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Non-bonded poly(ethylene oxide) polymer-coated column for protein separation by capillary electrophoresis

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Abstract

A simple method to coat a non-ionic hydrophilic polymer onto the inner wall of a bare fused-silica capillary is established. The capillary is first filled with 1 *M* HCl solution, then flushed with 0.2% poly(ethylene oxide) solution containing 0.1 *M* HCl, and finally rinsed with the electrophoretic buffer. Each step takes only 5 min. For regeneration of the coating, this procedure is repeated. Four basic proteins were separated by this method around pH 3–7 with phosphate buffer. The separation efficiency in terms of peak shape was excellent compared to bare fused-silica capillaries and comparable to many other more sophisticated column-treatment methods. The reproducibility of migration times of proteins was less than 3% R.S.D. at pH 6 by manual operation of the coating process. Titration of the fused-silica surface with acid and the structure of poly(ethylene oxide) are important factors to form a stable coating via hydrogen bonding to the surface silanol groups.

Keywords: Poly(ethylene oxide)-coated columns; Capillary columns; Proteins

1. Introduction

In the past decades, capillary electrophoresis (CE) has become one of the most powerful tools to analyze protein mixtures [1]. Proteins, however, often adsorb onto the inner surface of fused-silica capillaries (FSC), because of diverse interactions. Proteins have a wide variety of characteristics and functionality, which mainly originate from their high-order structure. Various forces such as hydrophobic interaction, coulombic interaction, hydrogen bonding, and van der Waals interaction are included [2]. Especially when basic proteins are analyzed, there exists a considerable amount of adsorption because silica normally are negatively charged above pH 3 [3]. This interacts with the positive sites of proteins and degrades the separation. Firstly, adsorption causes tailing of the sample peak, which leads to loss of resolution and sensitivity. Secondly, it will change the ζ-potential of the capillary which affects the electroosmotic flow (EOF) rate, giving improper migration times and poor reproducibility. Also non-uniform distribution of the ζ-potential caused by local adsorption of proteins causes additional peak broadening [4,5].

In order to avoid adsorption problems, the inner wall of FSC has been coated by various methods [5–28]. These can be categorized into two groups, i.e., covalent bonding and adsorption methods. So far, the former is most frequently used in the

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separation of proteins in CE. The strategy is to immobilize polymers [5,6,10–19] as well as small units [7–9] such as C_{18} groups. Hydrophilic polymers such as polyacrylamide [10–12], poly(ethylene glycol) [13–16], poly(ethylene–propylene glycol) [16], poly(vinylpyrrolidinone) [17], and epoxy polymer [18,19], polysaccharides [5,13,16] have been coated via bifunctional anchors such as 3-methacryloxypropyltrimethoxysilane [5,10,17], polyvinylmethylsiloxanediol [12], 3-glycidoxypropyltrimethoxysilane [14,15,18,19], 3-aminopropyltriethoxysilane [13], and Grignard reagent [11]. These methods, however, have some disadvantages. For example, the coating process usually consists of multistep reactions, which are quite difficult to control, laborious, and time consuming. Also, if the siloxane bonding is used to fix polymers, it gradually degrades due to hydrolysis in aqueous buffer media.

Comparatively, adsorption methods are less difficult for preparing coatings. In order to adsorb the coating material, the solution is introduced and passed through the capillary and/or is added in the running buffer. Normally polymers [20–25] or surfactants [26–28] are employed as coating materials because these are strongly adsorbed onto the silica surface by way of coulombic interaction, hydrogen bonding, or Van der Waals force. When cationic polymers [20–22] or surfactants [26,27] are used they produce excess positive charges on the surface and reverse EOF direction. Anionic species cannot be separated because they are attracted and adsorb onto the positive sites on the capillary wall. On the other hand, non-ionic surfactants below the critical micellar concentration can be added in the electrophoretic buffer. These are adsorbed onto a C_{18} bonded capillary to form the coating via hydrophobic interaction [28]. Similarly, pluronic polymers are adsorbed onto pre-derivatized capillaries with silylating agents [25]. Recently poly(vinyl alcohol) (PVA) is added to the electrophoretic buffer, which is used to coat FSC dynamically [23]. This coating scheme, however, can be used only at a pH less than 4. For separation at higher pH, thermal treatment was necessary to fix PVA permanently on the capillary. Very recently, cellulose acetate, which is not water soluble, is physically adhered onto FSC by passing through an acetone solution followed by drying with He gas [24]. This is a fairly simple method and can be applied for the separation of proteins below pH 7.5.

Recently, solutions of entangled linear polymers were demonstrated as effective new sieving materials in CE for biopolymers such as DNA and proteins [31,32]. Compared to conventional cross-linked polymers, e.g. polyacrylamide, there are some benefits in the utilization of non-cross-linked linear polymers, such as ease of preparation, replaceability of the sieving matrices, and higher reproducibility. We discovered that PEO as an effective sieving material for CE of DNA [29,30] has some advantages over linear polyacrylamide, i.e., higher separation performance, higher separation speed, and lower viscosity. Very recently, it has been revealed that bare FSC could be used to separate DNA fragments at high speed when PEO solution is used in a fresh capillary [30]. The separation performance is identical to that obtained by polyacrylamide-coated capillaries. There seems to be the spontaneous formation of a coating of PEO which considerably suppresses EOF. For repeated usage, on the other hand, this fresh state of the capillary surface is regenerated by titrating the inner wall with 0.1 *M* HCl [30]. In this case EOF seems to be completely suppressed, because the migration times for DNA fragments are even shorter than those obtained in fresh FSC. The adsorption of PEO on FSC surface seems to prevent the dissociation of silanol groups under higher pH. In this study, PEO is investigated as a coating material to prevent both adsorption of proteins and EOF. A simple way for making stable PEO via adsorption is developed. Basic proteins are employed as a test sample in order to evaluate the performance of the coating.

2. Experimental

2.1. Materials and equipment

Four basic proteins, lysozyme (chicken egg white), cytochrome *c* (bovine heart), ribonuclease A (bovine pancreas), and α-chymotrypsinogen A (bovine pancreas), were obtained from Sigma (St. Louis, MO, USA). PEO of M_r 8 000 000 was purchased from Aldrich (Milwaukee, WI, USA). Hydroxyethylcellulose (HEC, QP-100 MH) and hydroxypropylmethyl-

cellulose (HPMC, viscosity of 2% aqueous solution: ca. 50 cP at 25°C) were obtained from Union Carbide (Danbury, CT, USA) and Sigma, respectively. All other chemicals are of analytical-reagent grade. Deionized water was used throughout this study. Electrophoresis was run by an ISCO Model 3850 electropherograph (Lincoln, NE, USA) with fused-silica capillaries (effective length, 30 cm; total length, 50 cm, 75 μm I.D., 365 μm O.D., Polymicro Technologies, Phoenix, AZ, USA). Data collection and processing were accomplished with a DAS 801 A/D converter (sampling rate 1 Hz, Keithley Instruments, Taunton, MA, USA) and an IBM PC/AT compatible personal computer. Phosphoric acid was titrated by NaOH solution to adjust pH for the preparation of electrophoretic buffers. Protein sample solution was prepared by dissolving the four proteins in water at concentrations of approximately 0.2 mg/ml. The sample was injected by electromigration method at 15 or 10 kV for 3–5 s. Detection wavelength was set at 220 nm. Electrophoresis was run at 15 kV (unless indicated otherwise) and room temperature.

2.2. Capillary wall treatment

Prior to the first run of the day or of a new set of conditions, the inner capillary was washed with a mixture of 0.1 *M* NaOH and 0.1 *M* sodium dodecylsulphate solution and thoroughly rinsed with water. Coating solution was made by adding polymer powder into water being stirred vigorously in an Erlenmeyer flask and heated at 95°C in a water bath by using a magnetic stirrer and hot plate. After complete dissolution and cooling, concentrated HCl was added if necessary. For HEC solution, the heating process is omitted in order to avoid clouding. The composition of the coating material is 0.2 or 2.0% (w/v) polymer and 0, 0.1 or 1.0 *M* HCl in water. (Percentage for polymer concentration is w/v.)

Typical coating protocol is as follows: the solution of 1.0 *M* HCl was forced to flow into the bare silica capillary by a syringe, then followed by the coating solution containing the polymer. Finally, the capillary was flushed with the electrophoretic buffer. Each step took 5–15 min. This procedure was repeated before the next run for protein separation.

3. Results and discussion

3.1. Establishment of coating protocol

Fig. 1 shows the electropherograms produced by various capillary treatment protocols. As shown, an untreated bare silica capillary, which has been washed with a NaOH–SDS mixture, gave two unresolved peaks for the four proteins (Fig. 1a). From the pH dependence of electroosmotic mobility, μ_{EOF} [33,34], a large fraction of the silanol groups

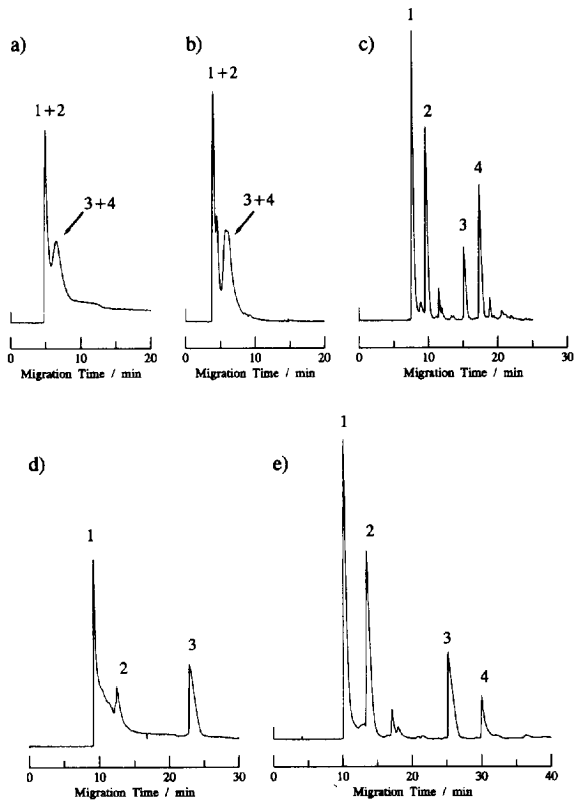

Fig. 1. Electropherograms of the separation of four basic proteins in various treated capillaries and electrophoretic buffers. (a) Untreated capillary, 10 kV, 32 μA; (b) capillary treated with 1.0 *M* HCl for 5 min, 15 kV, 48 μA; (c) capillary treated with 1.0 *M* HCl then 0.2% PEO solution containing 0.1 *M* HCl for 5 min each, 15 kV, 47 μA; (d) untreated capillary, 15 kV, 47 μA; and (e) capillary treated with 1.0 *M* HCl for 5 min, 15 kV, 43 μA. Electrophoretic buffer: $[H_3PO_4]_T$=30 m*M*, titrated to pH 6.0 with NaOH for (a), (b), and (c). For (d) and (e), 0.2% PEO was added. Sample injection: 15 kV for 5 s. Peaks: 1=lysozyme; 2=cytochrome c; 3=ribonuclease A; and 4=α-chymotrypsinogen A.

on the silica surface are dissociated to produce negative charges at pH 6.0. These proteins have positive charges at pH 6.0, because the isoelectric point, p*I*, is reported as around 9–11 [35]. Therefore they are likely adsorbed strongly onto the silica surface through coulombic interactions to cause peak tailing.

Next, the inner wall of the capillary is flushed with 1.0 *M* HCl solution for 5 min before introducing the electrophoretic buffer. This treatment showed virtually no improvement in peak profile (Fig. 1b). Fig. 2 shows the observed electrophoretic mobility, μ_{Obs}, of lysozyme in the electropherograms in Fig. 1. Here μ_{Obs} consists of μ_{EOF} and electrophoretic mobility of the protein, μ_{EP}. Namely,

$$\mu_{Obs} = \mu_{EOF} + \mu_{EP} \quad (1)$$

where μ_{EOF} and μ_{EP} have the same sign because the directions of electroosmosis and electrophoresis are the same. Between Fig. 1a and Fig. 1b, μ_{EP} should be almost the same because there is no difference in the conditions of electrophoresis except for the electrical current. Also the degree of interaction with the walls seemed to be similar due to the similarity of the peak shapes. Therefore the slightly smaller μ_{Obs} of b in Fig. 2 implies that μ_{EOF} is also smaller in Fig. 1b. This is caused by the initial protonation of the silanol groups by HCl, which however gradually degrades as the surface is exposed to higher pH solution during electrophoresis.

When a bare capillary is treated only with 2% PEO for 15 min, it also gave tailing of the peaks.

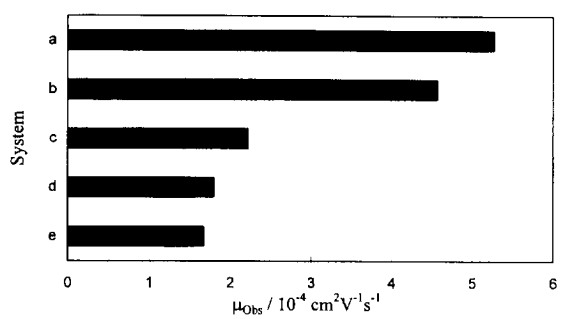

Fig. 2. Observed electrophoretic mobility for lysozyme in various systems. Systems a to e correspond to the electropherograms shown in Fig. 1a–e.

This implies that PEO treatment is not effective in preventing adsorption of proteins onto the wall if there exists a dissociated silanol group on the silica surface. In contrast, treatment with 1.0 *M* HCl followed by 0.2% PEO solution containing 0.1 *M* HCl for 5 min each clearly exhibited four well resolved peaks of the basic proteins, as well as the impurity peaks (Fig. 1c). Also it should be noted that μ_{Obs} is considerably lower than those of systems a and b (Fig. 2). Under this condition, it is very likely that the silica surface is effectively coated by PEO to suppress negative charges, thus preventing both protein adsorption and electroosmotic flow.

The effect of PEO and HCl concentration on peak profile and separation performance is investigated. Coating with 2% PEO gave the same results as 0.2%, indicating that the latter is sufficient to cover the entire surface of the capillary. On the other hand, HCl concentration seems to be an important factor for reproducibility. Table 1 lists the relative standard deviation (R.S.D.) of migration times of each protein obtained by two sets of treatment protocol. The R.S.D. obtained by treatment with 1.0 *M* HCl followed by PEO coating is practically acceptable. In fact these values are rather good if we consider that each step of treatment is operated manually by using syringes. Even if the time of the treatment step is fixed quite accurately, the flow-rate of HCl, coating solution, and buffer solution may have some variance. The R.S.D. can probably be improved if the procedure is fully automated. At present, capillaries which have already been bonded with non-ionic hydrophilic polymers are commercially available. One report mentioned that the reproducibility of migration times of basic proteins is 0.4–1.9% R.S.D. at pH 6 [36]. That was based on run-to-run reproducibility. It should be noted that coating is regenerated after every run in our case. Therefore the reproducibility here corresponds to a batch-to-batch situation.

In the case of 0.1 *M* HCl treatment, R.S.D. values were larger. It has been reported that acid–base equilibrium of silanol groups on FSC surface is a relatively slow phenomenon [37]. The formation or deformation of a silica gel layer, which are slow processes, always accompanies deprotonation or protonation [34]. From a kinetic point of view, HCl concentration is one of the crucial factors which will

Table 1
Average and relative standard deviation of migration times obtained by two coating methods[a]

Peak No.	Protein	1.0 *M* HCl and 0.2% PEO (n=5, i=49.6 μA)		0.1 *M* HCl and 0.2% PEO (n=4, i=51.0 μA)	
		t_m/min	R.S.D. (%)	t_m/min	R.S.D. (%)
1	Lysozyme	7.0_8	1.4	7.5_5	3.2
2	Cytochrome *c*	8.8_4	1.8	9.3_6	8.0
3	Ribonuclease A	13.9_6	2.9	15.3_4	8.2
4	α-Chymotrypsinogen A	15.9_6	3.4	17.6_1	9.5

[a] Coating procedure: 1.0 *M* or 0.1 *M* HCl was introduced into the bare silica capillary, followed by 0.2% PEO solution containing 0.1 *M* HCl. Finally, the capillary is flushed with electrophoretic buffer. Each step took 5 min. This procedure is repeated before the next run. Applied voltage: 15 kV. Electrophoretic buffer: $[H_3PO_4]_T$=30 m*M*, pH 6.0 with NaOH. t_m and i are averaged values.

affect the overall rate of the titration reaction. Therefore in the case of 1.0 *M* HCl titration, reprotonation seemed to be completed within 5 min, while reprotonation has not been completed by 0.1 *M* HCl over the same period.

An alternative way of coating was studied. Namely, the PEO solution pretreatment step is eliminated but PEO is added to the electrophoretic buffer. This method is frequently called 'dynamic coating'. Fig. 1d shows the separation with an untreated bare silica capillary. Compared to Fig. 1a, there are more discrete peaks. Importantly, the μ_{Obs} of lysozyme is even smaller than that obtained in system c (Fig. 2). This does not necessarily mean the coverage of the silica surface is sufficient. The large peak tailing of lysozyme is evidence of residual negative charges on the surface. Theoretically, μ_{EOF} and μ_{EP} are given by Eq. 2 [38] and Eq. 3 [39],

$$\mu_{EOF} = -\frac{\epsilon\zeta_c}{\eta} \quad (2)$$

$$\mu_{EP} = \frac{2\epsilon\zeta_p}{3\eta} f(\kappa a) \quad (3)$$

where ϵ is the dielectric constant, and η is the viscosity of the buffer medium. ζ_c (<0) and ζ_p (>0) are the ζ-potentials of the inner wall of FSC and the protein, respectively. $f(\kappa a)$ is a function dependent upon the shape, κ, reciprocal of the double layer thickness, and a, radius of the protein. These equations predict that increase of η reduces both μ_{EOF} and μ_{EP} and thus μ_{Obs}. Qualitatively, 0.2% PEO solution appears syrupy and therefore has a larger η. In conclusion, the small μ_{Obs} can be attributed not only to a smaller ζ_c-potential but also to a larger viscosity.

On the other hand, when the capillary was titrated with 1.0 *M* HCl for 5 min before use, four well resolved peaks are observed (Fig. 1e). This result indicates the necessity of acid titration of the silanol groups on the silica surface before polymer solution is introduced. The observed electrophoretic mobility of the solutes are smaller than those in Fig. 1c (for lysozyme, see Fig. 2). Again there seems to be the effect of viscosity on μ_{EOF} and μ_{EP} as given by Eqs. 2 and 3.

By comparison of Fig. 1c and Fig. 1e, the former is superior from a practical point of view, because it is faster even when the time required for column treatment is taken into account. The lack of PEO in the running buffer eliminated the effects of viscosity in Fig. 1c. In summary, the established protocol for coating is as follows: (1) 1.0 *M* HCl was forced to flow through the bare silica capillary; (2) then the capillary is flushed with 0.2% PEO solution containing 0.1 *M* HCl; and (3) finally the capillary is flushed with electrophoretic buffer. Each step takes 5 min.

3.2. Plausible mechanism of adsorption

Fig. 3 shows the electropherograms obtained by using capillaries treated by established protocols with other polymer materials such as hydroxyethylcellulose (HEC) and hydroxypropylmethylcellulose (HPMC), as well as PEO. For HEC, the peak tailing of lysozyme and broadening of ribonuclease A and

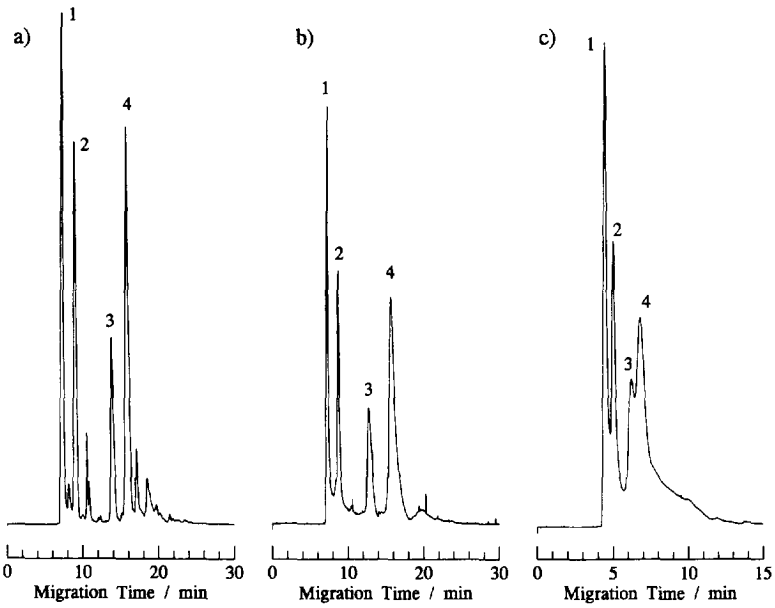

Fig. 3. Separation of basic proteins by capillaries coated with various polymer materials. (a) PEO, 51 μA; (b) HEC, 50 μA; and (c) HPMC, 52 μA. Conditions and peak assignment are identical to Fig. 1a.

α-chymotrypsinogen A are observed. In the case of HPMC, the migration times of all proteins are shorter than for PEO and HEC. Resolution is also insufficient. These results indicate that cellulose derivatives have low affinity for absorption onto silica surfaces and/or are easily desorbed during electrophoresis. These may be caused by the hydroxyl groups which possess higher hydrophilicity than PEO [16]. These cellulose-based polymers have often been added to the electrophoretic buffer in order to suppress EOF [40]. This type of dynamic coating mode was also attempted by using a HCl-titrated capillary filled with buffer containing 0.2% HEC or HPMC. These separations, however, showed similar resolution as in Fig. 3b and Fig. 3c except that μ_{Obs} values are reduced to about half. This seemed to originate from viscosity effect on μ_{EP} (see Eqs. 1 and 3).

From these comparisons of polymer material as well as the necessity of HCl titration, a likely scheme of the coated surface with PEO can be illustrated as in Fig. 4. As shown, the poly(oxyethylene) chain is bound to silanol hydrogen effectively through hydrogen bonding. The coating by PEO should be stable both thermodynamically and kinetically. In other words, the degree of coverage when the polymer solution is introduced in a capillary is determined by the free energy of adsorption under equilibrium condition. On the other hand, after the polymer solution is flushed out, there is no PEO in the bulk solution and there arises a driving force to desorb PEO. In this situation, kinetic stability becomes the most important factor. Unless desorption kinetics are slow, the PEO will be immediately removed from

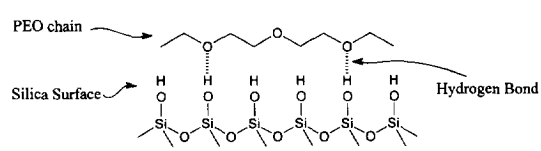

Fig. 4. Schematic diagram of adsorption of PEO onto silica surface.

the surface during the separation. Kinetic stability is described by the activation energy of desorption. For both free energy of adsorption and activation energy of desorption, the binding mode is a vital factor. This is strongly related to the molecular structure of PEO. As shown in Fig. 4, it has ether oxygen which works as a hydrogen acceptor, while cellulose-based polymers are rich in hydroxyl groups which normally act as hydrogen donors. This is the cause of energetically favorable PEO coating.

For kinetic stability, the length of the polymer is also important. Clearly some segments of PEO will have enough energy to exceed the activation energy to break hydrogen bonds. Unless the segment is located at the terminal of the PEO chain, it has a good chance to rebind to the silica surface. If it is at the terminal, it can activate adjacent groups because the terminal is stabilized due to solvation after it is detached from the silanol group. In this way, desorption of a PEO molecule likely begins from its terminal group. If two columns are compared which are coated with the same concentration of PEO but at different molecular mass, the one with longer PEO has a smaller number of terminals and thus is less labile. For this reason, PEO of M_r 8 000 000 is employed in this study, which has the highest molecular mass among the commercially available PEO. There is no available data on the M_r values of HPMC and HEC used here. They, however, have bulky structures based on glucose units and have shorter lengths per M_r value compared to PEO.

In another context, the importance of molecular structure should be considered, namely the balance of hydrophobicity and hydrophilicity [16]. Proteins can undergo mainly three types of interactions: coulombic, hydrophobic, and hydrogen bonding interactions. If the polymer is too hydrophobic like polyethylene, it will adsorb proteins via hydrophobic interaction. If it is too hydrophilic, it may form hydrogen bonds with proteins. The best protein separations with capillaries chemically bonded by hydrophilic polymers are those based on poly(ethylene glycol) or poly(ethylene–propylene glycol) [16], which share the same partial structure with PEO. In conclusion, PEO is one of the better materials because of its coating properties and its neutrality to proteins.

3.3. pH dependence

Fig. 5 shows electropherograms obtained at the buffer pH range of 3.0 to 7.0. The ionic strength was maintained constant at 47 m*M* for Fig. 5a–e. The established protocol was used for coating. The observed electrophoretic mobilities of proteins as well as the one of lysozyme obtained with untreated and HCl-treated columns are plotted against pH in Fig. 6. As shown, peak shapes of proteins are good around pH 3–6 but poor at pH 7. In coated columns, μ_{Obs} decreases uniformly (Fig. 6), which is mainly caused by the loss of positive charge on the protein. At pH 4, the peaks of lysozyme and cytochrome *c* overlap and the order changes. This implies that p*I* can only be used as an indicator of the magnitude of net charge in the pH region close to p*I*. Discrepancies between μ_{Obs}^{HCl} and μ_{Obs}^{None} show that the equilibration process of acid dissociation reaction at FSC surface is slow, as discussed above.

In Fig. 6, the difference between the μ_{Obs} of lysozyme obtained by untreated and coated columns corresponds to the electroosmotic mobility suppressed by PEO coating. Namely,

$$\mu_{EOF}^{Suppressed} = \mu_{Obs}^{None} - \mu_{Obs}^{Coated} \quad (4)$$

From Fig. 6, $\mu_{EOF}^{Suppressed}$ increases greatly around pH 5.0 and has its maximum ($3.6 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}) at pH 7.0. This behavior is quite similar to the pH dependence of EOF [33,34]. Typically, the reported value of μ_{EOF} for FSC is around $5 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} at pH 7.0. This is evidence that there is no serious degradation of the coating at pH 7, even though there appeared tailing and broadening of the peaks (Fig. 5e). At the same time, this confirms that acid dissociation of uncovered silanol groups can cause protein adsorption. In other words, even though the ζ_c-potential is suppressed substantially, the small increase in charge density on the wall has an overwhelming effect compared to the decrease of positive charges on protein.

In this system, ionic strength of the buffer plays quite an important role. For instance, if the phosphate concentration in the buffer is increased from 26 m*M* to 30 m*M* at pH 7.0, which leads to the

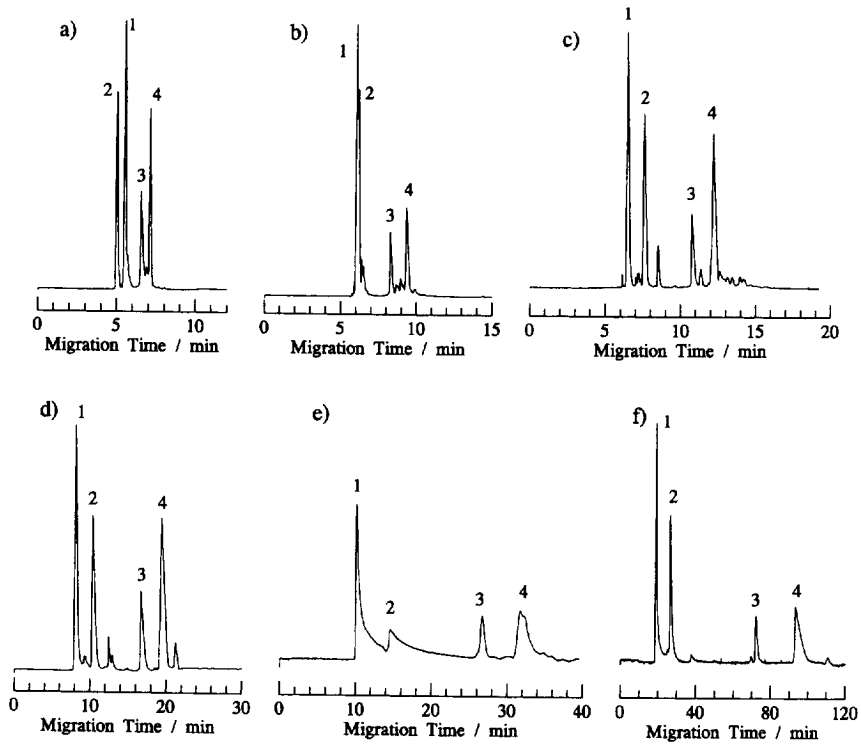

Fig. 5. Electropherograms of separation at various pH obtained in capillaries prepared with the optimized coating protocol. Total phosphate concentration in electrophoretic buffer, pH, and current are: (a) 54 m*M*, pH 3.0, 65 μA; (b) 47 m*M*, pH 4.0, 61 μA; (c) 46 m*M*, pH 5.0, 63 μA; (d) 42 m*M*, pH 6.0, 64 μA; (e) 26 m*M*, pH 7.0, 68 μA; and (f) 30 m*M*, pH 7.0, 54 μA. pH of buffer was adjusted with NaOH. Ionic strength was set to be 47 m*M* for all pH except (f) 54 m*M*. All separations were run at 15 kV except (f) 10 kV. Sample injection: 15 kV for 3–5 s for (a)–(e) and 10 kV for 6 s for (f). Peak assignment is given in Fig. 1.

increase of ionic strength from 47 m*M* to 54 m*M*, the peak shape improved especially for lysozyme, cytochrome c, and ribonuclease A (Fig. 5f). It has been reported that higher ionic-strength buffers can resolve proteins well [41,42]. This is due to increased cationic components such as sodium ions which compete with proteins for adsorption sites on FSC.

Fig. 7 shows the resolution, R_s, estimated by Eq. 5, between two adjacent peaks obtained by the three methods.

$$R_s = \frac{\sqrt{2 \ln 2}\Delta t_m}{HW_1 + HW_2} \quad (5)$$

where Δt_m is the difference of migration time between two peaks, and HW_1 and HW_2 are full width at half maximum of each peak for the three methods, i.e. the established protocol, 1.0 *M* HCl titration only, and no treatment. In all cases, the resolution increases in the order of: no treatment<HCl treatment<HCl and PEO treatment. Obviously, the treatment with both HCl and PEO is required for optimum resolution. At pH 3.0, there seems to be no difference in the resolution among the three methods. This is because the pH is near the point of zero charge of silica gel [3], which no longer attracts proteins via coulombic force. When the pH becomes higher, to 7.0, the R_s for untreated and HCl-treated columns approaches zero. In contrast, R_s obtained with PEO-coated columns has its maximum around pH 5.0.

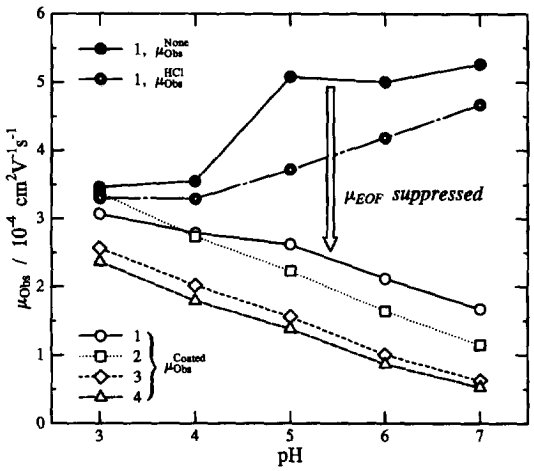

Fig. 6. The observed electrophoretic mobility for basic proteins in Fig. 5a–e as well as one of lysozyme obtained in untreated and HCl-treated columns. The numbers correspond to the proteins assigned in Fig. 1. Closed circle: untreated column, double circle: 1 *M* HCl-treated column, open symbols: PEO-coated column. The difference between μ_{Obs} of closed and open circles indicates the magnitude of μ_{EOF} suppressed by the coating. Electrophoretic conditions are given in Fig. 5.

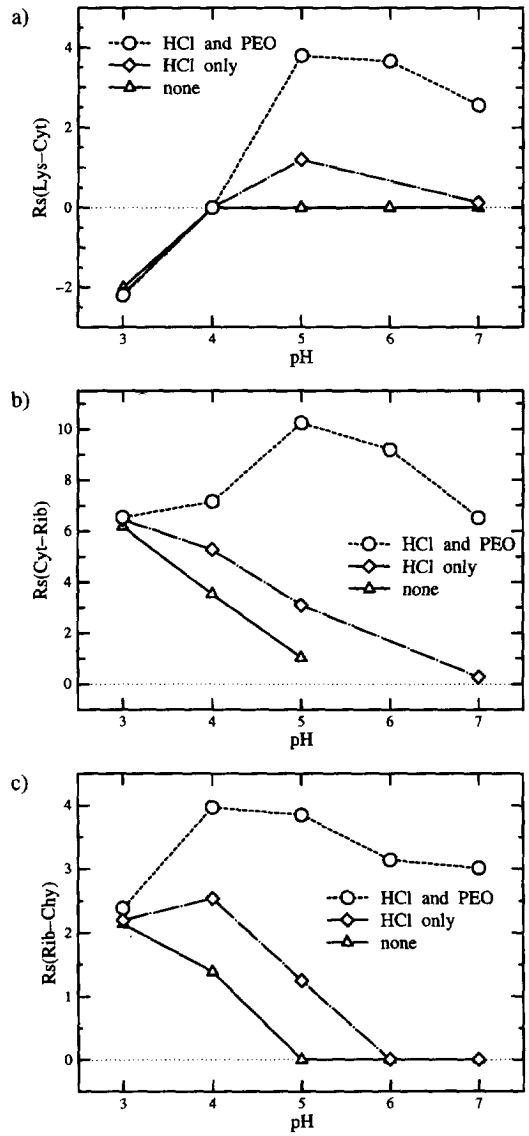

Fig. 7. The resolution between peaks by various capillary treatment methods. (a) Lysozyme–cytochrome c pair; (b) cytochrome c–ribonuclease A pair; and (c) ribonuclease A–α-chymotrypsinogen A pair. Electrophoretic buffer: $[H_3PO_4]_T$=26–53 m*M*; pH, 3.0–7.0 with NaOH; ionic strength, 47 m*M*. Running condition: 15 kV, 61–69 μA.

4. Conclusions

In this study, methods to coat the inner wall of capillaries, which are able to reduce EOF and adsorption of basic proteins, are established. The separation efficiency in terms of peak shape and reproducibility is fairly good. No laborious procedure for immobilizing reaction of the polymer is required. Regeneration of the coating is also easy. With further research, one should be able to reduce the time required for reconditioning the column walls to make the procedure more practical. Recently there have appeared some reports of coating methods which are based on the adsorption of non-ionic polymers. However, they require special techniques such as silanized columns, thermal treatment, or organic solvent and evaporation [23–25]. This is then the first report of coating capillaries by direct adsorption of non-ionic polymer onto bare FSC from aqueous solution. The structure of PEO plays a very important role in forming the coating via hydrogen bonding to the surface silanol group. Nowadays, commercial CE instruments are able to change buffer reservoir automatically. By using these, the present coating procedure can be programmed to allow fully automated operation. No investments on commercially available coated columns, which are usually

expensive, and little added labor and time for preparing these coated columns will be necessary.

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