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Novel poly(vinyl alcohol)-based column coating for capillary electrophoresis of proteins

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ABSTRACT

A novel and simple method for the preparation of chemically bonded poly(vinyl alcohol) (PVA) coating to silica capillary inner wall was developed, and the PVA-coated capillary columns were employed for capillary electrophoresis (CE). The coating procedure included pretreatment of the capillary inner wall, silanization, aldehyde group functionalization and PVA immobilization. Electroosmotic flow of the coated capillary was almost suppressed over a wide pH range (pH 3–10). High-efficiency separations of cationic proteins (including cytochrome *c*, lysozyme, α-chymotrypsinogen A) at pH 3.0–5.0 and of anionic proteins (including myoglobin and trypsin inhibitor) at pH 10.0 were achieved with the PVA-coated capillary. Moreover, a "dual-opposite-injection" approach was adopted for simultaneous separations of both cationic and anionic proteins at neutral pH with the prepared column. In this CE mode, positively charged proteins migrated from one end of the column to the detector while negatively charged proteins from the other end to the detection window. Good run-to-run repeatability was obtained in all of the protein CE separations performed in this work. The PVA-coated column can also afford long-term stable uses for protein separations, as demonstrated in 100 repeated uses using a single capillary with the relative standard deviation values of the retention times less than 0.9%. Moreover, good column-to-column reproducibility was demonstrated by protein CE separation with 10 different columns. The results indicate that the present method for PVA-coated capillary preparation is promising for protein CE applications.

1. Introduction

Protein separation has become an important aspect in protein drug analysis, clinic examination as well as the rapidly developing fields of proteomics. Having the advantages of high separation efficiency, short separation time, lower consumption of both samples and reagents as well as ease of automation, capillary electrophoresis (CE) has become a powerful separation technique for proteins [1–3]. However, CE in protein analysis is still limited due to the irreversible adsorption of proteins to naked silica capillary inner walls. The limitations usually pose many problems, such as loss of efficiency, tailing of peak, instability of baseline as well as lack of reproducibility of the migration time [4,5]. Coated capillaries are always applied to prevent or minimize the protein-wall interaction [6–10].

Capillary coatings for protein separations can be classified into two major categories: (1) dynamic coatings, in which the coating is reversibly adsorbed to the capillary inner wall [11–13]; (2) permanent coatings, in which the coating materials are chemically bonded to the surface or otherwise irreversibly immobilized as films on the capillary walls [7–10,14–18]. Compared with dynamic coatings, permanent coatings are favorable for protein separations since no additives are needed to introduce to both the sample solution and the running buffer. So far, many materials have been applied to produce permanent capillary coatings, such as polyethyleneimine [19–21], hydroxypropyl cellulose [22], polyacrylamide [7–8,23], polyvinyl alcohol (PVA) [9–10,24] and so on. Among all those coatings described in the literatures, PVA is considered to be the most hydrophilic polymer coating material [10]. The first PVA-based capillary coating was introduced by Gilges et al. [9]. In their work, the coating was generated by initially adsorption of PVA from an aqueous solution and then immobilization of the adsorbed polymer film through thermal treatment. To further improve the PVA coating stability, Belder et al. prepared a cross-linked PVA coating by pretreating the capillary with a glutaraldehyde cross-linking agent followed by a solution of PVA [10]. The polymer films prepared by the above methods were physically adsorbed to the capillary inner wall, so they may not as stable as that chemically coupled to

Abbreviations: CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid; CE, capillary electrophoresis; Cyt-*c*, cytochrome *c*; CZE, capillary zone electrophoresis; DMSO, dimethylsulfoxide; DOI, dual-opposite-injection; EOF, electroosmotic flow; Lys, lysozyme; MB, myoglobin; PVA, poly(vinyl alcohol); RSD, relative standard deviation; TI, trypsin inhibitor; α-Chy-A, α-chymotrypsinogen A.

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the capillary surface. To prepare a chemically bonded PVA coating, Karger and Goetzinger fabricated a poly(vinyl acetate) coating in a vinyl group modified capillary column [24]. PVA coating was then obtained by hydrolysis of the initially prepared coating. A drawback of the approach is that they are based on complicated multistep chemical reactions.

In this work, a novel and easy method for the generation of chemically bonded PVA coating for protein CE was presented. Electroosmotic flow (EOF) of the prepared capillary column was almost fully suppressed over a wide pH range from 3.0 to 10.0. Therefore, high-efficient separation of proteins was easily achieved with the PVA-coated capillary. Moreover, the prepared column was also used for simultaneous separation of both cationic and anionic proteins at neutral pH with the Dual-opposite-injection capillary zone electrophoresis (DOI-CZE) method. Besides, run-to-run and column-to-column reproducibilities of the prepared column in protein CE were also discussed in this work.

2. Materials and methods

2.1. Materials and instrumentation

Lysozyme (Lys), α-chymotrypsinogen A (α-Chy-A), myoglobin (MB), trypsin inhibitor (TI) and PVA (Av. Mol. Wt. 70,000–100,000) were purchased from Sigma (St. Louis, MO, USA). Cytochrome *c* (Cyt-*c*) was from Lianxing Chemical Reagents Company (Tianjin, China). (3-Aminopropyl)trimethoxysilane (ATPS) (97%) were obtained from Jingchun Chemical Reagents Company (Shanghai, China). Glutaraldehyde was bought from Guangfu Institute of Fine Chemical Industry (Tianjin, China). Other chemicals were all analytical reagent grade from local sources. Deionized water was used in all of the experiments. Untreated fused-silica capillaries of 50 μm ID and 375 μm OD were provided by Yongnian Optic Fiber (Hebei, China).

The CE experiments were performed on a P/ACE system MDQ capillary electrophoresis instrument (Beckman Coulter, Fullerton, CA, USA), as described previously [25].

2.2. Preparation of PVA coating

The chemical reactions involved in the fabrication of the PVA-coated capillary column are schematically shown in Fig. 1. The detailed synthesis method of the PVA-coated capillary was described in our recent publication [26].

2.3. EOF measurements

Capillaries (including both PVA-coated and uncoated capillaries) of 20 cm in effective length and 30 cm in total length were used in the experiments. Dimethylsulfoxide (DMSO) was selected as the EOF marker. Phosphate buffers (20 mM) of pH 3.0–9.0 were prepared to determine EOF at different pH values.

Two methods were used to measure the EOF. For the uncoated capillary column, EOF was determined by EOF marker injection (0.4 psi, 3 s) and a subsequent application of a high voltage (15 kV). For EOF measurement of the PVA-coated column, this method was not useful because the EOF of a coated column was extremely low, resulting in a very long migration time of EOF marker. In this work, a "twice-injection" method was developed for the measurement of the extremely low EOF. It was a modified method similar to that reported by Williams and Vigh [27], including the following three steps. First, EOF marker was injected to the column. Then, a high voltage (E) of 20 kV (used for cathodic EOF) or −20 kV (used for anodic EOF) was applied for 5–20 min. In this step, the injected marker migrated in the capillary driven by the EOF. In the third step, a second injection of marker was performed, and two marker bands were pushed to the detector by pressure (0.5 psi). EOF measured with this method can be calculated with the following equation.

$$\mu_{EOF} = \frac{L_t L_e (t_2 - t_1)}{t_2 t_e E}$$

where L_t is total length of the capillary (m), L_e the effective length of the capillary (m), t_2 the migration time of the second peak detected in step 3, t_1 the migration time of the first peak detected in step 3, t_e the voltage applying time in step 2, and E the applied voltage in step 2.

2.4. CE of proteins

A protein mixture containing 0.1 mg/ml Lys, 0.1 mg/ml Cyt-*c* and 0.1 mg/ml α-Chy-A was first prepared as the sample. Phosphate buffers (40 mM) of pH 3.0, 4.0 and 5.0 were used as the running solutions. Both PVA-coated and uncoated capillaries (L_e = 40 cm, L_t = 50.2 cm) were used for the CE of the protein sample. The applied voltage was 15 kV. Protein sample containing 0.5 mg/ml TI and 0.5 mg/ml MB was then prepared and separated with PVA-coated and uncoated capillary columns (L_e = 40 cm, L_t = 50.2 cm) using 20 mM NaOH/3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) of pH 10.0 as the running buffer. The applied voltage was −20 kV.

The DOI-CZE method was used for the simultaneous separation of cationic and anionic proteins at neutral pH in a single run. The protein mixture containing Lys, Cyt-*c* and TI was selected as the sample. A PVA-coated capillary of 30.2 cm in total length (20 cm from one end to the detection window and 10.2 cm from the other end to the detection window) was used as the column. The column was first washed with the running buffer (60 mM phosphate buffer, pH 7.0) for 10 min. Then, it was equilibrated with the running buffer under 15 kV until both the baseline and current strength were stable. Sample injection was performed by immerging both ends of the column into the sample vials and applying 5 kV for 4 s. During this step, positively charged proteins migrate into the capillary from the anodic end, while negatively charged proteins move into the column from the cathodic end. Thereafter, both ends of the capillary column were switched into the running buffer. A high voltage (15 kV) was then applied to the column for protein CE.

3. Results and discussion

3.1. Design of chemically bonded PVA coating

There are two methods for the fabrication of a chemically bonded polymer coating, namely, the "graft to" and "graft from" methods [28,29]. In the "graft from" method, the coated polymer grows from the capillary inner wall by polymerization. High temperature and complicated operations are usually required in the coating procedures. For example, Karger and Goetzinger [24] selected vinyl acetate as the monomer, and fabricated a poly(vinyl acetate) coating in a vinyl group modified capillary column by in situ polymerization at 75 °C. PVA coating was then obtained by hydrolysis of the initially prepared coating. High reaction temperature may induce solvent volatilization at both ends of the capillary column during the reaction, thereby resulting in coating inhomogeneity. In addition, complicated coating procedures may compromise the column-to-column reproducibility.

In this work, we developed a "graft to" method for the preparation of chemically bonded PVA coating. In the "graft to" method, the polymer was directly coupled or coated to the capillary inner wall. As can be seen in Fig. 1, after the capillary treatment with APTS and glutaraldehyde, PVA polymer was chemically grafted to the capillary inner wall through a simple aldehyde and hydroxyl groups reaction. Compared with the classical method of generating chemically bonded PVA coating [24], the present coating method