

Electrokinetic studies of inorganic coated capillaries

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Abstract

The procedures for the preparation of silica capillaries coated with titanium oxide or aluminum oxide are developed. These inorganic coated capillaries are studied for their applicability in capillary electrophoresis. The points of zero charge are measured as pH 5 and pH 7 for titanium oxide- and aluminum oxide-coated capillaries, respectively. Both titanium oxide and aluminum oxide coatings give better protein separations in comparison to the use of fused-silica capillaries. Separation efficiency of lysozyme as model protein is measured in the range of 20 000 theoretical plates/m of inorganic coated capillaries. However, the hydrophobic interaction between proteins and modified capillary wall possibly contributes to the tailing of observed protein peaks.

1. Introduction

Silica capillaries are the most commonly used tubing in capillary electrophoresis (CE) and exhibit the electroosmotic flow toward the cathodic end when the solution pH is above 2 [1]. Reported attempts for affecting the ζ potential and the electroosmotic flow at the silica-aqueous interface included the use of surface-active agents, buffer pH, buffer composition, temperature, or chemical derivatization of the surface [2–6]. In addition, a recent approach involving the use of a radial electric potential gradient across the capillary wall for the direct control of the ζ potential and the electroosmosis was demonstrated [7–11].

Clearly, a surface with a wider and/or differ-

ent range of the ζ potential and the electroosmosis from silica substrate is desirable and can provide alternatives in the applications of CE. For example, capillaries made from organic synthetic materials such as polyfluorocarbon, polyethylene or poly(vinyl chloride) were studied for their electrokinetic behavior in the aqueous-organic solvent mixture [12]. It is suggested that the dependence of the surface charge on the solution pH in these capillaries was caused by the protolysis of carboxylic groups located at the surface [12]. The ζ potential and the electroosmosis was about half of that of fused-silica capillary under comparable conditions.

In this study, silica capillaries coated with titanium oxide or aluminum oxide are prepared. It has been reported that Ti–O–Si and Al–O–Si bonds are chemically stable [13] and should be appropriate for most applications in CE. With the application of radiotracer adsorption method, the point of zero charge (PZC) for titanium

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oxide and that for aluminum oxide were measured to be 5.9 and 7.6, respectively [14,15]. It is anticipated that the ζ potential and the electroosmosis on these inorganic coated surfaces should be much lower than those of silica substrate at neutral pH. The electrokinetic behavior of inorganic coated silica surfaces are studied with the Gouy–Chapman–Stern–Grahame (GCSG) model (see ref. 16) and compared with the measured electroosmotic mobilities at various solution pH values. Preliminary protein adsorption studies on these newly generated inorganic surfaces are presented and discussed.

2. Experimental

2.1. General

A complete CE system with a UV detector as described elsewhere was used in this study [10]. Fused-silica capillaries of 50 μm I.D. \times 150 μm O.D. were obtained from Polymicro Technologies (Phoenix, AZ, USA) in the preparation of titanium oxide- or aluminum oxide-coated tubings. A constant electric field of 200 V/cm across the buffer solution in a 40 cm long capillary was applied for driving the electroosmotic flow. A 0.5% dimethyl sulfoxide solution was used as the neutral marker for monitoring the direction and flow-rate of the electroosmosis [17]. The distance between injection end and UV detector was 24 cm. The electroosmotic mobility was assigned as positive when the direction of flow was toward the cathodic end.

All chemicals were purchased from Aldrich (Milwaukee, WI, USA). Proteins such as lysozyme and myoglobin were obtained from Sigma (St. Louis, MO, USA). The running solution was prepared as 10 mM sodium acetate for solution $\text{pH} \geq 4$ or 10 mM acetic acid for solution $\text{pH} \leq 3$. The pH of the solution was adjusted with 0.1 M hydrochloric acid or 0.1 M sodium hydroxide. The solutions were formulated to contain appropriate amounts of sodium chloride for maintaining constant ionic strength.

2.2. Preparation of inorganic coated silica capillaries

Fused-silica capillaries were washed with 1 M NaOH at 80°C for 30 min followed by distilled water at 80°C for another 30 min. The tubings were then dried with nitrogen for 1 h. A solution of titanium isopropoxide–toluene (30:70, v/v) was passed through the tubings with dry nitrogen at 50°C for 90 min. Titanium isopropoxide reacted with surface-bound water and underwent hydrolysis. The hydrolyzed product, titanium hydroxide, would then react with the surface silanol groups to form a stable titanium oxide coating. The reaction solution was allowed to remain in the tubings for 10 min before it was replaced with fresh solution. This refurbish of reaction solution was repeated during the 90-min reaction period. At the end of the reaction period, the excess reagent in the capillaries was removed by nitrogen and the tubings were then kept at 200°C overnight. The same coating reaction process was repeated again with the modified capillaries in the next day. The titanium oxide-modified capillaries were washed with 0.1 M NaOH for 30 min followed by distilled water and the running buffer for 30 min of each step before the electrokinetic studies were carried out.

To obtain aluminum oxide-coated silica capillaries, a reaction solution of aluminum isopropoxide in toluene (0.2 M) was prepared. Following the same coating procedures as described above, the aluminum oxide-modified capillaries was kept at 200°C for one week to obtain a stable surface coating. The capillaries were washed with the same steps as described above before the electroosmotic mobility was measured.

3. Results and discussion

To provide a basis for the comparison with inorganic coated tubings, the electroosmotic mobilities of fused-silica capillary at various solution pH values in 10 mM acetate were measured. The

Table 1
Modeling parameters used in the GCSG model

Substrate	Dissociation constant		Binding constant of		Surface site density N_s (sites/cm ²)
	pK_{a1}	pK_{a2}	Cations, pK_M	Anions, pK_A	
Silica oxide	-0.2	5.8	6.8		$5 \cdot 10^{14}$
Titanium oxide	2.7	9.1	4.5	7.2	$12.2 \cdot 10^{14}$
Aluminum oxide	5.2	11.8	9.2	7.9	$8 \cdot 10^{14}$

measured electroosmotic mobilities were then compared with the predictions based on the GCSG model [16]. The modeling parameters used in the calculation such as the surface density of silanol groups, the dissociation constants of silanol groups, and the binding constant of counter-ions at the silica–aqueous interface were reported by Scale *et al.* [18] and summarized in Table 1. As shown in Fig. 1, the predictions were in good agreement with the measured electroosmotic mobilities. The results shown in Fig. 1 support the use of a GCSG model for investigating the electrokinetic behavior in the inorganic coated capillaries.

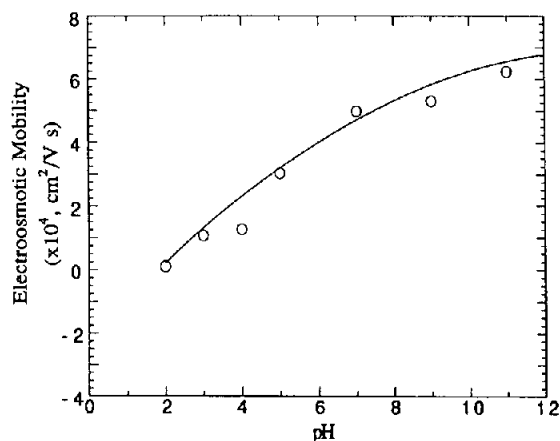


Fig. 1. Plot of the electroosmotic mobility, μ_{eo} , in a fused-silica capillary against the solution pH in 10 mM acetate. The open circles and the solid line represent the measured electroosmotic mobilities and theoretical prediction, respectively. The error in the measurement of electroosmotic mobility was about 2–5% for over 5 runs at various solution pH values.

3.1. Titanium oxide-coated capillaries

As shown in Fig. 2, the measured electroosmotic mobility on the titanium-coated surface was about $-3.0 \cdot 10^{-4}$ cm²/V s at pH 3 and leveled off to $2.9 \cdot 10^{-4}$ cm²/V s at pH 11. The PZC was shifted from pH 2 for the fused-silica capillary to about pH 5 for the titanium oxide-coated capillary. The measured PZC was smaller than pH 5.9 as reported in the literature for sol material [14].

The modeling parameters including the dis-

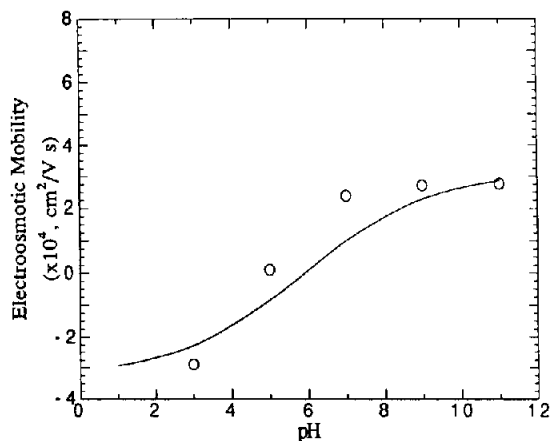


Fig. 2. Plot of the electroosmotic mobility, μ_{eo} , in a TiO₂-coated capillary against the solution pH in 10 mM acetate. The open circles and the solid line represent the measured electroosmotic mobilities and theoretical prediction, respectively. The error in the measurement of electroosmotic mobility was about 2–5% for over 5 runs at various solution pH values.

sociation constants, pK_{a1} and pK_{a2} , the binding constants of counter-ions, pK_M and pK_A , and the surface site density, N_s , were obtained for rutile titanium oxide [19,20] and summarized in Table 1. As shown in Fig. 2, the predictions based on the GCSG model were in good agreement with the measured results and supported the existence of titanium oxide coating on the silica surface. In addition to smaller PZC observed in this study, the measured electroosmotic mobilities at the solution pH above PZC were all larger than the predictions. These observations might indicate that the dissociation constant of pK_{a2} was smaller than the literature value used in the calculation. It is possible that the less hydrated surface obtained in this study results in a larger dissociation constant as explained by Nawrocki [13].

3.2. Aluminum oxide-coated capillaries

As shown in Fig. 3, the measured electroosmotic mobility in the aluminum oxide coated silica capillary was varied from $-2.3 \cdot 10^{-4} \text{ cm}^2/\text{V s}$ at pH 3 to $2.8 \cdot 10^{-4} \text{ cm}^2/\text{V s}$ at pH 11. The modeling parameters reported for γ -aluminum oxide [21] were used in the calculation and summarized in Table 1. As shown in Fig. 3, the

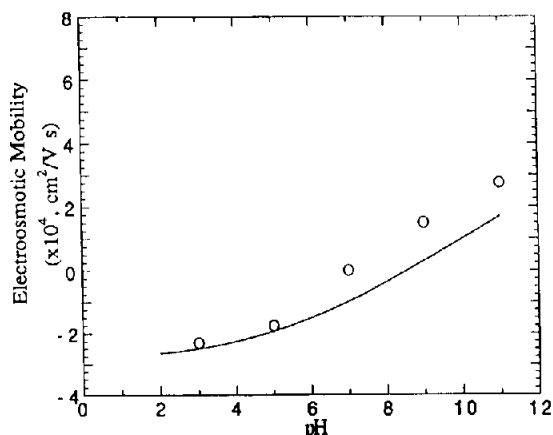


Fig. 3. Plot of the electroosmotic mobility, μ_{eo} , in a Al_2O_3 -coated capillary against the solution pH in 10 mM acetate. The open circles and the solid line represent the measured electroosmotic mobilities and theoretical prediction, respectively. The error in the measurement of electroosmotic mobility was about 2–5% for over 5 runs at various solution pH values.

excellent agreement between the predictions based on the GCSG model and the measured electroosmotic mobilities indicated the existence of aluminum oxide coating on the silica surface.

The PZC was shifted from pH 2 for the fused-silica capillary to near pH 7 for the aluminum oxide-coated tubing. The measured PZC was smaller than the reported value of 7.6 [15]. The lower PZC observed in this study could be attributed to the smaller pK_{a2} in the coated surface than the literature value used in the calculation. It is possible that the less hydrated surface as the outcome of our coating procedure results in a larger dissociation constant as explained by Nawrocki [13].

3.3. Protein adsorption

Both titanium oxide- and aluminum oxide-coated silica capillaries as prepared in this study have low or near zero ζ potential at or close to neutral solution pH. Significant reduction of protein adsorption at these modified capillary surfaces in comparison with fused-silica capillaries is therefore anticipated. Two model proteins, lysozyme (pI 11) and myoglobin (pI 7.2) with the concentration of 1 mg/ml for each protein in the mixture were prepared in 10 mM acetate buffer at the solution pH of 5 and 7. As shown in Fig. 4, a satisfactory result for the separation of lysozyme and myoglobin in the titanium-coated capillary was observed at 10 mM acetate buffer and pH 5. The measured separation efficiency of lysozyme was in the range of 20 000 theoretical plates/m of capillary. This separation efficiency was better than the result obtained from fused-silica capillary which was below 5000 theoretical plates/m of capillary (not shown) with significant peak tailing.

As shown in Fig. 4, there was tailing for lysozyme separated in the titanium oxide-coated capillary at pH 5. In addition, the tailing of lysozyme peak was observed for the separations in the aluminum oxide-coated capillary at both pH 5 and 7 (not shown). At pH 5, the aluminum oxide-coated silica capillary exhibited a strong anodic electroosmosis and indicated the presence of a net positive surface charge. The observation

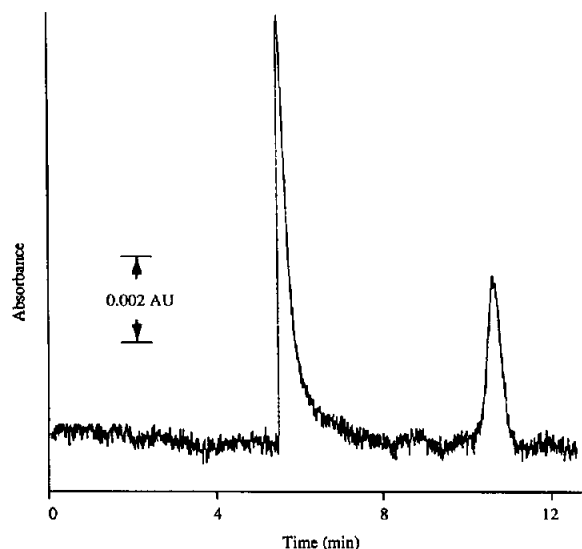


Fig. 4. Electropherogram of lysozyme and myoglobin in a TiO_2 -coated silica capillary at 10 mM acetate and pH 5. Elution order: lysozyme and then myoglobin.

of lysozyme tailing was probably due to the hydrophobic interaction of protein with the modified surfaces.

It has been reported that titanium oxide and aluminum oxide were suitable as bases for the strong adhesion with polyethylene glycol and carbohydrates [22]. In contrast, these substances did not attach well to untreated glass substrate [22]. The hydrophilic polymer such as polyethylene glycol (PEG) with various molecular masses (3350, 5000 and 15 000) was therefore added in the running buffer for the reduction of protein adsorption onto the modified surfaces. Before the protein separation, the modified capillary was loaded with the running buffer containing 5% (w/w) of PEG overnight. During the protein separation, both protein samples and running buffer contain 0.5% (w/w) of PEG. However, no significant improvement for lysozyme tailing was observed with the addition of PEG of molecular masses varying from 3350 to 15 000.

Finally, the inorganic coated silica capillaries prepared in this study were very stable under alkaline conditions. 0.1 M NaOH followed by distilled water and the running buffer was used

routinely during the measurement of electroosmotic mobility at various solution pH values. These inorganic coated surfaces with different range of the ζ potential and the electroosmosis from silica substrate may provide alternatives in the applications of CE.

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