Journal of Chromatography, 594 (1992) 325–334
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 859

Capillary modification and evaluation using streaming potential and frontal chromatography for protein analysis in capillary electrophoresis

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(First received August 26th, 1991; revised manuscript received November 1st, 1991)

ABSTRACT

The modification of capillary surfaces in capillary electrophoresis requires accurate chemical characterization of the synthesized materials. A three-step procedure was developed for the evaluation of capillaries used for protein separations in capillary electrophoresis. These measurements include ζ potential determination via streaming potential measurements, adsorption characteristics using frontal chromatography measurements of test solutes, and overall performance under running conditions using selected test solutes. The ζ potential determined from the streaming potential reflects the degree to which the capillary wall can undergo electrostatic interactions with proteins as well as the magnitude of the electroosmotic flow. Frontal chromatography measurements with selected probe proteins can indicate both the amount of adsorption as well as the probable types of interactions involved in the adsorption. Kinetic information can also be obtained in some instances. Electrophoresis with test solutes reflects the overall effect of adsorption.

For this study, three types of capillaries were evaluated: (1) bare fused-silica capillaries, (2) capillaries coated with several thickness of cross-linked polyethylene glycol and (3) cross-linked polyethyleneimine. The polyethylene glycol column displayed much weaker electrostatic and similar hydrophobic and/or hydrogen bonding interactions as compared with underivatized fused-silica columns. The polyethyleneimine column exhibited poor performance for the test proteins used. Good electrophoretic performance seems to be possible only if adsorption measurements were below 0.2 ng/cm^2 (corresponding to 0.1% of available surface area) for all of the test proteins.

INTRODUCTION

Electrophoresis has long been an important separation technique for proteins. When high-performance capillary electrophoresis (HPCE) was developed in early 1980s, it was anticipated that HPCE would result in dramatically improved protein separations [1], and many successful separations have been reported. In the past several years, capillary zone electrophoresis [2–6], capillary gel electrophoresis [7] and capillary isoelectric focusing [8–10] have been applied to protein separations with good results. However, despite these successes, the routine application of HPCE to proteins and other macromolecules remains hindered by solute adsorption to the capillary walls.

In order to overcome the problems of protein adsorption, various methods have been applied, such as pH selection [2,3], high salt concentration [4] and surface modification [5,6]. The degree of adsorption is usually measured indirectly by the evaluation of column efficiency. However, separation efficiency alone can be an inaccurate indicator of adsorption problems, since column efficiency is affected not only by adsorption, but also by sample concentration [1,11], injection conditions [12,13] and applied voltage [3]. Second, even when efficiency problems are due to adsorption effects, little or no insight is gained as to the mechanisms of adsorption. To measure protein adsorption on capillaries, Green and Jorgenson [4] employed an elution chromatographic method and monitored the peak capacity factors (k') of proteins. This method was more reliable than column efficiency, but the

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accuracy of the method was limited, and it was difficult to measure weakly adsorbed solutes with $k' < 0.02$. Zhu *et al.* [14] reported a flow injection procedure to measure the residual adsorption of protein. This procedure was convenient but suffered from the low sensitivity inherent to on-column detection. Recently, Towns and Regnier [15] described a dual-detector method to monitor the peak area change of proteins in a capillary.

An important property of a capillary is its ζ potential. The ζ potential is generated by surface charges of the capillary. Therefore, the ζ potential reflects the potential for electrostatic interactions, as well as the expected capillary electroosmotic flow. The ζ potential can be estimated from the resulting electroosmotic flow, but more reliably by direct measurements of the streaming potential [16–18]. The streaming potential is a well-known electrokinetic property of a charged surface, and can be considered as the inverse property of electroosmotic flow. When a solution of ions flows through a charged tube, an electric potential, *i.e.*, the streaming potential, will arise. The relationship of the ζ potential to the streaming potential is [16]

$$\zeta = (4\pi\eta E_s/\varepsilon p)(K_b + 2K_s/R) \quad (1)$$

where η and ε are the viscosity and dielectric constant in the diffuse double layer, respectively, E_s is the streaming potential, p is the pressure difference across the tube, K_b and K_s are the specific bulk and surface conductance, respectively, and R is the radius of the tube. For large inner diameter capillaries operated under moderate electrolyte concentrations, $K_s/R \ll K_b$, and grouping the 4π constant and the units into a single constant, eqn. 1 can be simplified as follows:

$$\zeta = 4\pi\eta E_s K_b/\varepsilon p = 84.9 \cdot 10^7 \eta K_b E_s/\varepsilon p \quad (2)$$

In practice, η and ε are assumed to be equal to bulk values. η is in poises, K_b in $cm^{-1}\ \Omega^{-1}$, E_s in mV, p in cmHg and ζ in mV. Although the streaming potential measurements have been applied to evaluate capillaries used for HPCE [17,18], the precision (about 5 mV) was unsatisfactory, and no report on capillaries below 100 μm inner diameters was found.

During the course of research in our laboratory in the synthesis of new capillary bonding materials, it became necessary to evaluate surfaces for their adsorption characteristics and overall performance potential in HPCE. The evaluation procedures must be sensitive to adsorption, but insensitive to operating conditions. The procedures should also present information to distinguish among different interactions in the protein adsorption (hydrophobic, electrostatics, hydrogen bonding, etc.). In this paper, a three-step evaluation procedure was developed using streaming potential measurements, frontal chromatography of selected test solutes and overall column performance measurements under capillary zone electrophoresis (CZE) operating conditions. The capillaries bonded with polyethylene glycol (PEG) and polyethyleneimine (PEI) as well as bare fused silica were evaluated by the procedure.

EXPERIMENTAL

Streaming potential

The experimental setup is shown in Fig. 1. The nitrogen pressure was controlled by a Varian 3700 gas chromatograph (Sunnyvale, CA, USA), and was read out from a laboratory constructed mercury manometer. The nitrogen pressure could be applied to either reservoir by switching the six-port valve (Rheodyne 7000, Cotati, CA, USA) in order to alternate the flow direction. The volume of the reservoir was about 18 ml. The electrode was 0.5 mm O.D. Ag wire treated in 5 M HCl (+1.5 V for 10 min) [19]. The reservoirs and the capillary (typically length of 6–7 cm) were put into a metal thermostatic jacket which also functioned as a Faraday cage. The streaming potential was measured with a Keithley

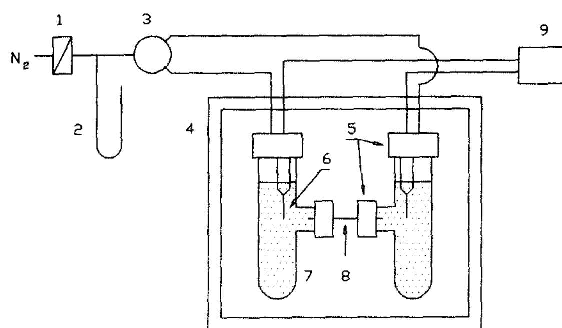

Fig. 1. Apparatus for streaming potential measurements. 1 = Pressure control valve; 2 = mercury manometer; 3 = 6-port valve; 4 = metal thermostatic jacket (Faraday cage); 5 = cap; 6 = Ag/AgCl electrode; 7 = glass reservoir with electrolyte solution; 8 = capillary; 9 = electrometer.

616 digital electrometer (Cleveland, OH, USA) with 0.01 mV resolution.

A typical electrolyte solution was 18 mM KCl + 0.5 mM KH_2PO_4 + 0.5 mM K_2HPO_4, using NaOH solution to adjust the pH to 7.2 and applying one drop of 0.5% $AgNO_3$ solution into 100 ml solution. Before measuring, capillaries were equilibrated overnight with the electrolyte solution, then the solution was pumped back and forth (about 10 min each time) until the difference of potential readings was below 0.1 mV. The streaming potential was measured at 4–5 different pressure values (typically, from 15 to 40 cmHg), and at each pressure, two readings with different flow directions were taken. From these data, the E_s/p ratio was determined from the regression equation of E_s–p plot.

Frontal chromatography

Fig. 2 shows a schematic graph of the frontal chromatograph used in this research. The nitrogen pressure was controlled by the Varian 3700 gas chromatograph, the split needle valve functioned as low pressure (below 10 cmHg) controller. The reservoir was a 4-ml glass vial and could be quickly changed. On-column detection was accomplished using a modified Spectroflow 757 absorbance detector (Kratos Analytical, Ramsey, NJ, USA) at 200 nm. The signal was recorded with an OmniScribe A5111-5 recorder (Houston Instrument, Austin, TX, USA). The typical effective length of capillary was 40 cm, the total length was 50 cm.

Three buffers were used, *i.e.*, 5 mM KH_2PO_4 + 5 mM K_2HPO_4–NaOH(pH 7.2), 20 mM acetic acid–NaOH(pH 4.7) and 20 mM H_3PO_4(pH 2.1). The dead time was determined by injection of water (for pH 7.2 and 4.7 buffers) or acetone solution (for pH 2.1 buffer), and the linear flow-rate was controlled within 0.9–1.1 mm/s.

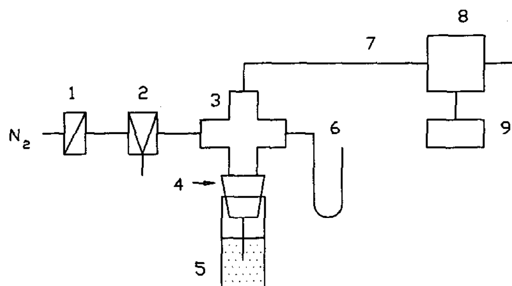

Fig. 2. Frontal chromatograph. 1 = Pressure control valve; 2 = split needle valve; 3 = four-way connector; 4 = rubber stopper; 5 = glass reservoir; 6 = mercury manometer; 7 = capillary; 8 = detector; 9 = chart recorder.

Three proteins (albumin, lysozyme and hemoglobin) were used as probes, and were dissolved separately in the measuring buffer at *ca.* 0.01 or 0.1 mg/ml. Two measuring procedures were performed: procedure A used the same capillary for all proteins, procedure B employed one capillary for just one protein. Both procedures start with fresh capillaries.

Procedure A. After a stable baseline was obtained with the buffer, a protein solution was flowed through the column until saturation was achieved and the breakthrough volume measured. Then, the column was cleaned as follows: 2–3 V_0 (column volume) of distilled water followed by about 15 V_0 1% sodium dodecyl sulfate (SDS, for negatively charged columns) or 1% cetyltrimethylammonium bromide (CTAB, for positively charged columns) followed by 2–3 V_0 distilled water then followed by about 15 V_0 buffer. Following this cleaning procedure, the next protein would be measured on the same column.

Procedure B. A single fresh column would be dedicated to one type of protein, with three identical columns from a single bonding being studied. For these studies, after breakthrough, the flow of protein solution was continued for about 1 V_0, then the flow was switched back to the buffer solution and keep running for at least 1.5 V_0. Then the columns were washed as follows: applying 7 p.s.i. nitrogen pressure for a 50 cm long column, wash cycles were 2–3 min with distilled water, 5 min with methanol, 15 min with methylene chloride, 5 min with methanol and 2–3 min with distilled water. Prior to any measurements, columns were equilibrated overnight with the appropriate buffers.

CZE performance evaluations

A Spectra Phoresis 1000 with a SP4400 integrator (Spectra-Physics, Reno, NV, USA) was used during capillary performance evaluations. All columns were 75 μm I.D. × 360 μm O.D., 35 cm effective length and 42.5 cm total length. The column temperature was 25°C. Running voltage was 12 kV. Injection conditions were 1 s for hydrodynamic or 5 s × 5 kV for electrokinetic injection. Detector wavelength was 200 nm with 0.5 s rise time.

Capillary modification

The capillary modifications using PEG 8M-10 and PEI-3M were produced by thermally cross-linking according to the temperature protocols recommended by the manufacturer. Prior to bonding, fused-silica capillaries (75 μm I.D. × 360 μm O.D., Polymicro Technologies, Phoenix, AZ, USA) of about 2 m in length were washed with a 0.1 *M* NaOH solution at 80°C for 1–1.5 h. After cooling, they were washed with distilled water followed by methanol. They were dried with nitrogen at 130°C for at least 4 h. The capillary was then filled to a length 12–15 cm with PEG 8M-10 or PEI-3M–methylene chloride solution (5–20%, w/v) and the solution was slowly pushed through the capillary with nitrogen. The capillary was then placed in the column oven of a Varian 3700 gas chromatograph under high-purity nitrogen flow. For PEG bonding, the temperature program was 30°C → 225°C at 5°C/min, holding at the upper temperature overnight (> 12h). For PEI bonding, the same procedure was used, but with a final temperature of 175°C. Following this cross-linking, each column was washed for half hour with methylene chloride, followed by methanol. The window on the modified capillary was created with hot sulfuric acid.

Reagents and supplies

PEG 8M-10 and PEI-3M polymer solutions were obtained from Innophase Corporation (Portland, CT, USA), bovine serum albumin (A-7030), chicken egg lysozyme (L-6876) and human hemoglobin (H-7379) from Sigma (St. Louis, MO, USA). A protein test mixture normally used for high-performance liquid chromatography was obtained from Applied Biosystems (Foster City, CA, USA), SDS from Bio-Rad Labs. (Richmond, CA, USA), acetic acid, phosphoric acid and sulfuric acid from J. T. Baker (Phillipsburg, NJ, USA), and all other chemicals from Fisher (Fair lawn, NJ, USA).

RESULTS AND DISCUSSION

Streaming potential

There are two requirements for the application of eqn. 2: a laminar flow and $K_s/R \ll K_b$. The flow situation in a capillary can easily be established by measuring the flow-rate and calculating the Reynolds number (R_e) as follows:

$$R_e = \rho u d/\eta \tag{3}$$

where ρ is the density of the solution in g/cm^3, u is the linear flow velocity in cm/s, d is the inner diameter of the capillary in cm, and η is the viscosity of the solution in poises. For example, applying 30 cmHg pressure, the flow-rate in a 6 cm long × 75 μm I.D. capillary is 33 μl/min or 12.4 cm/s, thus, $R_e = 9.3$. Since a turbulent flow corresponds to a Reynolds number >2000 in a straight open tube, all measurements could safely be assumed to in the laminar region.

When the inner diameter of a capillary is larger than 100 μm, it is usually believed that $K_s/R \ll K_b$. However, when the inner diameter is reduced to 50 or 25 μm, K_s/R term may no longer be negligible. To establish the magnitude of the K_s/R term, two capillaries (A and B) with different inner diameters must be used. Assuming both capillaries have the same ζ potential, from eqn. 1, we have

$$(E_s/p)_A(K_b + 2K_s/R_A) = (E_s/p)_B(K_b + 2K_s/R_B) \tag{4}$$

after rearrangement and for convenience letting $S_A = (E_s/p)_A$, $S_B = (E_s/p)_B$,

$$K_s = (1 - S_A/S_B)K_b/2(S_A/(S_BR_A) - 1/R_B) \tag{5}$$

In this research, a 100 μm and a 25 μm I.D. capillary were used to estimate the surface conductance, and in the pH 7.2 buffer (ionic strength, 20 m*M*), K_s is $1.5 \cdot 10^{-7}\ \Omega^{-1}$ according to eqn. 5. For a 25 μm I.D. capillary, K_s/R is $1.2 \cdot 10^{-4}\ (\Omega\ \text{cm})^{-1}$ and corresponds to 4.7% of the bulk conductance [$K_b = 2.53 \cdot 10^{-3}\ (\Omega\ \text{cm})^{-1}$]; 50 μm, 2.4%, and 100 μm, 1.2%. These results represent the most severe possible error from this source in regards to eqn. 2. There is also possible error in the K_s value due to differences in the ζ potential of the two capillaries, despite their being made of the same material, and being pre-treated equally. Nevertheless, the magnitude of these possible errors are still relatively small, and for capillaries with diameters on the order of 50–100 μm I.D., the surface conductance is negligible in the streaming potential measurement, although for 25 μm I.D. capillaries it can start to become significant.

The quality of the electrode has a great effect on the streaming potential measurement. Poor electrodes show potential differences and drift. It was found that an acceptable electrode could be made with freshly sanded silver wire, and that the potenti-

al difference between two electrodes could be kept below 0.02 mV. The flow direction has been reported to affect the streaming potential [16]. In this research, differences in potentials measured in different flow directions were also observed. However, the magnitude of the effect was only about 2%, and by taking average measurements, the effect of this difference on the E_s/p value is negligible. Moreover, the direction reversal operation is still necessary in order to keep the liquid level even in the reservoirs, and thus prevent syphon-induced flow.

Using the streaming potential to calculate the ζ potential was found to be convenient and reliable, especially for the modified capillaries with very low ζ potential, which would result in low electroosmotic flows. Measurements required only about 1 h (excluding capillary equilibration time). The only limitation of the method is the need for a precision electrometer, and reduction of extraneous noise through the use of a Faraday cage. The evaluation results of some modified capillaries are summarized in Table I.

Frontal chromatography

Potential interactions between proteins and the capillary wall are very complex, with various mechanisms such as electrostatic attraction, hydrophobic interaction and hydrogen bonding, as well as protein-specific geometries, all contributing to the magnitude of adsorption. No single measurement could ever reliably predict capillary performance under all conditions. The ζ potential is only an indicator for the possibility of electrostatic interactions, and more information can be obtained from the direct measurement of adsorption.

An accurate and convenient method to measure adsorption is that of frontal chromatography, which has long been applied to both gas–solid and liquid–solid adsorption measurements [20,21]. Frontal chromatography involves the transportation of a continuous flow of adsorbate solution through a column containing the adsorbent by a inert carrier. Upon saturation of the column, the adsorbed material is eluted by the carrier alone, and the concentration rises to the bulk adsorbate concentration. A typical signal profile of the frontal chromatography is shown in Fig. 3. From frontal elution measurements, four kinds of information can be obtained:

(1) The quantity of solute adsorbed. It is obvious that the sample loss between t_0 and t_R is caused by adsorption in the column, and the amount of adsorption (Q_{ad}) is [21]

$$Q_{ad} = (t_R - t_0)FC_0 \tag{6}$$

where t_R and t_0 are the retention and dead time, respectively, F is the flow-rate, and C_0 is the sample concentration. For capillaries, Q_{ad} is easily converted into adsorption per unit surface area, since the surface area of the adsorbent is equal to the inner surface of the capillary.

(2) The residual adsorption. This parameter is measured from the concentration difference between the b and c lines (Fig. 3), and can be expressed in percentage relative to the sample concentration. Irreversible adsorption will lead to a high residual percentage.

TABLE I

STREAMING POTENTIAL (E_s) AND ζ POTENTIAL OF COLUMN

Column	pH[a]	E_s (mV)	ζ (mV)
Bare	7.2	2.97–12.42	−73.1
5% PEG	7.2	1.14–2.78	−24.4
10% PEG	7.2	–	−16.0
20% PEG	7.2	0.06–0.14	−0.6
20% PEI	7.2	1.56–3.83	−22.7
20% PEI	4.7	2.18–5.72	+47.0

[a] Ionic strength = 20 mM.

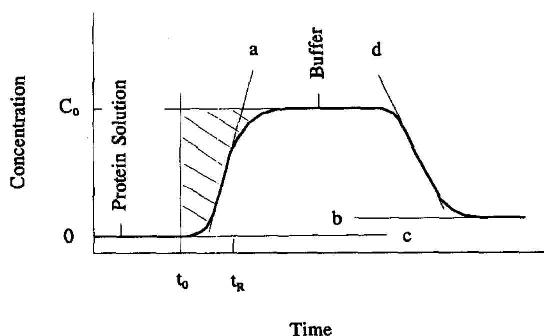

Fig. 3. Signal profile of frontal chromatography. a = Adsorption slope; b = ending baseline; c = beginning baseline; d = desorption slope. t_R is determined from the shaded area. C_0 = sample concentration.

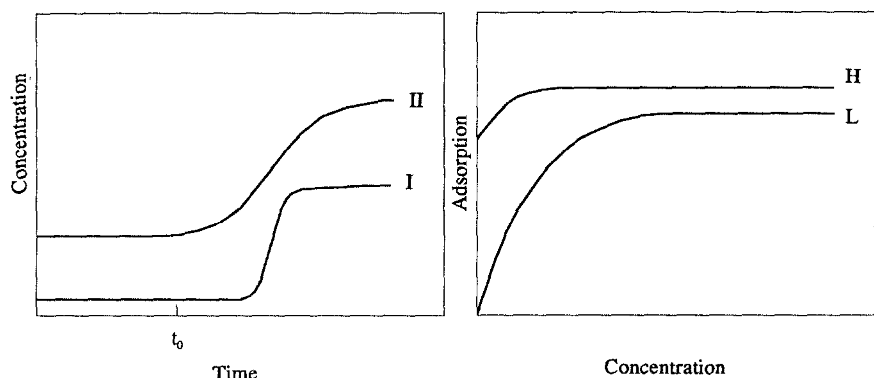

Fig. 4. Frontal chromatographic curves and adsorption isotherm types. Curve I corresponds to H-type isotherm, curve II to L-type isotherm.

(3) The ratio of desorption slope over adsorption slope, which is related to the adsorption isotherm and/or kinetic rate [22,23]. The slope is determined at the inflection point.

(4) Estimation of the shapes of adsorption isotherms and the overall adsorption rates [22,24], especially for the recognition of strong interaction. For example, in Fig. 4, a delayed and steep frontal chromatographic curve (curve I) corresponds to a H-type isotherm, which indicates a very strong adsorption onto part of the surface [22].

In adsorption measurements, the selection of probe proteins are critical. Ideally, each protein should be most sensitive to one type of interactions. Some properties of the probe proteins used in this research are summarized in Table II. As a probe, albumin tends to indicate the hydrophobic interaction as well as hydrogen bonding, provided that the capillary is of either same sign charge or neutral. Lysozyme is positively charged at pH values less than *ca.* 11, and thus will be sensitive to the electrostatic interactions with anionic sites. Hemoglobin was chosen because of its high surface activity [28].

The preliminary results of adsorption evaluations using these test proteins are listed in Table III. In neutral buffer, the adsorption of albumin on the bare capillary is quite weak, while lysozyme and hemoglobin demonstrate very strong adsorption. When the capillary is modified with PEG, the adsorption of albumin remained approximately constant, while the adsorption of lysozyme and hemoglobin were substantially reduced. Further more, the thicker the PEG coating, the weaker the adsorption. This change indicates that the dominant interaction under these conditions is probably elec-

TABLE II

PROPERTIES OF PROBE PROTEINS

Protein	Mol.wt.[a]	pI[a]	Hydrophobicity[b]	Carbonyl point[c]
Bovine albumin	66 000	4.7	2729	77
Egg lysozyme	14 000	11	25	19
Human hemoglobin	64 500	6.9–7.4	–	–

[a] Refs. 2, 6 and 25.
[b] Ref. 26 (measured by fluorescence probe method using *cis*-parinaric acid).
[c] Refer to the number of carbonyl surface contacts per molecule (ref. 27).

TABLE III

ADSORPTION OF PROTEIN ON COLUMN

Column	pH	Adsorption (ng/cm^2)[a]		
		Albumin	Lysozyme	Hemoglobin
Bare	7.2	0.6	206	254
5% PEG	7.2	<0.4	55.2	7.2
20% PEG	7.2	0.4	3.6	1.9
20% PEI	7.2	73.2	181	273
20% PEI[b]	4.7	328	14.5	50.8

[a] Measured by procedure A, 0.1 mg/ml protein.
[b] Measured by procedure B, 0.1 mg/ml protein.

trostatic, since the ζ potentials are progressively lower from the bare to 20% PEG bonding capillaries. It should be emphasized that even at its p*I*, hemoglobin still has obvious electrostatic interaction with the capillary. The PEI column is negatively charged at pH 7.2, but the adsorption of albumin (also negatively charged) is much stronger than that on the bare column. Therefore, strong hydrophobic interaction or hydrogen bonding must take place on the PEI column. Interactions other than electrostatic can also explain the significantly higher amount of adsorption of lysozyme and hemoglobin on the PEI column than those on the 5% PEG column, since both columns have similar ζ potentials (see Table I). At pH 4.7, the PEI column is positive charged, the adsorption of lysozyme and hemoglobin is reduced, especially lysozyme, but it is still higher than that on the 20% PEG column, while the adsorption of albumin is greatly increased. These adsorption characteristics indicated that under the condition studied, PEI column was generally poor for protein separations.

From Table III, it is found that the 20% PEG column produced the weakest adsorption. A more critical evaluation (procedure B, 0.01 mg/ml protein) was performed, with the results being tabulated in Table IV. On the 20% PEG column and at pH 7.2, all three proteins demonstrate similar adsorption. The equal amount of adsorption of albumin and lysozyme implies that the electrostatic interaction is negligible. The obvious difference of lysozyme relative to albumin and hemoglobin is its low slope ratio, *i.e.*, its desorption slope is much shallower than its adsorption slope. A low slope ratio is known to be caused by a non-linear adsorption isotherm [22] or by a slow desorption rate [23], and will deform the peak shape and lower the column efficiency. The relationship of the slope ratio to the modification layer is currently unknown.

TABLE IV

ADSORPTION OF PROTEIN ON 20% PEG BONDING COLUMN

Measured by procedure B, 0.01 mg/ml protein.

Protein	pH	Adsorption (ng/cm^2)	Slope ratio	Residual
Bovine albumin	7.2	1.0	0.96	−2%
Egg lysozyme	7.2	1.0	0.60	0%
Human hemoglobin	7.2	0.85	1.05	5%
Bovine albumin	2.1	0.24	0.93	0%
Egg lysozyme	2.1	0.22	0.89	0%
Human hemoglobin	2.1	0.09	0.96	0%

At pH 2.1, the adsorption of all proteins is reduced further, and the slope ratio of lysozyme is improved. From the amount of adsorption and the dimension of each molecule, the fractional surface coverage can be calculated. An adsorption of 0.24 ng/cm^2 of albumin (30 × 150 Å) [29] corresponds to 0.10% coverage, 0.22 ng/cm^2 of lysozyme (45 × 30 Å) [30], 0.14%, and 0.09 ng/cm^2 of hemoglobin (64 × 55 Å) [31], 0.03%.

CZE performance evaluation

From the adsorption measurements, the most suitable column appeared to be that producing the lowest adsorption measurements. However, factors other than simple adsorption can affect the actual efficiency of the CZE capillary. Also, it was not clear by this data what the minimum adsorption value must be in order to produce acceptable separations, and whether any such lower adsorption limit would be true for a variety of proteins. Therefore, the final test of capillary surfaces was the efficiency and detectable concentration of test molecules under operating conditions.

From Table V, it can be seen that, at pH 7.2, although the protein on the 20% PEG column is only about 1 ng/cm^2 (see Tables III and IV), the CZE separation of proteins is still significantly degraded. At pH 2.1, the adsorption is reduced to 0.2 ng/cm^2 and column efficiency improved dramatically. It is believed that the reduction of adsorption is the major factor which contributes to the efficiency improvement, although it may not be the exclusive one. Fig. 5 shows the CZE separation of the probe proteins at pH 2.1 on bare and 20% PEG capillaries, while Fig. 6 presents the CZE separation of a test mixture of proteins frequently used for reversed-phase liquid chromatography. The similar column efficiency of lysozyme on the PEI column and albumin on the bare column cannot be explained from the amount of adsorption.

The behavior of lysozyme on the 20% PEG column was unexpected. At pH 7.2, lysozyme should produce efficiencies similar to albumin since they

TABLE V

COLUMN EFFICIENCY MEASURED FROM PROBE PROTEIN

0.1 mg/ml protein. The values in the Table are theoretical plate numbers.

Protein	Bare		20% PEI	20% PEG	
	pH 7.2	pH 2.1	pH 4.7	pH 7.2	pH 2.1
Bovine albumin	$3.0 \cdot 10^3$	$1.3 \cdot 10^3$	No peak	$0.5 \cdot 10^3$	$10 \cdot 10^3$
Egg lysozyme	No peak	$24 \cdot 10^3$	$4.0 \cdot 10^3$	Irreversible	$70 \cdot 10^3$
Phenol(neutral)	$55 \cdot 10^3$	–	–	–	–

have a similar degree of adsorption and no irreversible adsorption. However, during CZE operation, lysozyme demonstrates irreversible adsorption on the PEG column at pH 7.2 and gives a signal like that of frontal chromatography. The reasons are unclear at present.

In addition to the column efficiency, adsorption also affects the detectable concentration in CZE analysis. The direct measurement of the detectable concentration seems not convenient. Instead, the intercept of the plot of concentration *vs.* signal in the linear range of detection can be the alternative parameter that is parallel to the detectable concentration. For example, on 20% PEG column, at pH 2.1, the regression equation for albumin (0.02–0.2 mg/ml) is

$$C = 0.00626 + 0.00521\,A\ (r^2 = 0.9996) \tag{7}$$

and for lysozyme (0.02–0.2 mg/ml)

$$C = 0.000196 + 0.00634\,A\ (r^2 = 0.9996) \tag{8}$$

where C is the sample concentration in mg/ml and A is the peak area in 10^4 count. The intercepts imply that when the concentrations of albumin and lyso-

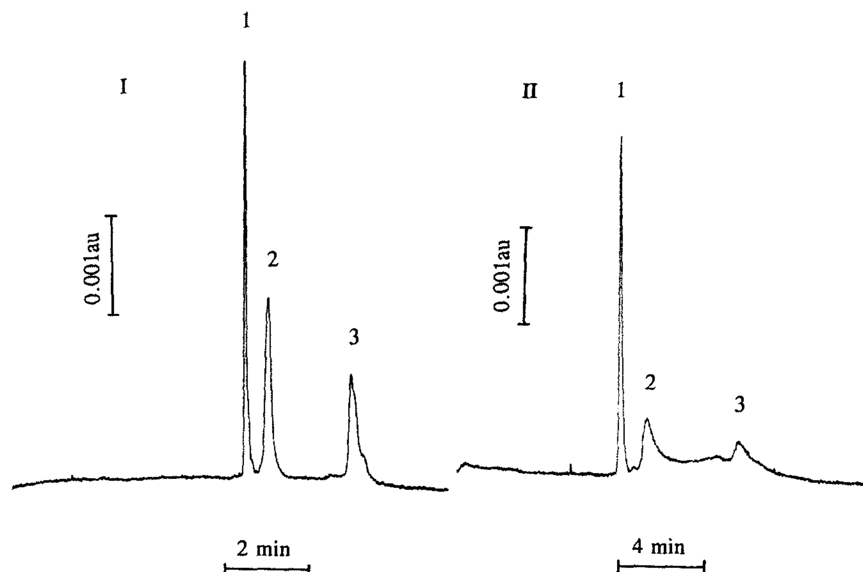

Fig. 5. Electropherograms of probe proteins. 20 mM H_3PO_4, pH 2.1. 5 s × 5 kV injection and + 12 kV running. Peaks: 1 = lysozyme; 2 = albumin; 3 = hemoglobin; 0.1 mg/ml each. (I) 20% PEG bonding capillary; (II) bare fused-silica capillary.

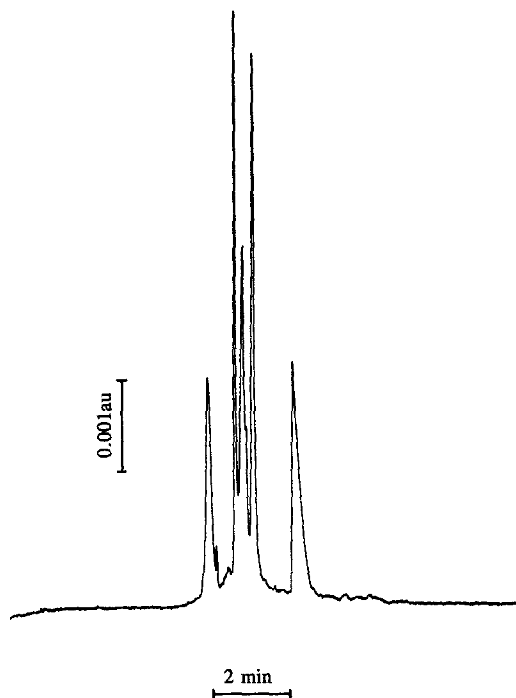

Fig. 6. Separation of protein mixture for reversed-phase high-performance liquid chromatography (insulin, cytochrome *c*, lactalbumin, carbonic anhydrase and ovalbumin). 20% PEG column, 20 m*M* H_3PO_4–NaOH, pH 2.5. 5 s × 5 kV injection and +15 kV running.

zyme are below 0.00626 and 0.00196 mg/ml, respectively, no peak can be detected. Although factors other than adsorption also contribute to non-zero intercepts, intercepts may be used to roughly estimate the degree of adsorption measured under the same conditions.

CONCLUSIONS

A three-step procedure is proposed to evaluate capillaries used for protein separations in capillary electrophoresis. The ζ potential determined from the streaming potential characterizes potential for capillaries to participate in electrostatic interactions and the expected electroosmotic flow. A simple streaming potential measuring apparatus was designed, which is able to measure capillaries with 25–100 μm I.D. and with submillivolt precision. Frontal chromatographic measurement with selected probe proteins can indicate the amount of adsorption, the major interactions in the adsorption and some thermodynamic or kinetic properties of the adsorption. The simple frontal chromatographic device used in this research is able to measure adsorption as low as 0.1 ng/cm^2. During CZE operation, the adsorption is evaluated by peak efficiencies and the intercept of the plot of concentration *vs.* peak area.

Using the above procedures, both bare and bonded capillaries were evaluated. Generally, the 20% PEG column displays much weaker electrostatic and similar hydrophobic and/or hydrogen bonding interactions compared with the bare capillary, and performed quite well at pH 2.1. However, at pH 7.2, lysozyme demonstrated irreversible adsorption on the PEG column. PEI-bonded surfaces showed generally poor behaviour for proteins separation. It appears that protein adsorption as measured by frontal chromatography should be below 0.2 ng/cm^2 or *ca.* 0.1% of the capillary surface area in order to obtain good performance in CZE analysis.

ACKNOWLEDGEMENT

This research is supported by the Center for Biotechnology, SUNY-Stonybrook and by Spectra-Physics Analytical Division. We also thank Dr. A. Yacynych at Rutgers University for his advice on the preparation of the electrodes.

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