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Small-sized dense magnetic pellicular support for magnetically stabilized fluidized bed adsorption of protein

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Abstract

A small-sized dense magnetic pellicular support (MPS) for magnetically stabilized fluidized bed (MSFB) adsorption of protein has been fabricated by introducing magnetic colloids and glass beads during the emulsification of 4% agarose solution. The MPS was identified with a mean density of 1.40 g/ml and a size range of 70–130 μm. The support has a good magnetization property with little residual magnetization after the field was removed. After cross-linking and reducing, the MPS was coupled with diethylaminoethyl (DEAE) groups to create an anion exchanger DEAE–MPS. The fluidization behavior of the support was examined in an MSFB with transverse magnetic field and the results were compared with those obtained in expanded bed. As a result, an optimum field intensity of 250 Gs was found to give the lowest axial dispersion of the MSFB. At the optimum field intensity, BSA adsorption at 208 and 283 cm/h showed that the dynamic binding capacity in MSFB was about 60% higher than that in expanded bed mode. It was attributed to the reduced backmixing in the MSFB compared to that in expanded bed. The results indicate that the dense MPS is promising for MSFB protein adsorption at elevated flow rates.

Keywords: MSFB; Magnetic support; Magnetic field; Agarose gel; Protein adsorption; Glass bead

1. Introduction

Magnetically stabilized fluidized bed (MSFB) uses an externally applied, axially or transversely aligned magnetic field to independently control the magnetic particles movement in a column. Even at high liquid-phase flow velocities, magnetic stabilization can serve to suppress mixing of the solid particles, thus, MSFB exhibits combined advantages of fixed bed and fluidized bed in a liquid fluidized bed system (Siegell, 1987; Liu et al., 1991; Webb et al., 1996; Hou and Williams, 2002; Tong and Sun, 2003). This enables the concentration and separation of soluble substances from crude feedstock containing fine particles. This technology is expected to show potential applications in various areas such as bioseparations and immobilized enzyme systems (Dekker, 1989; Kondo and Fukuda, 1997, O'Brien et al., 1997; Diettrich et al., 1998; Zhang et al., 1999).

Early research of the rheology in MSFB concentrated more on gas-fluidized beds, which usually leads to enhanced mass transfer. However, with ion exchangers in liquid MSFB, a reduction of the mass transfer coefficients has been recognized (Hausmann et al., 2000; Franzreb et al., 2001). Thereby, MSFB has been recommended for application in high flow velocity process, which is consistent with one of its advantages over conventional fluidized bed or expanded bed (EB). That is, it is possible for an MSFB to be operated at elevated flow velocities with less risk of elutriation of the particles. When high flow velocity is applied, the mass transfer limit within the liquid film surrounding the supports can be reduced (Thömmes, 1997), and the treatment of viscous feed with large debris becomes convenient. Thus, suitable adsorbents for high flow

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velocity operation in MSFB have aroused the interest of biochemical engineers.

In the previous work of our laboratory (Tong and Sun, 2003), a magnetic agarose support has been fabricated for MSFB adsorption of protein. Due to the low density of the magnetic agarose support, however, its MSFB had to be operated at a very low flow velocity (45 cm/h) to retain a suitable bed expansion. Thus, in this article, we have attempted to prepare a dense magnetic agarose support. For this purpose, the concept of dense pellicular matrix (Pålsson et al., 2000; Tong and Sun, 2002a) was introduced in the fabrication procedure. That is, the magnetic support was fabricated by coating glass bead with agarose gel in which magnetic colloids were dispersed. The dense support was then coupled with diethylaminoethyl (DEAE) groups to make an ion-exchange adsorbent. Properties of the ion exchanger and the behavior of its MSFB for dynamic protein adsorption at high flow rates were investigated to evaluate its usefulness in protein chromatography in MSFB mode.

2. Materials and methods

2.1. Materials

Agarose was obtained from Gene Co., Ltd. (HK, China). Bovine serum albumin (BSA) was purchased from Sigma (St. Louis, MO, USA). Glass bead was from YongYing Glass Product Company (Langfang, Hebei, China). The glass bead was screened and washed with NaOH (1.0 mol/l), HCl (1.0 mol/l) and ethanol to remove any fouling before use. 2-(diethylamino)ethyl hydrochloride (DEAE-HCl) was purchased from Aldrich (Milwaukee, WI, USA). Sorbitan monooleate (Span 80) was obtained from Tianhai Fine Chemicals (Tianjin, China). Two permanent magnets ($90 \times 50 \times 20$ mm, maximum intensity of the field 4.0×10^3 Gs) were provided by the Research Institute of Rare Earth Elements (Baotou, China). Edible soybean oil was purchased from a local market. All other chemicals were of analytical grade from local sources.

2.2. Preparation of magnetic colloids

Agarose-stabilized magnetic colloids were synthesized by a modified coprecipitation method (Cuyper and Joniau, 1991; Tong and Sun, 2001). In a typical procedure, 7.2 g $FeSO_4$ and 14.0 g $FeCl_3$ were dissolved in 150 ml water and the solution was added to a 500-ml round-bottom glass flask. The solution was heated to 90 °C in a water bath and 40 ml of 25% ammonia was slowly added. After 5 min reaction under stirring at 1000 rpm, 20 ml of 4% agarose solution was slowly added to the flask and the reaction continued for 1 h at 90 °C. Then, the magnetic colloids in the suspension were collected as precipitate under magnetic field and washed with excessive amount of water to remove soluble salts. The magnetic precipitate was stored at room temperature for further use.

2.3. Fabrication of magnetic pellicular support

The water-in-oil emulsification method was utilized to prepare the magnetic agarose gel coated glass bead. The method was similar to those previously reported for the preparation of dense pellicular matrices (Pålsson et al., 2000; Tong and Sun, 2002a; Zhou et al., 2004), but was modified due to the difference in the properties of the mixture of glass bead and magnetic colloids as solid-phase that was coated. The procedure is briefly described below.

A 500-ml round-bottom glass flask equipped with a half-moon paddle fitting to the flask bottom was used to prepare water-in-oil emulsion. Soybean oil (240 ml) containing 5% Span 80 was added to the vessel and heated to 90 °C in a water bath. Then, 8 g of preheated glass beads (40–78 μm) and magnetic colloids (2 g) were suspended to 40 ml of 4% agarose solution at 90 °C. The suspension was slowly poured into the oil phase stirred at 1400 rpm. After 5 min, the vessel was rapidly cooled below 15 °C with cold water in the water bath, and the magnetic pellicular support (MPS) was recovered by the permanent magnet and washed with water and acetone to remove any adsorbed oil.

2.4. Cross-linking and DEAE coupling

The MPS was cross-linked and reduced by the method described earlier (Tong and Sun, 2002a). Before coupling DEAE, the particles were screened with standard sieves of 200 and 300 mesh. The particle size distribution of the screened MPS and glass bead was measured with the Mastersizer 2000 unit (Malvern Instruments, UK), and its image was recorded with an XS-18 optical microscope (Jiangnan Scientific Instruments, China) fitted with a QuickCam Pro 4000 camera (Logitech, Switzerland). The magnetization curve of the MPS was measured with an LDJ-9600-1 vibrating sample magnetometer (LDJ Electronics, Inc., MI) at room temperature.

The procedure for DEAE coupling is described as follows. The MPS particle was drained to remove the interparticle fluid, and the drained solid phase was mixed with twice volumes of 3 mol/l DEAE–HCl in a 250 ml flask. After agitation at 170 rpm in water bath of 40 °C for 30 min, NaOH was introduced to a final concentration of 1.5 mol/l, and the agitation was continued for additional 1 h. Then, the solid phase was routinely washed with deionized water, 25% ethanol, 0.5 mol/l NaCl solution. The ion exchange capacity of the DEAE–MPS was determined by the acid–base titration method (Qian and Liu, 1984).