ONE–STEP PREPARATION OF AMINO-PEG MODIFIED PMMA MICROCHIPS FOR ELECTROPHORETIC SEPARATION OF BIOGENIC COMPOUNDS

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ABSTRACT

This paper reports a chemical surface modification of poly(methyl methacrylate) microchips with amino-poly(ethyleneglycol) (PEG-NH₂) by nucleophilic addition–elimination reaction for a microchip electrophoresis (MCE) analysis of biogenic compounds. The PEG chains were robustly immobilized only by introducing an aqueous solution of PEG-NH₂ into the microchannel. The electroosmotic mobilities on the modified chips remained constant during over 50 runs. The PEG-NH₂ modified chips provided an efficient MCE separation of proteins with a wide variety of pI within 15 s. Furthermore, the modified chip was applied to affinity MCE using bovine serum albumin, which gave a good chiral separation of racemic amino acids.

KEYWORDS: Microchip electrophoresis, PEG, Surface modification, Chiral separation

INTRODUCTION

In microchip electrophoresis (MCE) analysis of proteins, sample adsorption onto the surface of a separation microchannel should reduce the separation efficiency and the analytical reproducibility. It has been known that dynamic coating of several polymers is one of the useful approaches for suppressing the protein adsorption. However, desorption of the coated polymers from the surface of the microchannel is sometimes problematic. In the covalent bonding methods, on the other hand, troublesome and time-consuming modification processes are often required. Thus, a stable modification with simple procedures is still desired in MCE.

In our previous study, poly(methyl methacrylate) (PMMA) chip was modified with poly(ethyleneimine) (PEI) by nucleophilic addition–elimination reaction for the MCE analysis of cationic proteins [1]. In this modification, an aqueous solution of coating polymer is only introduced into the PMMA microchannel. Although the surface adsorption of cationic proteins could be well suppressed on the PEI modified PMMA chip due to the electrostatic repulsion force, the modified chip could not be applied to the MCE analysis of acidic proteins. In this study, to overcome this limitation, we conducted the one-step and covalent immobilization of amino-poly(ethyleneglycol) (PEG-NH₂) onto the inner surface of PMMA microchannel. The reaction between acylcarbon of PMMA and primary amino group in PEG-NH₂ dissolved

![Figure 1. Covalent immobilization of PEG-NH₂ (n = 680, Mw = 30000) onto the PMMA surface.](image-url)
in a basic aqueous solution is expected to proceed as shown in Figure 1.

EXPERIMENTAL

A PMMA microchip with a simple cross-type channel (100 µm width × 30 µm depth) with a total separation length of 38 mm was employed. To Immobilize PEG-NH\(_2\) onto the PMMA surface, a solution of 10% (w/w) PEG-NH\(_2\) in borate buffer (pH 12.5) was pumped through the microchannel using a syringe pump, followed by rinsing with deionized water for 20 min. The modified PMMA microchip was then dried at 30 °C overnight. Sample proteins and amino acids were labeled with fluorescein isothiocyanate (FITC) for laser-induced fluorescence detection.

RESULTS AND DISCUSSION

To optimize the immobilization condition of PEG-NH\(_2\), the reaction time and temperature were varied. The electroosmotic mobility (\(\mu_{eo}\)) and its relative standard deviation (RSD) were decreased with increasing the modification time from 2 h to 12 h at higher temperature, which indicated the increase in the immobilization amount of PEG-NH\(_2\). In this study, the reaction time and temperature were set at 6 h and 70 °C, respectively. The durability of the PEG-NH\(_2\) coating onto the PMMA surface was investigated. The \(\mu_{eo}\) remained almost constant during 37 runs within 35 days. The day-to-day reproducibility of \(\mu_{eo}\) was acceptable with the RSD of 9.6% (\(n = 4\) within 35 days) in the dry storage condition. This longer stability of the prepared microchip indicated that the loss of PEG-NH\(_2\) was successfully suppressed by the immobilization through the covalent bond.

In the MCE analysis of BSA, a sharper peak was obtained on the PEG-NH\(_2\) modified chip (Figure 2). A 10-fold increase in the efficiency and better reproducibility were attained by the PEG-NH\(_2\) modification. By fluorescence imaging, it was also confirmed that the PEG-NH\(_2\) modification onto the PMMA chip could apparently reduce the surface adsorption of BSA as shown in Figure 3. On the PEG-NH\(_2\) modified microchip, BSA (pI 4.0), myoglobin (MYO, pI 7.2) and ribonuclease A (RIB, pI 9.2) were successfully separated within 15 s only utilizing a separation length of 5 mm (Figure 4). The RSDs of the detection time were 4–18% on the

![Figure 2. Electropherograms of 100 ppm BSA on the (a) bare and (b) PEG-NH\(_2\) modified PMMA microchips. Modification condition, 10% (w/w) PEG-NH\(_2\), pH 12.5, 70 °C, 6 h; running buffer, 10 mM borate buffer (pH 9.8); distance of detection point from the injection cross, 5 mm.](image)

![Figure 3. Fluorescent images of the (a) bare and (b) PEG-NH\(_2\) modified PMMA microchips after 10 consecutive runs of fluorescently derivatized BSA.](image)
bare PMMA chip, whereas on the PEG-NH2 modified chip the RSD values were improved to less than 4% (Table 1).

The PEG-NH2 modified chips were also applied to affinity MCE analysis of racemic Trp by using BSA as an affinity ligand. At fourth run, the chiral separation of Trp was attained on the modified PMMA chip, while on the bare chip the labeled Trp could not be separated due to unstable surface adsorption of BSA (Figure 5). These results indicated that the PEG chains immobilized on the PMMA surface could avoid the irreversible adsorption of proteins, which provided a high performance analysis medium for biogenic compounds.

**CONCLUSIONS**

To reduce irreversible adsorption of proteins, one-step immobilization of PEG-NH2 onto the surface of the PMMA microchips was developed on the basis of nucleophilic addition-elimination reaction. By using the modified microchips, efficient and reproducible MCE analyses of proteins and racemic amino acids were attained.

**REFERENCE**