Whey Protein Concentrate Hydrogels as Bioactive Carriers

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ABSTRACT: Two sets of heat-induced hydrogels were prepared from whey protein concentrate (WPC), one set at a constant concentration [15% (w/v)] and varying pHs (pH 5.1–10.0) and the other set at a constant pH (10.0) and varying concentrations (12%, 15%, and 18%). At a given pH, the higher the protein concentration, the shorter was the gelation time and the larger were the equilibrium storage modulus (G'_∞) and failure stress. For a given protein concentration, the gelation kinetics and mechanical properties of WPC hydrogels were strongly pH dependent. The swelling behavior of WPC gels was studied at 37.5°C ± 0.5°C. The equilibrium swelling ratio (*SR*) was at the minimum when pH of the swelling medium was close to the isoelectric point (*pI*) of the whey protein, and when the swelling medium pH was far from the *pI* (from 6.0 to 10.0), the *SR* increased. In particular, when the pH was higher than the *pI*, the swelling was highly pH sensitive. The higher the WPC concentration used in preparing the hydrogel, the lower was the *SR*. The controlled drug release properties of the WPC hydrogels were studied using caffeine as the model drug. Consistent with the swelling behavior of the gels, release was slower when the pH of the medium was lower (pH 1.8) than when it was higher (pH 7.5). The *SR* and the drug release rate decreased significantly when the gels were surface-coated with alginate. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 99: 2470–2476, 2006

Key words: controlled release; gelation; hydrogels; proteins; stimuli-sensitive polymers; swelling

INTRODUCTION

A hydrogel can be defined as a three-dimensional network that exhibits the ability to swell in water and retains a significant fraction of water in its structure. A wide variety of hydrogels are made from natural and synthetic polymers. They can absorb water because they have hydrophilic groups such as —OH, —CONH—, —CONH$_2$, —COOH, and —SO$_3$H.[1] Hydrogels can be neutral or ionic in nature. Because of their potential applications in controlled and site-specific drug release, the swelling behavior of hydrogels has been studied extensively. The driving force for swelling comes from the contribution of the water–polymer thermodynamic mixing energy to the overall free energy, which is coupled with a contribution from an elastic polymer.[2] For ionic hydrogels, the ionic interaction between charged polymer and free ions also contributes to swelling.[3]

Over the last decade many hydrogels have been developed as controlled drug release carriers using water-soluble, biodegradable polymeric materials[4–8] including synthetic or natural polymers. Among the natural polymers that have been used to develop pH-sensitive hydrogels are alginates[6,7] and chitosan.[8,9] The latter is usually crosslinked with other polymers such as poly(vinyl alcohol)[8] or polyether[9] using glutaraldehyde to produce semi-interpenetrating networks. Park et al.[7] reported that pH-sensitive hydrogels can be prepared from egg albumin simply through heat-induced gelation. They investigated the effect of gel preparation conditions, particularly the initial pH of the protein solution, on the swelling of dried albumin gel in phosphate buffer solutions. The albumin hydrogels exhibited pH-sensitive swelling behavior; the degree of swelling was low around the protein isoelectric point (*pI*; pH 4) and increased with pH.

Whey proteins are those proteins remaining soluble at pH 4.6 and 20°C after removal of caseins from milk.[10] The main whey proteins, constituting approximately 70% of all whey proteins, are β-lactoglobulin and α-lactalbumin. β-Lactoglobulin, the main whey protein component and its principal gelling agent, is a globular protein of 162 amino acid residues, with a monomer molecular weight of 18.3 kDa, two disulfide bridges (between residues 106–119 and 66–160), and a free thiol group (SH) at residue 121. These provide a potential for intermolecular and intramolecular disulfide link interchange during conformational changes associated with pH alterations, heat, or pressure treatment.

Although the physicochemical properties of whey proteins suggest that they may be suitable for developing pH-sensitive hydrogels for the controlled delivery of biologically active substances, reported research in this field is still nonexistent. Strong or weak heat-induced gels with high or low water-holding capacity may be prepared from whey protein solutions simply by adjusting several of the gelation variables. Thus, it is possible to design heat-induced whey protein gels with good pH sensitivity, tailored permeability, and mechanical properties that can be used as drug carriers. The advantages of using whey protein–based gels as potential devices for the controlled release of pharmaceuticals is that they are completely biodegradable and their preparation does not require any chemical crosslinking agents. These are two of the major requirements for wide use of hydrogels not only in the pharmaceutical area but also in many food and bioprocessing applications.

The objective of this investigation was to prepare and characterize the swelling and drug release behavior of whey protein concentrate hydrogels.

EXPERIMENTAL

Gelation

Whey protein concentrate (WPC) powder obtained from a commercial source (Davisco Foods Inc., Eden Prairie, MN) was used. This powder was composed of 82.5% protein, 6.8% fat, 3.4% ash, and 6.5% lactose (data supplied by the manufacturer). Two sets of WPC gels were prepared: Set 1—WPC concentration = 15% (w/v), pH = 5.1, 5.7, 6.2, 6.8, 7.2, and 10.0; Set 2—pH = 10.0, WPC concentration = 12%, 15%, and 18%. Details of gel preparation methods were previously reported by Xiao.[11]

The gelation of WPC was investigated using a dynamic rheometer (Bohlin CVOR, Bohlin Instruments Inc., East Brunswick, NJ). the protein solution was poured directly into the couette system and covered with a thin layer of paraffin oil to prevent water evaporation during the experiment. The solution was heated to 80°C for 1 h and then cooled to 25°C at a rate of 2°C/min. During the entire thermal treatment, the storage modulus (G'), loss modulus (G''), and phase angle (δ) of the samples were measured at a frequency of 1 Hz and a maximum target strain of 0.01.

Gel mechanical properties

Gels were also prepared in cylindrical stainless-steel tubes (inner diameter of 10 mm, length of 40 mm) by filling them with protein solutions. The tubes were closed with rubber stoppers, sealed with vinyl electrical tape, and placed vertically in a water bath and heated isothermally at 80°C $\pm$ 0.5°C for 1 h. The gels

were cooled to room temperature and left at 4°C overnight before being removed from the tubes and cut into cylindrical specimens corresponding to a height-to-diameter ratio of 0.6 (10 mm in diameter, 6 mm in length).

Uniaxial compression tests were performed using a universal testing machine (Synergie 200; MTS System Corporation, Cary, NC) equipped with a 50-N load cell. The gel specimens were deformed to 80% of their initial height at a constant crosshead speed of 1 mm/s (the test was stopped if the gel collapsed before 80% deformation). The compressive force at failure and the corresponding deformation were determined from the force-deformation data.[12]

Gel tablet preparation

The gels were cut into tablets 10 mm in diameter and 2 mm thick and dried in an enclosed desiccator until the tablets reached a constant mass (i.e., within ± 0.001 g). The gels also were prepared with an encapsulated model drug. Caffeine (Aldrich Chemical Company, Inc., F.W. 194.19, Milwaukee, WI) was chosen as the model drug because of these desirable properties: its UV absorbance was easy to detect; it was thermally stable at 80°C, the gelling temperature of WPC; it was readily water soluble; and it did not interact with WPC. Caffeine (0.15 g) was dissolved in a 15% WPC solution (3.0 g of WPC in 17.0 g of a pH 10.0 buffer solution) to get a 1:20 drug/WPC mass ratio. The drug-loaded WPC gel tablets (10 mm in diameter, 2 mm thick) were prepared and dried as described above.

A portion of the WPC gels (prepared at a 15% concentration and pH 10) and caffeine-encapsulated WPC gels were alginate-coated by placing the tablets in 1% sodium alginate solution for 2 min.[13] The gels were then cured to a gel alginate on the surface in a 0.1M CaCl$_2$ solution for 15 and 30 min. Additional layers of alginate coating were applied by repeating the procedure two (for two alginate layers) or four (for four alginate layers) times, as needed. The thickness of each alginate layer was measured using a micrometer as about 37.5 $\pm$ 1.0 μm. The alginate-coated gels were washed twice using deionized water and dried in a desiccator. The alginate coating was desirable with protein gels in order to prevent gel hydrolysis by proteolytic enzymes in the stomach (e.g., pepsin).

Swelling experiment

Triplicates of dried gel tablets were weighed and placed in 20 mL of pH 1.8, 2.9, 5.8, 6.6, 7.6, 10.1, and 11.4 phosphate buffer solutions (ionic strength = 0.2M). Temperature was maintained at 37.5°C $\pm$ 0.5°C in an incubator (Model 2005; VWR Scientific Inc., West Chester, PA). Gels were removed from the

TABLE I
Effect of Whey Protein Concentrations (12%, 15%, and 18%) on Gelation Kinetics and Mechanical Properties of WPC Gels Prepared at pH 10.0

Property	WPC concentration (%)		
	12	15	18
Gelation time (t_{gel}, s)	500 ± 36^a	160 ± 14^b	60 ± 5^c
Rate of increase in G' [b value in eq. (2), s^{-1}]	0.0010	0.0012	0.0023
Equilibrium modulus (G'_∞, kPa)	0.2 ± 0.04^a	0.85 ± 0.09^b	3.3 ± 0.13^c
Compressive force at failure (N)	5.4 ± 0.3^a	11.4 ± 0.8^b	17.2 ± 1.6^c
Hencky strain at failure	1.04 ± 0.03^a	1.24 ± 0.05^b	1.06 ± 0.04^a
True stress at failure (kPa)	28.0 ± 0.9^a	45.7 ± 1.8^b	83.4 ± 3.6^c

Superscripts a, b, and c in each row indicate significant differences between concentrations ($p = 0.05$).

buffer solution periodically, blotted dry with tissue paper. and weighed. The swelling ratio (SR) was calculated from the mass measurements of wet gel (m_w) and dry gel (m_d) as follows:

$$SR = \frac{m_w - m_d}{m_d} \qquad (1)$$

In vitro drug release experiment

The *in vitro* drug release tests were carried out using the USP 23n.1 dissolution test apparatus (Model 2000; Distek Inc., North Brunswick, NJ) fixed with six rotating baskets. A basket apparatus was used in order to reduce the variability of the hydrodynamic conditions of the test and to overcome the problem of gel possibly sticking to the wall of the dissolution apparatus. The dissolution medium was USP phosphate buffer, pH 7.5 (300 mL, 37.5°C ± 0.5°C), and the speed of rotation was 100 ± 1 rpm. Three dried tablets were placed into each of three baskets, and 3-mL samples were collected from the release medium at regular intervals. After each sample collection, the same amount of fresh release medium at the same temperature was added back. The amount of drug released was monitored by a UV-vis spectrophotometer (DMS 300; Varian Inc., Palo Alto, CA) at 272 nm. The caffeine UV standard absorbance curve was established first. In the concentration range investigated (2×10^{-5} to 6×10^{-5} M), UV absorbance obeyed Beer's law.[11]

RESULTS AND DISCUSSION

Gelation kinetics and gel mechanical properties

On heating whey protein solutions at 80°C, G' rapidly increased with time and then tended to reach a plateau according to the following equation:

$$G'(t) = G'_\infty(1 - e^{-bt}) \qquad (2)$$

where t is the time, b is an empirical parameter that measures the rate of increase in gel strength (i.e., G')

with time, and G'_∞ is the equilibrium storage modulus. This pattern was similar to those of other protein gels. The gelation time[14] (t_{gel}), G'_∞, and b values were strongly dependent on WPC concentration at pH 10.0 (Table I). The increase in failure force, failure stress, and failure strain with increasing WPC concentrations, listed in Table I, was consistent with published results on protein gels.[15–17]

The effect of pH was found to agree with that reported in the literature. That is, gelation occurred faster at pH 10.0 (t_{gel} = 160 s) than at pH 7.1 (t_{gel} = 210 s) because a high pH is favorable to protein denaturation. At pH 10.0, SH reactivity increased so rapidly that the SH—S—S interchange reaction occurred even without heating. Also, at a given concentration, G'_∞ was higher at pH 7.1 than at pH 10.0. This was because of the difference in the network structure of gels formed at pH 7.1 and those formed at ph 10.0.[18–20] At pH 5.0, the gel was very weak (small G', high tan δ, negligible failure stress), and it was rather difficult to determine the gelation time.

Effect of swelling medium pH on kinetic and equilibrium swelling

The swelling kinetics of 15% WPC hydrogel denatured at pH 10.0 kept in swelling media with different pHs are shown in Figure 1(a). The SR of WPC hydrogels was sensitive to the pH of the swelling medium—the higher the pH of the swelling medium, the faster the swelling occurred. At pH 10.0 the gels reached an equilibrium SR in about 50 min, whereas at pH 1.8 it took almost twice as long.

The kinetics of swelling may be understood by considering several simultaneous effects. The contours of the time-versus-penetrant uptake curve deviated more often from the classical Fickian model. When this occurred, the sorption process was not passive diffusion of the solvent molecules into the void spaces of the network but included concomitant relaxation of the network segments that resulted from the advancing solvent front, leading to plasticization of the material and a large increase in volume.
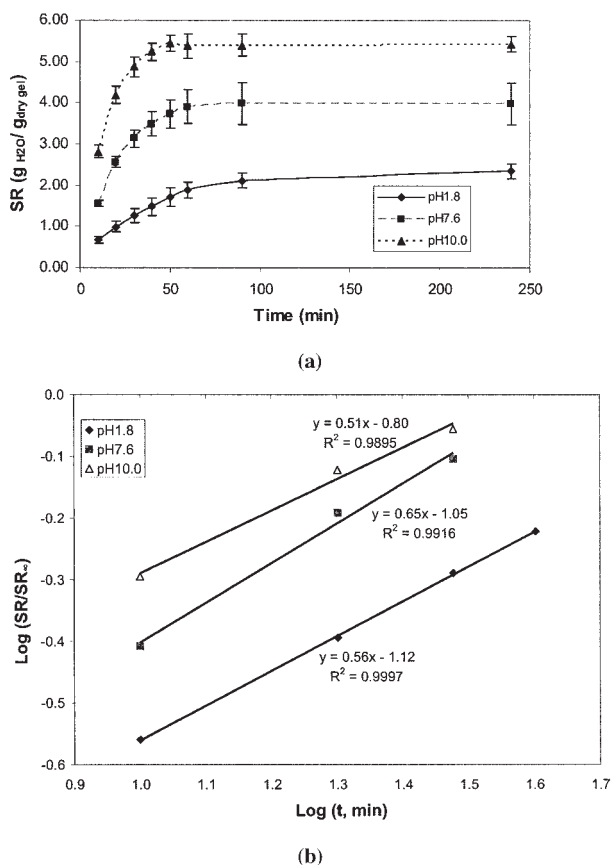
Figure 1 (a) Swelling kinetics of 15% whey protein hydrogels prepared at pH 10.0 with different pHs of the swelling media; (b) fitting of swelling kinetics of 15% whey protein prepared at pH 10.0 with different pHs of swelling media by the power law, $SR/SR_\infty = Kt^n$ (the values of n are shown in the figure).

The generalized semiempirical equation used to describe the swelling kinetics was[21–23]

$$\frac{SR_t}{SR_\infty} = Kt^n \qquad (3)$$

where K is a characteristic constant of the system, that is a function of the geometry of the hydrogel tablet and the diffusion constant. Eq. (3) is valid when (SR_t/SR_∞) < 0.6. On the basis of the value of the exponent n, this equation has been used to distinguish three types of sorption behavior: case I, case II, and anomalous.[24] Case I sorption is typified by $n \sim 0.5$. It represents a perfect Fickian process, during which the rate of solvent penetration is slower, as it is the rate-determining step, than the chain relaxation rate. For case II sorption, $n = 1.0$, that is, the mobility of the penetrant is substantially faster than the chain relaxation rate, and the solvent uptake is directly proportional to time. Anomalous sorption occurs when $0.5 < n < 1.0$, in which case, the rate of penetrant mobility and segmental relaxation are comparable.

Thus, the relative importance of solvent diffusion and polymer matrix relaxation effects can be analyzed by examining exponent n of the power law. As shown in Figure 1(b), at pH 10.0, n was 0.51, and the process could be considered an example of diffusion-controlled case I sorption, whereas at pH 1.8 and 7.6, n was 0.56 and 0.65, respectively, examples of anomalous sorption. This kind of kinetic behavior can be understood by considering the network structure of the WPC hydrogel. At pH 10 polymer chain relaxation was greatly decreased because of strong electrostatic repulsion of negative charges at the surface of the gel microstructure. So water diffusion was faster than relaxation of the polymer chain, and swelling turned out to be diffusion controlled. In contrast, when the pH of the swelling medium pH was 7.6, most of the net negative charges were neutralized by the positive charges from the swelling medium, so a smaller number of net charges existed in the hydrogel. As a result, electrostatic repulsion strongly decreased and polymer chain relaxation increased to become comparable with water diffusion, resulting in an anomalous sorption mechanism.

Hydrogel swelling also is governed by ionization of negatively charged groups. When the swelling medium pH = 10, the number of negatively charged groups was greatest, so the equilibrium SR (= 5.5) was the highest because of strong electrostatic repulsion. When the swelling medium pH = 7.6, the protons from the swelling medium neutralized most negatively charged groups, so the equilibrium SR (= 3.8) was lower because of the reduced electrostatic repulsion. When the swelling medium pH = 1.8, all negatively charged groups were neutralized, and there were some positive amine groups, instead. Because there were fewer amine groups than carboxyl groups in the hydrogel, there were few net charges, so the equilibrium SR (= 2.2) was very low.

Effect of concentration and pH on equilibrium swelling

The equilibrium swelling ratios of hydrogels at different whey protein concentrations (12%, 15%, and 18%) versus the pH values of the swelling media are shown in Figure 2(a). At all swelling media pH values, 12% WPC hydrogels took up the most water, whereas 18% WPC gels took up the least. This can be explained by Flory's swelling theory.[1] At a higher concentration the density of the protein network was high and because of this, the equilibrium SR should have decreased with increasing protein concentration.
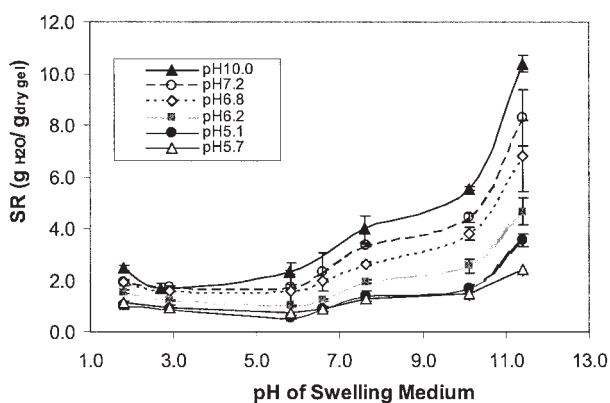
The equilibrium swelling ratios of 15% WPC hydrogels prepared at different pH values versus the pHs of the swelling media are shown in Figure 2(b). At all swelling medium pHs, the general trend was that the higher the gelation pH, the higher the equilibrium SR.
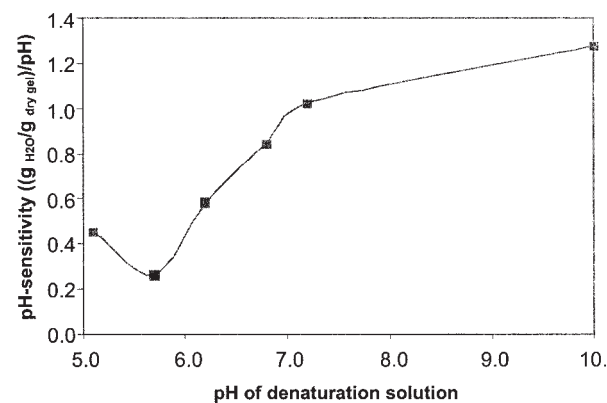
(a)
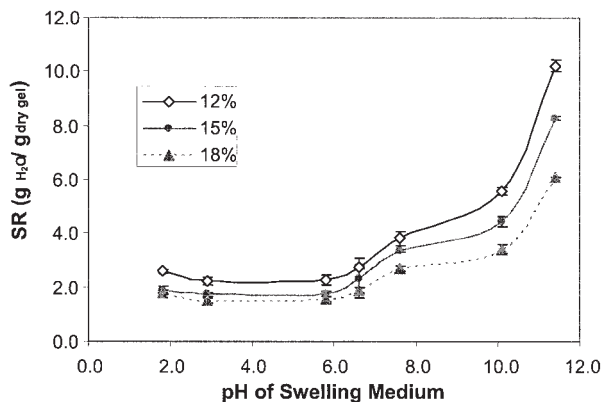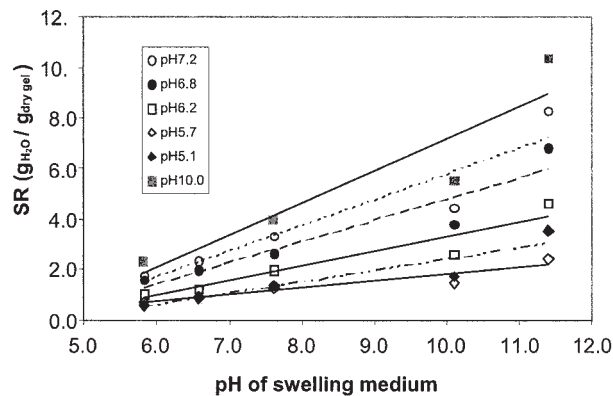
(b)

Figure 2 Equilibrium swelling ratio (*SR*) of WPC gels: (a) of different concentrations (12%, 15%, 18%) prepared at pH 10.0; (b) of different protein preparation pHs (5.1, 5.7, 6.2, 6.8, 7.2, and 10.0) at a WPC concentration of 15%.

(a)

(b)

Figure 4 Equilibrium swelling ratio (*SR*) of 15% WPC gel: (a) prepared at a pH of 10.0 with the swelling medium having different pHs; (b) pH sensitivity of 15% whey protein hydrogel versus the pH of the preparation. pH sensitivity was defined as the slope $[d(SR)/d(\text{pH})]$ of the first-order regression shown in (a).

The structure of thermally denatured protein gels depends on the pH of the protein solution (see schematic illustration in Fig. 3). When pH < *pI*, in an acidic swelling medium the increase in *SR* was very small
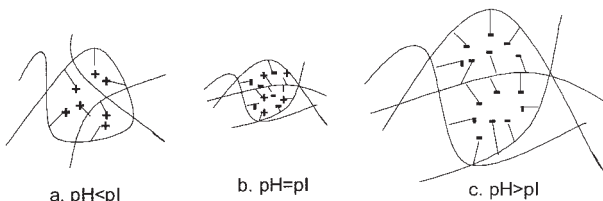

Figure 3 Schematic illustration of whey protein swelling mechanism at different swelling pHs: (a) at pH < *pI*, swelling resulted from the electrostatic repulsion between positively charged groups, which were in limited amounts in the whey proteins; (b) at pH = *pI*, swelling was at a minimum because of zero net charge; (c) at pH > *pI*, swelling increased to a greater extent because of the stronger electrostatic repulsion between larger amounts of negatively charged amino groups compared to the positively charged groups in the whey protein.

because there were very few amine groups at protein chains, so the positive charges were very limited [Fig. 3(a)]. *SR* reached its minimum when swelling medium pH was close to the *pI* of the whey protein (= 5.4). This is because the net charge of the whey protein molecules was close to zero at *pI*, which means there was almost no electrostatic repulsion between chains in thermally denatured whey protein and minimum *SR* [Fig. 3(b)].

On the other hand, when pH > *pI*, there were a lot of negatively charged groups in the protein chains [Fig. 3(c)], so the gels would contain many net charges when a swelling medium with a high pH value was used, resulting in an increased equilibrium *SR*. The higher the pH, the greater was the number of surface charges, the higher was the electrostatic repulsive force, and the higher was the equilibrium *SR*.

Linear regressions of equilibrium *SR* of WPC denatured at various pHs (5.1, 5.7, 6.2, 6.8, 7.2, and 10.0) are presented in Figure 4(a). The slope of these lines represents pH sensitivity, which is plotted against the pH
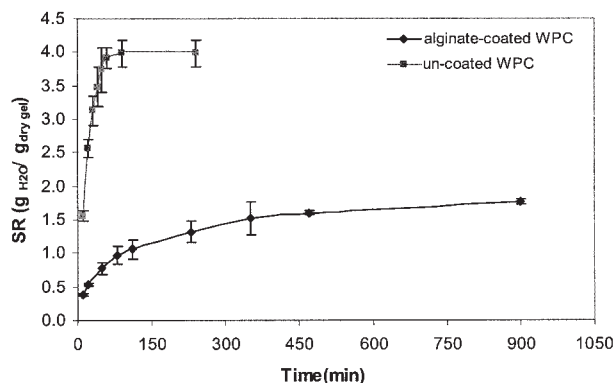
Figure 5 Comparison of selling ratio (*SR*) of alginate-coated WPC gel (15%; gel preparation, pH 10.0; swelling medium, pH 7.5) with and without alginate coating.

Figure 6 Effect of pH of release medium on the *in vitro* release profile of caffeine from the WPC gel tablet.

of the 15% WPC solution used for gel preparation shown in Figure 4(b). The WPC hydrogels denatured at a higher pH showed higher pH sensitivity. As mentioned before, the gels denatured at higher pH values had higher surface net charges or negative charges, so electrostatic repulsion between the charges led to a higher equilibrium *SR* and higher pH sensitivity.

Effect of alginate coating on swelling

Alginate can form a very stable gel in the presence of Ca^{++}, and it is widely used for the coating of polymer matrices used in controlled drug delivery systems.[13] It is well known that alginate coating lowers the diffusion of solvent and encapsulated drug release. Figure 5 shows such an effect of alginate coating on the swelling of 15% WPC gel prepared at pH 10.0. The equilibrium *SR* and the rate of swelling decreased dramatically after coating whey protein gel with alginate compared with that of the gel without coating. It is well known that alginate gel formed through calcium ion bridges is very rigid and does not swell readily.[25] Furthermore, the *n* value determined [per eq. (3)] for the alginate-coated gel was 0.44. Thus, it can be said that alginate coating not only lowers the diffusion rate but also alters the swelling kinetics from anomalous sorption (*n* = 0.65 before alginate coating) to diffusion-controlled sorption.

Effect of pH of release medium on drug release

Caffeine release profiles from the 15% WPC hydrogel prepared at pH 10.0 in release media with pHs of 7.6 and 1.8 are shown in Figure 6. At pH 7.6 caffeine release was substantially faster than at pH 1.8. The slower release at pH 1.8 is a result of fewer net charges and electrostatic repulsion. This is consistent with pH-sensitive swelling behavior. The *n* values determined [per eq. (3)] were 0.5 at pH 7.6 and 0.47 at pH 1.8.
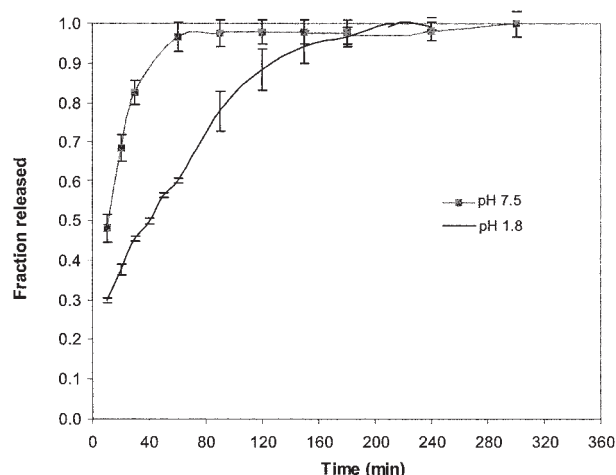
These *n* values suggest that the release at both pHs was diffusion controlled.

Effect of alginate coating on drug release

The caffeine release profile from 15% WPC hydrogel prepared at pH 10.0 with alginate coating is shown in Figure 7. The release profile from whey protein gel without alginate coating also is shown for comparison. It is obvious that the caffeine release rate was reduced significantly by alginate coating, which is consistent with the results of the swelling study. The Ca^{++}-induced alginate gel was very strong, rigid, and hard to swell, so the diffusion through this coating was the rate-limiting step for swelling and drug release. The release was prolonged by additional alginate layers on the hydrogel surface. The release profile of the sample with a coating of four alginate layers was interesting
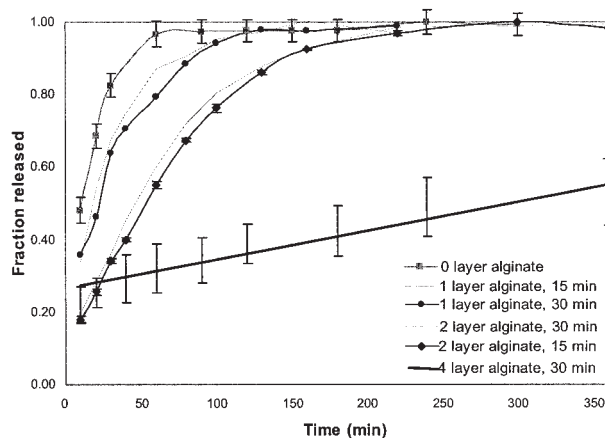

Figure 7 *In vitro* release profiles (pH 7.5 in swelling medium) of caffeine from WPC gel tablets with 0, 1, 2, and 4 layers of alginate coating cured for 15 and 30 min.

in that not only was the release rate significantly lower, but also the release kinetics changed to zero order. Similar results have been reported by others.[26,27] The curing time of alginate gel seems to have no significant effect on the drug release behavior, indicating that alginate coating completely cured within 15 min.

CONCLUSIONS

Various factors affecting the gelation and/or swelling of WPC hydrogels were studied. These factors include concentration, pH value of the swelling medium, pH value of the whey protein solution before gelation, and alginate coating at the surface of whey protein hydrogel tablets. The swelling of whey protein hydrogels was pH sensitive; the equilibrium swelling ratio reached the minimum when the pH of the swelling medium was close to the whey protein pI; and when the pH of the swelling medium was far from the pI (from 6.0 to 10.0), the equilibrium SR increased. When the pH of the swelling medium was higher than the pI, the swelling was highly pH sensitive. The effect of protein concentration on the equilibrium SR also was significant: the higher the concentration, the stronger was the gel matrix, and the lower was the equilibrium SR. The gels formed at high pH had a higher equilibrium SR. WPC hydrogel swelling can be described by a power law; the value of exponent n varied with pH between 0.44 and 0.76. The alginate coating of the gel surface decreased the SR significantly and possibly altered the swelling kinetics.

Caffeine release from WPC hydrogels is pH dependent. When the pH of the release medium was lower (1.8), the release was slower than when the release occurred in a medium with a higher pH (7.5), consistent with the swelling behavior. The release kinetics for both cases were diffusion controlled. The release rate of caffeine also was lowered by alginate coating. The release kinetics changed from diffusion controlled without alginate coating ($n = 0.5$), to anomalous when a one- or two-layer alginate-coating was applied ($n = 0.65$), and to zero-order or time-independent release when four layers of alginate-coating was applied ($n = 1$).

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