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Microporous Whey Protein Isolate Gel Sorbent

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Abstract: Microporous adsorbents made of whey protein isolate (WPI) gel were developed and tested as a cation exchanger using copper ions as a challenge material. Isotherms as a function of pH were also determined and described by a modified Langmuir-style isotherm, with values of q_m, K_m, and k_1 of 68 mg/g, 1.4 mg/mL and 7.634×10^{-3} M, respectively.

Keywords: Whey protein isolate, Cation exchange material, Copper adsorption

INTRODUCTION

Adsorbents are widely used in many different types of industrial applications wherein solutes are selectively transferred from the fluid phase to the stationary phase of sorbents. This selective transfer of material is facilitated by the property of the stationary phase; for example, cationic sorbents will adsorb/exchange anionic species, anionic sorbents will adsorb/exchange cationic species, and phobic sorbents will adsorb phobic molecules. Germane to this paper is the adsorption of metal ions from aqueous streams, a topic of importance for wastewater management. Currently many different adsorbents exist that are employed in such a setting, with commercial materials principally polymeric in nature. Interest in green technology suggests developing alternate adsorbent materials through either environmentally benign

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processing or through the use of biological-based materials. It is the latter that the subject of this paper is about, the preparation of adsorbent materials from, in our case, a by-product of cheese manufacturing.

The newly developed microporous WPI gel (1–4) is an unusual sorbent that contains functionalities associated with side chain amino acid chemistry. The presence of primary amine, hydroxyl, and carboxyl groups on WPI means that the protein gel can be used as a generic adsorbent (Table 1). This study evaluates the use of microporous WPI gel as a cationic sorbent. The low cost of WPI gel (<\$5/L) and the presence of about 27 carboxyl groups per β-lactoglobulin molecule (major component of WPI) justifies the belief that WPI gel may be useful as an inexpensive cationic sorbent for heavy metal cleanup in industrial waste stream. Adsorption isotherms of cupric chloride from water were developed in this study to probe the capacities of WPI sorbent.

MATERIALS AND METHODS

WPI was provided by NZMP (North America), Inc. (32W895). Dry WPI contains 97.4% protein, 1.6% ash, 0.4% fat, and 0.6% lactose.

Table 1. Amino acid composition of bovine β-lactoglobulin

Amino acids	Count	Classification
Pro	8	
Gly	3	
Ala	14	Aliphatic
Val	10	Aliphatic
Ile	10	Aliphatic
Leu	22	Aliphatic
Tyr	4	Aromatic
Phe	4	Aromatic
Trp	2	Aromatic
Asp	11	Acid
Glu	16	Acid
Lys	15	Basic
His	2	Basic
Arg	3	Basic
Asn	5	Polar uncharged
Ser	7	Polar uncharged
Gln	9	Polar uncharged
Thr	8	Polar uncharged
Cys	5	Sulfur containing
Met	4	Sulfur containing

The apparatus shown in Fig. 1 was used for the determination of pI and extractable of WPI gel.

Formation of WPI Gel Sorbent

Protein solution was prepared by dissolving 28 weight percent of WPI powder in 0.075 M $CaCl_2$ buffer at room temperature. A very small amount of 5 M HCl was used to adjust the pH of the protein solution to 6.15. The protein solution was then centrifuged at about 160x*g* to remove bubbles. It is important to maintain a low speed such that the settling of protein aggregates could be avoided. The protein solution was then transferred carefully onto a flat 5-in nonstick cooking pan (Teflon® coated) and drawn to an even thickness of about 1 mm by spin-coating the pan with the protein solution. Excess protein solution was removed from the pan (about 13 mL protein solution needed per pan). The pan was then covered with aluminum foil completely and heated in a steam autoclave at 121°C to initiate and complete the gelation process. The autoclave was exhausted at the end of 1 h and the protein gel was removed and immediately immersed in water, by pouring DI water directly into the pan, to prevent the hot gel from drying. The gel was cooled to room temperature and was gently peeled away from the nonstick pan. The air-contacting side of the protein gel was regarded as the front of the gel whereas the pan-contacting side was treated as the back of the gel. The gel was then immersed in about 5 times its own weight of 20% formaldehyde solution in water overnight to further strengthen the gel by cross-linking. The cross-linked gel was then washed and soaked in 10 times its own weight of DI water overnight, and the washing and soaking were repeated two more times.

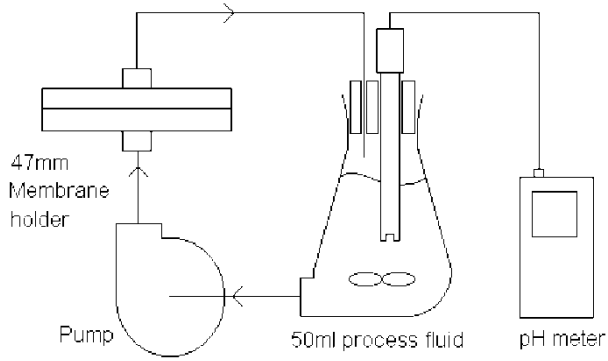

Figure 1. Setup for pI and extractable evaluation.

Determination of Isotherms, Membrane pI, and Extractables

Skin layers formed on the front and back surfaces of the WPI gel are due to the adsorption of protein molecules at interface (1), and will resist fluid flow into the porous gel phase. Removal of the skin layer will not change the shape of the adsorption isotherms of the protein gel but will allow the process to reach equilibrium faster. Both front and back skins were removed by wet abrasion with 1500-grit silicon carbide sandpaper until the shiny skin is completely removed from the gel. The gel was broken into pieces for the characterization of adsorption isotherms. To determine isotherms, samples of gel were placed in the challenge solutions ($CuCl_4$ in water) and allowed to shake gently overnight at room temperature.

For the study of pI and extractable, removal of the skin layer was similarly performed to reduce pressure needed to pump the liquid through the membrane (Fig. 1). This design permits one to titrate charged residues in a simple method by observing pH rise as base is added to the recirculation loop. More importantly, it allows one to determine the amount of membrane loss (if any) due to attrition from pumping fluid by providing an undiluted sample. Typical experimental conditions were room temperature with a recirculation rate of 5 mL/min. Extra WPI gels were stored wet at about 4°C and used within a month.

For determination of isotherms, a known weight of WPI gel (particle size was approximately 2 mm) was added into various $CuCl_2$ solutions of different concentrations and incubated with shaking for 24 h at room temperature to allow for equilibrium uptake of cupric ions. The equilibrium concentrations of $CuCl_2$ in the solutions were measured using a spectrophotometer, and the amount of cupric ions lost in the solutions was assumed adsorbed onto the protein gel.

RESULTS AND DISCUSSION

Material Preparation

The formation of WPI gel is different from the conventional concentration-induced or temperature-induced phase separation methods. Formation via gelation can be described as a two-step process where the protein molecules first associate with each other with an intensity determined by the electrostatic forces, followed by gelation induced by heating (5–7). The gelation of protein solution is attributed to the formation of covalent disulfide bonds between cysteine residues that are revealed when protein molecules unfolded near the denaturation temperature (7, 8). Since protein molecules possess amphiphilic characteristics, it will also unfold and orient itself at the interface to

reduce interfacial tension. Subsequently, a layer of adsorbed protein is associated with the interface, providing a dense layer once the material sets.

The morphology and characteristics of the WPI gel adsorbent are strongly influenced by electrostatic forces, controllable by pH and ionic strength of the protein solution (5–9). At extremely high repulsive environment, protein molecules repel each other, making the formation of protein network impossible. With the reduction of repulsive forces by adjusting the solution pH closer to the isoelectric point (pI), a low degree of molecule association occurs, forming a gel with very fine structure (i.e., nonporous) after gelation. At pI, protein molecules are neutral, and they form aggregates driven by electrostatic attractive forces. This dispersed aggregate solution will give rise to a porous protein gel with aggregate structure after gelation. Addition of salt neutralizes charge carried by the protein molecules and thus enhances the association of protein molecules into aggregates (5–10). The SEM image shown in Fig. 2 represents a typical WPI gel made in this study. Note that the skin layers formed on the top and bottom surfaces were removed via abrasion.

Determination of pI and Extractables

Extractables are defined as WPI returning to solution either through dissolution or flaking, with quantification a measure of integrity. For the determination of extractables from WPI gel made with 28wt% WPI and 0.075 M $CaCl_2$, 50 mL

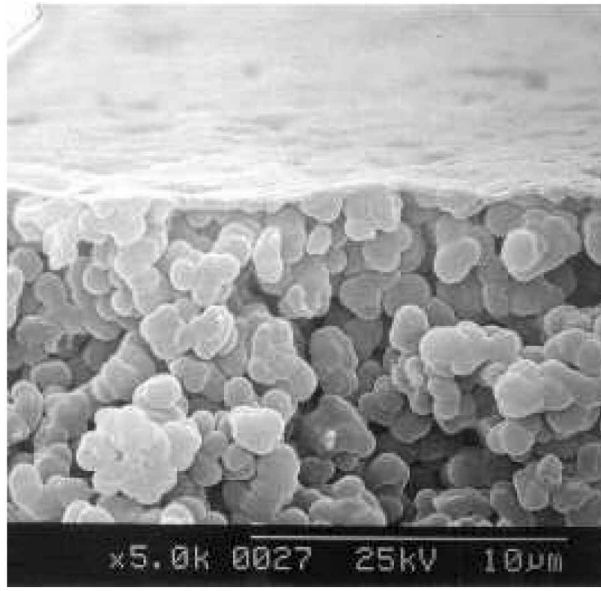

Figure 2. Representative WPI membrane with the skin layer intact.

of sterilized MilliQ® water was recirculated through a WPI membrane. After 12 h, protein content in solution was determined.

The WPI concentration at the end of the challenge was measured and gave an average value of 89 μg/mL. The high solubility of WPI in aqueous solution permitted the determination of extractables in this batch system under pseudosteady-state conditions, with the driving force of mass transfer unaffected by the small quantity of WPI dissolved. Therefore, the process of recirculation is equivalent to filtering 3.6 L with an extractable concentration of 1.0 nanogram WPI membrane lost/mL filtrate · gram WPI gel.

The pI of the protein gel was obtained in a similar way. The WPI sorbent was first equilibrated in 0.015 M of HCl solution before loading into the membrane holder. The system was then filled with 100 mL of 0.015 M HCl with fluid recirculated until constant pH was measured. An aliquot of 1 M sodium hydroxide was added and the system was allowed to reach a new equilibrium pH. Addition of sodium hydroxide was repeated, generating a titration curve (Fig. 3). A similar titration curve for water is included in Fig. 3 for comparison.

The isoelectric point of the membrane was estimated to be a value between 5.0–5.5. The membrane pI was a similar value to that belonging to β-lactoglobulin, the principal component of WPI. Such information is important from an application standpoint, since the pI of charged based separation materials dictate the pH at which adsorption will occur, and regeneration pH.

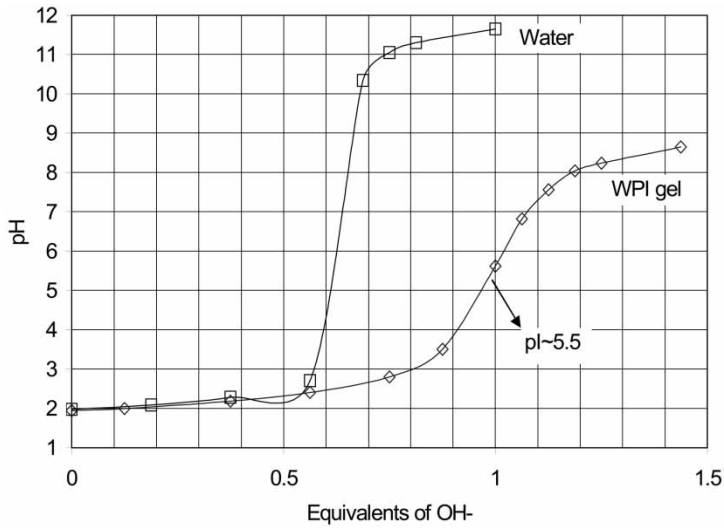

Figure 3. Isoelectric point of WPI gel.

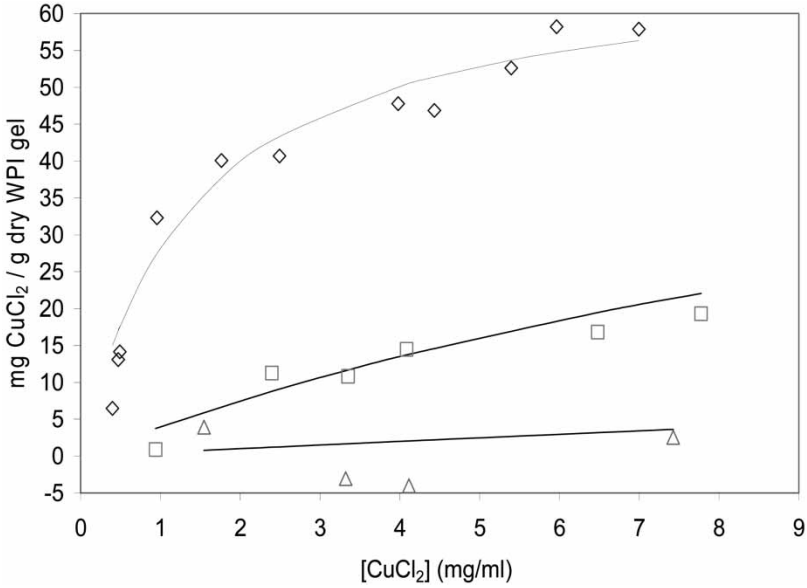

Figure 4. Adsorption isotherms of cupric chloride at different concentration of HCl solutions: water (◇), 0.08 M HCl (□), 0.71 M HCl (△).

Adsorption Isotherm of Cupric Chloride in Water

WPI gel contains many carboxyl groups that can function as a weak cation exchanger when the system pH is above the isoelectric point of the carboxyl. At acidic conditions, the carboxyl groups on the WPI gel will be protonated and reduce its exchange capacity for positively charged ions. To illustrate the uptake of cations, adsorption isotherms of Cu(II) was examined at different pH values.

A modified Langmuir adsorption model that takes into the consideration of H^+ concentration (pH) was used to describe the adsorption behavior of WPI:

$$q = \frac{q_m C}{K_m\left(1 + \frac{[H^+]}{k_1}\right) + C} \tag{1}$$

where,

q = amount adsorbed per g of dry WPI gel, mg/g dry WPI;

q_m = maximum amount adsorbed per g of dry WPI gel, mg/g dry WPI;

K_m = constant, mg/mL;

C = concentration, mg/mL;

k_1 = constant, M; and

$[H^+] = H^+$ concentration, M.

Figure 4 presents the result of cupric chloride adsorption isotherms along with the result obtained using modified Langmuir adsorption isotherm. The q_m, K_m, and k_1, were found to be 68 mg/g, 1.4 mg/mL, and 7.634×10^{-3} M, respectively. At around neutral pH (water), WPI gel has a maximum adsorption capacity of about 68 mg $CuCl_2$ per gram of dry WPI gel. At acidic condition of 0.08 M HCl, the adsorption capacity is reduced to about 25%, and at about 0.71 M HCl, the WPI gel does no adsorb $CuCl_2$ appreciably. When viewed as a whole, the results indicate that WPI gel may be useful for the removal of metal ions from water.

CONCLUSION

WPI gel was shown to be a useful sorbent for Cu(II). It has a low level of extractable at about 1 ng WPI membrane lost/mL·g WPI membrane, and an isoelectric point of 5.0–5.5. At appropriate pH values WPI gel behaved as a cation exchanger with a maximum exchange capacity of 68 mg cupric chloride per gram dry WPI gel membrane due to the presence of carboxyl groups on amino acid side chains.

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