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Fabrication and characterization of aligned macroporous monolith for high-performance protein chromatography

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ABSTRACT

In the present study, a freeze casting method combined with particle accumulation was applied to fabricate the aligned macroporous monolith for high-performance protein chromatography. For the preparation, the reactive colloids were first prepared by using glycidyl methacrylate and ethylene glycol dimethacrylate as monomers. Subsequently, these colloids accumulated regularly and polymerized into the aligned macroporous monolith. The aligned porous structure of the monolith was characterized by SEM, mercury intrusion, and flow hydrodynamics. The results revealed that the generated monolith was possessed of aligned macropores in size of about 10 μm and high column permeability. Finally, after being modified with sulfonated groups, the monolith was evaluated for its chromatographic performance. It demonstrated that the aligned macropores endowed the monolith with excellent adsorption capacity and high column efficiency.

1. Introduction

In recent years, much attention has been directed to the development of porous monolith, which has been proved to be an excellent material for protein chromatography. For the preparation, a mixture of vinyl monomers and initiator is polymerized into colloids in an inert solvent, and the accumulation of colloids transforms into diffusive pores and heterogeneous macropores [1]. Generally, the bimodal porous system is the key factor that determines the chromatographic performance, because it not only allows for fast mass transport through the monolith but also provides high specific surface area for protein adsorption. Until now, numerous strategies (living radical polymerization, high internal phase emulsion polymerization, TEMPO-mediated living radical polymerization, template-assisted radical polymerization, cryotropic gelation technique, atom transfer radical polymerization, transition metal-catalyzed polymerization, and electron-beam initiated polymerization) have been developed to construct various porous monoliths for high-performance chromatography [2–10]. However, since the abovementioned methods are all based on organic phase separation technique, the produced macropores are often random and irregular. With such irregular macropores, some molecules would move more slowly and lag behind in the monolith, thereby compromising column efficiency and permeability [11–13]. Hence, how to design and construct the porous structure, becomes a critical issue to develop a high-performance monolith for protein chromatography.

Compared with irregular pores, aligned macropores are considered as suitable pore structure for high-performance chromatography. These aligned macropores not only provide flow-through paths for fast mass transfer but also facilitate uniform liquid transport by reducing eddy diffusion [14]. With such aligned macropores, the monolith enjoys the advantages of high column efficiency and good permeability. However, it is still a challenge to explore efficient methods for the production of monolith with desired aligned porous structure.

The cryotropic gelation is a simple and versatile route to fabricate organic monolith with large pores for chromatography. For the typical preparation, a monomer-contained solution is frozen in liquid nitrogen, in which the crystals grow into a continuous frozen framework and are interspersed with the non-frozen monomer phase. After being polymerized, the bicontinuous pore system is preserved and a highly porous monolith is formed [5]. Unfortunately, the constructed monoliths, despite their high porosity, still contain heterogeneous porous structure and low specific surface area. This deficiency is ascribed to the irregular crystal growth and the lack of diffusion pores on the monolith skeleton. Theoretically, the crystals can be originated into a regular shape in a solution or colloidal suspension by a directional freeze casting process, and the subsequent removal of irregular crystals can generate the aligned

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porous structure. For example, Lozinsky has reported the preparation of poly(vinyl alcohol) based aligned porous material and its potential application for bioseparation [15,16]. Meanwhile, the accumulation of particles can transform into porous material with high specific surface area. However, the constructed aligned porous material generally exhibits the poor mechanical strength and the inert surface [15–17]. Given that, this material cannot meet the requirement of stationary phase for the chromatographic application.

In this context, a preparation strategy toward aligned porous monolith was proposed by combining freeze-casting method together with particle accumulation. In this strategy, two points should be taken into account to meet the requirement of high-quality monolith for chromatography. One is the choice of reactive monomers (glycidyl methacrylate and ethylene glycol dimethacrylate), which were often used to prepare the chromatographic support. The other is the synthesis and post polymerization of the reactive colloids. With the post polymerization, the accumulated colloids are chemically linked each other and then contributed the monolith with strong mechanical strength and diffusional pores. Under the optimized conditions, the aligned porous monolith was prepared and then was evaluated in detail for protein chromatography.

2. Experimental

2.1. Materials

Glycidyl methacrylate (GMA) and ethylene glycol dimethacrylate (EDMA) were obtained from Yuanji Chemical Corp. (Shanghai, China). Benzoyl peroxide (BPO) (95%) was obtained from Damao Chemical Corp. (Tianjin, China) and recrystallized before use. Lysozyme (MW 14kd, pI11.0) and IgG (MW 160kd, pI8.0) were purchased from Sigma (MO, USA). Tripropylene glycol, polyethylene glycol, dodecanol, cyclohexanol, 1,4-dioxane, and other solvents were of analytical grade and obtained from local sources.

2.2. Preparation and modification of aligned macroporous monolith

The fabrication process of aligned macroporous monolith is described in detail as follows. First, the polymerization system was prepared by dissolving initiator (BPO, 1.0% of monomers in weight) and reaction monomers (GMA and EDMA) in porogenic solvent (1,4-dioxane, tripropylene glycol, dodecanol, and cyclohexanol). The proportions of all components were 15 wt.% for GMA, 10 wt.% for EDMA, 20 wt.% for 1,4-dioxane, 17.5 wt.% for tripropylene glycol, 25 wt.% for dodecanol, 12.5 wt.% for cyclohexanol, respectively. After being purged with nitrogen for 30 min, the mixture was heated in a water bath at 60 °C for 6 h with stirring to produce a milk-like suspension. Subsequently, the suspension was poured into a blank 50 mm × 5 mm I.D. column and frozen unidirectionally from the column bottom upward by immersing the column into a liquid nitrogen bath at a constant rate of 3 cm h^{-1}. After being completely frozen, the frozen sample was heated to 80 °C quickly and kept for 12 h to solidify. Then, the monolith-filled column was connected with a peristaltic pump to remove the porogenic solvent, which led to the generation of aligned porous monolith. Then, the aligned porous monolith was modified with sulfonic groups as follows [17,18]. In brief, the solution of Na_2SO_3/2-propanol/water (in a weight ratio of 10/15/75) was pumped through the epoxy-anchored monolith at 0.2 ml min^{-1} and 37 °C for 12 h. This process converted the epoxy groups into sulfonic acid groups within the aligned porous monolith. Subsequently, the functionalized monolith was rinsed repeatedly with water to remove the reaction residues and then stored in 20% aqueous ethanol solution for the next chromatographic evaluation.

2.3. Characterization of aligned macroporous monolith

Scanning electron microscopic (SEM) images were recorded on an XL 30 ESEM (Philips, Netherlands) to investigate the microscopic morphology of the aligned macroporous monolith. A thin gold film was sputtered on the samples before SEM measurement. Pore size distribution was analyzed by mercury porosimeter (Quantanchrome Corporation, USA). The chromatography experiments were carried out by the Waters HPLC system (Waters, Milford, MA, USA) with a model 600E multi-solvent delivery system, a Rheodyne 7725i injector valve (Rheodyne, Cotati, CA, USA), and a 2748 UV detector. The sulfonated ligand density of aligned porous monolith was determined by titrating with 1 mol L^{-1} NaOH solution [19].

2.4. Flow hydrodynamics

The flow hydrodynamics of aligned macroporous monolith was investigated by analyzing the variation of back pressure of column along with flow rate of pure water [19]. The column pressure values with and without the monolith were recorded at each flow rate, and the difference between the two values was determined as the back pressure through monolith. Based on these hydrodynamic data, the hydraulic permeability of aligned macroporous monolith was calculated by using Darcy's model [5].

$$\frac{\Delta p}{L} = \frac{u\mu}{B_0} \tag{1}$$

where Δp stands for back pressure (Pa), L for column length (m), u for velocity (m s^{-1}), μ for mobile phase viscosity (Pa s), B_0 for hydraulic permeability (m^2).

2.5. Frontal analysis and dynamic binding capacity

Frontal analysis is based on the breakthrough curve of proteins and often used to determine the dynamic binding capacity of the chromatographic support [5]. To highlight the role of aligned macropores on adsorption efficiency, varied protein solutions (2 mg mL^{-1} lysozyme or 1 mg mL^{-1} IgG) in buffer A (20 mM phosphate solution, pH 6.0) were applied for the breakthrough experiment. Prior to each protein feeding, the aligned macroporous monolith was equilibrated with buffer A until the absorbance at 280 nm reached the baseline. After reaching the adsorption equilibrium, the protein solution in buffer A was continuously loaded into the aligned macroporous monolith and the protein concentration in the outlet stream was assayed using the UV monitor at 280 nm. When the breakthrough was completed, the protein loading was stopped and eluted with buffer B (1.5 M NaCl in 20 mM phosphate). Subsequently, the amount of eluted proteins was determined based on the protein concentration and volume in the eluent. The protein recovery was obtained based on the ratio of the eluted and adsorbed proteins. The whole porosity of aligned macroporous monolith (system void volume) was determined by using pulse chromatography with acetone as the non-retained solute [5]. All experiments were performed in triplicate under each set of tested conditions. The protein dynamic binding capacity (DBC) on the aligned porous monolith was determined as a mean value of three assays by the following equation [5]:

$$q_x = \frac{c_0(V_x - V_0)}{V_b} \tag{2}$$

where q_x is the dynamic binding capacity (mg mL^{-1}) at x% breakthrough, c_0 is feed IgG concentration (mg mL^{-1}), V_b, V_x and V_0