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Epoxy resin coatings for capillary zone electrophoretic separation of basic proteins

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Abstract

Epoxy resins, combining with a cross-linker, were coated on organosilanes modified fused-silica capillaries. After cross-linking, a tough three-dimensional network was attached to capillary surface via Si–O–Si–C bonds. The most effective coating consisted of epoxy resins cross-linked with diaminodiphenylmethane, which was stable over the pH range 2–12 and suppressed protein adsorption effectively. The electroosmotic flow was also greatly reduced and maintained constant over the pH range 6–10. High resolutions and separation efficiencies for basic proteins were obtained in the pH range 3–10 and reproducible separations were achieved from run to run and day to day.

Keywords: Capillary electrophoresis; Coating methods; Column coating; Proteins; Basic proteins

1. Introduction

Capillary zone electrophoresis (CZE) is rapidly developing as a powerful separation tool for biopolymers, superior in separation power to other techniques such as LC [1]. Theoretically, more than 10^6 plates/m could be obtained in the separation of proteins. However, negatively charged fused-silica capillaries have a great tendency to adsorb charged species, especially some basic proteins. Such adsorption can lead to many problems, such as peak broadening, lower efficiency than theoretically predicted, altered electroosmotic flow (EOF) and poor reproducibility [2].

Many strategies have been employed to eliminate the wall adsorption of proteins. The use of buffers of high [3] or low pH [4,5] can suppress the interaction between proteins and the capillary surface. This method has the disadvantage that it can potentially cause protein degradation and limit the operational pH range for the optimization of separation. Another approach was to use a buffer of high salt concentration whereby protein adsorption can be eliminated by a cation-exchange mechanism on the silica surface [6]. A highly efficient separation of proteins was obtained when short capillaries of small inner diameters were used [7]. However, in this approach an additional cooling system must be applied to remove Joule heat and it may also introduce difficulties in injection and detection. The use of additives in the sample [8] and buffer [9–12] has also had some success in reducing protein adsorption. Some additives can form a dynamic coating on the capillary surfaces to

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prevent protein adsorption effectively [13–17]. However, a high concentration of additives must be used in the running buffer which may interact with proteins and contaminate the sample collected or make it unsuitable for on-line capillary electrophoresis–electrospray mass spectrometry (CE–ES-MS).

Alternative approaches to circumvent this problem include chemical modification of the fused-silica surface, which has the advantage of greater flexibility with respect to the choice of buffer [2,19–27]. Many coatings have been developed for the separation of proteins in CZE, such as glycol [2], aryl pentafluoro (APF) [19], polyethylene glycol (PEG) [20–22], poly(methyl glutamate) [23], carbohydrate [24] and polyacrylamide [25] types. Although each achieved some success in reducing protein adsorption, these coatings are easy to hydrolyse under basic condition (pH > 8) and the column performance deteriorates rapidly. Treatment of capillaries with an epoxy polymer can further extend the column lifetime and pH range of operation [26]. Although highly efficient separations could be achieved over the pH range 5–10, production of the coating involved a five-step process, making the preparation difficult. Hjerten and Kubo [27] reported a pH- and detergent-stable coating which can withstand short washes of capillaries with 0.05 *M* NaOH and 1 *M* HCl between runs. However, this highly stable coating eliminates both adsorption and EOF at the same time. The oppositely charged species cannot be separated in one run. Hence it is very important to prepare stable coatings which can maintain a sufficient EOF while reducing protein adsorption over a wide pH range.

Epoxy resins contain more than one epoxy groups per molecule and can be polymerized through these epoxy groups, using a cross-linking agent, to form a tough three-dimensional network [28]. When the resins are cured, they have the well known properties of outstanding toughness, adhesion to many substrates, chemical resistance and high mechanical strength. Their versatility has led to many applications, including surface coatings, adhesives, monolithic flooring, laminates and sealing compounds for more than 60 years.

In this work, our goal was to provide a permanent coating with the characteristics of (1) ease for preparation, (2) stable and high efficiency in the pH range 2–11 and (3) constant, moderate EOF over a wide pH range. As epoxy resins have excellent chemical resistance (stable against 25% HCl and 50% NaOH for 180 days at 82°C) [29], we had examined the utility and stability of epoxy coatings in CZE for the separation of proteins over a wide pH range (2–12) [30]. However, strong adsorption of basic protein still occurred above pH 9.0 and the coating stability was also unsatisfactory. This may be because the ethylenediamine (EDA) used to cross-link epoxy resins is a room-temperature curing agent. The cross-linking reaction was so fast that the coatings were brittle and easily cracked owing to the substantial shrinkage of the cured resins, leaving some areas of the silica surface uncoated. In this work, another curing agent, diaminodiphenylmethane, was used to improve the coating stability and the results was compared with those for the coatings described previously [30]. The effect of a flexible polysulphide modifier on the coating properties were also investigated. Further work consisted of extensive evaluation of the coatings in terms of EOF, efficiency, long-term stability and reproducibility.

2. Experimental

2.1. Apparatus

The CE system was purchased from Beijing Institute of New Technology Applications (Beijing, China). Fused-silica capillaries (Herbei Yongnian Optical Fibre Factory, Herbei, China) of 50 μm I.D. were used to prepare the capillaries. The capillaries were 66 cm long with a separation length of 44 cm. Proteins were detected at 214 nm.

2.2. Reagents

Protein samples were purchased from Shanghai Biochemical (Shanghai, China), except for α-chymotrypsinogen, which was purchased from

Sigma (St. Louis, MO, USA). Bisphenol A, resins and polysulphides (R_2–SH in Fig. 1) were gifts from the Beijing Institute of Aeronautical Materials. All other reagents were of analytical-reagent grade.

2.3. Electrophoresis

Protein solutions of 1.0 mg/ml were introduced into the capillary by siphoning for a fixed time (5 s) at a fixed height (10 cm). Dimethylsulphoxide (DMSO) was used as the neutral marker. Between runs, the capillary was flushed with separation buffer for 3 min.

2.4. Preparation of capillary

Pretreatment of capillaries

Capillaries were first treated with 1.0 mol/l NaOH for 30 min, followed by 30 min of washing with deionized water. Residual water was evaporated from the capillaries in a gas chromatographic oven at 100°C for 1 h under a nitrogen pressure of 400 kPa. Then the capillaries were dynamically coated with γ-glycidoxypropyltrimethoxysilane and heated at 90°C for 1 h.

Preparation of epoxy resin coatings

Cured by aliphatic amines (ER-I). The details of this procedure have been reported elsewhere [30]. A mixture of the bisphenol epoxy resins (700 mg/ml) and ethylenediamine (49 mg/ml) in methylene chloride was deposited dynamically on the capillary surface. The resins were cured at room temperature for 2 h and heated at 100°C for 1 h. Then the capillaries were washed with water for 24 h before use.

Cured by aromatic amines (ER-II). A thin film of epoxy resins was deposited dynamically on the surface of pretreated capillaries by aspirating a mixture of bisphenol epoxy resins (500 mg/ml) and 4,4′-diaminodiphenylmethane (DDM) (140 mg/ml) in acetone. Residual solvent was removed from polymer film in a gas chromatographic oven at 30°C for 30 min under a nitrogen pressure of 200 psi. The resins were cured at 80°C for 2 h, 150°C for 1 h and 180°C for 3 h. Then the capillaries were washed with water for 24 h before use.

Preparation of epoxy and polysulphide copolymer coatings (EP)

The pretreated capillary were filled with a polymer solution of DEBA (250 mg/ml), polysulphides (250 mg/ml) and triethyleneamine (TEA) (38 mg/ml) in methylene chloride. A thin film of polymer were precipitated on capillaries by moving the polymer solution under 200 psi nitrogen pressure and cross-linked at room temperature for 24 h. Then the resins were further cured at 100°C for 2 h and washed with water for 24 h before use.

3. Results and discussion

3.1. Capillary modification

Epoxy resins can be cross-linked though the oxirane ring by use of polyfunctional curing agents, such as polyamines, into a three-dimensional infusible network. The cured resins have outstanding adhesive properties towards a wide variety of structural materials, including metals, glass, ceramics, wood and many types of plastics. During the curing process, epoxy adhesive is adsorbed either via the formation of covalent bonds at active sites on the adsorbent surface or via Van der Waals forces reinforced by dipole–dipole interaction [31]. Sometimes the cohesive strength of a properly cured resin within the glue line is so high that failure under stress occurs within the material being bonded rather than within the glue line itself. However, clean, untreated glass surfaces present an unusual adhesion problem in that the high affinity of water for glass results in preferential wetting of the glass by moisture during exterior exposure, with subsequent debonding of the epoxy adhesive. For this reason, the capillary surfaces were pretreated with a silane coupling agent, γ-glycidoxypropyltrimethoxysilane (GOX), so as to render it more chemically reactive towards the epoxy adhesive molecules. Furthermore, the oxirane ring in GOX can participate in the curing reaction of resins to generate stable Si–O–Si–C

bonds between the silica surface and epoxy resins, which will further increase the cohesive strength of the coatings. It should be noted that only a thin layer of GOX is necessary, or they will cross-link by themselves, which consequently destroys the adhesion between the epoxy resin and capillary surface.

The coating properties attained by an epoxy resin are largely dependent on the curing agents used to convert the resin into a thermoset product. Here we prepared two kinds of epoxy resin coatings cured by aliphatic amines and aromatic amines. The curing reactions of the epoxy resin coatings are shown schematically in Fig. 1a.

EDA is a room-temperature curing agent for epoxy resins. After curing at room temperature for 2 h, more than 50% conversion of epoxide groups may be achieved. By increasing the temperature to 100°C, the resins could be further cross-linked. However, the partly polymerized resins had already become rigid and some unreactive oxirane rings had been trapped in the resins, and as a consequence the epoxy resins could not be fully cross-linked. Alternatively, aromatic amines such as DDM are less reactive towards epoxy resins and require elevated temperature cures. At room temperature, the curing reaction did not occur within at least 48 h. When the temperature was raised to 80°C, both the epoxy resins and DDM changed into liquid form

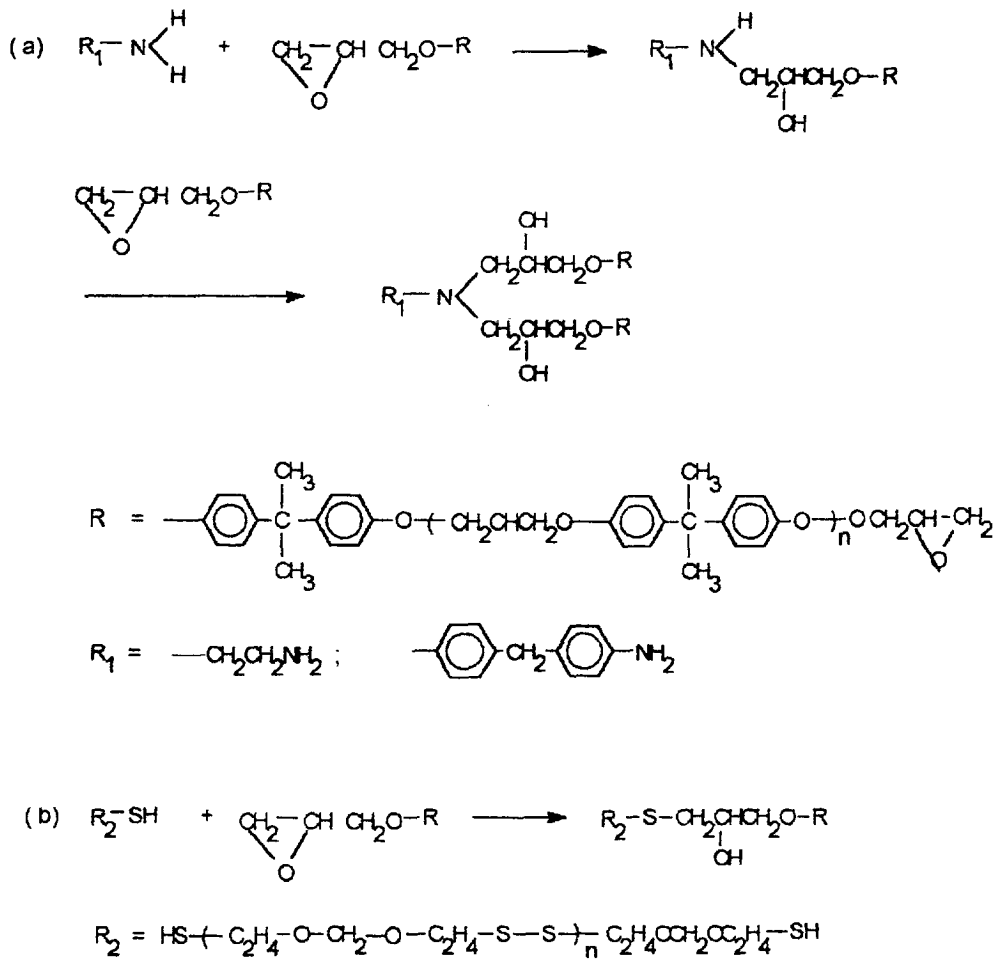

Fig. 1. Typical reaction scheme used in the preparation of an epoxy polymer coating.

while the rate of the cross-linking reaction was still very slow, so they could be mixed thoroughly on the silica surface. On further increasing the temperature to 180°C, the cross-linking proceeded nearly to completion, so that a denser and more homogeneous film was deposited on the capillary surface.

Although epoxy resins have outstanding adhesive properties, the cured resins are hard and brittle, and easily crack owing to curing shrinkage. Modifying epoxy resins with polysulphides can reduce the shrinkage on curing and improve the toughness or increase flexibility. Further, the outstanding chemical resistance of polysulphides may further increase the stability of the resins against strongly alkaline and inorganic acids [32]. Principally the polysulphides are able to react with epoxy resins via the thiol group (Fig. 1b), but the cross-linking reaction was slow and the copolymers could not be fully cross-linked unless an amine catalyst such as TEA was added. In addition, TEA is also a curing agent for epoxy resins and can react with the epoxy groups of the resins in the usual way.

3.2. Evaluation of coated capillaries

The coated capillaries were evaluated with a mixture of four basic proteins, lysozyme, cytochrome *c*, ribonuclease A and chymotrypinogen A. Fig. 2 shows a typical electropherogram protein mixtures at pH 7.0 on epoxy resin-coated capillaries ER-I and ER-II. Some performance parameters for the two capillaries are given in Table 1. The protein mixtures can be separated successfully in these epoxy resin-coated capillaries, which indicates that a substantial decrease in protein adsorption has been achieved. Higher efficiency and better resolution and stability were obtained on the ER-II-coated capillary (Table 1). This is because the cross-linking reaction between EDA and epoxy resins was so fast that the ER-I coatings were brittle and easily cracked owing to the great shrinkage of the cured resins, leaving some areas of the silica surface uncoated. Further, as a much higher degree of cross-linking was achieved, a denser and more homogeneous layer was deposited on ER-II-coated capillaries to further mask surface silanols.

It should be noted that the ionic strength of the electrolyte also has some effects on the ER coatings (Table 2). With a low concentration of running electrolyte (<0.05 *M* phosphate buffer), the efficiency of lysozyme above pH 8.0 is low, which indicates that there was still some surface and solutes interaction. This may be due to the interaction between proteins and the coating itself, leading to some protein adsorption. How-

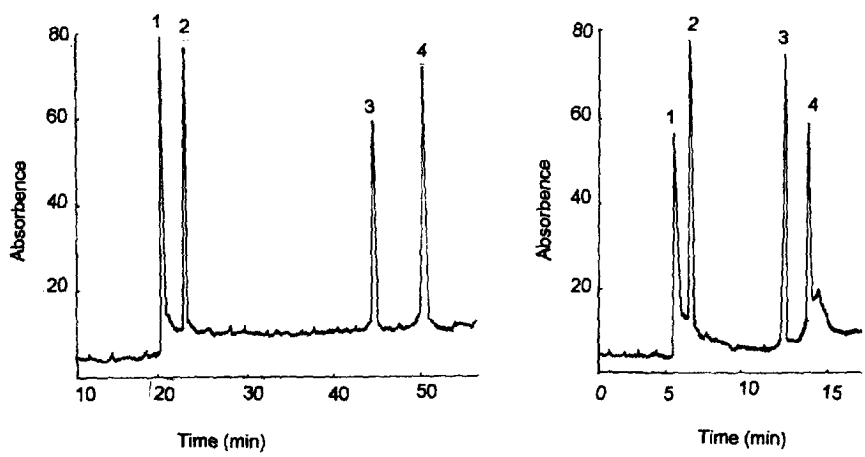

Fig. 2. Capillary electrophoretic separation of basic protein mixture using (A) ER-I- and (B) ER-II-coated silica capillaries. Electrophoretic condition: capillaries, 66 cm (44 cm to detector) × 50 μm I.D.; 0.07 *M* phosphate buffer (pH 7.0); hydrodynamic injection, 5 s at 10 cm height; applied voltage, 19 kV. Peaks: 1 = lysozyme; 2 = cytochrome *c*; 3 = ribonuclease A; 4 = chymotrypsinogen.

Table 1
Separation parameters and stabilities of epoxy resin-coated capillaries

Capillary	EOF (10^{-8} m^2 N · s)	pH range used	Original plate number	Plate number after immersion for 48 h	
				pH 9	pH 12
Untreated	4.24		–		
ER-I coating	0.89	2–10	81450	58730	–
ER-II coating	–[a]	2–10	92670	91500	89120
EP coating	0.97	2–5	111330	106870	101970

Eletrophretic condition: capillaries, 66 cm (44 cm to detector) × 50 μm I.D.; 0.05 *M* phosphate buffer (pH 5); applied voltage, 19 kV for protein separation, 24 kV for EOF; lysozyme used to evaluate column efficiency (pH 5), DMSO as neutral marker. Coating stability was investigated after the capillaries had been immersed in 0.05 *M* sodium phosphate (pH 9) and 0.01 *M* NaOH for 48 h.
[a] DMSO cannot be detected after 1.5 h.

ever, when using 0.07 *M* phosphate buffer, the protein adsorption can be depressed and high efficiencies were obtained.

Theoretically, epoxy and polysulphide copolymer coatings (EP) can show excellent chemical resistance and stability, as the flexibility of the resins was greatly improved [32]. Fig. 3 shows the separation of basic proteins on EP-coated capillaries at pH 5.0. When high-molecular-mass polysulphides was added, the strong hydrophilicity of the epoxy resins decreased. As a result, the separation efficiency was improved as a result of the prevention of hydrogen bonding between proteins and the coating [33]. The column efficiency was the highest on the EP-coated capillaries at pH 5.0, among all the columns evaluated (Table 1). However, the separation efficiency dropped quickly and lysozyme showed long tailing at pH 7.0. The reason is unknown. Further studies will be made to investigate the influence of resin structure on protein adsorption.

Table 2
Effect of ionic strength on separation efficiency (plate number)

Protein	Buffer concentration (*M*)		
	0.03	0.05	0.07
Lysozyme		45600	62043
Cytochrome *c*	54110	98660	106560
Ribonuclease A	89650	124700	170010
Chymotrypsinogen A	46700	50670	58520

Electrophoretic conditions: capillary with ER-II coating, 66 cm (44 cm to detector); phosphate buffer (pH 8.3); applied voltage, 19 kV.

3.3. Electroosmotic flow

Reducing protein adsorption by modification of the silica surface would be at the expense of EOF, which pumps both positively and nega-

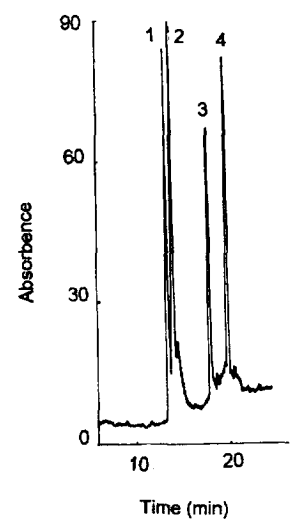

Fig. 3. Capillary electrophoresis of four basic proteins in EP-coated capillary at pH 5. Conditions as in Fig. 2. Peaks: 1 = lysozyme; 2 = cytochrome *c*; 3 = ribonuclease A; 4 = chymotrypsinogen.

tively charged samples to the detector. Therefore, it was necessary to maintain a moderate EOF while reducing the negative silanol charges on the capillary surface that cause protein adsorption.

The effect of pH on EOF was examined over the pH range 2–11, using DMSO as a neutral marker. Fig. 4 shows the results obtained for treated and untreated capillaries. Both ER-coated capillaries exhibit substantial decreases in EOF over the pH range 2–11 while the EOF increased rapidly above pH 5.0 on the EP-coated capillaries. For the two kinds of ER-coated capillaries, the electroosmotic flow characteristics are also different. ER-II coatings drastically minimized the contribution of the surface silanols to the observed EOF. Below pH 4.0, the capillary surfaces were positively charged owing to the tertiary amine groups in the cured resins, which resulted in an anodic EOF. In the pH range 4–5, the surface was neutral and EOF could not be detected after 1.5 h when 24 kV were applied to these capillaries. On further increasing the buffer pH, a moderate cathodic EOF was generated and remained constant over the pH range 6–10. This constant EOF makes it possible to change the pH freely between 6 and 10 without a significant change in capillary-dependent separation variables. The optimum pH can be employed to give maximum selectivity without adversely affecting the analysis time. When the buffer pH was increased to 11, the EOF greatly increased, probably because of further ionization of hydroxyl groups. However, the EOF characteristics of ER-I are different from those of ER-II, although they have similarly coating structures. The EOF of ER-I remaintained near zero over the pH range 2–5 and still showed a large increase over the pH range 5–11. This is because the degree of cross-linking in ER-I coatings was low so that it could not form a dense and homogeneous film on the capillary surface to shield the surface silanols effectively. The EOF is still mainly charged by silanol ionization of the silica surface, which in turn is dependent on the buffer pH.

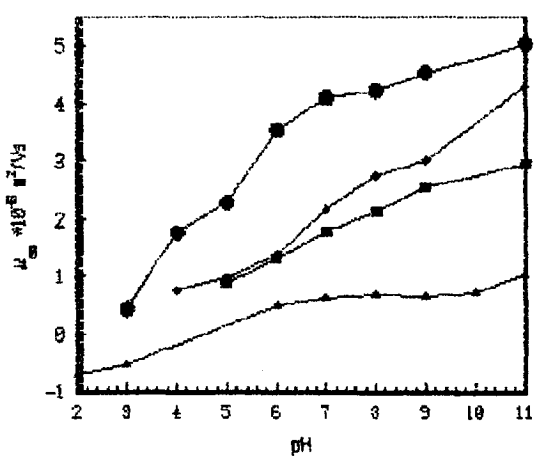

Fig. 4. Dependence of EOF on pH for (○) uncoated, (□) ER-I-coated, (△) ER-II-coated and (◇) EP-coated silica capillaries. Conditions: ER-II-coated capillary, 66 cm (44 cm to detector) × 50 μm I.D.; 0.07 *M* phosphate buffer; operating voltage, 24 kV.

The effect of film thickness on EOF was also investigated, using 30%, 50% and 80% epoxy resins in acetone solution to coat the capillary. The film thickness was calculated with the empirical equation

$$d_f = \frac{c}{200r}(0.265u + 0.25)$$

where c is the concentration of polymer solution in acetone, r is capillary inner radius and u is the velocity of the coating plug [34]. Fig. 5 illustrates the observed EOF of ER-II-coated capillaries with three different film thicknesses. At low film thickness (below 50 nm), a pH dependence of EOF is observed because the film, although being homogeneously coated on the capillary, was too thin to shield all the electric charges of the silica surface. At high film thicknesses (>100 nm), the EOF changed from anodic to cathodic over the pH range 2–11 and remaintained constant in the pH range 6–10. On further increasing the film thickness (>150 nm), no significant change was observed in the EOF. This indicated that surface silanols had already been shielded effectively at a film thickness of 100 nm, hence the surface properties are fully dependent on the coatings themselves and do not undergo any change with increasing film thickness. However, at large film thicknesses the separation efficiency will decrease owing to the radial diffusion of the

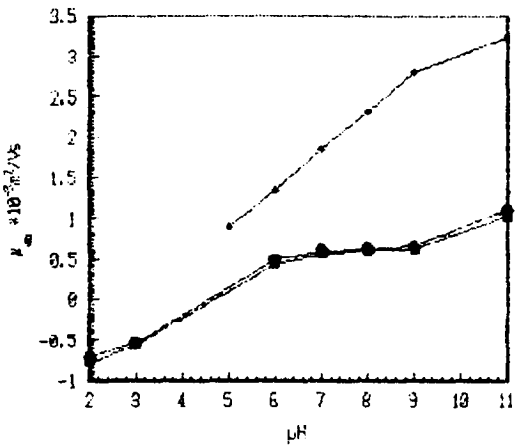

Fig. 5. Effect of film thickness on EOF plot. Conditions: ER-II-coated capillary, 66 cm (44 cm to detector) × 50 μm I.D.; 0.07 *M* phosphate buffer; operating voltage, 24 kV; DMSO as neutral marker. Film thickness: (◇) 50 ; (○) 100; (□) 130 nm.

proteins into the coating layer [33]. Hence the optimum film thickness was 80–100 nm.

3.4. Effect of pH on separation

According to the literature [6–11], many coatings based on derivatization with simple organosilanes are easily destroyed above pH 7.0 owing to hydrolysis of the Si–O–Si–C bonds. In this work, the coatings were also connected to capillaries via Si–O–Si–C bonds, but the polymer monolayer was further cross-linked with polyamines to form a three-dimensional network. These coatings were found to be stable over a wide pH range (2–11). Both types of ER-coated capillaries provided successfully separation of proteins over the pH range 3–10 whereas the EP coatings still had a great tendency to adsorb basic proteins above pH 5 owing to its unreactive thiol groups. As expected, the efficiency of ER-II is much higher than that of ER-I because of the more effective shielding of surface silanols in ER-II coatings (Table 1). Further, better resolution was achieved for basic proteins in ER-II-coated capillaries owing to its special EOF characteristics. Since the EOF was reduced eightfold and remained constant over the pH range 6–10, the resolution could easily be optimized by modifying the electrolyte pH while providing constant-flow transport across the capillary channel. Below pH 5, although the differences among the electrophoretic mobilities of basic proteins becomes smaller, the EOF changes to zero or even changes direction, so good resolution and efficiencies were achieved at acidic pH. Fig. 6 shows the separation of proteins in ER-II-coated capillaries over the pH range 2–10. Sharp peaks and good efficiencies were obtained at all pH values used. Table 3 gives the plate numbers for four basic proteins at different pH. It was found that excellent efficiencies and separation were achieved in the pH range 3–10. Below pH 3, although the efficiency was very high, protein denaturation occurred. Above pH 10, the efficiencies dropped rapidly and lysozyme showed long tailing, which may be due to the ionization of hydroxyl groups in the coatings.

3.5. Reproducibility and stability of coatings

Table 4 summarizes the run-to-run, day-to-day and column-to-column (for the ER-II coating only) migration reproducibilities expressed in terms of relative standard deviation (R.S.D.) with four basic proteins at pH 5.0. The column-to-column reproducibility for the ER-II coating reflects columns coated with the use of 50% epoxy resin solution in acetone. All the coatings exhibited good run-to-run and day-to-day reproducibility over the pH range used. The column-to-column reproducibility for the ER-II coating was unsatisfactory. This may be because the coating speed and temperature cannot be controlled strictly with a sample dynamic coating device. As a result, the film thicknesses for three capillaries will differ slightly, thus influencing the protein migration time. A flow restriction device which can stabilize the coating speed is being investigated so that good reproducibility of film thickness can be achieved. However, the protein adsorption was greatly suppressed in all of these capillaries. The column efficiencies of three capillaries for lysozyme were above a plate height of 50 000.

Although the Si–O–Si–C bonds are easily

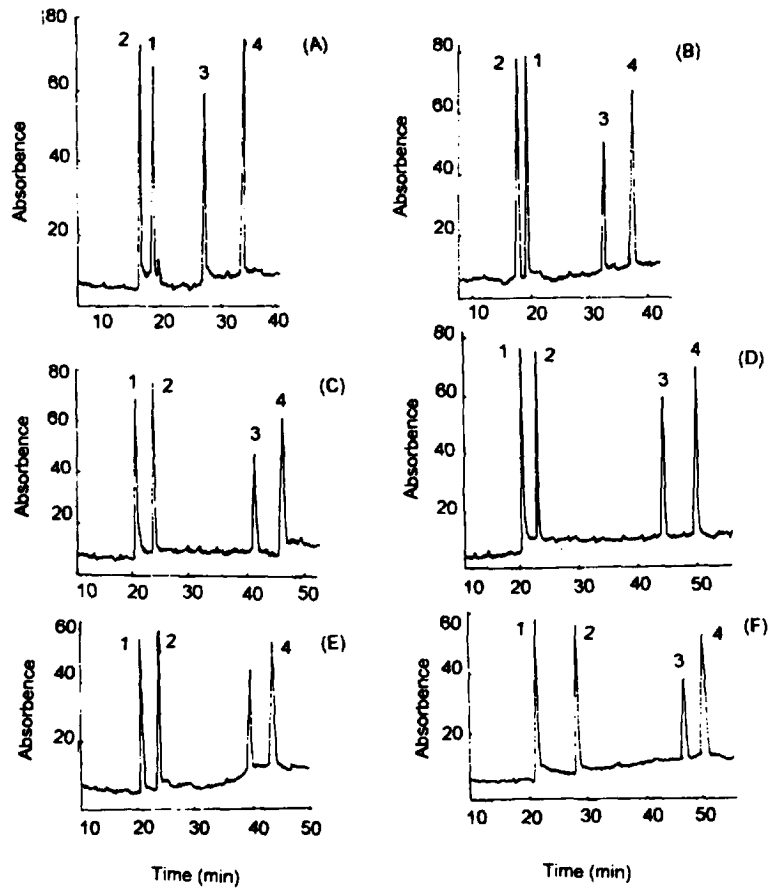

Fig. 6. Separation of basic proteins over the pH range 3–10 on an ER-II-coated capillary. Conditions: ER-II-coated capillary, 66 cm (44 cm to detector) × 50 μm I.D.; 0.07 *M* phosphate buffer; operating voltage, 19 kV; pH: (A) 3.0; (B) 5.0; (C) 6.0; (D) 7.0; (E) 8.3; (F) 9.5. Peaks: 1 = lysozyme; 2 = cytochrome *c*; 3 = ribonuclease A; 4 = chymotrypsinogen.

Table 3
Efficiency (plate number) for four basic proteins at various pH values

Protein	pH					
	2.9	5.0	7.0	8.3	9.5	7.0[a]
Lysozyme	86470	81450	67200	62043	58690	58730
Cytochrome *c*	119170	108820	107155	106560	99687	96380
Chymotrypsinogen A	87720	51060	83900	58520	106200	67520
Ribonuclease A	197250	196990	160350	170010	176310	104370

Electrophoretic conditions: capillary with ER-II coating, 66 cm (44 cm to detector); 0.07 *M* phosphate buffer; applied voltage, 19 kV.

[a] After the coated capillary had been immersed in 0.01 *M* NaOH for 48 h.

Table 4
Reproducibility of migration times of proteins on epoxy resin-coated capillaries

Protein	R.S.D. (%)						
	ER-I coating		ER-II coating		Column to column ($n = 3$)	EP coating	
	Run-to-run ($n = 6$)	Day-to-day ($n = 5$)	Run-to-run ($n = 6$)	Day-to-day ($n = 5$)		Run-to-run ($n = 6$)	Day-to-day ($n = 5$)
Lysozyme	1.2	2.0	1.4	1.8	4.1	1.2	1.6
Cytochrome *c*	1.4	2.3	1.4	2.0	5.2	1.6	2.2
Chymotrypsinogen A	2.7	3.6	3.2	3.8	14.3	2.5	3.4
Ribonuclease A	1.8	2.4	2.6	3.2	21.8	2.0	2.6

Capillaries, 66 cm (44 cm to detector) × 50 μm I.D.; 0.07 *M* phosphate buffer (pH 5); applied voltage, 19 kV; n = number of determinations.

hydrolysed under basic conditions, the cross-linked layer, which has an infusible three-dimensional network structure, can prevent the electrolytes from attaching these bonds in sublayers. In general, the denser and more homogeneous the cross-linked layer, the more stable and chemically resistant the coatings will be. Table 1 gives some efficiency parameters of three coated capillaries after immersion in 0.05 *M* sodium phosphate (pH 9.0) and 0.01 *M* NaOH (pH 12) for 48 h. The ER-I coating was stable in 0.05 *M* sodium phosphate (pH 9.0) but fully destroyed in 0.01 *M* NaOH, which indicated this coating was still not stable enough under basic conditions. The ER-II coating, which has a denser and more uniform cross-linked layer, was even stable at pH 12 (Table 3). Only slight changes in performance and migration times were observed after the capillary had been immersed in 0.01 *M* NaOH for 48 h. Therefore, 0.01 *M* NaOH can be used to release adsorbed solutes from the capillaries. It should be noted that the coating will generate a slight surface dullness after immersion in NaOH solution; consequently, the EOF will maintain its value at pH 12 and cannot revert to its normal value for at least 24 h on changing to separation buffer in the capillary channel. Although the EP coating had a tendency to adsorb basic proteins above pH 5.0, it was stable over the pH range 2–12, as no change in performance was observed after the capillaries had been immersed in basic electrolytes (Table 1). This is probably because the flexibility and chemical resistance of EP coatings were improved by the polysulphides, thus resulting in excellent stability towards basic electrolytes.

4. Conclusions

Capillaries coated with epoxy resins can produce a stable, hydrophilic cross-linked layer. It was found that the coating characteristics were greatly dependent on the cross-linking agents. The best results in terms of efficiency, resolution and stability were obtained when aromatic amines were used as cross-linkers. Protein adsorption were greatly suppressed while the EOF changed from anodic to cathodic over the pH range 2–12 and remained constant at pH 6–10. High resolution, separation efficiency and reproducibility can be achieved over a wide pH range (3–10). Epoxy and polysulphide copolymer coatings with lower hydrophilicity can provide the highest separation efficiencies in the pH range 3–5. Above pH 5, substantial protein adsorption still existed, but the coating was stable over the pH range 2–11.

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