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Characterization and performance of a neutral hydrophilic coating for the capillary electrophoretic separation of biopolymers

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ABSTRACT

Polyvinylmethylsiloxanediol (50% vinyl) was synthesized and combined with a cross-linker for static coating onto fused-silica columns. After cross-linking and binding to the surface, linear polyacrylamide was grafted to the double bonds of the siloxanediol; subsequently, this linear polymer matrix was cross-linked with formaldehyde. The grafted neutral polymeric layer provided suppression of electroosmotic flow and minimized adsorption. This combination yielded successful open tube and polymer network separations of proteins, peptides and DNA molecules. Very high efficiencies (*ca.* $1 \cdot 10^6$ plates/m) were achieved for open tube protein separations, and hundreds of consecutive runs were performed with minimal change in migration times.

INTRODUCTION

Capillary zone electrophoresis (CZE) is rapidly developing as a powerful separation tool [1]. One of the major applications of CZE is the analysis of biopolymers such as peptides, proteins, DNA fragments and carbohydrates where CZE can provide ease of operation, speed and high separation efficiency [2]. Unfortunately, many macromolecules, and proteins in particular, tend to adsorb to the surface of the capillary tube due to coulombic and hydrophobic interactions [3]. Adsorption leads to significant zone broadening, non-reproducible migration times, and errors in quantitation.

Two different approaches to preventing the interaction between proteins and the fused-silica surface are currently being explored. In one approach, high [4,5] or low pH [3,6], high salt concentrations [7,8] and/or specific additives [9–13] have been applied to adjust the buffer composition. In a number of cases, the additives become an adsorbed coating to modify the fused-silica surface [14,15]. High or low pH of the background electrolyte can potentially cause sample degradation and limit the operational pH range for optimization of the separation. Furthermore, high salt concentrations might result in extra band broadening due to Joule heating. Using smaller inner diameters will minimize heating effects but may also introduce additional difficulties in injection and detection. Additives may interact with the sample, potentially distorting results when intermolecular interactions are important, *e.g.* affinity capillary electrophoresis [16], or may obliterate the sample signal in capillary electrophoresis–mass spectrometry (CE–MS). Furthermore, in this regard,

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high salt concentrations can destabilize the electrospray.

A second strategy involves the chemical alteration of the fused-silica surface by a fixed coating [17–26]. In principle, this approach may be less limiting than the previous one in allowing the separation conditions to be adapted more freely to the needs of the analytical problem. We have taken this approach in this work and have set the following goals for a permanent coating: (1) ease of preparation; (2) high batch-to-batch reproducibility; (3) high efficiencies over a wide pH range of the buffer; (4) good pH stability and (5) negligible electroosmotic flow (EOF).

There are three reasons we have sought to minimize EOF. First, migration time reproducibility should in principle be high, since the lower the EOF, the less susceptible migration times will be to changes in bulk flow. Second, in all columns with EOF (coated and uncoated), there could be local differences in flow, creating electroosmotic mixing of the zones, and resulting in band broadening [27]. Finally, columns with negligible electroosmotic flow will have broad applicability in open tube and polymer network separations and potentially in IEF. One disadvantage of negligible flow will be the inability to separate positive and negative species in one run.

The basic strategy of the surface modification was to divide the coating into two polymeric layers (Fig. 1). The sublayer, a polymeric film, was present to provide a stable surface with high grafting capacity as a substitute for the fused-silica wall. The dense hydrophilic top layer was present to prevent adsorption and to minimize electroosmotic flow by means of its high viscosity. We have thus developed a coating composed of a vinyl siloxanediol as the sublayer where the OH groups were used for cross-linking and the vinyl groups for grafting the top layer of linear or cross-linked polyacrylamide. Moreover, the usual pretreatment of the fused-silica surface could be neglected, and coating and cross-linking of the sublayer could be performed in one single step at room temperature without further manipulation, *e.g.* thermal treatment. The second part of this work consisted of the extensive evaluation of the coating in terms of EOF,

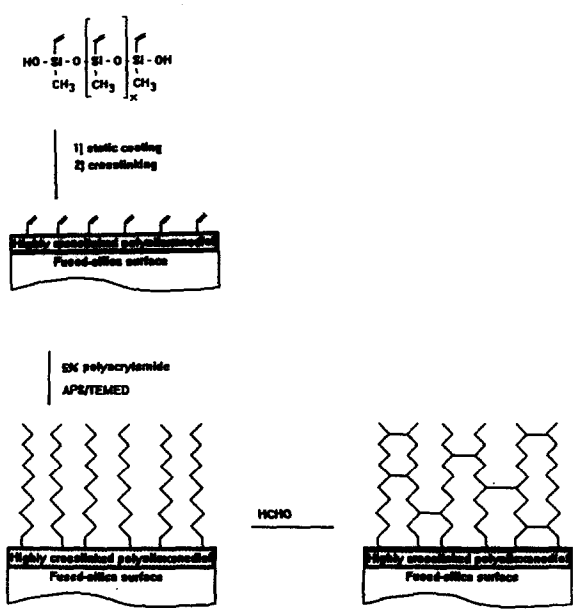

Fig. 1. Synthetic scheme for the neutral hydrophilic coating.

efficiency, long-term stability, and batch-to-batch reproducibility.

EXPERIMENTAL

Instrumentation and electrophoresis

A P/ACE 2100 capillary electrophoresis instrument controlled by System Gold software (Beckman, Fullerton, CA, USA) was used for protein and peptide separations. For the DNA separations and the electroosmotic flow measurements, the system consisted of a Spectra 100 spectrophotometer (Spectra Physics, San Jose, CA, USA) and a Series EH high-voltage power supply (Glassman High Voltage, Whitehouse Station, NJ, USA). Proteins and peptides were detected at 214 nm, whereas DNA and acetone, as the neutral electroosmotic flow marker, were detected at 260 nm. The proteins were dissolved in the separation buffer at a concentration of 0.1 mg/ml. Peptides were dissolved in water to a concentration of 0.013 mg/ml. Between runs the columns were rinsed by high pressure with the separation buffer for 2.5 min. Electoosmotic flow was measured at 270 V/cm and pH 8.8 (20 m*M* TAPS/AMPD; see *Reagents and materials* for

abbreviations). Buffer in the vials was changed after every 27 runs.

Reagents and materials

Anhydrous ether, glacial acetic acid (HAc), formaldehyde (37%, w/v solution in water), and spermine were all obtained from Aldrich (Milwaukee, WI, USA). N,N,N',N'-tetramethylethylenediamine (TEMED), ammonium persulfate (APS) and electrophoresis-grade acrylamide were from ICN (Cleveland, OH, USA). Proteins, peptides, cetyldimethylethylammonium bromide, cacodylic acid, N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS), and N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) were supplied by Sigma (St. Louis, MO, USA). The 123 base pair ladder was received from GIBCO BRL (Bethesda, MD, USA). 6-Aminocaproic acid (EACA) and 2-amino-2-methyl-1,3-propanediol (AMPD) were from Fluka (Ronkonkoma, NY, USA). Ultra-pure electrophoresis grade Tris-base was obtained from Schwarz/Mann Biotech (Cambridge, MA, USA). Fused-silica capillaries of 50 and 75 μm I.D. were purchased from Polymicro Technologies (Phoenix, AZ, USA).

Coating procedure

An amount of 50 mg of the polymethylvinylsiloxanediol, prepared according to ref. 28, and a silane cross-linker was dissolved in 4.6 ml anhydrous ether and filled into the untreated fused-silica column (70 cm × 75 μm). The column was closed at one end with a septum, and a vacuum was applied at the other end (static method). After complete evaporation of the solvent, the septum was removed, and cross-linking took place overnight. The calculated film thickness was 0.2 μm [29].

To form the top layer, a solution of 50 mg acrylamide in 1 ml water was degassed for 30 min by bubbling through helium. Aliquots of TEMED (10 μl) (10%, w/v in water) and APS (10 μl) (10%, w/v in water) were then added, and the mixture was immediately pushed by means of a syringe into the siloxanediol-coated column. After 12 h the polymerized but not grafted part of the acrylamide was pushed out of the column. For cross-linking the polyacrylamide (PAA), a coated column was filled with a 37% solution of formaldehyde which was adjusted to pH 10 with NaOH. After three hours, the column was rinsed with water and was then ready for use.

RESULTS AND DISCUSSION

Surface modification

The chemistry used for the highly cross-linked hydrophobic polymeric siloxane sublayer (Fig. 1) is well-established for sealants and adhesives and is known as the room temperature vulcanizing (RTV) method [30]. The inner wall of the separation capillary was coated with a mixture of a polysiloxanediol (containing 50% vinyl and 50% methyl groups) and silane cross-linker dissolved in anhydrous diethyl ether. Cross-linking and binding to the silica surface took place in one step at room temperature: the cross-linker reacted with the Si–OH groups of the siloxanediol and the silica surface via the formation of Si–O–Si bonds. No prior treatment (*e.g.* etching) of the capillary wall was necessary since the amount of OH groups already present on the silica surface was sufficient for the effective binding of the polymer to the capillary wall. Moreover, column etching did not result in improved column performance.

Since cross-linking of the siloxane was performed at room temperature instead of at the commonly used elevated temperature [31], a high concentration of the temperature-sensitive vinyl groups were available for the dense grafting on the final hydrophilic top layer. A dense and highly viscous top layer should strongly suppress the EOF [17] and minimize protein adsorption. It must be further noted that the cross-linking of the siloxanediol was also important for the mechanical strength of the sublayer. Coatings with a non-cross-linked siloxanediol sublayer were destroyed by the shear force created when the highly viscous non-grafted excess of polyacrylamide was removed after polymerization.

The top layer, covalently attached to the polysiloxane sublayer, consisted of polymethylol acrylamide which could, in principle, be prepared in one step by direct polymerization inside

the capillary. However, the final polymer tended to cross-link spontaneously [32], preventing its subsequent removal from the capillary. Thus, it was decided to form the cross-linked top layer in two steps. In the first, linear polyacrylamide was grafted to the polysiloxane layer, and in the second, polyacrylamide chains were converted into polymethylol acrylamide by reacting with formaldehyde at pH 10 [32]. In this way a dense, cross-linked, hydrophilic layer was formed. Bisacrylamide, which is usually added for the cross-linking of acrylamide, was not used in this approach because polymerization of the acrylamide took place throughout the entire column volume. Even small amounts of bisacrylamide ($< 0.2\%$) resulted in the formation of a rigid gel which could not be pushed out from the column, unless careful control of the conditions and time of polymerization were established.

Electroosmotic flow

As mentioned in the introduction, one of the goals in developing this coating was to minimize electroosmotic flow. Unfortunately at present there is no agreed method for assessing the extent of electroosmotic flow, making it difficult to compare the results of different coatings. Our recommended procedure is to use sensitive conditions of buffers with high pH (*ca.* 9) and low ionic strength (*ca.* 20 m*M*), since the contribution of the buffer to the suppression of the EOF is then minimized. At pH 8.8, essentially all of the Si–OH groups on the fused-silica surface are deprotonated, and the EOF of bare fused silica is maximized [34]. Low ionic strength buffers further enhance the electroosmotic flow by increasing the zeta potential [18].

Columns coated with only the polysiloxanediol sublayer showed almost the same strong electroosmotic flow as plain fused-silica columns (under basic pH conditions). However, because the polysiloxane film was thicker than the electric double layer on the capillary wall, it is assumed that the flow was not caused by the negatively charged Si–OH groups on the fused silica surface. Given a uniform coating, one possible explanation is the fact that an electric double layer is always formed at the solid/liquid interface. For example, even PTFE capillaries exhibit electroosmotic flow in spite of the fact that no functional groups are present on the PTFE surface for deprotonation [34]. A second possible explanation is that the siloxanediol layer possessed some negative charge.

After attachment of the top polymeric layer the EOF was substantially decreased. At pH 8.8 and 20 m*M* buffer, the EOF for the linear polyacrylamide coating was found to be 300 times less than that of a plain fused-silica column and the polymethylol acrylamide coating showed an initial 150-fold reduction in EOF (see Fig. 5). It is reasonable to conclude that during the separation time of an analysis, the small EOF played no significant role. The major decrease in EOF indicated the dense grafting of the top layer to the polymeric siloxanediol sublayer. The difference in EOF of a factor of two between the linear and cross-linked polyacrylamide top layers was possibly caused by the hydrolysis of a small percentage of the amide bonds during the formaldehyde treatment at pH 10.

Other important quantitative parameters for the characterization of a coating are: run-to-run migration time reproducibility, efficiency, and batch-to-batch reproducibility. To examine the stability of the coating in terms of migration time and band width reproducibility, we have used the following sensitive testing conditions: high electric field (*ca.* 500 V/cm), acidic and basic buffers (pH 4.4, 8.0 and 8.8) with low ionic strengths (20 m*M*).

Protein separations at acidic pH

Basic proteins are known for being sensitive probes of the quality of the capillary wall treatment due to their positive net charge and the typical negative charge on the walls. On uncoated columns or poorly shielded surfaces such proteins tend to interact strongly with the negatively charged fused-silica surface, leading to irreversible adsorption and poor reproducibility in migration times. On the present coated columns no indications for interactions with the capillary wall could be observed for the separation of a basic protein test mixture at pH 4.4. The absolute change in migration times after 600 consecutive injections (150 hours of continuous run time) was negligible, and the relative stan-

TABLE I

REPRODUCIBILITY OF MIGRATION TIMES FOR CONSECUTIVE RUNS OF BASIC PROTEINS AT pH 4.4

Conditions as in Fig. 2. t_m = Migration time.

Peak number	Protein	t_m	R.S.D. (%)
1	Lysozyme	5.94[a]	0.31
		5.91[b]	0.34
2	Cytochrome *c*	6.04	0.29
		6.01	0.31
3	Myoglobin	8.64	0.36
		8.62	0.39
4	Trypsinogen	9.09	0.39
		9.08	0.39
5	α-Chymotrypsinogen A	9.26	0.41
		9.23	0.40

[a] $n = 100$ (run No. 1–100).
[b] $n = 100$ (run No. 500–600).

dard deviations (R.S.D.s) of the migration times were still less than 0.5% after the 600 runs (Table I, Fig. 2).

High efficiencies were obtained ranging between $4 \cdot 10^5$ and $6 \cdot 10^5$ plates per meter (N/m) (Table II, Fig. 2). Moreover, no loss in efficiency was found during the 600 consecutive injections [Table II]. It must be added that the initial peaks widths were caused to a certain extent by the widths of the injection plugs, since this sample of standard proteins was dissolved in the running buffer. By dissolving the sample in deionized water and making use of focusing techniques by sample stacking [1], the efficiencies could be increased up to $2 \cdot 10^6$ plates per meter.

The batch-to-batch reproducibility of the coating was evaluated with three batches, each batch consisting of three columns coated in the same manner. The columns were evaluated using the basic protein test mixture (conditions as in Fig. 2). The R.S.D.s for the migration times of the basis proteins were less than 1%, and the R.S.D. values for the efficiencies were less than 14%, confirming excellent reproducibility of the coating.

Protein separations at basic pH

To examine next the long-term performance of

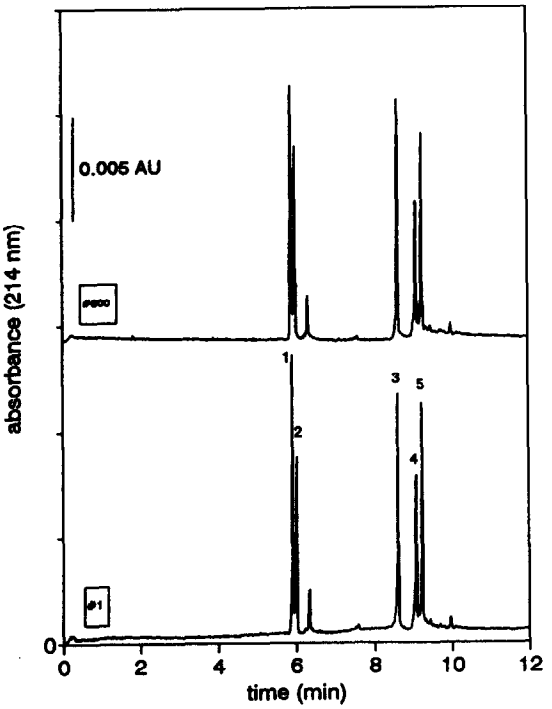

Fig. 2. Proteins as in Table I, 0.1 mg/ml in buffer; 8 s pressure injection; 20 mM EACA–HAc, pH 4.4; 214 nm; 526 V/cm, 17 μA, $l = 50$ cm (migration length), $L = 57$ cm (total length), 75 μm I.D.

the coating at basic pH, an acidic protein test mixture was run at pH 8.0. The R.S.D.s of migration times of the acidic proteins were again very low, less than 0.7% even after 600 consecutive runs (150 h of continuous run time) (Table III, Fig. 3). The efficiencies were as high as

TABLE II

COLUMN EFFICIENCIES FOR CONSECUTIVE RUNS OF BASIC PROTEINS AT pH 4.4

Conditions as in Fig. 2.

Peak number	Protein	N/m ($\cdot 10^3$)	
		Run No. 1	Run No. 600
1	Lysozyme	433	336
2	Cytochrome *c*	597	594
3	Myoglobin	515	575
4	Trypsinogen	472	605
5	α-Chymotrypsinogen A	592	735

TABLE III

REPRODUCIBILITY OF MIGRATION TIMES FOR CONSECUTIVE RUNS OF ACIDIC PROTEINS AT pH 8.0

Conditions as in Fig. 3.

Peak number	Protein	t_m	R.S.D. (%)
1	Glucose-6-phosphate dehydrogenase	5.53[a]	0.42
		5.45[b]	0.39
2	Trypsin inhibitor	6.47	0.48
		6.38	0.50
3	*l*-Asparaginase	10.05	0.42
		10.01	0.49
4	α-Lactalbumin	10.45	0.70
		10.41	0.69

[a] $n = 100$ (run No. 1–100).
[b] $n = 100$ (run No. 500–600).

$1 \cdot 10^6$ plates per meter at the beginning of the study, with only a 25% average decrease in efficiency found after the 600 runs (Table IV).

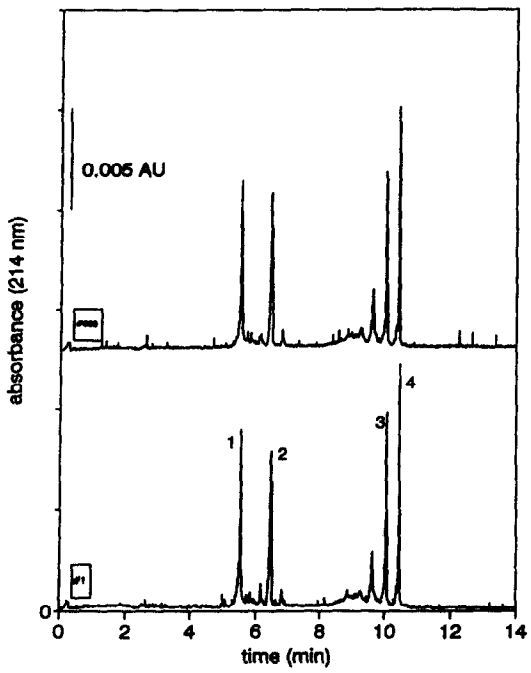

Fig. 3. Proteins as in Table III, 0.1 mg/ml in buffer; 8 s pressure injection; 20 m*M* TAPS–Tris, pH 8.0; 214 nm; 526 V/cm, 6 μA, $l = 50$ cm, $L = 57$ cm, 75 μm I.D.

TABLE IV

COLUMN EFFICIENCIES FOR CONSECUTIVE RUNS OF ACIDIC PROTEINS AT pH 8.0

Conditions as in Fig. 3.

Peak number	Protein	N/m ($\cdot 10^3$)	
		Run No. 1	Run No. 600
1	Glucose-6-phosphate dehydrogenase	295	240
2	Trypsin inhibitor	–[a]	–[a]
3	*l*-Asparaginase	820	610
4	α-Lactalbumin	975	694

[a] Not evaluated due to presence of double peak.

Nevertheless, the plate counts after the 600 runs was still greater than $6 \cdot 10^5$ plates per meter. The EOF was measured at pH 8.8 before and after the 150-h test period, and it was found that the EOF changed by 25% from an initial 160 times less than on a plain fused-silica column to 120 times less after the 600 runs.

From the results in Tables I–IV and measurements performed between pH 2 and 3 (results not shown) it can thus be concluded that the coating can be used for an extended period of time without significant loss in performance. The stability of the coating is probably the result of the cross-linked polymeric structure of the siloxanediol linker, which stabilizes the coating even if some damage to the sublayer occurs, and also to the formaldehyde cross-linking of the top layer, which decreases the rate of base hydrolysis of the amide bonds.

For the further investigation of the stability of the coating at basic pH, the coated column was studied with an acidic protein test mixture at pH 8.8. Even at this pH with 600 consecutive injections, the R.S.D.s of migration times for the first 100 runs were less than 0.7% and still less than 1.6% for the last 100 injections (runs No. 500–600) (Table V, Fig. 4). Furthermore, the absolute change in migration time over the 600 runs was less than 1%. Initially, efficiencies were as high as $1.1 \cdot 10^6$ plates per meter, but after 600

TABLE V

REPRODUCIBILITY OF MIGRATION TIMES FOR CONSECUTIVE RUNS OF ACIDIC PROTEINS AT pH 8.8

Conditions as in Fig. 4.

Peak number	Protein	t_m	R.S.D. (%)
1	Glucose-6-phosphate dehydrogenase	6.16[a]	0.39
		6.19[b]	0.48
2	Trypsin inhibitor	7.43	0.38
		7.47	0.57
3	β-Lactoglobulin B	8.30	0.43
		8.50	0.95
4	l-Asparaginase	10.87	0.44
		11.01	0.99
5	α-Lactalbumin	11.60	0.67
		11.73	1.55

[a] $n = 100$ (run No. 1–100).
[b] $n = 100$ (run No. 500–600).

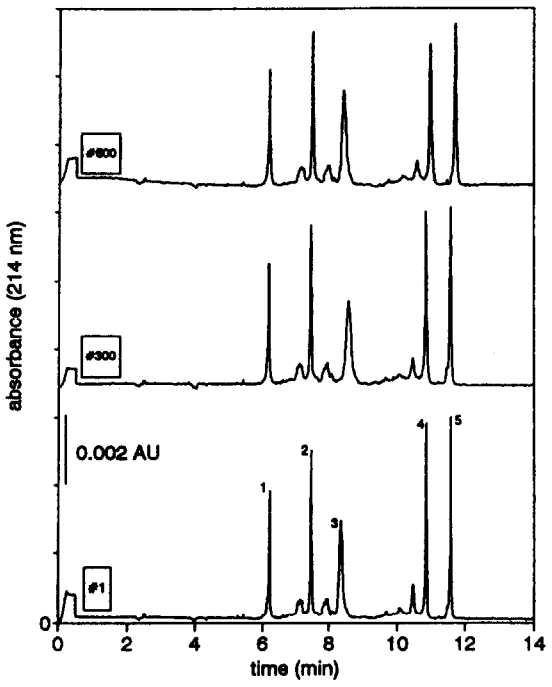

Fig. 4. Proteins as in Table V, 0.1 mg/ml in buffer; 8 s pressure injection; 20 m*M* TAPS–AMPD, pH 8.8; 214 nm; 526 V/cm, 14 μA, $l = 50$ cm, $L = 57$ cm, 75 μm I.D.

consecutive runs (150 h of continuous run time), and average loss in plate count of about 60% was observed (Table VI). However, as seen in Fig. 4, despite this decrease, the efficiencies were still sufficient for high resolution. (The low efficiency of β-lactoglobulin B (Fig. 4, Peak No. 3) was most likely due to the heterogeneity of this protein.) Furthermore, by changing the buffer after these 600 runs to pH 4.4, an average of $5 \cdot 10^5$ plates per meter could still be achieved for the basic protein test mixture.

Because of the decrease in efficiency found during the long term operation of pH 8.8, we decided to continuously monitor the EOF at this pH to see if changes in flow could be correlated with the observed loss in column performance. Both coatings, the linear and the crosslinked polyacrylamide, showed a slow increase in EOF over time, but the relative increase in the EOF for the polymethylol acrylamide coating was remarkably less than for the non-modified polyacrylamide coating (Fig. 5). After 156 h of continuous use at pH 8.8, the EOF of the polymethylol acrylamide coating was still about 100 times less than for a plain fused-silica column and remained constant at this value for another 160 h of continuous operation at pH 8.8. Moreover, as already discussed above, only a 25% change was observed for the polymethylol acrylamide coating at pH 8.0 during the 150-h testing period. The increase in EOF over time indicated

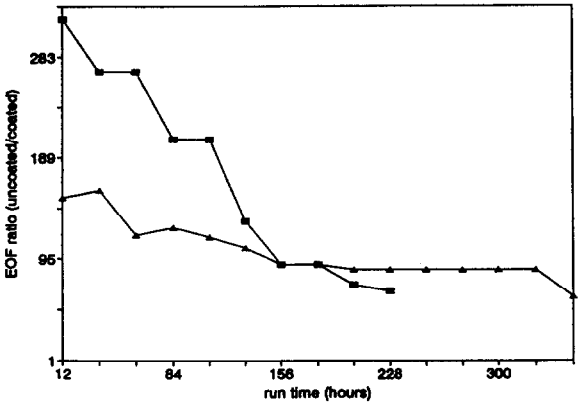

Fig. 5. EOF of (▲) cross-linked and (■) non-cross-linked polyacrylamide top layer with siloxanediol as sublayer. 20 m*M* TAPS–AMPD, pH 8.8; 7 μA, 268 V/cm; acetone used as EOF marker.

a gradual change on the capillary surface which might have been caused by the hydrolysis of amide bonds of the top layer and/or by the cleavage of Si–O–Si bonds of the siloxanediol sublayer [35]. The higher stability of the polymethylol acrylamide coating is probably the result of partial cross-linking along with the alkyl substitution of amide bonds slowing down the basic attack. The strong suppression of the EOF even after 150 h of continuous operation at pH 8.8 is in good agreement with the negligible changes in migration times observed. Furthermore, based on the results in Fig. 5, there would appear to be a relationship between the hydrolysis of the coating and the decrease in efficiency.

Coating stability and electric field

To test if the actual use of a column under high electric field operation had an influence on the stability of the coating, a coated column was stored without an applied electric field for 168 h filled with the pH 8.8 buffer. The comparison of the electropherograms run before and after this storage with the electropherograms of the column continuously run at pH 8.8 clearly demonstrated that the rate of deterioration depends on the operation of the electric field (Tables VI and VII). Indeed, little change in performance was observed for the column that was simply stored. A change in the pH of the buffer in the vials, caused by the redox processes occurring at the electrodes [33], can be excluded as an explanation for the effect of the electric field since all the electrolytes were well buffered and replaced after every 27 runs. One possibility is that the high electric field interacted with the permanent dipoles of the amide bonds, aligning the polyacrylamide fibers and thereby opening the three dimensional structure of the top layer for base attack. From the results of Table VI and VII it can be concluded that lifetime testing of a column should be on the basis of high electric field operation.

TABLE VI

COLUMN EFFICIENCIES FOR CONSECUTIVE RUNS OF ACIDIC PROTEINS AT pH 8.8

Conditions as in Fig. 4.

Peak number	Protein	N/m ($\cdot 10^3$)		
		Run No. 1	Run No. 2	Run No. 600
1	Glucose-6-phosphate dehydrogenase	544	469	266
2	Trypsin inhibitor	785	226	184
3	β-Lactoglobulin B	62	29	40
4	*l*-Asparaginase	1127	520	288
5	α-Lactalbumin	928	593	371

TABLE VII

INFLUENCE OF COLUMN STORAGE WITHOUT AN APPLIED ELECTRIC FIELD ON EFFICIENCY AT pH 8.8

Peak number	Protein	N/m ($\cdot 10^3$)	
		Before storage	After 168-h storage
1	Glucose-6-phosphate dehydrogenase	715	743
2	Trypsin inhibitor	642	613
3	β-Lactoglobulin B	98	92
4	*l*-Asparaginase	1112	980
5	α-Lactalbumin	1268	624

Protein separations at neutral pH

So far, only separations at acidic and basic pH have been discussed. However, since the selectivities of proteins in open tube CZE is governed mainly by charge differences, the coating should allow operation close to neutral pH. Moreover, this pH region is especially interesting since proteins often exist in native structures, and sample degradation is thus minimized in this region.

The test mixture of acidic proteins was separated over the pH range from 6 to 9 with high efficiencies in low ionic strength buffers (Fig. 6). For the basic protein test mixture, it was found

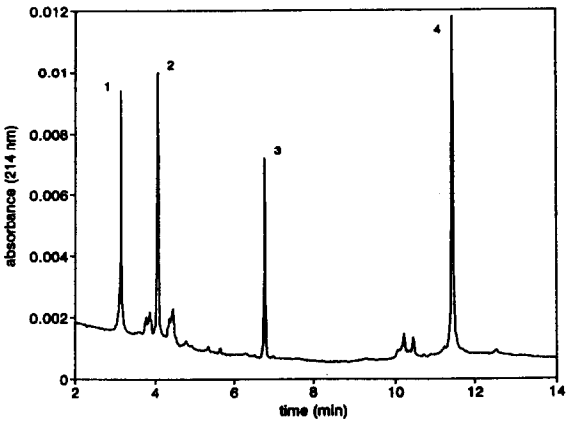

Fig. 6. (1) Glucose-6-phosphate dehydrogenase, (2) trypsin inhibitor (3), *l*-asparaginase, (4) α-lactalbumin, 0.1 mg/ml in water, 10 s pressure injection; 20 m*M* cacodylic acid + Bis-Tris, pH 6.2; 214 nm; 810 V/cm, 13 μA; $l = 37$ cm, $L = 30$ cm, 50 μm I.D.

that the addition of minute amounts of cationic additives to the low ionic strength background electrolyte were necessary for high separation efficiencies at pH 7. Fig. 7 depicts the separation of the basic proteins at this pH (10 m*M* phosphate–NaOH) in the presence of $2 \cdot 10^{-4}$ *M* spermine. It might be noted that not all of the peaks at pH 7 were totally symmetrical since the mobilities of several of the proteins were too low at this pH (*e.g. ca.* $4 \cdot 10^{-5}$ cm^2/Vs for the last peak) to match the mobilities of the buffer ions. Electropherograms similar to that in Fig. 7 were also obtained at pH 6.2 when $5 \cdot 10^{-5}$ *M* cetyldimethylethylammonium bromide was added to the running buffer (20 m*M* Tris–cacodylic acid) (results not shown). Since the conductivities of the background electrolytes were still low at neutral pH, very high electric fields could be applied (up to 1100 V/cm), keeping the separation times of the protein test mixtures to less than ten minutes. Moreover, the positively charged additives did not generate electroosmotic flow toward the anode as usually observed when such additives are used in uncoated columns [11,13].

Other applications

The coating has also been successfully used for open tube peptide and polymer network DNA separations. The peptides were separated in less than 80 s since very high fields (1100 V/cm) and a short column ($L = 27$ cm) could be utilized (Fig. 8). In the 123 bp DNA ladder polymer network separation very high resolution was achieved with efficiencies up to $1 \cdot 10^7$ plates per meter (peak No. 15) (Fig. 9). With replaceable linear polyacrylamide matrices, more than 500 DNA separations could be performed (pH 8.4)

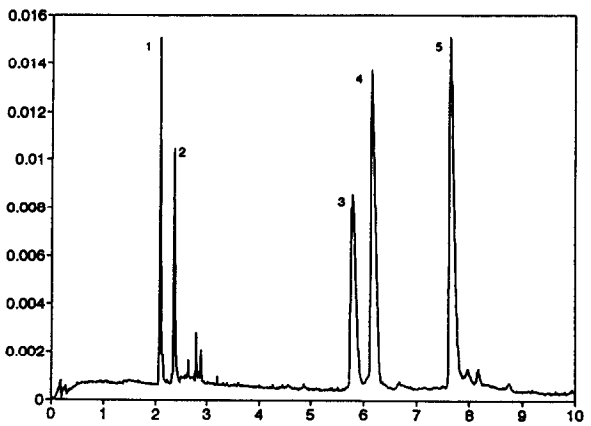

Fig. 7. (1) Lysozyme, (2) cytochrome *c*, (3) ribonuclease A, (4) α-chymotrypsinogen, (5) trypsinogen, 0.1 mg/ml in water; 8 s pressure injection; 10 m*M* H_3PO_4–NaOH, $2 \cdot 10^{-4}$ *M* spermine, pH 7.0; 214 nm; 1111 V/cm, 32 μA; $l = 20$ cm, $L = 27$ cm, 50 μm I.D.

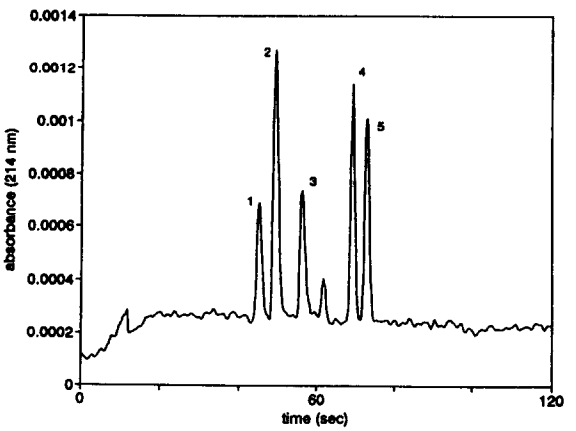

Fig. 8. (1) K-R-T-L-R-R, 0.05 mg/ml; (2) K-R-Q-H-P-G, 0.006 mg/ml; (3) R-P-K-P, 0.02 mg/ml; (4) R-P-K-P-Q-Q-F-F-G-L-M, 0.013 mg/ml; (5) R-P-K-P-Q-Q-F-F-G, 0.013 mg/ml all in water; 8 s pressure injection; 50 m*M* HAc, pH 3.0; 214 nm; 1111 V/cm, 8 μA; $l = 20$ cm, $L = 27$ cm, 50 μm I.D.

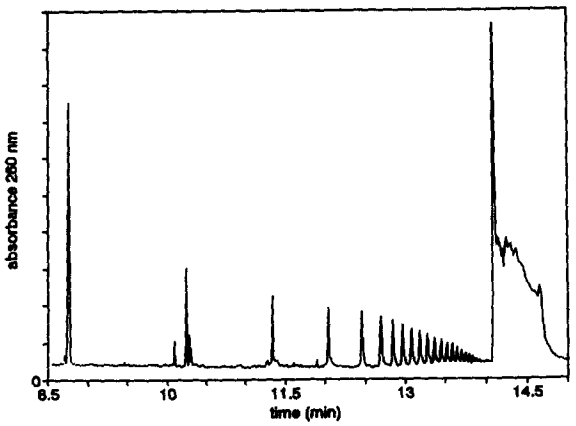

Fig. 9. 123 bp DNA ladder, 100 μg/ml in water; 2 s electrokinetic injection at 8 kV; 3%T PAA, 0.1 *M* Tris–boric acid, pH 8.4; 260 nm; 300 V/cm, 15 μA; $l = 30$ cm, $L = 40$ cm, 75 μm I.D.

with a single column before changes in migration times or efficiencies were noticeable.

CONCLUSIONS

A simple coating procedure has been developed with a high batch-to-batch reproducibility in which the EOF was greatly suppressed. Very high efficiencies were achieved for both acidic (pH 8–9) and basic proteins (pH 4.4) with low ionic strength buffers. Acidic proteins could be rapidly separated over the pH range from 6 to 9. For basic proteins, very small amounts of additives were necessary at or near neutral pH to achieve high performance. At both pH 8.0 and 4.4, hundreds of consecutive runs could be performed with excellent reproduciblities in migration times and efficiencies. The coating also showed high performance in peptide separations and for DNA mixtures using polymer network matrices. The recommended working range of the coating is between pH 2–9. Work is continuing on developing coatings that are stable for extended periods of time at extreme pH values.

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