Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1003 (2003) 217–221
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Novel polyamine coating providing non-covalent deactivation and reversed electroosmotic flow of fused-silica capillaries for capillary electrophoresis

Emilia Hardenborg[a], Aida Zuberovic[a], Sara Ullsten[a], Lennart Söderberg[b], Eva Heldin[a], Karin E. Markides[a,*]

[a]*Department of Analytical Chemistry, Uppsala University, Box 599, SE-751 24 Uppsala, Sweden*
[b]*Center for Surface Biotechnology, Uppsala University, Box 577, SE-751 23 Uppsala, Sweden*

Received 15 July 2002; received in revised form 10 April 2003; accepted 28 April 2003

Abstract

A new polycationic coating for use in capillary electrophoresis has been developed that enables chemical modification of fused-silica capillary surfaces for analysis of compounds like basic proteins. The cationic polyamine, containing short aliphatic blocks of combined 2 and 3-carbon length, was physically adsorbed onto the negatively charged fused-silica surface through ionic interaction by flushing the capillary with a polyamine solution, followed by a self-stabilization step. The polyamine coated capillaries generated an anodal electroosmotic flow that was independent of pH in the investigated range of pH 4–8. The capillary performance was demonstrated by fast separations of basic proteins with peak efficiencies in the range of 265 000–584 000 plates.

Keywords: Coated capillaries; Polyamine coating; Proteins

1. Introduction

In capillary electrophoresis (CE), fused-silica is the most commonly used capillary material due to its good thermal conductivity, ultraviolet (UV) transparency and uniform inner diameter. The chemical properties of the capillary surface play an important role in the separation process and fused-silica exposes a hydrophobic, negatively charged, pH dependent surface. These features of fused-silica can be problematic, especially when analyzing biomolecules like proteins, due to analyte–wall interactions [1,2]. Proteins might be adsorbed, irreversibly or with slow desorption, causing bad reproducibility and impaired efficiencies. Proteins with an isoelectric point, p*I*, above 8 and/or with a mass, M_r, larger than 50 000 have been identified as most difficult to analyze on bare fused-silica capillaries [3].

A successful way to overcome analyte adsorption problems is chemical modification of the fused-silica capillary internal wall and several reviews have been published about coating of capillaries for CE [4–6]. The most successful methods are those whereby the silanol groups are deactivated by a polymer layer.

*Corresponding author. Tel.: +46-1-8471-3691; fax: +46-1-8471-3692.
E-mail address: karin.markides@kemi.uu.se (K.E. Markides).

0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)00739-8

Several polymers have been covalently attached via bifunctional silylating reagents thus producing a hydrophilic surface with suppressed electroosmotic flow (EOF) [7–12]. These coating procedures are, however, often laborious and the siloxane bond is hydrolytically unstable at high pH [13]. As an alternative approach cationic polymers can simply be physically adsorbed to the capillary surface by a multisite ionic interaction. When the polymer has a higher density of charged sites than the surface below, the net charge of the newly created surface will become positive and thus reverse EOF compared to conventional CE using un-coated fused-silica. This is advantageous compared to coatings with suppressed EOF because fast separations can still be performed. In addition, the resolution of analytes of similar mobility can be enhanced when the direction of migration is opposed to the EOF. To date, a large flora of cationic polymers, such as polyethyleneimine (PEI) [2,3,14–16], polybrene [3,17–22], poly-(diallyldimethylammonium chloride) (PDADMAC) [3,23,24], polyarginin [18] and chitosan [25,26] have been used for non-covalent deactivation of fused-silica surfaces. The produced positive surface will repel cationic analytes, providing more or less useful alternatives for the separation of compounds like basic proteins.

In this study, a new polycationic polymer, PolyE-323, was introduced. The synthesis and capillary coating procedure is described. The performance of the coated capillaries in CE was investigated by short and long term measurements of the electroosmotic flow at different pH values. The level of surface coverage was tested by separation of basic proteins with pI>8.

2. Materials and methods

2.1. Instrumentation

The protein separations were carried out using a home-built capillary electrophoresis system, consisting of a Plexiglas cage, a high voltage power supply (Bertan ARB 230, Bertan High Voltage, Hicksville, NY, USA) and a UV detector (Lambda 1000, Bischoff, Leonberg, Germany). For the pH and stability studies, a Beckman P/ACE instrument with Karat Software (Beckman Coulter, Fullerton, CA, USA) was used.

2.2. Chemicals and materials

For polymer synthesis 1,2-bis(3-aminopropylaminoethane (CAS Number [10563-26-5]) of practical grade was purchased from LabKemi (Stockholm, Sweden) and epichlorohydrine ''puriss'' grade from Fluka (Buchs, Switzerland). All other chemicals were of analytical-reagent grade and obtained from Merck (Darmstadt, Germany). Water was purified with a Milli-Q purification system (Millipore, Bedford, MA, USA). Fused silica capillary tubing of 50 μm I.D.×365 μm O.D. was obtained from Polymicro Technologies. (Phoenix, AZ, USA). Lysozyme (chicken egg white L-6867), cytochrome *c* (bovine heart C-2037), ribonuclease A (bovine pancreas R-5500) and α-chymotrypsinogen (bovine pancreas C-4879) were purchased as lyophilized powders from Sigma–Aldrich (Steinheim, Germany) and used without further purification. Stock solutions of proteins were made by dissolving proteins in Milli-Q water at a concentration range of 1–2 mg/ml. The solutions were stored at −20 °C and warmed to room temperature prior to use. Samples were made by mixing the proteins to a concentration of 0.25 mg/ml of each and adding a small amount of ethanol as electroosmotic flow marker.

2.3. Synthesis of PolyE-323

In a 250 ml E-flask, 17.65 g (0.10 mol) of 1,2-bis(3-aminopropylamino)ethane was mixed with 20 g Milli-Q water and 9.3 g (0.10 mol) epichlorohydrine during intensive magnetic stirring. The flask was sealed and the mixture was continuously stirred at room temperature for 48 h while the reaction mixture was thickened. An additional 100 g Milli-Q water was added. Finally the equilibration reaction was allowed to continue for 1 week. The polymer solution was stored at +8 °C and used without further purification.

2.4. Coating procedure

In order to make a detection window, the external

polyimide coating was burned off over a length of 3 mm, before coating the capillary with PolyE-323. The fused-silica capillary was thereafter activated by flushing with 1 *M* NaOH for 30 min at 690 kPa followed by Milli-Q water for 3 min at the same pressure. The capillary was further treated with a diluted PolyE-323 solution (200 μl of the polyamine solution was mixed with 1000 μl 0.2 *M* acetic acid, giving a pH of about 7) for 1 min at 690 kPa (corresponding to approximately 20 capillary volumes) and the solution was thereafter left in the capillary for 1 h. The capillary was further flushed with an ammonium acetate buffer of pH 7 for 5 min. The capillary preparation was performed in an enclosed air-free device utilizing nitrogen gas to push liquids through capillaries from clean glass vials. In these initial tests, re-coating of the capillary after 5 days enhanced the stability of the polyamine-coated surface. The capillary was thus refilled with polymer solution (1 min flow at 690 kPa) and the solution was left in the capillary for 1 h. Thereafter the capillary was flushed with running buffer for 5 min at 690 kPa. The coating procedure is presently being optimized with an emphasis to shorten coating time.

3. Results and discussion

The use of polyamines to deactivate fused-silica capillaries in CE is attractive due to the speed and simplicity of the coating procedure. By simply flushing capillaries with polyamine solution, a reproducible coating is formed by multisite ionic interaction. In this study a new polyamine, PolyE-323, is introduced and the structure is schematically depicted in Fig. 1. The nitrogen atoms in the backbone are separated by carbon chains of predominantly three atoms length, which is in contrast to other polymers commonly used for capillary coatings, like the highly branched polyethyleneimine with repeating two-carbon units or polybrene, in which the nitrogens are separated by six carbon atoms. The length of the spacer arm between the nitrogen atoms will affect polymer properties like flexibility, hydrophobicity and protonation behavior [27]. PolyE-323 also contains hydroxyl groups, which can contribute to polymer immobilization on the capillary wall by hydrogen bonding. The structure of PolyE-323 is thus purposely constructed to have mixed bonding characteristics. An in-depth study to further understand the surface binding characteristics and architecture of PolyE-323 is being planned.

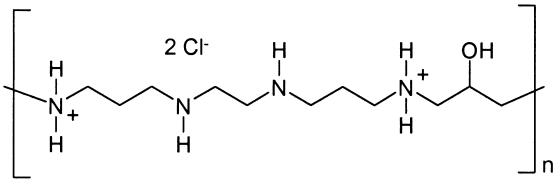

Fig. 1. Schematic structure of PolyE-323 depicted at a degree of protonation of 50%.

3.1. Electroosmotic flow at different pHs

It is clear that PolyE-323 has a high density of positive charges that will interact strongly with the negatively charged silanol groups on the fused-silica surface. The excess of positive surface charges gives an electroosmotic flow towards the anode (so called anodic EOF) of opposite direction compared to bare fused-silica. In order to investigate the surface behavior, the electroosmotic flow generated by PolyE-323 coated capillaries was measured at different pHs and was compared to bare fused-silica as shown in Fig. 2. Fused silica surfaces show an increased EOF with increasing pH due to the well known increased ionization of silanol groups at higher pH values. For the polyamine coated capillary, on the other hand, the net positive charge was constant from pH 4 to 8, giving a stable electroosmotic flow over this pH range. This property is a great asset that will provide reproducible migration behavior and also a possibility to optimize pH for best separation without altering the electroosmotic flow.

3.2. Coating stability

In order to investigate the reproducibility of the capillary coating procedure, five capillaries were prepared the same day and four capillaries were coated on different days, according to the coating procedure described. The electroosmotic flow, re-

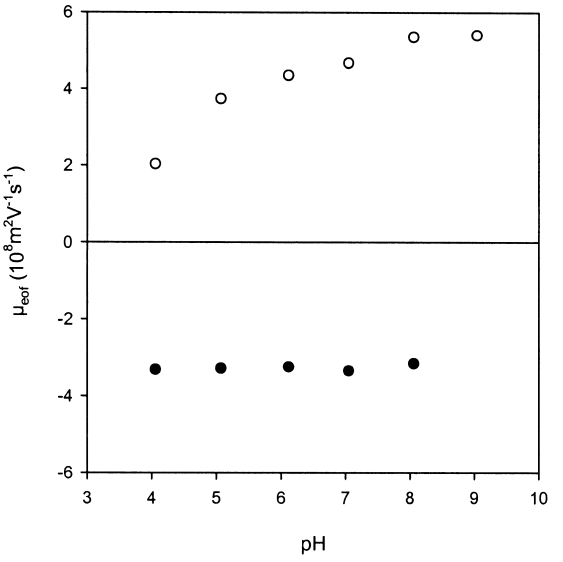

Fig. 2. Electroosmotic flow at different pHs for (●) a polyE-323 coated capillary and (○) a bare fused-silica capillary. Conditions: capillaries of 31 cm (21 cm to detection window)×50 μm I.D.×365 μm O.D.; background electrolyte at pH 4, 5 and 6 was sodium acetate and at pH 7 and 8 Tris–HCl. All buffers were of 20 m*M* ionic strength; UV detection at 190 nm; Milli-Q water was used as EOF marker. Applied voltage of −10 kV using PolyE-323 coated capillaries and +10 kV using fused-silica capillaries. Each value had an RSD of <0.4% ($n=5$) for the polyE-323 coated capillary.

flecting surface chemistry, was used as evaluation parameter. The relative standard deviation was 2.0% for the capillaries made the same day and 3.4% for capillaries prepared on different days [calculated from six injections of the flow marker dimethyl sulfoxide (DMSO)].

The lifetime of PolyE-323 coated capillaries was investigated by consecutive measurements of the electroosmotic flow 80 times at pH 7. The running buffer was changed every 15th run in order to minimize problems with buffer depletion [28]. The EOF was found to be extremely stable for the first 60 injections with an RSD of 0.8%. The electroosmotic flow was thereafter decreased with 7%. In a long-term stability test, PolyE-323 coated capillaries were tested 6 months after they were prepared and the performance was found to be the same as when used immediately. In a preliminary study the capillaries were also subjected to drying by purging nitrogen gas through them for 20 min and thereafter leaving the capillaries dry for a week. When the capillaries were refilled with buffer and tested, the resulting electroosmotic flow was found to be at the same value as before drying. This possibility of capillary drying makes handling of coated capillaries easier, and the PolyE-323 deactivation more attractive, since there is no need for capillaries to be stored in buffers when not in use.

3.3. Separation of basic proteins

The positively charged wall of PolyE-323 coated capillaries will not only generate an anodal electroosmotic flow but also hinder analyte–wall interactions of basic analytes by electrostatic repulsion. The performance of PolyE-323 coated capillaries was investigated by separating a test sample of four basic proteins with p$I>8$ that cannot be analyzed on untreated fused-silica [1,2]. A typical electropherogram of a separation performed at pH 7 is shown in Fig. 3. The coating appears to be efficient in reducing analyte–wall interactions of basic proteins,

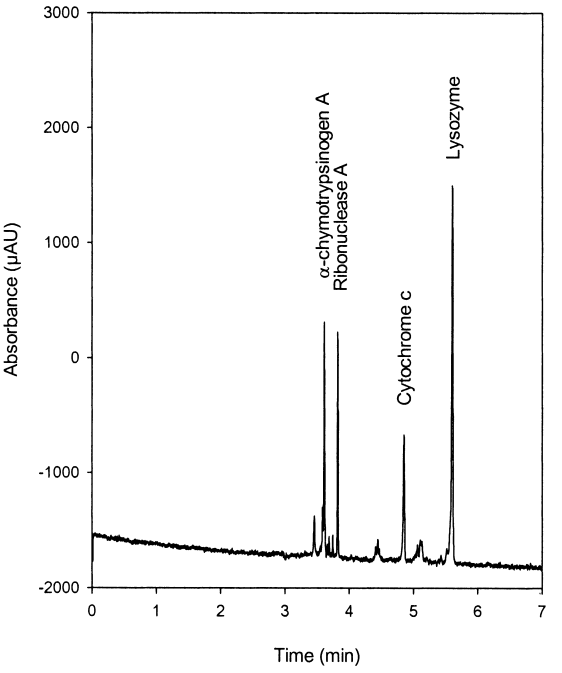

Fig. 3. CE separation of four basic proteins on a PolyE-323 coated capillary. Conditions: PolyE-323 coated capillary, 42 cm (32 cm to detection window)×50 μm I.D.×365 μm O.D.; background electrolyte: Tris–HCl, pH 7, 25 m*M* ionic strength; applied voltage of −15 kV; hydrodynamic injection at 8 cm×3 s; UV detection at 220 nm, protein concentration of 0.25 mg/ml of each protein.

resulting in good peak shape and high plate numbers; 4.00, 5.84, 2.64 and $4.03 \cdot 10^5$ plates, respectively. In addition, the separation was completed in less than 6 min, indicating that this new coating can become useful in fast protein analysis.

Due to the cationic nature of adsorbed coatings under alkaline conditions, PolyE-323 coated capillaries can be used in protein separations performed at physiological pH. This is in contrast to covalent, cationic coatings based on silanization (e.g. 3-aminopropyltriethoxysilane) where a charge reversal occurs at a pH of approx. 5–6 [13,29–31]. Further, the stable EOF of PolyE-323 coated capillaries in the range of pH 4–8 also provides the ability to optimize separation pH without altering the electroosmotic flow. At a background electrolyte ionic strength of 20 m*M*, the magnitude of the generated anodal electroosmotic flow was typically 125 nl/min. This is sufficiently high to be used in sheathless electrospray ionization when mass spectrometric detection is used. A study on using on-line capillary electrophoresis mass spectrometry of basic proteins using PolyE-323 coated capillaries is underway.

4. Conclusions

In this study, a novel cationic polymer, PolyE-323, for non-covalent deactivation of fused-silica capillaries in CE was introduced. The polyamine coated capillaries generated a stable anodal electroosmotic flow that was independent of pH over the investigated range of pH 4–8. The performance of the coated capillaries was demonstrated by separating basic proteins, obtaining efficiencies of 265 000–584 000 plates. The ease of capillary coating in combination with the stable electroosmotic flow and good deactivation properties makes PolyE-323 an attractive polymer for use in analysis of basic biomolecules.

Acknowledgements

Financial support from The Swedish Research Council project K-1439-326, and from the Swedish Foundation for Strategic Research is gratefully acknowledged. Beckman Coulter is acknowledged for loan of instrumentation.

References

[1] J.K. Towns, F.E. Regnier, Anal. Chem. 63 (1991) 1126.
[2] J.K. Towns, F.E. Regnier, Anal. Chem. 64 (1992) 2473.
[3] E. Córdova, J. Gao, G.M. Whitesides, Anal. Chem. 69 (1997) 1370.
[4] I. Rodriguez, S.F.Y. Li, Anal. Chim. Acta 383 (1999) 1.
[5] J. Horvath, V. Dolnik, Electrophoresis 22 (2001) 644.
[6] C.-Y. Liu, Electrophoresis 22 (2001) 612.
[7] S. Hjertén, J. Chromatogr. 347 (1985) 191.
[8] B.J. Herren, S.G. Shafer, J. Van Alstine, J.M. Harris, R.S. Snyder, J. Colloid Interface Sci. 115 (1987) 46.
[9] G.J.M. Bruin, R. Huisden, J.C. Kraak, H. Poppe, J. Chromatogr. 480 (1989) 339.
[10] A. Fridström, N. Lundell, L. Nyholm, K.E. Markides, J. Microcol. Sep. 9 (1997) 73.
[11] W. Nashabeh, Z. El Rassi, J. Chromatogr. 559 (1991) 367.
[12] J.T. Smith, Z. El Rassi, Electrophoresis 14 (1993) 396.
[13] M.A. Moseley, J.W. Jorgenson, J. Shabanowitz, D.F. Hunt, K.B. Tomer, J. Am. Soc. Mass Spectrom. 3 (1992) 289.
[14] J.K. Towns, F.E. Regnier, J. Chromatogr. 516 (1990) 69.
[15] F.B. Erim, A. Cifuentes, H. Poppe, J.C. Kraak, J. Chromatogr. A 708 (1995) 356.
[16] M.S. Nutku, F.B. Erim, J. High Resolut. Chromatogr. 21 (1998) 505.
[17] Y.J. Yao, K.S. Khoo, M.C.M. Chung, S.F.Y. Li, J. Chromatogr. A 680 (1994) 431.
[18] R.W. Chiu, J.C. Jimenez, C.A. Monnig, Anal. Chim. Acta 307 (1995) 193.
[19] J.F. Kelly, S.J. Locke, L. Ramaley, P. Thibault, J. Chromatogr. A 720 (1996) 409.
[20] M.X. Li, L. Liu, J.-T. Wu, D.M. Lubman, Anal. Chem. 69 (1997) 2451.
[21] J.J. Pesek, M.T. Matyska, S. Swedberg, S. Udivar, Electrophoresis 20 (1999) 2343.
[22] M. Dong, R.P. Oda, M.A. Strausbauch, P.J. Wettstein, J.P. Landers, L.J. Miller, Electrophoresis 18 (1997) 1767.
[23] Y. Wang, P.L. Dubin, Anal. Chem. 71 (1999) 3463.
[24] Q. Liu, F. Lin, R.A. Hartwick, J. Chromatogr. Sci. 36 (1997) 126.
[25] P. Sun, A. Landman, R.A. Hartwick, J. Microcol. Sep. 6 (1994) 403.
[26] Y.J. Yao, S.F.Y. Li, J. Chromatogr. A 663 (1994) 97.
[27] R.C. van Duijvenbode, A. Rajanayagam, G.J.M. Koper, M. Borkovec, W. Paulus, U. Steuerle, L. Häußling, Phys. Chem. Chem. Phys. 1 (1999) 5649.
[28] M.S. Bello, J. Chromatogr. A 744 (1996) 81.
[29] Y. Guo, G.A. Imahori, L.A. Colón, J. Chromatogr. A 744 (1996) 17.
[30] M. Thorsteinsdóttir, R. Isaksson, D. Westerlund, Electrophoresis 16 (1995) 557.
[31] D. Figeys, R. Aebersold, J. Chromatogr. B 695 (1997) 163.