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# Protein adsorption in supermacroporous cryogels with embedded nanoparticles

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**Abstract**

Supermacroporous cryogel with embedded nano-size adsorbent particles is a new media suggested for separation and purification of biomolecules. In order to reveal the protein adsorption characteristics in this novel cryogel, several polyacrylamide-based cryogel beds with embedded $Fe_3O_4$ nanoparticles were prepared by radical cryo-copolymerization of acrylamide, *N*,*N*′-methylene-bis-acrylamide, allyl glycidyl ether and surfactant-stabilized $Fe_3O_4$ nanoparticles under freezing variation conditions in glass columns of inner diameters 16 and 26 mm. Bovine serum albumin (BSA) was used as model protein to study the cryogel adsorption behaviors in the cryogels. Experimental measurements of the isothermal and kinetics adsorption of BSA were carried out and the protein adsorption capacities were determined. Breakthrough curves of BSA in NaAc–HAc buffer at different liquid flow rates were obtained to reveal the protein adsorption behaviors. Effect of liquid velocity, as well as the pore-structures and liquid dispersion on the protein breakthrough performance were investigated and analyzed.


*Keywords:* Supermacroporous cryogel; Nanoparticle; Bovine serum albumin; Protein; Isothermal adsorption; Dynamic adsorption; Breakthrough curve

## 1. Introduction

Supermacroporous cryogel monolith chromatography is recently suggested as an efficient separation method for recovery of recombinant proteins, enzymes, microbial cells, viruses and other bioproducts in downstream processes [1–13]. The monolithic cryogel has sponge-like structure with interconnected supermacropores of diameters of several to hundreds micrometers and it can be prepared by cryogenic copolymerization of polymer monomers under frozen condition followed by a thawing step. This cryogel sponge not only has founded its applications as an attractive stationary phase in bioseparation, but also has potential application as a porous biocompatible matrix for mammalian cell growth in tissue engineering [14].

As a new chromatographic media, however, the binding capacity of cryogel for proteins or enzymes is low due to the existing of large pores within the gel matrix [6]. Recently, Savina et al. [15] developed cryogel monoliths with high amount of functional groups for protein adsorption by graft polymerization of *N*,*N*′-dimethylaminoethyl methacrylate (DMAEMA) onto the polyacrylamide-based cryogel pore surface initiated using potassium diperiodatocuprate and the BSA binding capacity of the obtained cryogel was increased. We developed a novel polyacrylamide-based cryogel monolith by embedding nano-size $Fe_3O_4$ adsorbent particles in cryogel matrix with their partial surfaces exposed to the liquid in cryogel pores [16]. These nanoparticles have large specific area and high protein loadings [17–19] and served as effective binding sites for protein adsorption. It was found that BSA adsorption capacity of this cryogel was also improved due to these nano-size adsorbent particles [16].

In this work, isothermal and kinetic adsorption and chromatography performance of BSA under various conditions in these cryogel beds were investigated. Measurements of BSA breakthrough curves in NaAc–HAc buffer at different liquid flow rates were carried out. Influences of liquid velocity as well as the pore-structures and liquid dispersion on protein breakthrough behaviors were studied and analyzed.

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**Nomenclature**

| | |
|---|---|
| $A$ | cross-section area of cryogel monolith ($m^2$) |
| $c$ | equilibrium concentration of protein in the batch adsorption ($kg/m^3$) |
| $c_b$ | protein concentration in the bulk phase at time $t$ ($kg/m^3$) |
| $c_0$ | initial protein concentration in the batch adsorption ($kg/m^3$) |
| $C(t)$ | measured concentration of protein in chromatography ($kg/m^3$) |
| $C_0$ | feed concentration of protein in chromatography ($kg/m^3$) |
| $L$ | length of cryogel bed (m) |
| $K_a$ | adsorption constant ($m^3/kg$) |
| $K_L$ | overall mass transfer coefficient for protein from liquid phase to pore surfaces (1/s) |
| $m$ | mass of dried matrix (kg) |
| $\Delta p_w$ | pressure drop (Pa) |
| $q$ | binding density of protein (kg/kg dried matrix) |
| $q_{max}$ | adsorption capacity (kg/kg dried matrix) |
| $q_t$ | total amounts of dynamic absorbed protein (kg) |
| $Q_w$ | water flow rate ($m^3/s$) |
| $t$ | time (s) |
| $U_L$ | superficial liquid velocity (m/s) |
| $V$ | volume of the feed solution used in the finite batch adsorption ($m^3$) |
| $V_f$ | volume of feed applied to column ($m^3$) |

*Greek letter*

| | |
|---|---|
| $\varphi$ | cryogel bed porosity |

## 2. Experimental

### 2.1. Materials

Acrylamide (AAm, 99.9%) was from Biobasic (Toronto, Canada). *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (TEMED, 99%) and bovine serum albumin (BSA, 98%) were from Amresco (Ohio, USA). *N*,*N*′-Methylene-bis-acrylamide (MBAAm, 99%) and allyl glycidyl ether (AGE, 99%) were from Sigma–Aldrich (Steinheim, Germany). Ammonium persulfate (APS, 98%), $FeCl_2{\bullet}4H_2O$ (98%), $FeCl_3{\bullet}6H_2O$ (99%), decanoic acid (>98.5%), ammonium hydroxide (28% $NH_3$ in water) and other chemicals used (analytical grade) were from local sources. All reagents were used as received.

### 2.2. Preparation of cryogel beds with embedded $Fe_3O_4$ nanoparticles

Free radical cryo-copolymerization method was used to prepare the cryogel beds with embedded $Fe_3O_4$ nanoparticles, as detailed described in [16]. The monomers used were AAm, MBAAm and AGE. The weight of AAm/MBAAm = 4.6/1 and AAm/AGE = 6.2/1 in all the preparation experiments and the total concentration of monomers for different beds was 7%. The amount of TEMED and APS were 0.5 and 1.2% of the total weight of monomers. The weight of $Fe_3O_4$ nanoparticles in the reactive mixture was 0.92% (w/w). In a typical preparation, surfactant-stabilized $Fe_3O_4$ nanoparticles coated with decanoic acid were synthesized by chemical co-precipitation according to [20] and dialyzed using dialysis membrane (MWCO10000) in deionized water for 24–48 h. The obtained dialyzed suspension was mixed with the aqueous solution of monomers under vigorous stirring conditions followed by adding TEMED and APS. The reactive mixture was poured into glass columns with different inner diameter (diameters of 16 and 26 mm, length of 200 mm) and frozen in a program controlled refrigerated bath system (THCD-01, Tianheng Instrument Factory, Nibo, China) under freezing routes A or B shown in Fig. 1. Finally, the columns were thawed and washed with acetone solution and water and used for further experiments.

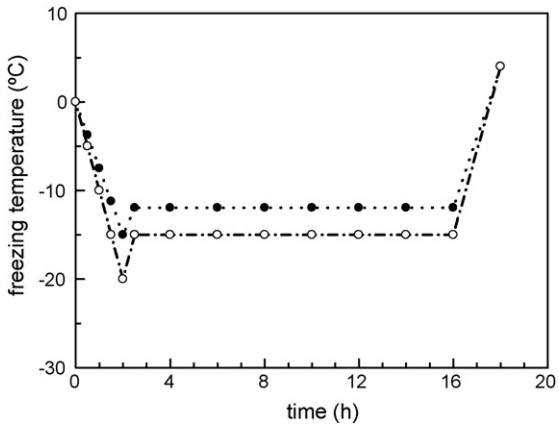


Fig. 1. Variations of freezing-temperature with time in the cryogel preparation. Freezing (●) route A and (◯) route B.

### 2.3. Measurements of cryogel bed properties

The axial dispersion of cryogel beds were tested by measuring residence time distributions (RTDs) using tracer pulse method at different flow rates. The total theoretical plate numbers ($N$) were calculated by the obtained data of RTDs and the values of height equivalent to theoretical plate (HETP) were calculated together with the column length [8]. A chromatography system combining on-line flow-through UV spectrometer at 280 nm (HD-21-88, Qite Analytical Instruments, Shanghai, China) and switching valve (Knauer, Berlin, Germany) were used in the measurements. The flow resistance of cryogel beds was evaluated by measuring the relationship of water flow rate versus hydrostatic pressure across columns at different hydrostatic pressure drops. Porosities of cryogel beds were estimated by the content of free water and the cryogel volume of a given sample. Morphology photograph of gel structure was examined using a scanning electron microscope (XL-30-ESEM, Philips, Netherlands) and morphology of the embedded $Fe_3O_4$ nanoparticles in cryogels was examined using a JEOL JEM-1230 transmission electron microscopy. Detailed experimental procedures for