ELSEVIER

Journal of Chromatography A, 832 (1999) 265–271

JOURNAL OF
CHROMATOGRAPHY A

Short communication

Use of micellar partition in capillary isotachophoretic focusing

Miroslava Št'astná, Karel Šlais*

Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, Veveøí 97, 611 42 Brno, Czech Republic

Received 22 September 1998; received in revised form 27 November 1998; accepted 30 November 1998

Abstract

The influence of micelles of nonionogenic surfactant in the leading electrolyte on improvement of separation of dinitrophenyl and dansyl derivatives of amino acids and position isomers of nitrophenols by isotachophoretic focusing was studied. The use of micelles enabled or improved the separation of above mentioned substances. The method combines in a single step the advantage of improvement in separation efficiency by use of micelles, applied for this purpose mostly in capillary zone electrophoresis (micellar electrokinetic chromatography), with the advantage of analyte focusing by the isotachophoretic technique. The experiments were carried out in instrumentation with a tapered channel in the separation compartment. At least a 10-fold increase of concentration of analytes in detector in comparison with sample and complete analyses at applied voltage up to 1 kV was achieved. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Isotachophoresis; pH gradients; Conductivity gradients; Buffer composition; Amino acids; Nitrophenols; Phenols

1. Introduction

Use of surfactants is quite common in capillary electrophoretic (CE) techniques. They are usually applied for the modification of inner capillary surface in order to suppress the undesirable electroosmotic flow and/or to prevent the sorption of analytes on the capillary wall [1–3].

In micellar electrokinetic capillary chromatography (MEKC) [4,5] which can be considered as a modification of high-performance capillary zone electrophoresis (CZE), an improvement of the separation efficiency is made by using micelles which are formed when the surfactant concentration in the running buffer is above its critical micellar concentration (CMC). Micelles create a so-called micellar pseudophase [6] or pseudo-stationary phase [7] and both neutral and ionic analytes can be separated on the basis of differential partition between electrophoretically migrating micellar pseudophase and the surrounding aqueous phase and according to their different effective mobilities, respectively. Many micelle structures have been proposed. The simplest and generally accepted model of an aqueous micelle is a sphere with a hydrophobic core and hydrophilic groups located on the micellar surface. More complicated micellar structures which better explain the micelle–analyte interaction in the separation process and micelle shape changes with their increasing concentration have also been reported [6,8]. In both CZE and MEKC a fundamental drawback appears: the analyte zone is diluted during its migration towards detector and therefore a large injection

*Corresponding author. Tel.: +42-5-7268211; fax: +42-5-41212113.

0021-9673/99/$ – see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(98)01016-4

volume is needed to obtain the reliable solute detection of diluted sample. This problem is eliminated in isotachophoresis (ITP), where the zone is focused during the transport to the detector and a smaller injected concentration of sample in comparison to CZE is sufficient.

Particularly in ITP, cyclodextrins or crown ethers have been applied in order to improve the separation process through the inclusion mechanism [9–13]. Using micelles for this purpose still has not been reported in ITP to date.

In the present paper the recently suggested and experimentally verified method of ITP with combined pH and conductivity gradient generated by isotachophoretic moving of synthetic carrier ampholytes [14,15] called isotachophoretic focusing (ITF) [16] was applied. In this method the Gaussian zones are detected in detector, which is similar to CZE but it is an advantage in comparison to standard ITP. ITF can be used for separation and focusing of both weak and strong electrolytes [15].

To demonstrate the influence of micelles in ITF, we have used known and well characterized compounds which were also well separated by MEKC, CZE or EKC with cyclodextrins, namely dinitrophenyl (DNP-) amino acids [17], dansyl (Dns-) amino acids [18,19] and nitrophenols [20–22].

The aim of this contribution was to show the influence of the micellar phase and its increasing concentration on separation performance of above mentioned weak and strong anions by ITF.

2. Experimental

2.1. Apparatus

Instrumentation for CE was assembled and partly constructed in our laboratory and was described in more detail previously [23,24]. Separation compartment of apparatus included the tapered channel [25–28] of volume 3 μl (inlet 0.8 mm I.D., outlet 0.4 mm I.D.) fabricated into organic glass followed by capillary of volume either 4 μl (50×0.32 mm I.D.) or 8 μl (100×0.32 mm I.D.) and detection fused-silica capillary of volume 2.5 μl (50×0.25 mm I.D.) to the detector. The total effective capillary length in separation compartment related to the 0.25 mm I.D. of detection fused-silica capillary was either 190 or 280 mm. When it is not indicated otherwise, all experiments were performed with capillary of effective length 190 mm. A tapered channel was used to decrease of total applied voltage [23,26] and capillary inserted between tapered channel and detection fused-silica was applied in order to increase the total volume of the separation compartment [15]. The reservoir of terminating electrolyte (TE) is separated from separation compartment by cellophane membrane, so the apparatus is closed at one end. The electroosmotic flow was suppressed by dynamic modification of capillary wall by polyethylenimine (PEI) and hydroxypropylmethylcellulose (HPMC) added to the leading electrolyte (LE). Other details of this instrumentation were described in previous papers [23]. A modified LCD 2082 UV detector (ECOM, Prague, Czech Republic) with on-column optical fibers arrangement was used to detect analytes [29]. High-voltage Spellman power supply (CZE 1000R, New York, USA) connected to the electrodes in LE and TE was applied.

2.2. Reagents

All chemicals used were of analytical-reagent grade. Trihydroxymethyl-aminomethane hydrochloride (Tris·HCl) and 2-(cyclohexylamino)-ethanesulfonic acid (CHES) were from Merck (Darmstadt, Germany). Trihydroxymethylaminomethan (Tris) and KOH were obtained from Lachema (Brno, Czech Republic), HPMC from Sigma (St. Louis, MO, USA) and PEI from Serva (Heidelberg, Germany). The solution of synthetic carrier ampholytes ampholine, pH 3.5–10.0, was purchased from Pharmacia (Uppsala, Sweden). Tergitol NPX (ethoxylated nonylphenol), *o*-nitrophenol, *m*-nitrophenol and *p*-nitrophenol, DL-asparagine were from Fluka (Buchs, Switzerland), DL-leucine, L-proline, L-hydroxyproline from Lachema and DL-aspartic acid, DL-threonine from Merck.

Chemicals used for derivatization of amino acids 5-dimethylamino-1-naphtalenesulfonyl chloride (dansyl chloride), acetonitrile were from Merck, 2,4-dinitrofluorobenzene (DNFB) from International Enzymes (Windsor, UK), Li_2CO_3, Na_2CO_3, HCl and ethanol from Lachema.

Solutions of amino acids derivatives were pre-

pared in our laboratory using either dansyl chloride according to the procedure described in [30] or DNFB according to the procedure developed earlier [31].

2.3. Electrolyte systems

LE (pH 8) consisted of 10 mmol l^{-1} solution of Tris·HCl, 10 mmol l^{-1} solution of Tris, 0.06% HPMC and 2.5 μg ml^{-1} PEI. TE was solution of 50 mmol l^{-1} CHES and 5 mmol l^{-1} KOH. Although the pH values in zones of anions following the LE zone were expected to be above pH 8, we have used the same kind of carrier ampholytes, i.e. ampholine 3.5–10.0 (8% solution), in order to be consistent with our previous experiments [23,24]. Solutions of Dns- and DNP-amino acids were injected after appropriate dilution.

3. Results and discussion

Figs. 1–3 illustrate the dependencies of separation efficiency and selectivity of nitrophenols, DNP- and Dns-amino acids on the increasing concentration of nonionic surfactant Tergitol in LE in the range 20–80 mmol l^{-1}. CMC of Tergitol is 0.2 mmol l^{-1} (average number of ethoxy groups equals 9). Thus, most of surfactant is in the form of micelles for concentrations applied here. It can be seen that with increasing Tergitol concentration the retention time prolongs and the shape and resolution of peaks improve. The time shift observed for the different surfactant concentration could probably be explained by the rest electroosmosis on membrane between TE and separation channel. It is known [33] that cellulose membrane has the small electroosmosis dependent on the surrounding solution. Another explanation of the overall time shift may be in the increase in the LE conductivity by ionic admixtures in tenside. Indeed, we have found that the 50 mmol l^{-1} water solution of our sample of Tergitol has conductivity 0.12 mS. Thus, the focused peaks always migrate within the carrier ampholyte gradient. Since the migrations times change differently for different peak pairs and both electrophoretic migration and electroosmosis move the all zones uniformly under steady state, the selectivity changes can be explained only by incorporation of analytes into the micelles. The pH window of the carrier ampholytes gradient was calculated from 8.07 to 8.96 pH units for our experimental conditions by means of Becker's procedure [32]. The conductivity of TE was calculated about 10-fold lower than that for LE. The time window of the carrier ampholytes was found to be about 15 min, following from the measurements of the total voltage course at constant electric current and from UV record of the background at 230 nm. We choose nitrophenols as example of focusing of weak acids (Fig. 1). With use of LE with pH 8 the nitrophenols are partially dissociated in their zones with degrees of dissociation between 0.2 and 0.4. Since they have the identical ionic mobilities, μ, see Table 1, their migration order should follow their dissociation constants, pK_a. However, *o*-nitrophenol and *p*-nitrophenol have values of dissociation constants too close and hence they do not separate without micelles. It corresponds to the situation in Fig. 1a. By addition of Tergitol micelles at a concentration of 20 mmol l^{-1} these two nitrophenols start to separate (Fig. 1b) and separation efficiency improves up to 80 mmol l^{-1} Tergitol concentration in LE. The order of separated nitrophenols is in agreement with their octanol–water partition coefficients, P_{oct} (Table 1). Since the log P_{oct} value for *p*-nitrophenol is the higher than that for *o*-nitrophenol, *p*-nitrophenol migrates more slowly with increasing Tergitol concentration, which contributes to the separation. To avoid the possible speculations about influence of other effects than micelles on improvement in separation efficiency (i.e. the increase in resolution by influence of Tergitol on the electroosmotic flow and thus longer migration times of analytes similarly to counter-flow techniques), we also performed ITF in a capillary of total effective length 280 mm. This higher effective length means elongation of separation time by about 20 min. Thus, migration times of analytes for shorter capillary arrangement with the use of 80 mmol l^{-1} Tergitol concentration in LE roughly agree with these migration times for 280 mm long capillary without Tergitol in LE (see Table 2). Table 2 also presents differences in migration times for *o*- and *p*-nitrophenol for both 190 and 280 mm total effective capillary lengths. No separation of *o*- and *p*-nitrophenol occurs without presence of Tergitol in either

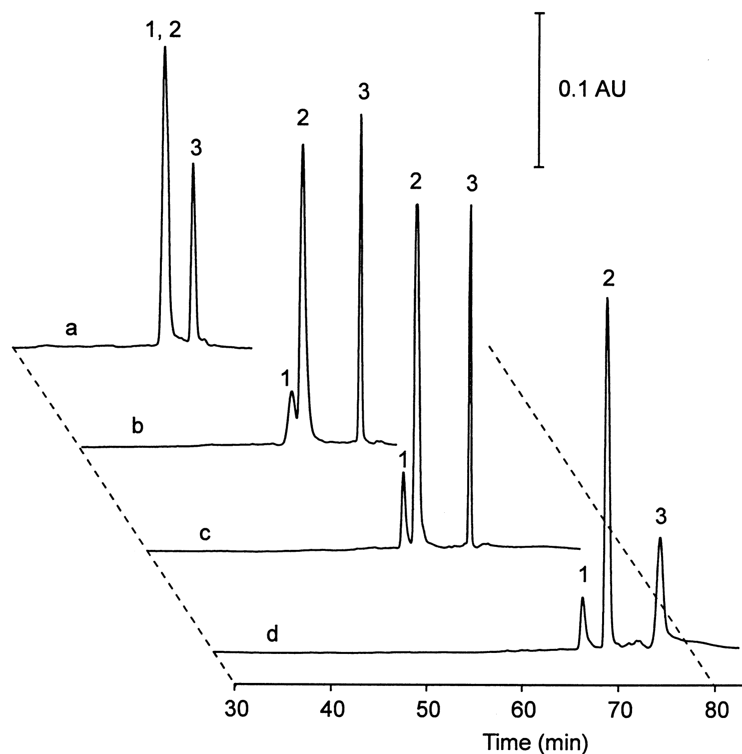

Fig. 1. Separation of nitrophenols. 1, *o*-nitrophenol (400 ng), 2, *p*-nitrophenol (40 ng), 3, *m*-nitrophenol (104 ng). Conditions: leading electrolyte (pH 8), 10 mmol l^{-1} Tris·HCl and 10 mmol l^{-1} Tris, 0.06% HPMC, 2.5 μg ml^{-1} PEI; terminating electrolyte, 50 mmol l^{-1} CHES and 5 mmol l^{-1} KOH; background, 8% ampholine pH 3.5–10.0; sample volume, 500 nl; background volume, 400 nl; electric current, 7 μA; λ=330 nm. Concentration of Tergitol in LE in mmol l^{-1}: a=0, b=20, c=40, d=80.

arrangement. The separation improved in a similar way with the presence of Tergitol in LE in both cases. It can be seen that differences of migration times $t_{1,2}$ are similar, which also implies that analytes are in a steady state in the shorter separation compartment (see Fig. 1). These experiments support our assumption that better separation and selectivity is mostly due to micellar partition and similar improvement cannot be reached, i.e., by use of longer separation capillary.

In the ITF method used here the conductivity gradient contributes to the separation and focusing of analytes in addition to the pH gradient [15]. Derivatized amino acids behave as strong electrolytes by using LE with pH 8, so only the conductivity part of

Table 1
List of physicochemical constants of nitrophenols

	pK_a^a	$\mu \cdot 10^{9a}$ ($m^2 V^{-1} s^{-1}$)	log P_{oct}^b
o-Nitrophenol	7.23	−33.4	1.73
m-Nitrophenol	8.40	−33.4	2.00
p-Nitrophenol	7.15	−33.4	1.91

[a] According to [34].
[b] From [35].

Table 2
Migration times of *o*-nitrophenol (t_1) and differences in migration times between *o*- and *p*-nitrophenol ($t_{1,2}$) without and with the presence of 80 mmol/l^{-1} Tergitol concentration in LE ($c_{Tergitol}$) in shorter and longer separation capillary

$c_{Tergitol}$ (mmol/l^{-1})	Effective length 190 mm		Effective length 280 mm	
	t_1 (min)	$t_{1,2}$ (min)	t_1 (min)	$t_{1,2}$ (min)
0	36.08	0	56.07	0
80	58.99	2.14	77.97	2.40

continuous gradient influences the separation. Separation is on the basis of different ionic mobility and/or different size of molecules. Since the size of the molecules can be considered to be very similar and we can assume their similar ionic mobility, separation is expected to be poor. It corresponds to the analyses in Figs. 2a and 3a. With addition of Tergitol micelles from 20 to 80 mmol l^{-1} to LE, the electrophoretic mobilities of derivatized amino acids incorporated to the micelles decrease according to the increase in the hydrophobicity of analytes. Thus, the separation of both DNP- and Dns-amino acids improves due to differential partition between buffer and micelles, see Fig. 2b to d and Fig. 3b to d. Similarly to the case of separation of nitrophenols, the same experiments were made with two total effective capillary lengths in separation compartment with similar results (i.e. no separation without Tergitol in LE and the similar selectivities with increase in Tergitol concentrations) for both capillary lengths.

In our case it was calculated that the injected volume was 500 nl and the volume of Gaussian zones in detector was less than 50 nl, it means at least 10-fold focusing, which is similar to previous work [15].

4. Conclusion

This paper shows that micelles can be successfully used for improvement of separation efficiency and selectivity in ITF. Use of micellar separation mechanism can extend applicability of this method as has been done in capillary zone electrophoresis. The merit of this work is the achievement of focusing of analyte zones simultaneously with the selectivity control by micelles. Experiments with a narrower inner diameter of the fused-silica capillary will be studied in the future.

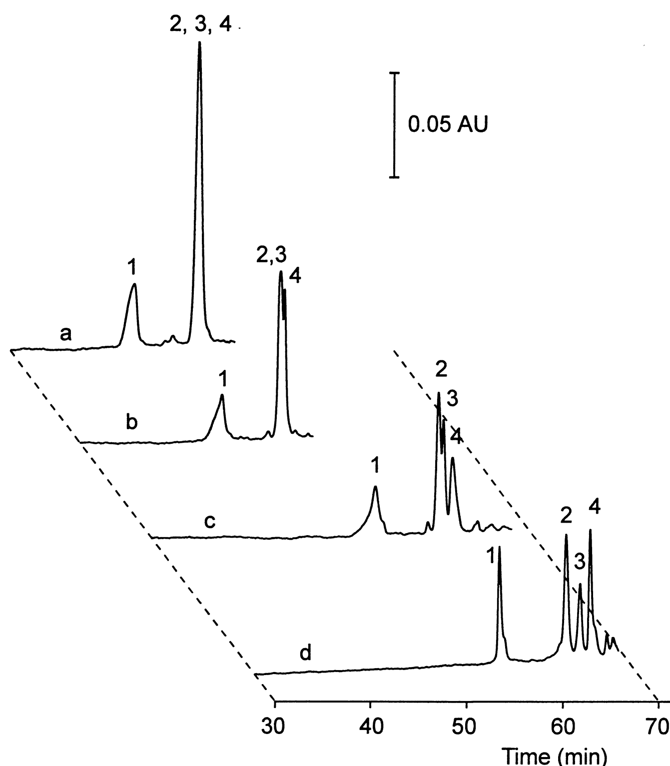

Fig. 2. Separation of DNP-amino acids. 1, DNP-DL-aspartic acid (30 ng), 2, DNP-L-hydroxyproline (15 ng), 3, DNP-DL-asparagine (15 ng), 4, DNP-DL-threonine (15 ng). Experimental conditions see Fig. 1.

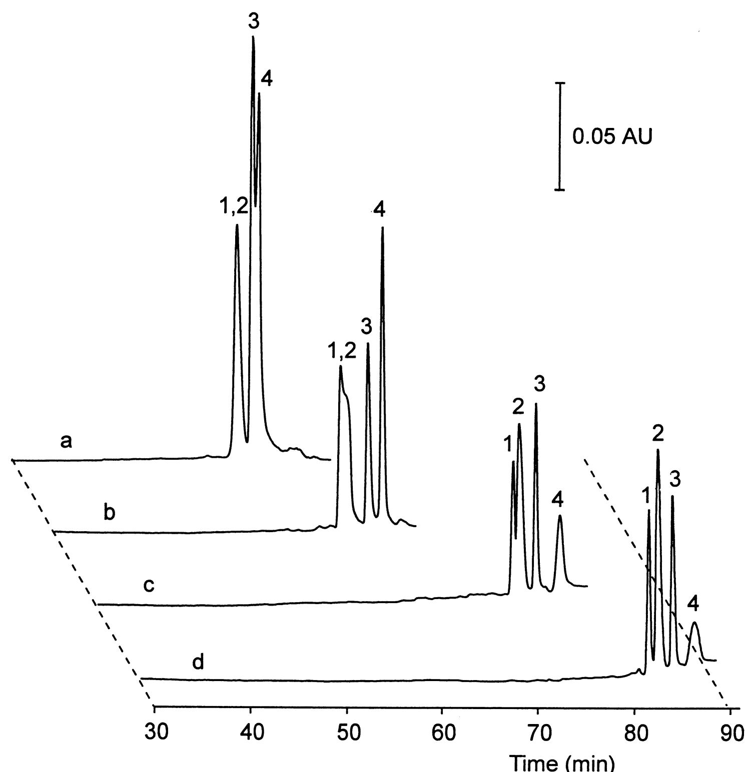

Fig. 3. Separation of Dns-amino acids. 1, Dns-OH (product of hydrolysis of dansyl chloride) (20 ng), 2, Dns-DL-asparagine (35 ng), 3, Dns-L-proline (30 ng), 4, Dns-DL-leucine (50 ng). Concentration of Tergitol in LE in mmol l^{-1}: a=0, b=20, c=60, d=80. Other experimental conditions as in Fig. 1.

Acknowledgements

This work was partially supported by the Grant Agency of the Academy of Sciences of the Czech Republic, grant No. A 4031504.

References

[1] F.M. Everaerts, J.L. Beckers, Th.P.E.M. Verheggen, Isotachophoresis, Elsevier, Amsterdam, 1976.

[2] P. Boček, M. Deml, P. Gebauer, V. Dolník, Analytical Isotachophoresis, VCH, Weinheim, 1988.

[3] F. Foret, L. Křivánková, P. Boček, Capillary Zone Electrophoresis, VCH Weinheim, 1993.

[4] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834.

[5] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.

[6] J. Snopek, I. Jelínek, E. Smolková-Keulemansová, J. Chromatogr. 452 (1988) 571.

[7] P.G. Muijselaar, K. Otsuka, S. Terabe, J. Chromatogr. A 780 (1997) 41.

[8] S.A. Shamsi, I.M. Warner, Electrophoresis 18 (1997) 853.

[9] S. Tanaka, T. Kaneta, M. Taga, H. Yoshida, H. Ohtaka, J. Chromatogr. 587 (1991) 364.

[10] J. Snopek, I. Jelínek, E. Smolková-Keulemansová, J. Chromatogr. 438 (1988) 211.

[11] J. Snopek, E. Smolková-Keulemansová, J. Dohnal, J. Klinot, E. Klinotová, J. Chromatogr. 450 (1988) 373.

[12] J. Snopek, H. Soini, M. Novotný, E. Smolková-Keulemansová, I. Jelínek, J. Chromatogr. 559 (1991) 215.

[13] K. Fukushi, K. Hiiro, J. Chromatogr. 523 (1990) 281.

[14] K. Šlais, J. Chromatogr. A 764 (1997) 309.

[15] T. Rejtar, K. Šlais, J. Chromatogr. A 798 (1998) 223.

[16] R. Charlionet, A. Bringard, Ch. Davrinche, M. Fontaine, Electrophoresis 7 (1986) 558.

[17] Y.F. Yik, S.F.Y. Li, Chromatographia 35 (1993) 560.

[18] C.P. Ong, C.L. Ng, H.K. Lee, S.F.Y. Li, J. Chromatogr. 559 (1991) 537.

[19] E. Skočir, J. Vindevogel, P. Sandra, Chromatographia 39 (1994) 7.

[20] S. Terabe, H. Ozaki, K. Otsuka, T. Ando, J. Chromatogr. 332 (1985) 211.

[21] D. Kanianský, E. Krčmová, V. Madajová, M. Masár, Electrophoresis 18 (1997) 260.
[22] C.P. Ong, C.L. Ng, N.C. Chong, H.K. Lee, S.F.Y. Li, J. Chromatogr. 516 (1990) 263.
[23] M. Šťastná, V. Kahle, K. Šlais, J. Chromatogr. A 730 (1996) 261.
[24] M. Šťastná, K. Šlais, J. Chromatogr. A 768 (1997) 283.
[25] K. Šlais, J. Chromatogr. 684 (1994) 149.
[26] K. Šlais, J. Microcol. Sep. 7 (1995) 127.
[27] K. Šlais, J. Chromatogr.A 730 (1996) 247.
[28] K. Šlais, J. Electrophoresis 16 (1995) 2060.
[29] F. Foret, M. Deml, V. Kahle, P. Boček, Electrophoresis 7 (1986) 430.
[30] E. Tapuhi, N. Miller, B.L. Karger, J. Chromatogr. 205 (1981) 325.
[31] F. Sanger, Biochem. J. 39 (1945) 507.
[32] J.L. Beckers, F.M. Everaerts, J. Chromatogr. 68 (1972) 207.
[33] Z. Ryšlavý, P. Boček, M. Deml, J. Janák, J. Chromatogr. 147 (1978) 446.
[34] T. Hirokawa, M. Nishino, N. Aoki, Y. Kiso, Y. Sawamoto, T. Yagi, J. Akiyama, J. Chromatogr. 271 (1983) D1–D106.
[35] A. Leo, C. Hansch, D. Elkins, Chem. Rev., 71 (1971).