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Preparation of a thermoresponsive polymer grafted polystyrene monolithic capillary for the separation of bioactive compounds

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ABSTRACT

To develop aqueous microseparation columns for bioactive compounds, a thermoresponsive polymer grafted polymer monolith was prepared inside silica capillaries having an I.D. of 100 μm by polymerization of styrene (St) with *m/p*-divinylbenzene (DVB) in the presence of polydimethylsiloxane as porogen, followed by surface-initiated atom transfer radical polymerization (SI-ATRP) of *N*-isopropylacrylamide (NIPAAm). SEM analysis indicated that the resulting poly(*N*-isopropylacrylamide) (PNIPAAm) grafted polystyrene monolith had a consecutive three-dimensionally interconnected structure and through-pores, similar to the base polystyrene (PSt) monolith. The elution behavior of steroids with different hydrophobicity was evaluated using micro-high-performance liquid chromatography in sole aqueous mobile phase. Temperature dependent interaction changes were observed between steroids and the PNIPAAm modified surfaces. Furthermore, the interaction between bioactive compounds and the PNIPAAm grafted PSt surfaces was controlled and eventually separate these molecules with different hydrophobicities by simple temperature modulation in aqueous environment. The PNIPAAm grafted PSt monolithic capillary showed improved separation properties of bioactive compounds, compared with a PNIPAAm grafted hollow capillary in aqueous environment.

1. Introduction

Stimuli-responsive polymer modified matrices have been developed to control the behavior of biomolecules on material surfaces under temperature [1,2], pH [3,4], and light [5,6] stimulation in the biomaterials field. In particular, thermoresponsive polymer modified surfaces have the advantage that they undergo rapid and easy changes in their surface properties upon temperature variations [7]. Poly(*N*-isopropylacrylamide) (PNIPAAm) is a typical thermoresponsive polymer that undergoes a reversible, temperature responsive solubility change in aqueous solutions at 32 °C, which is its lower critical solution temperature (LCST) [8]. Moreover, the PNIPAAm-modified surfaces exhibit reversible thermoresponse to change their surface properties and control the interaction between surfaces and molecules, including proteins and drug molecules, and even cells [9–11].

One of the applications of PNIPAAm modified surfaces is the development of thermoresponsive chromatography matrices for separation technology of biomolecules preserving their biological activity, using an aqueous mobile phase [12–14]. Thermoresponsive chromatography matrices are helpful to evaluate the interaction of biomolecules with biointerfaces. In particular, PNIPAAm grafted matrices prepared by surface-initiated atom transfer radical polymerization (SI-ATRP) were found to improve the separation of bioactive compounds owing to the induction of hydration/dehydration alterations of the PNIPAAm brushes with high density and controlled molecular weight [15]. Furthermore, silica capillaries modified with dense PNIPAAm brushes showed potential as thermoresponsive chromatography matrices in the microanalysis of biomolecules due to the reduction of sample vol-

Abbreviations: St, styrene; DVB, *m/p*-divinylbenzene; NIPAAm, *N*-isopropylacrylamide; SI-ATRP, surface-initiated atom transfer radical polymerization; PNIPAAm, poly(*N*-isopropylacrylamide); PSt, polystyrene; PNIPAAm-PSt, PNIPAAm grafted PSt; LCST, lower critical solution temperature; μHPLC, micro-high-performance liquid chromatography; AIBN, 2,2′-azobis(isobutyronitrile); TREN, tris(aminoethyl)amine; CuCl, copper (I) chloride; $ZnCl_2$, zinc (II) chloride; Me_6TREN, tris(*N*,*N*-dimethylaminoethyl)amine; MAPTMS, 3-methacryloxypropyltrimethoxysilane; CTCS, 2-(m/p-chloromethylphenyl)ethyltrichlorosilane; PDMS, Polydimethylsiloxane; CMME, chloromethyl methyl ether; NaOH, sodium hydroxide; THF, tetrahydrofuran; PSt-CH_2Cl, chloromethylated PSt; EDTA, ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid disodium salt; SEM, scanning electron microscopy; ATR-FTIR, attenuated total reflectance Fourier transform infrared; cor, cortisone; dx, dexamethasone; tes, testosterone.

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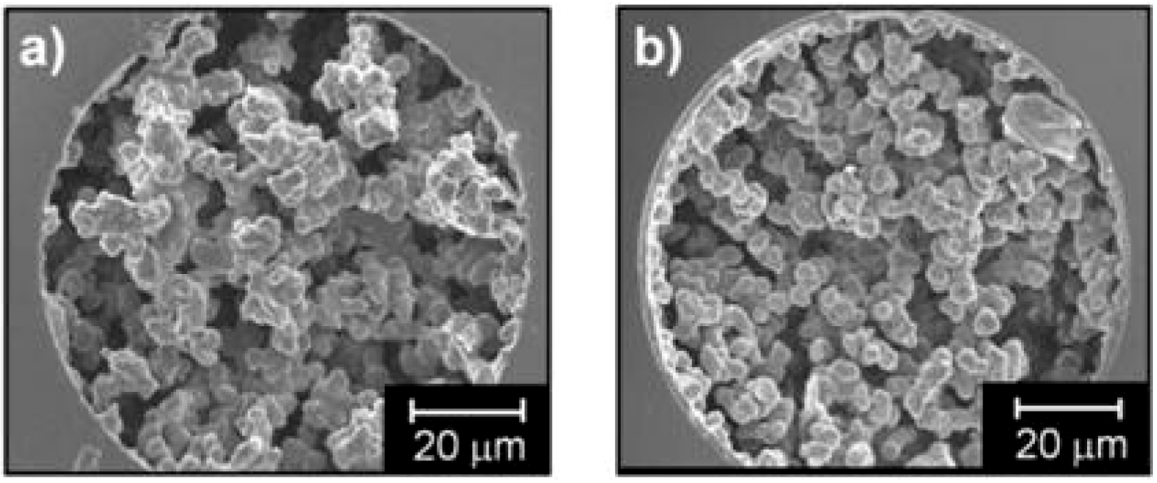

Fig. 1. SEM images of the cross section of a) PSt monolithic capillary and b) PNIPAAm-PSt monolithic capillary. The size of particle and pore of PSt monolith were 2.3 ± 1.1 μm and 10.3 ± 2.5 μm, respectively (n = 3, mean ± standard deviation).

ume and analysis time compared to traditional thermoresponsive chromatography [16]. Therefore, these thermoresponsive capillary chromatography matrices are expected to find applications in microanalysis and separation technique of biomolecules present in small amounts within biological samples [16].

The silica based stationary phase including silica capillary, however, may be limited by instable separation repeatability under alkaline conditions due to a possible erosion of the silica base [17]. PNIPAAm grafted polymer beads have been developed as thermoresponsive chromatography matrices resistant to alkaline conditions to give stable separation reproducibility of bioactive compounds [18]. However, the dense packing of the PNIPAAm modified polymer beads into a silica capillary is known to be challenging.

Thus, we focused our interest on the use of polymer monoliths as packing materials, in analogy to silica monoliths [19–21], for improving both flow and chemical resistance within the capillary. Polymer monoliths as packing materials for capillaries were reported to be capable of microseparation of organic compounds and biomolecules with the same advantages associated to the use of monolithic silica [22], such as usability of in situ preparation of packing material with high permeability in the narrow capillary [23,24] and convenience of the surface modification of functional polymer including the thermoresponsive polymer [22,25].

Although a thermoresponsive polymer grafted polymer monolith is a good candidate for the microseparation of biomolecules in capillary chromatography, the density of grafted PNIPAAm on the packing material is important to avoid the adsorption of bioactive compounds for chromatographic analysis. Peters et al. [25] reported pioneering work on PNIPAAm grafted poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) monolithic column, but the structure of grafted PNIPAAm on their monolith was partially cross-linked due to the modification of PNIPAAm in presence of cross-linker. We thus challenged to graft PNIPAAm brush with the definite structure onto the polymer monolith in the narrow capillary by applying SI-ATRP technique. The dense PNIPAAm brush grafted surface produced by SI-ATRP was reported to prevent biomolecules from the irreversible adsorption even if the PNIPAAm was grafted onto hydrophobic polymer surface [18]. However, so far only few reports about the modification of PNIPAAm brushes on a polymer monolith surface inside a conventional stainless steel column by SI-ATRP appeared in the literature [26,27]. In particular, in report by Zhang et al. [27], the authors changed only salt concentration to modulate protein interaction and eventually no temperature-dependent interaction change was demonstrated.

Thus, in this article, we prepared the PNIPAAm brush modified polystyrene (PSt) monolith into narrow capillaries for regulated interaction of biomolecules by changing only temperature using aqueous mobile phase. We also tried to characterize the PNIPAAm grafted PSt (PNIPAAm-PSt) monolithic capillary as a column of micro-high-performance liquid chromatography (μHPLC) using only an aqueous mobile phase without the use of organic solvents. In this study, a PSt monolith was first prepared within methacrylated silica capillaries (100-μm I.D.) by copolymerization of a styrene (St) and divinylbenzene (DVB) mixture in the presence of polydimethylsiloxane as porogen. A Friedel-Crafts reaction was employed to introduce a chloromethyl group as initiator for atom transfer radical polymerization (ATRP) on the PSt surface. The PNIPAAm-PSt monolithic capillaries were then prepared by SI-ATRP of NIPAAm onto the PSt surface. The elution behavior of several steroid molecules through the modified capillary was measured using μHPLC systems to investigate the interaction between bioactive compounds and PNIPAAm modified surfaces.

2. Experimental

2.1. Materials

St, DVB, 2,2′-azobis(isobutyronitrile) (AIBN), 1,3,5-trimethylbenzene, NIPAAm, tris(aminoethyl)amine (TREN), copper (I) chloride (CuCl), zinc (II) chloride ($ZnCl_2$), cortisone, dexamethasone, and testosterone were all purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ethylenediamine-*N,N,N′,N′*-tetraacetic acid, disodium salt (EDTA) were purchased from Dojindo Laboratories (Kumamoto, Japan). St was purified by distillation under reduced pressure and under a nitrogen atmosphere, and the fraction boiling at 39 °C/1.6 kPa was collected. DVB was purified according to a previous report [28]. NIPAAm was purified by recrystallization from hexane and dried under vacuum. AIBN was purified by recrystallization from methanol. Tris(*N,N*-dimethylaminoethyl)amine (Me_6TREN), used as ligand in the SI-ATRP, was synthesized from TREN according to a previous report [29]. 3-Methacryloxypropyltrimethoxysilane (MAPTMS) and 2-(*m/p*-chloromethylphenyl)ethyltrichlorosilane (CTCS) were purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Polydimethylsiloxane (PDMS) (nominal MW = 9400) was purchased from Aldrich (MO, USA). Chloromethyl methyl ether (CMME) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The fused silica capillary (inner diameter: 100 μm) was purchased