ARTICLE

PMOXA/PAA Brushes toward on-Line Preconcentration for BSA in Capillary Electrophoresis

Xiao-ru Liu, Chao Pan, Yan-mei Wang*

CAS Key Laboratory of Soft Matter Chemistry, Department of Polymer Science and Engineering, University of Science and Technology of China, Hefei 230026, China

(Dated: Received on May 31, 2018; Accepted on June 7, 2018)

In this work, a binary-mixed-brushes-coated (BBC) capillary with switchable protein adsorption/desorption properties was developed and applied for on-line preconcentration of proteins. Firstly, amine-terminated poly(2-methyl-2-oxazoline) (PMOXA-NH_2) and thiol-terminated poly(acrylic acid) (PAA-SH) were synthesized by using cationic ring-opening polymerization (CROP) and reversible addition fragmentation chain transfer (RAFT) polymerization, respectively. Then, the BBC capillary based on poly(2-methyl-2-oxazoline) (PMOXA) and poly(acrylic acid) (PAA) was prepared by sequentially grafting of PMOXA-NH_2 and PAA-SH onto fused-silica capillary inner surface through poly(dopamine) (PDA) as an anchor. The obtained PMOXA/PAA coating formed on the capillary or capillary's raw material was characterized in terms of the thickness, surface chemical composition by using scanning electron microscope (SEM) and X-ray photoelectron spectrum (XPS). The switchable protein adsorption/desorption performance of the BBC capillary was investigated by using fluorescence microscope under different solutions with certain pH and ionic strength (I). The results showed that bovine serum albumin (BSA) could be adsorbed on BBC capillary at pH=5.0 (I=10^{-5} mol/L), and then the adsorbed BSA could be released at pH=9.0 (I=0.1 mol/L). This switchable protein adsorption/desorption property of coated capillary was then used to preconcentrate proteins on-line for increasing the detection sensitivity of BSA in capillary electrophoresis (CE). With this method, a sensitivity enhancement factor (SEF) more than 5000 for BSA detection was obtained.

Key words: Mixed brush coating, Switchable properties, On-line preconcentration, Capillary electrophoresis, Detection sensitivity

I. INTRODUCTION

Analysis of proteins is of great importance in clinical diagnostics and the rapidly developing field of proteomics [1]. Over the years various analytical techniques have been developed, and capillary electrophoresis (CE) is at the forefront of the most powerful analytical tools [2–5] for protein separation due to its high separation efficiency, high resolving power, short analysis time, and low mass detection limit [6]. In commercial CE instrument, ultraviolet (UV) detector is the most commonly used one [7–9] because it is cheaper and more convenient than laser induced fluorescence detector, mass spectrometry and others. However, it often suffers from the poor detection sensitivity owing to its short optical path length and small injection volume [4, 10, 11]. In order to overcome these shortcomings, some approaches have been developed [12]: (i) use of alternative capillary geometry to increase the optical path length, (ii) concentration of the sample solution before CE performance, and (iii) sample preconcentration strategies during CE performance. The first approach frequently suffers from limited improvement because of the difficulty in designing detectors with other geometry. The second approach, usually called off-line sample preconcentration, could concentrate the diluted sample by using filtration, centrifugation, distillation and so forth. However, it has to bear massive organic solvent consumption, cumbersome operation, environmental pollution, *etc.* The last one, commonly called on-line sample preconcentration, which can enrich the concentration of the sample in the process of CE, is particularly worthy of investigation since it can be easily accomplished by carefully controlling the operation conditions on a commercial CE instrument equipped with a UV detector [10–12].

A number of on-line sample preconcentration [13–18] techniques have been developed to improve the detection sensitivity of CE over these years, including stacking, sweeping, capillary isotachophoresis (CITP), and so on. Stacking is one of the most widely used techniq-

*Author to whom correspondence should be addressed. E-mail: wangyanm@ustc.edu.cn, Tel: +86-551-63607652

ues in on-line sample preconcentration due to its diversity (*i.e.*, field-amplified sample stacking, dynamic pH junction, large-volume sample stacking with reversed polarity, *etc.*) [19, 20]. Though the sample stacking method could improve the detection sensitivity of CE, it can not be used for analyzing very dilute sample since its maximum injection volume should be less than the column volume. Therefore, on-line sample preconcentration combined with the on-line sample extraction method has been used to overcome the drawbacks mentioned above. For example, Li *et al.* [21] have developed a negatively charged sol-gel coating based on sulfonate groups for on-line preconcentration of myoglobin (MB, pI≈7.07) in CE. The negatively charged sol-gel coated capillary was used to extract myoglobin through electrostatic interaction with a buffer solution of pH value lower than the pI value of MB firstly. The extracted analytes were then desorbed and focused by a buffer solution whose pH higher than the pI value of MB under normal electroosmotic flow (EOF). In their work, the sample volume that could be used for analytes enrichment was not limited due to the enhanced surface area and appropriate surface charge of negatively charged so-gel coated capillary. The successful combination of on-line extraction in full length of the prepared capillary and dynamic pH junction has provided a sensitivity enhancement factor (SEF) of about 3000 for MB. Xu *et al.* [1] have prepared a negatively charged tentacle-type polymer-coated capillary column. Then the polymer-coated capillary was used to extract the MB through the electrostatic attraction between the positively charged MB and the negatively charged tentacle-type polymer coating when the pH value of background electrolyte solution (BGE) was lower than pI value of MB. The extracted analytes were desorbed and focused by a buffer solution whose pH value was higher than the pI value of MB under EOF in the opposite direction to the sample injection flow. By the method, more than 1500-fold sensitivity enrichment was obtained for MB, because the loading capacity of capillaries was not limited by the capillary volume. The above studies have improved the detection sensitivity of protein effectively through changing the electrostatic interaction between the negatively charged polymer coated capillary and protein by varying pH values of BGE (mobile phase), which could control the predominant ionic form of proteins.

Recently, some stimuli-responsive polymer brushes have drawn more attention due to their reversible conformational changeable ability with external stimuli (temperature, pH, light, ionic strength (I), *etc.*) [22, 23], which could lead to the variations in the physical and chemical properties of the polymers, such as volume, shape, surface area, and protein adsorption, *etc.* This feature enables the stimuli-responsive polymer brushes to have applications in the capture/release of proteins in a defined time.

Among stimuli-responsive polymers, poly(acrylic acid) (PAA) as a weak polyelectrolyte, is widely studied for its swelling-deswelling behavior upon pH and I change. Meanwhile, the switchable properties of PAA brushes toward protein adsorption generated by the swelling-deswelling behavior of PAA chains have been well investigated. For instance, at pH=5.0 and low I, PAA chains are swollen, enabling a large amount of human serum albumin (HSA, pI≈4.8) to be adsorbed on the PAA brush modified surface [24]. At pH=9.0 and high I, both HSA and PAA were negatively charged (PAA has collapsed conformation), which drastically limits the adsorption percentage of HSA on the PAA brush modified surface. However, proteins are slightly difficult to be repelled by the shrunken brushes when they are adsorbed by swollen PAA brush, whatever the isoelectric point of the protein is [25, 26]. To deal with the issue, mixed polymer brushes with a protein adsorbing and a protein repelling parts seem to be a favorable method. Delcroix *et al.* [25] have developed a mixed coating (mixed brushes of PAA and poly(ethylene oxide) (PEO)) on the gold substrate for controlling adsorption of HSA. PEO builds the protein repelling part, while the PAA acts as the stimuli-responsive one that can adsorb and desorb proteins upon pH and I change. The mixed polymer coating could adsorb high amounts of HSA at pH=5 and I=10^{-5} mol/L, and 86% of the adsorbed HSA could be desorbed at pH=9 and I=0.1 mol/L, indicating a largely improved regulation for protein adsorption and desorption compared with pure PEO or PAA brushes. Though PEO has been used extensively as protein-repellent polymer, it is well known that PEO could undergo degradation by (auto-)oxidation to form aldehydes and ethers, resulting in the fact that PEO coatings can lose their function when being used in physiological environment [27]. Poly(2-oxazoline)s, especially the water soluble poly(2-methyl-2-oxazoline) (PMOXA), a kind of peptidomimetic polymer, have lately drawn increasing attention for their excellent protein resistance. In comparison to PEO, PMOXA has similar properties (*e.g.*, hydrophilicity, protein resistant property, and biocompatibility) and better structural stability against biological degradation because of their structure of peptidomimetic structure [28, 29]. Therefore, we have prepared the binary mixed brushes based on PMOXA and PAA via sequentially grafting of amine-terminated PMOXA (PMOXA-NH_2) and thiol-terminated PAA (PAA-SH) onto poly(dopamine) (PDA) coated substrates [30]. Concerning the protein adsorption/desorption behaviour, in comparison to the pure PAA (PDA/PAA) brushes, the binary mixed brushes (PDA/PMOXA/PAA) coating could adsorb a mass of bovine serum albumin (BSA) at pH=5.0 (I=10^{-5} mol/L), and then the most adsorbed BSA could be released at pH=9.0 (I=0.1 mol/L) when the chain length of PAA is longer than that of PMOXA [30], exhibiting a synergetic effect of the protein resistance of PMOXA and pH and I sensitivity of PAA. As had been anticipated the obtained binary mixed brushes

DOI:10.1063/1674-0068/cjcp1805130

based on PMOXA and PAA revealed a smart surface, which is able to be well switched toward protein adsorption/desorption upon pH and I change.

We further evaluated the efficacy of binary mixed brushes based on PMOXA and PAA coating for developing a simple method to improve the BSA detection sensibility in CE. To assess the efficacy of the binary mixed brushes based on PMOXA and PAA coating, the adsorption of BSA on the binary-mixed-brushes-coated (BBC) capillary was investigated by fluorescence spectroscopic measurements, and the switchable property toward protein adsorption of the BBC capillary was confirmed. Subsequently, the performance of the binary mixed coating about on-line preconcentration of proteins in CE was studied, and the key experimental factors that influence the accurate assay of BSA, such as injection time and separation voltage were investigated carefully.

II. EXPERIMENTS

A. Materials

2-Methyl-2-oxazoline (MOXA, 99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dried by refluxing over CaH_2 and subsequently distilled prior to use. Acrylic acid (AA) was purchased from Sinopharm Chemical Reagent Co., Ltd. (SCRC, Shanghai, China) and purified by distillation under reduced pressure before use. 2,2-Azobis(2-methylpropionitrile) (AIBN) was recrystallized from methanol. Dopamine and BSA (MW≈6.7 kDa, pI=4.7) were purchased from Sigma-Aldrich. Fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA) was prepared according to previously published procedures [31]. All the chemicals were of analytical grade or higher. Fused-silica capillary was purchased from Yongnian Ruifeng Chromatographic Device Co. (Hebei, China). The capillary's raw material was friendly supplied by Yongnian Ruifeng Chromatographic Device Co.

All aqueous solutions were prepared freshly with deionized (DI) water just before use unless otherwise stated. The dopamine, PMOXA-NH_2, and PAA-SH solutions were prepared using Tris-HCl buffer of pH=8.5 (10 mmol/L). The phosphate buffer solutions with determined pH and I were prepared by adjusting 0.02 mmol/L $NaH_2PO_4{\cdot}2H_2O$, 1.0 mol/L H_3PO_4 to desired pH=5.0 and I=10^{-5} mol/L, and by adjusting 0.1 mmol/L $Na_2HPO_4{\cdot}2H_2O$, 1.0 mol/L NaOH and NaCl to desired pH=9.0 and I=0.1 mol/L. The phosphate buffer solution of pH=5.0 and I=10^{-5} mol/L was used to prepare the BSA solution and FITC-BSA solution.

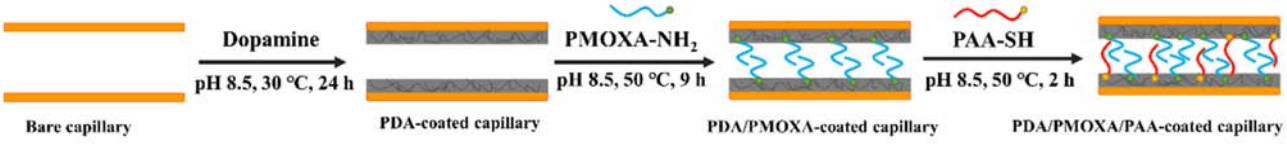
B. Synthesis of the polymers

According to previous reports [32, 33], PMOXA was synthesized by cationic ring-opening polymerization (CROP) of MOXA with methyl trifluoromethanesulfonate (MeOTf) as an initiator (the molar ratio of monomer to initiator was 25:1) and then end-capped with potassium phthalimide. After dealing with hydrazine monohydrate, the end group of phthalimide was removed, and the amine-terminated PMOXA (PMOXA-NH_2) was obtained, the detail is shown in Scheme S1 (see supplementary materials). PAA was synthesized by reversible addition fragmentation chain transfer (RAFT) polymerization with AIBN as initiator and S-1-dodecyl-S′-(α,α'-dimethyl-α''-acetic acid) trithiocarbonate (DDMAT) as chain transfer agent (the molar ratio of AA, DDMAT, to AIBN was 50:1:0.25). After dealing with ethanolamine in nitrogen atmosphere, the thiol-terminated PAA (PAA-SH) was obtained. The detailed steps are shown in Scheme S2 (see supplementary materials). The degree of polymerization (DP) of the polymers was determined by ^{1}H NMR (FIG. S1 in supplementary materials).

C. Preparation of the capillary coating

First of all, the fused-silica capillary was pretreated by flushing with 1 mol/L HCl and DI water for 20 min and 15 min, respectively, and then followed with rinses of 1 mol/L NaOH and DI water for another 20 min and 15 min respectively, and finally dried with air. The pretreated capillary was then filled with oxygen in advance [34], after that the dopamine in Tris-HCl buffer (10 mmol/L, pH=8.5) with the concentration of 6 mg/mL was exposed to air and introduced into the pretreated capillary with a syringe when it turned brownish, according to the procedure reported in our previous work [28, 35, 36]. The capillary was kept at 30 °C for 24 h with both ends sealed with silicon rubber. After that, the capillary was rinsed with DI water for 5 min to remove the dopamine physically attached to the tube surface and dried with the nitrogen stream for 10 min, and then PDA-coated capillary was obtained. Subsequently, the Tris-HCl solution of PMOXA-NH_2 with the concentration of 2 mg/mL was introduced into the PDA-coated capillary and the capillary was kept at 50 °C for 9 h with both ends sealed using silicon rubber. After that, the residuary solution in the capillary was flushed out and the capillary was dried with nitrogen flow, and then the PDA/PMOXA coated capillary was obtained. Finally, the Tris-HCl solution of PAA-SH with the concentration of 2 mg/mL was injected into the PDA/PMOXA coated capillary, and the capillary was kept at 50 °C for 2 h with both ends sealed using silicon rubber. Then, the residuary solution in the capillary was flushed out with DI water flow and the capillary was dried with nitrogen stream, and the PDA/PMOXA/PAA coated capillary was obtained. A schematic illustration of preparation of PDA/PMOXA/PAA coated capillary is shown in Scheme 1. In addition, the PDA, PDA/PMOXA, and

DOI:10.1063/1674-0068/cjcp1805130



Scheme 1 The procedure of PDA/PMOXA/PAA coating grafted onto the fused-silica capillary inner wall.

PDA/PAA coated capillaries were also prepared as the procedure above. Meanwhile, the capillary raw material was modified using the same procedure for XPS detection.

D. Characterization

Scanning electron microscope (SEM) was employed to measure the thickness of polymer coatings deposited on the inner wall of the capillary. SEM images were taken by field emission scanning electron microscope SIRION 200 (FEI, Netherlands). The bare or coated capillary was cut into segments of 5 mm, then was sputtered by using gold in a vacuum for 90 s, finally the image of cross section of capillary was obtained by SEM.

The X-ray photoelectron spectroscopy (XPS) was used to characterize the surface chemical composition and the capillary's raw material was used to facilitate the characterization. The take-off angles of the photoelectrons were 90°, spot size maintained at 500 μm. XPS data were collected on a VG ESCALAB MK II X-ray Photoelectron Spectrometer (VG Scientific Instruments, England) with an Al Kα X-ray source (energy 1486.6 eV). All spectra were measured at room temperature.

E. Adsorption/desorption of BSA on the capillary

BSA was chosen as the model protein for the evaluation of protein adsorption and desorption on the bare and polymer coated capillary [37]. All fluorescence spectroscopy measurements were carried out on an optical microscope, Olympus BX81 (Olympus, Japan) equipped with a halogen lamp, filter U-MNG2 (λ_{exit}=470−490 nm, λ_{exit}>510 nm) and camera type DP72. The phosphate buffer solution of FITC-BSA with the concentration of 1.0 mg/mL at pH=5.0 (I=10^{-5} mol/L) or pH=9.0 (I=0.1 mol/L) was freshly prepared and used for the tests. Firstly, the bare or polymer coated capillary was filled with the phosphate buffer solution (pH=5.0, I=10^{-5} mol/L) for 30 min. Then, the capillaries were flushed with FITC-BSA solution (0.5 mL) of pH=5.0 and I=10^{-5} mol/L at the speed of 10 μL/min by using a syringe pump, after that, the capillary was washed with the phosphate buffer solution (pH=5.0, I=10^{-5} mol/L) for 10 min to remove the weakly bound proteins, and dried using air flow. Then, a segment with the length of about 5.0 mm was cut off from the capillary, and external polymer surface of the segment was split. In the next moment, the rest of the capillary was incubated in the phosphate buffer solution (pH=9.0, I=0.1 mol/L) for 30 min, after that, the capillary was flushed with the phosphate buffer solution (pH=9.0, I=0.1 mol/L) for 10 min and dried using air. Another segment with the length of about 5.0 mm was cut off from the capillary. The fluorescence images of capillary segments under the conditions of pH=5.0 (I=10^{-5} mol/L) and pH=9.0 (I=0.1 mol/L) were examined using an optical microscope, respectively.

F. On-line preconcentration of BSA

All CE experiments were carried out on a Beckman P/ACE MDQ system (Beckman, Coulter Instruments, Fullerton, CA, USA) with temperature set at 25 °C and detection wavelength at 214 nm with a UV detector, and data were acquired with the 32 Karat software (Beckman, Coulter Instruments, Fullerton, CA, USA). The BSA solutions with the certain concentration used in experiments were prepared freshly. For the procedure of on-line preconcentration in CE, the bare or polymer coated capillaries were rinsed with the phosphate buffer solution (pH=5.0, I=10^{-5} mol/L) at 20.0 psi for 5.0 min and pre-run under the voltage of 20.0 kV for 2.0 min, firstly. Then the capillary was injected with the BSA solution of pH=5.0 and I=10^{-5} mol/L with certain concentration at 0.5 psi for certain time and then kept for 10.0 min (the procedure of injection). And rinsed with the phosphate buffer solution (pH=5.0, I=10^{-5} mol/L) at 20.0 psi for 6 s (the procedure of rinse). Repeat the procedures of injection and rinse for three times. After that, the capillary was injected with phosphate buffer (pH= 9.0, I=0.1 mol/L) at 0.5 psi for 5.0 s and then kept for 5.0 min, subsequently, the BSA was separated under certain voltage. This whole procedure mentioned above (including the injection at pH=5.0, I=10^{-5} mol/L and separation at pH=9.0, I=0.1 mol/L) is referred to as the separation under the condition of pH=9.0 (I=0.1 mol/L) in the following description. Separation of BSA on bare or coated capillary at pH=5.0 (I=10^{-5} mol/L) directly was also performed as the method mentioned above (this procedure is referred to as the separation at the condition of pH=5.0 (I=10^{-5} mol/L) in the following description). The separation efficiency was evaluated by the theoreti-

DOI:10.1063/1674-0068/cjcp1805130

cal plate numbers (N) and peak height (H). The values of N were calculated with the following equation:

$$N = 5.54 \times \left(\frac{t_{\mathrm{m}}}{W_{1/2}}\right)^2 \quad (1)$$

where N is theoretical plate number, t_{m} is migration time and $W_{1/2}$ is the peak width at half height of selected peak.

The peak area (A) and peak height (H) of BSA obtained with preconcentration were used as the peak parameters to calculate the sensitivity enhancement factor (SEF) using the following equation [38]:

$$\mathrm{SEF} = \frac{A_1(H_1)}{A_0(H_0)} \times e \quad (2)$$

where area (A_1) or height (H_1) was obtained at pH=9.0 (I=0.1 mol/L) for the BSA with lower concentration in the polymer coated capillary and A_0 or H_0 was obtained at pH= 9.0 (I=0.1 mol/L) for the BSA with high concentration in the bare capillary. e is dilution factor which is the ratio of high concentration to lower concentration.

III. RESULTS AND DISCUSSION

A. Synthesis and characterization of the polymers

In this work, PMOXA-NH_2 was first synthesized through CROP of MOXA with MeOTf as an initiator. The successful end functionalization was confirmed by ^{1}H NMR spectroscopy, as shown in FIG. S1(A) (see supplementary materials). PAA was synthesized by RAFT polymerization with DDMAT as a chain transfer agent. The chemical shifts of the protons in PAA can be clearly identified in the ^{1}H NMR spectrum, as shown in FIG. S1(B) (see supplementary materials). The degrees of polymerization (DP) of PMOXA-NH_2 and PAA-SH were figured out to be about 23 and 44 respectively by ^{1}H NMR. The corresponding contour chain length for PMOXA-NH_2 and PAA-SH chains were about 8.34 and 12.06 nm calculated by the method in previous work [30, 39], respectively, which indicated that the contour chain length of PAA chains was about 1.45 times of that of PMOXA chains.

B. Characterization of the polymer coating

FIG. 1 illustrates SEM images of the cross section of the bare capillary (A) and PDA/PMOXA/PAA coated capillary (B). Comparing FIG. 1(A) and FIG. 1(B), a film with 350 nm thickness was clearly observed on the PDA/PMOXA/PAA coated capillary, indicating the formation of a polymer coating on the bare fused silicon capillary.

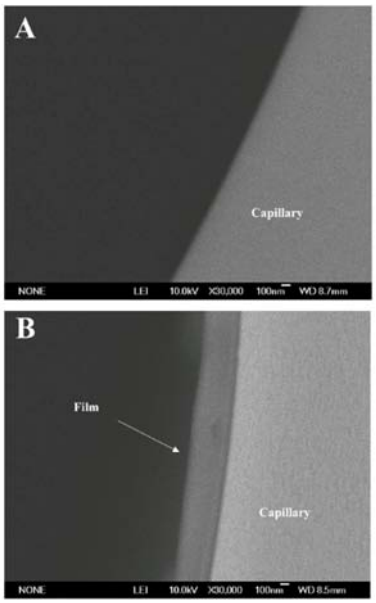


FIG. 1 SEM images of bare (A) and PDA/PMOXA/PAA (B) coated capillary with the magnification of ×30000.

FIG. 2 represents the XPS spectra of wide scan for the bare, PDA-coated, PDA/PMOXA-coated, PDA/PAA-coated, and PDA/PMOXA/PAA-coated capillary raw materials, and Table I shows the corresponding average element composition of the surface. As shown in FIG. 2 and Table I, the peaks at the binding energies of about 102.18, 284.80, 400.02, and 532.20 eV are assigned to Si2p, C1s, N1s, and O1s, respectively. The Si2p signal characteristic decreased from 30.21% on the bare capillary raw material to 9.71% on the PDA-coated capillary raw material, then to 6.25% on the PDA/PMOXA-coated capillary raw material, finally to 4.65% on the PDA/PMOXA/PAA-coated capillary raw material. Meanwhile, the obvious increase of C1s signals and appearance of N1s signals on the coated capillary raw materials compared with the bare capillary raw materials demonstrated that the polymers have grafted on the bare capillary raw materials through PDA anchor successfully. Appearances of S signal (163.63 eV) on the PDA/PAA and PDA/PMOXA/PAA coated capillary raw materials further confirmed that the PAA-SH chains have been grafted to the PDA coating. As shown in Table I, the highest ratio of N/O (0.413) is presented on the PDA/PMOXA-coated capillary raw materials, suggesting the successful graft of nitrogenous PMOXA on PDA-coated interface. And the ratio of N/O decreased to 0.350 on the PDA/PMOXA/PAA-coated capillary raw materials, between the ratio N/O of PDA/PMOXA and PDA/PAA coated capillary raw materials, indicating the simultaneous appearance of PAA and PMOXA. These results suggest that the PMOXA-NH_2 and PAA-SH could be grafted on the capillary raw material surface with certain proportion.

DOI:10.1063/1674-0068/cjcp1805130

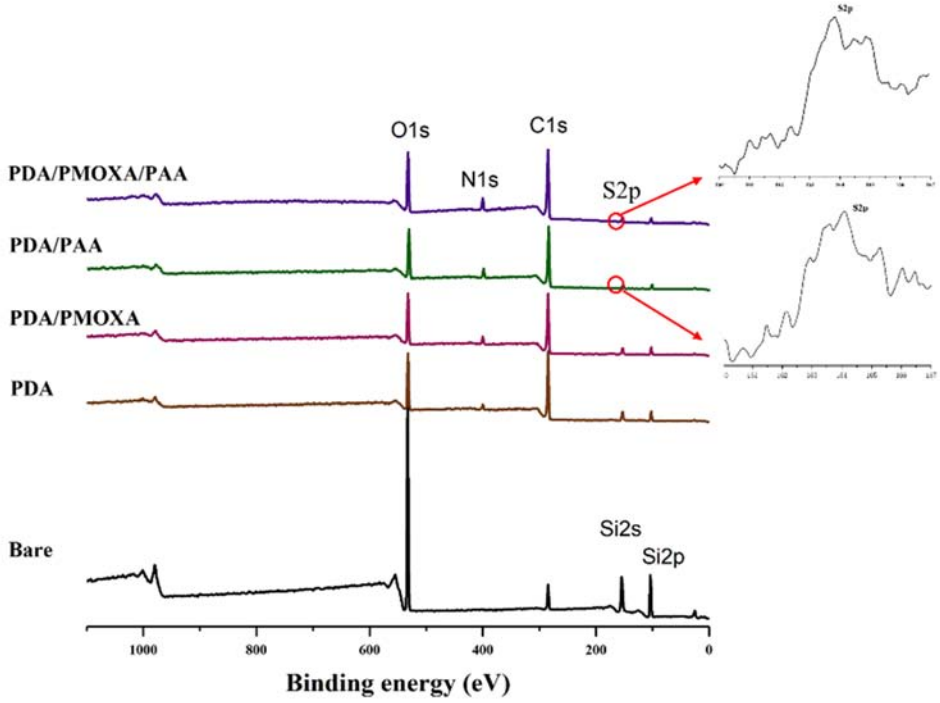


FIG. 2 XPS spectra of wide scan for the bare, PDA, PDA/PMOXA, PDA/PAA, and PDA/PMOXA/PAA coated capillary raw materials.

TABLE I The atomic percentage of elements on the bare and polymer coated capillary raw materials determined by XPS.

Coating surfaces	Composition[a]/(atom%)					Molar ratio of N/O
	C	N	O	Si	S	
Bare	16.11±2.03	0.50±0.09	53.18±2.89	30.21±1.63	0	0.009
PDA	65.06±1.20	4.32±0.15	20.91±1.54	9.71±2.18	0	0.207
PDA/PMOXA	64.73±3.08	8.48±3.027	20.54±0.95	6.25±0.67	0	0.413
PDA/PAA	67.64±1.67	6.15±2.16	21.43±5.23	4.40±1.65	0.37±0.09	0.287
PDA/PMOXA/PAA	65.92±1.53	7.54±0.58	21.55±2.46	4.65±2.06	0.34±0.12	0.350

[a] Expressed as mean ±SD (n=3).

C. Controlled adsorption of FITC-BSA in capillary

To assess qualitatively the protein adsorption on the inner wall of bare or the polymer-coated capillaries, a fluorescence test was performed using FITC-BSA as model protein. FIG. 3 shows the images of the bare and polymer coated capillaries after adsorption of FITC-BSA solution (pH=5.0 and I=10^{-5} mol/L, 1.0 mg/mL) and images of corresponding capillaries after rinse using the phosphate buffer solution (pH=9.0 and I=0.1 mol/L). For the bare capillary, intense green fluorescence is observed under the two conditions mentioned above. It indicated that plenty of FITC-BSA was adsorbed on the inner surface of bare capillary and nearly no protein was desorbed from the surface with the change of pH and I. When the inner surface of capillary was modified with PDA, decreased fluorescence intensity was observed compared with the bare capillary, and the fluorescence intensity almost does not change with the variation of pH and I for the PDA coated capillary. For PDA/PMOXA coated capillary, the sharp decrease of fluorescence intensity under two conditions happened due to the excellent protein repelling property of PMOXA. Interestingly, very intense fluorescence was observed at pH=5.0 (I=10^{-5} mol/L), and very weak fluorescence was observed at pH=9.0 (I=0.1 mol/L) when PAA chains were grafted on the PDA coated capillary. It suggested that PDA/PAA coated capillary could adsorb high amounts of BSA at pH=5.0 (I=10^{-5} mol/L), and part of the adsorbed proteins could then be desorbed at pH=9.0 (I=0.1 mol/L). For PDA/PMOXA/PAA coated capillary, very intense fluorescence was also observed at pH=5.0 (I=10^{-5} mol/L), and the fluorescence was almost invisible while changing pH to 9.0 and I to 0.1 mol/L. The above phenomenon implied that large amounts of BSA were able to be

DOI:10.1063/1674-0068/cjcp1805130

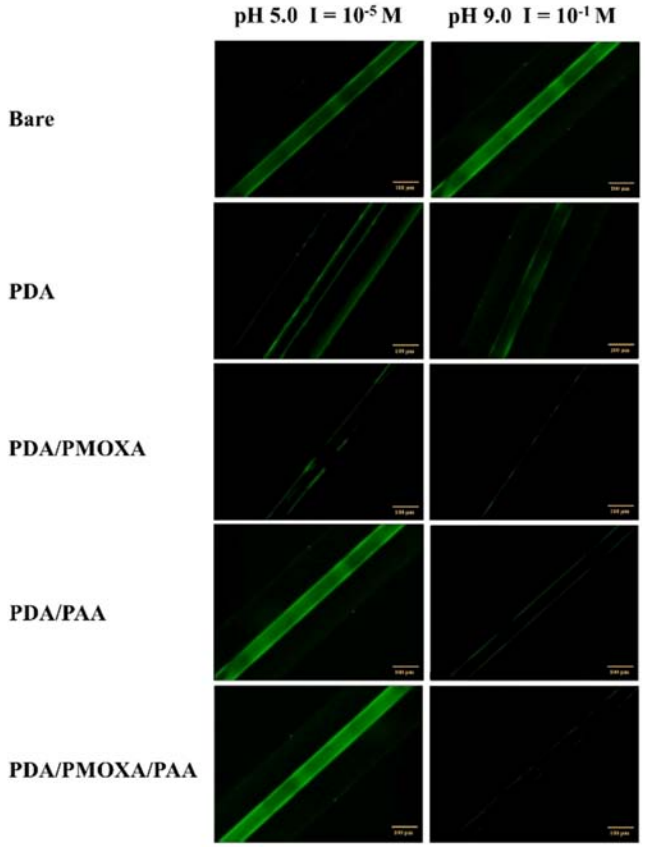


FIG. 3 Fluorescence microscope images of bare, and PDA, PDA/PMOXA, PDA/PAA, and PDA/PMOXA/PAA coated capillaries after adsorption of FITC-BSA solution (pH=5.0 and I=10^{-5} mol/L, 1.0 mg/mL) and images of corresponding capillaries after rinse using the phosphate buffer solution (pH=9.0 and I=10^{-1} mol/L). The scale bar is 100 μm.

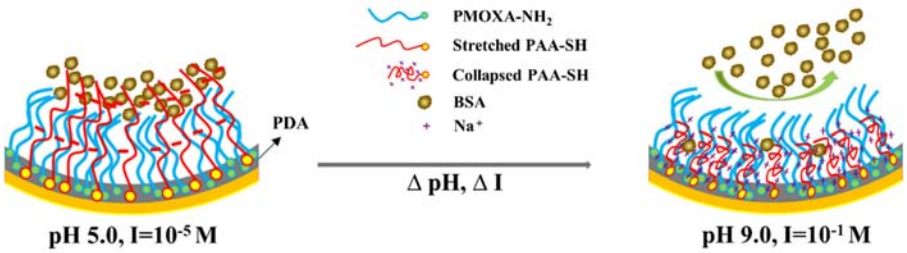
adsorbed on the PDA/PMOXA/PAA coated capillary at pH=5.0 and I=10^{-5} mol/L when the chain length of PAA (contour chain length for PAA-SH chains was 12.06 nm) was a little longer than that of PMOXA (contour chain length for PMOXA-NH_2 was 8.34 nm), and most of the adsorbed proteins would then be desorbed at pH=9.0 and I=0.1 mol/L. These results were in line with our previous work [30]. In our previous work, the fluorescence intensity increased largely at pH=5.0 and I=10^{-5} mol/L on the PMOXA/PAA mixed brush modified glass wafer, while sharply decreased fluorescence intensity was observed at pH=9.0 and I=0.1 mol/L when the chain length of PAA in the PMOXA/PAA brush was a little longer than that of PMOXA.

Generally, at pH=5.0 and low I (10^{-5} mol/L), PAA chains are supposed to be slightly charged and swollen since few counterions from medium could participate the charge screening [40, 41]. These PAA chains could therefore adsorb large amounts of BSA into the brush due to the increase of entropy sourced from the release of counterions caused by the interaction of negative charge of the PAA chains with the patches of positive charges on BSA [42]. At pH=9.0 and I=0.1 mol/L, both BSA and PAA are strongly negatively charged, and the PAA chains are shrunk since the negative charges of PAA chains are screened by counterions from the solution, leading to a high electrostatic repulsion and volume exclusion, and therefore a lot of BSA are desorbed [25]. The brush coating used in our BBC capillary is composed of two parts, one is PMOXA (with theoretical contour chain length of 8.34 nm) with protein-repelling ability, and the other one is PAA (with theoretical contour chain length of 12.06 nm), with the ability of protein adsorption/desorption varying with pH and I. At pH=5.0 and low I (10^{-5} mol/L), PAA chains are partially negatively charged, and swollen in solution (as shown in Scheme 2). And the PAA chains are partially exposed at the outermost surface, enabling proteins in the solution to contact with the PAA chains exposed outermost easily, and hence the coating could adsorb a high quantity of BSA. At pH=9.0 and high I (0.1 mol/L), PAA chains are collapsed (as shown in Scheme 2) due to the entrapping of counterions from solution, which would eject part of adsorbed BSA; meanwhile, PMOXA chains are more exposed at outer surface than PAA causing desorption of BSA. Therefore the desorption of BSA at pH=9 and high I (0.1 mol/L) takes place under the synergetic effect of pH and I responsive properties of PAA as well as the strong protein resistant properties of PMOXA.

D. The study of experiment conditions on preconcentration

The results of fluorescence experiment showed that the PDA/PMOXA/PAA coated capillary could adsorb lots of BSA at pH=5.0 (I=10^{-5} mol/L) and then the most adsorbed BSA could be desorbed at pH=9.0 (I=0.1 mol/L). In addition, our previous work [30] revealed that the BSA adsorbed (794.5 ng/cm^2) from diluted solution during 2000 s on the PDA/PMOXA/PAA brushes modified surface of plasma resonance (SPR) sensor could be desorbed (709.7 ng/cm^2) in 70 s. The results above implied that PDA/PMOXA/PAA coating could capture the proteins from diluted solution and then release them through varying solution (mobile phase) pH and I values, which could increase the instantaneous concentration of proteins in a short time. Therefore, PMOXA/PAA binary brushes could be used as protein extraction and preconcentration coating to enhance the protein detection sensitivity of CE. Optimization of CE experiment conditions is of great importance for improving the accuracy and sensitivity, thus the injection time of BSA and separation voltage were studied in this work. The injection time and separation voltage of BSA in CE were investigated using PDA/PMOXA/PAA coated capillary. And the electrophoretograms of BSA sep-

DOI:10.1063/1674-0068/cjcp1805130



Scheme 2 Mechanism of adsorption and desorption of BSA on the PDA/PMOXA/PAA coated capillary.

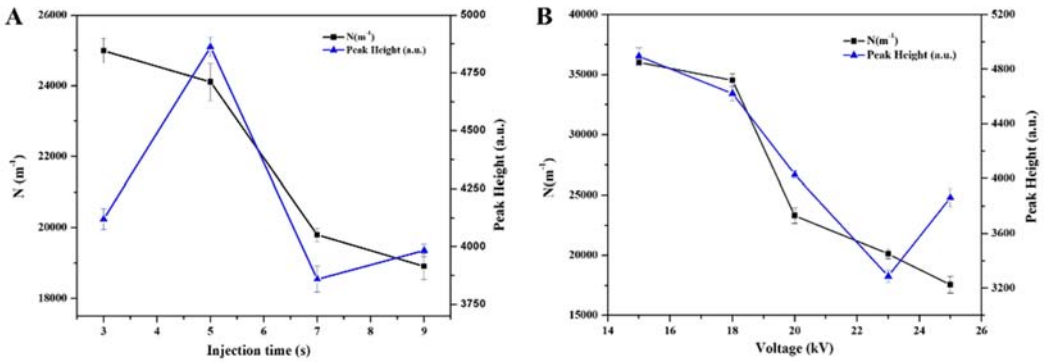


FIG. 4 Effects of CE conditions with on-line preconcentration procedure of standard BSA solution (0.5 mg/mL) using PDA/PMOXA/PAA coated capillary at pH=9.0 (I=0.1 mol/L) (n=5). (A) The effect of injection time, (B) the effect of separation voltage. Experimental conditions: temperature of 25 °C, detection of 214 nm.

arated at pH=9.0 (I=0.1 mol/L) at different injection time or different separation voltage are shown in FIG. S2 and FIG. S3 (see supplementary materials), respectively. The results showed that the bigger absorption peaks compared with the absorption peaks obtained at pH=5.0 (I=10^{-5} mol/L) (FIG. S2(A) and FIG. S3(A) in supplementary materials) were obtained at pH=9.0 (I=0.1 mol/L) (FIG. S2(B) and FIG. S3(B) in supplementary materials). It suggested that large amounts of the BSA could be adsorbed on the PDA/PMOXA/PAA coated capillary at pH=5.0 (I=10^{-5} mol/L), and then the adsorbed BSA could be released from the PDA/PMOXA/PAA coated capillary at pH=9.0 (I=0.1 mol/L). Thus, the results obtained at pH=9.0 (I=0.1 mol/L) after adsorption at pH=5.0 (I=10^{-5} mol/L) (*i.e.*, the sample extracted at pH=5.0 (I=10^{-5} mol/L) and desorbed at pH=9.0 (I=0.1 mol/L)) were used to analyze the separation efficiency. The N and peak height, calculated from the obtained peaks at pH=9.0 (I=0.1 mol/L) at different injection time (FIG. S2(B) in supplementary materials) and separation voltage (FIG. S3(B) in supplementary materials) are shown in FIG. 4.

From FIG. 4(A), the peak height presents an increase with the increase of injection time from 3.0 s to 5.0 s (having a maximum of 4863 a.u. at 5.0 s), but a sharp decrease from 5.0 s to 9.0 s. And when the injection time was 5.0 s, the N also had the relatively large value (24110 m^{-1}). Therefore, the time of 5.0 s was selected as the best injection time.

The separation voltage was also investigated. As shown in FIG. 4(B), the N and the corresponding peak height both decreased with the increase of separation voltage. Both the peak height and N have the maximum values (4896 a.u. and 36015 m^{-1}, respectively) at 15 kV. Finally, the voltage of 15 kV was selected as the separation voltage.

Finally, the optimal CE conditions for on-line precongcentration were obtained with 30 cm effective length (40 cm total length) and 50/365 μm id/od capillary, 15 kV separation voltage, 0.5 psi for 5.0 s as the best injection time.

E. On-line preconcentration of BSA

Under optimal CE conditions, the bare and polymer coated capillaries were used to preconcentrate a test protein (BSA). FIG. 5 shows the electropherotograms of BSA (0.5 mg/mL) in the bare and PDA, PDA/PMOXA, PDA/PAA, PDA/PMOXA/PAA coated capillaries at pH=5.0 (I=10^{-5} mol/L) (FIG. 5(A)) and at pH=9.0 (I=0.1 mol/L) (FIG. 5(B)). For bare capillary, almost no peak was detected at pH=5.0 (I=10^{-5} mol/L) or pH=9.0 (I=0.1 mol/L)), revealing the fact that a large quantity of BSA have been adsorbed on the bare capillary and almost all of the absorbed BSA could not be released from the bare

DOI:10.1063/1674-0068/cjcp1805130

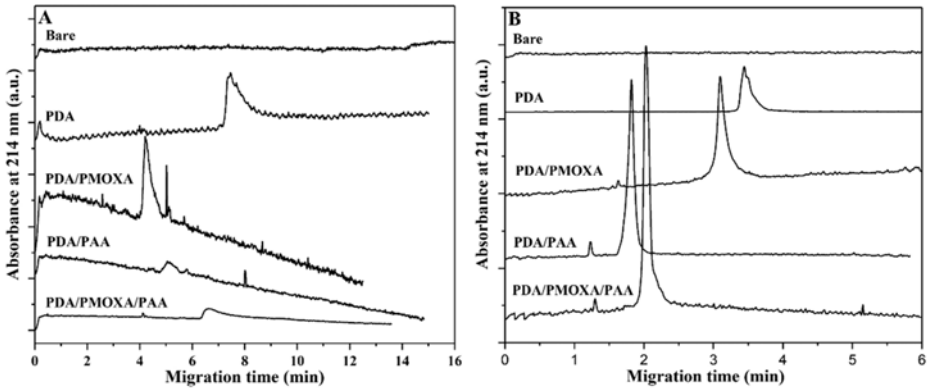


FIG. 5 Separation of BSA (0.5 mg/mL) in the bare and polymer coated capillaries at pH=5.0 (I=10^{-5} mol/L) (A) and separation at pH=9.0 (I=0.1 mol/L) (B) in CE. Experimental conditions: temperature of 25 °C, injection of 5.0 s at 0.5 psi, separation voltage of 15 kV, detection of 214 nm.

capillary. This result was similar to the results of fluorescent test (FIG. 3), in which the intense fluorescence could be observed under both pH=5.0 (I=10^{-5} mol/L) and pH=9.0 (I=0.1 mol/L) in bare capillary. For the PDA coated capillary, the obtained peak area and peak height at pH=5.0 (I=10^{-5} mol/L) were 736 (a.u.×min) and 4176 a.u, respectively; and a similar peak was also detected at pH=9.0 (I=0.1 mol/L) (The ratio of peak area and peak height for pH=9.0 (I=0.1 mol/L) to pH=5.0 (I=10^{-5} mol/L) (extraction) was 1.13 and 1.38, respectively). When the PDA coated capillary was further modified by PMOXA, the peak area (624 a.u.×min) and an increased peak height (5963 a.u.) compared with bare and PDA coated capillary were obtained at pH=5.0 (I=10^{-5} mol/L). And an analogous peak was observed at pH=9.0 (I=0.1 mol/L). The ratio of peak area and peak height for pH=9.0 (I=0.1 mol/L) to pH=5.0 (I=10^{-5} mol/L) (extraction) was 1.43 and 2.02, respectively. This phenomenon suggested that the adsorption of BSA on PDA/PMOXA coated capillary was insignificant under either protein extraction condition or preconcentation condition due to the protein repelling property of PMOXA. For the PDA/PAA coated capillary, the peak area of 608 (a.u.×min) and the peak height of 1021 a.u. were observed at pH=5.0 (I=10^{-5} mol/L). And a big peak with the peak area (6284 a.u.×min) and the peak height (21529 a.u.) were obtained at pH=9.0 (I=0.1 mol/L). The ratio of peak area and peak height for pH=9.0 (I=0.1 mol/L) (preconcentration) to pH=5.0 (I=10^{-5} mol/L) (extraction) was 10.34 and 21.09, respectively. These results indicated that the absorbed BSA on the PDA/PAA coated capillary could be released upon pH and I change owing to the existence of PAA chains. For PDA/PMOXA/PAA coated capillary, the area and peak height were 7328 (a.u.×min) and 32046 a.u. at pH=9.0 (I=0.1 mol/L), respectively, which is 17.02 time of peak area and 33.24 time of peak height obtained at pH=5.0 (I=10^{-5} mol/L). These results are in accordance with those obtained by fluorescent test as shown in FIG. 3, indicating the fact that desorption amount of BSA could be improved by PMOXA existing in the binary mixed brushes. The results above indicated that when BSA is passed through the PDA/PMOXA/PAA coated capillary at pH=5.0 (I=10^{-5} mol/L), BSA could be captured by swollen PAA brushes, which was partially negatively charged and exposed at the outermost surface thanks to its longer contour chain length than PMOXA, leading to their extraction. Using the present method, sample was introduced into PDA/PMOXA/PAA coated capillary at 0.5 psi for 5.0 s for three times, which corresponded to more than 15 capillary volumes of the sample passed through the capillary for on-line extraction [43]. It implied that sample volume is not limited by one capillary volume in our present work. At pH=9.0 (I=0.1 mol/L), under the synergetic effect of collapsed PAA chains and exposed PMOXA chains, the extracted proteins were desorbed from the PDA/PMOXA/PAA coated capillary. Therefore, the desorbed protein could be further focused and analyzed.

F. Preconcentration for BSA with different concentrations

FIG. S4 in supplementary materials presents the electropherograms of BSA obtained before and after preconcentration in the bare and PDA, PDA/PMOXA, PDA/PAA, and PDA/PMOXA/PAA coated capillaries with the BSA solution of 10-fold and 100-fold lower concentration using the described procedure above. The peak area and peak height obtained in the five different capillaries all decreased with decreasing the BSA concentrations due to the decrease of the injection sample amount. Based on the results shown in FIG. 5 and FIG. S4 (see supplementary materials), sensitivity enhancement factors (SEFs) calculated according to Eq.(2) with different BSA concentration (0.5, 0.05, and 0.005 mg/mL, respectively) are shown in Table II. SEFs

DOI:10.1063/1674-0068/cjcp1805130

TABLE II SEFs for BSA with different concentrations c in the bare and polymer coated capillaries.

c/(mg/mL)	Bare		PDA		PDA/PMOXA		PDA/PAA		PDA/PMOXA/PAA	
	SEF(A)	SEF(H)	SEF(A)	SEF(H)	SEF(A)	SEF(H)	SEF(A)	SEF(H)	SEF(A)	SEF(H)
0.5	1	1	23	56	45	118	153	211	176	314
0.05	-	-	145	212	189	379	603	781	983	1335
0.005	52	47	1634	1356	871	1665	1082	1570	3649	5498

TABLE III The repeatability of preconcentration process for BSA in the PDA/PMOXA/PAA coated capillary[a].

	Peak area/(a.u.×min)	Peak area RSD/%	Peak height/a.u.	Peak height RSD/%
Run to run[b]	6731±234	3.48	29016±667	2.29
Day to day[c]	5782±261	4.51	28426±1124	3.95
Capillary to capillary[d]	7318±658	8.99	30042±1623	5.40

[a] Experimental conditions: temperature of 25 °C, injection of 5.0 s at 0.5 psi, separation voltage of 15 kV, detection of 214 nm, and sample of 0.5 mg/mL.
[b] Expressed as mean ±SD (n=20), obtained from one capillary for 20 times.
[c] Expressed as mean ±SD (n=10), obtained from one capillary for one month, and tested every three days.
[d] Expressed as mean ±SD (n=5), obtained from five capillaries.

based on the peak area and peak height were boosted greatly in PDA/PMOXA/PAA coated capillary compared with bare capillary. For BSA of 0.005 mg/mL, the result based on peak heights provided SEF values of 47 and 5498 for the bare and PDA/PMOXA/PAA coated capillary, respectively. Similar calculations made on the basis of peak area gave the SEF values of 52 and 3649 for the bare and PDA/PMOXA/PAA coated capillary, individually. It suggests that the protein load ability of the capillary was greatly increased after modification with the mixed brushes of PMOXA/PAA.

G. Repeatability and reproducibility of the coating

To study the repeatability of presented method, the consecutive on-line preconcentration of BSA was performed in PDA/PMOXA/PAA coated capillary by monitoring the relative standard deviation (RSD) values of the peak area and peak height. The results are summarized in Table III. The RSD values of run-to-run repeatability in terms of peak area and peak height were 3.48% and 2.29%, respectively; the day-to-day RSDs based on peak area and peak height were 4.51% and 3.95%, respectively; the capillary to capillary RSDs based on peak area and peak height were 8.99% and 5.40%, respectively. The presented RSD values indicated that the repeatability of the preconcentration method is quite good due to the long-term stability and the good durability of PDA/PMOXA/PAA coating. Compared with other work [1, 21], the method used for on-line preconcentration of protein in this work is convinient and effective due to its simple manipulation; furthermore, the detection sensitivity of proteins in CE-UV could be improved due to the increase of instantaneous concentration of proteins, which is owing to the switchable abililty of BBC capillary upon pH and I change.

IV. CONCLUSION

In this work, a novel method was developed for the preparation of BBC capillary based on PMOXA and PAA with switchable protein adsorption/desorption properties upon pH and I change. On-line preconcentration for BSA in CE was performed in this coated capillary. The presented on-line preconcentration method provided a significant enhancement in detection capability of a standard CE system equipped with a UV detector due to its maximum injection volume higher than the column volume. A SEF value of more than 5000 was obtained for BSA. On-line preconcentration method presented in this work might prove to be a promising approach in trace analysis of protein by CE.

Supplementary materials: The detailed syntheses of PMOXA-NH_2 and PAA-SH as well as the corresponding ^{1}H NMR spectra are avaliable. The electrophoretograms of BSA at different pH values and in different coated-capillaries are also avaliable.

V. ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (No.21674102).

[1] L. Xu, X. Dong, and Y. Sun, Electrophoresis **30**, 689 (2009).
[2] S. Sun, Y. Wang, X. Liu, R. Fu, and L. Yang, Talanta **180**, 90 (2018).

DOI:10.1063/1674-0068/cjcp1805130

[3] Y. Huang, C. Huang, C. Hu, and H. Chang, Electrophoresis **27**, 3503 (2006).
[4] F. Kitagawa, M. Kamiya, Y. Okamoto, H. Taji, S. Onoue, Y. Tsuda, and K. Otsuka, Anal. Bioanal. Chem. **386**, 594 (2006).
[5] V. Dolnk, Electrophoresis **29**, 143 (2008).
[6] L. Xu and Y. Sun, J. Chromatogr. A **1183**, 129 (2008).
[7] E. Varesio, S. Rudaz, K. Krause, and J. Veuthey, J. Chromatogr. A **974**, 135 (2002).
[8] K. Tsukagoshi, Y. Shimadzu, T. Yamane, and R. Nakajima, J. Chromatogr. A **1040**, 151 (2004).
[9] M. Kato, H. Kinoshita, M. Enokita, Y. Hori, T. Hashimoto, T. Iwatsubo, and T. Toyo, Anal. Chem. **79**, 4887 (2007).
[10] X. Xie, Y. Yang, H. Zhou, M. Li, and Z. Zhu, Talanta **179**, 822 (2018).
[11] Y. Wen, J. Li, J. Ma, and L. Chen, Electrophoresis **33**, 2933 (2012).
[12] M. Albin, P. D. Grossman, and S. E. Moring, Anal. Chem. **65**, 489 (1993).
[13] W. Li, D. Fries, A. Alli, A. Malik, S. Florida, and E. F. Avenue, Anal. Chem. **76**, 218 (2004).
[14] M. M. Hsieh and H. T. Chang, Electrophoresis **26**, 187 (2005).
[15] Y. Lin, C. Huang, and H. Chang, Anal. Bioanal. Chem. **376**, 379 (2003).
[16] T. Wang, J. H. Aiken, C. W. Huie, and R. A. Hartwick, Anal. Chem. **63**, 1372 (1991).
[17] P. Britz-mckibbin and S. Terabe, J. Chromatogr. A **1000**, 917 (2003).
[18] Z. K. Shihabi, J. Chromatogr. A **902**, 107 (2000).
[19] S. Dziomba, M. Biernacki, I. Oledzka, E. Skrzydlewska, T. Baczek, and P. Kowalski, Talanta **125**, 1 (2014).
[20] Z. K. Shihabi, Electrophoresis **23**, 1612 (2002).
[21] W. Li, D. Fries, and A. Malik, J. Sep. Sci. **28**, 2153 (2005).
[22] W. Li, J. Wang, J. Ren, and X. Qu, Angew. Chem. **52**, 6726 (2013).
[23] E. Bittrich, M. Kuntzsch, K. J. Eichhorn, and P. Uhlmann, J. Polym. Sci. **48**, 1606 (2010).
[24] C. Czeslik, G. Jackler, T. Hazlett, E. Gratton, R. Steitz, A. Wittemann, and M. Ballauff, Phys. Chem. Chem. Phys. **6**, 5557 (2004)
[25] M. F. Delcroix, G. L. Huet, T. Conard, F. E. Du Prez, and J. Landoulsi, Biomacromolecules **14**, 215 (2013).
[26] C. Czeslik, G. Jackler, and R. Steitz, J. Phys. Chem. B **108**, 13395 (2004).
[27] E. Vlassi, A. Papagiannopoulos, and S. Pispas, Eur. Polym. J. **88**, 516 (2017).
[28] H. Du, C. Zhang, K. Mao, and Y. Wang, Talanta **170**, 275 (2017).
[29] R. Konradi, B. Pidhatika, A. Mu, and M. Textor, Langmuir **24**, 613 (2008).
[30] C. Pan, X. Liu, K. Gong, F. Mumtaz, and Y. Wang, J. Mater. Chem. B **6**, 556 (2018).
[31] I. Munaweera, D. Levesque-bishop, Y. Shi, A. J. Di Pasqua, and K. J. Balkus, ACS Appl. Mater. Interf. **6**, 22250 (2014).
[32] H. M. L. Lambermont-Thijs, F. S. van der Woerdt, A. Baumgaertel, L. Bonami, F. E. Du Prez, U. S. Schubert, and R. Hoogenboom, Macromolecules **43**, 927 (2010).
[33] R. Obeid and C. Scholz, Biomacromolecules **12**, 3797 (2011).
[34] H. Lee, S. M. Dellatore, W. M. Miller, and P. B. Messersmith, Science **318**, 426 (2007).
[35] Y. Zhang, L. Chen, C. Zhang, S. Liu, H. Zhu, and Y. Wang, Talanta **150**, 375 (2016).
[36] R. Zeng, Z. Luo, D. Zhou, F. Cao, and Y. Wang, Electrophoresis **31**, 3334 (2010).
[37] W. J. Yang, T. Cai, K. G. Neoh, E. T. Kang, G. H. Dickinson, S. L. M. Teo, and D. Rittschof, Langmuir **27**, 7065 (2011).
[38] J. P. Quirino and S. Terabe, Anal. Chem. **72**, 1023 (2000).
[39] O. Pop-georgievski, D. Verreault, M.O. Diesner, V. Proks, S. Heissler, F. Rypaćek, and P. Koelsch, Langmuir **28**, 14273 (2012).
[40] C. C. Dupont-gillain, Colloids Surf. B Biointerfaces **124**, 87 (2014).
[41] A. M. Peterson and D. G. Shchukin, Biomacromolecules **13**, 3120 (2012).
[42] A. L. Becker, N. Welsch, C. Schneider, and M. Ballauff, Biomicromolecules **12**, 3936 (2011).
[43] D. J. Rose and J. W. Jorgenson, Anal. Chem. **60**, 642 (1988).

DOI:10.1063/1674-0068/cjcp1805130