ELSEVIER

Journal of Chromatography A, 879 (2000) 189–196

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Modified capillary electrophoresis system for peptide, protein and double-stranded DNA analysis☆

Boris G. Belenkii, Galina E. Kassalainen*, Dmitry G. Nasledov[1]

Institute for Analytical Instrumentation, Russian Academy of Sciences, 26 Rizhsky Pr., St. Petersburg 198103, Russia

Received 24 January 1999; received in revised form 31 January 2000; accepted 7 February 2000

Abstract

The results of high-performance capillary electrophoresis (HPCE) studies of peptide, protein and double-stranded DNA separations on a laboratory-made HPCE system are presented. Parameters of the HPCE system are given. The new method of capillary surface modification by grafting poly(glycidyl methacrylate) is described. The problems of HPCE biopolymer analysis connected with the sample–wall interactions are discussed.

Keywords: Capillary columns; Adsorption; DNA; Peptides; Proteins; Poly(glycidyl methacrylate)

1. Introduction

The separation of biopolymers is one of the most important high-performance capillary electrophoresis (HPCE) applications [1–7]. Theory predicts efficiencies up to 10^6 theoretical plates per meter of a capillary. Although the level of $5 \cdot 10^5$ theoretical plates in uncoated capillaries was demonstrated [1,8], in many applications a modification of the CE system was necessary to achieve the required efficiencies. The main reason for low efficiency and reproducibility is the partially irreversible adsorption of macromolecules on the inner surface of a capillary caused by electrostatic and hydrophobic interactions [2,3,9–11], conformational change of macromolecules [12,13], and physical adhesion mechanisms enhanced by the presence of defects on a capillary surface [14–16]. For example, the irreversible adsorption on the capillary surface becomes significant for biological samples, such as cells, where analyte is associated with multimolecular complexes [3,17]. These problems are especially important for traditional HPCE systems with UV-spectrophotometric detection where the analyte concentration should be maintained at 0.1–1.0 mg/ml.

Many methods of buffer and capillary surface modification were successfully used to prevent electrostatic and hydrophobic interactions, such as pH and ionic strength varying [17–19], buffer additives [20–27], physical and chemical coating [4,16,28–33]. At the same time the adsorption, caused by conformational changes, and physical adhesion of large molecules dramatically increased by capillary surface inhomogeneity were hardly overcome. The study of capillary surface quality [15]

☆Presented at the 12th International Symposium on High Performance Capillary Electrophoresis and Related Microscale Techniques, Palm Springs, CA, 23–28 January 1999.

*Corresponding author. Present address: Colorado School of Mines, Department of Chemistry and Geochemistry, 1500 Illinois Street, Golden, CO 80401, USA.

[1]Present address: Institute of Macromolecular Compounds, 31 Bolshoj Pr. V.O., St. Petersburg 199004, Russia.

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00226-0

showed the presence of significant defects in commercial capillaries with a surface layer of about 0.5 μm. The proteins preferably adsorbed at the surface defects where the contact area was maximum. Also, the influence of capillary surface characteristics on the coating stability was observed in Ref. [16].

This study was devoted to the HPCE device and capillary modification to achieve successful separations and minimize the adsorption of biomolecules, such as peptides, proteins and double-stranded DNA fragments.

2. Experimental

2.1. Equipment

The improved, in comparison with previous publications [34,35], laboratory-made HPCE system was applied for carrying out experiments on biomolecules. A stabilized 0–±30 kV power supply (±0.01%) was used for continuous field electrophoresis. A UV-spectrophotometric detector (wavelength range 214–360 nm, wavelength accuracy 3 nm, wavelength band 15 nm, noise $5 \cdot 10^{-5}$ AU, drift 10^{-4} AU/h) was operated in the on-line mode. The prealigned deuterium lamp was used as a light source. The beam with the wavelength of interest was selected with the monochromator and focused on the capillary ''detection window''. The part of a light beam was used as a reference signal. The photocurrents were measured in the detection block based on hybrid microcircuits. Then, data were processed in real time with a microprocessor and recorded with a personal computer compatible computer and a tape-recorder. Response time was 0.2, 0.5 and 1 s. The computer program provided data presentation, noise reduction, baseline shift compensation, peak characterization and comparison of electropherograms. The sampler, arranged as a precisely positioned carousel, included 20 vials with volumes ranging from 30 μl for biosamples to 150 μl for rinsing liquids. For biosample protection, the carousel was additionally provided with vial caps automatically removed during the vial lift. The electrode blocks held the biocompatible platinum electrode and a capillary inlet or outlet end. The air under pressure up to 2 atm was used for pumping liquids through the capillary (1 atm=101 325 Pa). The system allowed one to inject sample in electromigration, hydrostatic (height difference), and pneumatic (under air pressure) modes. Electrophoresis was performed with bare fused-silica capillaries of 50–60 μm I.D.×360 μm O.D. obtained from the Institute of Silica and Glass (St. Petersburg, Russia) or with the modified with poly(glycidyl methacrylate) (PGMA) capillaries.

Experiments with peptides and proteins were performed at ambient temperature. For the separation of DNA fragments in linear polymer solutions, the temperature of a capillary was maintained at 20°C (±0.1%) by means of the water jacket 25 cm in length connected to the external bath-circulator. This modification was caused by the enhanced gas content in the viscous polymer solution even after completing bubble removal by vacuum degassing. Also, the moderate viscosity of chosen polymer solutions allowed one to operate at this temperature and avoid increasing DNA adsorption on the silica observed at elevated temperatures [36].

2.2. Chemicals

Tris, boric acid, EDTA (ethylenediaminetetraacetic acid) used for the TBE buffer (89 m*M* Tris, 89 m*M* boric acid, 2 m*M* EDTA, pH 8.3), hydroxypropylmethylcellulose (HPMC) (M_r 86 000, viscosity of 2% solution equals 4000 cP), and proteins were obtained from Sigma (St. Louis, MO, USA). All buffers were prepared in distilled deionized water and filtered through 0.22-μm pore size filters (Millipore, Bedford, MA, USA). The 0.5% HPMC solution was prepared in the filtered buffer at 70°C with slow mixing and then degassed under vacuum. Peptides were obtained from Serva (Heidelberg, Germany) and the Institute of Macromolecular Compounds (St. Petersburg, Russia). Peptide samples were dissolved in the 10-fold diluted buffer for stacking. Protein samples were dissolved in the operating buffer. λ-Hind III DNA restriction fragment mixture was obtained from New England Biolabs (Beverly, MA, USA). All DNA samples were used without dilution. Glycidol, dioxane, H_2SO_4 were obtained from Reahim (St. Petersburg, Russia), 3-glycidoxypropyltrimethoxysilane (GPTMS), glycidyl methacrylate (GMA), $(NH_4)_2Ce(NO_3)_6$ were obtained from

Aldrich (Milwaukee, WI, USA). BF_3 was obtained from Fluka (Buchs, Switzerland). A 5% aqueous solution of acetone was used as a neutral marker for electroosmotic flow (EOF) rate measurement.

2.3. Capillary modification

The new method of neutral covalent coating of the inner surface of a fused-silica capillary was developed to reduce the EOF rate and, mainly, to prevent the adsorption of macromolecules by masking the charged sites and inducing steric hindrance repulsion. For this purpose, the surface of a capillary was covered with the diol groups of high surface density. The final step of coating included grafting PGMA. These mechanisms will be discussed in the next section.

The schematic representation of chemical reactions is given in Fig. 1.

First, the capillary was rinsed with 1.0 *M* NaOH solution during 15 min at ambient temperature and then with water up to neutral pH to remove impurities. Secondly, the reaction with 10% aqueous solution of GPTMS, pH 3, was allowed to proceed for 30 min at 90°C to form the diol groups on the surface. After that, rinsing with water and, then, with dioxane was carried out. Thirdly, the 25% glycidol solution in dioxane with 0.3% BF_3 as a catalyst was introduced into the capillary for 4 h (first, at 5–10°C, then at 50°C) to coat the wall with an oligomeric diol

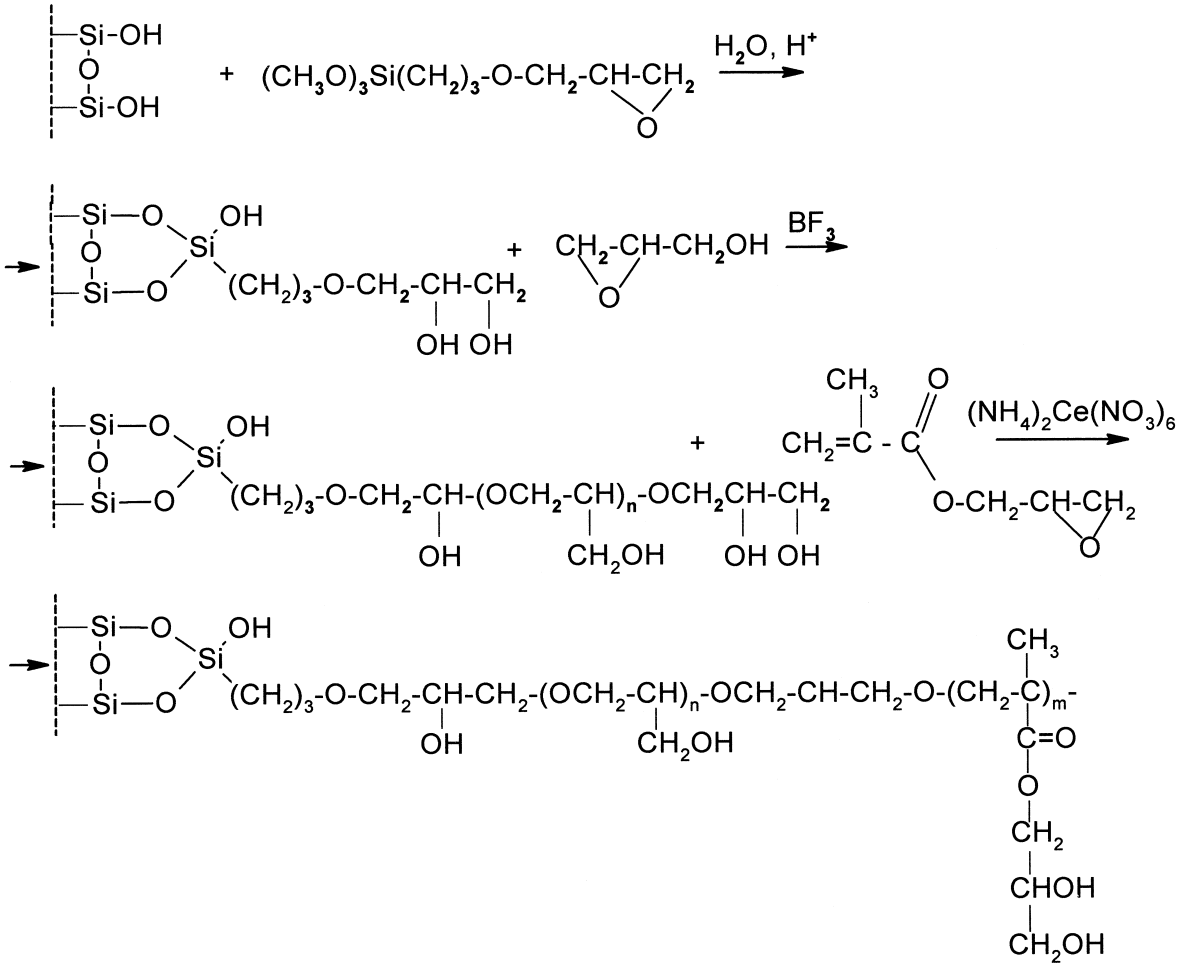

Fig. 1. Scheme of PGMA coating.

layer. Then, after washing with dioxane and water, the reaction with 1.5% water solution of GMA [0.1% $(NH_4)_2Ce(NO_3)_6$ as a catalyst] was carried out during 2 h at ambient temperature for grafting GMA with double bonds and forming a polymeric layer of high density. All reagents were pumped through the capillary. Finally, after washing with water, the treatment with H_2SO_4 solution, pH 2.5, during 30 min at 90°C was carried out to hydrolyze the epoxy groups of PGMA. After that, the capillary was rinsed with water up to neutral pH, then with ethanol, and dried.

3. Results and discussion

The best example of HPCE advantages is the separation of peptides. Theoretically predicted efficiency reaches 10^5 theoretical plates per meter of a capillary. At the same time the molecules of peptides are quite small and mobile to minimize the physical adsorption (adhesion) on a capillary surface. Two modes of capillary zone electrophoresis (CZE) can be used for peptides in uncoated capillaries. In the first case, at pH 7–9, negatively charged peptides move toward the anode with different velocities but the strong EOF replaces them to the cathode. For our separation (see Fig. 2) we obtained an efficiency of about $3\cdot10^4$ theoretical plates (RSD=1%). It should be noticed that the strong EOF is not optimal for the resolution. Let us examine the relationship for the resolution of two species, R_s, in CZE:

$$R_s = \frac{\Delta t}{2(\sigma_{t1} + \sigma_{t2})} = \frac{l/(v_{eo} + v_{ep2}) - l/(v_{eo} + v_{ep1})}{2(\sigma_{t1} + \sigma_{t2})} \quad (1)$$

where Δt is the difference between migration time for two species, v_{eo} and v_{ep1}, v_{ep2} are, subsequently, electroosmotic and electrophoretic velocities for the first and second species, σ_{t1} and σ_{t2} are standard deviations for two peaks, and l is the capillary length. Assuming $v_{ep1} = v_{ep} + \Delta v/2$ and $v_{ep2} = v_{ep} - \Delta v/2$, we can rewrite it as:

$$R_s = \frac{l}{2(\sigma_{t1} + \sigma_{t2})} \cdot \frac{\Delta v}{v_{eo}^2 + 2v_{eo}v_{ep} + v_{ep}^2 - (\Delta v/2)^2} \quad (2)$$

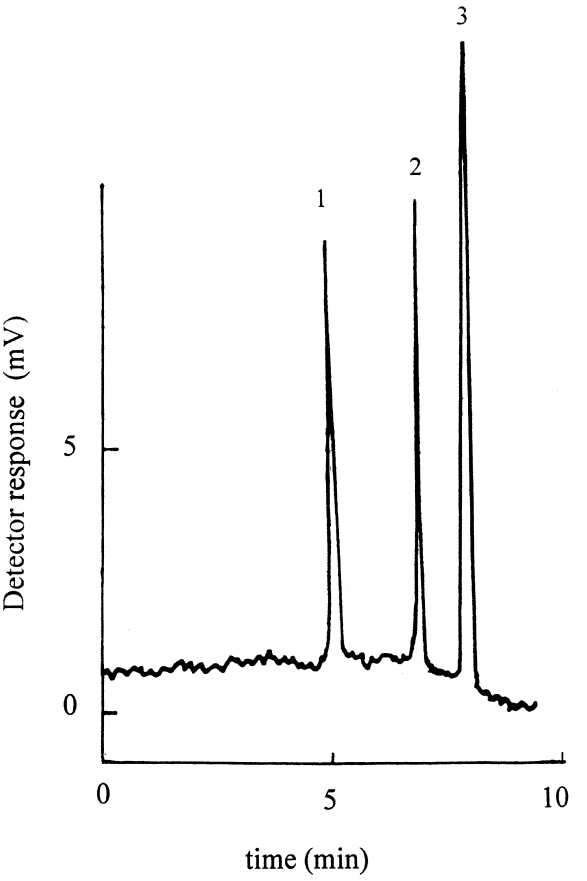

Fig. 2. Capillary zone electrophoresis of peptides at pH 7.5. Conditions: capillary 50 cm (43 cm effective length)×50 μm; voltage 20 kV; detection wavelength 254 nm; electrolyte 0.02 *M* Tris–boric acid buffer, pH 7.5; electromigration injection: 8 s at 20 kV. Peaks: 1=bradykinin, 2=met-enkephalin, 3=substrate for elastase (succinyl–Ala–Ala–Ala–4-nitroanilide) (concentrations 0.2–0.3 mg/ml).

where $\Delta v = v_{ep1} - v_{ep2}$ and $v_{ep} = (v_{ep1} + v_{ep2})/2$.

Under the condition $v_{eo} >> v_{ep}$ Eq. (2) may be simplified:

$$R_s = \frac{l\Delta v}{2(\sigma_{t1} + \sigma_{t2})v_{eo}^2} \quad (3)$$

From Eq. (3), we can conclude that resolution is inversely proportional to the square of the electroosmotic velocity.

The second CZE mode utilizes buffers at pH 2–4 allowing one to suppress the EOF and electrostatic

interactions. In this case, positively charged peptides move toward the cathode in accordance to their electrophoretic mobility. In this mode we achieved theoretically predicted efficiencies about 10^5 theoretical plates per meter (RSD = 1%) and separated peptides with very similar structure. The peptide corresponding to the third peak in Fig. 3 differs from the second one only by the substitution in the side chain of methionine giving methionine sulfoxide, Met[O]. Also we obtained two well-resolved peaks for met-enkephalin and its admixture (peaks 4 and 5 in Fig. 3) eluted as one narrow peak in the previous case (Fig. 2). We did not observe significant adsorption and band broadening even for positively charged peptides.

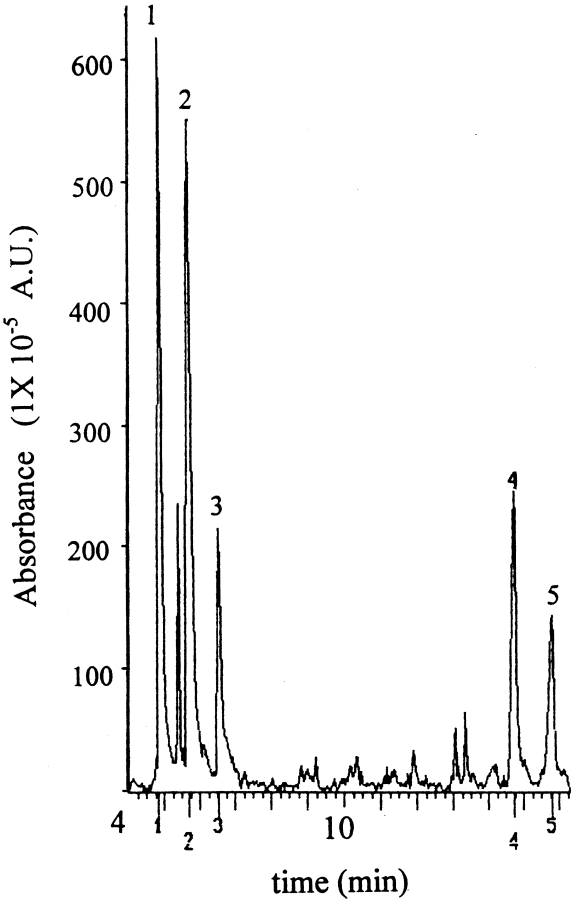

Fig. 3. Capillary zone electrophoresis of peptides at pH 3.6. Conditions: capillary 45 cm (37 cm effective length)×50 μm; voltage 15 kV; detection wavelength 214 nm; electrolyte 0.025 *M* citric acid–Na_2HPO_4 buffer, pH 3.6; electromigration injection: 10s at 5 kV. Peaks: 1 = anisoyl–Arg–Arg–Ile–Arg–Leu–His–Val–NH_2, 2 = anisoyl–Arg–Arg–Ile–Arg–Pro–Met–Leu–NH_2, 3 = anisoyl–Arg–Arg–Ile–Arg–Pro–Met[O]–Leu–NH_2, 4, 5 = met-enkephalin and its admixture (concentrations 0.1–0.3 mg/ml).

For proteins, the situation was changed. Even for negatively charged proteins at pH 9 we obtained only about 10^3 theoretical plates in the uncoated capillary. The low protein recovery was measured in Ref. [11] for similar conditions. This result may be explained by presence of adsorption associated with conformational changes of protein molecules under action of short-range forces [12,13]. This adsorption is energetically favorable even for both negatively charged proteins and surfaces but affected by surface quality and hydrophilicity. In accordance with Ref. [15], the preferential adsorption of protein molecules occurred near the capillary surface defects observed in CE commercial capillaries. The covalent surface coating with a hydrophilic polymer was shown to be the most stable with higher efficiencies and protein recoveries [9,31,33]. At the same time, the study of hydrophilic polyacrylamide (PAA) coating [37] showed the presence of protein adsorption even at negligible EOF. From the above described point of view, even multilayer [16] or cross-linked [33,38] polymer coatings can not fully eliminate protein adsorption.

The effective method to prevent protein adsorption on the capillary surface is the formation of the regular ''brush'' polymer structure with the distance between polymer chains comparable to the protein size [13]. The steric hindrance repulsion between polymer chains and proteins does not permit a protein molecule to approach the near surface position where the short-range interactions are significant. The success of coating with derivatized polystyrene nanoparticles [30] was probably caused by this effect. At present, it is difficult to obtain the controllable polymer ''brush'' inside a micron size capillary channel. But the traditional chromatographic method of grafting [39] may be the logical approach to solving this problem.

The new method of coating with grafting PGMA is described in Section 2.3. Literature data showed [38] that hydrophilic epoxy polymer coatings provided the good efficiency of protein separations and stability in the wide pH range. In our procedure we avoided the polymer cross-linkage and developed the

method of grafting PGMA to the CE capillary surface. This coating is particularly good not only to suppress hydrophobic interactions and EOF but to prevent adsorption caused by short-range interactions. The test showed more than 1000 h stability of this coating. The EOF rate in the coated capillary at neutral pH decreased 4.2-times in comparison with the uncoated capillary. Using PGMA-coated capillaries we achieved efficiencies up to $5 \cdot 10^4$ theoretical plates for the positively charged protein mixture (RSD = 2%) (Fig. 4). Not very high efficiency can be caused by residual electrostatic attraction. Another explanation is high protein concentrations (1–2 mg/ml) used for this study. In accordance with Ref. [33], decreasing protein concentration from 1 to 0.1 mg/ml can result in a fivefold efficiency increase.

Another biological macromolecule, double-stranded DNA, is very different from proteins in its adsorption behavior. First, the DNA molecule is negatively charged in the TBE buffer (pH 8.3). Hence, the electrostatic repulsion with negatively charged silanol groups on a capillary surface takes place. Secondly, the hydrophobic sites of DNA are located in the interior of a double helix. Thirdly, the conformational changes are restricted by the relatively rigid structure of a double helix [36]. As a result, the adsorption of double-stranded DNA on the silica surface is unfavorable in low ionic strength buffers near and above neutral pH [36,40]. At the same time, the size of DNA molecules is sufficient for the strong adhesion.

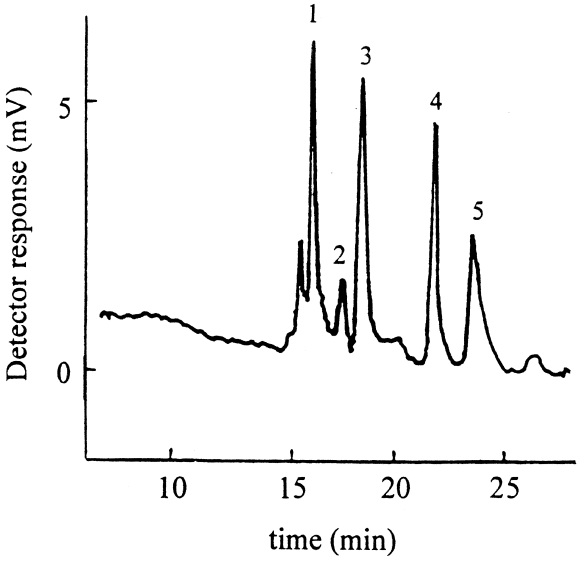

Fig. 4. Capillary zone electrophoresis of basic proteins in the PGMA-coated capillary. Conditions: capillary 50 cm (43 cm effective length) × 60 μm; voltage 18.5 kV; detection wavelength 230 nm; electrolyte 0.02 *M* citric acid–Na_2HPO_4 buffer, pH 4.2; electromigration injection: 10 s at 18.5 kV. Peaks: 1 = cytochrome *c*, 2 = ribonuclease A, 3 = sperm whale myoglobin, 4 = horse myoglobin, 5 = conalbumin (concentrations 1–2 mg/ml).

It is known that DNA fragments cannot be separated by free solution CZE due to the linear charge-to-size ratio. HPCE in noncrosslinked polymer solutions provides size-based DNA separation due to interaction with linear randomly entangled polymers in solution [4,5,41,42].

After the preliminary study, the 0.5% HPMC solution was chosen as having moderate viscosity and providing dynamic coating of a capillary surface [25,43]. The first separation experience was negative. We could not detect the highly purified λ-Hind III DNA Digest sample because it was fully adsorbed in the capillary. It was clear that in this case the adhesion of DNA molecules took place. The method of capillary treatment [42], applied with a few modifications, permitted one to achieve the reproducible separation of a standard DNA mixture in the uncoated capillary (RSD = 2%) (Fig. 5). The new capillary was treated with 1 *M* NaOH for 3 h and then rinsed with water. Then, between experiments, the capillary was rinsed, first, with 1 *M* NaOH for 10 min, then with 0.1 *M* NaOH for 10 min, then with water for 10 min, then with diluted HPMC solution (about 30% of final HPMC concentration) for 10 min, and, finally, filled with the viscous operating buffer. As can be seen, the treatment is much longer than the electrophoresis itself. Besides removing adsorbed DNA molecules, this treatment repetition had the same effect as the hydrofluoric acid etching applied in Ref. [15] for smoothing the surface and, hence, diminishing adhesion. The treatment of a capillary with 0.1 *M* HCl utilized in Ref. [43] can cause the time dependence of EOF that is difficult to control.

The neutral covalent coatings used for DNA separations [4,31,32] have a questionable effect on DNA adsorption. These coatings serve to suppress EOF. At the same time, eliminating the negative capillary surface charge decreases the repulsion of

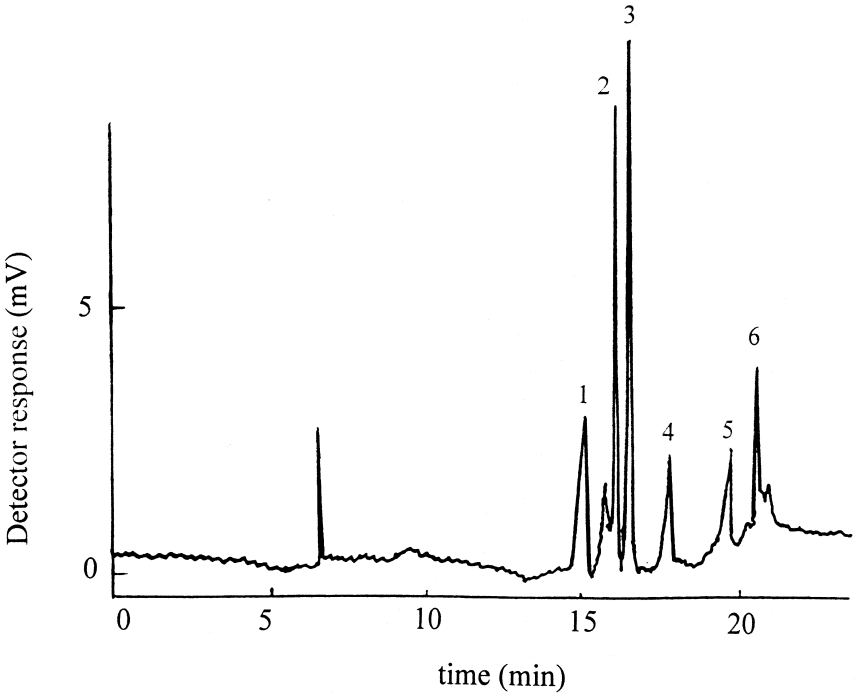

Fig. 5. HPCE of λ-Hind III DNA digest in noncrosslinked polymer solutions. Conditions: capillary 50 cm (43 cm effective length)×50 μm; voltage 20 kV; detection wavelength 254 nm; electrolyte 0.5% hydroxypropylmethylcellulose in TBE; electromigration injection: 29 s at 20 kV. Peaks: 1=23 130 base pairs (bp), 2=9416 bp, 3=6557 bp, 4=4361 bp, 5=2322 bp, 6=2027 bp.

DNA. Also, the treatment of a capillary surface with cleaning liquids is not available. Finally, the thin polymer layer can not smooth the capillary surface. From this point of view, the multilayer negatively charged coatings with desired EOF rate would be useful for double-stranded DNA separation over a wide range of molecular mass.

4. Conclusion

After this series of experiments we can conclude that HPCE of biopolymers is a very powerful technique. The high efficiencies can be achieved for pure samples. At the same time the negative influence of chemical adsorption and adhesion of macromolecules on a capillary surface enhanced by the presence of surface defects is significant. CE performance, reproducibility and recovery, especially, for practical applications, such as blood proteins, cellular DNA and environmental samples can be problematic. In the case of proteins the methods of capillary coating by grafting with hydrophilic polymer chains can be used to prevent adsorption caused by short-range interactions. For double-stranded DNA, the capillary surface quality plays the dramatic role in the prevention of adhesion. We suppose that improved capillary quality control during the industrial process together with further development of HPCE technique and capillary surface modification procedures may be the way to reach the wide admittance of HPCE for biological and environmental applications.

Acknowledgements

The work of G.E.K. on the paper preparation and presentation at the HPCE‘99 conference were supported in part by the grant from ACTR/ACCELS with funds provided by the US Information Agency.

References

[1] J.W. Jorgenson, K.D. Lukacs, Science 222 (1983) 266.
[2] C. Schoneich, A.F.R. Huhmer, S.R. Rabel, J.F. Stobaugh, S.D.S. Jois, Anal. Chem. 67 (1995) 155.
[3] N.A. Guzman, L. Hernandez, S. Terabe, in: Cs. Horváth, J.G. Nikelly (Eds.), Analytical Biotechnology – Capillary Electrophoresis and Chromatography, ACS Symposium Series, No. 434, American Chemical Society, Washington, DC, 1990, p. 3.
[4] O. Salas-Solano, E. Carrilho, L. Kotler, A.W. Miller, W. Goetzinger, Z. Sosic, B.L. Karger, Anal. Chem. 70 (1998) 3996.
[5] N.J. Dovichi, Electrophoresis 18 (1997) 2393.
[6] V. Dolnik, Electrophoresis 20 (1999) 3106.
[7] I. Rodriguez, S.F.Y. Li, Anal. Chim. Acta 383 (1999) 1.
[8] H.H. Lauer, D. McManigill, Anal. Chem. 58 (1986) 166.
[9] M. Chiari, N. Dell'Orto, A. Gelain, Anal. Chem. 68 (1996) 2731.
[10] Z. Zhao, A. Malik, M.L. Lee, Anal. Chem. 65 (1993) 2747.
[11] J.K. Towns, F.E. Regnier, Anal. Chem. 64 (1992) 2473.
[12] W. Norde, Adv. Colloid Interface Sci. 25 (1986) 267.
[13] M.K. Ko, J.J. Pellegrino, R. Nassimbene, P. Marko, J. Membr. Sci. 76 (1993) 101.
[14] S.C. Beale, Anal. Chem. 70 (1998) 279R.
[15] S. Kaupp, R. Steffen, H. Watzig, J. Chromatogr. A 744 (1996) 93.
[16] S. Hjerten, K. Kubo, Electrophoresis 14 (1993) 390.
[17] T.M. McNerney, S.K. Watson, J.H. Sim, R.L. Bridenbaugh, J. Chromatogr. A 744 (1996) 223.
[18] F.A. Chen, J. Chromatogr. 559 (1991) 445.
[19] T. Bergman, B. Agerberth, H. Joerenvall, FEBS Lett. 283 (1991) 100.
[20] F.A. Chen, L. Kelly, R. Palmieri, R. Biehler, H. Schwartz, J. Liq. Chromatogr. 15 (1992) 1143.
[21] M.J. Gordon, K.-J. Lee, A.A. Arias, R.N. Zare, Anal. Chem. 63 (1991) 69.
[22] H. Linder, W. Helliger, A. Dirschimayer, M. Yaquemar, B. Puschendort, J. Biochem. 283 (1992) 467.
[23] C. Stathakis, E.A. Arriaga, D.F. Lewis, N.J. Dovichi, J. Chromatogr. A 817 (1998) 227.
[24] C. Stathakis, E.A. Arriaga, N.J. Dovichi, J. Chromatogr. A 817 (1998) 233.
[25] M. Gilges, M.H. Kleemiss, G. Schomburg, Anal. Chem. 66 (1994) 2038.
[26] E.L. Hult, A. Emmer, J. Roeraade, J. Chromatogr. A 757 (1997) 255.
[27] K.K.C. Yeung, C.A. Lucy, Anal. Chem. 69 (1997) 3435.
[28] S. Hjerten, J. Chromatogr. 347 (1985) 191.
[29] E. Cordova, J. Gao, M. Whitesides, Anal. Chem. 69 (1997) 1370.
[30] G. Kleindienst, C.G. Huber, G.K. Bonn, Electrophoresis 19 (1998) 262.
[31] C. Gelfi, M. Curcio, P.G. Righetti, R. Sebastiano, A. Citterio, H. Ahmadzadeh, N.J. Dovichi, Electrophoresis 19 (1998) 1677.
[32] Y. Liu, W.G. Kuhr, Anal. Chem. 71 (1999) 1668.
[33] K. Srinivasan, C. Pohl, N. Avdalovic, Anal. Chem. 69 (1997) 2798.
[34] B.G. Belenkii, Yu.V. Belov, G.E. Kasalainen, J. Anal. Chem. (Transl. of Zh. Anal. Khim.) 51 (1996) 753.
[35] B.G. Belenkii, Yu.V. Belov, G.E. Kasalainen, M.I. Medvedeva, S.T. Khor, J. Anal. Chem. (Transl. of Zh. Anal. Khim.) 53 (1998) 291.
[36] K. Melzak, C.S. Sherwood, R.F.B. Turner, C.A. Haynes, J. Colloid Interface Sci. 181 (1996) 635.
[37] M.A. Strege, A.L. Lagu, J. Chromatogr. 630 (1993) 337.
[38] J.K. Towns, J. Bao, F.E. Regnier, J. Chromatogr. 599 (1992) 227.
[39] B.G. Belenkii, E.S. Gankina, G.E. Kasalainen, M.B. Tennikov, in: M. Potschka, P.L. Dubin (Eds.), Strategies in Size Exclusion Chromatography, ACS Symposium Series, No. 635, American Chemical Society, Washington, DC, 1996, p. 274.
[40] V. Balladur, A. Theretz, B. Mandrand, J. Colloid Interface Sci. 194 (1997) 408.
[41] E. Carrilho, M.C. Ruiz-Martinez, J. Berka, I. Smirnov, W. Goetzinger, A.W. Miller, D. Brady, B.L. Karger, Anal. Chem. 68 (1996) 3305.
[42] A.E. Baron, W.M. Sunada, H.W. Blanch, Biotech. Bioeng. 52 (1996) 259.
[43] E.N. Fung, E.S. Yeung, Anal. Chem. 67 (1995) 1913.