tryptophan (Serva, Heidelberg, Germany), benzene-1,3-disulphonic acid (Fluka, Buchs, Switzerland) and (+)-diacetyl-L-tartaric anhydride (Aldrich, Steinheim, Germany) were all reagent grade; polyvinylpyrrolidone 15 (mean molecular mass 11 000) (Serva); $NaH_2PO_4 \cdot H_2O$, $Na_2HPO_4 \cdot 12H_2O$, NH_4OH (30%), HCl (37%) and NaOH were all "purum" quality (all from Carlo Erba, Milan, Italy). Bidistilled water was used (Menichelli, Rome, Italy). Acetonitrile was of "HiPerSolv" quality (for HPLC, BDH, Poole, UK). For the coating of the capillary 3-(trimethoxysilyl) propylmethacrylate (purum, Fluka), acrylamide, $K_2S_2O_8$ and N,N,N′,N′-tetramethylethylenediamine (TEMED) (all of electrophoresis purity; Bio-Rad Labs., Richmond, CA, USA) were used.

Apparatus

The zone electrophoretic measurements were carried out using the apparatus previously described [5], consisting of a separation capillary (kept at ambient temperature without thermostating equipment), a high-voltage power supply (Series EH; Glassmann, Whitehouse Station, NJ, USA) and a variable-wavelength UV detector (Model 2250; Varian, Palo Alto, CA, USA), connected to an integrator (Chromatopac C-R5A; Shimadzu, Kyoto, Japan).

The fused-silica separation capillary (Polymicro Technologies, Phoenix, AZ, USA) had the following dimensions: 100 μm inner diameter, 54 cm total length, 38 cm effective length to the detector. It was coated to eliminate electroosmosis [16].

The injection of the sample was carried out hydrodynamically by keeping both ends of the capillary for 5–10 s (depending on the viscosity of the buffer solution) at a height difference of about 10 cm.

Procedures

D-, L- and DL-tryptophan were reacted with (+)-diacetyl-L-tartaric anhydride to form the

corresponding tartaric acid (mono)amides using a procedure similar to that described in ref. 17: 10 m*M* tryptophan was dissolved in 25 ml of acetonitrile, and water was removed by distillation under water-jacket vacuum at 40°C for 1 min. After cooling, 20 m*M* (+)-diacetyl-L-tartaric anhydride was added and the reaction mixture was kept at 50°C for 20 h. Then acetonitrile was removed by distillation, and to the residue a 3% aqueous solution of NH_4OH was added, followed by redistillation. The final product was dried in a desiccator.

Suppression of electroosmosis was controlled by the mobility values measured from at least day-to-day injections of a reference compound (benzene-1,3-disulphonic acid) and comparison of the obtained values with the literature [18]. The constancy of this value and its agreement with the literature values allowed the conclusion that electroosmotic flow was negligible.

RESULTS AND DISCUSSION

The influence of the concentration of PVP on the migration times of the diastereomers of D- and L-tryptophan and of the reference compound benzene-1,3-disulphonate can be seen from Table I. All migration times increase with increasing concentration of PVP. The variation in the migration time of the reference component reflects, at least in part, the change in viscosity of the solution due to the addition of the polymer. The most important effect, however, is the induced difference in the migration behaviour of the diastereomers at PVP concentrations higher than 1%: the L-isomer migrates more slowly than the D-isomer. This leads to selectivity coefficients, r_{ij}, for the diastereomeric compounds i and j, larger than 1.

These selectivity coefficients (Table I) continuously increase from 1.00 at 0% PVP to 1.018 at 6% PVP. The value of 1.00 (0% PVP) is obtained within the measuring error at the pH of the separation electrolyte system; under this condition the diastereoisomers could not be separated. As the separation efficiency changes only moderately upon the addition of PVP (about a 30% decrease in the plate numbers was measured for the higher PVP concentrations), the increase in selectivity results in the separation of the analytes. It can clearly be observed from Fig. 1 that the resolution continuously increases with increasing concentration of PVP, leading finally to baseline separation of the two diastereomeric derivatives at 6% PVP.

The diastereomers, which have equal effective mobilities at 0% PVP under the given conditions, are separated by the different strength of their interaction with the polymer chains. This kind of mechanism, demonstrated here with tryptophan derivatives as analytes and PVP as a physical network, is supposed to be of wide

TABLE I

MIGRATION TIMES, t_{Ri}, AND SELECTIVITY COEFFICIENTS, r_{LD}, OF THE DIASTEREOMERIC D- AND L-TRYPTOPHAN DERIVATIVES DEPENDING ON THE CONCENTRATION OF POLYVINYLPYRROLIDONE (PVP)

Concentration of PVP (%, w/v)	t_{Ri}			r_{LD} [a]
	D-Derivative	L-Derivative	Benzene-1,3-disulphonate	
0	10.8[b]		5.61	1.00
1	10.9[b]		5.90	1.00
2	12.08	12.18	7.14	1.008
3	11.52	11.65	6.68	1.011
4	12.11	12.27	6.85	1.013
5	13.50	13.72	7.35	1.016
6	13.67	13.91	7.81	1.018

[a] The selectivity coefficients are defined as the ratios of the effective mobilities of the separands.
[b] The migration times of the diastereomers cannot be distinguished within the measuring error.

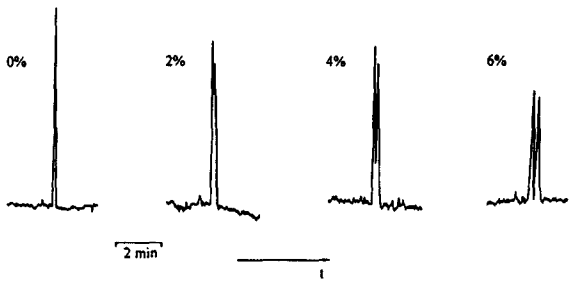

Fig. 1. Influence of the concentration of polyvinylpyrrolidone in the buffering electrolyte on the separation of the diastereoisomeric D- and L-tryptophan diacetyl-L-tartaric acid monoamides. The concentration of polyvinylpyrrolidone (mean molecular mass 11 000) is given in %(w/v), as indicated. Separation conditions: phosphate buffer pH 6.35, concentration 0.016 mol/l; coated capillary: total length 54 cm (38 cm to detector); applied voltage 10 kV; temperature 24°C; analyte concentration (in water): 10^{-4} mol/l; detection: UV absorbance at 233 nm.

applicability to other interactive additive/analyte systems, and is analogous to that operating in chromatographic reversed-phase systems.

This approach in fact seems to exhibit a general significance for the adjustment of selectivity in capillary electrophoresis far beyond the separation of diastereomeric compounds. It is an example of the important features of physical networks (polymers and gels) in CZE besides their discrimination properties according to the size of large analytes (as applied, for example, as a sieving medium for the separation of SDS complexes of proteins [19]).

ACKNOWLEDGEMENT

Financial support for this work by the "Austrian Academic Exchange Service" (project funded by the technical-scientific agreement between Austria and Italy) is acknowledged.

REFERENCES

1 J. Snopek, I. Jelinek and E. Smolkova-Keulemansova, *J. Chromatogr.*, 452 (1988) 571.
2 J. Snopek, I. Jelinek and E. Smolkova-Keulemansova, *J. Chromatogr.*, 609 (1992) 1.
3 R. Kuhn and S. Hoffstetter-Kuhn, *Chromatographia*, 34 (1992) 505.
4 A.S. Cohen, S. Terabe, J.A. Smith and B.L. Karger, *Anal. Chem.*, 59 (1987) 1021.
5 S. Fanali, L. Ossicini, F. Foret and P. Bocek, *J. Microcol. Sep.*, 1 (1989) 190.
6 J. Snopek, I. Jelinek and E. Smolkova-Keulemansova, *J. Chromatogr.*, 438 (1988) 211.
7 S. Fanali, *J. Chromatogr.*, 474 (1989) 441.
8 S. Fanali, *J. Chromatogr.*, 545 (1991) 437.
9 A.S. Cohen, A. Paulus and B.L. Karger, *Chromatographia*, 24 (1987) 15.
10 S. Terabe, M. Shibata and Y. Miyashita, *J. Chromatogr.*, 480 (1989) 403.
11 A. Dobashi, T. Ono, S. Hara and J. Yamaguchi, *J. Chromatogr.*, 480 (1989) 413.
12 K. Otsuka, J. Kawahara, K. Tatekawa and S. Terabe, *J. Chromatogr.*, 559 (1991) 209.
13 H. Nishi, T. Fukuyama and M. Matsuo, *J. Microcol. Sep.*, 2 (1990) 234.
14 I.S. Lurie, *J. Chromatogr.*, 605 (1992) 269.
15 A.D. Tran, T. Blanc and E.J. Leopold, *J. Chromatogr.*, 516 (1990) 241.
16 St. Hjerten, *J. Chromatogr.*, 347 (1985) 191.
17 W. Lindner, Ch. Leitner and G. Uray, *J. Chromatogr.*, 316 (1984) 605.
18 Ch. Schwer and E. Kenndler, *Chromatographia*, 33 (1992) 331.
19 A. Widhalm, Ch. Schwer, D. Blaas and E. Kenndler, *J. Chromatogr.*, 549 (1991) 191.