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Thermo- and pH-responsive polymer brushes-grafted gigaporous polystyrene microspheres as a high-speed protein chromatography matrix

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ABSTRACT

Dual thermo- and pH-responsive chromatography has been proposed using poly(*N*-isopropylacrylamide-*co*-butyl methacrylate-*co*-*N*,*N*-dimethylaminopropyl acrylamide) (P(NIPAM-*co*-BMA-*co*-DMAPAAM)) brushes grafted gigaporous polystyrene microspheres (GPM) as matrix. Atom transfer radical polymerization (ATRP) initiator was first coupled onto GPM through Friedel–Crafts acylation with 2-bromoisobutyryl bromide. The dual-responsive polymer brushes were then grafted onto GPM via surface-initiated ATRP. The surface composition, gigaporous structure, protein adsorption and dual-responsive chromatographic properties of the matrix (GPM-P(NIPAM-*co*-BMA-*co*-DMAPAAM) were characterized in detail. Results showed that GPM were successfully grafted with thermoresponsive cationic polymer brushes and that the gigaporous structure was well maintained. A column packed with GPM-P(NIPAM-*co*-BMA-*co*-DMAPAAM presented low backpressure, good permeability and appreciable thermo-responsibility. By changing pH of the mobile phase and temperature of the column in turn, the column can separate three model proteins at the mobile phase velocity up to 2528 cm h^{-1}. A separation mechanism of this matrix was also proposed. All results indicate that the dual thermo- and pH-responsive chromatography matrix has great potentials in 'green' high-speed protein chromatography.

1. Introduction

The interest in and demand for bioproducts in biotechnology, biochemistry and medicine has contributed to an increased exploitation of separation and purification technology of biomacromolecules. Liquid chromatography (LC) has been an important technique and a necessary procedure for the separation and purification of such macromolecules owing to its high resolution and mild separation conditions [1]. Temperature-responsive chromatography developed in recent years has drawn considerable attention in protein chromatography [2–6]. This new emerging chromatography technology is environment-friendly because it can be operated under one aqueous mobile phase by a change in temperature in the absence of organic solvents or high salt concentration. PNIPAM is a commonly used polymer in preparation of temperature-responsive stationary phase, which shows a hydrophilic–hydrophobic property transition in response to water temperature changes across a lower critical solution temperature (LCST) at 32 °C. Aside from temperature-responsive stationary phases, dual pH- and thermo-responsive chromatography media have also been evaluated as 'greener' matrices for the separation of bioactive compounds [7–10]. These environment-responsive chromatographic systems offer promise for cost-effective separation of biomolecules with low loss of bioactivity.

However, some limitations in these environment-responsive stationary phases still exist when it comes to industrial applications. The supports so far reported are dominated by silica packing materials and polysaccharide gels. Silica-based sorbents are chemical-instability at high pH and lack of operational clean-in-place robustness under the alkaline conditions often employed for the industrial cleaning of equipment [9,11]. As for polysaccharides soft gels, the poor mechanical strength of particles restricts their application in large-scale operation. In addition, the pore size of most conventional packing materials is in the range of 10–30 nm,

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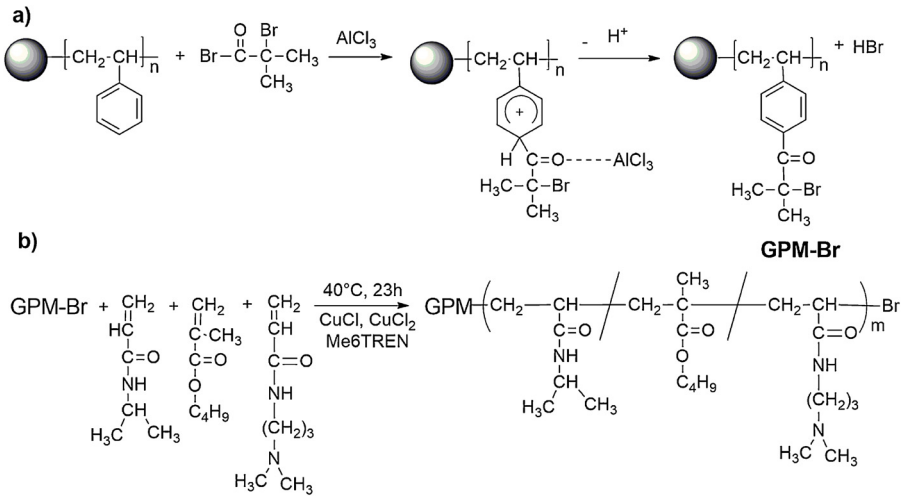

Scheme 1. (a) 2-Bromoisobutyrylation of GPM and (b) reacting route of GPM-Br grafted with P(NIPAM-*co*-BMA-*co*-DMAPAAM) brushes.

which results in stagnant mass transfer problems during the separation of biomacromolecules [12]. There has been little published literature examining the purification of large proteins.

GPM with pore diameter of about 300–500 nm developed by Zhou et al. exhibited great potentials in high-speed protein chromatography after hydrophilic modification and derivatization [13–16]. The main advantages of GPM lie in their strong mechanical strength, good chemical stability and high mass transfer rate in high flow velocities. We have recently developed a high-speed thermoresponsive medium by grafting PNIPAM brushes onto GPM via ATRP technique [17]. The introduction of thermoresponsive copolymer material not only changes the microspheres to be sensitive to temperature but simultaneously increases the hydrophilicity of the surface of the microspheres. Such a column can successfully separate protein mixtures model at the mobile phase velocity up to 2167 cm h^{-1} by changing only the external column temperature.

The purpose of the present paper is to develop a new stationary phase for the effective separation of biomacromolecules, which cannot be separated only through hydrophobic–hydrophilic interactions. We synthesized and characterized a thermo- and pH-responsive anion-exchange matrix using GPM as base supports. The successful separation of three model proteins was achieved at high flow-rate of the mobile phase. In addition, a clear separation mechanism of the dual pH- and thermo-responsive medium for model proteins is proposed.

2. Experimental

2.1. Chemicals and materials

The GPM were prepared by suspension polymerization processes as reported in our previous paper [18]. The specific surface area was 22.69 m^2 g^{-1}, the average diameter was 65 μm, and the average pore size was 280 nm. The microspheres were extracted with acetone in Soxhlet extractor for 24 h, and then dried under vacuum at 40 °C.

N-Isopropylacrylamide (NIPAM, >98%) was ordered from Tokyo Chemical Industry Co., Ltd. (Japan). Butyl methacrylate (BMA, 99%), *N*,*N*-dimethylaminopropyl acrylamide (DMAPAAM), ethylenediamine tetraacetic acid (EDTA, AR) and 2-bromoisobutyryl bromide (CP) were from Chengdu Xiya Chemical Reagent Co., Ltd. (China). Bovine serum albumin (BSA), myoglobin (MYO) and trypsin (TRY) were from Amresco (USA). Anhydrous aluminum chloride ($AlCl_3$, AR), carbon disulfide (CS_2, AR), copper(II) chloride ($CuCl_2$, 98%) and copper(I) chloride (CuCl, 98%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). High purity argon and liquid nitrogen were ordered from Qingdao Tianyuan Gas Manufacturing Co., Ltd. (China). Tris[2-(dimethylamino)ethyl]amine (Me_6TREN) was synthesized according to literature procedures [19]. Other reagents were of analytical grade from local sources.

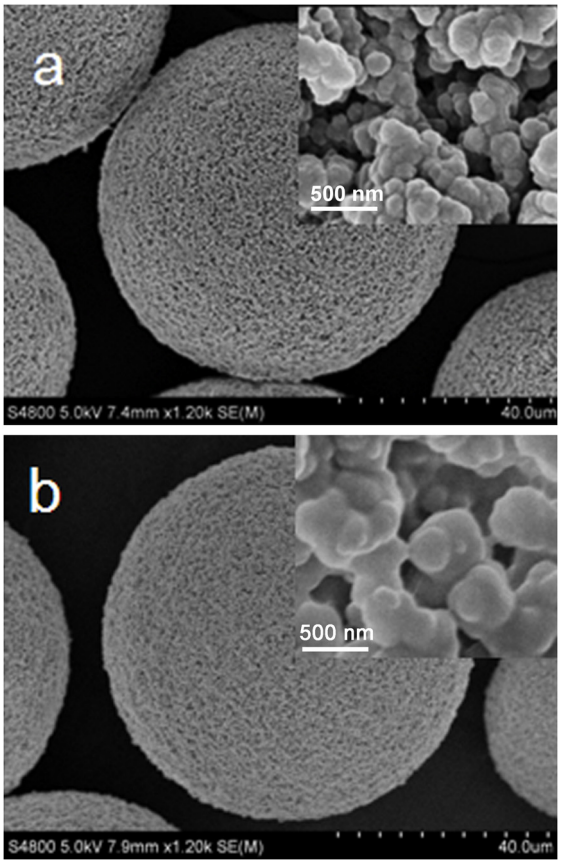

Fig. 1. SEM images of (a) GPM and (b) GPM-P(NIPAM-*co*-BMA-*co*-DMAPPAM).

2.2. Instrumentation

SEM (S-4800, Hitachi, Japan) was employed to observe the gigaporous structure of GPM and GPM-P(NIPAM-*co*-BMA-*co*-DMAPAAM). The porosity of the microspheres before and after