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Permanent coated capillaries with reversed electroosmotic flow for anion analysis[1]

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Abstract

Positively charged capillaries for CE with reversed EOF have been prepared by copolymerization of trimethylammoniumstyrene chloride with vinyl groups bonded covalently onto the capillary surface. The EOF was reversed up to pH values of 6 and amounted to the size achievable with uncoated fused-silica capillaries at pH 9, however in the opposite direction. By the addition of acrylamide to the polymerization reactions capillaries could be prepared reproducibly where the EOF was in the anodic direction and was zero at pH values above 7. The coating is stable up to pH of 10. Application of these capillaries for anion separations are shown. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Coated capillaries; Electroosmotic flow; Reversed electroosmotic flow; Anion-exchangers; Anions

1. Introduction

Capillary zone electrophoresis (CZE) is a versatile method for the analysis of small ions and molecules. The indirect detection technique allows also the analysis of ions without chromophores. Applying this technique the separation of alkali and alkaline earth ions can be achieved in less than a minute [1] and detection sensitivities are in the lower ppb range [2]. These cations are moving in front of the electroosmotic flow (EOF), which contributes in this coelectroosmotic technique to the fast migration of these ions [3].

The separation of fast and slow moving anions in the same run is more complicated than the separation of cations. It is easy to analyse the fast migrating anions in the counterelectroosmotic technique by field reversal, however the slower anions (migration velocity smaller than the EOF) are transported by the EOF to the cathodic buffer reservoir. In the coelectroosmotic technique only the slow migrating anions can be detected. To analyze both slow and fast migrating anions the EOF has to be suppressed or better reversed.

Various approaches have been described to reduce or reverse the EOF. The addition of polyamines like spermine [4] to the buffer or coating the capillary surface with a polymeric cations either by physical adsorption, like with polybrene [5] or permanent coating with polyethyleneimine [6] leads to the formation of positively charged surfaces resulting in a reversed EOF, permitting the separation of slow and fast migrating anions in a single run.

Quarternary ammonium salts with at least one long alkyl chain have also been used successfully to reverse the EOF. Here a double layer of the detergents is formed with positive charges reaching into the buffer solution. The anions are then separated in the coelectroosmotic way, resulting in short

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analysis times. Many applications applying these quarternary ammonium ions like CTAB (cetyltrimethylammoniumbromide) have been described in literature [3,7–12].

The formation of the double layer is a dynamic process, consequently the tensides have to be present in the running buffer. There they can form ion pairs with the background electrolyte which may increase buffer viscosity or even leed to the precipitation of ion pairs plugging the capillary. Ion pair formation with sample anions of course affects ion mobilities and hence resolution. For this reasons permanantly bonded charges on the capillary surfaces are wanted. Here the problem of bonding stability arises. Many attempts on coating or permanently bonding positive charges to the capillary surfaces have been described [4,6,13–15]. With physically adsorbed polymers, wich have not to be present in the running buffer reconditioning steps of the polymeric coating are required between each run [16,17]. With chemically bonded phases stability of the coating is one problem [13], on the other hand the reversal of the electroosmotic flow does not always occur over the whole pH range [15]. In the most cases flow reversal is only observed at pH<6, which shows that the surface coverage of the silanole groups is incomplete.

Recently in our laboratory a method has been developed [18] which resulted in stable permanently bonded hydrophilic coatings for the separation of biopolymers in CE. In a two step reaction the capillary surface was first coated with a permanently bonded layer of vinyl groups. In a second step these vinyl groups have been copolymerized with acrylamide. This technique is used here to prepare capillary coatings with permanently bonded positive charges by copolymerization of the covalent fixed vinyl groups with TAMS (trimethylammoniumstyrene chloride). The reaction scheme for the preparation of these capillaries is shown in Fig. 1.

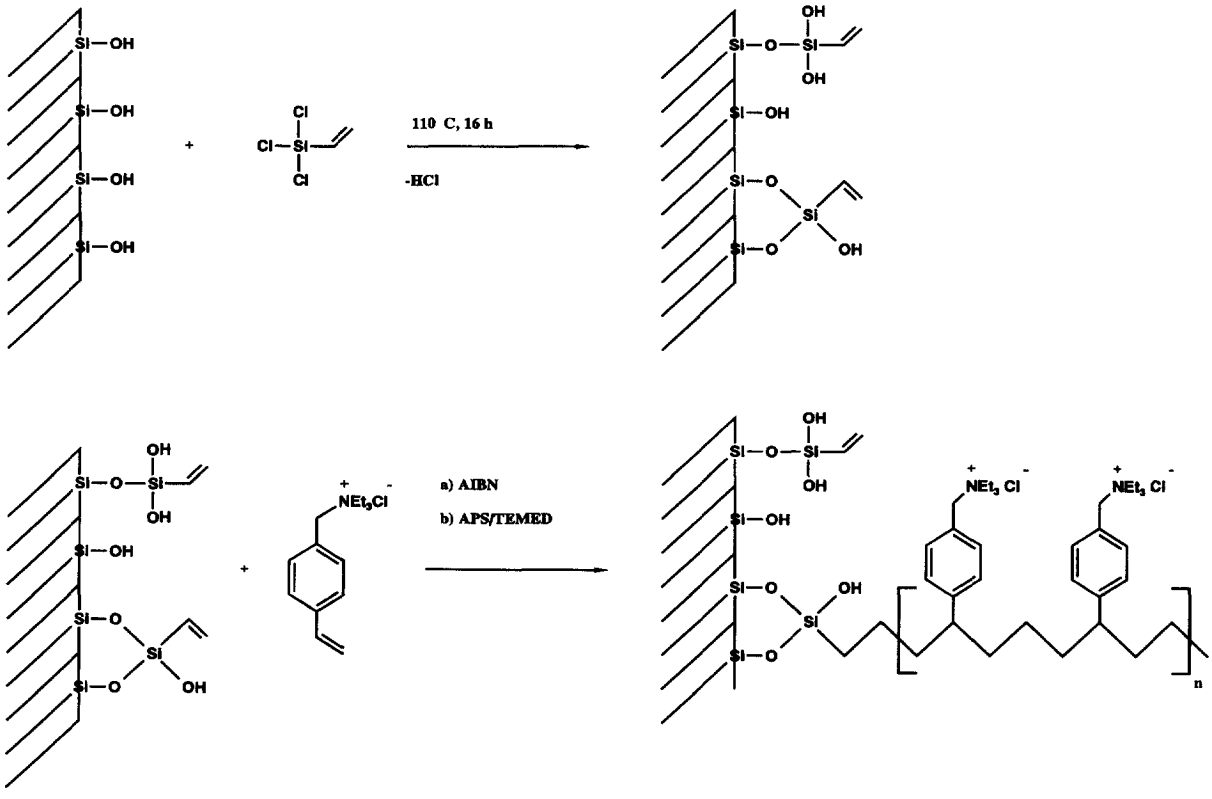

Fig. 1. Reaction scheme for the preparation of TAMS/acrylamide capillaries.

The behaviour of the EOF with these capillaries, their stability even under alkaline conditions and theirs applications in anion separation by CE will be discussed.

2. Experimental conditions

2.1. Materials

Fused-silica capillaries were obtained from Polymicro Technologies (German distributor: Laser 2000, Munich, Germany). Reagents for surface modification were commercially available or prepared in the laboratory. Vinyltrichlorsilane, AIBN were purchased by Fluka (Neu-Ulm, Germany). Ammonium persulfate (APS) and *N,N,N',N'*-tetramethylethylenediamine (TEMED) were obtained from BDH Laboratory Supplies (Poole, Dorset BH15ITD, UK). Acrylamide was purchased from BioRad (Munich). The monomer triethylammonium styrenchloride (TAMS) was prepared by the reaction of chlormethylstyrene and triethylamine (Fluka, Neu-Ulm, Germany) in dry acetone [19]. The mixture was heated for two hours. After cooling, the TAMS was precipitated by the addition of diethylether at 0°C. The product was stored at −18°C.

All chemicals used as electrolytes were at least p.a. grade and purchased from Fluka. The solutions of the samples were prepared with deionized water from a Milli-Q System from Millipore (Eschborn, Germany).

2.2. Coating procedures

Capillary pretreatment and the reaction with trichlorovinylsilane was performed as described [20]. For the copolymerization of the vinyl groups in the capillary with the TAMS-monomer the capillary was filled with a solution of 1% (w/v) monomer and 10% (w/w monomer) AIBN as initiator in isopropanol. The capillaries were sealed at both ends and heated for reaction over night at 80°C in a GC-oven. For the preparation of the coatings using APS and TEMED the vinyl capillaries were filled with a solution of 1% (w/v) monomer and 10% (w/w monomer) APS and TEMED in water. The capillaries were kept over night at room temperature. The surplus of the monomer and the not covalently bonded parts of the polymer were flushed out of the capillary using the same solvent as used for polymerization.

2.3. Apparatus

For all measurements a Spectraphoresis 1000™ from Thermo Separation Products Darmstadt, Germany) was used. The data acquisition was accomplished with a personal computer using the Spectraphoresis 1000 V1.05b software.

3. Results and discussion

3.1. Characterization of the coating efficacy

Because of the extremely low surface of capillaries the classical physical chemical methods for surface characterization fail. The only way to measure changes on surface induced by chemical reactions is the determination of the amount and direction of the EOF. By this technique the changes of the amount of charges on the surface can be determined. As EOF marker dimethylsulfoxide was used and in the pH range from 3 to 9 phosphate buffers (10 m*M*) have been used exclusively.

In Fig. 2 the EOF curves of the initial silica capillary and the vinylcoated capillary are compared to those obtained for TAMS coated capillaries

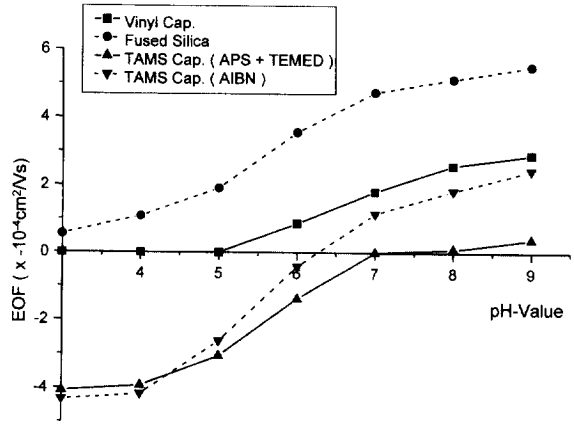

Fig. 2. EOF curves of cationic coating: Conditions: L=20,5/28 cm; I.D.=50 μm; buffer: 10 m*M* phosphate; field: ±1071 V cm^{-1}; neutral marker: dimethylsulphoxide.

prepared with different polymerization starters. Both capillaries prepared with TAMS showed at low pH a reversed EOF which amounted to the same speed as achieved with untreated fused-silica at high pH. With the capillary prepared with AIBN as initiator the EOF changed direction at a pH value above 6, whereas with the capillary where the polymerization was initiated with APS and TEMED the EOF changed direction at pH 7 and was at higher pH values smaller than with the capillary prepared using AIBN. This indicates that the coating efficacy, i.e. the disguise of surface silanols was better with the APS and TEMED started polymerization. In this Figure also the EOF characteristic of the vinyl capillary is included. The vinyl groups block a certain amount of surface silanols, but their dissociation at pH>5 contribute to the EOF. At pH 9 the vinyl capillary shows approximately half the EOF of the untreated capillary. As can be also seen, the capillary treated with AIBN shows at pH 9 almost the same EOF as the vinyl capillary.

The increase of the EOF under alkaline conditions can be explained by an incomplete coverage of the capillary surface. At high pH values the surface silanols are fully deprotonated and the bonded polymer is not able to shield the negative charges. The structure of the polymer is influenced by the reaction temperature, the solvent and the initiators used. It seems that the combination of APS and TEMED as initiators yields a polymer which is better fitted to shield the surface silanols as when AIBN is used as initator.

In a cross experiment TAMS was polymerized in an untreated fused-silica capillary (no vinyl groups bonded!). Also here a reversed EOF could be observed due to adsorption of the polymer. However, the observed EOF was only half of that achieved with covalently bonded TAMS polymer. The flow direction changed already to normal at pH 5. After going back to pH 3 the EOF was again directed to the anode but its mobility was much smaller, indicating a removal of the polymer during the experiments. Consequently in order to achieve stable coatings a binding of the TAMS polymer to the surface is essential.

3.2. Improvement of surface silanol shielding

With vinyl coated capillaries and acrylamide stable coatings could be achieved, where the influence of surface silanols was neclectable even under alkaline conditions. These capillaries could be used for efficient separation of biopolymers [21]. In order to improve the shielding of the surface silanols and to generate a reversed EOF, acrylamide and TAMS have been copolymerized in a vinyl coated capillary with APS and TEMED as initiators. The ratio of the monomers have been varied between 1:1 and 1:4 (TAMS: acrylamide). The EOF characteristics of these capillaries are depicted in Fig. 3. The mobility of the reversed EOF decreased with increasing amount of acrylamide in the mixture and varied between $2.8 \cdot 10^{-4}$ ($cm^2\ V^{-1}\ s^{-1}$) and $3.6 \cdot 10^{-4}$ ($cm^2\ V^{-1}\ s^{-1}$). With increasing acrylamide content the surface shielding improved. With a concentration of 1:2, TAMS: acrylamide the surface silanols were totally shielded. The EOF did not switch to its normal cathodic direction. Almost no EOF could be observed (dimethylsulfoxide did not reach the detector within 1 h) at pH values above 6. The best results (highest reversed EOF, no EOF at pH>6) could be achieved with capillaries prepared with a TAMS: acrylamide ratio of 1:2. Capillaries discussed in the following have been prepared with this combination, which leads to an effective coverage of the surface silanols even under alkaline conditions.

The reproducibility of the capillary preparation was also determined. Starting from the identical batch of fused-silica the variation of the EOF mobility varied between 3.2 and $3.5 \cdot 10^{-4}$ $cm^2\ V^{-1}\ s^{-1}$ with a 3.8% R.S.D. ($n=5$).

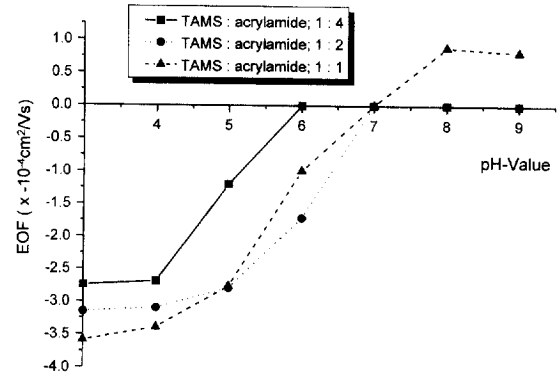

Fig. 3. Influence of the TAMS/acrylamide ratio on the mobility of the EOF. Conditions: See Fig. 2.

3.3. Stability of the coating

As found for the acrylamide coatings also the TAMS capillaries exhibited a good long time stability as can can be seen in Fig. 4. This is valid for the TAMS capillary as well as for the TAMS/acrylamide capillary. The latter showed slightly improved stability. Within 50 consecutive analysis no significant change in the EOF could be observed. Also storage of the capillaries at pH values of 10 and 3 for more than 10 days did not destroy the coating. The mobility of the EOF measured at pH 3 did not change. This demonstrates that acrylamide coating suppresses the EOF and stabilizes the binding through excessive surface shielding. The addition of TAMS to the polymerization mixture generates at low pH the anodic (reversed) EOF.

3.4. Applications

For the separation of inorganic anions chromate has proven as the most versatile background electrolyte for indirect detection. In Fig. 5 the separation of anions with a TAMS/acrylamide capillary is shown. The separation at pH 7.7 was completed within 9 min. The migration order is identical with that observed with dynamic CTAB coating. No baseline fluctuations could be observed. The variation of migration times of the sample ions in five consecutive injections was below 0.25% R.S.D. Fig. 6 shows the separation of the herbicide glufosinate from its main metabolites. The solution shows also great differences in their mobilties, so that they can only be separated in the coelectroosmotic way. Using pyridine-2,6-dicarboxylic acid the variations in migration times were below 0.3% R.S.D. for $n=20$.

As already discussed the capillaries show also good stability at high pH values. Even under alkaline conditions highly efficient separations of anions can be achieved. In Fig. 7 the separation of inorganic anions at pH 9 using a borate buffer is shown. Direct detection at 200 nm has been applied. The precision

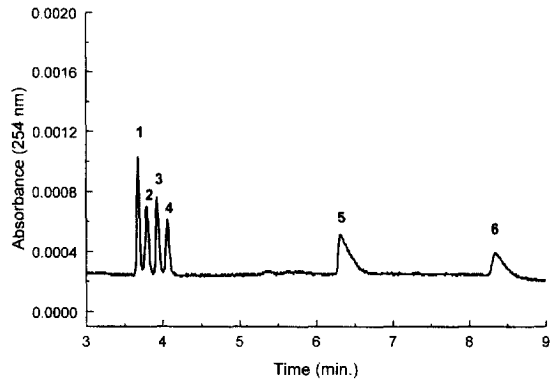

Fig. 5. Separation of inorganic anions. Conditions: TAMS/acrylamide capillary, L=59,5/67 cm, I.D.=50 μm; electrolyte: 5 m*M* chromate, pH 7,7; injection: 0.1 bar, field: −448 V cm^{-1}; samples: 1: chloride, 2: sulfate, 3: nitrite, 4: nitrate, 5: phosphate, 6: carbonate.

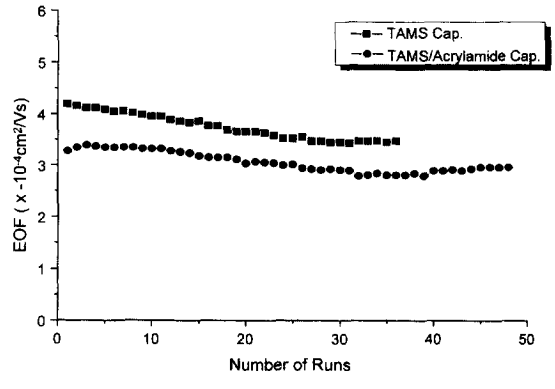

Fig. 4. Comparison of the stability of TAM213S- and TAMS/acrylamide capillaries. Conditions: L=20,5/28 cm; I.D.=50 μm; buffer: 10 m*M* phosphate, pH 3.0; field: −1071 V cm^{-1}; injection: 0.1 bar, 5 s, neutral marker: dimethylsulphoxide.

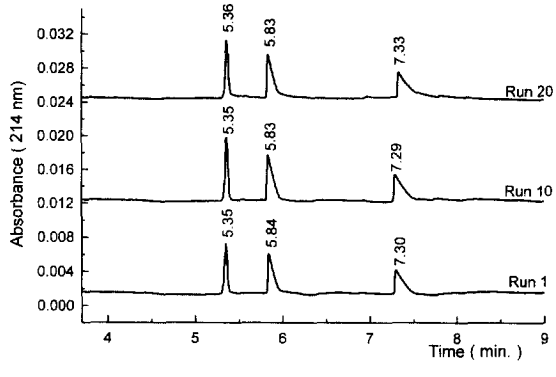

Fig. 6. Separation of the herbizide glufosinate from its metabolites. Conditions: TAMS/acrylamide capillary L=27,5/35 cm, I.D.=50 μm; electrolyte: 5 m*M* pyridine-2,6-dicarboxylic acid, pH 4.3; injection: 0.1 bar, field: −448 V cm^{-1}; samples: 1: metabolite A, 2: metabolite B, 3: glufosinate.

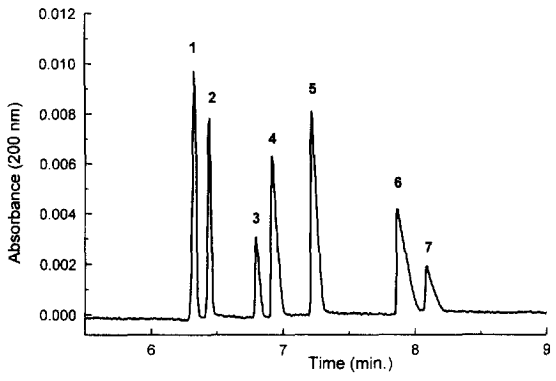

Fig. 7. Separation of anions under alkaline conditions. Conditions: TAMS/acrylamide capillary L=82,5/90 cm, I.D.=50 μm; buffer: 40 m*M* borate, pH 9.0; injection: 0.1 bar, field: −333 V cm^{-1}; samples: 1: bromide, 2: iodide, 3: chromate, 4: nitrite, 5: nitrate, 6: thiocyanate, 7: molybdate.

of migration times was very good. They varied between 0.22% for bromide and 0.36% for molybdate (n=20). This demonstrates that with the TAMS/acrylamide capillaries even at high pH values efficient and reproducible separations can be achieved.

4. Conclusion

The preparation of stable positively charged permanent coatings by copolymerization of TAMS and acrylamide with vinyl groups bonded to the capillary surface permits highly efficient separations of anions in the coelectroosmotic way even at high pH values in the indirect as well as in the direct detection technique. The preparation of the coating can be done highly reproducible, and the capillaries show good stability even at high pH values due to efficient shielding of the surface silanole groups by the polymeric layer. Due to this efficient coverage of the surface silanole groups also efficient separation of basic proteins should be possible with the described capillaries.

References

[1] W. Beck, H. Engelhardt, Chromatographia 33 (1992) 313.
[2] A. Weston, P.R. Brown, P. Jandik, A.L. Heckenberg, W.R. Jones, J. Chromatogr. 608 (1992) 395.
[3] P. Jandik, G. Bonn, Capillary Electrophoresis Of Small Molecules And Ions, Verlag Chemie, 1993.
[4] J.E. Wiktorowicz, US Patent 1991, No. 5 015 350.
[5] J.E. Wiktorowicz, J.C. Colburn, Electrophoresis 11 (1990) 769.
[6] F. Regnier, J.K. Towns, J. Chromatogr. 516 (1990) 69.
[7] W.R. Jones, P. Jandik, Am. Lab. 22 (1990) 51.
[8] W.R. Jones, P. Jandik, J. Chromatogr. 546 (1991) 445.
[9] G. Gutnikov, W. Beck, H. Engelhardt, J. Microcol. Sep. 6 (1994) 565.
[10] W.R. Jones et al., United States Patent, 1992, No. 5 104 506.
[11] R. Stahl, J. Chromatogr. A 686 (1994) 143.
[12] M.T. Galcern, L. Puignou, M. Diez, J. Chromatogr. A 732 (1996) 167.
[13] H. Burt, D.M. Lewis, K.N. Tapley, J. Chromatogr. A 739 (1996) 367.
[14] K. Cheng, Z. Zhao, R. Garrik, F.R. Nordmeyer, M.L. Lee, J.D. Lamb, J. Chromatogr. A 706 (1995) 517.
[15] R.J. Xu, C. Vidal-Madjar, B. Sébille, J.C. Diez-Masa, J. Chromatogr. A 730 (1996) 289.
[16] P.J. Oefner, Electrophoresis 16 (1995) 46.
[17] C. Stathakis, R.M. Cassidy, Anal. Chem. 66 (1994) 2110.
[18] H. Engelhardt, M.A. Cuñat-Walter, J. Chromatogr. A 717 (1995) 15.
[19] F. Th. Hafner, Master Thesis, Saarbrücken, 1995.
[20] J. Kohr, H. Engelhardt, J. Microcol. Sep. 3 (1991) 491.
[21] H. Engelhardt, M.A. Cuñat-Walter, J. Chromatogr. A 716 (1995) 27.