Chemical Engineering Journal 235 (2014) 244–251

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Development of functionalized mesoporous silica for adsorption and separation of dairy proteins

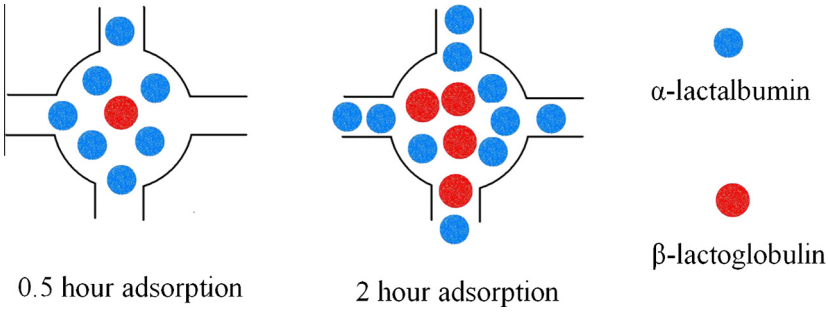
Mehdi Nasiri Sarvi [a,b,1], Tony Budianto Bee [a], Chuen Kang Gooi [a], Brad W. Woonton [c], Michelle L. Gee [b], Andrea J. O'Connor [a,*]

[a] Department of Chemical and Biomolecular Engineering, University of Melbourne, Victoria 3010, Australia
[b] School of Chemistry, Particulate Fluids Processing Centre, University of Melbourne, Victoria 3010, Australia
[c] CSIRO Food and Nutritional Sciences, Victoria 3030, Australia

HIGHLIGHTS

- α-Lactalbumin and β-lactoglobulin were adsorbed on mesoporous silica FDU-12.
- The protein adsorption depends on the pore entrance size and surface functionality.
- Adsorption kinetics and capacity were increased by amine functionalization.
- Modeling showed intraparticle protein diffusion can be rate limiting.
- Separation of these dairy proteins is feasible under non-equilibrium conditions.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 May 2013
Received in revised form 26 August 2013
Accepted 8 September 2013
Available online 18 September 2013

Keywords:
α-Lactalbumin
β-Lactoglobulin
Mesoporous silica
FDU-12
Protein separation

ABSTRACT

A class of mesoporous silica material, FDU-12, was synthesized with different pore sizes and amine functionalized to determine its potential to differentially adsorb and separate dairy proteins. The adsorption behavior of the dairy proteins α-lactalbumin and β-lactoglobulin on the amino surface functionalised and unfunctionalised material was examined. It was shown that the pore size of unfunctionalised FDU-12 is an important factor in the protein adsorption capacity. Amine functionalization of FDU-12 significantly increased protein adsorption for larger pore size materials, with a marked difference in the rate of uptake between the two proteins. The kinetics of adsorption were investigated as a function of temperature and were modeled using pseudo second order kinetics and an intraparticle diffusion model, showing that intraparticle protein diffusion can be rate limiting. These results indicate that functionalised FDU-12 has potential for the selective separation of proteins with similar size and isoelectric point, under dynamic processing conditions.

1. Introduction

Templated mesoporous silica materials [1] are synthetic materials with uniformly sized mesopores that can be tailored in the range of 2–50 nm. They have a high surface area and porosity and a variety of interconnected pore structures that are controllable through various synthesis conditions and starting reagents. These characteristics make them ideal for many applications including separation, catalysis, and sorption.

There have been many studies of the potential to use mesoporous silica materials for biomolecule adsorption [2–11]. It has been shown that ordered mesoporous materials are useful for stable entrapment of biomolecules and the stabilization of biologically interesting molecules under different conditions [12–14].

* Corresponding author. Tel.: +61 3 83448962; fax: +61 3 83444153.
E-mail addresses: mnsarvi@cc.iut.ac.ir (M.N. Sarvi), brad.woonton@gmail.com (B.W. Woonton), mlgee@unimelb.edu.au (M.L. Gee), a.oconnor@unimelb.edu.au (A.J. O'Connor).
[1] Current address: Department of Mining Engineering, Isfahan University of Technology, Isfahan, Iran.

1385-8947/$ - see front matter
http://dx.doi.org/10.1016/j.cej.2013.09.036

However, challenges remain in the design and optimization of adsorbents suitable for industrial separations, due to problems including pore blockages, slow adsorption kinetics due to hindered intraparticle diffusion and lack of selectivity. FDU-12 is one such mesoporous silica material, first reported by Fan et al. [15]. Its three dimensional, interconnected porous structure is advantageous for bio-adsorption since it reduces the likelihood of pore blockage and facilitates higher diffusion rates during the separations process [16]. Furthermore, recent studies have shown that FDU-12 can be synthesized with different pore size distributions [17,18]. In this study, FDU-12 was selected to study the adsorption and separation of target biomolecules, specifically milk proteins, and kinetic models used to analyze protein adsorption within its pores.

Milk and its associated products such as cheese and whey are important in the human diet. In particular, whey proteins are often used in food products due to their high nutritional value in, for example, infant formula, and as gelling agents and emulsifiers [19]. The two major whey proteins in bovine milk are α-lactalbumin and β-lactoglobulin, which are found in milk and whey at concentrations of approximately 1.2 g/L and 3.2 g/L, respectively [20]. In comparison, human breast milk does not contain any β-lactoglobulin [21] and approximately 2.4 g/L α-lactalbumin, which constitutes approximately 10–20% of the total proteins in human breast milk [22]. Infant formula manufactures are continuously striving to replicate the composition of human breast milk, which requires the addition of α-lactalbumin and the removal of β-lactoglobulin. Simple and cost effective methods to isolate α-lactalbumin from bovine milk and whey will offer infant formula manufacturers the ability to manufacture products that more closely replicate human breast milk [21].

Methods used to isolate proteins from milk and whey include diafiltration, size-exclusion chromatography [23], precipitation [24–26], heat coagulation, ion exchange adsorption, ion exchange chromatography [27,28] and ultrafiltration [29,30]. Although these fractionation techniques can provide good protein purification there are still problems in separation of the two most important whey proteins (α-lactalbumin and β-lactoglobulin) due to the similar isoelectric point and size of these proteins.

Here we present a study of the application of mesoporous silicates for the separation of dairy proteins. Specifically we have tuned the pore size of the materials to facilitate protein uptake after functionalization of the pore surfaces. The pore surfaces were functionalized with amino groups to regulate their uptake capacity and the kinetics of protein adsorption were analyzed to assess the rate controlling steps in the uptake as a function of temperature and surface functionalization using FDU-12. Using these insights, the selective adsorption and separation of the milk proteins α-lactalbumin and β-lactoglobulin from a model milk solution were investigated under dynamic conditions. Our results show the potential of amino-functionalised FDU-12 for the selective separation of proteins with similar physicochemical properties.

2. Material and methods

2.1. Chemicals

The triblock copolymer poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide), ($EO_{106}PO_{70}EO_{106}$, Sigma–Aldrich), tetraethyl orthosilicate (TEOS, 98% purity, Sigma–Aldrich), 1,3,5-trimethylbenzene (TMB, 99% purity, Acros), potassium chloride (99.5% purity, Merck), (3-aminopropyl)triethoxysilane (APTES, 99% purity, Sigma–Aldrich), iso-propanol (analytical reagent, Merck), α-lactalbumin from bovine milk (⩾85% purity, Sigma–Aldrich), β-lactoglobulin from bovine milk, calcium depleted (⩾90% purity, Sigma–Aldrich), 2-(N-Morpholino)-ethane-sulphonic acid (MES) hydrate (Sigma, 99.5%), and sodium MES (Sigma–Aldrich, 99%) were used as received. Hydrochloric acid (2 M) was made from 35% w/w HCl (AR grade, Merck). All water used was purified (Millipore Simplicity unit, France, purified to a resistivity of ⩾18.2 MΩ cm). Toluene (Ajax, 99%) was pre-dried over 4 Å activated molecular sieves (Ajax Finechem) overnight, followed by distillation over sodium. The distilled toluene was kept in a dry bottle containing activated molecular sieves before use for no more than a few hours.

2.2. FDU-12 synthesis

FDU-12 was synthesized as previously reported [15]. In a typical synthesis, 2.0 g of $EO_{106}PO_{70}EO_{106}$ was dissolved in 120 mL of 2 M HCl followed by dissolution of KCl (5.0 g). The solution was mixed using a magnetic stirrer hot plate to keep the mixture at 38 °C. After approximately 12 h, 5.5 g of TMB was added drop wise and vigorous mixing was continued at this temperature for 24 h. Next, 8.2 g of TEOS was added drop wise to the solution with continued mixing at 38 °C for another 24 h. The mixture was transferred to an autoclave and hydrothermally treated at 100 °C for 72 h. The solid product was collected by vacuum filtration on a Buchner funnel, washed with water and calcined in air at 550 °C for 6 h to remove all organic species, heating at 1.5 °C per minute [15]. In order to synthesise enlarged pore FDU-12, the same procedure was followed but the hydrothermal treatment was performed at 120 °C. In what follows, the FDU-12 samples are denoted by FDU-12-100 and FDU-12-120, according to their hydrothermal treatment temperature.

2.3. Amino functionalization

FDU-12 was amino-functionalised using (3-aminopropyl)triethoxysilane (APTES) in dry toluene to avoid undesirable side reactions of the APTES with water [31]. Typically, 2.0 g of FDU-12 was added to 75 mL of dry toluene. APTES (16 mL) was added and the solution was heated under reflux for 18 h under nitrogen. The functionalized sample was then collected and washed sequentially with dry toluene, iso-propanol and water through a Buchner funnel. The filtered sample was suspended in 50 mL of water, incubated at 25 °C for 3 h and filtered again to remove any unreacted APTES. Finally, the FDU-12 sample was vacuum dried overnight at 100 °C. The amino functionalized samples are given the suffix -NH_2.

2.4. Characterization of FDU-12

The surface areas and pore size distributions of the FDU-12 samples were estimated using nitrogen sorption in a Micromeritics ASAP 2000 gas adsorption analyzer (Norcross GA, USA). Samples were degassed at 150 °C for 5 h prior to analysis [32]. The entrance and cage size distributions of the FDU-12 samples were calculated by the BJH method using the desorption and adsorption branches of the isotherm, respectively [15]. To estimate the surface areas, the BET model was used [33].

2.5. Protein adsorption

The batch adsorption kinetics of α-lactalbumin and β-lactoglobulin onto unfunctionalized and functionalized FDU-12 samples were examined in 0.05 M MES buffer solution at pH 6.9. These conditions are typical of the ionic strength and pH of milk. To determine the kinetics of adsorption, aliquots (10 mL) of protein