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Development of Functionalized Mesoporous Silica for Adsorption and Separation of Dairy Proteins

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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]. However, challenges remain in the design and optimisation 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 analyse 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 1.2g/L and 3.2g/L, respectively [20]. In comparison, human breast milk does not contain any β-lactoglobulin [21] and approximately 2.4g/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 functionalisation 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 functionalisation 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 $M\Omega.cm$). Toluene (Ajax, 99%) was pre-dried over 4Å 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 2M HCl followed by dissolution of KCl (5.0g). The solution was mixed using a magnetic stirrer hot plate to keep the mixture at 38 ℃. After approximately 12 hours, 5.5 g of TMB was added drop wise and vigorous mixing was continued at this temperature for 24 hours. Next, 8.2 g of TEOS was added drop wise to the solution with continued mixing at 38 ℃ for another 24 hours. The mixture was transferred to an autoclave and hydrothermally treated at 100 ℃ for 72 hours. The solid product was collected by vacuum filtration on a Buchner funnel, washed with water and calcined in air at 550 ℃ for 6 hours to remove all organic species, heating at 1.5 ℃ per minute [15]. In order to synthesise enlarged pore FDU-12, the same procedure was followed but the hydrothermal treatment was performed at 120 ℃. 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 hours 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 ℃ for 3 hours and filtered again to remove any unreacted APTES. Finally, the FDU-12 sample was vacuum dried overnight at 100 ℃. 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 ℃ for 5 hours 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 0.05M 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 solution (α-lactalbumin or β-lactoglobulin at 1.5 mg/mL) were mixed with 100 mg samples of FDU-12-100, FDU-12-120 or FDU-12-120-NH$_2$. The solutions were mixed at either 5℃, 15℃ or 25 ℃ using an incubator orbital shaker (Thermoline Scientific TLM 570) at 120 rpm for up to 72 hours. After 0.5, 1, 2, 3, 4, 5, 23 and 51 hours, the solutions were centrifuged at 4500 g for 4 minutes. Protein concentrations in 0.5mL samples of the supernatants were measured by UV-visible spectroscopy (Varian Cary 1E UV-VIS spectrophotometer) at a detection wavelength of 280 nm. Samples were returned to the bulk solutions and mixing continued until the next sample was taken for analysis.

Equilibrium adsorption amounts of α-lactalbumin and β-lactoglobulin were determined by suspending 20 mg samples of adsorbent (FDU-12-120-NH$_2$) in 15 mL centrifuge tubes containing 0.05M MES buffer solution with different protein concentrations. The tubes were shaken at 25 ℃ using the incubator shaker for a period sufficient to reach equilibrium, as determined from the kinetics experiments. Analysis of protein uptake was performed as above.

2.6. Protein separations

Protein separations were examined using solutions containing equal concentrations of α-lactalbumin and β-lactoglobulin (each at 1.5 mg/mL), or different concentrations of α-lactalbumin and β-lactoglobulin (1.2 and 3.2 mg/mL, respectively) comparable to the actual concentrations of these proteins in milk. All solutions were buffered at pH 6.9 using 0.05M MES. Aliquots of 10 mL protein solutions were each mixed with 100 mg of FDU-12-120-NH_2. The solutions were mixed at 25 ℃ in the incubator shaker for protein uptake. After 0.5, 2 and 5 hours the solutions were centrifuged at 4500 g for 4 minutes and the quantity of α-lactalbumin and β-lactoglobulin in the supernatant measured using the HPLC method reported by Taylor and Woonton [34]. The results presented are the mean of three measurements for each sample. The standard error was less than 2%. The amount of each protein adsorbed to the FDU-12 material was calculated by difference and the selectivity of separation was calculated using equation 1:

$$S = \frac{L_\alpha}{L_\alpha + L_\beta} \qquad eq\ (1)$$

where S is the selectivity of adsorption of α-lactalbumin, L_α is the percentage of adsorbed α-lactalbumin and L_β is the percentage of adsorbed β-lactoglobulin.

3. RESULTS AND DISCUSSIONS

3.1. Characterization of FDU-12 samples

The nitrogen adsorption and desorption characteristics of the FDU-12 materials were analysed using DataMaster software (Micromeritics, Norcross GA, USA). The unfunctionalised materials showed type IV isotherms with type H_1 hysteresis (Figure 1a), as is typical of enlarged pore FDU-12 materials [33],[15]. The entrance and cage size distributions showed a single peak with significantly larger sizes for the sampled prepared with a hydrothermal treatment temperature of 120°C (Figures 1b and c). The characteristics of FDU-12-120-NH_2 (Fig 2) are of the same form as the parent unfunctionalized material, showing that the mesoporous structure was maintained

post-functionalisation. The average dimensions from these analyses for each FDU-12 sample are summarized in Table 1. Note there was a small decrease in the entrance and cage sized upon functionalization. This indicates that functionalization has occurred inside the pores without losing the mesoporosity. The marked drop in BET surface area is most likely dominated by the loss of microporosity formed from the high level of hydration around the ethylene oxide block of the copolymer during synthesis and subsequently blocked by amino-functionalisation. This has been observed previously for MCF materials.[35]
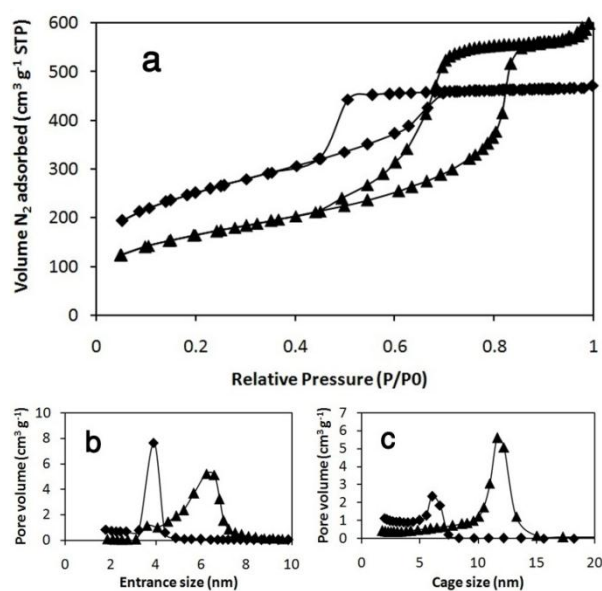

Figure 1. Volume of nitrogen adsorbed (cm^3 g^{-1}) onto FDU-12 samples as a function of relative pressure (a). The entrance size distributions are calculated from the desorption branch of isotherm using the BJH model (b) and the cage size distributions are calculated from the adsorption branch of isotherm using the BJH model (c); FDU-12-100 (◆) and FDU-12-120 (▲).

Table 1. The average entrance size, cage size, BET surface area and pore volume of FDU-12-100, FDU-12-120, and FDU-12-120-NH$_2$ silicas.

Sample	Entrance size [a] (nm)	Cage size [a] (nm)	BET surface area (m^2 g^{-1})	Pore volume (cm^3 g^{-1})
FDU-12-100	3.9	6.0	899	0.73
FDU-12-120	6.3	11.6	593	0.93
FDU-12-120-NH$_2$	6.1	10.2	326	0.63

[a] the entrance and cage sizes were calculated by applying the BJH method using the desorption and adsorption branch of the isotherm respectively [15]
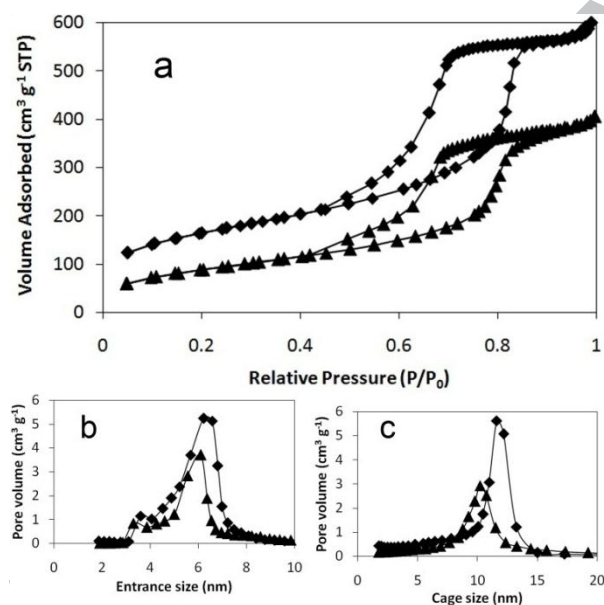

Figure 2. Volume of nitrogen adsorbed (cm^3 g^{-1}) onto FDU-12 samples as a function of relative pressure (a). The entrance size distributions are calculated from the desorption branch of isotherm using the BJH theory (b) and cage size distributions are calculated from the adsorption branch of isotherm using the BJH theory (c); FDU-12-120 (◆) and FDU-12-120-NH$_2$ (▲).

3.2. Protein adsorption onto FDU-12

Figure 3 shows the kinetics of adsorption of both α-lactalbumin and β-lactoglobulin on unfunctionalised FDU-12. These data show that FDU-12-100, i.e. FDU-12 ages at 100°C, is less effective at adsorbing these proteins than FDU-12-120, over the 51 hours examined. This is attributed to the fact that FDU-12-100 has much smaller windows (3.9 nm) than FDU-12-120 (6.3 nm). The entrance size of FDU-12-120 (6.3 nm) is greater than all but one of the molecular dimensions of α-lactalbumin and β-lactoglobulin (Table 2). The dimer form of β-lactoglobulin is larger in one dimension than the entrance size of the FDU-12-120 but might still penetrate into the pores, in contrast to the smaller pored FDU-12-100. .
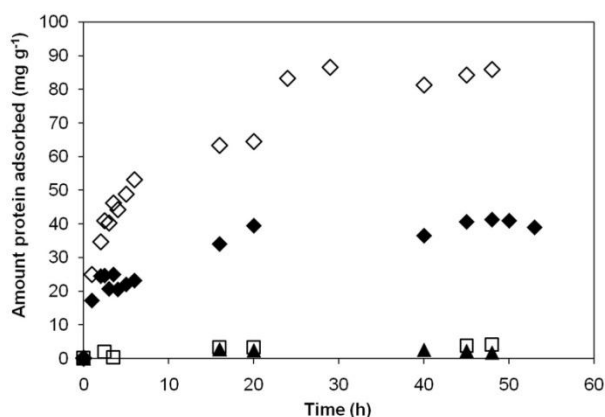

Figure 3. Quantities of α-lactalbumin adsorbed onto FDU-12-100 (▲) and FDU-12-120 (◇) and β-lactoglobulin adsorbed onto FDU-12-100 (□) and FDU-12-120 (◆) over 51 hours. An aliquot (10mL) of each protein solution (1.5 mg/mL) was incubated at 25ºC with 100mg of each FDU-12 material at pH 6.9.

Table 2. The molecular weight, molecular dimensions and isoelectric points of the α-lactalbumin monomer, the β-lactoglobulin monomer and the β-lactoglobulin dimer. [36]

Protein	Configuration	Molecular Weight (Daltons)	Molecular Dimensions (nm)	Isoelectric Point (pI)
α-lactalbumin	Monomer	14200	2.2 x 4.4 x 5.7	4.5-4.8
β-lactoglobulin	Monomer	18400	2.9 x 3.4 x 4.0	5.2
β-lactoglobulin	Dimer	36800	2.9 x 3.4 x 8.0	5.2

The data in Figure 3 also show that FDU-12-120 adsorbed twice as much α-lactalbumin than β-lactoglobulin despite their similar molecular weights. This is likely to be due to the different aggregation states of the two proteins. α-lactalbumin does not readily form complex quaternary structures, and its monomer molecular dimensions (Table 2) would allow it to easily diffuse into the FDU-12-120 pores and adsorb. However, β-lactoglobulin exists as a monomer, dimer or octamer, with the equilibrium between the different forms influenced by the medium conditions (concentration, temperature, pH and ionic strength) [37]. At the conditions employed, there would have been an equilibrium between the monomeric and dimeric forms of β-lactoglobulin . The monomeric form would not suffer size exclusion effects. However, the dimeric form is ellipsoidal and its length is larger than the FDU-12-120 entrance size (8 nm versus 6.3 nm), it would have less chance of entering the pores and its diffusion would be hindered inside the pores.

The effects of temperature on adsorption kinetics were investigated using the larger pored FDU-12-120 (Figure 4). The data are surprisingly sensitive to temperature over this small 20°C variation (expected to lead to only 7% faster diffusion based on the Stokes-Einstein equation). This implies that protein sizes are changing significantly over this temperature range. To better understand the mechanism of adsorption, two kinetics models were fitted to the data: a pseudo second order model and an intraparticle diffusion model [38]. The pseudo second order model yields a rate constant, k_2, given by:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \qquad (2)$$

where q_t is the amount adsorbed at time t and q_e is the adsorption capacity at equilibrium. The resulting fits to the data shown in Figures 5a and 5b, provide a reasonably good fit for both proteins. The resulting rate constants and equilibrium adsorption capacities are shown in Table 3.
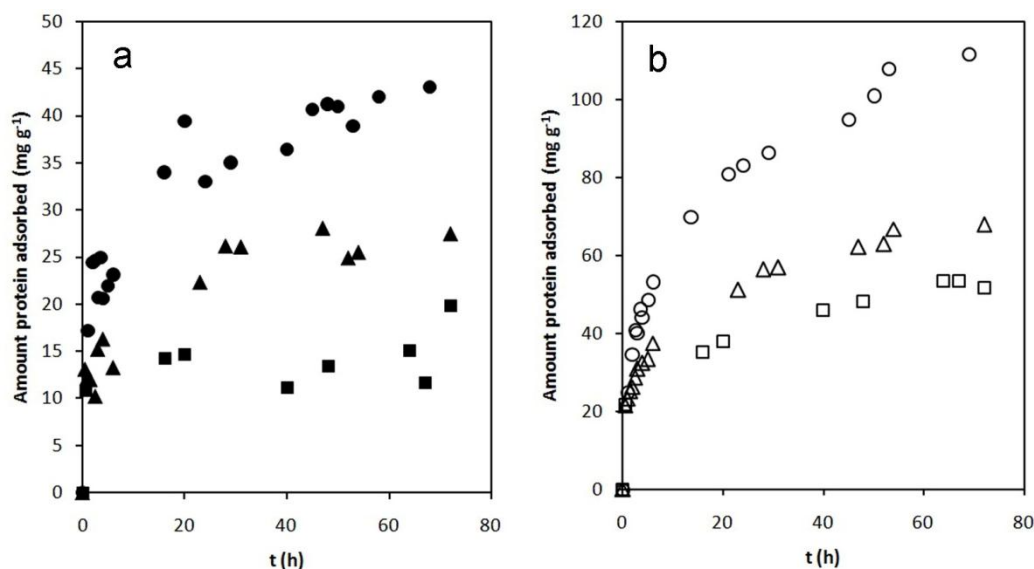

Figure 4. Kinetics of adsorption of a) β-lactoglobulin on FDU-12-120 at different temperatures (■: 5 °C, ▲: 15 °C, ●: 25 °C).; and b) α-lactalbumin on FDU-12-120 at different temperatures (□: 5 °C, △: 15 °C, ○: 25 °C)

The intraparticle diffusion model yields a rate constant, k_i, given by:

$$q_t = x_i + k_i t^{1/2} \qquad (3)$$

where x_i is a constant proportional to the mass transfer boundary layer thickness around the particle [39]. If this model holds, the amount of protein adsorbed should vary linearly with $t^{1/2}$, which is tested in Figures 5c and 5d. There are two linear regions for each data set, which indicates two different stages in the adsorption process. In the initial stage, protein adsorption depends on film diffusion and is expected to occur primarily on the external surfaces of the particles at a relatively high rate [39]. Interestingly, the extent of adsorption at early time points on the smaller pore FDU-12-100 (Figure 3) at 25 °C was much lower than that on FDU-12-120. This effect of pore size on the initial adsorption rate suggests that the proteins do penetrate the pores of the larger pore material even in the early stages, possibly adsorbing just within the pore

mouths. In the second stage of adsorption, intraparticle diffusion dominates with a reduction in the adsorption rate as proteins need to penetrate into the porous structure to access further adsorption sites[39]. The rate constants for both these stages are shown in Table 3. The data fit the model well for α-lactalbumin and reasonably well for β-lactoglobulin, although there was much more scatter in the data of the latter (Figure 5). It is possible that the scatter in the β-lactoglobulin data is a result of a mixed population consisting of monomers and dimers [37], which would have different diffusion rates and be affected differently by hindered diffusion within the FDU-12 pores.
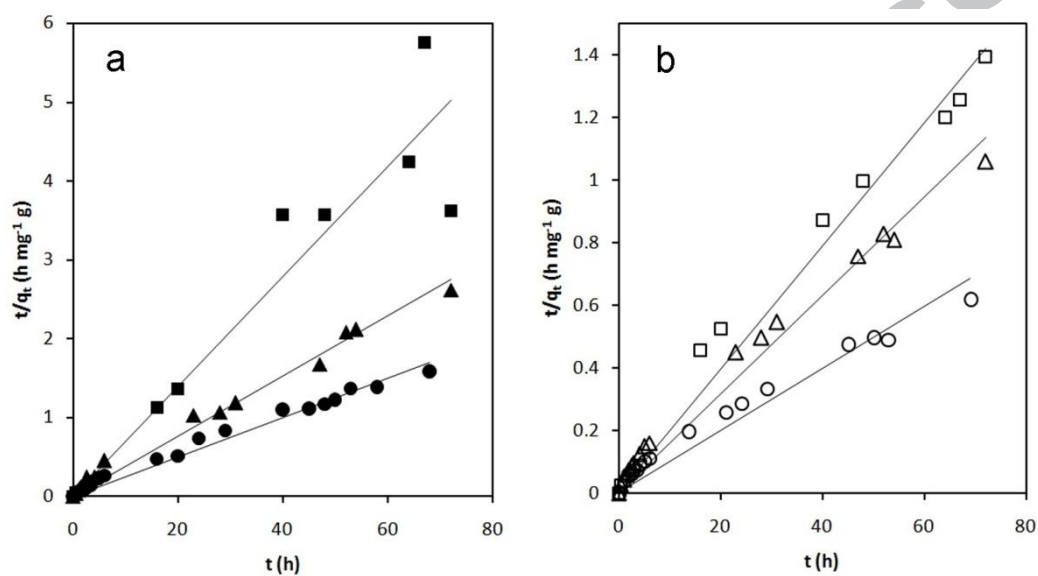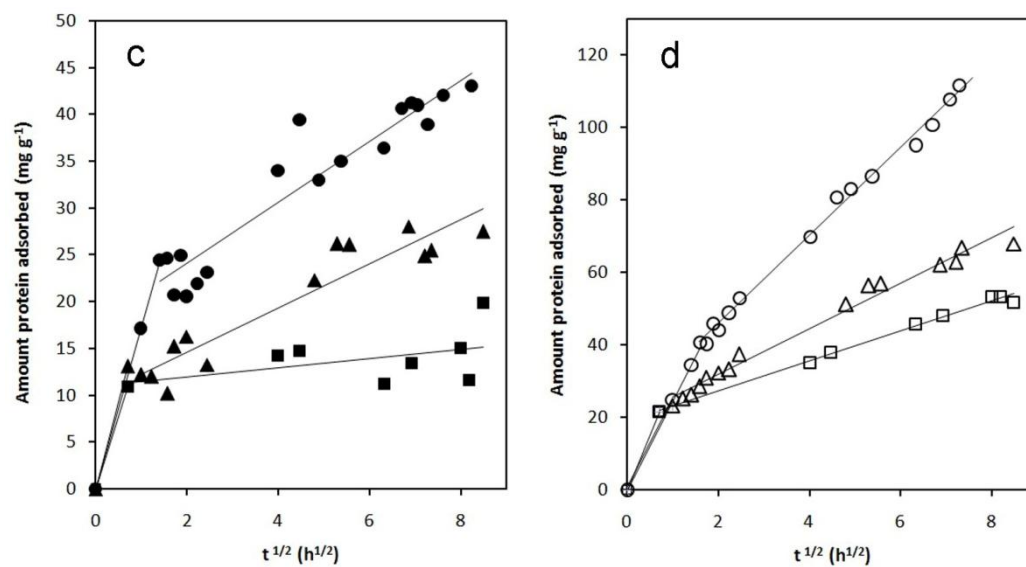
Figure 5. The pseudo-second-order model (a and b) and the intraparticle diffusion model (c and d) plots for adsorption of β-lactoglobulin (■: 5 °C, ▲: 15 °C, ●: 25 °C) and α-lactalbumin (□: 5 °C, △: 15 °C, ○: 25 °C) on FDU-12-120.

Table 3. Rate constants determined for the pseudo second order and intraparticle diffusion models for adsorption of α-lactalbumin and β-lactoglobulin onto FDU-12-120 and FDU-12-120-NH$_2$

Adsorbent	Protein (initial concentration, mg.mL^{-1})	Temperature (°C)	Pseudo-second-order model			Intraparticle diffusion model			
			q$_e$ (mg.g^{-1})	k$_2 \times 10^{-3}$ (h.g.mg^{-1})	R^2	k$_{i1}$ [a] (mg.g^{-1}.h$^{1/2}$)	R$_{i1}^2$	k$_{i2}$ [b] (mg.g^{-1}.h$^{1/2}$)	R$_{i2}^2$
FDU-12-120	α-lactalbumin (1.5)	5	55	4	0.98	30.5	1	4.14	0.99
		15	71	3.8	0.99	25.7	0.95	6.27	0.98
		25	125	1.3	0.99	25.20	1	12.1	0.99
	β-lactoglobulin (1.5)	5	15	30.3	0.88	15.4	1	0.50	0.21
		15	28	13.9	0.98	18.52	1	2.37	0.89
		25	42	8.5	0.98	17.22	1	3.35	0.90
FDU-12-120-NH$_2$	α-lactalbumin (1.5)	25	167	900	1.00	176.9	0.93	0.35	0.98
	α-lactalbumin (3.0)	25	333	1800	1.00	175.8	0.98	10.4	0.94
	β-lactoglobulin (1.5)	25	143	2.8	0.99	57.92	0.97	10.3	0.97

[a] The intraparticle diffusion model rate constants for the first and [b] second adsorption regions.

3.4. Protein adsorption onto amino-functionalized FDU-12

The impact of amino-functionalisation of FDU-12-120 on its adsorption kinetics for α-lactalbumin and β-lactoglobulin at 25°C are shown in Figure 6, where adsorption of the funnctionalised material is compared with the unfunctionalised material. Amino-functionalisation clearly increases the adsorption capacity for both proteins . Electrostatic attraction between the proteins and the amino-functionalised surface drives more rapid

adsorption. α-lactalbumin has a lower isoelectric point than β-lactoglobulin (Table 2) so will have a greater net negative charge than β-lactoglobulin at the solution pH employed (pH 6.9), increasing its attraction to the surface.
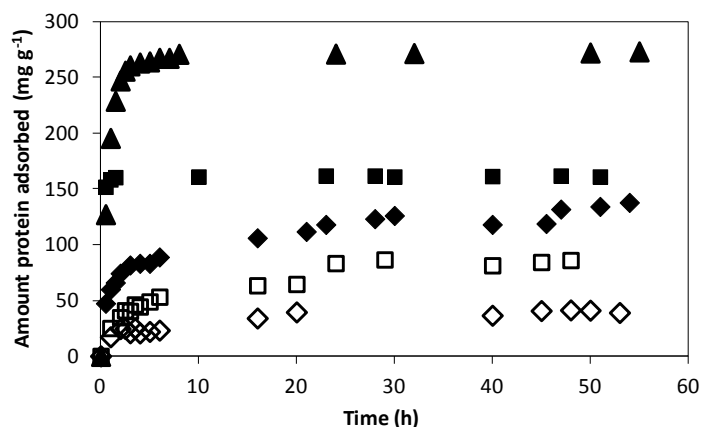

Figure 6. Kinetics of adsorption at 25 °C of α-lactalbumin onto FDU-12-120 (□) and FDU-12-120-NH$_2$ (■) and β-lactoglobulin adsorbed onto FDU-12-120 (◇) and FDU-12-120-NH$_2$ (◆) with an initial protein concentration of 1.5 mg/mL and adsorption of α-lactalbumin to the FDU-12-120-NH$_2$ (▲) with an initial concentration of 3 mg/mL.

The pseudo-second-order model fitted these kinetic data reasonably well (Figure 7 and Table 3), suggesting that adsorption might involve changes in protein conformation.[40] However, intraparticle diffusion model plots for adsorption from 1.5 mg/mL protein solutions onto amino-functionalised FDU-12-120 appeared to fit the data better and showed two steps in the adsorption mechanism (Figure 7). The first step is attributed to adsorption onto the external surface the particles or at the mouths of the pores. The second step is due to intraparticle diffusion, as described above, except for adsorption of α-lactalbumin from a 1.5 mg/mL solution, on the functionalized material, which appears to reach equilibrium rapidly. This was tested by monitoring adsorption at the higher starting α-lactalbumin concentration of 3 mg/mL. In this case, intraparticle diffusion was observed. This implies that the plateau observed at 1.5 mg/mL is not a rapid equilibrium but rather due to solution depletion of protein. At 3 mg/mL, three linear regions are observed in the data. The final plateau is once again likely due to solution depletion.
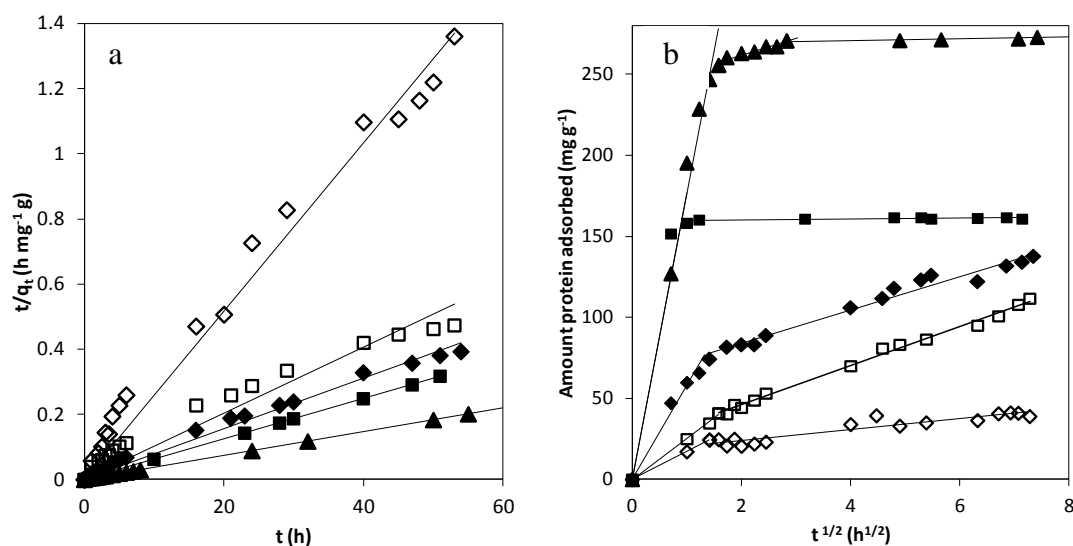
Figure 7. The pseudo-second-order model (a) and the intraparticle diffusion model (b) fitted to the adsorption at 25 °C of α-lactalbumin to the FDU-12-120 (□) and FDU-12-120-NH$_2$ (■) and the β-lactoglobulin to the FDU-12-120 (◇) and FDU-12-120-NH$_2$ (◆) all at the same initial protein concentration (1.5 mg/mL) and adsorption of α-lactalbumin to the FDU-12-120-NH$_2$ (▲) with a higher initial concentration (3 mg/mL).

For β-lactoglobulin, both the rate constants for adsorption on the external surface / pore mouths and intraparticle diffusion increased after functionalization of FDU-12-120 as a result of the electrostatic attraction between the protein and the surface. The initial rate was not high enough to cause substantial solution depletion, so intraparticle diffusion was found to be significant even at the lower initial solution concentration of 1.5 mg/mL. The rate constant for the second region of adsorption fitted to the intraparticle diffusion model for β-lactoglobulin onto FDU-12-120-NH$_2$ was greater than that for the same region for the α-lactalbumin.

3.5. Adsorption isotherms onto amino functionalized FDU-12-120

The isotherm adsorptions of two proteins on FDU-12-120-NH$_2$ were recorded and fitted to the Langmuir model (Figure 8). The loading of protein on functionalized FDU-12-120 increased up

to a plateau and fitted the Langmuir model well. Based on the Langmuir equation, the maximum capacity, Q, and the affinity parameter, K_L, for α-lactalbumin were found to be 310 mg α-lactalbumin/g FDU-12-120-NH$_2$ and 414 mL/mg, and for β-lactoglobulin were 173 mg β-lactoglobulin/g FDU-12-120-NH$_2$ and 8.3 mL/mg, respectively. The large K_L value was brought about by strong binding strength due to the electrostatic interactions between the protein and FDU-12-120-NH$_2$. Furthermore, the Langmuir isotherm obtained approached a rectangular shape, often defined as an irreversible isotherm. This suggests that the adsorption of α-lactalbumin on FDU-12-120-NH$_2$ may involve chemisorption and may cause difficulties in regeneration of the adsorbent.
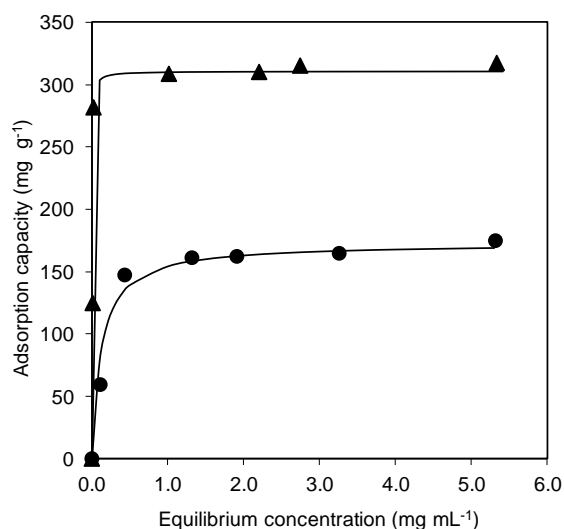

Figure 8. Adsorption isotherms for α-lactalbumin (▲) and β-lactoglobulin (●) on the FDU-12-120-NH$_2$ and the Langmuir equation fits to the experimental data (the solid lines).

3.6. Separation of proteins with amino functionalized FDU-12-120

The selectivity of the amino-functionalised FDU-12-120 was tested for mixtures of α-lactalbumin and β-lactoglobulin. At equal initial concentrations of α-lactalbumin and β-lactoglobulin, the quantity of protein adsorbed increased with contact time (Figure 9). However, as expected a greater quantity of α-lactalbumin adsorbed to the FDU-12-120-NH$_2$ than β-

lactoglobulin at all time points examined. This may be attributed to the lower isoelectric point and the smaller size of α-lactalbumin, as discussed above. As contact time increased, the selectivity of FDU-12-120-NH$_2$ for α-lactalbumin, calculated using Equation 1, decreased (Table 4). This may be attributed to the decreasing rate of α-lactalbumin adsorption onto FDU-12-120-NH$_2$ over time due to the significant reduction in the amount of α-lactalbumin remaining in solution, as discussed above. However the adsorption rate of the β-lactoglobulin does not reduce as markedly because its solution concentration is not depleted as much in the early stages of uptake. Hence the selectivity reduced with contact time.
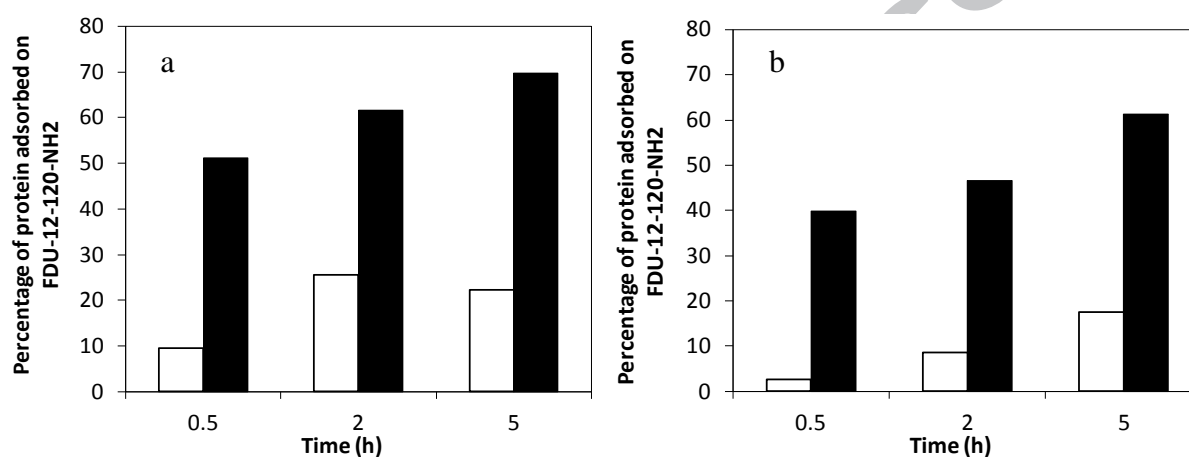

Figure 9. The amount (percentage of feed) of α-lactalbumin (black) and β-lactoglobulin (white) adsorbed to FDU-12-120-NH$_2$ after different contact times. Proteins were initially at identical concentrations of 1.5 mg/mL (a), or at concentrations close to bovine milk (b) (1.2 mg α-lactalbumin/mL and 3.2 mg β-lactoglobulin /mL).

Table 4. Reduction in protein selectivity with increasing contact time

	Contact time (hr)	Selectivity [a]
A: identical initial concentrations of 1.5 mg/mL for both proteins	0.5	0.84
	2	0.71
	5	0.76
B: initial concentration of 1.2 mg/mL for α-lactalbumin and 3.2 mg/mL for β-lactoglobulin	0.5	0.94
	2	0.84
	5	0.78

[a]: the selectivity of adsorption of α-lactalbumin was calculated using Equation 1.

When the proteins were prepared at concentrations similar to those found in bovine milk (Figure 9), similar trends were observed. However, compared to the experiment undertaken with equal concentrations of α-lactalbumin and β-lactoglobulin, the quantity of each protein adsorbed to FDU-12-120-NH$_2$ was lower. The lower adsorption of α-lactalbumin was expected as its initial concentration was lower (1.2 mg/mL versus 1.5 mg/mL). However, the lower adsorption of β-lactoglobulin was not expected as it was at a much higher concentration (3.2 mg/mL versus 1.5 mg/mL). This may be explained by the fact that at higher concentrations of β-lactoglobulin the protein might coagulate and block some of the pore entrances of the FDU-12-120-NH$_2$ [41]. Such blockages would reduce the loading capacity for each protein on FDU-12-120-NH$_2$. Furthermore, this potential coagulation of β-lactoglobulin could also contribute to the observed higher selectivity at each contact time.

The greatest difference in α-lactalbumin and β-lactoglobulin adsorption was observed at the first time point of 0.5 h with a selectivity of 0.94. Using protein concentrations similar to those found in milk, 3% of the β-lactoglobulin and 40% of α-lactalbumin adsorbed onto the FDU-12-120-NH$_2$. These results suggest that FDU-12-120-based materials may be employed to effectively separate α-lactalbumin and β-lactoglobulin under non-equilibrium conditions. Optimisation of the surface functionalisation and processing conditions to permit efficient solute desorption and regeneration of the adsorbent will be needed. With further research using model and real dairy

systems that contain other major dairy proteins and economical scale-up of the synthesis of the mesoporous materials, these findings have potential to be employed by the dairy industry to isolate α-lactalbumin.

4. CONCLUSIONS

It was shown that the adsorption of the dairy proteins α-lactalbumin and β-lactoglobulin to mesoporous silica FDU-12 is highly dependent on the entrance size of the pores and the surface functionality of the silica. By increasing the entrance size of the FDU-12 the amount of both proteins adsorbed increased significantly. The protein adsorption rates could also be increased by raising the solution temperature.

Amine functionalization of the silica surfaces increased the adsorption of both proteins by creating conditions for electrostatic attraction to the surfaces. The amount of α-lactalbumin adsorbed onto FDU-12 was almost double the amount of β-lactoglobulin adsorbed at equilibrium. Moreover the rate of adsorption of α-lactalbumin was significantly greater. After functionalization, α-lactalbumin adsorbed rapidly and the intraparticle diffusion model indicated that adsorption to the external surfaces of the particles dominated at lower initial solution concentrations. When more protein was present intraparticle diffusion became significant at longer contact times. Separation of α-lactalbumin and β-lactoglobulin from a mixture was achieved using the amino-functionalized FDU-12 for short contact times. Again α-lactalbumin adsorbed much faster than β-lactoglobulin, which could be attributed to the protein sizes, isoelectric points and potential coagulation of β-lactoglobulin at higher concentrations. The highest selectivity (0.94) was achieved with a contact time of 0.5 h at initial concentrations of α-lactalbumin and β-lactoglobulin based on bovine milk composition. These results suggest that functionalised FDU-12 silica materials may be employed to effectively separate α-lactalbumin and β-lactoglobulin under non-equilibrium conditions.

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5. REFERENCES

[1] C.T. Kresge, M.E. Leonowicz, W.J. Roth, J.C. Vartuli, J.S. Beck, Ordered mesoporous molecular-sieves synthesized by a liquid-crystal template mechanism Nature, 359 (1992) 710-712.

[2] J. Deere, E. Magner, J.G. Wall, B.K. Hodnett, Mechanistic and structural features of protein adsorption onto mesoporous silicates, Journal of Physical Chemistry B, 106 (2002) 7340-7347.

[3] A. Katiyar, L. Ji, P. Smirniotis, N.G. Pinto, Protein adsorption on the mesoporous molecular sieve silicate SBA-15: effects of pH and pore size, Journal of Chromatography A, 1069 (2005) 119.

[4] A. Katiyar, L. Ji, P.G. Smirniotis, N.G. Pinto, Adsorption of bovine serum albumin and lysozyme on siliceous MCM-41, Microporous and Mesoporous Materials, 80 (2005) 311.

[5] A. Katiyar, S. Yadav, P.G. Smirniotis, N.G. Pinto, Synthesis of ordered large pore SBA-15 spherical particles for adsorption of biomolecules, Journal of Chromatography A, 1122 (2006) 13.

[6] J.M. Kisler, A. Dahler, G.W. Stevens, A.J. O'Connor, Separation of biological molecules using mesoporous molecular sieves, Microporous and Mesoporous Materials, 44 (2001) 769.

[7] A.J. O'Connor, A. Hokura, J.M. Kisler, S. Shimazu, G.W. Stevens, Y. Komatsu, Amino acid adsorption onto mesoporous silica molecular sieves, Separation and Purification Technology, 48 (2006) 197-201.

[8] H.H.P. Yiu, C.H. Botting, N.P. Botting, P.A. Wright, Size selective protein adsorption on thiol-functionalised SBA-15 mesoporous molecular sieve, Phys. Chem. Chem. Phys., 3 (2001) 2983-2985.

[9] R. Brady, B. Woonton, M.L. Gee, A.J. O'Connor, Hierarchical mesoporous silica materials for separation of functional food ingredients -- A review, Innovative Food Science & Emerging Technologies, 9 (2008) 243-248.

[10] S.A. El-Safty, M.A. Shenashen, M. Khairy, Bioadsorption of proteins on large mesocage-shaped mesoporous alumina monoliths, Colloids and Surfaces B: Biointerfaces, 103 (2013) 288-297.

[11] J. Kisler, G.W. Stevens, A.J. O'Connor, Adsorption of Proteins on Mesoporous Molecular Sieves, Materials Physics and Mechanics, 4 (2001) 89-93.

[12] M. Hartmann, Ordered Mesoporous Materials for Bioadsorption and Biocatalysis, Chemistry of Materials, 17 (2005) 4577.

[13] M. Hartmann, D. Jung, Biocatalysis with enzymes immobilized on mesoporous hosts: the status quo and future trends, J. Mater. Chem., 20 (2010) 844-857.

[14] Z. Zhou, M. Hartmann, Recent Progress in Biocatalysis with Enzymes Immobilized on Mesoporous Hosts, Topics in Catalysis, 55 (2012) 1081-1100.

[15] J. Fan, C.Z. Yu, T. Gao, J. Lei, B.Z. Tian, L.M. Wang, Q. Luo, B. Tu, W.Z. Zhou, D.Y. Zhao, Cubic mesoporous silica with large controllable entrance sizes and advanced adsorption properties, Angew. Chem.-Int. Edit., 42 (2003) 3146-3150.

[16] H.X. Li, F. Zhang, H. Yin, Y. Wan, Y.F. Lu, Water-medium isomerization of homoallylic alcohol over a Ru(II) organometallic complex immobilized on FDU-12 support, Green Chem., 9 (2007) 500-505.

[17] J. Fan, C.Z. Yu, J. Lei, Q. Zhang, T.C. Li, B. Tu, W.Z. Zhou, D.Y. Zhao, Low-temperature strategy to synthesize highly ordered mesoporous silicas with very large pores, Journal of the American Chemical Society, 127 (2005) 10794-10795.

[18] T. Yu, H. Zhang, X. Yan, Z. Chen, X. Zou, P. Oleynikov, D.Y. Zhao, Pore Structures of Ordered Large Cage-Type Mesoporous Silica FDU-12s, J. Phys. Chem. B, 110 (2006) 21467-21472.

[19] J.M. Lozano, G.I. Giraldo, C.M. Romero, An improved method for isolation of beta-lactoglobulin, International Dairy Journal, 18 (2008) 55-63.

[20] B. Ribadeaudumas, R. Grappin, Milk protein analysis, Lait, 69 (1989) 357-416.

[21] R. Jost, J.C. Maire, F. Maynard, M.C. Secretin, Aspects of whey protein usage in infant nutrition, a brief review, International Journal of Food Science and Technology, 34 (1999) 533-542.

[22] J.G. Jackson, D.B. Janszen, B. Lonnerdal, E.L. Lien, K.P. Pramuk, C.F. Kuhlman, A multinational study of alpha-lactalbumin concentrations in human milk, Journal of Nutritional Biochemistry, 15 (2004) 517-521.

[23] M. Liang, V. Chen, H.L. Chen, W.L. Chen, A simple and direct isolation of whey components from raw milk by gel filtration chromatography and structural characterization by Fourier transform Raman spectroscopy, Talanta, 69 (2006) 1269-1277.

[24] H. Alomirah, I. Alli, Y. Konishi, Charge state distribution and hydrogen/deuterium exchange of alpha-lactalbumin and beta-lactoglobulin preparations by electrospray ionization mass spectrometry, Journal of Agricultural and Food Chemistry, 51 (2003) 2049-2057.

[25] C. Bramaud, P. Aimar, G. Daufin, Whey protein fractionation: Isoelectric precipitation of alpha-lactalbumin under gentle heat treatment, Biotechnol. Bioeng., 56 (1997) 391-397.

[26] C. Bramaud, P. Aimar, G. Daufin, Thermal isoelectric precipitation of α-lactalbumin from a whey protein concentrate: Influence of protein–calcium complexation, Biotechnology and Bioengineering, 47 (1995) 121-130.

[27] S.J. Gerberding, C.H. Byers, Preparative ion-exchange chromatography of proteins from dairy whey, Journal of Chromatography A, 808 (1998) 141-151.

[28] P.J. Skudder, Evaluation of a porous silica-based ion-exchange medium for the production of protein fractions from rennet- and acid-whey, Journal of Dairy Research, 52 (1985) 167-181.

[29] B. Cheang, A.L. Zydney, Separation of alpha-lactalbumin and beta-lactoglobulin using membrane ultrafiltration, Biotechnol. Bioeng., 83 (2003) 201-209.

[30] V. Espina, M.Y. Jaffrin, L.H. Ding, Extraction and Separation of alpha-lactalbumin and beta-Lactoglobulin from Skim Milk by Microfiltration and Ultrafiltration at High Shear Rates: A Feasibility Study, Separation Science and Technology, 44 (2009) 3832-3853.

[31] S. Boskovic, A.J. Hill, T.W. Turney, M.L. Gee, G.W. Stevens, A.J. O'Connor, Probing the microporous nature of hierarchically templated mesoporous silica via positron annihilation spectroscopy, Prog. Solid State Chem., 34 (2006) 67-75.

[32] S. Boskovic, A.J. Hill, T.W. Turney, G.W. Stevens, M.L. Gee, A.J. O'Connor, Micropore Characterization of Mesocellular Foam and Hybrid Organic Functional Mesocellular Foam Materials, J. Phys. Chem. C, 113 (2009) 21283-21292.

[33] S.J. Gregg, K.S.W. Sing, Adsorption, Surface Area and Porosity, 2 ed., Academic Press, London, 1982.

[34] C.M. Taylor, B.W. Woonton, Quantity and carbohydrate content of glycomacropeptide fractions isolated from raw and heat-treated milk, International Dairy Journal, 19 (2009) 709-714.

[35] S. Boskovic, Hill, A.J., Turney, T.M., Gee, M.L., Stevens, G.W., O'Connor, A.J., Probing the microporous nature of hierachically templated mesoporous silica via positron annihilation spectroscopy, Progress in Solid State Chemistry, 34 (2006) 67-75.

[36] A.L. Zydney, Protein separations using membrane filtration: New opportunities for whey fractionation, International Dairy Journal, 8 (1998) 243-250.

[37] M. Verheul, J.S. Pedersen, S.P.F.M. Roefs, K.G. de Kruif, Association behavior of native β-lactoglobulin, Biopolymers, 49 (1999) 11-20.

[38] Y.S. Ho, G. McKay, Pseudo-second order model for sorption processes, Process Biochemistry, 34 (1999) 451-465.

[39] T.P.B. Nguyen, J.W. Lee, W.G. Shim, H. Moon, Synthesis of functionalized SBA-15 with ordered large pore size and its adsorption properties of BSA, Microporous and Mesoporous Materials, 110 (2008) 560-569.

[40] F. Fang, J. Satulovsky, I. Szleifer, Kinetics of Protein Adsorption and Desorption on Surfaces with Grafted Polymers, Biophysical Journal, 89 (2005) 1516-1533.

[41] U.M. Elofsson, M.A. Paulsson, T. Arnebrant, Adsorption of beta-lactoglobulin A and B in relation to self-association: Effect of concentration and pH, Langmuir, 13 (1997) 1695-1700.
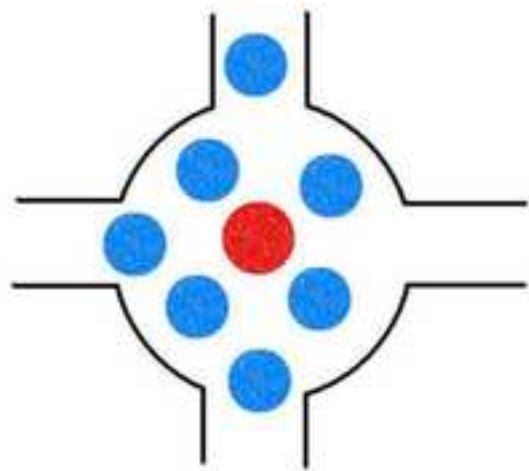
0.5 hour adsorption
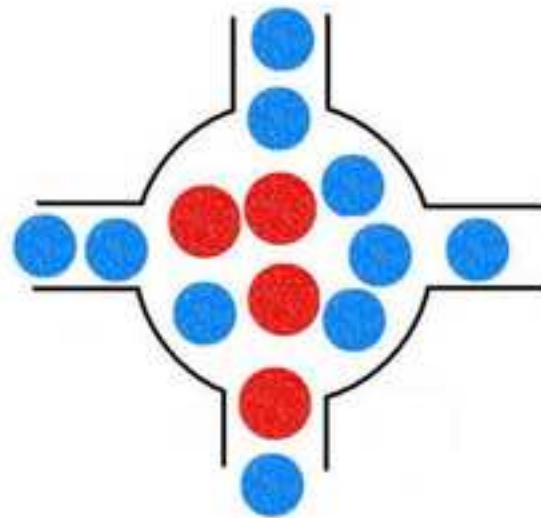
2 hour adsorption

α-lactalbumin

β-lactoglobulin

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

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