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Supermacroporous Support Having Dye for Reuseable Enzyme Immobilization

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Abstract

Poly(acrylamide-allyl glycidyl ether) [poly(AAm-AGE)] cryogel was prepared by bulk polymerization which proceeds in an aqueous solution of monomers frozen inside a glass column (cryo-polymerization). After thawing, the monolithic cryogel contains a continuous polymeric matrix having interconnected pores of 10-100 μm size. Cibacron Blue F3GA was attached by covalent binding onto poly(AAm-AGE) cryogel via epoxy groups. Poly(AAm-AGE) cryogel was characterized by FTIR and scanning electron microscopy (SEM). The equilibrium swelling degree of the poly(AAm-AGE) monolithic cryogel was 6.84 g H_2O/g cryogel. Poly(AAm-AGE) cryogel containing 68.9 μmol Cibacron Blue F3GA/g was used in the adsorption of β-casein from aqueous solution. The effects of initial concentration, pH, ionic strength and flow rate on the adsorption efficiency of cryogel were studied in a column system. The non-specific adsorption of β-casein was very low (1.26 mg/g polymer). The maximum amount of β-casein adsorption from aqueous solution in acetate buffer was 44.7 mg/g at pH 4.0. Up to 99% of the adsorbed β-casein was eluted when 0.1 M Tris/HCl buffer containing 0.5 M NaCl was used as elution agent. We concluded that Cibacron Blue F3GA attached poly(AAm-AGE) cryogel could be repeatedly applied for β-casein adsorption without significant losses in the adsorption capacity.

Key Words: β-Casein; Cryogels; Dye-affinity Chromatography; Protein Purification.

INTRODUCTION

Affinity techniques present an efficient method of protein purification [1-3]. The molecule having a molecular recognition capability is immobilized on a suitable insoluble support, which is usually a polymeric material in bead or membrane form, in affinity chromatography [4-6]. A wide variety of biomolecules, including proteins, enzymes, hormones, antibodies, nucleic acids, oligopeptides and oligonucleotides may be used as ligands in the design of novel adsorbents [7-10]. These ligands are extremely specific in most cases. However, they are expensive, due to the high cost of production and/or extensive purification steps. In the process of biospecific adsorbent preparation, it is difficult to immobilize them onto the supporting matrix with retention of their original biological activity [11]. High precautions are also needed in their use and storage.

Recently, dye-ligand affinity chromatography has gained a considerable attention in the purification of proteins, both in laboratory and large scale applications, assuring higher specificity, purity and

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recovery in a single chromatographic step as well as cost efficiency, safety and adsorbent capacity [12–16]. Dyes are known to bind many types of proteins showing different levels of affinity and specificity. This affinity results from the flexibility of dye, which can assume the polarity and geometry of the surface of a variety of competitive biomolecules [17,18]. They can easily be immobilized on matrices especially carrying hydroxyl groups. Dyes interact with the active sites of many proteins and enzymes by mimicking the structure of their substrates, cofactors or binding agents for those biomolecules [19]. A number of textile dyes, known as reactive dyes, have been used for protein purification. Most of these reactive dyes consist of a chromophore (either azo dyes, anthraquinone, or phathalocyanine), linked to a reactive group (often a mono- or dichloro-triazine ring). The interaction between the dye ligand and proteins can be by complex combination of electrostatic, hydrophobic, hydrogen bonding [20]. Conventional packed-bed columns possess some inherent limitations such as the slow diffusional mass transfer and the large void volume between the beads [21]. Although some new stationary phases such as the non-porous polymeric beads [22] and perfusion chromatography packings are designed to resolve the problems, these limitations cannot be overcome in essence [23]. Recently, cryogel materials are considered as a novel generation of stationary phases in the separation science [24-30]. Cryogels are a very good alternative to protein purification with many advantage including large pores, short diffusion path, low pressure drop and very short residence time for both adsorption and elution. Cryogels are also cheap materials and they can be used as disposable avoiding cross-contamination between batches [31].

Caseins from milk and β-casein in particular are surface active. Caseins are excellent glues and have been used for paint binders since early times [32]. Even today, studies on β-casein has continued such as investigations on competitive protein adsorption between β-casein and β-lactoglobulin during spray-drying in food industry [33]. For instance, beer bottle labels are still glued with casein and shadow masks for TV-tubes are still produced using casein [34]. β-casein is a small, flexible linear polyelectrolyte of not too high molecular weight (Mw 24 kDa) carrying a moderate net charge (-15 e) at neutral pH [35]. Its isoelectric point is at pH 4.9–5.2. Its highly non-uniform distribution of hydrophilic and hydrophobic amino-acid residues produces a distinctly amphiphilic molecular structure. The distinctly amphiphilic nature of the sequence confers high surface activity, contributing to good foaming and emulsifying actions [36]. The separation of caseins has long been one of the fundamental subjects of casein studies [37,38]. Chromatographic procedures (e.g., DEAE-cellulose) using urea buffers are widely used at present. However, traditional precipitation procedures are still attractive in some respects. For example, there are no requirements for special equipment and there is the capability to scale-up to obtain a large quantity of final products [39]. Depending on their final application, these proteins are required in different degrees of purity.

The goal of this study is to prepare Cibacron Blue F3GA attached poly(AAm-AGE) cryogel for efficient adsorption of β-casein. Poly(acrylamide-allyl glycidyl ether [poly(AAm-AGE)] cryogel was prepared by bulk polymerization which proceeds in an aqueous solution of monomers frozen inside a glass column (cryo-polymerization). Cibacron Blue F3GA, was covalently attached to the poly(AAm-AGE) cryogel. β-Casein adsorption properties of cryogel from aqueous solutions were investigated at different experimental conditions in a continuous column system. Elution of β-casein and reusability of the adsorbents were also tested.

EXPERIMENTAL

Materials

Acrylamide (AAm, more than 99.9% pure, electrophoresis reagent), allyl glycidyl ether (AGE, 99%), N,N'-methylene-bis(acrylamide) (MBAAm), amonium persulfate (APS) and β-casein were supplied from Sigma (St Louis, USA). N,N,N',N'-tetramethylene diamine (TEMED) was obtained from Fluka A.G. (Buchs, Switzerland). Cibacron Blue F3GA was obtained from Polyscience (Warrington, USA) and used without further purification. All other chemicals were obtained from Merck as analytical grade. All water used in the experiments was purified using a Millipore Corporation (France) Elix 5® reverse osmosis unit with an electrodeionisation (E.D.I.) module (Elix® 5 Water Purification System) followed by a Milli-Q Synthesis A10 Ultrapure® (Milli-Q® Ultrapure Water Purification Systems) organic/colloid removal and ion exchange packed bed system. Laboratory glassware was kept overnight in a 5% nitric acid solution. Before use the glassware was rinsed with deionised water and dried in a dust-free environment.

Preparation of poly(AAm-AGE) cryogel

Production of poly(AAm-AGE) cryogel was performed using the Arvidsson et al's procedure [40]. AGE was selected in order to insert reactive epoxy groups in the cryogel. Briefly, monomers (10 mL of AAm, 1 mL of AGE) were dissolved in deionized water and the mixture was degassed under vacuum for about 5 min to eliminate soluble oxygen. Total concentration of monomers was 6% (w/v). The cryogel was produced by free radical polymerization initiated by TEMED (120 µl) and APS (100 mg). After adding APS (1% (w/v) of the total monomers) the solution was cooled in an ice bath for 2-3 min. TEMED (1% (w/v) of the total monomers) was added and the reaction mixture was stirred for 1 min. Then, the reaction mixture was poured into a plastic syringe (5 mL, id. 0.8 cm) with closed outled at the bottom. The polymerization solution in the syringe was frozen at - 12°C for 24 h and then thawed at room temperature. Extensive cleaning procedure for removal of unconverted monomers and initiator was performed.

Cibacron Blue F3GA attachment

Cibacron Blue F3GA attachment studies were carried out in a recirculating system equipped with a water jacket for temperature control. The cryogel was washed with 30 mL of water. Then, 100 mL of Cibacron Blue F3GA solution (5 mg/mL) containing NaOH (5 g) was pumped through the glass column under recirculation at 80°C for 2 h. Under these experimental conditions, a chemical reaction took place between the chloride group of the Cibacron Blue F3GA and the epoxide group of the poly(AAm-AGE) cryogel. The adsorption was followed by monitoring the decrease in UV absorbance at 630 nm. After incubation, the Cibacron Blue F3GA-attached poly(AAm-AGE) cryogel was washed with distilled water and methanol until all the physically adsorbed Cibacron Blue F3GA were removed. The modified cryogel was then stored at 4°C with 0.02% sodium azide to prevent microbial contamination.

Characterization of cryogel

The swelling degree of the cryogel (S) was determined as follows: cryogel sample was washed on porous filter until washing was clear. Then it was sucked dry and then transferred to pre-weighed vial and weighed (mwet gel). After drying to constant mass in the oven at 60°C, the mass of dried sample was determined (mdry gel). The swelling degree was calculated as:

$$S = (m_{wet\ gel} - m_{dry\ gel}) / m_{dry\ gel} \quad (1)$$

The morphology of a cross section of the dried cryogel was investigated by scanning electron microscope (SEM). The sample was fixed in 2.5% glutaraldehyde in 0.15 M sodium cacodylate buffer overnight, post-fixed in 1% osmium tetroxide for 1 h. Then the sample was dehydrated stepwise in ethanol and transferred to a critical point drier temperated to 10°C where the ethanol was changed for liquid carbon dioxide as transitional fluid. The temperature was then raised to 40°C and the pressure to ca. 100 bar. Liquid CO_2 was transformed directly to gas uniformly throughout the whole sample without heat of vaporization or surface tension forces causing damage. Release of the pressure at a constant temperature of 40°C resulted in dried cryogel sample. Finally, it was coated with gold-palladium (40:60) and examined using a JEOL JSM 5600 scanning electron microscope (Tokyo, Japan).

FTIR spectra of the Cibacron Blue F3GA, the poly(AAm-AGE) cryogel and Cibacron Blue F3GA-attached poly(AAm-AGE) cryogel were obtained by using a FTIR spectrophotometer (FTIR 8000 Series, Shimadzu, Japan). The dry cryogel (about 0.1 g) was thoroughly mixed with KBr (0.1 g, IR Grade, Merck, Germany), and pressed into a tablet, and the spectrum was then recorded.

To evaluate the attachment amount of Cibacron Blue F3GA, poly(AAm-AGE) cryogel was subjected to elemental analysis using a Leco Elemental Analyzer (Model CHNS-932, USA).

Adsorption-Desorption Studies with β-Casein

β-Casein was selected as a model protein. The β-casein adsorption studies were carried out in a column system equipped with a water jacket for temperature control. The cryogel was washed with 30 mL of water and then equilibrated with 25 mM phosphate buffer containing 0.1 M NaCl (pH 7.4). Then, the prepared β-casein solution (50 mL of the aqueous β-casein solution) was pumped through the column for 2 h. The adsorption was followed by monitoring the decrease in UV absorbance at 280 nm. Effects of β-casein concentration, pH of the medium and ionic strength on the adsorption capacity were studied. The flow rate of the solution was changed in the range of 0.2-2.0 mL/min. To observe the effects of the initial concentration of β-casein on adsorption, it was changed between 0.1-2.0 mg/mL. To determine the effect of pH on the adsorption, pH of the solution was changed between 4.0 and 8.0 using of different buffer systems (0.1 M CH_3COONa-CH_3COOH for pH 4.0–6.0, 0.1 M K_2HPO_4-KH_2PO_4 for pH 7.0–8.0). To observe the effects of ionic strength, NaCl solution was used at ionic strength values of 0.01 and 0.5.

Desorption and Repeated Use

In all cases adsorbed β-casein molecules were desorbed using 0.1 M Tris/HCl buffer containing 0.5 M NaCl. In a typical desorption experiment, 50 mL of the desorption agent was pumped through the cryogel at a flow rate of 0.2 mL/min for 2 h. The final β-casein concentration in the desorption medium was determined by spectroscopic measurements. When desorption was achieved, the cryogel was cleaned with 1 M NaOH and then re-equilibrated with 25 mM phosphate buffer containing 0.1 M NaCl (pH 7.4). The desorption ratio was calculated from the amount of β-casein adsorbed on the cryogel and the final β-casein concentration in the desorption medium. In order to test the repeated use of Cibacron Blue F3GA-attached poly(AAm-AGE) cryogel, β-casein adsorption-desorption cycle was repeated for ten times using the same cryogel column. In order to regenerate and sterilize, after the desorption, the cryogel was washed with 1 M NaOH solution.

RESULTS AND DISCUSSION

A supermacroporous monolithic cryogel was produced by polymerization in the frozen state of monomers, acrylamide (AAm) and allyl glycidyl ether (AGE) with N,N'-methylene-bis(acrylamide) (MBAAm) as a cross-linker in the presence of amonium persulfate (APS)/N,N,N',N'-tetramethylene diamine (TEMED) as initiator/activator pair. The functional epoxy groups on the surface of the pores in monolithic cryogels allowed their modification with the ligand, Cibacron Blue F3GA. The scanning electron micrograph of the internal structure of the monolithic cryogel is shown in Figure 1. Poly(AAm-AGE) cryogel produced in such a way have non-porous and thin polymer walls, large continuous inter-connected pores (10-100 μm in diameter) that provide channels for the mobile phase to flow through. Pore size of the matrix is much larger than the size of the protein molecules, allowing them to pass easily. The molecular size of β-casein is 10 nm x 30 nm. As a result of the convective flow of the solution through the pores, the mass transfer resistance is practically negligible. The equilibrium swelling degree of the poly(AAm-AGE) monolithic cryogel was 6.84 g H_2O/g dry cryogel. Poly(AAm-AGE) monolithic cryogel is opaque, sponge like and elastic. This cryogel can be easily compressed by hand to remove water accumulated inside the pores. When the compressed piece of cryogel was submerged in water, it soaked in water and within 1-2 s restored its original size and shape.

Cibacron Blue F3GA was used as the affinity dye-ligand for specific binding of β-casein. It is reported that Cibacron Blue F3GA has no adverse effect on biochemical systems [41]. However, all commercial reactive dyes (including Cibacron Blue F3GA) contain various impurities which may affect their biochemical and related use [41-43]. Reactive dyes have been purified by a number of chromatographic procedures such as thin-layer chromatography,

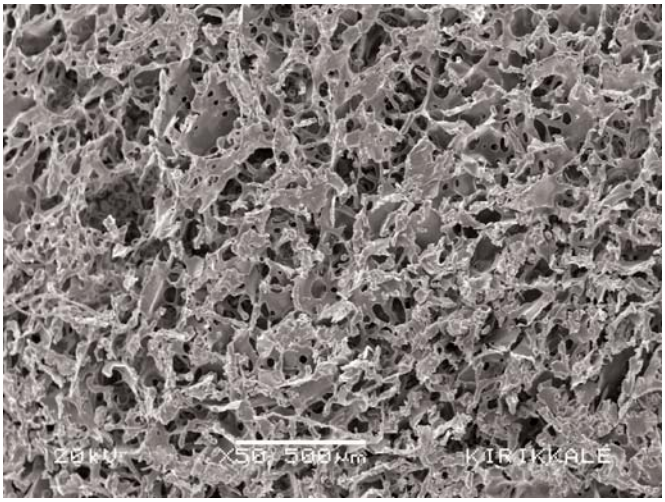

Figure 1. Scanning electron micrograph of the inner part of the supermacroporous poly(AAm-AGE) monolithic cryogel matrix.

high-performance liquid chromatography and column chromatography on Silica gel or Sephadex [44]. However, it is suggested that purification of reactive dyes is necessary only when free dyes are used [42]. In cases where immobilized dyes are used, purification of the dye before immobilization is not likely to be necessary, because few of the contaminants will be immobilized on the support matrix, and proper washing of the matrix should remove adsorbed contaminants [42].

Biomimetic dye-ligand Cibacron Blue F3GA is covalently attached on poly(AAm-AGE) cryogel. The FTIR bands observed at 1160 cm^{-1} was assigned to symmetric stretching of S=O, as also pointed out on the chemical structure of the Cibacron Blue F3GA (Figure 2). The split of the band at 3300-3500 cm^{-1} indicates also SO_3H and NH_2 groups. These bands show the attachment of Cibacron Blue F3GA within the poly(AAm-AGE) cryogel. The visual observations (the colour of the cryogel) ensured attachment of dye molecules. The dye content was 68.9 μmol/g dry cryogel. Note that AAm, AGE and other chemicals in the polymerization formula do not contain sulphur. This sulphur amount determined by elemental analysis originated from only immobilized dye into the polymeric structure.

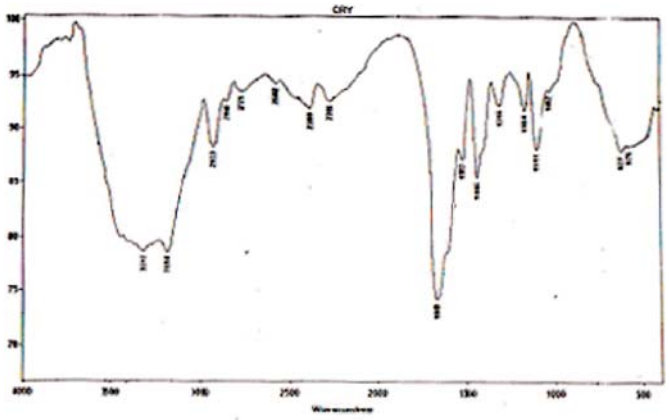

Figure 2. FTIR spectrum of Cibacron Blue F3GA attached poly(AAm-AGE)

β-Casein Adsorption from Aqueous Solutions

Effects of pH: The amount of β-casein adsorbed onto Cibacron Blue F3GA-attached poly(AAm-AGE) cryogel as a function of pH is shown in Figure 3. The maximum adsorption of β-Casein observed at pH 4.0. With the increase of pH above and below the pH 4.0, the β-casein adsorption capacity decreased.

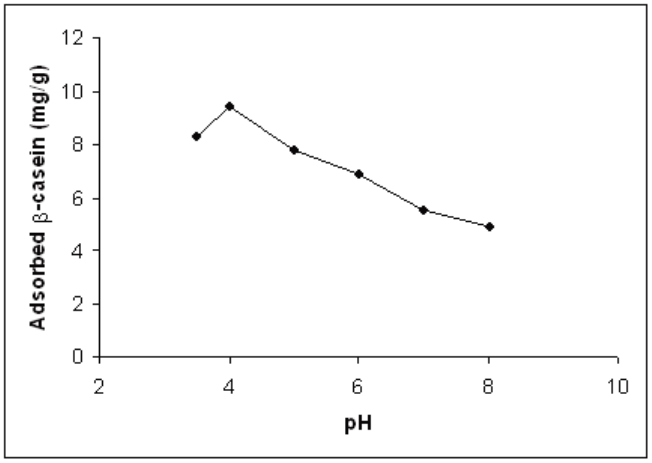

Figure 3. Effect of medium pH on the β-casein adsorption: Cibacron Blue F3GA content: 68.9 μmol/g; β-casein concentration: 0.1 mg/mL; Flow rate: 0.2 mL/min; T: 20°C. Each point is as average of five parallel studies.

The decrease in the β-casein adsorption capacity can be attributed to electrostatic repulsion effects between the identically charged groups. In addition, these interactions between the dye and protein molecules may result both from the ionization states of several groups on both the ligands (i.e., Cibacron Blue F3GA) and amino acid side chains in β-casein structure, and from the conformational state of protein molecules at pH 4.0. It should be also noted that non-specific adsorption (i.e., adsorption on poly(AAm-AGe) cryogel) was independent of pH and it was observed at the same at all the pH values studied.

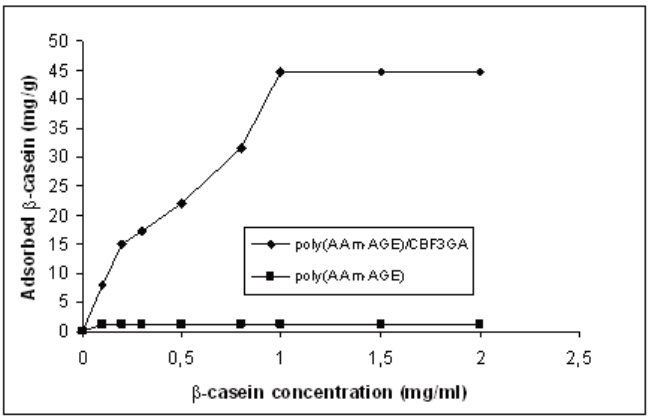

Figure 4. Effect of the concentration of β-casein on the β-casein adsorption: Cibacron Blue F3GA content: 68.9 μmol/g; pH: 4.0; Flow rate: 0.2 mL/min; T: 20°C. Each point is an average of five parallel studies.

Effects of β-Casein Concentration

Figure 4 shows the effects of the initial concentration of β-casein adsorbed. As can be seen in this figure, with increase β-casein concentration in solution, the adsorbed amount of β-casein per unit mass of cryogel increases until about 1.5 mg.mL^{-1}, and then approaches saturation. Note that one of the main requirements in dye-affinity chromatography is the specificity of the affinity adsorbent for the target molecule. The non-specific interaction between the support, which is the poly(AAm-AGE) cryogel in the present case, and the molecules to be adsorbed, which are the β-casein molecules here should be minimum in order to consider the interaction as

specific. As seen in this figure, negligible amount of β-casein was adsorbed non-specifically on the poly(AAm-AGE) cryogel, which was 1.26 mg/g dry cryogel. Cibacron Blue F3GA attachment increased the β-casein adsorption capacity of the cryogel (up to 44.74 mg/g). This increase in the β-casein binding capacity may have resulted from cooperative effect of different interaction mechanisms such as hydrophobic, electrostatic and hydrogen bonding caused by the acidic groups and aromatic structures on the Cibacron Blue F3GA and by groups on the side chains of amino acids on the β-casein molecules. It should be mentioned that Cibacron Blue F3GA is not very hydrophobic overall, but it has planar aromatic surfaces that prefer to interact with hydrophobic groups in the β-casein structure.

Adsorption isotherms

Two important physico-chemical aspects for evaluation of the adsorption process as a unit operation are the kinetics and the equilibria of adsorption. Modelling of the equilibrium data has been done using the Langmuir and Freundlich isotherms [45]. The Langmuir and Freundlich isotherms are represented as follows Equation 2 and Equation 3, respectively.

$$1/q_e = (1/q_{max}) + [1/(q_{max}\, b)]\, (1/C_e) \quad (2)$$

$$\ln q_e = 1/n\, (\ln C_e) + \ln K_F \quad (3)$$

where, b is the Langmuir isotherm constant, K_F is the Freundlich constant, and n is the Freundlich exponent. 1/n is a measure of the surface heterogeneity ranging between 0 and 1, becoming more heterogeneous as its value gets closer to zero. The ratio of qe gives the theoretical monolayer saturation capacity of magnetic beads.

Some model parameters were determined by nonlinear regression with commercially available software and are shown in Table 1. Comparison of all theoretical approaches used in this study shows that the Langmuir equation fits the experimental data best.

Table 1. Adsorption constants of Langmuir and Freundlich isotherms.

Langmuir Adsorption Isotherm		Freundlich Adsorption Isotherm	
Q_{max}, mg/g	53.48	K_F	41.42
b	3.46	n	0.47
R^2	0.977	R^2	0.956

Adsorption dynamics

In order to quantify the extent of uptake in adsorption kinetics, the kinetic models (Pseudo-first- and second-order equations) can be used in this case assuming that the measured concentrations are equal to adsorbent surface concentrations [46]. The first-order rate equation of Lagergren is one of the most widely used for the adsorption of solute from a liquid solution. It may be represented as follows:

$$\log (q_e - q_t) = \log (q_{1cal}) - (k_1 t)/2.303 \quad (4)$$

where qe is the experimental amount of β-casein adsorbed at equilibrium (mg/g); qt is the amount of β-casein adsorbed at time t (mg/g); k_1 is the equilibrium rate constant of first order adsorption (1/min); and q_{1cal} is the adsorption capacity calculated by the pseudo-first-order model (mg/g).

The rate constant for the second-order adsorption could be obtained from the following equation:

$$(t/q_t) = (1/k_2 q_{2cal}^2) + (1/ q_{2cal})\, t \quad (5)$$

where k_2 is the equilibrium rate constant of pseudo-second-order adsorption (g/mg.min); q_{2cal} is the adsorption capacity calculated by the pseudo-second- order kinetic model (mg/g).

The results given in the Table 2 shows that the second order mechanism is applicable (R^2 values are the highest). These results suggest that the pseudo-second order mechanism is predominant and that chemisorption might be the rate-limiting step that controls the adsorption process. The rate-controlling mechanism may vary during the course of the biosorption process with three possible mechanisms rate-limiting occurring [47]. There is an external surface mass transfer or film diffusion process that controls the early stages of the adsorption process. This may be followed by a reaction or constant rate stage and finally by a diffusion stage where the adsorption process slows down considerably [48].

Table 2. The second-order kinetic constants for Cibacron Blue F3GA attached poly(AAm-AGE) cryogel.

β-Casein Concentration	q_e	Second order kinetic		
(mg/ml)	(mg/g)	k_2 (g/mg min)	q_e (mg/g)	R^2
0.1	8.60	0.0035	10.6	0.987
0.2	15.43	0.0027	17.86	0.995
0.3	17.46	0.0043	19.27	0.995
0.4	21.91	0.0049	23.47	0.998
0.75	31.22	0.0045	33.22	0.997
1.0	44.70	0.0027	47.62	0.997
1.5	44.75	0.0076	46.08	0.999
2.0	44.59	0.0121	45.25	0.999

Effect of Flow-Rate

The breakthrough curves at different flow-rates are given in Figure 5. Results show that the β-casein adsorption capacity onto the poly(AAm-AGE)/ Cibacron Blue F3GA cryogel decreases when the flow-rate through the column increases. The adsorption capacity decreased significantly from 8.52 mg/g to 0.99 mg/g polymer with the increase of the flow-rate from 0.2 mL/min to 2.0 mL/min. An increase in the flow rate reduces the solution volume treated efficiently until breakthrough point and therefore decreases the service time of cryogel column. This is due to decrease in contact time between the β-casein molecules and the poly(AAm-AGE)/Cibacron Blue F3GA cryogel at higher flow rates. When the flow-rate decreases the contact time in the column is longer. Thus, β-casein molecules have more time to diffuse to the pore walls of cryogel and to bind to the ligand, hence a better adsorption capacity is obtained. In addition, for column operation the cryogel is continuously in contact with a fresh protein solution. Consequently the concentration in the solution in contact with a given layer of cryogel in a column is relatively constant.

Effect of Ionic Strength

The effect of ionic strength (adjusted by adding NaCl) on β-casein adsorption is presented in Figure 6, which shows that the adsorption capacity decreases with increasing ionic strength of the binding buffer (acetate buffer at pH 4.0). The adsorption amount of β-casein decreased by about 58.72% as the NaCl concentration changes from 0.01 to 0.5 M. Increasing the NaCl concentration could promote the adsorption of the dye molecules to the polymer surface by hydrophobic interaction. Moreover, the hydrophobic interactions between the immobilized dye molecules themselves would also become strong, because it has been observed that the salt addition to a dye solution caused the stacking of the free dye molecules. Thus, the numbers of the immobilized dye molecules accessible to β-casein would decrease as the ionic strength increased, and the adsorption of the β-casein to immobilized dye became difficult. It is also suggested that an increase in NaCl concentration

result in the reduction of electrostatic interactions [49].

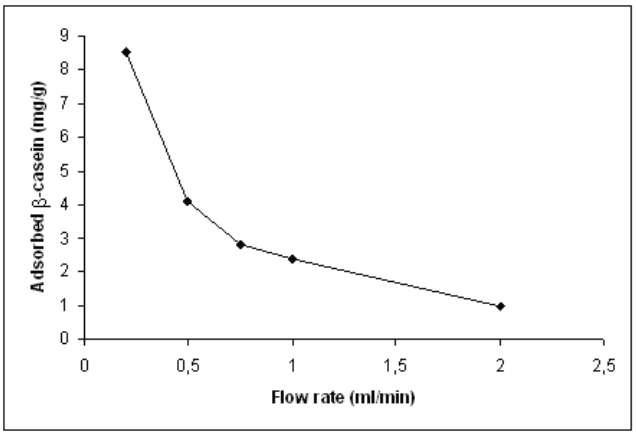

Figure 5. Effect of flow-rate on β-casein adsorption: Cibacron Blue F3GA content: 68.9 μmol/g; pH: 4.0; β-casein concentration: 0.1 mg/mL; T: 20°C. Each point is an average of five parallel studies.

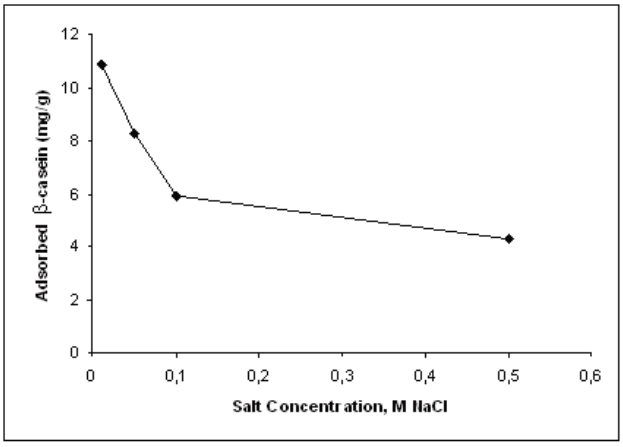

Figure 6. Effect of the NaCl concentration on β-casein adsorption: Cibacron Blue F3GA content: 68.9 μmol/g; β-casein concentration: 0.1 mg/mL; pH: 4.0; Flow rate: 0.2 mL/min; T: 20°C. Each point is an average of five parallel studies.

Desorption and Reusability Studies

Desorption of β-casein from the Cibacron Blue F3GA-attached poly(AAm-AGE) cryogel was also carried out in column system. The desorption of β-casein is expressed in % of totally adsorbed β-casein. Up to 99% of the adsorbed β-casein was eluted when 0.1 M Tris/HCl buffer containing 0.5 M NaCl was used as elution agent. The addition of elution agent reduced electrostatic interactions, resulting in the release of the β-casein molecules from the attached dye-molecules. Note that there was no Cibacron Blue F3GA release in this case which shows that dye-molecules are bonded strongly to poly(AAm-AGE) cryogel. With the desorption data given above we concluded that 0.1 M Tris/HCl buffer containing 0.5 M NaCl is a suitable desorption agent, and allows repeated use of the affinity cryogel used in this study.

In order to show the reusability of the Cibacron Blue F3GA-attached poly(AAm-AGE) cryogel, the adsorption-desorption cycle was repeated ten times using the same dye-affinity poly(AAM-AGE) cryogel. As shown in Figure 7, the adsorption capacities for the cryogel did not noticeably change during the repeated adsorption-desorption operations. By taking into account the different experimental parameters studied above, it should be possible to scale up the process of β-casein separation by increasing the cryogel size by dye-affinity chromatography on Cibacron Blue F3GA-attached poly(AAm-AGE) cryogel.

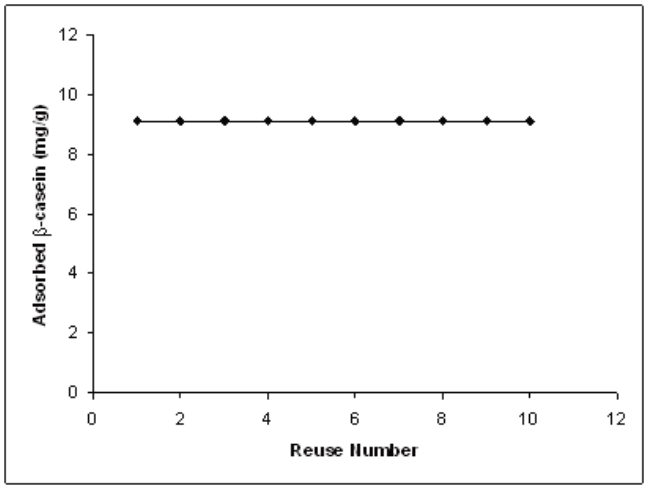

Figure 7. Repeated use of Cibacron Blue F3GA attached poly(AAm-AGE) cryogel. Cibacron Blue F3GA content: 68.9 μmol/g; β-casein concentration:0.1 mg/mL.

CONCLUSION

Recently, cryogel materials are considered as a novel generation of stationary phase in the separation science [24-30, 38]. Cryogels are a very good alternative to protein purification with many advantages [31]. Casein is well known as a good protein emulsifier and β-casein is the major component of casein and commercial sodium caseinate [32]. Today, caseins are hard to replace in a number of applications. For instance, beer bottle labels are still glued with casein and shadow masks for TV-tubes are still produced using casein [33]. Cibacron Blue F3GA attached poly(acrylamide-allyl glycidyl ether [poly(AAm-AGE)] cryogel was used in this study to provide an alternative matrix in column seperations. Poly(acrylamide-allyl glycidyl ether [poly(AAm-AGE)] cryogel was prepared by bulk polymerization which proceeds in an aqueous solution of monomers frozen inside a glass column (cryo-polymerization). Cibacron Blue F3GA was attached by covalent binding onto poly(AAm-AGE) cryogel via epoxy groups. Adsorption/desorption studies of β-casein on Cibacron Blue F3GA attached poly(acrylamide-allyl glycidyl ether [poly(AAm-AGE)] cryogel led to the following results. The maximum amount of β-casein adsorption from aqueous solution in acetate buffer was 44.74 mg/g at pH 4.0. Adsorbed β-casein was desorbed up to 99% by using 0.1 M Tris/HCl buffer containing 0.5 M NaCl as elution agent. Repeated adsorption/elution processes showed that Cibacron Blue F3GA attached poly(AAm-AGE) cryogel is very suitable for β-casein adsorption.

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