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Microchannel liquid-flow focusing and cryo-polymerization preparation of supermacroporous cryogel beads for bioseparation

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ABSTRACT

Polymeric cryogels are sponge-like materials with supermacroporous structure, allowing them to be of interest as new chromatographic supports, cell scaffolds and drug carriers in biological and biomedical areas. The matrices of cryogels are always prepared in the form of monoliths by cryo-polymerization under frozen conditions. However, there are limited investigations on the production of cryogels in the form of adsorbent beads suitable for bioseparation. In this work, we provide a new approach by combining the microchannel liquid-flow focusing with cryo-polymerization for the preparation of polyacrylamide-based supermacroporous cryogel beads with a narrow particle size distribution. The present method was achieved by introducing the aqueous phase solution containing monomer, cross-linker and redox initiators, and the water-immiscible organic oil phase containing surfactant simultaneously into a microchannel with a cross-shaped junction, where the aqueous drops with uniform sizes were generated by the liquid shearing and the segmentation due to the steady flow focusing of the immiscible phase streams. These liquid drops were in situ suspended into the freezing bulk oil phase for cryo-polymerization and the cryogel matrix beads were obtained by thawing after the achievement of polymerization. By grafting the polymer chains containing sulfo binding groups onto these matrix beads, the cation-exchange cryogel beads for protein separation were produced. The results showed that at the aqueous phase velocities from 0.5 to 2.0 cm/s and the total velocities of the water-immiscible phase from 2.0 to 6.0 cm/s, the obtained cryogel beads by the present method have narrow size distributions with most of the bead diameters in the range from 800 to 1500 μm with supermacropores in sizes of about 3–50 μm. These beads also have high porosities with the averaged maximum porosity of 96.9% and the mean effective porosity of 86.2%, which are close to those of the polyacrylamide-based cryogel monoliths. The packed bed using the cryogel beads with mean diameter of 1248 μm, as an example, has reasonable and acceptable liquid dispersion, but high water permeability (4.29×10^{-10} m^2) and high bed voidage (90.2%) owing to the supermacropores within the beads, enhanced the rapid binding and separation of protein from the feedstock even at high flow velocities. The purity of the obtained lysozyme from chicken egg white by one-step chromatography using the packed bed was in the range of about 78–92% at the flow velocities of 0.5–15 cm/min, indicating that the present cryogel beads could be an effective chromatographic adsorbent for primary bioseparation.

1. Introduction

Cryogels have attracted intensive attention as a new class of chromatographic adsorbents for bioseparation due to their advantages like high permeability, high porosity and supermacropores with sizes from several to hundreds of microns, which permit the rapid isolation of target biomolecules from crude feedstocks like microbial fermentation broths or cell homogenates [1–5]. These sponge-like materials also have potential applications

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as immobilization matrices in biotechnology [6–9], as scaffolds in tissue engineering [10–14], and as drug delivery carries in pharmaceutical fields [15]. Monolithic cryogels with various functional ligand groups for biochromatography have been prepared successfully by cryo-polymerization and graft method, and the mechanisms regarding the cryo-polymerization process as well as the chromatographic performance of biomolecules have also been investigated [16–32]. However, from the application view each monolith cryogel has its own shape (e.g., disk or rod) and scales (e.g., length and diameter) as its original form and could only be packed within the matched column for chromatographic applications.

Traditional particle-based adsorbents are suitable to be packed in columns with different scales, and thus popular and convenient for applications in downstream processes. These adsorbents, however, always have pore sizes below micron and the beds packed with them suffered from some disadvantages like the high back pressure and the requirement of clarified feedstocks. In recent years, macroporous adsorbents with pore sizes of hundreds of nanometers or close to micron-scale have attracted a growing interest of researchers, which could permit the fast isolation of biomolecules from various feedstocks [33–37]. The macropores within these adsorbents were generated using porogens like solvents [33,37,38], polymers and oligomers [33], solid particles [39,40] or even ionic liquids [41]. In fact, it is also possible to prepare cryogels in bead form with a similar method as that for monoliths, i.e., by freezing-thawing the aqueous droplets containing gel-forming monomers. The droplets can be generated by dropping the aqueous gel-forming mixture into water-immiscible solvents. Polyvinyl alcohol (PVA), alginate, agarose–alginate or DNA based cryogel beads have been prepared successfully by freezing–thawing the droplets generated using syringes [42,43], capillary pipettes [44,45], or by the liquid jet cutting [46] and splintering [47] methods. Most of these methods are convenient to operate and suitable for the preparation of cryogel beads mainly for immobilization purpose, but suffer some challenges either in the accurately controlling bead sizes or employing relatively complex equipments.

Microchannels and microfluidics have received great interest as a novel tool for the generation of monodisperse micro-scale drops and gel particles with precisely controllable diameters [48–52]. Due to the stable flow streams within microchannels, the segmentation of one fluid phase by the second immiscible liquid phase is very regular and the obtained drops or gel particles always have narrow size distributions. In our previous work, we also successfully prepared nano-sized lipid particles with a narrow size distribution by flow focusing in rectangle microchannels with T-shaped and cross-junctions [53,54].

Polyacrylamide (pAAm)-based cryogels are one popular class of supermacroporous monoliths for biotechnology applications [1–5]. However, there is a lack of precise methods for the production of pAAm-based cryogel beads with controllable diameters. In this work, we propose a new method by using microchannel liquid-flow focusing and cryo-polymerization of the aqueous solution containing gel-forming agents to produce pAAm-based cryogel beads. Fig. 1 shows a schematic diagram of this method. The aqueous phase mixture containing gel-forming agents was pumped into the main channel, while the water-immiscible phase solution containing surfactant was pumped simultaneously through the two branch channels into the main channel for flow focusing. The uniform aqueous monomer drops were then generated due to the focusing of the immiscible flow streams and in situ suspended into the freezing bulk water-immiscible phase to form frozen solid-state particles. The suspensions were filtered and the obtained particles were kept under frozen condition for cryo-polymerization. After the polymerization, the suspensions were thawed to get cryogel matrix beads. Controllable production of cryogel beads with a narrow diameter distribution is achieved by adjusting the flow rates of the aqueous phase and water-immiscible phase. Sulfo binding groups are grafted onto these cryogel bead matrices and the obtained cation-exchange adsorbents are applied for isolation of lysozyme from chicken egg white. The physical properties of these cryogel adsorbents like morphology, particle size distribution and porosity are measured. The permeability, the dispersion as well as the protein chromatography behaviors of the packed bed with these cryogel beads are also investigated.

2. Materials and methods

2.1. Materials

N,*N*′-methylene-bis-acrylamide (MBAAm, 99%), 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPSA, 99%), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, 99%), ammonium persulfate (APS) and lysozyme from chicken egg white were supplied by Sigma–Aldrich. Acrylamide (AAm, 99.9%), Coomassie Brilliant Blue R-250 and protein marker (a mixture of five proteins with molecular weights of 14.4, 31.0, 42.7, 66.2 and 97.4 kDa) were purchased from Biobasic (Toronto, Canada). Chicken eggs and other chemicals used (analytical grade) were obtained from local sources. All reagents were used as received.

2.2. Microchannels

The microchannel system used in this work was fabricated by using micro-milling cutter method and supplied by Wuhan Redywoods Bioengineering Co. Ltd. (Wuhan, China). The key rectangle microchannel has a cross-shaped junction fabricated on a 304 stainless steel slab with the thickness of 13 mm (Fig. 1). The main microchannel has the width, the depth and the length of 670 μm, 790 μm and 50 mm, respectively, while the branch channels of 20 mm length each located at the downstream position of 20 mm from the inlet of the main channel. The slab with microchannels was covered by a polypropylene plate (thickness of about 2 mm) followed by a milled and grinded stainless steel slab, similar as used in our previous work [53,54].

2.3. Preparation and characterization of cryogel beads

The pAAm cryogel matrix beads were prepared by the microchannel liquid-flow focusing and cryo-polymerization method. Typically, AAm and MBAAm were dissolved in deionized water and cooled to be about 3–5 °C. Then the amounts of TEMED and APS (both dissolved in deionized water) were quickly added into the solution with stirring and the obtained aqueous reaction solution was used as the disperse phase. The concentrations of AAm and MBAAm were 7.5% (w/w) and 1.5% (w/w), while the mass ratios of TEMED and APS to the total mass of AAm and MBAAm were 1.2% (w/w) and 0.5% (w/w), respectively. The water-immiscible phase was prepared by dissolving Span 80 in ethyl enanthate with a final concentration of 0.5% (v/v). The aqueous phase was injected into the microchannel at a given flow rate, while the water-immiscible phase was introduced from the two branch channels simultaneously for flow focusing [53,54], both by using precision syringe pumps (Model 11 plus, Harvard Apparatus Inc., Holliston, USA). The flow rate in each of the branch channels was kept the same. The suspension containing the aqueous drops and the water-immiscible phase at the outflow of the microchannel was introduced into the bulk ethyl enanthate solution containing 0.5% Span 80 in a glass beaker and stirred mechanically at 50 rpm. The temperature of the bulk water-immiscible phase (500 mL) was maintained at about −26 °C. During each run, series of the original aqueous solution phase fractions were prepared per 6 min in order