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Acidized Poly(vinyl alcohol) Coated Capillary in Electrophoresis†

ZHEN HUA MEI* and XIAO LIN LI
Key Laboratory of Eco-chemical Engineering, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao-266042, P.R. China
E-mail: mzh62@qust.edu.cn

In this work, a coated capillary is fabricated physically. The coating liquid contained poly(vinyl alcohol) and citric acid at the concentration of 1 % (w/v) and 0.1 mol/L, respectively. The capillary coating resulted in a good effect of preventing protein absorption on capillary wall as performing a capillary electrophoresis with amperometric detection.

Key Words: Coated capillary, Poly(vinyl alcohol), Capillary electrophoresis, Protein absorption, Peak tailing.

INTRODUCTION

After 1980, the analytical technology of capillary electrophoresis (CE) developed rapidly. Applications of capillary electrophoresis cover a wide range of analytes including small inorganic and organic species as well as large biomolecules. In particular, capillary electrophoresis technique plays a significant role in the growing field of bioanalytical studies[1-3]. According to Jorgenson's work[4], macromolecules as its small diffusion coefficient, would generate a greatest number of theoretical plates of 10^6/m also. However, due to the absorption of macromolecule (such as protein) to the bare capillary inner wall, it cannot achieve the theoretical separation efficiency practically. Especially, when regarded a basic protein as the analyte, a peak tailing would appear. It decreases the separation efficiency and sensitivity severely.

Several schemes was developed to avoid protein absorption: (1) adjusting pH of running buffer solution[5,6]; (2) adding additives or increasing the electrolyte concentration of running buffer[7,8]; (3) adhering neutral or positive molecules on capillary wall[9]; (4) coating the inner side of capillary prior[10-14]. Coated capillary is one of the effective methods for prevention of protein absorption in these methods. Continue researches in this field are still activated and necessary[15-23]. In the present paper, we attempted a mixed coating of poly(vinyl alcohol)[14,15] acidized with citric acid.

The coating techniques include three schemes: dynamic absorption[24,25], physical coating[16,18-20] and covalent binding method[14,17,21]. All of them have their own developable space. It is not certain which would method be more superior to the others. However,

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when contact electrochemical detection mode was used, the dynamic absorption coating method would not be adaptable because of the electrode-fouling coming from the mobile coating solutes. Therefore, physical coating method for present fabricating work is selected.

EXPERIMENTAL

The fused silica capillary of 50 μm i.d., 375 μm o.d. was purchased from Yongnian Reafine Chromatography Ltd. (Hebei, China). The import Nanofilm PAAm coated capillary was purchased from Sepax Technologies Inc (USA). 9323-HVPS type's of high-voltage power supply was purchased from Beijing Institute of New Technology (Beijing, China). Electrochemical analyzer (Model CHI800) was purchased from CH Instruments, Austin, TX, USA. Horseradish peroxidase (HRP, SERVA Electrophoresis GmbH), tris(hydroxymethyl) aminomethane (Tris, Chemical Reagent Co. of Medicine, China) and L-threonine (Tianjin Chemical Reagent company) are biological reagent. Poly(vinyl alcohol) (Tianjin Bodi Chemical Co. Ltd., China), citric acid (Shandong Laiyang Fine Chemical Plant, China) and 30 % hydrogen peroxid (Yantai Sanhe Chemical Reagent Co. Ltd., China) are analytical grade. All solutions were prepared by deionized water and kept overnight before use.

Coating procedure: (1) Preparing of coating solution, which contained poly(vinyl alcohol) and citric acid at the concentration of 1 % (w/v) and 0.1 mol/L, respectively and using HCl adjusted to pH 2.3; (2) Continuous injecting the coating solution into a dry and bare fused silica capillary (40 cm or so as needed) for a few minute by injector; (3) Continuous injecting dry air or nitrogen gas for about 48 h at the condition of vertical placement of the capillary at room temperature. In addition, the poly(vinyl alcohol) concentration of 1 % (w/v) in coating solution is a proper high concentration. When the concentration of poly(vinyl alcohol) is higher than 1 % (w/v) or it placed flatly, it would pluge the capillary during injection and coating process.

Running condition: The running solution contained tris 5 mmol/L, H_2O_2 5 mmol/L and L-threonine 100 mmol/L and adjusted to pH 4.0 by HCl. The substrate solution contained *o*-phenylenediamine and tris 20 mmol/L, respectively and adjusted to pH 4.0 by HCl. Sample solution contained free HRP 1 ng/L. The detecting parameters were separation voltage of 25 kV, detection potential of -0.6 V, electrical dynamic sample injecting time of 30 s at 25 kV, substrate reservoir height of 15 cm. The L-threonine used in running solution was used to shaping the peak.

RESULTS AND DISCUSSION

Comparison of cross-section view of capillaries: Fig. 1 showed the cross-section view of coated and bare capillaries. The coated capillary was measured with the inner diameter of 35.7 and 35.0 μm for homemade and import coated capillary, respectively, compared with the original bare capillary (50 μm i.d.). It showed the evident existence of coatings and that homemade and import coated capillaries had approximately the same thickness of coating at about 7.5 μm.

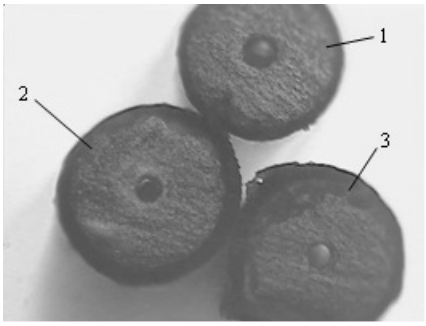

Fig. 1. Comparison of capillary cross-section: (1) bare capillary; (2) homemade-coated capillary; (3) import coated capillary. All of the three capillaries had the same original size of 50 mm i.d., 375 mm o.d.

Effect of coated capillaries: Aligned the coated capillary in equipment, running the electrophoresis with free HRP as the sample injection, the electropherograms were obtained. They were arranged together in Fig. 2, where as, curve 1, 2, 3 showed HRP signals with the use of bare capillary, poly(vinyl alcohol) coated capillary and acidized poly(vinyl alcohol) coated capillary, respectively. By measuring the peaks, the half-height peak width and bottom peak width were, respectively 18.8 and 93.6 s for peak 1; 8.4 and 73.6 s for peak 2; 4.6 and 28.0 s for peak 3. It is evident that the use of coated capillary has smaller peak tailing than uncoated bare one. And the effect of acidized coated capillary was again better than that of one fold poly(vinyl alcohol) coated capillary.

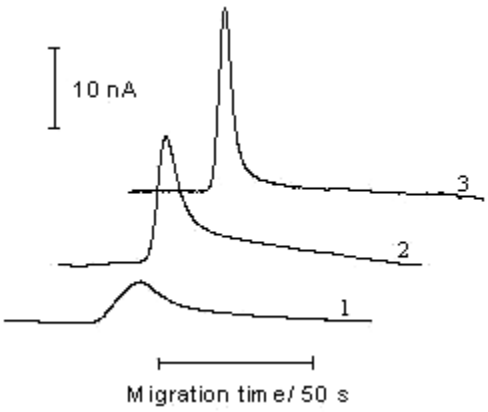

Fig. 2. Free HRP's electropherograms with: (1) bare capillary; (2) polyvinyl alcohol coated capillary; (3) acidized poly(vinyl alcohol) coated capillary. Running conditions: separation capillary size, 50 mm i.d. × 375 mm o.d. × 40 cm; reaction capillary length, 4 cm; detection mode, amperometry; reference electrode, Hg_2Cl_2/Hg; high voltage supply, 25 kV; detection potential, -0.6 V; injection time, 30 s; substrate reservoir height, 15 cm

The acidized coating had the advantages of neutralizing the external negative charge of fused silica in the inner wall of capillary, suppressing the absorption of positive molecules and without bothering with the turning buffer to an acid solution, *i.e.,* the running buffer could has its any necessary pH. The citric acid was chosen as the acidized reagent because of its buffer action at about pH 2.3 and not easily escaping from coating layer.

The acidized poly(vinyl alcohol) coated capillary has some stabilities. It could maintain its efficacy for about 40 days (details not showed here). When its lifetime is coming, it would decrease within 1 h. When an electrophoretic operation is over, the coated capillary should be maintained with the filling of acid solution at about pH 2.5.

Comparison of free HRP's electropherograms between homemade and import coating capillaries was shown in Fig. 3. It showed that the homemade-coated capillary had the same efficacy of desorption of HRP as compared with import coating capillary.

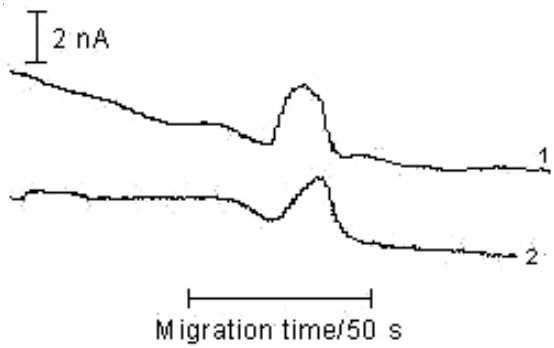

Fig. 3. Comparison of free HRP's electropherograms using: (1) homemade coated capillary; (2) import coated capillary. The running conditions were the same as in Fig. 2

Hydrophobic reverse of coating: After the coating, solution injected through the bare capillary, the wet coating need a proper drying treatment in order to prolonging the life time of coating capillary. Further experiments indicated that if the wet coating underwent an entirely drying at room temperature or especially at warmer temperature (50 ºC), the efficacy of coating disappeared. It gave a bad long peak tailing as shown in Fig. 4, curve 1. This might because of the change of conformation of solid poly(vinyl alcohol); the coating surface structure turned from hydrophilic state to hydrophobic state in hydrophobic gas environment (Fig. 4).

Fortunately, the hydrophobic reverse coating could be activated by hot water vapour. The coating surface structure turned back to hydrophilic state. Using the activated coating capillary, a sharp HRP peak appeared in the electroperogram as shown in Fig. 4, curve 2.

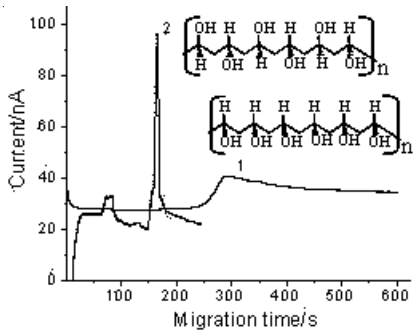

Fig. 4. Comparison of free HRP's electropherograms using: (1) hydrophobic reverse coating; (2) activated coating

Conclusion

Acidized poly(vinyl alcohol) coated capillary has an excellent property of preventing the absorption of protein on capillary wall. The coated capillary is simple to fabricate and has a certain period of lifetime. It offers alternative schemes in the field of coating capillary. In addition, the hydrophobic reverse property of poly(vinyl alcohol) may find its other used elsewhere.

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