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Synthesis and evaluation of epoxy polymer coatings for the analysis of proteins by capillary zone electrophoresis

John K. Towns, Jianmin Bao and Fred E. Regnier*

Department of Chemistry, Purdue University, West Lafayette, IN 47907 (USA)

ABSTRACT

This paper reports a diol-epoxy coating process to deactivate the inner surface of fused-silica capillaries for use in capillary zone electrophoresis. The most effective coating consists of an epoxy polymer covalently bonded to the silica surface through glycerylpropyl silane which eliminates a number of the negatively charged silanol groups. The epoxy polymer phase also further masks residual surface silanols and sterically limits interaction of proteins with the inorganic surface of the capillary.

Application of the coating was a multi-step process. In the inital step, glycidylpropyltrimethoxy silane was used to derivatize the surface of fused-silica capillaries. This was followed by deposition at the capillary wall of a film of multifunctional oxirane. Polymerization was achieved by either boron trifluoride or tertiary amine. These coatings were of sufficient thickness and hydrophilicity to reduce protein adsorption but still allow sufficient electroosmotic pumping to carry both positive and negative species past the detector. The coatings provided capillaries with lifetimes exceeding 120 h. Proteins were resolved quickly and efficiently with high recovery of both cationic and anionic species between pH 5 to 10.

INTRODUCTION

Dissipation of joule heat has been a major problem in electrophoresis for almost half a century. By carrying out electrophoretic separations in fused-silica capillaries this problem has now been substantially diminished [1]. Unfortunately, surface silanol groups in these high-surface-area systems have a large impact on electrophoretic separations. It has been seen that electroendosmosis is a major, if not the dominant, contributor to solute mobility above pH 4 in native fused-silica capillaries. This is due to the ionization of surface silanol groups [2–5]. Solute adsorption onto these surface silanols is a second major problem, particularly in the case of cationic species such as basic proteins [6]. Adsorption of charged species can change the properties of the wall and cause multiple problems, among these being broad asymmetrical peaks, poor efficiency, altered electroosmotic flow [EOF], reduced protein recovery, and poor reproducibility.

Many strategies have been examined to reduce the problem of protein adsorption. These include the use of high-pH buffers [7–9], low-pH buffers [10,11], amine additives [7,12], high concentrations of salt [13,14], zwitterionic buffers [15], additives to the sample [16] and surface coatings [6,11,14,17–24]. Each of these strategies have had varying degrees of success in reducing protein adsorption. Using coatings to reduce protein adsorption has the advantage that limits do not have to be placed on the sample and separation buffers in terms of salt concentrations, additives to compete for surface sites, and high or low pH. Manipulation of buffers has limitations such as the generation of heat, detector incompatibility, limited pH range, reduced sensitivity, denaturation of proteins, and decreased elution time reproducibility.

Although derivatization of capillary walls with simple organosilanes [11,17,19] greatly reduces surface silanol effects, silane derivatives leach from the capillary and performance deteriorates rapidly. Another approach is to apply a methylcellulose coating [21] to the capillary wall. This coating has been reported to totally control electro-endosmosis and apparently the protein adsorption problem. The

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disadvantage of this coating is that cationic and anionic species will be swept in opposite directions along the capillary axis. Two runs must be made and the polarity of the capillary reversed to analyze both the anionic and cationic compouds of a mixture. A third approach is to use non-ionic surfactants adsorbed to the surface of an octadecyl silane-derivatized capillary to control the adsorption problem [6]. The advantage of this solution is that the coating prevents protein adsorption while still allowing sufficient electroendosmosis to sweep both anionic and cationic species past a single detector. The disadvantage is that non-ionic surfactant must be used in the buffers to replace surfactant that leaves from the hydrophobic surface of capillaries during operation. Samples collected from capillaries will be contaminated with surfactant.

One of the first attempts to coat the inside of a capillary for the separation of proteins was by Jorgenson and Lukacs [17,24]. They followed the coating procedure of Chang *et al.* [25] for the preparation of high-performance liquid chromatography packing materials. The coating process involves the bonding of glycol groups to the surface of fused silica from an aqueous phase. They found that this coating was successful in reducing protein adsorption but had a lifetime of only a few days and exhibited separation efficiencies far below that predicted by theory. It was suggested that sample overloading was the most serious source of zone broadening for proteins. Bruin *et al.* [20] also analyzed the epoxy-diol type coating but carried out the derivatization in organic solvent instead of water. Below, pH 5, this coating produced broader peaks than those of a polyethylene glycol coating tested previously [19]. This was attributed to weak adsorption of the proteins to residual surface silanols. Above pH 5, peak shapes were very broad due to either strong interaction of the proteins with the residual silanols or possibly with the epoxy-diol layer.

This research examines the utility of cross-linking covalently bonded diol coatings with oxiranes [ethylene glycol diglycidyl ether (EGDE) and glycidol] in an attempt to extend the lifetime and pH range of diol based coated capillaries. The objective was to produce a thicker, more stable coating that would further mask silanol groups at the capillary wall. Further objectives were to reduce protein adsorption, extend the pH range of operation, increase resolution, and extend the lifetime of the capillaries. It was anticipated that this coating would also reduce the EOF due to a more complete masking of the silanol groups on the capillary.

EXPERIMENTAL

Apparatus

Capillary electrophoresis was performed on a laboratory-designed instrument which has been previously described [18]. Polyimide-coated fused-silica capillaries (Polymicro Technologies, Phoenix, USA, AZ) of 75 μm I.D. $\times$ 360 μm O.D. were used to prepare the capillaries. Capillaries were 60–100 cm long with separation length of 40 to 85 cm. Detection was achieved with a variable-wavelength UV absorbance detector (Model V4; Isco, Lincoln, NE, USA). When two detectors were employed, another Isco Model V4 UV detecor or UV-8 (Bioanalytical Systems, West Lafayette, IN, USA) was used. Protein elution was monitored at 200 nm when a single detector was used or 214 nm when double detectors were employed. The neutral marker mesityl oxide was detected at either 254 or 214 nm. Linear 2000 (Reno, NV, USA) strip chart recorders were used.

Reagents

Protein samples were purchased from Sigma (St. Louis, MO, USA). γ-Glycidoxy-propyltrimethoxy silane (Gox), EGDE, glycidol, 1,4-diazabicyclo[2.2.2]octane (DABCO), boron trifluoride etherate, mesityl oxide, toluene, triethylamine and methylene chloride were purchased from Aldrich (Milwaukee, WI, USA) as were buffer reagents. Electrophoresis buffers were prepared from laboratory-filtered (0.2 μm) double-distilled water.

Electrophoresis

Protein solutions of 1.0 mg/ml were introduced into the capillary by syphoning for a fixed time (1 to 3) at a fixed height (10–15 cm). Mesityl oxide was used as the neutral marker. Several buffer solutions were used to operate capillaries over the pH range of 3 to 11: 0.01 *M* acetate from pH 3 to 5, 0.01 *M* phosphate at pH 6 and 7, 0.01 *M* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) at pH 8, 0.01 *M* tris(hydroxymethyl)aminomethane (Trizma) at pH 9, and 0.01 *M* diaminopropane at pH 10

and 11. Salt was added to each buffer to give comparable ionic strengths and currents. During electrophoresis, current through the capillary never exceed 50 μA with all analyses being run at ambient temperature without temperature control. Between analyses, the capillary was flushed with doubled-distilled water and separation buffer for 1 min.

Preparation of capillaries ***I–VI***

Pretreatment of capillaries ***I.*** Capillaries were first treated with 1.0 *M* NaOH for 15 min followed by 15 min of washing with deionized water. Residual water was evaporated from the capillaries by connecting them to a gas chromatography oven at 100 °C for 2 h under a nitrogen pressure of 400 kPa. This uncoated capillary will be designated as **I**.

Preparation of 3-glycidoxy-propyltrimethoxy silane (GOX) ***II.*** The pretreated capillary **I** was filled with GOX containing 5% methylene chloride by aspiration with a syringe. The capillary was placed in an oil bath at 90°C for 3 h with fresh solution being pulled through the capillary every 15 min. After the 3-h silylation, the capillary was removed from the bath, wiped clean of oil, and residual GOX removed by pushing nitrogen through the capillary. The capillary was washed with several capillary volumes of methanol followed by a 10-min wash with filtered (0.2 μm), double-deionized water.

Preparation of the cross-linked ethylene glycol diglycidyl ether (EGDE) phase ***III.*** A fim of EGDE was deposited onto a 2-m section of the oxirane-derivatized capillary **II** by aspirating a volume of reagent sufficient to fill a 10 cm length of column into the capillary. Three different solutions were evaluated: 1% and 10% EGDE in methylene chloride, and 100% EGDE. The bolus of reagent was pushed through the capillary in a gas chromatography oven with nitrogen at 400 kPa. Nitrogen flow through the capillary was continued for 30 min to drive off the methylene chloride. Catalyst in the form of boron trifluoride vapor from an etherate solution was then sent through the capillary in a stream of nitrogen to cross-link the oxiranes. Catalyst was delivered from a 3-ml vial containing 1 ml BF_3 etherate that was capped with a rubber septum. The capillary was connected to this vessel by insertion through the septum. Careful attention was placed on making sure that the capillary cleared the BF_3 etherate solution by one half inch so that no liquid enters the capillary. A second capillary was connected to the nitrogen line and pushed through the rubber septum to the bottom of the vial so that nitrogen bubbled through the BF_3 etherate. The gaseous catalyst stream was forced through the capillary with nitrogen for 1 h at a rate of 2 ml/min. The coated capillary was cured in a gas chromatography oven for 2 h at 100°C under nitrogen pressure of 400 kPa.

Preparation of glycidol derivatized phase ***IV.*** A 2-m section of previously coated capillary **III** was further derivatized by depositing 10% glycidol in methylene chloride in a volume sufficient to form a 10-cm bolus. After excess glycidol was pushed out of the capillary, polymerization was catalyzed in the same fashion as described above for the phase **III**.

Esterification of the phase ***IV*** *coating* ***V.*** A 2-m section of phase **IV** coated capillary was further derivatized by sending diazomethane through the capillary in diethyl ether to methylate possible carboxyl groups generated in the previous coating steps. The diazomethane was generated by placing approximately 1 g of N-nitrosomethylurea in a polished round-bottom flask containing 50 ml each of diethyl ether and a 50% KOH solution. At the interface, the N-nitrosomethylurea is converted into diazomethane which extracts into the ether phase giving a yellow color. Diazomethane in diethyl ether was aspirated through the capillary for 10 min at 0.25 ml/min. The capillary was then washed with diethyl ether, water and running buffer.

Preparation of the base-catalyzed diol-EGDE cross-linked phase ***VI.*** A solution of 0.5% DABCO in CH_2Cl_2 was aspirated into a capillary with the diol-bonded phase **II** and allowed to ion-pair with residual silanol groups in the capillary for 5 min before removal with pressurized nitrogen. A 10% solution of EGDE was forced into the column and allowed to react for 30 min at room temperature before heating to 120°C and continuing the reaction for another 30 min. Non-bound monomer was pushed out of the column with pressurized nitrogen and the column washed with methanol. Heavier

coatings were obtained by repeating the cross-linking reaction with EGDE for an additional 3 h followed by a 12-h treatment at 120°C. Removal of non-bound material and washing was as described above.

Determination of protein recovery

Protein recovery was determined with a dual detector system as previously described [6]. Placing detectors at 20 cm and 70 cm from the capillary inlet allowed protein recovery to be measured over the 50 cm section of capillary between the detectors. Recovery was determined using 10 ng of protein and a non-adsorbed internal standard. Detectors were switched from front to back to accommodate for differences in detector response. Percent recoveries were determined by subtracting peak areas from each detector after taking into account differences in detector response.

RESULTS AND DISCUSSION

Reactions in the epoxy coating process are shown schematically in Fig. 1. It should be noted that the structures in this scheme do not represent the exact structure of the coating. GOX was coupled to sur-

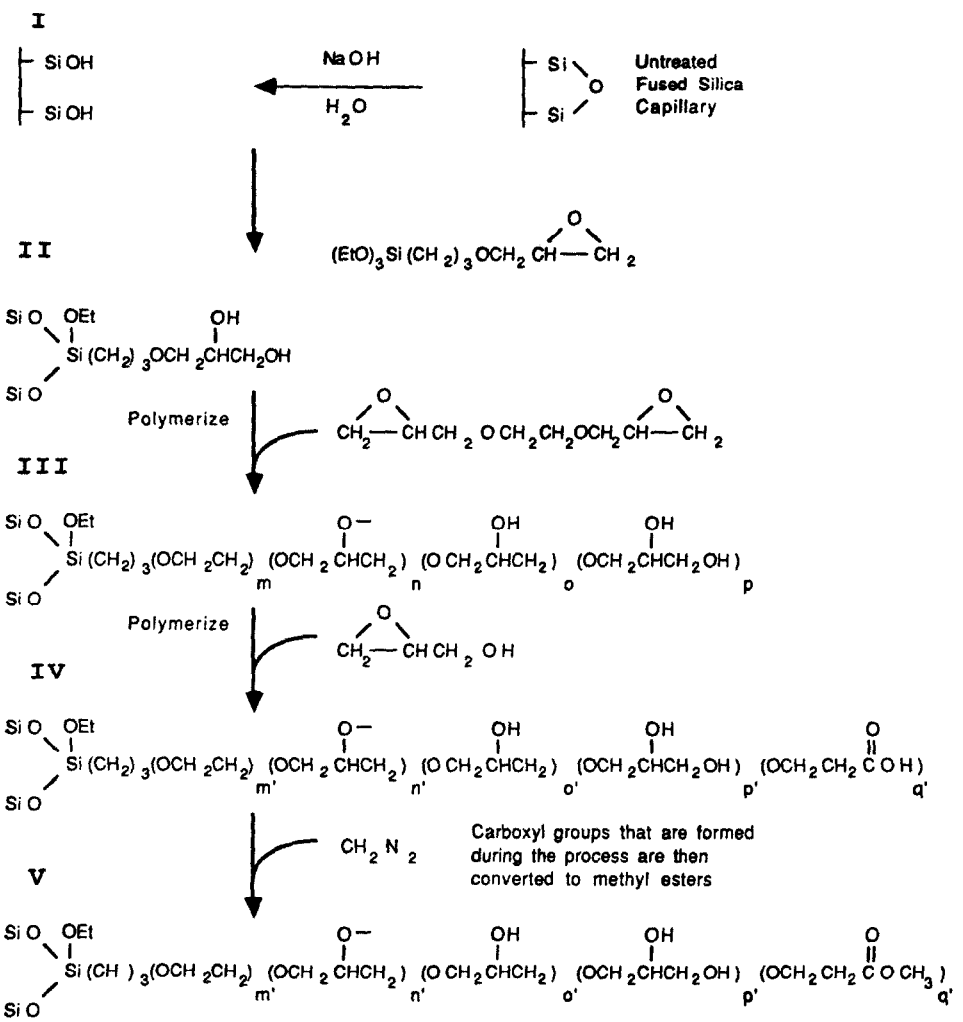

Fig. 1. Typical reaction scheme for the multi-step process used in the preparation of the diol-based expoxide coating. Et = Ethyl.

face silanols by the elimination of methanol in the first step. Since polymerization may occur with trimethoxy silanes, silane monomer solution was continuously pumped through capillaries to minimize polymerization. After the reaction was finished, excess GOX was washed from the capillary and the epoxy group at the capillary surface used in a second binding reaction. Copolymerization of EGDE and surface epoxy groups were used in the second step to form a highly crosslinked surface layer. Multifunctional oxiranes, such as EGDE, have been shown previously to produce highly cross-linked structures in preparing chromatographic media [16]. The resulting epoxy polymer has only C–O, C–C and C–H bonds and is stable in both acid and base. In addition, the high content of ether and hydroxyl groups in the polymer produced capillaries with very hydrophilic walls.

Evaluation of acid-catalyzed epoxy-based coatings

Fig. 2 shows the corresponding peak shape for each step of the acid-catalyzed coating process (pretreated uncoated capillary **I** with coated capillaries **II**–**V**). Separations were carried out with 0.01 *M* phosphate buffer (pH 7.0) using a 80 cm × 75 μm capillary (65 cm to detector) at 300 V/cm. Capillaries were evaluated in terms of EOF, peak efficiency, lysozyme recovery, peak skew and capillary lifetimes for each stage of the coating process (Table I).

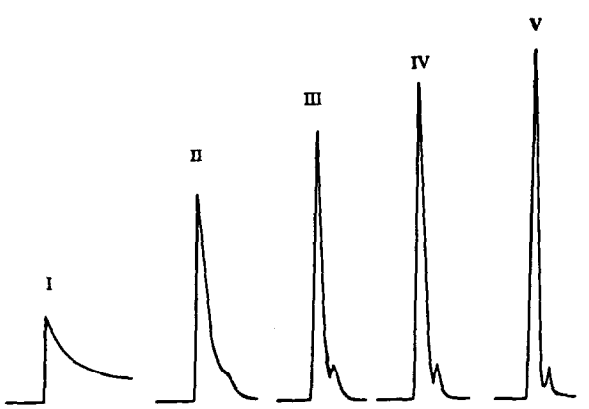

Fig. 2. Peak shape and migration times for lysozyme and a neutral marker (mesityl oxide) for each step of the acid-catalyzed epoxy coating process. Conditions: 0.01 *M* phosphate, pH 7.0, detection at 200 nm, 100 cm × 75 μm capillary (85 cm to detector), 28 kV, 30 μA.

The first three 10-ng injections of lysozyme were completely adsorbed to the wall before reaching the detector. The fourth injection, shown at the far left in Fig. 2 reached the detector but the peak tailed badly and eluted much later than expected. The long elution time of this positively charged protein (p*I* 11) is the result of adsorption to the capillary wall. Following lysozyme adsorption, the negative charge on the wall is reduced, modifying the double layer and significantly lowering EOF.

The first coating stage involved the bonding of glycidoxy-propylsilyl to the capillary wall. Silylation was achieved from both aqueous and non-aqueous solutions [17,20]. It was found that silylation in 5% methylene chloride gave improved results compared to an aqueous solution in terms of reduced EOF, increased efficiency, increased peak symmetry and improved resolution. In addition to reducing the concentration of negatively charged silanol groups, silylation also moves the plane of shear some distance from the wall by lowering the charge potential. These two effects cause a two-fold reduction in EOF compared to the uncoated capillary. Lysozyme in a 50-cm section of capillary increased to 80% with the diol coating **II**. This is a 15-fold increase in recovery relative to an uncoated capillary of the same length. Although the diol coating substantially improved performance compared to uncoated capillaries, plate efficiency is far below that predicted by theory and much less than has been observed with other coatings using similar conditions [6,18]. The diol coating **II** also suffers from severe peak tailing, lower protein recovery and shorter column lifetimes compared to results that have been reported by others [6,11,14,18,21,22].

With the objective of further masking the negatively charged wall and extending the lifetimes of the capillary, the diol coating **II** was cross-linked with EGDE to form the second stage of coating. The BF_3-catalyzed polymerization of the dioxirane monomers onto the diol ligand produced a very hydrophilic coating (**III**). These highly active monomers react rapidly with hydroxyl groups on the diol ligand and with each other to form a stabile coating. This coating further masks charges on the capillary wall.

To optimize concentration film deposition on the diol coating **II**, 1%, 10%, and pure EGDE were

TABLE I

SEPARATION PARAMETERS FOR EPOXY-COATED CAPILLARIES

EOF = Electroosmotic flow (0.01 *M* phosphate buffer, pH 7.0); plate number $N = 5.54 (t_e/w_{1/2})^2$ for lysozyme on a 100 cm × 75 μm capillary (where t_e = retention time and $w_{1/2}$ is peak width at half height); recovery is for lysozyme (p*I* 11.3) at pH 7.0 taken over a 50-cm section of capillary; peak skew = ratio of *a*/*b* at 10% peak height [18]; life time is the time required to deviate 10% from original electroosmotic flow.

Capillary	EOF (10^8 m^2/V s)	Efficiency	Recovery (%)	Peak skew	Life time (h)
Untreated					
I	5.62	–	0	–	–
Diol					
II	2.98	35 000	80	0.33	20
Acid catalyzed					
III	1.49	80 000	85	0.80	50
IV	0.92	85 000	87	0.90	60
V	0.67	92 000	90	0.93	>150
Base catalyzed					
VI	1.30	85 000	85	0.83	>100

evaluated. Table II shows the performance parameters for the three concentrations examined. The 10% EGDE solution was found to give the best results in terms of efficiency, lifetime, peak skew and lysozyme recovery while giving the lowest EOF and was used for the remainder of the study. This coating **III** showed increased efficiency, lower EOF, better resolution, reduced peak skew and a 5% increase in lysozyme protein recovery up to 85%. Lifetime of the capillary was also increased beyond the few days of the diol coating. This coating, however, still gives efficiencies less than expected and further masking was needed to increased separation performance.

To create a thicker hydrophilic coating, glycidol was bonded to phase **III**, resulting in a highly branched matrix. This coating **IV** showed further improvement in separation performance with respect to increased percent recovery, improved peak symmetry, and a more complete masking of the silanol groups as indicated by the further reduction in electro-osmotic flow. However, there was little improvement in efficiency.

A problem with both the **III** and **IV** coating is that EOF gradually increases. It is unacceptable for the elution time of solutes to change in this manner. This problem is attributed to the formation of aldehyde groups during the BF_3-catalyzed cross-linking step. Over time, these groups oxidize to carboxyl groups and increase the negative charge at the capillary walls. This results in increased EOF and a reduction in the separation performance of the capil-

TABLE II

EFFECT OF PERCENT EDGE IN METHYLENE CHLORIDE ON SEPARATION PARAMETERS FOR ACID-CATALYZED DIOL-EDGE COATING **III**

See Table I for definitions.

Coating (%)	EOF (10^8 m^2/V s)	Efficiency	Recovery (%)	Peak skew	Life time (h)
1%	2.78	41 000	82	0.68	30
10%	1.49	80 000	85	0.80	50
100%	2.92	45 000	81	0.72	35

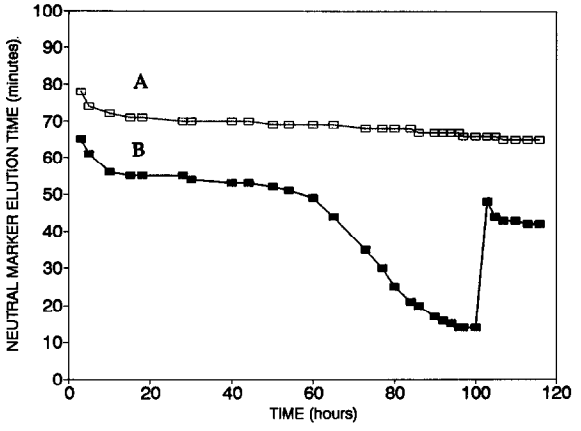

Fig. 3. Plot of neutral marker elution time *vs.* electrophoretic running time for (A) phase V coated capillary and (B) phase IV coated capillary treated after 100 h with diazomethane. Conditions as in Fig. 2 with the exception of detection at 254 nm.

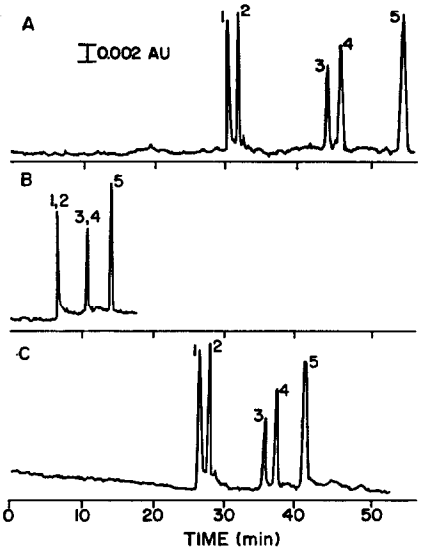

Fig. 4. Separation of five basic proteins on a phase IV coated capillary at (A) 5 h, (B) 100 h, and (C) after treatment with diazomethane. Peaks: 1 = lysozyme; 2 = cytochrome *c*; 3 = ribonuclease A; 4 = chymotrypsinogen; 5 = myoglobin. Conditions as in Fig. 2.

lary. This problem was alleviated by converting these acid groups to esters. Diazomethane was used for esterification because it is easily introduced into the capillaries.

The effect of the diazomethane treatment on neutral marker elution time (a measure of EOF) is shown in Fig. 3 for two coated capillaries. Line A shows the elution times of the neutral marker *vs.* electrophoretic running time for an IV coated capillary treated with diazomethane. This capillary exhibited long term stability past the 120-h, mark (run over a two week period). Diazomethane treatment further decreased EOF by methylation of carboxyl groups formed in the initial cross-linking steps. Line B shows the elution time of a neutral marker *vs.* electrophoretic running time for the phase IV coated capillary. After approximately 60 h EOF increases drastically, presumably due to the formation of negatively charged carboxyl groups in the coating surface. This was confirmed by esterification of the capillary with diazomethane. EOF was restored to a value comparable to that of the phase V coating. Fig. 4 shows the electropherograms of five basic proteins run on the phase IV capillary at the 5-h mark, the 100-h mark, and then after treatment with diazomethane. The electropherograms show the influence of the oxidation and removal of carboxyl groups on EOF.

Although phase V gives good results in terms of peak efficiency, lysozyme recovery and peak shape, there are still some drawbacks with this coating. Delivery of the catalyst into the capillary in the gas phase apparently limits the length of capillary that can be prepared during the cross-linking step. Capillary lengths above 3 m gave wide fluctuation in segment to segment reproducibility. The capillary end nearest the point of catalyst entry is exposed to too much catalyst. This appears to cause excess formation of aldehydes which are subsequently oxidized to carboxyl groups that increased the EOF. In contrast, the exit of the capillary was not exposed to enough catalyst and perhaps did not cross-link sufficiently. The mid-section of the capillary was suitable. This means that only there middle 3 m of a 10-m capillary would be coated properly. Another problem is the need to esterify the phase IV coating. Along with the time needed for this extra step, diazomethane is carcinogenic and explosive.

Evaluation of base-catalyzed epoxy-diol phase ***VI***

The problems associated with the acid catalyst were circumvented by the use of a base catalyst for polymerization. Using DABCO, the catalyst is sent

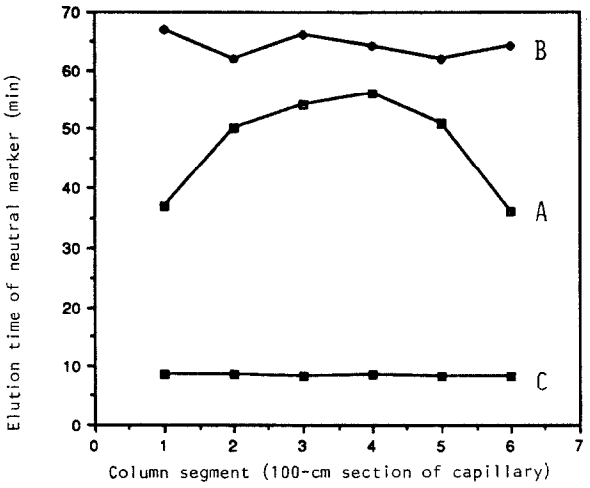

Fig. 5. Neutral marker elution time as a function of meter long sections of a 6-m length of coated capillary for (A) acid-catalyzed coating **IV**, (B) base-catalyzed coating **VI** and (C) uncoated capillary. Conditions as in Fig. 3.

through the capillary in solution, rather than the gaseous form. This allows capillaries of longer length to be coated without the adverse effects associated with the gaseous catalysis. Fig. 5 shows the difference in neutral marker elution time for both acid- and base-catalyzed epoxy-diol coated capillaries as a function of position in a 6-m capillary. The elution times of an uncoated capillary is shown for comparison. The curvature seen for the acid-catalyzed coating is indicative of sections over-exposed and under-exposed to catalyst. This is not the case with the base catalyzed process where all sections are evenly exposed to the catalyst. The percent difference of the first section to middle section of capillary for the acid and base catalyzed procedures are 36% and 3%, respectively.

An unusual feature of this base catalyzed coating process was that the DABCO catalyst and oxirane monomer were added sequentially. This was done because the capillary was positively charged after the coating process when they were added together. It is thought this is because the amine catalyst is incorporated into the coating. The success of sequential addition is attributed to the fact that residual silanols on the capillary will ion-pair with DABCO catalyst and retain a small amount of the amine even after displacement of the catalyst solution from the capillary. This small amount of catalyst is apparently sufficient to cross-link the multifunctional oxirane monomer. Catalyst concentration is important. When more than 1% DABCO was used, the coating was cationic and a negative EOF was detected at pH 7. This is because the extra amine ion-paired on the surface will be protonized and form positive charges on the surface. These positive charges will induce a negative layer in the buffer solution and reverse the zeta potential at the capillary wall and cause the EOF to move in the opposite direction. Although DABCO catalyst might be incorporated into the polymer in the sequential addition process, it probably is masked by the anionic silanol groups and the coating.

Capillaries coated with the hydrophilic epoxy layer (**VI**) give good separation of the positively charged protein test mixture (Fig. 6) previously run on the acid-catalyzed coating (**V**). The separation is comparable to coating **V**, but required fewer synthetic steps and much longer lengths of capillary could be coated. As was the case with the acid catalysis, coating **VI** maintains sufficient EOF at pH 7 to sweep both anionic and cationic species past the detector as seen in the separation of trypsinogen, myoglobin, conalbumin, carbonic anhydrase and α-amylase (Fig. 7). It was seen that less band broad-

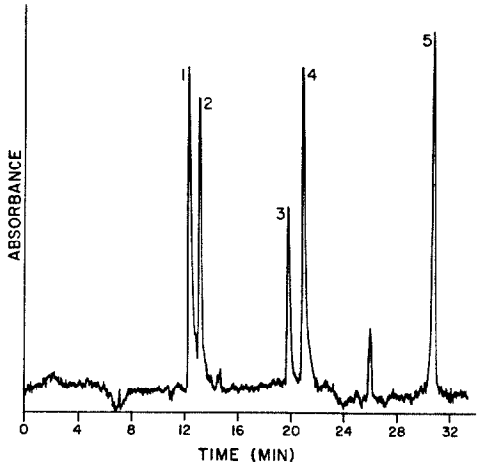

Fig. 6. Capillary electrophoretic separation of five basic model proteins on a base-catalyzed coated capillary (**VI**). Peaks: 1 = lysozyme; 2 = cytochrome *c*; 3 = ribonuclease A; 4 = chymotrypsinogen; 5 = myoglobin. Conditions: 85 cm × 50 μm I.D. (65 cm separation length); 0.01 *M* phosphate buffer, pH 7.0; 300 V/cm, 17 μA.

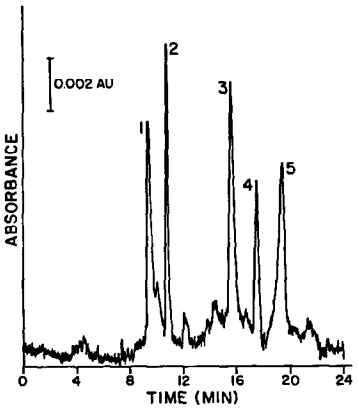

Fig. 7. Capillary electrophoretic separation of near neutral proteins on a base-catalyzed diol-epoxide coated capillary (**VI**). Peaks: 1 = trypsinogen; 2 = myoglobin; 3 = conalbumin; 4 = carbonic anhydrase; 5 = amylase. Conditions as in Fig. 6 with the exception of a shorter capillary (60 cm with a 42 cm separation length).

ening was experienced with positive proteins ($pI > 7$) than that with negative proteins ($pI > 7$). This has been attributed to the fact that positive proteins move in the direction of EOF whereas negative proteins migrated against the flow [6].

The utility of this coating in electrophoretic mobility studies was examined as a function of pH. It is well known that the net charge of a protein at a specific pH determines its migration rate under an applied electrical field. In general, positive charge on a protein will decrease as pH increases. Thus, lower electrophoretic mobility is expected at higher pH. This means that resolution will be difficult when EOF is large [6,18]. Fig. 8 shows that EOF is low and does not vary widely as a function of pH with the coated phase **VI** capillary. A pH *vs.* electrophoretic mobility map (Fig. 9) shows that there is a pH optimum for separating any set of proteins. It was easy to see from these electropherograms that, in contrast to uncoated capillaries, sharp peaks were obtained for all separations. This is a major advantage of this coating over uncoated capillaries where proteins can only be separated at either high pH (such as pH 9 or above) or low pH (such as pH 2).

A comparison of the base-catalyzed coating and acid-catalyzed coatings at neutral pH showed the former to be more stable coating in terms of run-to-run and day-to-day relative standard deviations (R.S.D.) and more reproducible in terms of segment

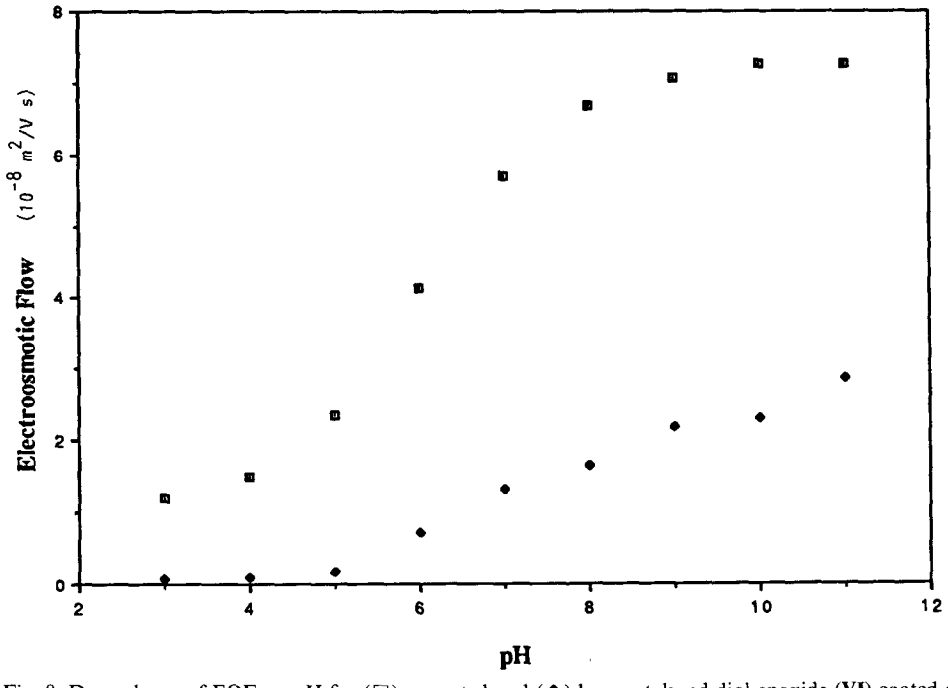

Fig. 8. Dependence of EOF on pH for (□) uncoated and (◆) base-catalyzed diol-epoxide (**VI**) coated capillaries.

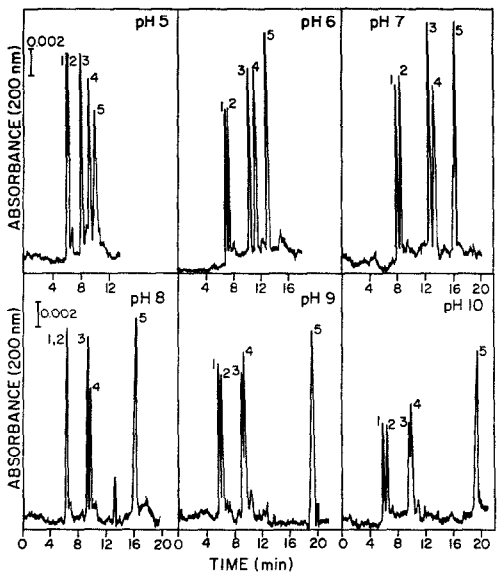

Fig. 9. Separation of five basic proteins at pH values ranging from 5 to 10 on a base-catalyzed coated (**VI**) capillary. Peaks: 1 = lysozyme; 2 = cytochrome *c*; 3 = ribonuclease; 4 = chymotrypsinogen; 5 = myoglobin. Capillary: 65 cm × 50 μm I.D. (40 cm separation length).

to segment R.S.D. values (Table III). The larger value for the segment-to-segment R.S.D. value of the acid-catalyzed coatings is again attributed to the uneven exposure of the gaseous catalyst as compared to the solution application with the base-catalyzed coating. The base-catalyzed coating process has the additional advantage of only two steps as compared to the four with the acid-catalyzed process.

The stability of the coatings was also examined under alkaline pH conditions (pH 10.0). The stability of the coatings at elevated pH were found to increase as subsequent layers of coating were applied (coated capillaries **II**–**V**). This extension in capillary lifetimes at high pH is presumably due to increased shielding of the siloxane bond from the alkaline buffer. The acid-(**V**) and base-(**VI**) catalyzed coatings gave similar stability results deviating 10% from their initial EOF values after 5 days (50 h, total running time). This deterioration over time is characteristic of organosilane coatings which are removed from the surface of the capillary under alkaline conditions. The increased cross-linking, however, was able to extend the lifetimes of these capillaries approximately 10-fold compared to that of the simple organosilane coating.

TABLE III

ACID-CATALYZED COATING (**IV**) *vs.* BASE-CATALYZED COATING (**VI**) NEUTRAL MARKER ELUTION TIME REPRODUCIBILITY

	Relative standard deviation (%)	
	Acid catalyzed	Base catalyzed
Run-to-run ($n=6$)	3.1	2.0
Day-to-day ($n=5$)	3.5	2.8
Segment-to-segment ($n=6$) (sections of same capillary)	18.3	3.2

CONCLUSIONS

Epoxy polymer layers bonded to the fused-silica capillary produce a hydrophilic coating that greatly reduces protein adsorption. These coated capillaries give high efficiency, symmetrical peaks and high protein recovery. Column lifetime exceed 150 h without significant loss in resolution or change in elution time. Base catalysis was most effective in producing stable, reproducible epoxy polymer-coated surfaces that can be used over a wide pH range with wide variation in EOF and still allow sufficient EOF for both negative and positive species under the same conditions.

The separations presented here demonstrate the applicability of covalently bonded phases in the separation of proteins by capillary zone electrophoresis. One of the most attractive features of capillary zone electrophoresis is the short times required both in terms of the separation and the time required to equilibrate the system when buffer pH is switched. The time that must be taken to bond coatings to the capillary wall are far outweighed by added freedom that is allowed with the separation buffer. This becomes increasingly important for example when coupling capillary electrophoresis to mass spectrometry where limited buffer compatibility is a major concern.

REFERENCES

1 J. W. Jorgenson and K. D. Lukacs, *Anal. Chem.*, 53 (1981) 1298.
2 T. Tsuda, K. Nomura and G. Nakagawa, *J. Chromatogr.*, 264 (1983) 385.

3 C. F. Simpson and K. D. Altria, *Anal. Proc.*, 23 (1986) 453.
4 C. S. Lee, W. C. Blanchard, and C. T. Wu, *Anal. Chem.*, 62 (1990) 1550.
5 W. J. Lambert and D. L. Middleton, *Anal. Chem.*, 62 (1990) 1585.
6 J. K. Towns and F. E. Regnier, *Anal. Chem.*, 63 (1991) 1126.
7 H. H. Lauer and D. McManigill, *Anal. Chem.*, 58 (1986) 166.
8 Y. Walbroehl and J. W. Jorgenson, *J. Microcolumn Sep.*, 1 (1989) 41.
9 M. Zhu, R. Rodriquez, D. Hansen and T. Wehr, *J. Chromatogr.*, 516 (1990) 123.
10 J. C. Grossman, H. H. Colburn, R. G. Lauer, R. M. Nielsen, G. S. Riggen, E. C. Rickard and G. S. Sittampalam, *Anal. Chem.*, 61 (1989) 1186.
11 R. M. McCormick, *Anal. Chem.*, 60 (1988) 2322.
12 R. G. Nielsen, G. S. Sittampalam and E. C. Rickard, *Anal. Biochem.*, 177 (1989) 20.
13 J. S. Green and J. W. Jorgenson,, *J. Chromatogr.*, 478 (1989) 63.
14 S. A. Swedberg, *Anal. Biochem.*, 185 (1990) 51.
15 M. M. Bushey and J. W. Jorgenson, *J. Chromatogr.*, 480 (1989) 301.
16 M. J. Gordon, K-J. Lee, A. A. Arras ans R. N. Zare, *Anal. Chem.*, 63 (1991) 69.
17 J. Jorgenson and K. D. Lukacs, *Science (Washington, D.C.)*, 222 (1983) 266.
18 J. K. Towns and F. E. Regnier, *J. Chromatogr.*, 516 (1990) 69.
19 G. J. M. Bruin, J. P. Chang, R. H. Kuhlman, K. Zegers, J. C. Kraak and H. Poppe, *J. Chromatogr.*, 471 (1989) 429.
20 G. J. M. Bruin, R. Huisden, J. C. Kraak and H. Poppe, *J. Chromatogr.*, 480 (1989) 339.
21 S. Hjertén, *J. Chromatogr.*, 347 (1985) 191.
22 K. A. Cobb, V. Dolnik and M. Novotny, *Anal. Chem.*, 62 (1990) 2478.
23 M. A. Mosely, L. J. Deterding, K. B. Tumer and J. W. Jorgenson, *Anal. Chem.*, 63 (1991) 109.
24 J. W. Jorgenson, *Trends Anal. Chem.*, 3 (1984) 51.
25 S. H. Chang, K. M. Gooding and F. E. Regnier, *J. Chromatogr.*, 120 (1976) 321.