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Dynamics of antimicrobial hydrogels in physiological saline

Ghadeer F. Mehyar [a], Zhiqiang Liu [b], Jung H. Han [b,*]

[a] Department of Nutrition and Food Technology, University of Jordan, Amman 111942, Jordan
[b] Department of Food Science, University of Manitoba, Winnipeg, Man., Canada R3T 2N2

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Abstract

It is critical in the design of hydrogel drug release system to understand the effect of drugs on the properties of hydrogel. Physically cross-linked hydrogels – pea starch (PS) gel formed by hydrogen bonding and calcium alginate (ALG) gel by ionic interaction were investigated in this paper. Antimicrobials added in the starch gel and alginate gel were trisodium phosphate (TSP) and acidified sodium chlorite (ASC), respectively. Solids loss, water uptake, antimicrobial release, and change in storage modulus for both blank and antimicrobial hydrogels immersed in a saline solution were evaluated as a function of time. The apparent diffusivities of total solids, water and antimicrobials in hydrogel were determined according to Fickian diffusion. It was found that TSP in PS gel simultaneously accelerated the loss of total solids and uptake of water in the hydrogel, entailing an increased reduction in the total solid content. Consequently, the storage modulus of the antimicrobial PS gel decreased faster than the blank PS gel. The diffusivities of antimicrobials, 2.72×10^{-9} m^2 s^{-1} for TSP in PS gel and 6.58×10^{-9} m^2 s^{-1} for ASC in alginate gel, were close to those of water but much lower than those of total solids, suggesting that gel swelling control the release of antimicrobials. Controlled antimicrobial release systems based on hydrogel are expected to see applications in preserving fresh carcass in the meat and poultry industries.

Keywords: Hydrogel; Diffusivity; Storage modulus; Antimicrobial; Starch; Alginate

1. Introduction

Hydrogel is a network of hydrophilic polymer chains, which are able to hold up water but are kept from dissolution by either physical or chemical cross-links. There has been an increasing interest in physically cross-linked hydrogel, in lieu of chemically cross-linked hydrogel which may involve the use of toxic agents. Several physical interactions have been exploited in the design of hydrogel, such as electrostatic attraction (Bodmeier, Chen, & Paeratakul, 1989; Bodmeier & Wang, 1993; Doria-Serrano, Ruiz-Trevino, Rios-Arciga, Hernandez-Esparza, & Santiago, 2001; Grant, Morris, Rees, Smith, & Thom, 1973; Ortega & Perez-Mateos, 1998; Seely & Hart, 1974), hydrogen bonding (Durrani & Donald, 1995; Goodfellow & Wilson, 1990; Liu & Han, 2005; Ring et al., 1987), antigen–antibody binding (Miyata, Asami, & Uragami, 1999). Basically, it is required that polymers possess an abundance of functional groups (e.g., -OH, -COO$^-$, -NH, -SH) to achieve inter- and intra-molecular interactions in the formation of hydrogel.

As a major storage polysaccharide in plants, starch is a compound of amylose and amylopectin, with its composition depending on the plant origin. Amylose is a nearly linear polymer of α-1,4 anhydroglucose units, with molecular weight of 10^5–10^6 (Durrani & Donald, 1995; Galliard & Bowler, 1987). In contrast, amylopectin is a highly branched polymer consisting of short α-1,4 chains linked by α-1,6 glucosidic branching points occurring every 25–30 glucose units, with molecular weight of 10^7–10^9 (Durrani & Donald, 1995; Galliard & Bowler, 1987). When heated in water at 60 °C or above, starch granules gelatinize, characterized by granular swelling, amylose exudation and disruption of long-order crystalline structure (Liu,

* Corresponding author. Address: Frito-Lay Inc., 7701 Legacy Drive, Plano, TX 75024, USA. Tel.: +1 972 334 4408; fax: +1 972 334 4444.
E-mail address: Jung.Han@FritoLay.com (J.H. Han).

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2005). Suspension of gelatinized starch starts gelling upon cooling as a result of inter- and intra-molecular hydrogen bonding of amylose and linear branches on amylopectin (Goodfellow & Wilson, 1990; Liu & Han, 2005). Macroscopically, starch gel is a three-dimensional network constructed mainly by helical strands of polymeric chains (Ring et al., 1987).

Alginate in a form of free acid or sodium salt is a collective term for a family of polysaccharide prepared mostly from brown algae (Smidsrød and Grasdalen, 1984). Chemically, alginate is a mixture of poly(β-D-mannuronate), poly(α-L-guluronate), and poly(β-D-mannuronate α-L-guluronate), with its exact composition depending on algal source. Similar to starch gel, alginate gel features a 3-D network structure (Ahearne, Yang, El Haj, Then, & Liu, 2005; Decho, 1999; Doria-Serrano et al., 2001; Walkenström, Kidman, Hermansson, Rasmussen, & Hoegh, 2003). However, alginate forms hydrogel by polymeric chains interacting with Ca^{2+} and other divalent and trivalent metal ions (Donati et al., 2005; Rees & Samuel, 1967), according to the so-called "egg-box" model (Grant et al., 1973). As a result of ionic interaction, the presence of di- or multivalent cations enable the formation of junction zones between guluronic blocks, those of mannuronic blocks, and those of mannuronic–guluronic blocks (Donati et al., 2005).

In addition to many other biomedical applications such as enzyme immobilization (Ortega & Perez-Mateos, 1998) and tissue engineering (Ahearne et al., 2005; Li, Ramay, Hauch, Xiao, & Zhang, 2005), hydrogel is useful for drug release (Bodmeier & Wang, 1993; Rajaonarivony, Vauthier, Couarraze, Puisieux, & Couvreur, 1993). Drug release from hydrogel occurs mainly due to gel swelling, which can be controlled by the formulation chemistry of polymeric network (e.g., functional groups, degree of cross-linking) and by the environmental conditions (e.g., pH, temperature, ionic strength, etc.) (Peppas, Huang, Torres-Lugo, Ward, & Zhang, 2000). The swelling of hydrogel in water permits the entrapped drug to diffuse throughout the entire network and release from the gel. The release rate is primarily determined the degree of swelling (Prokop, Kozlov, Carlesso, & Davidson, 2002).

Due to its ability to sustain the release of antimicrobials, hydrogel has become a potent carrier of antimicrobials in the meat and poultry industries (Natrajan & Sheldon, 2000a, 2000b). It is the purpose of this paper to examine the swelling and rheological properties of starch and alginate hydrogels in physiological saline and the release of antimicrobials from the hydrogels to the saline solution, which simulates the fluidic condition on the surfaces of chicken skin, pork and beef. Most importantly, this paper will elucidate the effect of entrapped molecules on the structure of host matrices, which is critical but often overlooked in the design of hydrogel for controlled or sustained drug release (Bodmeier & Wang, 1993; Rajaonarivony et al., 1993).

2. Materials and methods

2.1. Preparation of starch and alginate hydrogels

Three grams of pea starch (PS, 37% amylose, Nutri-Pea Ltd., Portage-la-Prairie, MB, Canada) was dispersed in 100 ml cold water. The dispersion was heated to boiling with agitation and held for 5 min until starch granules were almost fully gelatinized. The solution was then cooled to room temperature (23 °C), and 10 g of trisodium phosphate (TSP, Sigma Chemical Co., St. Louis, MO, USA) was added in, followed by homogenization with a Powergen-700 (Fisher Scientific International Inc., Whitby, ON, Canada) for 5 s at 20,000 rpm. The solution was then poured into two 200 ml beakers, with 50 ml solution for each beaker, and left overnight at room temperature to allow the stabilization of gel structure. PS hydrogel without TSP was also prepared and used as control.

Two solutions were used to prepare calcium alginate (ALG) hydrogel. Solution (a) was an acidified sodium chlorite (ASC) solution containing 1% w/v of calcium chloride ($CaCl_2$, Sigma Chemical Co.). The ASC solution was prepared by mixing equal portions of citric acid solution (900 ppm) and sodium chlorite solution (1100 ppm) (Sanova, Alcide Corp., Redmond, WA, USA), and was used within 30 min after preparation. Solution (b) contained 0.5% (w/v) sodium alginate (Product No. 180947, CAS 9005-38-3, Sigma Chemical Co.) dissolved in water at room temperature.

Calcium alginate gel was prepared using a plastic assembly consisting of a mold and two fixative rings (Fig. 1). A piece of $CaCl_2$ permeable membrane (Dialysis Tubing, Fisher Brand regenerated cellulose, Fisher Scientific, Nepean, ON, Canada) was first attached onto the mold by one

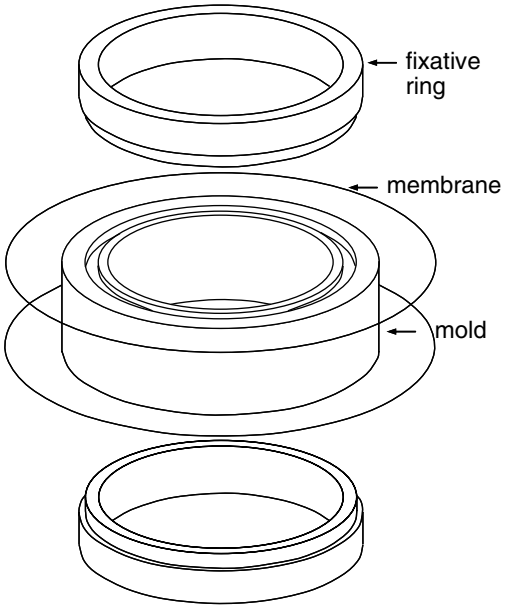

Fig. 1. Schematic assembly used for preparing calcium alginate gel.

fixative ring. Solution (b) of 50 ml was poured into the mold, and the mold was covered by another piece of the membrane, which was fixed onto the mold using the other ring. Two assemblies containing the sodium alginate solution were then immersed in solution (a) of 500 ml and taken out after 24 h. Self-standing calcium alginate gels containing ASC were obtained after the removal of the rings and membranes. Calcium alginate gels without ASC were also prepared by the same procedure except that solution (a) used was a pure $CaCl_2$ solution with the same concentration of 1% (w/v).

2.2. Rheological properties of hydrogels in air and in saline

Freshly prepared hydrogel was cut into a cylinder using a plastic borer with a height of 10 mm and internal diameter of 20 mm. The gel cylinder was then sliced into specimens with a thickness of 5 mm by a sharp blade. Rheological analysis was carried out using a controlled stress rheometer (AR-1000, TA Instruments Inc., New Castle, DE, USA) with 20 mm parallel plate geometry. After a specimen was centered on the base platen, the upper platen was programmed to move down at a decelerating speed until came in contact with the specimen in order to avoid any pre-loading deformation. Oscillatory stress sweeps from 0.1 to 10 Pa at a frequency of 1 Hz were done at a temperature of 25 °C to determine the linear viscoelastic range for hydrogels in air. Since both gels exhibited linear elastic regions at stress below 2 Pa, a stress of 1 Pa was chosen for the following time-sweep experiments.

A physiological saline was prepared by dissolving 0.8 g of sodium chloride (Sigma Chemical Co.) in 100 ml water, followed by adjusting pH to 6.8. After a gel specimen was centered in a bath (height 30 mm, inner diameter 34 mm, outer diameter 64 mm) glued to the base platen, the saline solution double the specimen's volume was filled inside the bath while the upper plate came in contact with the specimen. Oscillatory time sweeps (1 Pa at 1 Hz) were run for all specimens in saline. A series of dynamic storage moduli (G') as a function of immersion time (t) were obtained from the control software.

2.3. Determination of TSP and ASC concentrations in saline

After the specimen was loaded, saline solution of 0.5 ml was withdrawn periodically and collected in a vial. The samples, diluted 1000 folds, were analyzed by an ion chromatography system (Dionex Corporation, Sunnyvale, CA, USA). The injection volume and flow rate were maintained at 50 μl and 1 ml/min, respectively, throughout the analysis. External standards (0, 5, 25, 50, 75 and 100 μg/ml) for both phosphate and chlorite anions were used for calibration. NaOH solution of 30 mM was used as eluent for all samples. The concentration of antimicrobial (C) in the saline was determined by the peak area for the elution which was calculated by Chromeleon Chromatography Management Systems (Dionex Corporation). The concentration of ASC was determined by subtracting the peak area of chloride anions in the pre-load saline solution from the peak area of both chloride and chlorite anions in the samples containing ASC, since both chlorite anions from ASC and chloride anions from NaCl eluted at the same time (3 min). The concentration of TSP was determined directly by the peak area for phosphate anions in the samples. The concentration of antimicrobial after 12 h immersion in the saline solution was taken as the equilibrium concentration (C_∞).

2.4. Solids loss and water uptake during gel swelling

A freshly prepared gel sample (diameter 20 mm and thickness 5 mm) was weighed before (M_i) and after (M_d) drying at 105 °C to reach constant weight (about 5 h). The initial solid content (SC_0) of the fresh gel was determined as M_d/M_i. A gel sample, after weighing (M_0), was immersed in a saline bath (the sample size, the inner diameter of the bath, the concentration and amount of the saline solution were the same as those used in the rheological determination) for 8 h. The swollen gel was then weighed (M_{sw}), followed by drying at 105 °C to become constant weight (M_s). The amount of solids (M_{s0}) and water (M_{w0}) in the pre-swelling gel were M_0SC_0 and $M_0(1 - SC_0)$, respectively. The amount of solids (M_s) and water (M_w) in the post-swelling gel were M_s and $M_{sw} - M_s$, respectively. The solid content (SC) of the swollen gel was M_s/M_{sw}. All samples were duplicated.

2.5. Apparent diffusivities of solids, water and antimicrobials

Solids loss, water uptake and antimicrobial release were all assumed to follow Fickian diffusion. A simple form of the solution (Schwartzberg & Chao, 1982) is:

$$\frac{X - X_\infty}{X_0 - X_\infty} = C_1 \mathrm{Exp}\left(-\frac{q_1^2 Dt}{R^2}\right) \quad (1)$$

where X can be the amount of solids (M_s), amount of water (M_w), or the concentration of antimicrobial released (C). Subscripts 0 and ∞ stand for at zero and infinite time, respectively. The constants c_1 and q_1 are correlated. For infinite cylinder, $q_1 = \sqrt{4(\alpha+1)(\alpha - c_1)/c_1}/\alpha$, where the stripping factor $\alpha = 2$ (the volume of saline solution divided by the volume of gel). R is the radius of gel sample (10 mm), and t is the immersion time. The apparent diffusivity (D) of solids, water and antimicrobial were obtained by three parameter non-linear fitting of M_s/M_{s0} vs. t; M_w/M_{w0} vs. t; and C/C_∞ vs. t, based on Eq. (1).

3. Results and discussion

3.1. Solids loss and water uptake

Hydrogels in contact with solution lost solids and absorbed water (Figs. 2 and 3). After immersion in the

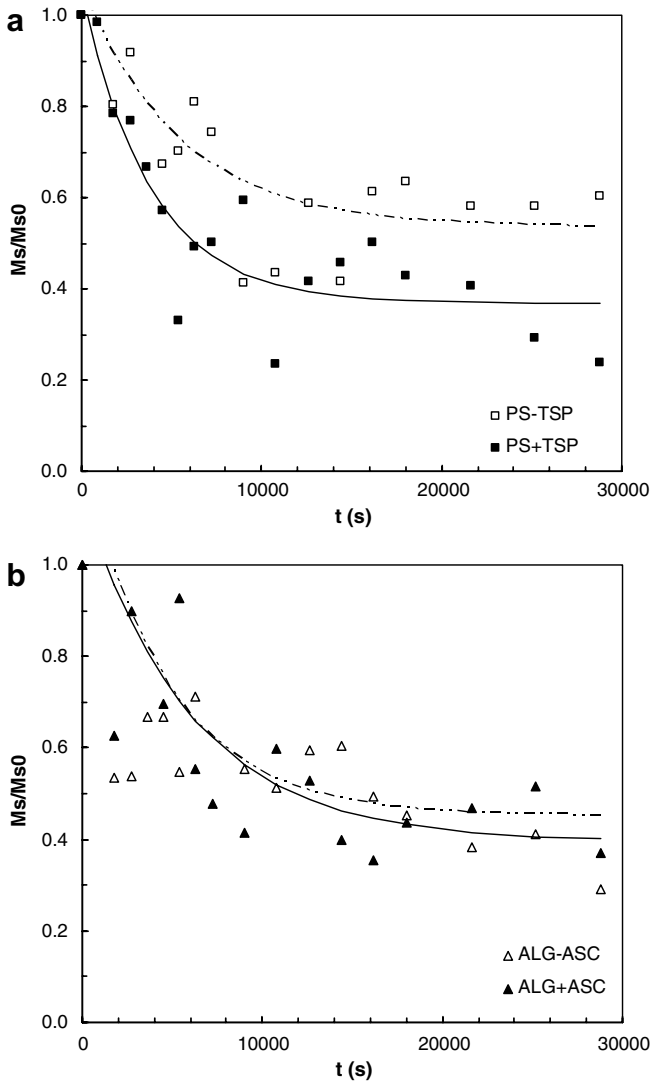

Fig. 2. Dimensionless mass of solids (M_s/M_{s0}) in starch gels (a) and alginate gels (b) as a function of time (t) of immersion in saline solution, with fitted curves based on Fickian diffusion.

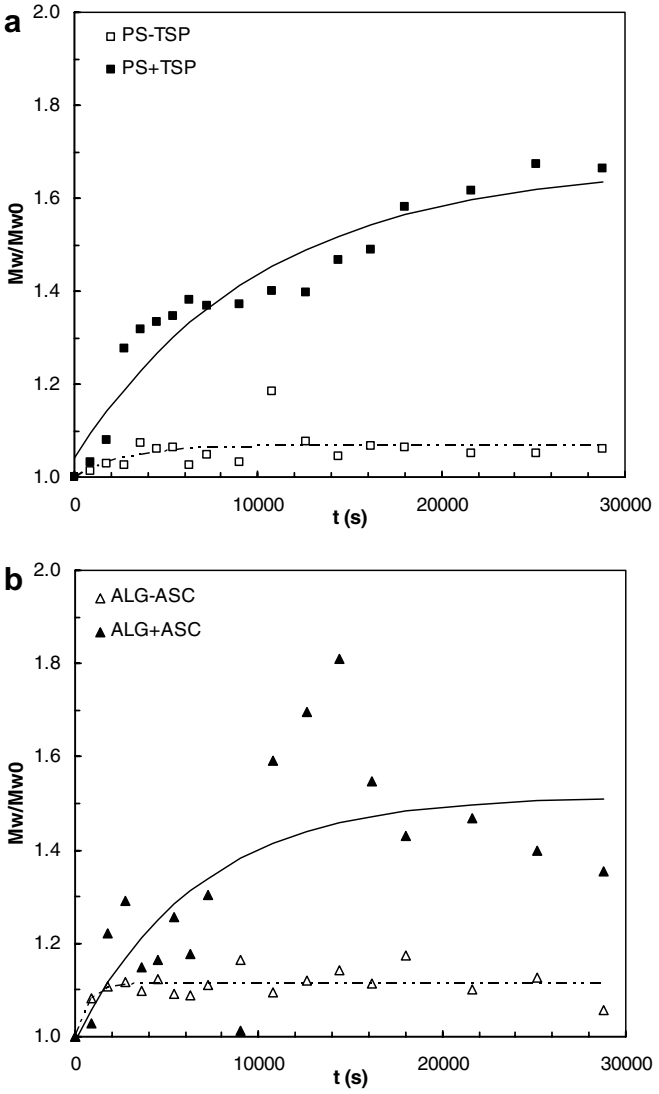

Fig. 3. Dimensionless mass of water (M_w/M_{w0}) in starch gels (a) and alginate gels (b) as a function of time (t) of immersion in saline solution, with fitted curves based on Fickian diffusion.

saline solution for 3 h, hydrogels released 40% of the initial solids or above while absorbing more water at the same time. The solids loss and water uptake largely followed Fickian diffusion, as shown by good fittings. However, the noticeable scattering of data points about the Fickian curves may imply the concurrent gel erosion and swelling in an oscillatory manner (Makino, Idenuma, & Ohshima, 1996). The presence of TSP in starch gel aggravated the release of solids (Fig. 2a), whereas ASC made little difference in the solid loss of alginate gels (Fig. 2b). In contrast, the presence of TSP or ASC in the gel substantially affected the degree of water absorption (Fig. 3). For example, gels with antimicrobials absorbed about 45% more water than those without antimicrobials after 3 h immersion in the saline solution. Due to their high charge density, phosphate anions also tend to structure water by hydrogen bonding (Jane, 1993), and facilitate the water absorption of starch gel. It is likely that those electrolytes by electrostatic interactions swell the cross-linked gel structures, which become more accessible to water molecules into the highly hydrophilic gels. Meanwhile, more solids would be lost in a more open gel structure, since it imposes less hindrance for small molecules (e.g., antimicrobials) and/or dangling clusters to leach out.

3.2. Antimicrobial release

As shown in Fig. 4, the release of antimicrobials from hydrogels into the saline solution followed Fickian diffusion. All R^2 values for the non-linear fitting were greater than 0.95. The apparent diffusivity for TSP in starch gel was $2.72 \times 10^{-9}\ m^2\ s^{-1}$, which is much lower than the apparent diffusivity of the solids in starch gel ($10.3 \times 10^{-9}\ m^2\ s^{-1}$) but close to the water diffusivity in starch gel ($2.88 \times 10^{-9}\ m^2\ s^{-1}$) that is caused by osmotic pressure. Similarly, the apparent diffusivity for ASC in algi-

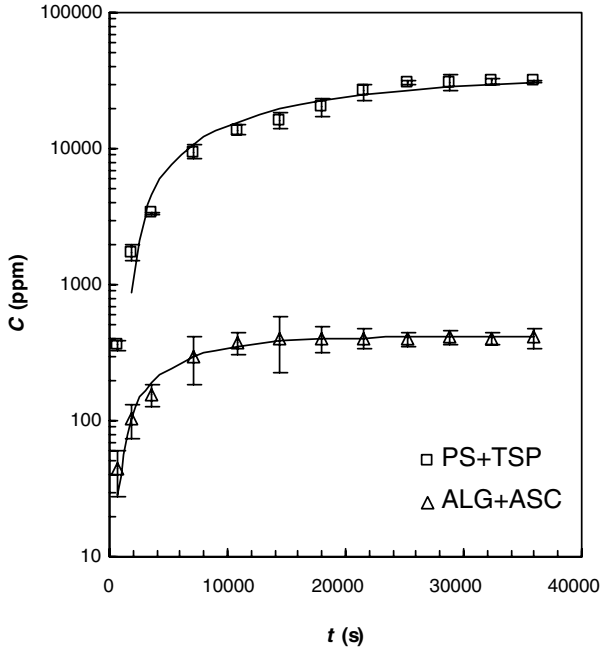

Fig. 4. Concentration of antimicrobials (C) released from PS+TSP and ALG+ASC gels into the saline solution, as a function of immersion time (t), with fitted curves based on Fickian diffusion.

nate gel (6.58×10^{-9} m^2 s^{-1}) was lower than the apparent diffusivity of the solids in alginate gel (9.22×10^{-9} m^2 s^{-1}) but fairly close to the water diffusivity in alginate gel (5.21×10^{-9} m^2 s^{-1}). On this basis, the antimicrobials were most likely unattached to polymer chains in the gel, but rather liberated in the water phase. Therefore, the release of antimicrobial TSP and ASC was resulted from the osmotic pressure, rather than dissolution of solids. Due to higher solid content of the starch gel compared to the alginate gel, the denser gel structure imposes a greater hindrance to the release of the antimicrobial (or to the absorption of water), entailing a slower release rate of TSP. Therefore, the PS+TSP gel would be of particular interest to applications where sustained release of the antimicrobial agent is required.

3.3. Storage modulus of hydrogel

The dimensionless storage modulus (G'/G'_0) of hydrogel in the saline solution decreased with immersion time in a trend of exponential decay (Fig. 5). Substantially decreased solid content (Fig. 6) due to both solid loss (Fig. 2) and water uptake (Fig. 3) was largely responsible for the softening of gels as the immersion prolonged. Since the solids content of PS+TSP gel decreased faster than that of the PS−TSP gel (Fig. 6a), it could be expected that storage modulus of the PS+TSP gel decreased faster than that of the PS-TSP gel (Fig. 5a). TSP interfered with the intermolecular hydrogen bond between starch polymers, which is the major gel-forming force. However, the ALG+ASC gel showed significantly slower modulus reduction than the ALG−ASC gel (Fig. 5b), even though both gels had little difference in the change in dimensionless solids content with time (Fig. 6b). The stabilization effect of ASC on the alginate gel presumably results from the immobilization of Ca^{2+} in the gel, which is promoted by citrate from ASC. Otherwise Ca^{2+} would be prone to ion exchange with Na^+ in the saline solution, as in the ALG−ASC gel. Due to the acidity of ASC components along with Ca^{2+}, the alginate gel has more negative charges and consequently higher gel strength than that without ASC.

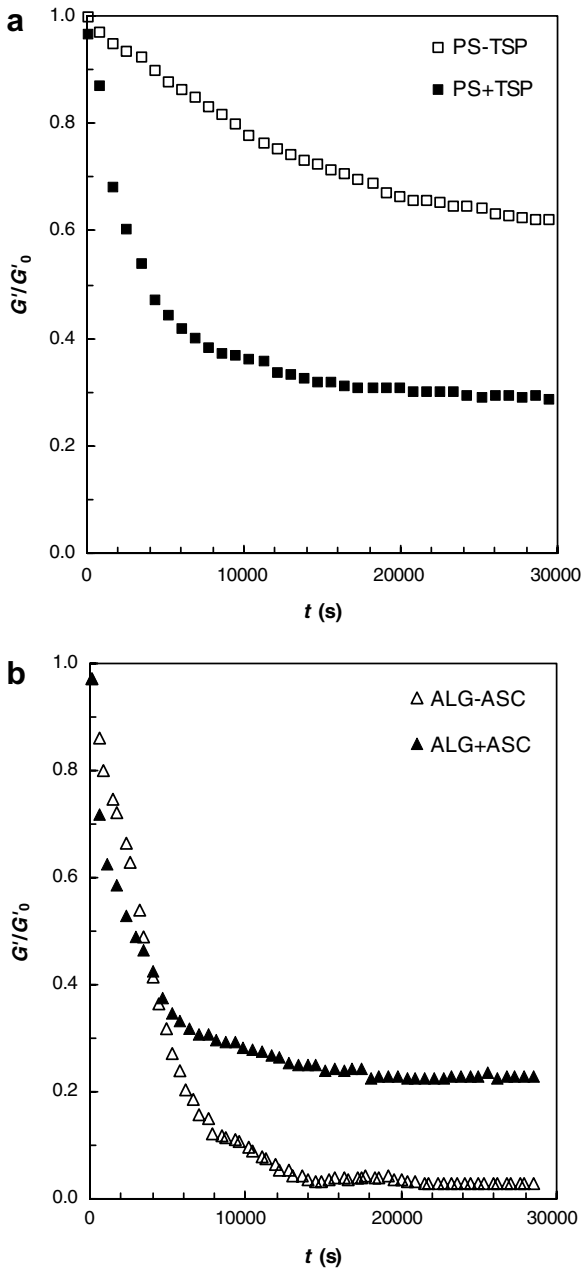

Fig. 5. Dimensionless storage moduli (G'/G'_0) for starch gels (a) and alginate gels (b) as a function of time (t) of immersion in saline solution.

4. Conclusions

The presence of antimicrobials substantially influenced the rheological properties of hydrogels by accelerating solid

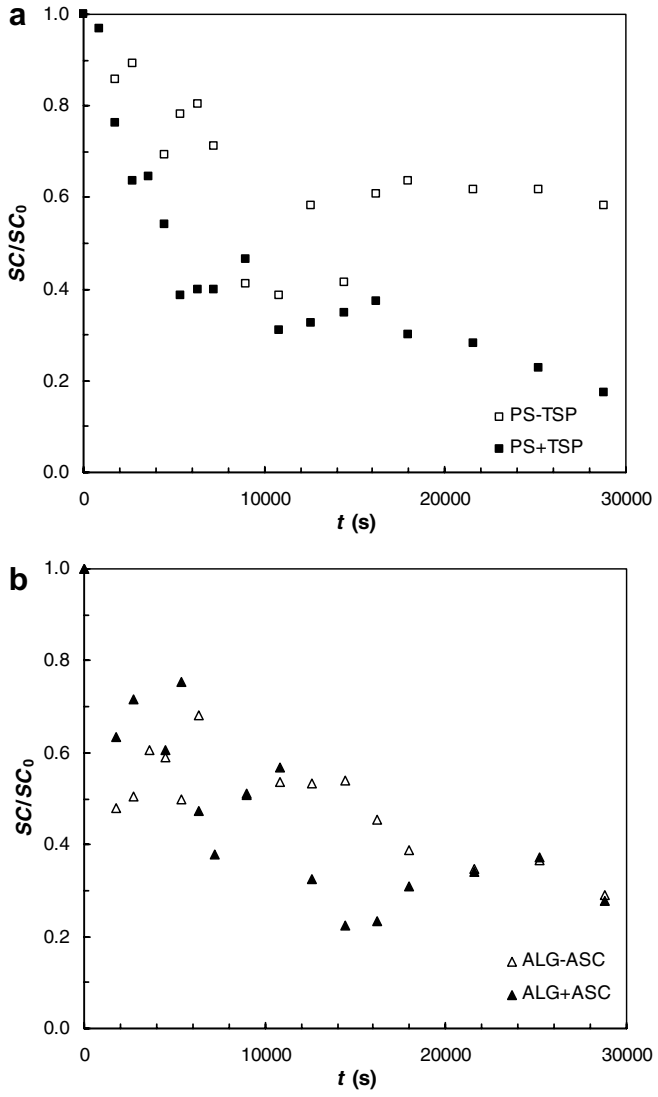

Fig. 6. Dimensionless solid content (SC/SC_0) of starch gels (a) and alginate gels (b) as a function of time (t) of immersion in saline solution.

release and water absorption. Since the release of antimicrobials was slower than the loss of total solids in the gel, and antimicrobials and water had the same level of diffusivity, it is suggested that the release of antimicrobial TSP from starch gel or ASC from alginate gel be largely controlled by osmotic-pressure-induced gel swelling (water in and ions out), rather than dissolution of polymer chains of the gel structure. This work implies that water diffusivity in hydrogel could be used as a monitor of the release of drugs or nutraceuticals when the releasing chemical is known not to strongly interact with polymer chains in the hydrogel.

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