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Capillary zone electrophoresis with fluid-impervious polymer tubing inside a fused-silica capillary

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Abstract

A fused-silica capillary was used as a mold in the in situ formation of, and as a sheath for, a highly crosslinked poly(styrene–divinylbenzene) inner tubing having 3–5 μm wall thickness. This 'tube-in-the-tube' construction with the thin-walled, fluid-impervious polymer inner tubing precluded contact between the aqueous buffer solution and the inner wall of the fused-silica capillary. As a result, this structure withstood long-term treatment with highly alkaline solutions without deterioration. In order to hydrophilize the inner surface of the polymer tube, a polyoxyethylene oligomer was grafted to its inner wall and subsequently crosslinked. The inner tube with such hydrophilic coating was also stable to hydrolytic attack by 1 *M* NaOH. Although the nonpolar polymeric inner surface generated electrosmotic flow as if it had some fixed negative charges, the flow velocity became almost negligibly small once it had been hydrophilized. As illustrated by electropherograms, the hydrophilization of the polymeric inner tube greatly facilitated the CZE of basic proteins in the pH range from 3 to 6 without the need for additives in the electrophoretic medium to mask the silanol groups at the surface of quartz capillaries. © 1997 Elsevier Science B.V.

Keywords: Capillary columns; Tube-in-the-tube capillaries; Poly(styrene–divinylbenzene) capillaries; Coating, of silica capillaries; Fluid-impervious polymer inner tubes; Hydrophilic coating; Electrosmotic flow; Proteins

1. Introduction

In many applications of capillary zone electrophoresis (CZE), such as the separation of proteins under non-denaturing conditions, it is essential to prevent untoward effects arising from the interaction of positively charged sample molecules with the negatively charged inner wall of the fused-silica capillary [1]. To alleviate such undesired interactions, the use of buffer additives [2], modification of the fused-silica capillary inner surface [3], and employment of plastic capillaries [4] have been proposed so far.

Among the above methods, the use of additives which preferentially interact with the capillary inner wall and thus mask the silanol groups has widest currency. This approach resembles the methods employed in HPLC to attenuate silanophilic interactions [5]. In the CZE of proteins, certain amino compounds appear to be the most effective buffer additives [6–11]. However, the additive may change the buffer properties, interact with the analytes or can cause problems with detection by UV or MS.

The second method entails the grafting of appro-

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priate hydrophilic functions to the silica surface via siloxane bridges [12–19]. Among others, polyacrylamide [16,20], hydroxylated polyether [18], polyvinylmethylsiloxanediol–polyacrylamide [21], and polyacryloylaminoethoxyethanol [22] have been described in the literature for masking the silanol groups at tube inner surface. The large number of publications on this subject notwithstanding, none of methods yields a coating that withstands treatment with caustic NaOH solution, often needed to clean the column in protein CZE.

Masking of the surface silanol groups was also brought about by depositing a water-soluble polymer of relatively high molecular mass to form a neutral and hydrophilic layer that strongly adheres to the capillary inner wall. Indeed coating the inner wall of fused-silica capillaries by poly(vinyl alcohol) [23], polyarginine [24], cellulose acetate [25], or poly(ethylene–propylene glycol) [26], did facilitate protein separation by CZE with high efficiency. Whereas such non-covalently bound 'skin coating' appears to offer a more effective silanol masking than the grafting method, the hydrolytic stability of such layers is generally not sufficient to withstand washing the column with strongly alkaline solutions. In most cases, capillaries coated by this method are limited to operation at pH lower than 8 [25].

The third approach [4,27] employs plastics instead of fused-silica capillaries. The surface of the plastics employed is not expected to be have fixed ionogenic groups and thus to generate electrosmotic flow (EOF) in a certain pH range of the background electrolyte. On the other hand, most plastic capillaries are not transparent to low-wavelength UV light to be used with on-column detection, and may enter into hydrophobic interactions with the analytes.

The goal of this work is to prepare capillary columns that combine the advantages of polymeric and silica capillaries by making in situ a fluid-impervious polymeric inner tube in a fused-silica capillary, conveniently called 'tube-in-the-tube' (TITT) as illustrated in Fig. 1, and thus preclude contact between the silica surface and the background electrolyte. The application of these capillaries with a hydrophilized inner surface was investigated in the CZE of proteins.

2. Experimental

2.1. Materials

Fused-silica capillary tubing with a polyimide outer coating, having 75 and 50 μm I.D. (375 μm O.D.) were purchased from Quadrex (New Haven, CT, USA). γ-(Trimethoxysilyl)propyl methacrylate was purchased from Polysciences (Warrington, PA, USA), 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) and ethylenediamine (99+%) from Aldrich (Milwaukee, WI, USA), monobasic, dibasic and tribasic sodium phosphate and dimethylformamide (DMF) (99.9%) from J.T. Baker (Phillipsburg, NJ, USA), styrene from Fluka (Ronkonkoma, NY, USA), divinylbenzene (DVB) (85%) and vinylbenzyl chloride (VBC) from Dow (Midland, MI, USA), azobisisobutyronitrile (AIBN) (98%) from Pfaltz & Bauer (Waterbury, CT, USA). Hypol PreMA G60 prepolymer was a gift from Hampshire Chemical Co. (Lexington, MA, USA). Lysozyme (chicken egg-white), cytochrome *c* (horse heart), ribonuclease A (bovine pancreas), and α-chymotrypsinogen A (bovine pancreas) were purchased from Sigma (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO), was purchased from Burdick & Jackson (Muskegon, MI, USA). Phosphoric acid (85%), hydrochloric acid (37%), hydrofluoric acid (48%), and sulfuric acid (98%) were all of analytical reagent grade from Mallinckrodt (Paris, KY, USA). Methanol, acetone and methylene chloride were of HPLC grade and purchased from Fisher (Fair Lawn, NJ, USA). Water was purified and deionized with a NANOpure system (Barnstead, Boston, MA, USA). Styrene and DVB were washed with 10% aqueous sodium hydroxide to remove the inhibitors before use. The other materials were used without further purification.

2.2. Apparatus and electrophoresis

Experiments were performed using a Model P/ACE 2200 capillary electrophoresis unit (Beckman, Fullerton, CA, USA) equipped with a UV-detection system and controlled by a NEC personal computer PowerMate SX/20 with Beckman System Gold software.

The total length of the fused-silica capillaries with

an inner tube made of polystyrene crosslinked with divinylbenzene (PS–DVB) was 37 or 47 cm. The window for on-column detection was made by burning off a small (3–5 mm) section of both the polyimide outer coating and the inner polymer coating with an Archer Torch Model B, a microtorch fueled with butane (Radio Shack, New Haven, CT, USA), while the tube lumen was purged with oxygen at 200 kPa. The capillaries were exactly cut to have an effective length (from the inlet to the window) of 30 or 40 cm.

In all experiments, 30 m*M* aqueous phosphate buffers were used. The protein sample contained 0.6 mg/ml cytochrome *c*, 0.4 mg/ml lysozyme, 1.0 mg/ml ribonuclease A and 0.5 mg/ml α-chymotrypsinogen A in deionized water and was injected at 0.5 p.s.i. for 1 s (1 p.s.i.=6894.76 Pa). Between runs the column was rinsed with 0.1 *M* HCl for 1 min and then with the background electrolyte for 3 min at 20 p.s.i. inlet pressure. The proteins and DMSO, used as the inert marker for EOF measurement, were detected at 214 nm. All experiments were carried out at room temperature in the range from 22 to 25°C.

2.3. Preparation of PS–DVB inner tube

2.3.1. Pretreatment

Fused-silica capillaries of 50 or 75 μm I.D. and 2 m in length were washed and filled with 1.0 *M* NaOH. The capillary ends were sealed with a butane flame produced by a Veriflo Air-Gas Torch (Macalaster Bicknell, New Haven, CT, USA). The tube was heated at 100°C for 2 h in the oven of a Sigma 2000 gas chromatograph (Perkin-Elmer, Norwalk, CT, USA) and thereafter was washed with deionized water for 30 min and then with acetone for 10 min. Subsequently the capillary was placed again in the oven at 120°C and purged with nitrogen for 1 h to remove residual water.

2.3.2. Silanization

A solution containing 50% (v/v) γ-(trimethoxysilyl)propyl methacrylate and 0.01% (w/v) DPPH in DMF was prepared, deaerated with helium for 15 min and filled into the pretreated capillary. Then after both ends were sealed it was placed in the oven at 120°C for 6 h. The capillary was then taken out and washed extensively with DMF, methanol and methylene chloride, and was blown dry with nitrogen.

2.3.3. In situ polymerization

A solution containing 10% (v/v) of styrene, 3–5% (v/v) DVB and 0.5 mg/ml of the initiator AIBN was filtered through a cellulose membrane having 0.22-μm pores (Millipore, Bedford, MA, USA). The solution was first purged with helium for 15 min, then filled into the silanized capillary. After both ends of the capillary were sealed, it was heated at 55°C for 16 h during which a 1–2 μm thick polymer annulus formed inside the capillary tube. Then, the residual solution of an oligomeric mixture was displaced by nitrogen, the capillary was washed with acetone and blown dry with nitrogen. The capillary was cut in two 90-cm long pieces for the subsequent treatment.

2.3.4. Dynamic coating [28]

A liquid prepolymer of styrene and DVB (1:1) was prepared by heating a mixture of 0.5 ml of styrene, 0.5 ml of DVB and 2 mg of AIBN in a 5-ml capped glass vial at 70°C for 3–7 min under nitrogen, and then rapidly cooled to room temperature. The mixture was filtered through a cellulose membrane filter having 0.22-μm pores as described above. The prepolymer was used to deposit dynamically a layer to form in situ the polymeric inner tube in the 90-cm long capillary by heating it at 75°C for 1 h with nitrogen purge. The coating procedure was repeated several times to obtain a polymer inner tube of 3–5 μm wall thickness. After the last treatment the tube was heated at 75°C overnight with nitrogen purge.

2.4. Hydrophilization of the PS–DVB inner wall

After the preparation of the PS–DVB inner tube described above its inner wall was dynamically coated with a mixture of VBC and DVB (75:25) containing 3 mg/ml of AIBN, and subsequently the capillary was heated at 75°C for 12 h with nitrogen purge. In this way the inner surface of the capillary was functionalized with chloromethyl groups. Thereafter the capillary was filled with a 50% (v/v)

solution of ethylenediamine in methanol, its ends were sealed and it was placed in the oven at 70°C for 30 min. After cooling to room temperature the capillary was washed with 1.0 *M* aqueous NaOH, water and acetone. Subsequently the capillary with an abundance of primary and secondary amino groups at the inner surface was treated by passing through a 2% (v/v) solution of Hypol PreMA G60 in acetone for 1 h. After washing the tube with acetone for 10 min and water for 15 min, it was heated at 70°C for 30 min with the lumen filled with water and both ends sealed. After this treatment the capillary was washed with acetone and then rinsed with the Hypol PreMA G60 solution for 30 min. After washing the tube with acetone and water again, it was finally treated with an aqueous solution containing 0.5 *M* $NaNO_2$ in 0.5 *M* H_2SO_4 for 1 h and washed with water extensively. The reaction schemes involved in this treatment are shown in Fig. 2.

2.5. Preparation of specimens for scanning electron microscopy

The fused-silica capillaries with PS–DVB inner tubing were examined by using scanning electron microscopy (SEM) and a typical electron micrograph of the capillary cross-section is shown in Fig. 3. The capillary was first fractured and the specimens were prepared by two methods: (i) the fractured ends were cut into approx. 2-mm long pieces for SEM; (ii) the fractured ends were immersed in aqueous hydrofluoric acid (48% HF) for 10 min, washed subsequently with water and cut to a length of approx. 2 mm. All the specimens were coated with gold under vacuum before the SEM.

3. Results and discussion

3.1. Column preparation

Before the in situ copolymerization of styrene and DVB to deposit a PS–DVB inner tube, the inner wall of the fused-silica capillary was silanized for two reasons. First, the treatment facilitated wetting by the solution of the monomer mixture. Second, the covalently bound vinyl functions at the surface offered anchoring sites for the polymer. Although grafting of the polymer to the silica surface is not essential for the preparation of the polymeric inner tube, we found that its uniformity and mechanical strength were enhanced by the preceding silanization treatment. The inhibitor, DPPH, was used to prevent thermal polymerization of the bifunctional silanizing agent containing methacrylic groups during silanization at elevated temperature.

After forming a 1–2-μm thick annulus of the polymer, the wall thickness of the inner tubing was further increased by dynamic coating with a styrene–DVB prepolymer and subsequent polymerization in situ. This method for enhancing the mechanical properties of the inner tube resembles that used for coating glass or fused-silica capillaries for use in gas chromatography [28]. In this work, however, a liquid plug of the prepolymer was forced through the vertically held capillary by gravity and due to the outstanding wetting of primary inner tube a highly uniform annular film of the prepolymer was left behind. This treatment and the following polymerization steps were repeated two or three times till the thickness of the inner tube reached 3–5 μm, as estimated by microscopic observation.

It seems to be appropriate to regard the in situ-formed highly crosslinked PS–DVB annulus as 'inner tube' rather than 'coating', because it is indeed a fluid-impervious polymer tube on its own that is stable both hydrolytically and mechanically, and has a thickness far greater than the usual coatings. In other words, the fused-silica capillary serves just as a mold in the preparation step and as a sheath to protect the integrity of the polymer inner tubing. Fig. 3 shows a fractured section of the typical PS–DVB inner tube having a wall thickness of approximately 3 μm in a fused-silica capillary. The employment of the TITT concept illustrated in Fig. 1 has several advantages in controlling the properties of the capillary inner wall in CZE. By preventing any contact between the silica surface and the aqueous electrophoretic medium, the undesirable properties of the siliceous inner surface of the raw quartz capillaries cannot manifest themselves.

By using appropriate chemistries developed for the preparation of stationary phases for chromatography, the PS–DVB inner tubing can be functionalized by methods employed in the synthesis of ion exchangers

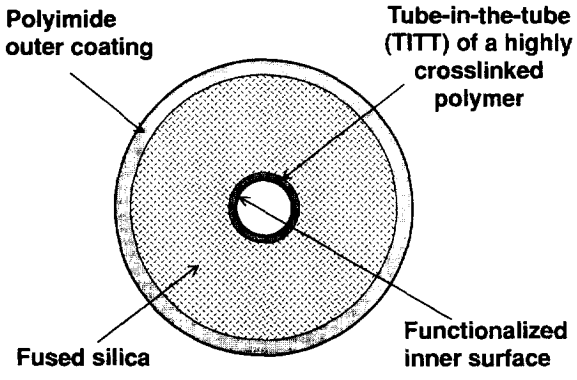

Fig. 1. Schematic illustration of the cross-section of a fused-silica capillary with a fluid-impervious polymeric inner tube representing the 'tube-in-the-tube' (TITT) concept.

[29] and chromatographic stationary phases for use in different applications [30–36]. For instance, the inner surface can have a high density of sulfonic acid or quaternary ammonium groups or a highly hydrophilic top layer with or without functional groups. In this work the PS–DVB inner surface was hydrophilized without any ionogenic or other type of functional groups for use of the capillaries in the CZE of proteins at close to neutral pH.

3.1.1. Hydrophilization

In order to render the surface of the PS–DVB inner tubing hydrophilic, first a thin film of poly(vinylbenzyl chloride) (PVBC) crosslinked with DVB was formed on the inner surface by the dynamic coating method [28]. The annular PVBC film adhered strongly to the PS–DVB support and could not be removed by methylene chloride, a very good solvent for the monomers. Furthermore, the crosslinked PVBC layer provided an abundance of chloromethyl groups at the surface to serve as anchoring groups for various functions having a nucleophilic reacting group. The chemical modification of the capillary inner surface by the chloromethyl functions to serve as the anchoring sites for the subsequent reaction steps is shown schematically in Fig. 2.

It is seen that first the chloromethyl groups are reacted with a large excess of ethylenediamine to anchor primary amino groups at the surface. After removal of the excess ethylenediamine, and the HCl formed in the reaction, by washing the tube with 1.0

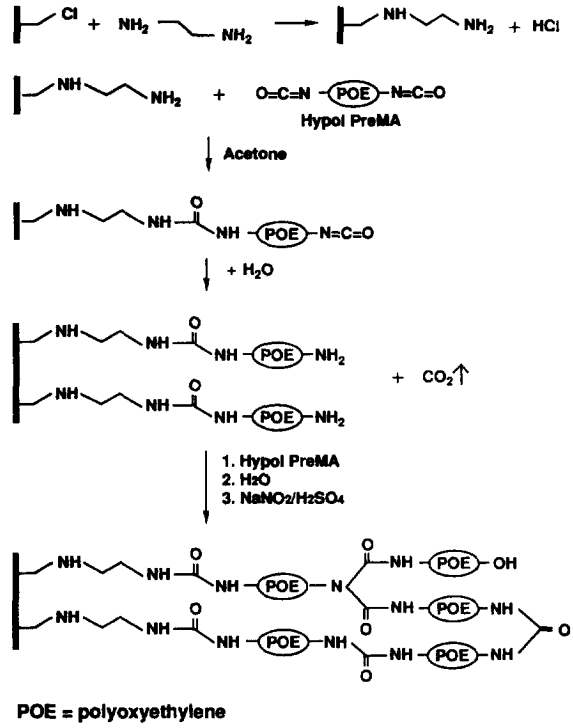

Fig. 2. Reaction scheme for the hydrophilic functionalization of the PS–DVB inner tube after it is coated with poly(vinylbenzyl chloride). The Hypol PreMA G series prepolymers used for hydrophilization contain polyoxyethylene (POE) chains and isocyanate functions. Its crosslinking with water and the conversion of the residual primary amino groups into hydroxyls result in a hydrogel layer of low protein adsorption.

M aqueous NaOH solution, water and acetone, the primary amino groups were reacted with Hypol PreMA G60, a hydrophilic prepolymer, that contains polyoxyethlene chains terminated with highly reactive isocyanate groups.

The crosslinking of the hydrophilic prepolymer is believed to occur in a reaction of the isocyanate functions with water via the unstable carbamic acid (not shown in Fig. 3), which decomposes to form primary amino groups and carbon dioxide [37], and finally by a reaction between the primary amino and the isocyanate groups. After the crosslinking polymerization is completed, the unreacted aliphatic primary amino groups were converted to hydroxyls by a treatment with sodium nitrite and sulfuric acid. Replacement of the residual ionogenic primary amino groups by hydroxyls renders the inner surface

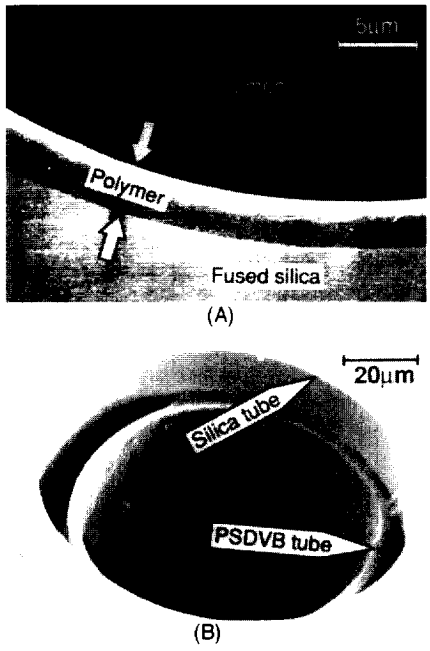

Fig. 3. Scanning electron micrographs of the fused-silica capillary with a PS–DVB inner tube formed by in situ polymerization. (A) A fractured section of the capillary showing the polymer inner tube; (B) the fractured section after etching away some fused silica with HF and thus revealing more of the tube in the tube.

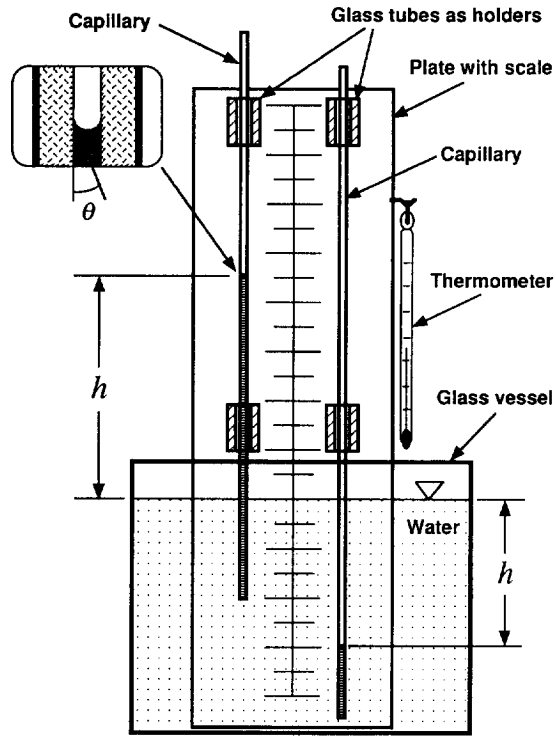

Fig. 4. Set-up for determining surface hydrophilicity by the capillary rise method with water. From the distance h the contact angle θ is calculated by Eq. (1).

of the capillary column more inert and more hydrophilic.

3.1.2. Wetting properties

The wetting of the capillary inner wall by water was measured at different stages of the treatment by using the capillary rise method [38] with the simple set-up shown in Fig. 4. The wetting test allows the evaluation of the contact angle, θ, from the capillary rise, h, by using the following equation

$$\cos\theta = (1/4)hd(\rho - \rho_v)g/\gamma \qquad (1)$$

where γ is the surface tension (dynes/cm), d the inner diameter of the capillary (cm), h the height of capillary rise (cm), i.e., the distance measured from the liquid level in the vessel to the meniscus in the capillary, ρ the density of the liquid (g/cm^3), ρ_v the density of the surrounding air saturated with the vapor of the liquid used (g/cm^3), and g is the acceleration of gravity (cm/s^2). It should be noted that the height, h, is negative when the meniscus in the capillary is lower than the liquid level in the vessel. This gives a contact angle greater than 90°.

In most cases we used water as the testing liquid because of our interest in the hydrophilicity of the capillary inner wall. Before each test of the capillary rise, the water in the vessel was degassed for 30 min by using a Model 8892-MTH 47 KHz sonicator (Cole-Parmer, Chicago, IL, USA). The position of the meniscus was measured at least 5 h after the lower end of the capillary was placed into the liquid in the vessel. This guaranteed that the meniscus reached its final position. For the calculation of the contact angle of water at 25°C by Eq. (1) the data were taken from the literature [39] as: $\rho = 0.997$ g/cm^3, $\rho_v = 1.1845 \times 10^{-3}$ g/cm^3, $\gamma = 71.97$ dynes/cm, and $g = 980.665$ cm/s^2. Typical results obtained with fused-silica capillaries after having received different treatments of the inner wall are listed in Table 1. It is seen that upon hydrophilization of the

Table 1
The rise of water meniscus and the contact angle of water in the capillary as measured at 25°C after major stages in the preparation of the 'tube in the tube' with a hydrophilic inner surface

Capillary	75 μm I.D.		50 μm I.D.	
	h (cm)	θ (°)	h (cm)	θ (°)
Fused silica[a]	36	24	54	24
Silanized[b]	10	75	15	75
PS–DVB[c]	−21	120	−33	120
Aminated[d]	20	62	32	61
Hydrophilized[e]	26	52	40	52

[a]Fused-silica capillaries rinsed with 1.0 *M* aqueous NaOH for 30 min and then washed with water and dried with N_2 at 120°C for 1 h.
[b]With γ-(trimethoxysilyl)propyl methacrylate.
[c]Approximately 2.5-μm thick styrenic inner tube after washing with methanol, acetone and methylene chloride and blowing dry with N_2.
[d]Chloromethylated surface reacted with ethylenediamine.
[e]Reacted with 2% (v/v) Hypol PreMA G60 prepolymer in acetone, as described in the text, and thoroughly washed with water.

PS–DVB surface, the contact angle of water was reduced from 120 to 52°. This is still higher than the contact angle of about 24° for water in uncoated fused-silica capillaries. By the use of a hydrophilizing agent more hydrophilic than the Hypol PreMA prepolymer the contact angle can be further reduced (unpublished results) nevertheless as we see later the treatment used here already gave satisfactory results in protein CZE. It should be noted that the hydrophilized capillary should be stored filled with an aqueous buffer containing glycerol.

3.2. Column evaluation

3.2.1. Separation of basic proteins

In the CZE of proteins wall adsorption, particularly that of basic proteins, has been a main impediment as discussed above, together with various approaches to enhance the separation efficiency when raw fused-silica capillaries are employed. We tested the usefulness of our approach employing capillary columns with a hydrophilized PS–DVB inner tube in protein CZE by the separation of four basic standard proteins: cytochrome *c*, lysozyme, ribonuclease A and α-chymotrypsinogen A. Fig. 5 shows typical electropherograms obtained at pH 4.5 and 6.0 with the protein mixture by using the

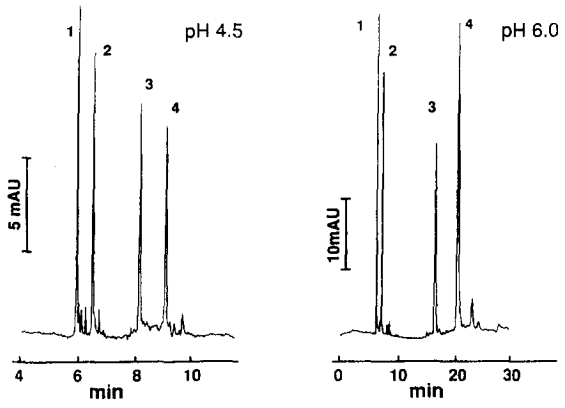

Fig. 5. Electropherograms of basic proteins obtained by using fused-silica capillaries with a fluid-impervious polymer inner tube having a hydrophilized inner surface. Conditions: fused-silica capillary, 30/37 cm×75 μm I.D.; thickness of the PS–DVB annulus plus the hydrophilized PVBC layer, 3.5 μm; 30 m*M* aqueous phosphate buffer, pH 4.5 and 6.0; applied voltage, 12 kV at pH 4.5 and 18 kV at pH 6.0; UV detection at 214 nm.

capillary with the hydrophilized inner surface. It is seen that even at pH 6.0 satisfactory separation can be obtained, albeit the plate efficiency drops significantly upon increasing the pH from 3.0 to 6.0, as seen from the data listed in Table 2.

3.2.2. Electrosmotic flow test and stability studies

In order to gain information on the zeta potential and other characteristics that determine the magnitude of the EOF in the capillaries, DMSO was chosen as the neutral marker for EOF, as its mobility was the same as that of acrylamide and gave sharp peaks. The mobility of DMSO was measured over a

Table 2
Effect of the pH on the plate efficiencies in the CZE of basic proteins with a fused-silica capillary having a hydrophilized PS–DVB inner tubing

Protein	Efficiency (plates/m)		
	pH 3.0	pH 4.5	pH 6.0
Cytochrome *c*	323 000	368 000	329 000
Lysozyme	364 000	502 000	326 000
Ribonuclease A	351 000	461 000	169 000
α-Chymotrypsinogen A	310 000	532 000	223 000

Conditions: fused-silica capillary, 47/40 cm×75 μm I.D.; thickness of the PS–DVB annulus plus the hydrophilized PVBC layer, 3.5 μm; 30 m*M* aqueous phosphate buffer, pH 3.0, 4.5 and 6.0; applied voltage, 20 kV; UV detection at 214 nm.

wide pH range in fused-silica capillaries with a fluid-impervious polymeric inner tube before and after hydrophilization of the inner surface. For comparison the EOF velocity was also measured in the raw fused-silica capillary under similar conditions. The results in Fig. 6 show that the mobility of DMSO representing the magnitude of EOF per unit field strength is very low in the hydrophilized PS–DVB inner tube. This is the reason for conducting the experiments with the hydrophilized capillary at a field strength higher than that employed in the measurements of EOF in the other tubes.

The results show that the EOF was reduced over the whole pH range studied in capillaries containing the PS–DVB inner tube with respect to the EOF obtained with raw fused-silica capillaries under identical conditions. The very low EOF velocity in the capillary with the hydrophilized inner surface suggested that either the zeta potential at the capillary inner surface was practically nil in the pH range investigated or the high viscosity of the hydrogel layer aborted EOF [15]. The relatively high EOF at alkaline pH in the capillary with the PS–DVB inner tube is in agreement with results reported in the literature that at sufficiently high electric field there is EOF in plastic capillaries [4,27,40]. No satisfactory explanation has been put forward yet for this phenomenon, and we resort to the trivial hypothesis that certain charged components of the electrophoretic medium may be preferentially adsorbed on and thus impart 'fixed' charges to the PS–DVB inner surface, and the double layer so established engenders EOF when a strong electric field is imposed on the capillary.

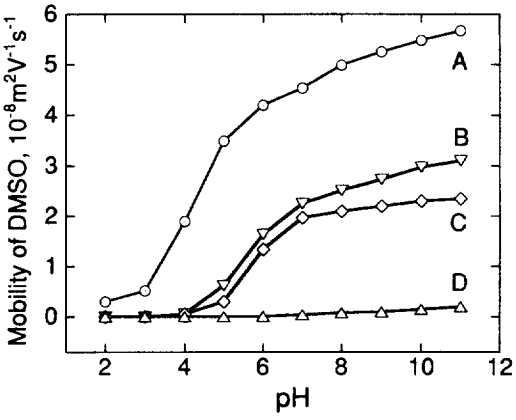

Fig. 6. Plots of the mobility of DMSO (neutral marker) for EOF measurement against the pH of the background electrolyte in various capillaries having different inner surface characteristics. (A) Raw fused silica; (B) fused silica with a fluid-impervious hydrophobic PS–DVB inner tube incubated with 1.0 *M* aqueous NaOH for 4 months at room temperature; (C) the same capillary before the long-term incubation; and (D) the same capillary, but with the PS–DVB inner tube hydrophilized by grafting with Hypol PreMA G60 prepolymer. Conditions: fused-silica capillary, 37/30 cm×75 μm I.D. (A); with PS–DVB inner tube of 3 μm wall thickness (B,C); with PS–DVB annulus plus the hydrophilized PVBC layer of 3.5 μm (D); 30 m*M* phosphate; pH 2.0–11.0; EOF marker, DMSO; applied voltage, 8 kV (12 kV for Hypol polymer-coated tube). EOF velocity was taken as zero when, in the pH range from 2 to 4, the electrophoretic migration time of DMSO in columns B, C and D was longer than 5 h.

The number of different commercially available coated fused-silica capillaries is rather small considering the coating technique galore described in the literature over the last few years. In most cases the major problem lies in the poor stability of the wall coating toward hydrolytic attack at high pH when the capillary is regenerated and cleaned by a strongly alkaline solution.

In this work, therefore, the stability of fused-silica capillaries with PS–DVB inner tubes was first tested by incubation with aqueous sodium hydroxide solution. A typical capillary having a PS–DVB inner tube of approximately 3 μm wall thickness was filled with 1.0 *M* NaOH and stored at room temperature for 4 months. After this treatment the capillary was rinsed with water, and upon drying the integrity of the polymeric inner tube was examined. Optical and scanning electron microscopy showed no eroding of, and no cracks in, the polymeric inner tube, and no gaps between the polymeric annulus and the fused silica, i.e., the PS–DVB inner tube was mechanically stable. As shown in Fig. 6 the EOF changed only slightly after 4 months of incubation with 1 *M* NaOH. The slight change in EOF is likely due to some degradation and oxidation of the polymer surface giving rise to anionogenic functions during the long-term incubation.

The results described above indicate that the PS–DVB inner tube serves indeed as a fluid-impervious barrier between the fused silica and a corrosive electrophoretic medium or cleaning agent. The long-term stability of the hydrophilized surface of the polymeric inner tube is under investigation. The

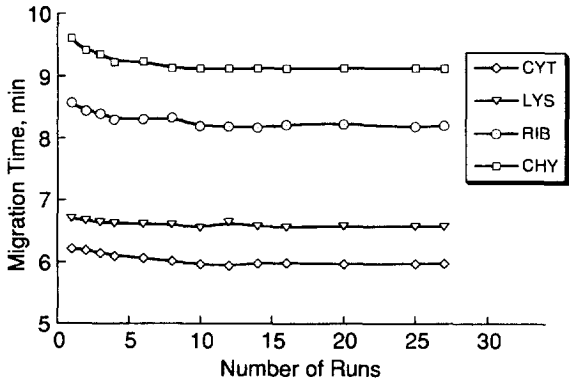

Fig. 7. Plots of the migration time of basic proteins in a fused-silica capillary with PS–DVB inner tube having a hydrophilized inner surface against the number of CZE runs with a 3-min long flushing of the column with 1.0 *M* aqueous NaOH between two consecutive runs. Fused-silica capillary, 37/30 cm×75 μm I.D.; wall thickness of the PS–DVB inner tube, 3 μm; 30 m*M* aqueous phosphate buffer, pH 4.5; applied voltage, 18 kV.

–C–O–C– bonds in the Hypol polymer due to its high polyoxyethylene content are known to be stable under alkaline conditions. The –C–N–C– bonds by which the hydrophilic Hypol polymer is grafted to the PS–DVB surfaces, as shown in Fig. 2, is also expected to be stable under alkaline conditions.

The stability of the hydrophilized inner surface of the inner tube was tested by making 27 consecutive runs to separate the four basic proteins at pH 4.5. Between each CZE run the capillary was flushed with 1.0 *M* NaOH for 3 min and then equilibrated with the running buffer. Fig. 7 shows plots of the migration time against the number of runs. It is seen that the migration times, apart from a slight drop at the beginning of the experiment, are about the same. This is in good agreement with the observation that the electrophoretic pattern, i.e. peak position, shape and sharpness, did not change in the course of these experiments. From this we can conclude that the column is stable even under such harsh conditions as the repeated exposure to 1.0 *M* NaOH. Although in separations of 'clean samples' the hydrophilic surfaces of the inner wall need possibly not be regenerated by a caustic solution, NaOH rinse is still an effective means to thoroughly cleanse the columns from time to time.

The run-to-run, day-to-day and column-to-column reproducibility of the migration times in the hydrophilized TITTs were also investigated at pH 4.5 and the results are shown in Table 3. A trend can be seen only in the slight decrease in the migration times in the experiments that were carried out in 3 consecutive days. The hydration of the gel layer at the surface may have increased with time and caused a concomitant reduction in protein adsorption.

4. Conclusions

It is shown that the formation of a highly cross-linked PS–DVB inner tube in a fused-silica capillary is a practicable solution for eliminating much of the problems arising from the peculiarities of its siliceous inner surface in protein CZE. Such a polymeric inner tube is fluid impervious, withstands contact with strong alkaline solutions, and is readily functionalized at the surface by the great variety of chemistries established for modification of polymeric

Table 3
Reproducibility of the migration times of proteins in a fused-silica capillary with hydrophilized polymeric inner tube

Protein	R.S.D. (%): run-to-run ($n=18$)	Migration times[a] (min)					
		Day			Column		
		1st	2nd	3rd	1st	2nd	3rd
Cytochrome *c*	0.31	3.80	3.78	3.66	3.80	3.72	3.67
Lysozyme	0.27	4.05	3.94	3.89	4.05	3.97	3.92
Ribonuclease A	0.36	5.13	5.09	5.07	5.13	5.14	5.11
α-Chymotrpsinogen A	0.61	5.69	5.67	5.63	5.70	5.75	5.71

Conditions: fused-silica capillary, 37/30 cm×50 μm I.D.; polymer layer thickness, approximately 2.5 μm for the polymeric inner tube; 30 m*M* phosphate buffer, pH 4.5; applied voltage, 20 kV.

[a]Mean value of five measurements for each day or each column.

surfaces. This approach employing such a composite capillary combines the advantages of the fused silica and highly crosslinked plastic materials of which surface is readily functionalized to meet the need of a particular CZE application. Due to the versatility of this procedure it can be used to prepare a large family of capillary columns that meet rigorously all the various requirements not only in CZE but also in capillary electrochromatography (CEC). In this work the polymer was hydrophilized by bonding and crosslinking a hydrophilic polymer at the surface for use in CZE, with the result of greatly reduced wall adsorption of basic proteins and enhanced stability against treatment with 1.0 *M* NaOH. Further work has shown (unpublished results) that by anchoring ionogenic functions in the hydrogel layer the scope of this approach can be further expanded.

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