Available online at www.sciencedirect.com
ScienceDirect
Science and Technology of Advanced Materials 7 (2006) 558–565
SCIENCE AND TECHNOLOGY OF ADVANCED MATERIALS
www.elsevier.com/locate/stam

One-step immobilization of cationic polymer onto a poly(methyl methacrylate) microchip for high-performance electrophoretic analysis of proteins

Fumihiko Kitagawa*, Kei Kubota, Kenji Sueyoshi, Koji Otsuka

Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan

Received 12 December 2005; received in revised form 27 February 2006; accepted 27 February 2006
Available online 28 September 2006

Abstract

One-step covalent immobilization of poly(ethyleneimine) (PEI) onto poly(methyl methacrylate) (PMMA) substrates was investigated to achieve an efficient separation of basic proteins in microchip electrophoresis (MCE). The PEI-treated PMMA chip showed the anodic electroosmotic flow and its rate was almost kept stable during 32 days with over 50 runs. This longer stability of the prepared microchip indicated that the loss of PEI was successfully suppressed by the immobilization through the covalent bond. Furthermore, the PEI modification onto the PMMA chip could apparently reduce the surface adsorption of cationic proteins. In the MCE analysis on the PEI-modified microchip, two proteins were successfully separated within 30 s only utilizing a separation length of 5 mm. While the migration time of the protein gradually increased during only four consecutive runs on an untreated PMMA chip, reproducible analyses were attained by using the PEI immobilized microchip. These results demonstrated that Coulombic repulsion force generated between cationic PEI and basic proteins could avoid the irreversible adsorption of the analytes onto the PMMA surface, which provided a high-performance analysis medium for biogenic compounds.

Keywords: Microchip electrophoresis; Protein analysis; Surface modification; Poly(methyl methacrylate)

1. Introduction

In recent years, on-chip analysis systems for biogenic compounds have undergone a steady progress and applied to the biological, medical, clinical, and pharmaceutical fields due to a potential for high-throughput parallel analysis of different samples, e.g., DNA, proteins, metabolites, sugar chains, and so on [1–4]. Among various on-chip analysis techniques, microchip electrophoresis (MCE), based on the separation principle of capillary electrophoresis (CE), has been widely used in the analysis of chemical and biogenic compounds because of its high separation efficiency, high-speed analysis and low consumption of samples in addition of the suitability for the parallel analysis on chips [5–8]. In the MCE analysis of biogenic compounds, especially proteins, however, it is well known that sample adsorption onto the surface of a separation microchannel due to electrostatic and/or hydrophobic interactions [9,10] often causes the reduction of the separation efficiency and the analytical reproducibility. Thus, various microchannel coating techniques have been developed to prevent the adsorption of biogenic compounds in MCE [11,12].

In the early stage of the development of MCE, inorganic materials like quartz, Pyrex glass and silicon were mainly employed as substrates. Since ionizable silanol groups (Si-OH) on these inorganic surfaces strongly attract with biogenic samples, considerable efforts have been devoted to develop the wall coating techniques for appropriate analytes. In the glass microchip, the wall modification is conducted by mainly two different methods: (i) physically adsorbed polymer coating (dynamic polymer coating) and (ii) covalently immobilized polymer coating. Dynamic coating which is conducted by adding surface active

*Corresponding author. Tel.: +81 75 383 2449; fax: +81 75 383 2450.
E-mail address: fkitagawa@mbox.kudpc.kyoto-u.ac.jp (F. Kitagawa).
URL: http://anchem.mc.kyoto-u.ac.jp/.

1468-6996/$ - see front matter © 2006 NIMS and Elsevier Ltd. All rights reserved.
doi:10.1016/j.stam.2006.02.021

polymers to a background solution (BGS) is the easiest technique for the surface modification. For this purpose, cellulose derivatives, e.g., hydroxyethylcellulose (HEC) [13], hydroxypropylmethylcellulose (HPMC) [14], and hydroxypropylcellulose [15], have been employed as buffer additives to suppress the adsorption of proteins. On the other hand, covalent attachment of polymers onto the glass surface based on silanization chemistry has been also applied. After the treatment with a silanization agent on the glass surface, polymers such as poly(acrylamide) [16,17], poly(vinylalcohol) [18] and poly(vinylpyrrolidone) [19] are covalently immobilized to reduce protein adsorption. Although stable coatings are obtained, siloxane bonds can be hydrolyzed in higher pH solution, which limits the use of the coating to acidic and neutral BGS.

On the other hand, polymer microchips have several advantages, e.g., suitability for mass production, less expensive, multiple methods of microchannel fabrication, and so on, which enables to be used as disposable analytical devices. Among various polymers, poly(methyl methacrylate) (PMMA) is one of the most popular substrates for the electrophoretic microdevices and several PMMA microchips are commercially available for the use in the MCE analysis. As well as glass microchips, however, the adsorption of proteins onto the surface of PMMA microchannel is often problematic for the electrophoretic separation, so that several coating techniques have been also introduced in PMMA chips. Dynamic coating of neutral polymers including HEC, HPMC, and poly(ethyleneglycol) (PEG), has been applied to the PMMA microchip, which provides a good separation of biogenic compounds [20,21]. However, desorption of coated polymers from the surface of the microchannel would reduce the analytical reproducibility. Thus, the application of covalently immobilized polymer coatings onto the PMMA surface is more appropriate for the MCE analysis.

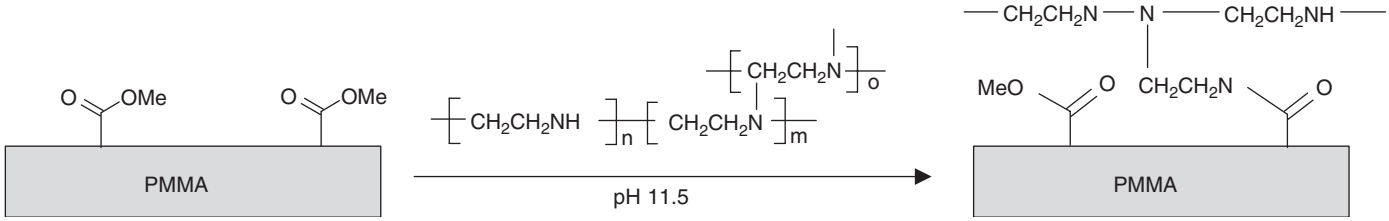
As for surface coating through a covalent bond, only a few groups have reported the development of chemical modification procedure for PMMA substrates to achieve a successful MCE analysis of biogenic compounds [22–24]. Henry et al. reported that chemical modification of PMMA substrate by using amidation reaction with lithiated diamine to introduce amino groups onto the surface [22]. The aminolyzed PMMA microchip can be modified with *n*-octadecane-1-isocyanate to obtain an ODS-like reversed stationary phase. Lee et al. [23] reported that the application of atom-transfer radical polymerization to graft PEG onto the brominated-PMMA surface. The PEG-grafted PMMA microchip could reduce non-specific adsorption of proteins, which brought a better separation efficiency compared to untreated PMMA. Stable coatings were obtained by covalent modifications, whereas these techniques required to use organic solvents as reaction media and/or rinsing liquids. It is well known that the resistance of PMMA to many organic chemicals, e.g., acetone, chloroform, *N,N′*-dimethylformamide (DMF), hexane, methanol, tetrahydrofuran, toluene, and so on, is generally low, so that the use of these materials would damage PMMA chips. Therefore, a modification method with no organic solvents should be introduced to the PMMA microchip.

In this study, to obtain a stable coating with easy manipulations, the covalent immobilization of cationic polymer with amino groups onto the surface of PMMA microchip by nucleophilic addition–elimination reaction [25] was investigated. This modification approach has been recently reported by Fixe et al. [26,27] to yield DNA chips based on PMMA substrates. However, the technique has been applied only to the immobilization of small oligonucleotide and hexamethylene-diamine, so that the application to larger polymers has not been reported. To achieve high-performance analysis of proteins, high-molecular-mass poly(ethyleneimine) (PEI) which has been employed to coat fused silica capillaries for the efficient separation of basic proteins in CE [28,29] was chosen as a surface modifier. High-molecular-mass PEI has a large number of amino groups and a positive net charge over a wide pH range, and thus the adsorption of cationic proteins can be reduced due to a strong electrostatic repulsion force generated between PEI and analytes. We anticipated that the one-step immobilization reaction between acylcarbon of PMMA and secondary amino groups in PEI dissolved in basic aqueous solution can proceed as shown in Scheme 1 and the PEI-treated PMMA chip was applied to the MCE analysis of proteins. Effects of the immobilization of PEI onto the PMMA surface on the adsorption and the separation efficiency of basic proteins were investigated.

2. Experimental

2.1. Chemicals

PEI (M_W 750 000) and rhodamine B isothiocyanate (RBITC) were obtained from SIGMA-ALDRICH (Tokyo,



Scheme 1. Covalent immobilization of PEI onto the PMMA surface.