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Capillary Modification by Noncovalent Polycation Adsorption: Effects of Polymer Molecular Weight and Adsorption Ionic Strength

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The strong polycation poly(dimethyldiallylammonium chloride) (PDADMAC) was noncovalently immobilized on fused-silica capillaries. The stability and efficiency of the adsorbed coating was studied as a function of polymer molecular weight and coating ionic strength. By monitoring the electroosmotic flow on PDADMAC-coated capillaries, we found that optimal surface coverage is achieved using high molecular weight polycations at high ionic strength. Such capillaries can be used to elute positively charged proteins. In this study, true protein mobilities were obtained by (1) extrapolation of effective mobility to desired conditions and (2) theoretical calculation from the Henry function. By comparing apparent protein mobility with true protein mobility, we demonstrated that the protein−polycationic capillary surface interaction could enhance the selectivity of the silica capillary for separation of BSA and *β*-lactoglobulin.

Protein adsorption is a major problem confronting bioanalytical capillary electrophoresis (CE).[1−3] Such adsorption is often observed at pH < p*I*, where proteins are positively charged and can be electrostatically adsorbed onto negatively charged capillary surfaces. Many methods have been developed to address this problem, often by[3] surface modification.[2−5] Traditional methods for capillary surface modification involve chemical derivation of silica using silylation reagents, such as (*γ*-(methacryloxy)propyl)-trimethoxysilane[6] and trimethylchlorosilane,[7] and subsequent chemical reaction of the chloro or acryl group with desired materials to provide the final surface coverage of the capillary.[8−10] However, it has been reported that such modifications may lead to several difficulties, including a lack of consistent performance from capillary to capillary, a loss of coating stability with time, and limited pH-application ranges.[10,11] Therefore, later efforts focused on polymeric coatings.[8,10−16]

Polymer coating has been proven successful for control of protein adsorption. It is usually achieved by chemically linking either monomeric or polymeric materials on the capillary surface.[10,12−16] Many polymers have been utilized to coat capillaries, such as polyacrylamide,[1,8] poly(vinylpyrrolidinone),[12] poly(ethylene glycol),[13] poly(dimethyldiallylammonium chloride) (PDADMAC),[14] cellulose,[15] and polyethyleneimine (PEI).[16]

An alternative method for coating capillaries with polymers is through physical adsorption. Such adsorption is possible if a strong interaction exists between polymers and capillary surfaces, as is the case for polycations, which can have a strong electrostatic interaction with a negatively charged surface, leading to noncovalent adsorption. Recently, Whitesides et al.[17] and Hartwick et al.[18] separately showed that polycations could be noncovalently adsorbed on capillary surfaces. Their results suggest further exploration of the advantages of noncovalent capillary modification with polycations. The use of a noncovalently adsorbed polycation to modify siliceous chromatography packings was also reported recently by us.[19]

Noncovalent polymer modification may provide several advantages over chemical modification: (1) Such modification is versatile, as a wide variety of polymers with different structures and functional groups can be introduced onto the capillary surface. (2) The procedure is economical and easy to carry out. (3) As shown by Whitesides,[17] a stable electroosmotic flow (EOF) can be obtained after adsorption of strong polycations. (4) If the polyelectrolyte can selectively interact with proteins, an additional mechanism can contribute to enhanced protein separation.

Recent studies have focused on the efficiency of the modified capillaries, neglecting the mechanism of polyelectrolyte adsorption. However, the phenomenon of polyelectrolyte adsorption has

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in fact been studied extensively, both theoretically[20−25] and experimentally.[26−32] Thus, the available fundamental principles can guide polyelectrolyte adsorption techniques. Particularly relevant are studies of the effects of ionic strength and polymer molecular weight on the amount and molecular configuration of noncovalently adsorbed polyelectrolytes. This information has not yet been applied to optimize polyelectrolyte adsorption in capillary electrophoresis.

Our purpose here is to consider the relationship between polyelectrolyte adsorption conditions and the bound state of the adsorbed polymer with the goal of using polyelectrolyte immobilization to prevent basic protein adsorption, achieve stable EOF, and improve protein separation. Although ref 17 involved the adsorption of different polycations, the selection of experimental conditions was somewhat arbitrary with respect to the polymer molecular weight and ionic strengths used for adsorption. Since a complete study of all these experimental variables for a variety of polycations would be a formidable task, we chose poly(dimethyldiallylammonium chloride) (PDADMAC) as a model polyelectrolyte to study the relationship between adsorption conditions and the structure of the immobilized polyelectrolyte as manifested in the CE performance of the treated capillaries. This polycation offers a number of advantages: (1) as a "strong polyelectrolyte", its charge is not pH dependent; (2) it is commercially available; (3) it is easily prepared with a range of molecular weights; and (4) we have prior experience in the coating of glass packings with this polymer. The optimization of the efficiency of the coated capillary was considered as a secondary issue, which can be the principal objective of future studies.

EXPERIMENTAL SECTION

Materials. PDADMAC of MW 428 000 or 30 000, with polydispersity on the order of $M_w/M_n \sim 1.5$, was obtained from Dr. W. Jaeger (Fraunhofer-Insitut, Teltow, Germany). Bovine serum albumin (BSA) (Lot 100062) was purchased from Boehringer Mannheim (Indianapolis, IN). β-Lactoglobulin (L2506) (a mixture of β-lactoglobulins A and B), lysozyme (L-6876), and ovalbumin (A-5503) were obtained from Sigma. All proteins were used without further purification. Mesityl oxide was purchased from Aldrich.

Capillary Coating. Fused-silica capillaries (27 cm × 50 μm i.d., Restek) were preconditioned with 0.1 N NaOH (40 min) and H_2O (20 min) before coating. A PDADMAC coating solution was prepared in pH = 8.0 Tris buffer at a selected ionic strength. To simplify, a column coated at high ionic strength (I), high polymer concentration (C), and low polymer molecular weight (m) is given the designation ICm; similar abbreviations are used for all other coating conditions; see Table 1. Coating procedures were similar to those in ref 17. PDADMAC adsorption was carried out by rinsing the capillary with the coating solution at alternating high (20 psi, 5 min) and low (0.5 psi, 120 min) pressures for 16 h. The high-pressure rinse was adopted to assist mixing; the long conditioning time was used because equilibration in the adsorption polydisperse polyelectrolytes is slow.[33] After coating, the capillary was washed with run buffer for 40 min before analysis.

Table 1. Capillary Coating Conditions[a]

abbreviation	ionic strength, M	polymer concn, g/L	polymer $M_n \times 10^{-3}$ [b]
ICM	0.50	20.0	428
Icm	0.50	0.10	30
iCM	0.0050	20.0	428
icM	0.0050	0.10	428

[a] pH = 8.0. [b] Polydispersity: $M_w/M_n \sim 1.5$.

Capillary Electrophoresis. All CE experiments were carried out at 25 ± 0.1 °C and a detection wavelength of 200 nm on a Beckman P/ACE 5500 instrument with a voltage of 8 kV used to maximize the separation efficiency.[34] The polarity of the electrodes was switched for the coated capillary due to the reversal of electroosmotic flow. All capillaries were equilibrated with running buffer for 40 min before use. Since proteins will adsorb to bare silica at pH < pI and to polycation-coated capillaries at pH > pI, it was, in general, necessary to employ two different run buffers: for an uncoated capillary, pH = 8 and I = 0.05 (tris); for a coated capillary, I = 0.05 (NaOAc−HOAc), adjusted to various neutral or acidic pH values. The electroosmotic flow was monitored with 0.05% w/w mesityl oxide. Protein samples were made at 0.5 g/L with 3 or 4 drops of 0.05% mesityl oxide added. The apparent protein mobility, μ_{app}, was calculated through eq 1, and the true

$$\mu_{app} = \frac{Ll}{V}\left(\frac{1}{t} - \frac{1}{t_0}\right) \quad (1)$$

protein mobility would be the same as the apparent protein mobility in the absence of protein−capillary interactions. In eq 1, L (cm) is the total capillary length, l (cm) is the capillary length from the injection end to the detection window, V (v) is the voltage, and t (s) and t_0 (s) are the elution times of the solute and the neutral marker. (It should be noted that the sign of μ_{app} agrees with the solute charge sign when solute migrates in the same direction as the EOF-determining ions, e.g. in the case of cationic solutes on bare silica, so that $t < t_0$, but does not agree with the solute charge sign when the solute migrates against the EOF. We have defined the signs of the mobility to be consistent with the true electrophoretic mobility, regardless of the direction of

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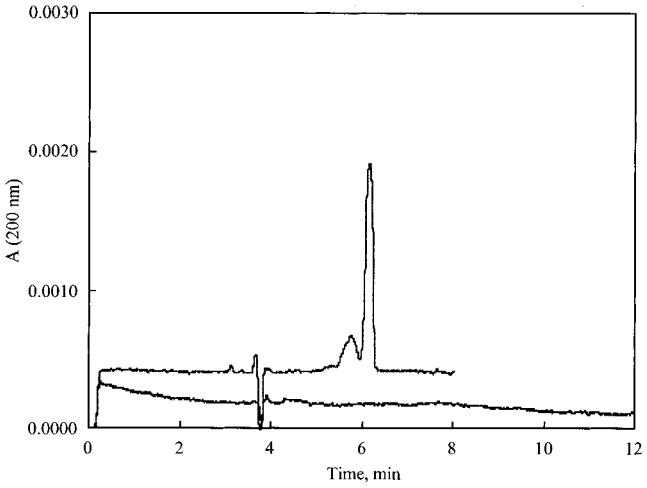

Figure 1. Electrophoregrams of lysozyme on both coated (above) and uncoated (below) capillaries. Run buffer: pH = 8, $I = 0.05$ (Tris buffer).

migration with respect to EOF.) The relative error for 10 repeated CE measurements was within 1%. The numbers of theoretical plates, determined by injection of the neutral marker mesityl oxide, were 6.8×10^3 and 3.1×10^4 for coated and uncoated capillaries, respectively.

RESULTS

The effects of polycation adsorption conditions were studied using ionic strength and polymer molecular weight and concentration as variables. To facilitate comparisons, capillary modification was carried out only at the extremes of these conditions; i.e. $I =$ 0.5 M vs 0.005 M ionic strength, polymer MW's of 4.28×10^5 and 3.0×10^4, and polymer concentrations of 20 and 0.1 g/L.

To demonstrate the effect of polycation treatment, Figure 1 shows the electrophoregrams of lysozyme at a concentration of 0.20 g/L, in a run buffer of pH = 8 and $I = 0.05$ (Tris buffer), injected into both treated and untreated capillaries. Since lysozyme has a p*I* of 11, it is positively charged in this buffer and, due to adsorption, does not elute on a native silica capillary, even after sustained times (not shown). However, lysozyme can be eluted from the polycation-coated capillary in this buffer following reversal of polarity which is required because of the reversal of EOF. These results suggested that PDADMAC was successfully immobilized on the capillary surface. Similar results for lysozyme were also obtained for capillaries modified under other conditions.

Effect of Polycation Adsorption Conditions. The polyelectrolyte coating influences the ζ potential (ζ) on the capillary surface.[17] Since the EOF is directly proportional to ζ^4, EOF measurements can be used to monitor the capillary surface coverage under different coating conditions.

Effects of polymer adsorption conditions were studied by monitoring the EOF on coated capillaries at pH 8 and pH 4, with the results summarized in Table 2. It was found that the EOF was independent of pH for capillaries modified with high-MW polymer ($I = 0.5$) but was dependent on pH on low-MW polymer-coated capillaries. A similar discrepancy was also observed for capillaries coated with polymer at different ionic strengths. EOF was found independent of pH on capillaries modified at high ionic strength (polymer MW = 4.28×10^5) but pH dependent on

Table 2. Electroosmotic Flow at Different Coating Conditions and Buffer pHs[a]

adsorption conditions		$10^4 \lvert\mu_{EOF}\rvert$, cm^2 V^{-1} s^{-1}	
MW $\times 10^{-3}$	ionic strength, M	pH 8	pH 4
b	*b*	3.63	*c*
428	0.50	3.54	3.54
30	0.50	2.98	3.66
428	0.0050	3.02	3.58

[a] Polymer adsorption was carried out at $C_p = 20.0$ g/L. Run buffer ionic strength was 0.05 M. EOF values are averages of two or more measurements. [b] Uncoated. [c] Too low to measure.

capillaries modified at low ionic strength. Only the capillaries coated at high ionic strength and with high-MW polymer yield constant μ_{EOF} between pH 8.0 and pH 4.0; all other capillaries show higher μ_{EOF} at pH 4.0 than at pH 8.0. This may be explained by insufficient surface coverage and the corresponding effect of silanol groups. At low pH, the decrease of silanol group ionization will increase the net positive surface charge density (since the charge of the polycation is not changed). Such an increment will increase the ζ potential and therefore increase μ_{EOF}. The constancy of EOF at pH 4.0 and pH 8.0 for a capillary coated with high molecular weight polymer at high ionic strength suggests a complete coverage of the capillary surface. The effect of polymer concentration was also studied, i.e. 20 g/L vs 0.1 g/L (polymer MW = 4.28×10^5, $I = 0.5$), but the same μ_{EOF} was observed.

Stability of the Polycation Coating. The stability of PDADMAC-coated capillaries was monitored by repeated EOF measurements at pH 4.0 and ionic strength 0.05 on a capillary coated at high ionic strength and high polymer concentration with high polymer MW (designated "ICM"). To compare our result with that obtained by Whitesides et al. [17] (different ionic strength and voltage were used), the mobility is normalized by dividing by the value of the first measurement ($\mu_{EOF}/\mu_{EOF,1}$). The normalized μ_{EOF} changed only 2% after 25 repetitions, compared with 6−9% μ_{EOF} variation for the polybrene-coated capillary in Whiteside's study. These results suggest that the PDADMAC-coated capillary is stable under experimental conditions. The capillaries coated under other conditions ("Icm", "iCM", and "icM") displayed a similar EOF stability at pH 4.0.

The charge on an "ICM" capillary was studied as a function of the pH of the run buffer. The pH dependence of the EOF is compared to that of an uncoated capillary in Figure 2. Between pH 5 and 7, a sharp increase in EOF is observed for the uncoated capillary, which suggests an apparent silanol pK_a around pH 5.5, as also reported by Righetti et al.[35] For the coated capillary, the EOF is reversed and is relatively constant in the range pH 2−8. However, a small decrease of EOF was found at $3 <$ pH < 4 (see insert in Figure 2), which suggests an apparent silanol pK_a pH $\cong$ 3 on the coated capillary. This pK_a shift is due to the presence of polycations on the capillary surface which stabilize the ionized state of silanol, thus making it a stronger acid. As a result, the EOF is rather stable between pH 4 and pH 8. The small EOF change below pH 4 suggests that the majority of the silanol groups form ion pairs with polycations.

The circumstances determining the pH dependence of the EOF for the coated capillary are complex. First of all, the dominant

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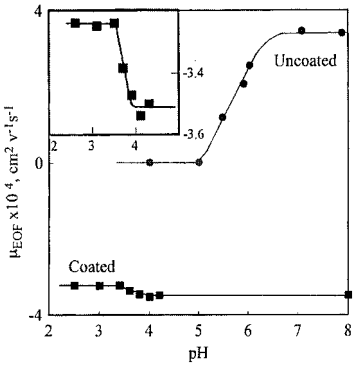

Figure 2. Effect of pH on μ_{EOF} for coated (■) and uncoated (●) capillaries, $I = 0.05$. Insert: effect of pH on μ_{EOF} for coated capillaries (■).

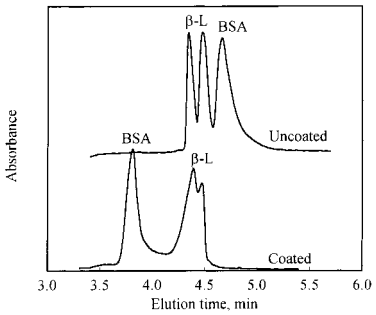

Figure 3. Electrophoregrams of BSA and β-lactoglobulin on coated and uncoated capillaries.

carrier of electroosmotic flow on coated capillaries is anions, which are distributed around the polycation chains. The distribution of such anions is very complicated, and the change of anion distribution due to the elimination of capillary surface charge upon decrease of pH is very difficult to predict. Second, the flow of anions could be affected by the configuration of the polymer chains which also might rearrange at low pH.

Protein Separation Using PDADMAC-Modified Capillaries. If proteins interact selectively with adsorbed polycations, enhancement of separation could be observed for coated capillaries. Direct comparison between the protein selectivity of coated and uncoated capillaries is complicated by the fact that the pH ranges of both columns are limited and necessitate comparison of resolution at different pHs. Since capillaries coated at high ionic strength and high MW (ICM) display an EOF similar to that of an uncoated capillary and provide stable EOF, selectivity was compared between ICM and uncoated capillaries.

Mixtures of BSA and β-lactoglobulins (A and B) were separated on both coated (pH = 4, $I = 0.05$) and uncoated (pH = 8, $I = 0.05$) capillaries, as shown in Figure 3. The lower resolution of β-lactoglobulins A and B on PDADMAC-coated capillaries results from the low efficiency of the coated capillaries (6.8×10^3 vs 3.1×10^4 plates). Despite this effect, the separation of BSA from the β-lactoglobulins is increased 3-fold on the coated capillaries: the resolution, given by $R = (t_2 - t_1)/(W_{1,1/2} + W_{2,1/2})$, is 0.88 on the uncoated capillary but 2.77 on the coated one.

DISCUSSION

Among the various theories that treat polyelectrolyte adsorption on charged surfaces are the lattice theory,[20,21] the single-chain statistic model,[24] and the scaling treatment,[23] all summarized in a review by Fleer et al.[20] It has been pointed out[20] that the configurations of adsorbed polyelectrolyes can be described in terms of trains, loops, and tails[20] and that these configurations are determined by polymer properties and adsorption conditions, e.g. polymer molecular weight and ionic strength. These theoretical findings may be used to explain some of the current results.

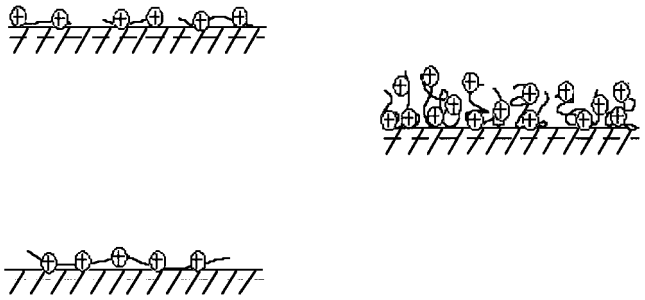

Figure 4. Schematic depiction of effects of polymer molecular weight and ionic strength on polyelectrolyte adsorption. (Upper left) low MW, low *I*; (lower left) high MW, low *I*; (right) high MW, high *I*.

Effect of Polymer Molecular Weight. As shown schematically in Figure 4, the low molecular weight PDADMAC in this study and in ref 17 (MW = 10 000) may not form the loops and tails that are mandated for effective charge inversion.[20] As pointed out in ref 17, PDADMAC is less flexible than typical vinylic polymers, so low-MW PDADMAC may adsorb in a relatively flat configuration. Such adsorption will only lead to charge neutralization, instead of charge inversion. As a result, the EOF for capillaries modified with a low-MW polycation will decrease dramatically, and thus neither neutral marker nor lysozyme peaks are observed within 20 min. A second difference between high- and low-MW polycations is that the adsorption of a high-MW polymer may be more energetically favorable if segment adsorption is cooperative. It should be noted that the EOF of our low-MW PDADMAC is still of reasonable magnitude; but this MW (30 000) is significantly larger than the one used in Whitesides' study, so that the EOF in that work might have been problematic because of low polymer MW.

Effect of Ionic Strength. The effect of ionic strength (I) on polyelectrolyte adsorption is apparent from EOF measurements (Table 2), which show that adsorption at high I is preferable. Thus, the ionic strength used in ref 17 (25 mM Tris + 192 mM Gly) may be too low. From the work of Esteso et al.,[36] who measured the activity coefficient of NaCl in the presence of various concentrations of glycine, we can conclude that 25 mM Tris/192 mM glycine is equivalent with respect to I to 37 mM NaCl; hence, the effective ionic strength used in ref 17 was less than 40 mM. As shown by van der Schee and Lyklema,[37] strong electrostatic repulsion between polymer chains at low I can lead to the depletion of polymers near the coating surface. In addition, low I is likely to result in adsorption in a flat configuration.[20,37] Thus, as illustrated in Figure 4, charge neutralization, instead of charge inversion, is likely to occur at low ionic strength. This leads to a low EOF so that proteins migrate away from the detector after the polarity of electrodes is switched.

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Table 3. Protein Titration Charge, Z^a and Mobility ($\mu \times 10^4$, cm^2 V^{-1} s^{-1})^b

pH 4.0, uncoated capillary				pH 8.0, ICM-coated capillary			
BSA		β-lactoglobulin		BSA		β-lactoglobulin	
Z	μ_{app}	Z	μ	Z	μ_{app}	Z	μ
27	0.64	19	1.02	−16	−1.12	−16	−1.19

[a] From ref 43. [b] EOF in all cases 3.50−3.63.

Selective Protein Separation. The protein selectivity of polyelectrolyte-modified porous glass has been interpreted as resulting from the interaction between proteins and the loops and tails of the immobilized polyelectrolytes.[19] The same explanation may also be applied to PDADMAC-coated capillaries.

The basis of the enhanced protein selectivity on coated capillaries can be investigated by comparing mobility-to-charge ratios (μ/z) for different proteins. Table 3 summarizes protein titration charge (z) and apparent mobility (μ_{app}) values. In the absence of protein−capillary interactions, $|\mu/z|$ should depend only on the free protein friction coefficient and thus be nearly constant. The calculated $|\mu/z|$ values are indeed all close to 0.06, except for that of BSA on the coated capillary (0.024). Because similar μ_{EOF} values were obtained for both coated (ICM) and uncoated capillaries, the relatively low $|\mu/z|$ for BSA on a coated capillary is likely due to interactions between BSA and the loops and tails of adsorbed polyelectrolytes.

Interactions between proteins and polyelectrolyte-coated capillaries were further studied by comparing the apparent protein mobility (μ_{app}) with the true mobility (μ). Negative deviations of μ_{app}/μ from unity are indicative of interactions between the protein and the polyelectrolyte-modified surface. Literature values of μ for BSA have been mainly obtained by the moving-boundary method, with substantial inconsistencies, probably due both to the limitation of the method and to the quality of the protein used.[38] However, the true protein mobility can be estimated using Henry's theory[39] of electrophoresis in the limits of low ζ potential ($\zeta e/kT < 1$)

$$\mu = \frac{Z_a e}{6\pi\eta r}\frac{H(\kappa r)}{1+\kappa r} \qquad (2)$$

where Z_a is the electrophoretic charge of the protein, η the viscosity of the medium, $H(\kappa r)$ Henry's function, κ the Debye parameter, and r the Stokes radius of the protein. Xia et al.[31] found a low value for ζ of BSA at pH ≈ pI. Therefore, we attempted to extrapolate the true mobility of BSA from reported literature values, correcting the ionic strength effect via eq 2. From measurements of the electrophoretic mobility of BSA as a function of pH in conjunction with eq 2, we calculated $\mu = 1.3 \times 10^{-4}$ cm^2 V^{-1} s^{-1}, at pH 4 and ionic strength 0.05. Hence, μ_{app}/μ of BSA ≅ 0.49 ≠ 1, which indicates an interaction between BSA and PDADMAC. Unfortunately, literature data at pH 4.0 for β-lactoglobulin were insufficient to calculate μ_{app}/μ.

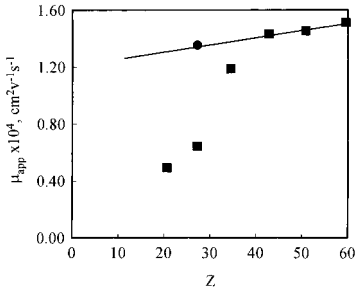

Figure 5. Electrophoretic mobility of BSA (■) as a function of protein charge on a PDADMAC-coated capillary. The line shows extrapolation from low pH to pH = 4.0 (●).

An alternate method to estimate the electrophoretic mobility of a protein from eq 2 has been proposed by Compton et al.[40] and others.[41] The structural net protein charge, Z_c, can be estimated via the Henderson−Hasselbalch equation

$$Z_c = \sum_{n=1,4}\frac{P_n}{1+10^{\mathrm{pH}-\mathrm{p}K(P_n)}} + \sum_{n=1,5}\frac{N_n}{1+10^{\mathrm{p}K(N_n)-\mathrm{pH}}} \qquad (3)$$

where P_n and N_n refer to the respective positively and negatively charged amino acids. Compton et al.[40] used a semiempirical expression for protein mobility

$$\mu = \frac{C_1 Z_a}{C_2 M^{1/3} + C_3 M^{2/3} I^{1/2}} \qquad (4)$$

where C_1, C_2, and C_3 are constants, Z_a is the electrophoretic protein charge, and M is the protein molecular weight. If $Z_a/Z_c = f$ is independent of pH at any given ionic strength, then from eq 4

$$\frac{\mu_1}{\mu_2} = \frac{Z_{c1}}{Z_{c2}} \qquad (5)$$

Consequently, the electrophoretic mobility at one pH can be calculated from the mobility measured at another pH. Since Z_c is influenced by specific ion adsorption and structural and environmental effects on the pK_a of individual amino acids, the above equations provide only an approximation of protein mobility. Douglas et al.[38] suggested that protein charge titration data, if available, should be used in eq 4 to calculate protein mobilities. Therefore, electrophoretic mobilities of protein were measured on a coated column at low pH where no protein−polyelectrolyte interaction existed and extrapolated as a function of protein titration charge to give μ at the desired conditions.

Electrophoretic mobilities of BSA at pH 3.2, 3.4, 3.6, 3.8, 4.0, and 4.2 are shown in Figure 5 as a function of protein charge (obtained from ref 42). The value of μ_{BSA} extrapolated to pH 4.0 ($Z = 27$) is 1.35×10^{-4} cm^2 V^{-1} s^{-1}. Therefore, $(\mu_{app}/\mu)_{BSA} \cong 0.47$,

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in excellent agreement with the value of 0.49 calculated from eq 2. A similar procedure was used to obtain μ for β-lactoglobulin at pH 4.0 as 1.20×10^{-4} cm^2 V^{-1} s^{-1}, leading to $(\mu_{app}/\mu)_{\beta-1} \cong 0.85$, somewhat closer to unity. The smaller value of μ_{app}/μ for BSA relative to β-lactoglobulin suggests a stronger interaction between BSA and the PDADMAC surface, accounting for the better separation of these two proteins achieved using coated capillaries. It should be noted that the early elution of BSA results from a dynamic interaction between BSA and the adsorbed polymer which significantly offsets the electrophoretic mobility (in the opposite direction of elution).

Li et al.[42] measured the electrophoretic mobility of β-lactoglobulin on a polybrene-coated capillary and found that μ was a linear function of pH between pH 3.3 and pH 4.3 (note that the protein titration charge is a linear function of pH in this pH range[43]) and that the dependence of $\mu_{\beta-1}$ on pH yielded a correct isoelectric point. Thus, contrary to our results, Li's data indicate no protein−polyelectrolyte interactions. This discrepancy is likely attributed to the different polyelectrolyte adsorption conditions. The low polyelectrolyte concentration and low ionic strength of the coating solution in ref 42, ca. $C_p = 0.05\%$ and $I = 0.02$, would result in relatively flat adsorption with few polyelectrolyte loops and tails.

CONCLUSIONS

The stability of the EOF of polycation-treated capillaries over a wide pH range is a consequence of the silanol group pK_a shift, which depends on the density and configuration of bound polycations. The formation of loops and tails in the adsorption layer is crucial for surface charge reversal. High polyelectrolyte MW and high ionic strength favor the formation of loops and tails. The stability and separation efficiency of capillaries coated with polycations such as PDADMAC thus depend on coating conditions.

Comparison of effective protein mobility to true mobility makes it possible to estimate the extent of the interaction between protein and immobilized polyelectrolyte. Such interaction contributes to the improved resolution. Although PDADMAC-coated capillaries display better separation of BSA from β-lactoglobulin than uncoated capillaries, peak broadening is greater for the coated capillary, a potential problem for further applications. This broadening could arise from the kinetics of partitioning of the solute between the bulk solution and the polymer adsorption layer, a hypothesis that could be tested by observing the effect of field strength and concomitant band velocity change. Reduction of such broadening could be addressed in future studies by properly controlling the polyelectrolyte adsorption layer thickness.

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