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Poly(ethylene–propylene glycol)-modified fused-silica columns for capillary electrophoresis using epoxy resin as intermediate coating

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Abstract

An epoxy resin was used for fused-silica capillary column pretreatment before coating with a hydrophilic polymer for capillary electrophoresis. The unique properties of the epoxy resins allow the deposition of a dense polymer layer on the column surface using the dynamic coating method. By using a free-radical cross-linking initiator, a hydrophilic polymer coating can be subsequently immobilized over the resin covered surface. The coating is of sufficient thickness and hydrophilicity to reduce protein adsorption and minimize electro-osmotic flow. Basic protein separations were achieved with high efficiency and reproducibility; one thousand consecutive runs on a single column gave an R.S.D. value of less than 2% in migration time. The coating was stable throughout the pH range 4–11. The coating provides a simple, stable, and reproducible means for deactivation of fused-silica columns for capillary electrophoresis.

Keywords: Capillary columns; Proteins; Ribonucleotides; Epoxy resins; Poly(ethylene–propylene glycol)

1. Introduction

Chemical modification of the fused-silica capillary surface is desirable to control the electro-osmotic flow (EOF) and to minimize the interaction of analyte molecules with the capillary walls in capillary electrophoresis (CE). Numerous approaches have been taken to achieve this goal. Mainly, these attempts fall into two categories: first is the use of ionic buffer additives, including extremes in high and low pH [1–11]; the second involves permanently coating the capillary wall with a material to which the analytes do not adsorb [1–24]. The latter approach represents the most successful and versatile method for avoidance of analyte adsorption, particularly for protein separations.

Among the efforts to achieve highly efficient and reproducible separations of biopolymers in CE using permanent coatings, polymethylvinylsiloxanediol has been bonded directly onto the column surface using the polymeric OH groups [23]. The other end of the polymer chain was covalently bonded to linear polyacrylamide, which in turn was bonded to the column surface. Then the polyacrylamide was immobilized using an alkaline formaldehyde solution. Without any further thermal treatment, the column prepared in this manner exhibited excellent reproducibility and stability for protein separations under acidic and basic conditions. Another example using the dynamic coating method involved poly(vinyl-alcohol) (PVA) coatings [24] which were used for protein separations on physically adsorbed or thermally fixed polymer layers on the fused-silica surface. The coating procedure involved no silanization

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step and was quite simple. In order to obtain good separations with the adsorbed coatings, the buffer pH must be below 4, and low concentrations of PVA additives must be introduced into the buffer. For the permanent coatings, high efficiencies were obtained for many separations. The lifetimes of the bonded columns were reported to be approximately 40 injections at a pH of 8.5. A combination of different coating procedures such as silanization and dynamic adsorption of nonionic surfactants for wall modification have been successfully applied as well [19]. Good protein recovery percentages were obtained with these columns. In general, these columns have shown superior performance over uncoated capillaries in terms of efficiency and reproducibility. However, in all of these column preparation procedures, either a series of chemical modifications or static coating methods were involved. Therefore, relatively long column preparation times were needed.

This work describes a new method for fast column preparation. It is based on the use of an epoxy resin to form a stable intermediate coating between the fused-silica surface and a cross-linked hydrophilic polymer top layer. The outstanding adhesive-bonding properties of epoxy resins were first recognized in 1944 (see Ref. [25]). Since the commercial introduction of epoxy resins, its use has been primarily for the production of adhesives. Epoxy resin adhesives feature a wide range of chemical functionality and remarkably low shrinkage on curing which have led to adhesive joints with low internal stress. A wide range of adhesive properties can be obtained, including but not limited to toughness, chemical resistance, high adhesive strength, excellent heat resistance, high electrical resistance and extreme mechanical properties. These properties depend on the chemical structure of the curing agent and the conditions at which the curing is performed. As a result of this versatility, these products have found use in protective coatings, adhesives for most substrates, body solders, caulking compounds, flooring, tooling compounds for molds, low-pressure molding resins, textiles and fibre-reinforced plastics. These interesting properties of epoxy resins have led us to explore the use of such materials for CE column preparation.

2. Experimental

2.1. Apparatus and electrophoresis conditions

A Dionex Model CES-1 CE system (Dionex, Sunnyvale, CA, USA) was used for all separations. Sample injection was performed by electromigration. Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 50 μm I.D.×360 μm O.D. were used with a total length varying between 45 and 60 cm. The capillaries were filled with the running buffer when not in use. Between runs the columns were rinsed with the separation buffer for 2 min. Analytes were detected with on-column UV absorbance at 215 nm for proteins and 260 nm for ribonucleotides. Data were collected by using a Dionex Advanced Computer Interface with a Model SP 4270 integrator (Spectra-Physics, San Jose, CA, USA).

2.2. Reagents and materials

Basic proteins, acidic proteins and Tris–HCl were obtained from Sigma (St. Louis, MO, USA); Ucon 75-H-900000 was obtained from Alltech (Deerfield, IL, USA); dicumyl peroxide (DCP) was obtained from Aldrich (Milwaukee, WI, USA); epoxy resin was obtained from Conap (Olean, NY, USA); and deionized water, which was used to prepare the buffer solutions and rinse the columns, was prepared from a Milli-Q water system (Millipore, Milford, MA, USA). All buffers were filtered through a 0.45-μm cellulose acetate membrane (Corning, Corning, NY, USA) and degassed before use.

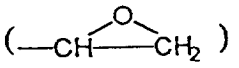
2.3. Capillary preparation

Approximately 1 ml of 0.5% (w/w) epoxy resin/methylene chloride solution was flushed through the column at a pressure of 100 p.s.i. (1 p.s.i.=6894.76 Pa) for 5 min, followed by nitrogen gas evaporation at 50 p.s.i. for 5 min. In the meantime, the epoxy resin proceeded to cure rapidly. A 4-ml methylene chloride coating solution containing 30 mg Ucon and 5 mg DCP was then forced through the column at a pressure of 150 p.s.i. for 5 min. The pressure was then released and the column was allowed to gradu-

ally empty. With a thin layer of coating deposited on the column surface, the column was heated from 40°C to final temperatures of 120, 160, 200, 220 and 240°C at 2°C min^{-1} and held at the final temperature for 45 min under a gentle nitrogen gas flow.

3. Results and discussion

3.1. Epoxy resin



Scheme 1. Epoxide functionality.

Epoxy resins are characterized by the presence of an epoxide functionality (see Scheme 1). The most widely used epoxy resins are the reaction products of epichlorohydrin and bisphenol-A. An idealized structure is shown in Fig. 1. In addition to the bisphenol-A base resins, there are several other types of epoxy resins of commercial significance, namely, the aliphatic and cycloaliphatic epoxy resins, the glycidated movolacs, and the tetraglycidyl ether of tetraphenylolethane. The latter two types of materials, having a high aromatic ring content combined with polyfunctionality, provide increased thermal stability for high-temperature applications. Epoxy resins can be cured by a large variety of chemical compounds. Because of their versatility, epoxy systems could be tailored to a myriad of performance features; combinations of various resins, modifiers, curing agents and accelerators have succeeded in providing us with remarkable products.

Although the tools are readily available to analyze commercial resin systems, suppliers still insist on keeping formulations proprietary because it is the resin chemistry that ultimately determines the applicability of a material and the strength and durability of the finished product. Until recent years, the actual chemical composition of the epoxy system was unknown [25], not only because of the proprietary nature imposed by formulators, but also because complete analysis required the successful use and data correlation of many time-consuming and complicated chemical and rheological techniques. Although reaching a complete understanding and full usage of the epoxy system may be more difficult by far, this does not limit their practical use.

3.2. Surface modification

The use of an epoxy resin as a first treatment was selected to produce a chemically stable coating over the fused-silica surface and to improve the ease of attachment for further modifications. From a surface chemistry viewpoint, the types of bonding of the epoxy resin to the silica surface can include Van der Waals attractions, hydrogen-bonding, and ionic, covalent or coordination bonds. Thus, with these anchoring groups, no particular covalent bonding to the silica surface was considered. Cross-linking and bonding to the silica surface took place in one step at room temperature. A thin film of epoxy resin was formed on the walls. By subsequently flushing the column with the second polymer solution, the top layer, poly(ethylene–propylene glycol) (Ucon) [26], which has been characterized as a weakly hydrophilic polymer and highly resistant to protein adsorption, attached readily to the epoxy resin layer. Furthermore, the top layer was further immobilized by cross-linking through methyl groups on the polymer chains under thermal treatment, thus improving its resistance to subsequent removal from the capillary wall under CE running conditions.

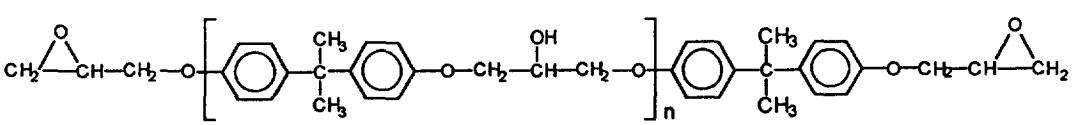


Fig. 1. Chemical structure of a typical epoxy resin.

3.3. Temperature control for immobilization

Immobilization of the polymer coating is necessary to generate a stable coating. It is known that the polymer coating becomes insoluble in water when the molecular mass increases sufficiently. In order to accomplish this, control of the cross-linking temperature is critical. The temperature should be high enough to initiate cross-linking and to maintain the subsequent polymerization without destroying the epoxy resin sublayer.

It was noted that when the column was coated only with the epoxy resin, separations of small molecules such as ribonucleotides were possible (Fig. 2A). However, the separation of proteins was unsuccessful because the epoxy resin contains a high percentage of benzyl groups which make it very hydrophobic. Bearing these facts in mind, a series of experiments were performed to find the optimized temperature for crosslinking the top layer.

Columns were prepared using cross-linking temperatures of 120, 160, 200, 220 and 240°C. They were then tested under alkaline buffer conditions for acidic protein separations. For the column cross-linked at 120°C, it was found that proteins only eluted in the first run. In order to confirm that this was due to the loss of the top layer rather than the epoxy resin sublayer, the same column was used for ribonucleotide separations. A typical electropherogram is shown in Fig. 2B. For comparison, the same sample mixture was analyzed on an untreated column (Fig. 2C). These results clearly indicate that high pH CE running conditions have little effect on the epoxy resin sublayer, and the loss of the top layer polymer network is responsible for the instability of the column coating under extreme pH running conditions. Therefore, a highly cross-linked dense polymer coating should improve the resistance to base hydrolysis.

All columns which were prepared in the same manner, but with different thermal treatment temperatures were examined under the same experimental conditions. A 50 m*M* borate buffer (pH 8.9) and a mixture of acidic proteins were used for column

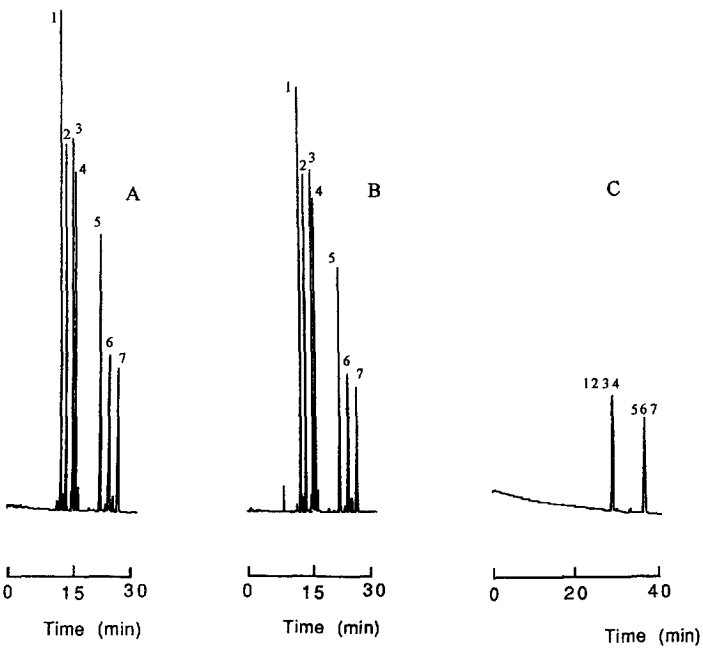


Fig. 2. Electropherograms of ribonucleotide separations using different capillary columns treated with (A) epoxy resin only, (B) Ucon/epoxy resin after being used under high pH conditions, and (C) untreated. Conditions: 50 cm×50 μm I.D. columns; 30 m*M* sodium potassium phosphate buffer (pH 6.0); 13 kV applied voltage; 260 nm detection. Peak identifications: (1) UTP, (2) ATP, (3) GTP, (4) CDP, (5) ADP, (6) GDP, (7) CMP.

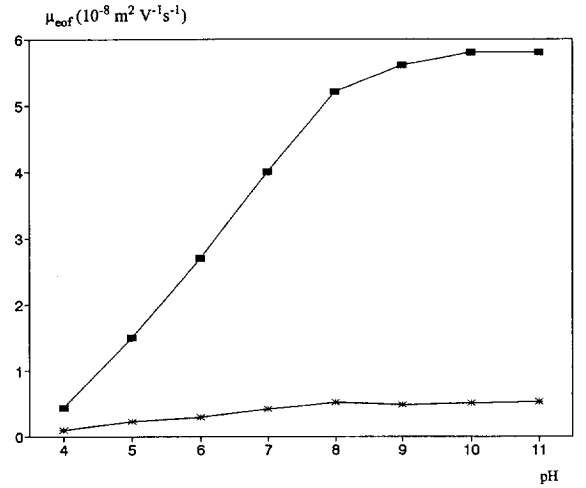


Fig. 3. Dependence of electro-osmotic flow on pH for (■) untreated and (*) Ucon/epoxy resin treated capillaries. Conditions: 45 cm×50 μm I.D. columns; 20 m*M* sodium potassium phosphate buffer; DMSO neutral marker; 215 nm detection.

testing. The column lifetime, which was represented by the effective run number for acidic protein separations, increased as the temperature of cross-linking increased from 120 to 220°C. However, columns prepared under 240°C cross-linking temperature were found to have lower efficiency and resolution. An optimum temperature of 220°C was determined from the above experiments.

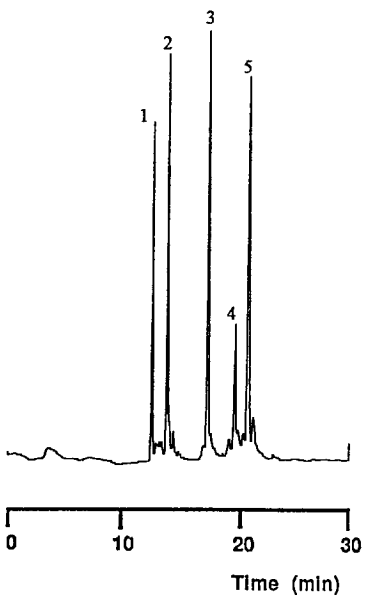


Fig. 4. Electropherogram of a basic protein separation on a Ucon/epoxy resin treated capillary. Conditions: 50 cm×50 μm I.D. column; 50 m*M* Tris–HCl buffer (pH 4.8); 215 nm detection. Peak identifications: (1) cytochrome *c*, (2) lysozyme, (3) ribonuclease A, (4) trypsinogen, (5) α-chymotrypsinogen A.

3.4. Electro-osmotic flow

The rate of EOF is dependent on the magnitude of the ζ potential across the interface, which is dependent on the charge density at the capillary wall. Thus, an increase in silanol ionization at higher pH would result in an increase in EOF. Fig. 3 illustrates the characteristic dependency of the EOF on pH for both uncoated and coated EOF capillaries over the pH range 4 to 11. There is a more than 10-fold increase in EOF when an uncoated capillary is used over this pH range, while there is little increase in EOF for the coated capillaries over the same pH range. Therefore, a favorable pH can be employed to give the optimum selectivity for a particular application without adversely affecting the analysis time.

3.5. Basic protein separations

An electropherogram of five basic proteins is shown in Fig. 4. The relative standard deviations (R.S.D.) of migration times and average theoretical plate numbers are listed in Table 1. They were determined from 1000 runs during a 2-month time period (examples shown in Fig. 5). Calculations were performed by first calculating a series of average migration times for run numbers from 1 to 100, 300 to 400, 600 to 700 and 900 to 1000. Thus, $x=(x_{1-100}+x_{300-400}+x_{600-700}+x_{900-1\,000})/4$. The standard deviation was calculated using $\sigma=(\Sigma\sigma_i^2)^{1/2}$. The R.S.D. values listed in Table 1 were obtained

Table 1
Reproducibility of migration times for consecutive runs of basic proteins[a]

Proteins	Efficiency ($\times 10^{-5}$ plates m^{-1})	t_m^b	R.S.D. (%)
Cytochrome *c*	3.56	11.83	1.45
Lysozyme	3.38	13.25	1.49
Ribonuclease A	3.14	16.51	1.55

[a] For conditions: see the legend to Fig. 4.
[b] Detailed calculations in text.

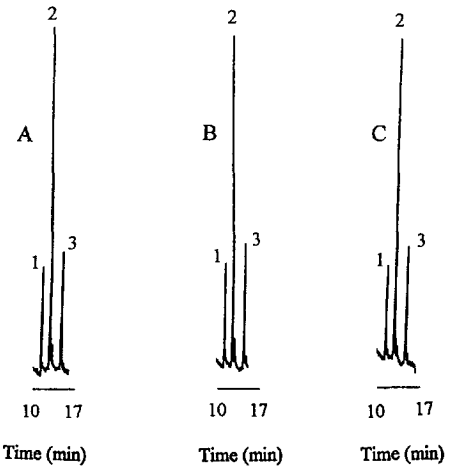


Fig. 5. Electropherograms obtained for three different runs during the lifetime study. (A) Run No. 1, (B) Run No. 500 and (C) Run No. 1000. Conditions: 60 cm×50 μm I.D. column; 50 m*M* Tris–HCl buffer (pH 4.8); 215 nm detection. Peak identifications: (1) cytochrome *c*, (2) lysozyme, (3) ribonuclease A.

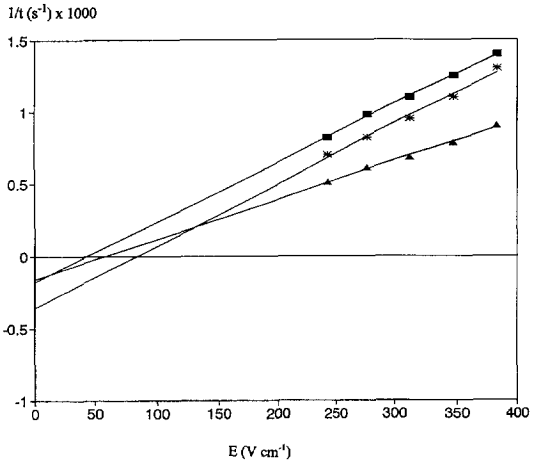


Fig. 6. Variation of $1/t$ vs. electric field strength (E). Conditions: 55 cm×50 μm I.D.; 50 m*M* Tris–HCl buffer (pH 4.8); 215 nm detection. Selected proteins: (■) cytochrome *c*, (*) lysozyme, (▲) ribonuclease A.

from the average of the different sets. These small R.S.D. values indicate that there was no apparent adsorption of solutes onto the column surface or leaching of coating into the buffer solution.

The batch-to-batch reproducibility of the coating was evaluated from four batches, each batch consisting of three columns coated in the same manner. The columns were evaluated using the basic protein test mixture. The R.S.D. values, migration times and efficiencies are given in Table 2. For 10 out of 12 columns, the R.S.D. values for migration times were less than 2%, and the R.S.D. values for efficiency

Table 2
Reproducibility of column preparation in terms of column efficiency and protein migration time[a]

Column No.	Efficiency ($\times 10^{-5}$ plates m^{-1})			Migration time (t_m) (min)		
	Cytochrome *c*	Lysozyme	Ribonuclease A	Cytochrome *c*	Lysozyme	Ribonuclease A
1	4.3	4.2	3.9	11.95	13.01	16.61
2	4.0	3.8	2.3	11.85	12.90	16.42
3	4.8	4.6	3.8	12.07	12.91	16.87
4	3.2	2.8	1.9	11.78	12.58	16.34
5	3.8	3.9	2.8	11.72	12.26	16.46
6	4.6	4.8	3.9	11.75	12.78	16.38
7	4.1	4.0	3.7	11.61	12.80	16.40
8	2.9	2.7	1.8	12.01	12.82	16.55
9	4.3	4.1	3.5	11.83	12.84	15.54
10	4.1	3.6	2.9	11.51	12.98	16.67
11	4.0	3.6	2.9	12.03	12.99	16.79
12	3.9	4.1	3.4	12.05	13.00	16.66
$\bar{x}$	4.0	3.85	3.10	11.85	12.81	16.47
σ_{n-1}	0.53	0.62	0.60	0.18	0.21	0.30
R.S.D. (%)	13.2	16.2	19.4	1.5	1.7	1.8

[a] For conditions, see the legend to Fig. 4.

Table 3
Interaction strength of selected proteins with the Ucon/epoxy resin coated capillary surface[a]

Protein	pI[b]	M_r[b]	Intercept (A)	Slope (B)	Linear regression coefficient (r)
Cytochrome c	10.2	12 500	−0.18	0.004	0.999
Lysozyme	11.0	14 000	−0.35	0.004	0.996
Ribonuclease A	9.3	13 500	−0.16	0.003	0.995

[a] For conditions, see the legend to Fig. 3, except for the actual applied voltage.
[b] Data were obtained from Ref. [19].

were less than 20%, confirming the high reproducibility of the coating method.

3.6. Surface charge characterization

One of the major factors causing degradation in protein separation efficiency and reproducibility in CE is coulombic interaction which results from the negative charges on the capillary walls [7]. The effect of these charges from silanol groups are markedly reduced by a neutral hydrophilic polymer coating. Here, we use a simple model developed by Cifuentes et al. [27] to quantify the interactions between proteins and the capillary wall:

$$\frac{1}{t} = \frac{\mu_p}{l} E - \frac{\mu_p Q_c}{l \epsilon x^2}$$

where t is the time needed for solutes to move a distance l from injection to detection, μ_p is the electrophoretic mobility of the solute, E is the electric field strength on the capillary, Q_c is the electrical surface charge on the capillary, ε is the dielectric constant of the buffer and x is the average distance between the surface and the particle. From this equation, a straight line of $1/t$ vs. E would be expected to give information about the surface charge of the capillary from the intercept. Three proteins were chosen to test the column according to this model. The $1/t$ vs. E curves in Fig. 6 show three straight lines. The intercepts, slopes, and linear regression coefficients for these lines are given in Table 3. There is a striking correlation between the pI values and intercepts of proteins. In particular, lysozyme has the highest pI value, thus it has the largest absolute value for its intercept. This suggests simply that the carriers of more electrical charge have stronger electrostatic interaction with the wall. The elution order of the proteins results from their charge to mass ratios. The results obtained here also coincide with what were obtained in McManigill and Swedberg's studies [28], in which they borrowed the concept of capacity factor (k') from chromatography. The lowest efficiency for lysozyme, which resulted from the strongest interaction with the wall, was also characterized by having the highest capacity factor among the three selected proteins.

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