ELSEVIER

Journal of Chromatography A, 894 (2000) 79–88

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Moderation of the electroosmotic flow in capillary electrophoresis by chemical modification of the capillary surface with tentacle-like oligourethanes

Susanne König, Thomas Welsch*

Department of Analytical and Environmental Chemistry, University of Ulm, Albert Einstein Allee 11, D-89069 Ulm, Germany

Abstract

The surface chemistry of the inner wall of fused-silica capillaries is one important means to control the magnitude as well as the direction of the electroosmotic flow and the adsorption activity. A method was developed to change the surface characteristics of fused-silica capillaries by binding tentacle-like oligourethane groups onto the inner surface. The electroosmotic flow at a buffer pH of 6–9 was reduced by 15 to 40% compared to that in a bare fused-silica tubing, dependent on the type of coating. Sample adsorption is diminished at the same time resulting in a separation of proteins with higher resolution and good migration time precision. At a pH below 4.5 the electroosmotic flow is reversed into the anodic direction, which offers further possibilities for the separation of positively charged analytes as demonstrated for the separation of aromatic and biogenic amines.

Keywords: Electroosmotic flow; Capillary columns; Surface treatment; Cheese; Food analysis; Oligourethanes; Proteins; Amines; Alcohols

1. Introduction

Fused-silica capillaries are predominantly used in capillary electrophoresis (CE) and related techniques as tube material. Based on negatively charged surface silanol groups an electric double layer at the interface of the inner capillary wall and the buffer solution is formed, which causes a cathodic electroosmotic flow (EOF) under the action of an electric field. The strength of the EOF is dependent on several parameters, mostly on the zeta-potential of the double layer, which in turn is influenced by the surface concentration of charged silanol groups, the buffer composition, its pH value and its ionic strength [1].

The initial silanol surface concentration of a (freshly drawn) fused-silica capillary is determined by the purity of the raw silica tubing and the parameters of the drawing process. Thus, the silanol concentration and hence the EOF in a non-treated fused-silica capillary varies not only from producer to producer but also from batch to batch [2]. The time of storage of non-sealed plain fused capillaries may also influence the resulting EOF. Flushing of the capillary with sodium hydroxide solution [3,4] or potassium hydroxide solution [5,6] mostly increases the concentration of free surface silanols and therefore leads to increased or restored electroosmotic flows.

Surface silanol groups, on the other hand, may act as sorption sites for analytes, especially for large positively charged molecules and/or impurities in the buffer [7–9]. That way the mobility of these

*Corresponding author. Tel.: +49-731-5022-754; fax: +49-731-5022-763.

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00718-4

molecules may be affected and the electroosmotic flow may be reduced. In addition, such sorption processes can directly affect the peak shape and hence the efficiency and can lead to the formation of a non-uniform zeta-potential over the length of the capillary which results in local variations of the electroosmotic flow with its negative consequences for the reproducibility of migration times [10–13].

Different methods have been developed to diminish or to block the action of silanols in CE separations which include silylation with common deactivation reagents [2,14–16], the dynamic or permanent coating of the surface with hydrophilic polymers such as polyacrylamide [3,4,7,17–28], polyvinyl alcohol [29–32], polyethylene/propylene glycols [5,33,34], polyvinyl-pyrrolidone [35,36], epoxy coatings [37,38], polyethylene oxide [39,40], or cationic polymers like polyethyleneimine [41–43] and the addition of a preferentially adsorbed additive to the buffer [10,11,44].

Those methods, however, also have some disadvantages: modification of the capillary surface by silylation often suffers from hydrolytic instability of the siloxane bonds at high pH values. Adsorbed neutral or cationic buffer additives are useful to deactivate the column surface but they also may change the properties of the buffer and often cause detection problems. A permanent coating of the capillaries with a hydrophilic polymer, e.g., with polyvinyl alcohol [30], results in a nearly total suppression of the EOF and a reduction of the adsorption activity. This is an advantage for the separation of proteins and peptides and the separation of other positively charged species yet it is a drawback for a number of other applications: Since the migration and the separation of positively charged analytes is completely based on their electrophoretic mobilities, analytes with a low electrophoretic mobility show long separation times. Therefore the application of such capillaries is limited to the separation of substances with high electrophoretic mobilities. In order to use a single (chemically modified) capillary for the separation of a wide range of different analytes, the possibility to adjust the EOF in both directions (cathodic or anodic) is desirable. So far only a few research groups have dealt with the surface modification of capillaries allowing a switchable EOF [45–47].

In this paper, we introduce a method to produce a long term stable chemically bonded tentacle-like coating which is based on urethane chemistry. Capillaries modified that way provide the possibility to switch the EOF in cathodic or anodic direction by changing the pH of the buffer. The coating diminishes analyte adsorption and reduces the cathodic EOF by 15 to 40% compared to bare silica tubing.

2. Experimental

2.1. Instrumentation

Capillary electrophoretic experiments were performed on a Hewlett-Packard HP ^{3D}CE system (Hewlett-Packard, Waldbronn, Germany). A HP ^{3D}CE chemstation served for instrumental control, data acquisition and data analysis.

Fused-silica capillaries of 50–60 cm (effective lengths 41.7–51.7 cm)×50 μm I.D. (MicroQuarz, Munich, Germany) were used for these studies.

2.2. Chemicals

Pentaerythritol, 1,4-butanediol, 2-hydroxymethyl-1,3-propanediol, Carbowax 20M, toluene-2,4-diisocyanate, benzyl alcohol, ω-phenylethanol, ω-phenylpropanol, ω-phenylbutanol and ω-phenylpentanol were obtained from Fluka, Neu-Ulm, Germany. 3-Isocyanatopropyltriethoxysilane was from ABCR, Karlsruhe, Germany. High-purity solvents and the test compounds 2-amino-4,6-dinitrotoluene, 2-methyl-3-nitroaniline, 2-methyl-5-nitroaniline, 4-amino-2-nitrotoluene, 2,4-diamino-6-nitrotoluene, and 2,6-diamino-4-nitrotoluene were from Promochem, Wesel, Germany. Phenylethylamine (hydrochloride), tyramine (hydrochloride), tryptamine (hydrochloride) and the proteins were from Sigma, Deisenhofen, Germany. All other chemicals were from Merck, Darmstadt, Germany.

2.3. Coating procedure

2.3.1. Capillary leaching

One-meter long pieces of the fused-silica capillary to be treated were immersed into a water bath kept at

a temperature of 70°C. One end of the capillary was connected to a custom-made coating reservoir which can be pressurized up to 20 bar. First, the capillaries were dynamically leached with a 1 mol/l sodium hydroxide solution for 3 h, followed by a 15 min rinse with a 2% hydrochloric acid solution. Then the capillaries were leached with an 18% hydrochloric acid solution for 3 h at 70°C, followed again by a rinse with a 2% hydrochloric acid solution for 15 min. The inlet pressure during all dynamic leaching processes was 0.8 bar. Finally, the capillaries were dried under a gentle flow of nitrogen at 200°C for 6 h.

The silylation reagent (PU I) which was used to form the first Si–O–Si bonded layer was synthesized as follows:

A 10 g amount of 1,4-butane was placed in a three-necked flask with magnetic stirrer and condenser. After addition of 5.5 g 3-isocyanatopropyltriethoxysilane in 5 g toluene the exothermic reaction started. The reaction was continued for 2 h at 60°C under nitrogen atmosphere. The obtained adduct PU I was then dried for 24 h under vacuum [48,49].

2.3.2. Surface coating

The surface coating was performed in the same manner as the dynamic leaching but at room temperature (23°C). The tentacle type coating was generated in several steps by forcing the different reagent solutions through the leached capillary. To chemically bind the first layer, a solution of PU I in tetrahydrofuran (THF) (1:1, v/v) was forced through the capillary for 1.5 h. After rinsing with THF for 30 min a solution of toluene-2,4-diisocyanate–toluene (1:1, v/v) and the diol/polyol component in THF, respectively, was alternately forced through the capillary for 1 h. The reaction plugs were separated by a plug of THF or toluene forced through the capillary for 30 min. An alcohol component was always applied as the final reaction plug. Before use the modified capillaries were flushed for 10 min with methanol, for 10 min with water, and finally with buffer solution as described below.

Additionally, a capillary was coated with polyvinyl alcohol (PVA) according to Gilges et al. [30] for comparison purposes.

Table 1 shows a list of the modified capillaries discussed in this paper.

2.4. Electrophoretic run conditions

Before each measurement of the electroosmotic mobilities, μ_{eo}, both plain or surface modified capillaries were flushed with the new buffer solution for 40 min (pressure 1000 mbar) and conditioned applying voltage for 15 min. Between each run the capillaries were flushed (pressure 1000 mbar) with the running electrolyte. The pH dependencies of μ_{eo} were measured going from the buffer with the highest pH to the buffer with the lowest pH. The buffer compositions and separation conditions are specified in the legends of the figures. Sample injection was performed hydrodynamically by applying a pressure of 50 mbar for 2 s. All mobility values are the mean of at least three measurements.

3. Results and discussion

In order to generate a chemically bonded surface coating which is most hydrolytically stable, deactivates the surface to a certain extent and allows one to switch the direction of the electroosmotic flow we considered chemically binding the oligourethanes onto the inner surface of the silica capillaries because the urethane group is fairly stable against hydrolysis and contains a protonable NR_2H group, which

Table 1
List of modified capillaries

Capillary	Diol/polyol component	No. of toluene-2,4-diurethane units in the tentacle	Compound used for termination
Tentacle-8	1,4-Butanediol	3	1,4-Butanediol
Tentacle-3-20M	1,4-Butanediol	1	Carbowax 20M
Tentacle-8-Hy	2-(Hydroxymethyl)-1,3-propanediol	3	2-(Hydroxymethyl)-1,3-propanediol
Tentacle-8-Pen	Pentaerythritol	3	Pentaerythritol

should enable an anodic electroosmotic flow at low pH values. The use of highly reactive isocyanates such as toluene-2,4-diisocyanate makes it possible to modify the capillaries under soft conditions, i.e., at room temperature without the use of a catalyst. The bulkiness and especially the ladder-type linkage of tentacles formed in case of triol- or polyol monomers are expected to shield the surface Si–O–Si bond from easy hydrolytic attack. A reaction scheme for the stepwise coating is given in Fig. 1.

The binding of the starting group (PU I, see Experimental) onto the silica surface was accomplished by silylation leaving enough unmodified surface silanols on the surface to provide a cathodic electroosmotic flow at high pH. The completion of the tentacle-like oligourethane chains occurred by alternate coating of the capillary with toluene-2,4-diisocyanate and a diol/polyol component.

The dependencies of the electroosmotic mobilities, μ_{eo}, on the pH value are shown in Fig. 2a and b for the different tentacle-type coated capillaries. For comparison purposes the EOF vs. pH plot of a plain fused-silica capillary and a polyvinyl alcohol-coated capillary are included in these Figures as well.

The plots of the Tentacle-8, Tentacle-3-20M, Tentacle-8-Hy and Tentacle-8-Pen coated capillaries (see Table 1) show a reduced electroosmotic mobility compared to plain fused-silica in the pH range of 6–9. The degree of EOF reduction obviously depends on the shielding effect of the tentacles which increases not only with the increasing number of OH groups of the alcoholic monomers [1,4-butanediol<

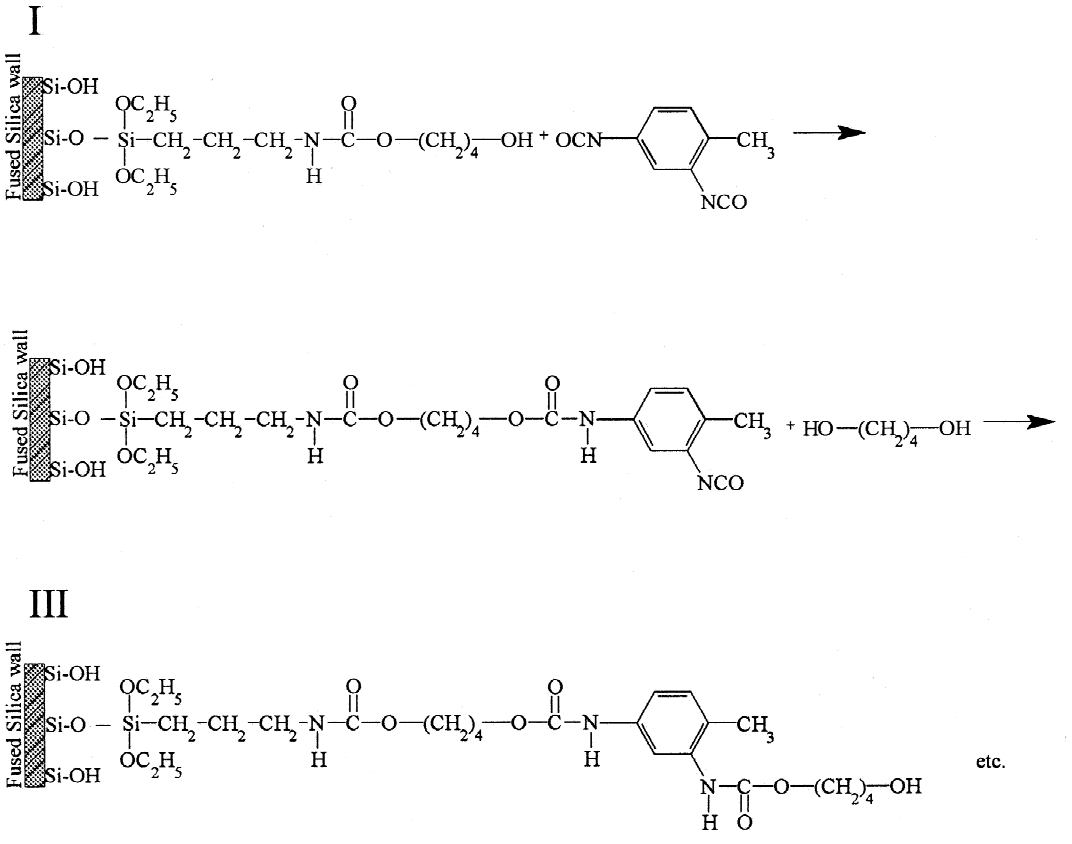

Fig. 1. Scheme of the formation of ''oligourethane tentacles'' on a fused-silica capillary surface with 1,4-butanediol as alcohol component. I=Surface modification after silylation with PU I; II=surface modification after reaction with toluene-2,4-diisocyanate; III=surface modification after further reaction with 1,4-butanediol; for further steps according to this scheme and for termination see Table 1.

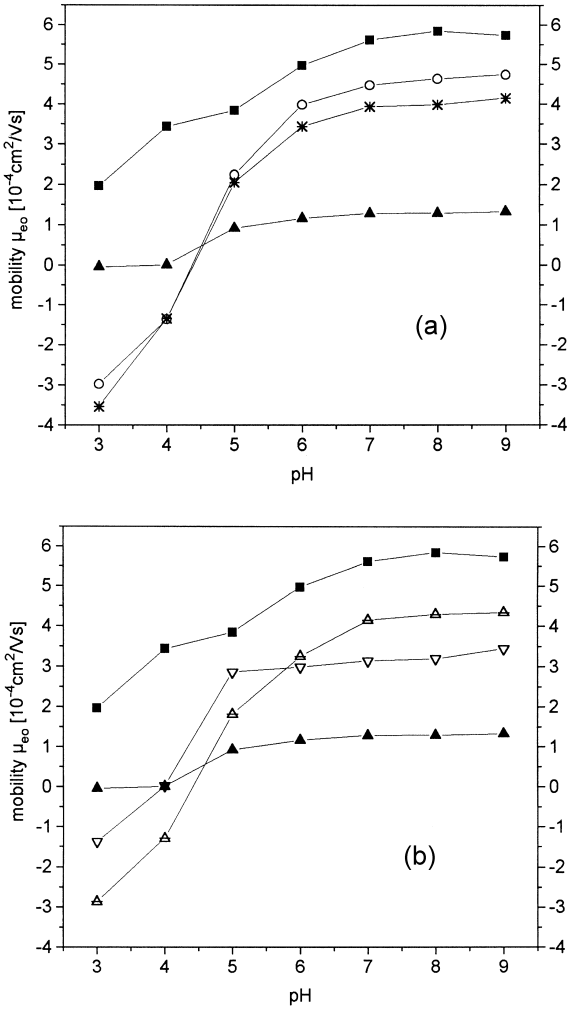

Fig. 2. EOF vs. pH for bare, PVA and oligourethane modified capillaries. Capillaries: 60 cm (effective length 51.7 cm)×50 μm I.D.; ■ bare fused-silica, ▲ PVA (75 μm I.D.); (a) ○ Tentacle-8, * Tentacle-8-Pen; (b) ▽ Tentacle-3-20M, △ Tentacle-8-Hy; conditions: 10 m*M* phosphate buffer; EOF marker=acetone; E=500 V/cm; injection: 100 mbar s; detection: UV at 254 nm.

2-(hydroxymethyl)-1,3-propanediol<pentaerythritol] and the degree of the ladder-type crosslinking directly connected with it, but also with the size of the tentacle terminating group. The largest reduction of the EOF (40%) is observed for the Tentacle-3-20M capillary. The bulky Carbowax 20M as terminating group causes the highest shielding effect of the charged surface silanols. Unlike in the case of the polyvinyl alcohol coated capillary a reversal of the EOF starts at a pH value around 4.5 for all tentacle-like oligourethane coated capillaries. This anodic EOF increases with decreasing pH and is maximum in case of the Tentacle-8-Pen capillary (Fig. 2a). This can be explained by the higher content of urethane groups within the coating because pentaerythritol has four hydroxy groups to react with toluene-2,4-diisocyanate yielding a high degree of ladder-type crosslinking of the tentacles.

The moderate reduction of the electroosmotic flow at pH 9 to 6 observed at all tentacle-like oligourethane coated capillaries can advantageously be used to increase the migration time window in electrokinetic chromatography (EKC). An example is presented in Fig. 3, where an EKC separation of a homologous series of ω-phenyl alcohols using a stable micelle [radically crosslinked sodium 10-undecenyl-1-oxybutane sulfonate (SUOBS)] as separation additive is shown. The peak capacity (for details on its calculation see Ref. [50]) measured between benzyl alcohol and ω-phenylpentanol is increased from 71 to 107, of course at the expense of a larger analysis time.

To test the degree of surface deactivation achieved by the different coatings and to examine the run-to-run migration time reproducibility and column-to-column reproducibility a protein mixture was analyzed, because proteins are known to be sensitive probes for the surface characteristic of the capillary due to their high affinity to wall adsorption [10,11]. In Fig. 4, the electropherograms of a mixture of myoglobin, β-lactalbumin, β-lactoglobulin B, β-lactoglobulin A, albumin, and trypsin inhibitor on a plain and a Tentacle-8-Hy fused-silica capillary illustrate both the improved efficiency (see the legend to Fig. 4) which can be attributed to reduced wall adsorption, and the better resolution as a result of increased migration times and the improved efficiency.

The reproducibility of the migration times of these proteins was determined evaluating seven consecutive runs. The results are summarized in Table 2. The relative standard deviation is below 1% for all modified capillaries. The best relative standard deviation (RSD) values are obtained with the Tentacle-8-Pen capillary. The high degree of ladder-type crosslinking and the shielding of the surface silanol groups connected with it may be a reason for this

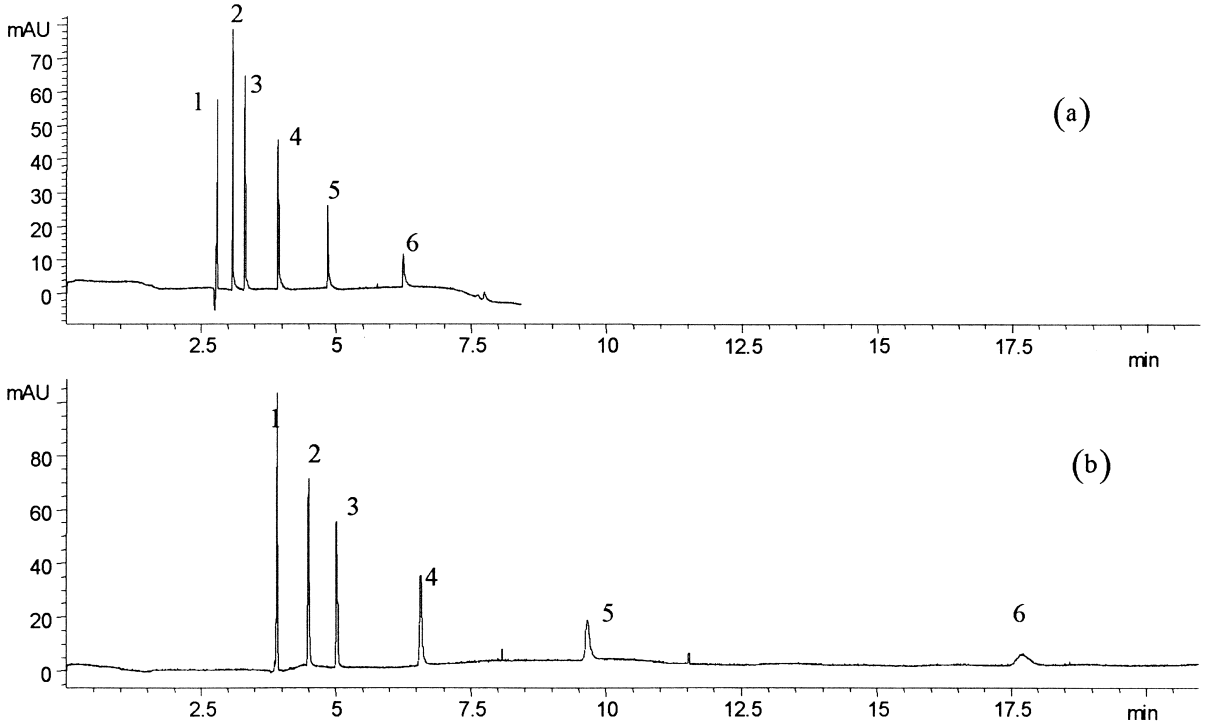

Fig. 3. MEKC separation of ω-phenyl alcohols. Capillaries: 60 cm (effective length 51.7 cm)× 50 μm I.D.; (a) bare fused-silica, (b) Tentacle-8; conditions: 10 m*M* phosphate buffer, pH 9, 1% SUOBS as micelle; E=500 V/cm; injection: 100 mbar s; detection UV at 205 nm; components: 1=thiourea, 2=benzyl alcohol, 3=ω-phenylethanol, 4=ω-phenylpropanol, 5=ω-phenylbutanol, 6=ω-phenylpentanol.

behavior. To examine the column-to-column reproducibility five Tentacle-8 modified columns were produced over a period of 1 month using two batches of bare fused-silica tubing (capillaries 1 and 2 were produced from one batch and capillaries 3, 4 and 5 from another batch). The effective mobilities of acetone as EOF marker and of the proteins were used as indicators. As the values in Table 3 illustrate, the reproducibility is acceptable with RSD values between 1.85 and 5.10%. Only the long term stability of the capillaries leaves something to be desired: after continued use for about 160 runs at a buffer pH of 9 some hydrolysis of the basic Si–O–Si bonds of the tentacles was indicated by an increase of the electroosmotic flow. Preliminary experiments showed that the lifetime of the coating can be improved to fare more than 300 runs if the first layer is generated by substituting an aminoalkylethoxysilane for PU I combined with an increase of the reaction temperature.

The reversed electroosmotic flow in the modified capillaries at low pH was used to separate positively charged analytes in the same way as negatively charged analytes are separated at high pH. As an example the separation of some aromatic amines is shown in Fig. 5. The separation of biogenic amines of a cheese sample at a reduced anodic EOF applying a relatively high concentrated glycine buffer (Fig. 6) is another example for the wide application range of the tentacle-like oligourethane coated capillaries.

4. Conclusions

The coating of fused-silica capillaries with tentacle-like oligourethanes results in capillaries which provide a reduced cathodic EOF at high pH values and a reversed anodic EOF at low pH values. Additionally, the adsorption activity of the inner wall surface of the fused-silica capillaries is diminished.

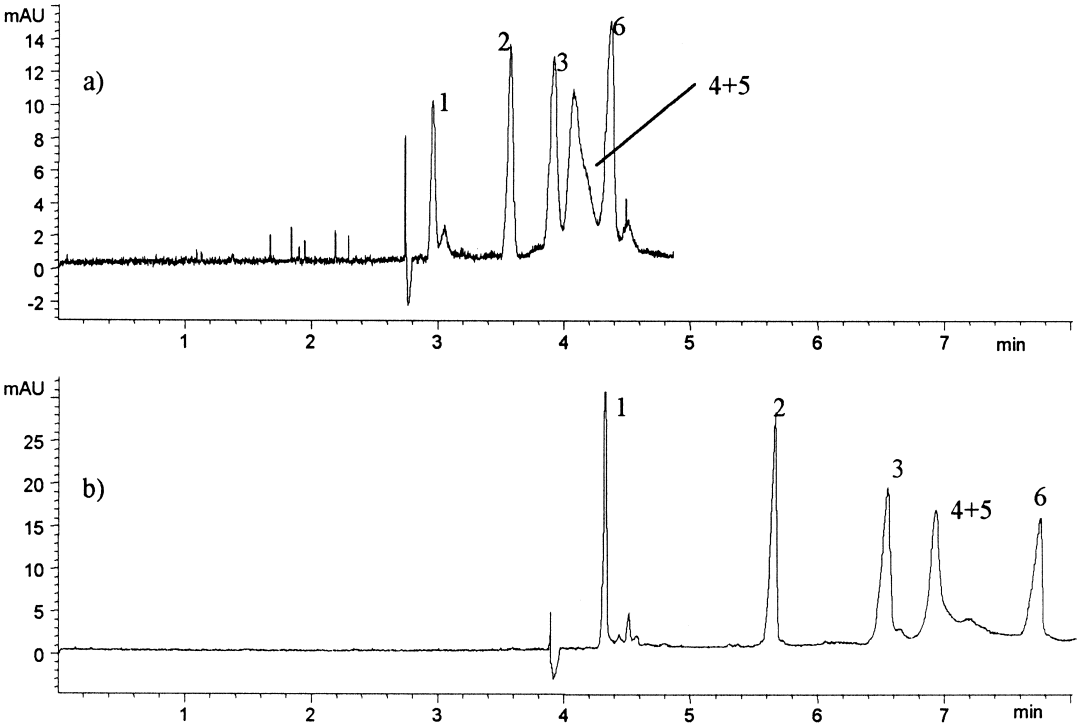

Fig. 4. Separation of acidic proteins. Capillaries: 60 cm (effective length 51.7 cm)×50 μm I.D.; (a) bare fused-silica; (b) Tentacle-8-Hy; conditions: 10 m*M* phosphate buffer, pH 9, E=500 V/cm; injection: 100 mbar s; detection: UV at 214 nm; components, (plates/m on capillaries a and b, respectively): 1=myoglobin (44 000/126 900); 2=β-lactalbumin (48 700/67 800); 3=β-lactoglobulin B (28 700/48 100); 4=β-lactoglobulin A (7000/33 700); 5=albumin (7000/33 800); 7=trypsin inhibitor (23 900/78 000).

Both, the well controlled EOF and the reduced adsorption activity ensure a good precision for migration time data of some proteins used as probes. Reduced EOF rates allow the enlargement of the migration time window and the peak capacity in electrokinetic chromatography. The reversed EOF at low pH has advantages for the separation of positively charged analytes such as amines. The developed surface modification procedure is very reproducible and the lifetime of the coated capillaries is acceptable. Experiments to further improve the long term stability of the capillaries are currently underway.

Table 2
Run-to-run (n=7) reproducibility of the migration times of acidic proteins[a]

Capillary	Myoglobin		Lactalbumin		β-Lactoglobulin B		β-Lactoglobulin A+albumin		Trypsin inhibitor	
	t_M (min)	RSD (%)	t_M (min)	RSD (%)	t_M (min)	RSD (%)	t_M (min)	RSD (%)	t_M (min)	RSD (%)
Tentacle-8	4.01	0.47	5.15	0.39	5.89	0.63	6.17	0.66	6.86	0.57
Tentacle-3-20M	5.67	0.36	8.46	0.52	10.81	0.99	11.84	1.16	14.52	1.58
Tentacle-8-Hy	4.30	0.84	5.72	1.08	6.52	1.32	6.91	1.35	7.89	1.54
Tentacle-8-Pen	4.63	0.51	6.30	0.55	7.47	0.39	8.01	0.32	9.17	0.26

[a] Capillaries: 60 cm (effective length 51.7 cm)×50 μm I.D.; conditions: 10 m*M* phosphate buffer, pH 9; E=500 V/cm; injection: 100 mbar s; detection: UV at 214 nm. t_M=Migration time.

Table 3
Column-to-column reproducibility of migration times[a]

	Mobility (10^{-4} cm^2/V s)					RSD
	Tentacle-8 (1)	Tentacle-8 (2)	Tentacle-8 (3)	Tentacle-8 (4)	Tentacle-8 (5)	(%)
Acetone	4.69	4.72	4.90	4.71	4.70	1.85
Myoglobin	4.19	4.30	4.52	4.34	4.33	2.75
Lactalbumin	3.22	3.34	3.52	3.37	3.40	3.20
β-Lactoglobulin B	2.76	2.93	3.11	2.98	2.98	4.30
β-Lactoglobulin A+albumin	2.58	2.79	2.97	2.84	2.85	5.08
Trypsin inhibitor	2.33	2.51	2.68	2.55	2.59	5.10

[a] Capillaries: Tentacle-8 (1, 3–5) 60 cm (effective length 51.7 cm)×50 μm I.D.; Tentacle-8 (2) 52 cm (effective length 43.7 cm)×50 μm I.D.; E=500 V/cm; injection: 100 mbar s; detection: UV at 214 nm.

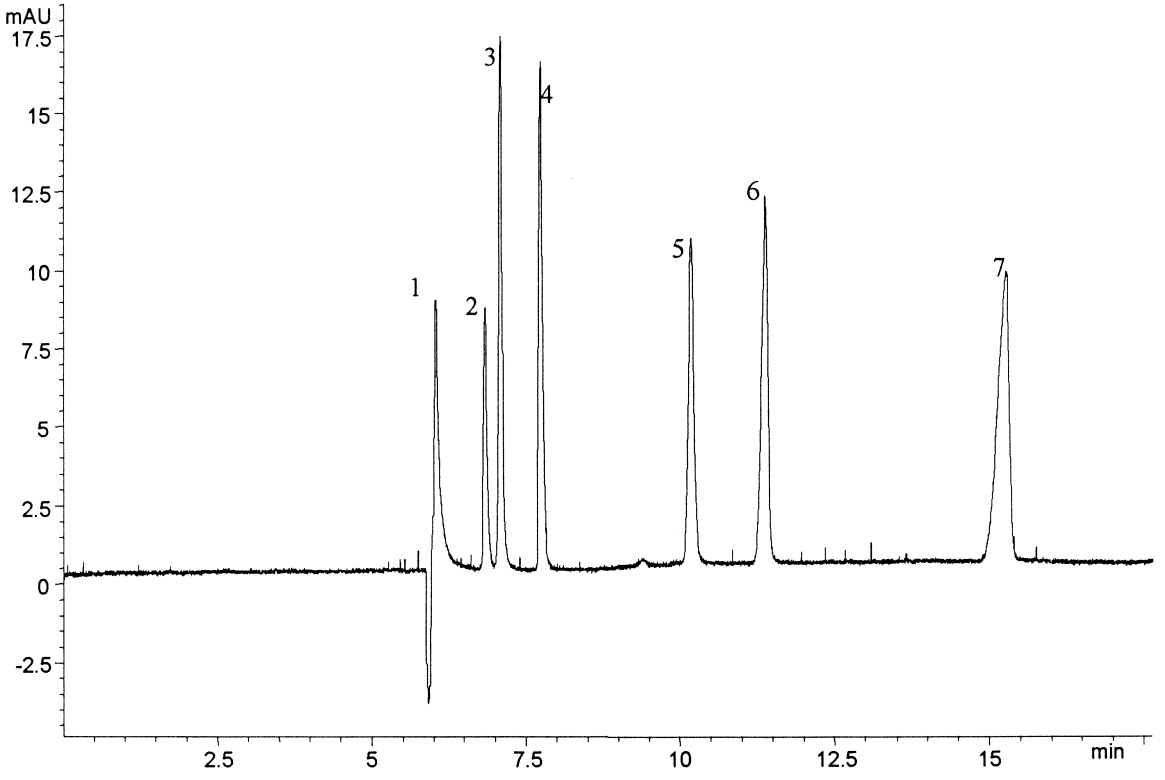

Fig. 5. Separation of aromatic amines. Capillary: Tentacle-8-Hy, 60 cm (effective length 51.7 cm)× 50 μm I.D.; conditions: 10 m*M* phosphate buffer, pH 3, E=−500 V/cm; injection: 100 mbar s; detection: UV at 215 nm; components: 1=2-amino-4,6-dinitrotoluene, 2=2-methyl-5-nitroaniline, 3=2-methyl-3-nitroaniline, 4=*o*-chloraniline, 5=4-amino-2-nitrotoluene, 6=2,6-diamino-4-nitrotoluene, 7=2,4-diamino-6-nitrotoluene.

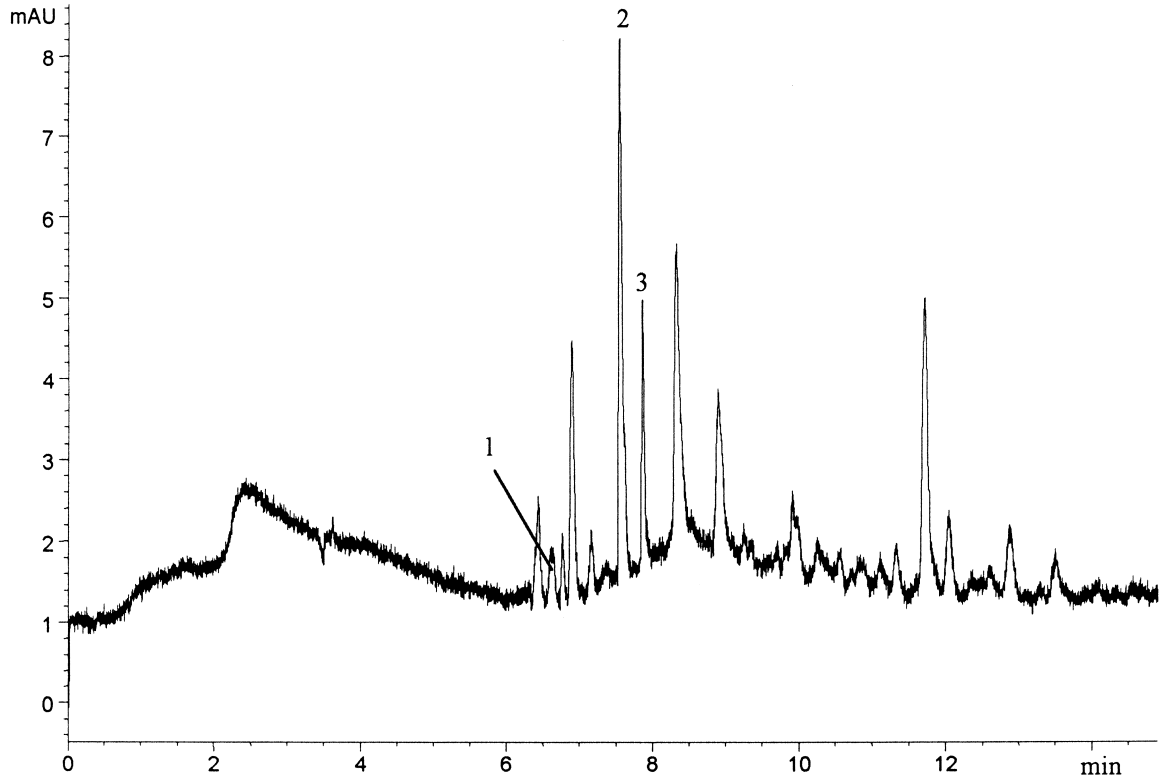

Fig. 6. Separation of biogenic amines in cheese. Capillary: Tentacle-8, 60 cm (effective length 51.7 cm)× 50 μm I.D.; conditions: glycine buffer (100 m*M* glycine; 100 m*M* sodium chloride), pH 3, *E*=500 V/cm; injection: 100 mbar s; detection: UV at 215 nm; sample: German Gouda cheese; identified components: 1=phenylethylamine, 2=tyramine, 3=tryptamine.

References

[1] J. Kohr, H. Engelhardt, in: N.A. Guzman (Ed.), Capillary Electrophoresis Technology, Chromatographic Science Series, Vol. 64, Marcel Dekker, New York, 1993, p. 357.
[2] G. Schomburg, in: N.A. Guzman (Ed.), Capillary Electrophoresis Technology, Chromatographic Science Series, Vol. 64, Marcel Dekker, New York, 1993, p. 311.
[3] M. Strege, A. Lagu, Anal. Chem. 63 (1991) 1233.
[4] K.A. Cobb, V. Dolnik, M. Novotny, Anal. Chem. 62 (1990) 2478.
[5] G. Bruin, J.P. Chang, R.H. Kuhlman, K. Zegers, J.C. Kraak, H. Poppe, J. Chromatogr. 471 (1989) 429.
[6] G. Bruin, R. Huidsen, J.C. Kraak, H. Poppe, J. Chromatogr. 480 (1989) 339.
[7] A. Cifuentes, J.M. Santos, M. de Frutos, J.C. Diez-Masa, J. Chromatogr. A 652 (1993) 161.
[8] S.V. Ermakov, M.Y. Zhukov, L. Capelli, P.G. Rigettthi, J. Chromatogr. A 699 (1995) 297.
[9] J.J. Bonvent, R. Barberi, R. Bartolino, L. Capelli, P.G. Righetti, J. Chromatogr. A 756 (1996) 233.
[10] K. Towns, F.E. Regnier, Anal. Chem. 63 (1991) 1126.
[11] K. Towns, F.E. Regnier, Anal. Chem. 64 (1992) 2473.
[12] M.R. Schure, A.M. Lenhoff, Anal. Chem. 65 (1993) 3024.
[13] B. Potocek, B. Gaš, E. Kenndler, M. Štedrý, J. Chromatogr. A 709 (1995) 51.
[14] J.W. Jorgenson, Science 222 (1983) 266.
[15] A.T. Balchunas, M.J. Sepaniak, Anal. Chem. 59 (1987) 1467.
[16] A.M. Dougherty, M.R. Schure, in: N.A. Guzman (Ed.), Capillary Electrophoresis Technology, Chromatographic Science Series, Vol. 64, Marcel Dekker, New York, 1993, p. 435.
[17] S. Hjertén, J. Chromatogr. 347 (1985) 191.
[18] K. Srinivasan, C. Pohl, N. Avdalovic, Anal. Chem. 69 (1997) 2798.
[19] C. Gelfi, P. de Besi, A. Alloni, P.G. Righetti, J. Chromatogr. 608 (1992) 333.

[20] D. Schmalzing, C.A. Piggee, F. Foret, E. Carrilho, B.L. Karger, J. Chromatogr. A 652 (1993) 149.
[21] M. Hunag, E. Dubrovcakova-Schneiderman, M.V. Novotny, H.O. Fatunmbi, M.J. Wirth, J. Microcol. Sep. 6 (1994) 571.
[22] M. Chiari, C. Micheletti, M. Nesi, M. Fazio, P.G. Righetti, Electrophoresis 15 (1994) 177.
[23] P. Sun, A. Landman, G.E. Barker, R.A. Hartwick, J. Chromatogr. A 685 (1994) 303.
[24] M. Chiari, M. Nesi, J.E. Sandoval, J.J. Pesek, J. Chromatogr. A 717 (1995) 1.
[25] K.W. Talmadge, M. Zhu, L. Olech, C. Siebert, J. Chromatogr. A 744 (1996) 347.
[26] M. Chiara, N. Dell'Orto, A. Gelain, Anal. Chem. 68 (1996) 2731.
[27] H. Engelhardt, M.A. Cunat-Walter, J. Chromatogr. A 716 (1995) 27.
[28] H. Engelhardt, M.A. Cunat-Walter, J. Chromatogr. A 717 (1995) 15.
[29] M. Gilges, H. Husmann, M.H. Kleemiß, S.R. Motsch, G. Schomburg, J. High Resolut. Chromatogr. 15 (1992) 452.
[30] M. Gilges, M.H. Kleemiss, G. Schomburg, Anal. Chem. 66 (1994) 2038.
[31] G. Schomburg, D. Belder, M. Gilges, S. Motsch, J. Cap. Electrophoresis 1 (1994) 219.
[32] U. Jegele, R. Grimm, H. Godel, R. Schuster, G. Ross, LC·GC Int. 10 (1997) 176.
[33] W. Nashabeh, Z. El Rassi, J. Chromatogr. A 559 (1991) 367.
[34] Ren, Y. Shen, M.L. Lee, J. Chromatogr. A 741 (1996) 115.
[35] R.M. McCormick, Anal. Chem. 60 (1988) 2322.
[36] R.J. Xu, C. Vidal-Madjar, B. Sebille, J.C. Diez-Masa, J. Chromatogr. A 730 (1996) 289.
[37] Y. Liu, R. Fu, J. Gu, J. Chromatogr. A 694 (1995) 498.
[38] Y. Liu, R.R. Fu, J. Gu, J. Chromatogr. A 723 (1996) 157.
[39] N. Iki, E.S. Yeung, J. Chromatogr. A 731 (1996) 273.
[40] X. Shao, Y. Shen, K. O'Neill, M.L. Lee, Chromatographia 49 (1999) 299.
[41] J.K. Towns, F.E. Regnier, J. Chromatogr. 516 (1990) 69.
[42] F.B. Erim, A. Cifuentes, H. Poppe, J.C. Kraak, J. Chromatogr. A 708 (1995) 356.
[43] E. Córdova, J. Gao, G.M. Whitesides, Anal. Chem. 69 (1997) 1370.
[44] M. Bushey, J.W. Jorgenson, J. Chromatogr. 480 (1989) 301.
[45] Y.F. Maa, K.J. Hyver, S.A. Swedberg, J. High Resolut. Chromatogr. 14 (1991) 65.
[46] J.T. Smith, Z. El Rassi, J. High Resolut. Chromatogr. 15 (1992) 573.
[47] Y. Guo, G.A. Imahori, L.A. Colón, J. Chromatogr. A 744 (1996) 17.
[48] K.W. Lee, T.J. McCarthy, Macromolecules 21 (1988) 3353.
[49] J. Wen, G.L. Wilkes, Polym. Bull. 37 (1996) 51.
[50] S. Kolb, T. Welsch, J.P. Kutter, J. High Resolut. Chromatogr. 21 (1998) 435.