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Efficient adsorption of hemoglobin from aqueous solutions by hybrid monolithic cryogel column

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ABSTRACT

In this study, a supermacroporous poly(2-hydroxyethyl methacrylate) (PHEMA)-based Cu^{2+}-attached bentonite particles embedded hybrid monolithic cryogel (Cu^{2+}-ABPs EHMC) was prepared by radical cryo-copolymerization of HEMA with *N,N′*-methylene-bis-acrylamide (MBAAm) as a cross-linker directly in a plastic syringe in the presence of Cu^{2+}-ABPs, and used for hemoglobin (Hb) separation from aqueous solution. Due to the naturally abundant hydroxyl groups on bentonite, Cu^{2}-attached bentonite particles (Cu^{2+}-ABPs) embedded hybrid monolithic cryogel (HMC) showed a good adsorption performance for Hb, covering an excellent adsorption capacity of 521.6 mg/g bentonite in phosphate buffer at pH 6.0 with a flow rate of 0.5 mL/min, fast binding stability within 60 min, well selectivity and reversibility. Compared with other adsorbent systems used for protein separation, this sorbent has many advantages such as excellent adsorption performance, ease of preparation, suitable, effective and environment-friendly to perform, which can serve as a more sustainable material in protein separation and purification.

1. Introduction

Macroporous cryogels have a 3D-interconnected flexible network with perfect mass fluidity as well as chemical and mechanical stability. These excellent properties such as short diffusion path, large pores, very short residence time and low-pressure drop [1] endow them remarkable feasibility in some area, including (bio)chromatography [2] biocatalysts or heterogeneous catalyst [3,4] etc. Whereas, the weakness of these materials is the low binding capacity due to the interconnected supermacropores with low surface area [2,5]. Therefore, improvements in the binding capacity of these cryogels have a great importance in biotechnology and separation science [6].

Particles embedding into cryogels are a special type of monolithic column to enlarge the surface area due to the combination of the unique properties of cryogels and particles. Up to now, many particle embedding studies with different successful applications have been presented in the literature [7–12]. The adsorbent with large surface area and greater pore volume offers high loading capacity. The large pores in the adsorbent facilitate the transport of biomolecules without harming 3D-structure nature of them.

Recently, as a competitor to the commercial counterparts, low-cost natural materials (leaves, clay and clay minerals, activated carbon fibres, fungi, etc.) with large surface areas which enable the high adsorption performance have been preferred [13]. Among these, clay and clay minerals (i.e., bentonite, diatomite) have been used frequently in many areas, such as enzyme adsorption [14], catalyst support [15], antibody purification [16] and the cosmetics and pharmaceuticals industry [17]. Due to the presence of the high surface negative charge and many exchangeable positive ions, bentonite may be used as a cation exchanger for heavy metal binding, like Cu^{2+} for a high protein loading.

Hemoglobin (Hb), a globular hemeprotein, is the iron-containing oxygen transport metalloprotein in the red blood cells in mammals. Hb that has a molecular weight of 64 kDa is composed of four polypeptide subunits (two α and two β), and each of them contains a heme (iron-porphyrin) as the active centre. The Hb is one of the most abundant protein for human and animals located in red blood cells. In some diseases such as sickle cell anemia, hemoglobinopathies and thalassaemia rupture, free Hb is found as high as 0.9 mg/mL in the blood volume by shielding low abundance proteins which are potential biomarkers in proteomic studies [18]. Up to now, some separation methods for Hb have been discussed such as imprinting technique [19], ion exchange chromatography [20], immobilized metal affinity chromatography [18,21], etc. In this study, we aim to develop a straightforward approach to prepare an adsorbent for Hb separation based on

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immobilized metal affinity chromatography with high capacity and fast binding stability. The preparation and characterization of Cu^{2+}-ABPs EHMC and the application of it for Hb adsorption from aqueous solutions were investigated and discussed here.

2. Experimental

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA), *N,N′*-methylene-bis-acrylamide (MBAAm) and bovine hemoglobin (Hb, mol wt: 64,500 D) were purchased from Aldrich (Munich, Germany) and used for both adsorption studies and SDS page as marker. *N,N,N′,N′*-Tetramethylethylenediamine (TEMED) and ammonium persulfate (APS) were supplied by Sigma (St. Louis, MO, USA). All other chemicals were of reagent grade and were purchased from Merck AG (Darmstadt, Germany). The natural bentonite was obtained from the locality of Ünye (Ordu, Turkey). The water used in the experiments was purified using a Barnstead (Dubuque, IA, USA) RO pure LP® reverse osmosis unit.

2.2. Preparation of bentonite particles (BPs)

The natural bentonite obtained from the locality of Ünye (Ordu-Turkey) was washed using distilled water to remove contaminants with the potential to affect the adsorption performance, and treated by the sedimentation method to obtain particle sizes of about 2 μm. The bentonite particles were warmed up to 150 °C for 5 h to remove unwanted organic impurities from the structure and surface. Due to this heating process, the surface area of the particles was enlarged.

2.3. Preparation of Cu^{2+}-attached bentonite particles (Cu^{2+}-ABPs)

Attachment of the Cu^{2+} ions onto the pumice particles was performed as described previously [22]. All studies were carried out in triplicate. The amount of attached Cu^{2+} ions onto the pumice particles was determined from the differences between the initial and final Cu^{2+} concentrations. The outflow amount of Cu^{2+} from the particles was also studied in the media with pHs varied from 4.0 to 8.0 containing 1.0 M NaCl.

2.4. Preparation of Cu^{2+}-ABPs embedded hybrid cryogel column

The polymerization steps before the embedding process were described in our previous study [11]. After the preparation of the polymer solution, 10 mg of Cu^{2+}-ABPs was added to the solution, and the prepared solution was poured into a plastic syringe (5 mL, i.d. 0.8 cm). Then it was frozen at −12 °C for 24 h, and afterwards thawed at room temperature. After thawing, the prepared column was respectively washed with diluted HCl solution and a water-ethanol mixture for removing possible impurities, such as unconverted monomers and initiator. The clearance of the matrix was observed by changes in the optical densities of the washing solutions. At the end of the washing process, the cryogels were left in a solution of 0.02% sodium azide at 4 °C till use. The Cu^{2+}-ABPs embedded cryogels for the adsorption of Hb are shown in Fig. 1.

2.5. Characterization of hybrid cryogel

The φ value was calculated to determine the free water amount in the cryogel sample and the volume. The saturation of a piece of cryogel was provided with deionized water. As a next step, this piece was immersed in water of volume V_1. Volume V_2 was determined as the total volume of the cylinder. The volume difference, $V_0 = V_2 - V_1$, was estimated to define the volume V_0, the volume of the water-saturated cryogel.

The water-saturated cryogel was weighed to determine the mass, m_w. Free water within the large pores of the cryogel was removed by squeezing the cryogel sample. For the next step, the cryogel sample not containing free water was weighted to determine the mass of the cryogel, m_s, as given before [11]. Eq. (1) was used to calculate the porosity of the cryogels:

$$\varphi = (m_w - m_s)/\rho_w \times V_0 \times 100 \quad (1)$$

where ρ_w is the deionized water density. To obtain totally dry cryogel, it was placed in an oven at 60 °C for 12–24 h and the m_d (dried cryogel mass) was obtained. Eq. (2) was used to estimate the total water fraction (*TWF*):

$$\mathrm{TWF} = (m_w - m_d)/\rho_w \times V_0 \times 100 \quad (2)$$

The microstructure and morphology of the hybrid cryogel were observed by scanning electron microscope (SEM). For this purpose, samples were swelled in water, and then put into absolute (98%) ethanol for displacing of water molecules with alcohol ones in the pores. After completion of alcohol diffusion into the pores, the samples were put into vacuum oven at 50 °C to remove alcohol from the samples without harming to structures. The sample obtained after the dehydration process was coated with gold-palladium (40:60) and examined for SEM (EVO LS 10 ZEISS 5600 SEM, Tokyo, Japan).

2.6. Hb adsorption studies from aqueous media

The experiments for the Hb adsorption onto the Cu^{2+}-ABPs embedded cryogel column were performed through a temperature controlled system of water jacket. The washing process was performed with 20 mL of distilled water, and the cryogels were equilibrated using 0.02 M phosphate buffer (pH 6.0). Then, the prepared Hb solution (i.e., 20 mL) was sent to the column via a peristaltic pump. The adsorption was followed by determining the decrease in the protein concentration using the

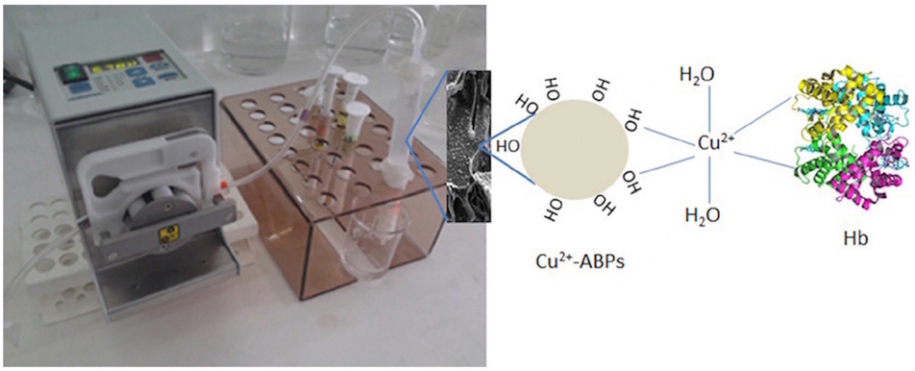

Fig. 1. Hybrid cryogel column embedded with Cu^{2+}-ABPs for Hb.