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Preparation and characterization of poly glycidyl methacrylete–zirconium dioxide–β-cyclodextrin composite matrix for separation of isoflavones through expanded bed adsorption

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ABSTRACT

The specially prepared adsorbent is most important in realizing the expanded bed adsorption (EBA) process. In the present work, a novel poly glycidyl methacrylete–zirconium dioxide–β-cyclodextrin (PGMA–ZrO_2–β-CD) composite matrix for EBA has been first prepared. Wet density, water content and pore properties of the composite beads have been investigated, which shows good expansion and stability in EBA. The application of custom-made adsorbent has been investigated to recover isoflavones from soy molasses. The recovery is up to 90% and the purity of isoflavones obtained is 75.4%. Compared with the traditional purification processes, EBA has the advantage of high efficiency and integrality, which leads to large reduction in operation time and cost.

1. Introduction

Much interest has been taken in soybeans and soy products in the past few decades, due to reports on their nutritious and health-promoting benefits. Soybean and soy products contain many phytochemicals, of which isoflavones, in particular, possesses disease resistance benefits [1–5].

In the present, the separation and purification of isoflavones are often carried out by solvents extraction or macroporous resins adsorption. Several studies have shown that the extraction efficiency of isoflavones can be increased by using solvents such as acetonitrile, methanol, ethanol and/or acetone in combination with 40–50% water or 10–20% acid, as well as ethyl acetate or butanol mixing with 44–54% water [6,7]. A high purity also can be achieved, but in the meanwhile, large usage of organic solvents may cause safety and environmental problem. Macroporous adsorption resins are widely employed in the separation of natural products from water extracts of Chinese herbal medicines. Some researchers have successfully obtained a high recovery of isoflavones by using macroporous resins successfully. However, purity of around 40% cannot always be satisfactory; moreover, the pretreatment processes are complicated, for example, centrifugation, filtration and extraction require 8–10 or more hours. In addition, more new techniques are being used in the separation and purification of soy isoflavones such as high speed countercurrent chromatography, ultrafiltration, membrane separation, solid phase extraction, supercritical fluid extraction and so on.

Recently, attention has been focused on the integrations of conventional purification operations. As one of the most practical and promising techniques among these integrated operations the expanded bed adsorption (EBA) chromatography is showing overwhelming potential advantages [8,9]. In an EBA process, the specially designed adsorbent particles with a defined size and density distribution are expanded by the upstream flow so as to form a loose stable 'classified' fluidized bed [8,10]. Hence it allows the unclarified feedstock, such as fermentation broth, cell homogenate or crude extracts, to pass unhinderedly through the column without the risk of blocking the bed while target molecules are adsorbed [11,12]. To some extent, EBA is a successful hybrid of the traditional packed bed and fluidized beds column chromatography [8,13]. Therefore, EBA makes it possible to integrate solid–liquid separation, concentration and primary purification into one processing step [14], and dramatically enhance process efficiency [15], effectively shorten operation time, cut running cost and advance the efficiency of downstream process compared to conventional methods [16,17].

In an EBA process, one of the important factors is the specially designed adsorbent. Two essential properties of EBA adsorbents are high density and relatively wide size distribution. Various kinds of EBA adsorbents have been developed during past few decades. GE Healthcares first developed the Streamline series in 1990s. Streamline adsorbents are based on 6% cross-linked agarose containing

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a crystalline quartz core as the densifier, with a mean density of 1.2 g/mL and the size distribution of 100–300 μm. Recently, higher density adsorbents, such as Streamline Direct and Fastline series have been introduced into the market by GE Healthcares and UpFront Chromatography A/S, respectively. Furthermore, some researchers have paid close attention to new material, such as cellulose–titanium oxide [18–20], cellulose–stainless steel [21], cellulose–nickel powder [22], etc. These adsorbents have proper density and size distribution, and lead to similar performance of EBA in both laboratory and industrial environment. However, these adsorbents are mainly designed for separation of enzyme, antibody, vector and other protein molecules. It confines EBA operations to separation of proteins and other biological molecules.

Up to now the EBA technique has been increasingly widely applied in many bioseparation cases such as purification of proteins and other biological molecules from microbial and mammalian cell culture. However, the literatures about separation and purification of natural products utilizing the EBA technique are scarce. Also the matrix for special separation of natural products has not been studied yet.

In the present work, a novel matrix for EBA has been prepared in our laboratory. The physicochemical properties of this matrix have been studied systematically. EBA has been first applied in the separation and purification of soy isoflavones from soy molasses using our matrix and different EBA parameters would be discussed.

2. Experimental

2.1. Materials

Glycidyl methacrylete (GMA), diethenyl benzene (DVB) was from Nankai University Chemical Factory (Tianjin, China). Zirconium dioxide (ZrO_2) with a high density of 4.7 g/cm^3 and a mean particle diameter of 40 nm was ordered from Shanghai Caiyu Nanotechnology Co., Ltd. (Shanghai, China). Benzoyl peroxide (BPO) was purchased from Shantou Xilong Chemical Factory (Guangdong, China). β-Cyclodextrin (β-CD) was purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Soy molasses was from QinHuangdao Jinhai Food Technology Co., Ltd. (QinHuangdao, China). Other reagents were of analytical reagent grade from Beijing Chemical Factory (Beijing, China).

2.2. Preparation of composite matrix

The composite matrix was prepared through the method of oil-in-water thermal suspension polymerization. Initially, a suitable amount of deionized water was put into a 500 mL 3-mouth flask with reflux condensation, and added, in proportion, by the gelatin, sodium chloride as a dispersed phase under continuously agitation at 400 rpm for 0.5 h at 50 °C, then heated up to 60 °C. After that appropriate amounts of GMA, DVB, toluene, *n*-heptane were blended with 0.3 g BPO, 2 g ZrO_2 as a polymerization phase in a 250 mL beaker under magnetic stirring at 200 rpm for 0.5 h at 60 °C. Then the polymerization phase was added to the dispersed phase in drops and keeping this reaction at 60 °C for 1 h. The suspension was heated to 80 °C, 85 °C, 95 °C, kept for 1 h, 2 h, 4 h under continuous stirring, respectively, then cooled down. Finally, approximately 50 mL resulting composite particles with a diameter of 100–300 μm were obtained by washing in turn with boiling water and acetone for several times, then reserved after drying in an oven.

2.3. Measurement of physicochemical properties

The shapes and structures of the composite matrices were observed by an optical microscope, DME (Leica, Wetzlar, Germany) and a scanning electron microscope (SEM), S-4700 (Hitachi, Tokyo, Japan).

The determination of several physicochemical properties has been reported in literatures [23–25]. Briefly, wet density (ρ_P, g/mL of wet matrix) is determined by water replacement in a gravity bottle, and the wet density of the composite matrix could be calculated as follows:

$$\rho_p = \frac{M_1 \rho_w}{M_1 + M_2 - M_3} \quad (1)$$

where ρ_P, ρ_w (g/mL) are the matrix wet density and water density, respectively. M_1, M_2, M_3 (g) are the mass of wet matrix, a gravity bottle filled with water and a gravity bottle with wet matrix full of water, respectively.

Water content (ω, %) was obtained by removing water of wet matrix at 105 °C to a constant mass and calculated as follows:

$$\omega = \frac{m_2 - m_3}{m_2 - m_1} \times 100\% \quad (2)$$

where m_1, m_2, m_3 (g) are mass of weighing bottle, the matrix and weighing bottle before drying, and the matrix and weighing bottle after drying.

It is assumed that all pores in the matrix were filled with water, porosity (P, %) representing percentage of pore volume per volume wet matrix and pore volume V (mL/g dried matrix) representing pore volume per gram dried matrix can be roughly estimated as follows:

$$P = \frac{\rho_p \omega}{\rho_w} \times 100\% \quad (3)$$

$$V = \frac{\omega}{(1-\omega)\rho_w} \quad (4)$$

Specific surface area (S, m^2/mL) was measured by the adsorption of methylene blue solution and calculated as follows:

$$S = \frac{(C_i - C)\nu\rho_p}{m} \times 2.45 \quad (5)$$

where C_i and C (mg/mL) are the initial and equilibrium concentrations of methylene blue solution, ν (mL) is the adding volume of methylene blue solution, and m is the mass of the matrix, respectively. The constant (2.45 m^2/mg methylene blue) represents the coverage area of 1 mg methylene blue under the monolayer adsorption.

Presuming that all pores were cylindrical structural models, the mean pore diameter D (nm) can be estimated by

$$D = 4 \times 1000 \times \frac{V(1-\omega)\rho_P}{S} \quad (6)$$

Apparent immobilization amount of β-CD (Q_S, μmol/g) onto the matrix was obtained through the classical phenol–sulfuric acid method and calculated as follows:

$$Q_S = \frac{C_G \times 50 \times 1000}{180 \times 7 \times m} \quad (7)$$

where C_G (g/L) expresses glucose concentration in the hydrolysate, m expresses the mass of the matrix after immobilizing β-CD. The constants of 180, 750 and 1000 show the molecular weight of glucose and number of glucose units in β-CD, volume and dilution multiple of hydrolysate, respectively.

2.4. Immobilization of β-CD onto composite matrix

The matrix from Section 2.2 was put into a 250 mL conical flask and was added with *N*,*N*-dimethylform amide (DMF), which would remove water completely, and was laid overnight. β-CD, NaH, DMF were mixed in a 250 mL conical flask according to a certain ratio and agitated to clarification. The pretreated matrix and this clear