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The cell release kinetics and the swelling behavior of physically crosslinked xanthan–chitosan hydrogels in simulated gastrointestinal conditions

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ABSTRACT

Xanthan gum and chitosan can form physically crosslinked hydrogels of high swelling capacity. Xanthan–chitosan polyelectrolyte complex gels have been studied as microcarriers mostly for the encapsulation of enzymes while the studies on the applicability of the system for bacterial cells are scarce. In this work, probiotic bacteria were encapsulated in xanthan–chitosan gels. The main goal of this study was to characterize the swelling and the release behaviors of xanthan–chitosan hydrogel system under simulated GI-tract conditions to be able to assess its potential as an enteric delivery system for probiotics. We found that the cell release in simulated gastric fluid (SGF) at pH 2.0 for 2 h was negligible, and the complete release of the cells from the capsules in simulated intestinal fluid (SIF) was achieved in 5 h. The pH of the SGF solution was found to be more critical in determining the release properties of the capsules than the presence of the enzyme. The cell release kinetics under GI-tract conditions was also characterized. Cell release from xanthan–chitosan capsules in SIF (after 2 h exposure to SGF at pH 2.0), exhibited a Super Case II transport mechanism regardless of the formulation used, meaning that the chain relaxation is the driving mechanism for the release. Moreover, xanthan–chitosan capsules were found to swell by a diffusion-controlled mechanism. Additionally, cell viability study showed that xanthan–chitosan encapsulation provides a good protection for the probiotics. These results may suggest that xanthan–chitosan capsules have a good potential for the delivery of the probiotics to the intestines.

1. Introduction

Hydrogels are three-dimensional networks of natural or synthetic polymers with the ability of absorbing water (Hennink & van Nostrum, 2002; Kim, La Flamme, & Peppas, 2003). They have gained attention in biomaterials research due to their biocompatibility with tissue and blood (Hoffman, 2002; Lee & Mooney, 2001; Slaughter, Khurshid, Fisher, Khademhosseini, & Peppas, 2009). One of the main areas that the hydrogels find application is the controlled release of the molecules and cells (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2011; Costa, Valente, Miguel, & Queiroz, 2011; Peppas, Bures, Leobandung, & Ichikawa, 2000; Rao & Taguchi, 2011; van Tomme et al., 2005). Hydrogels can be formed by physical or chemical crosslinking. When subjected to water, both chemically and physically crosslinked hydrogels swell due to the solvent penetration. Physically crosslinked hydrogels will eventually degrade in solvent resulting from the swelling stress whereas chemically crosslinked hydrogels stay intact unless covalent bonds are broken with certain treatments (Hennink & van Nostrum, 2002; Rao & Taguchi, 2011).

Xanthan gum and chitosan are two natural polymers that are capable of forming physically crosslinked hydrogels with reversible ionic linkages (Berger et al., 2004; Magnin, Lefebvre, Chornet, & Dumitriu, 2004). These hydrogels can absorb large quantities of water, many times more than their dry weight (Argin-Soysal, Kofinas, & Lo, 2009). Xanthan gum is a microbial exopolysaccharide consisting of a cellulosic backbone with side chains of two mannose and one glucuronic acid on every second glucose residue (Jannson, Kenne, & Lindberg, 1975; Melton, Mindt, Rees, & Sanderson, 1976). Due to the presence of glucuronic acid and pyruvate in its side chains, xanthan gum is considered to be an anionic polyelectrolyte (Richardson & Ross–Murphy, 1987). For this reason,

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xanthan gum can form polyelectrolyte complex (PEC) gels with cationic polymers such as chitosan. Produced by the alkaline deacetylation of chitin, chitosan, aka poly-β-(1 → 4)-D-glucosamine, is the only natural cationic polysaccharide (Muzzarelli, 1977; Sandford, 1989). As polyelectrolyte hydrogels, xanthan–chitosan complexes have been suggested as promising candidates for the targeted delivery, and the controlled release of encapsulated products for oral administration due to the facts that (1) only nontoxic metabolites are produced during degradation, (2) the complex has relatively high enzymatic resistance and also has pH-sensitive swelling characteristics (Chellat et al., 2000; Chu, Kumagai, & Nakamura, 1996; Chu, Sakiyama, & Yano, 1995). In weak acid-weak base polyelectrolyte complexes as in the case of xanthan–chitosan, the number of electrostatic bonds varies with the ambient pH because of the change in the degree of dissociation (Kubota & Kikuchi, 1999). The xanthan–chitosan complex swells in the range of pH = 10–12 in NaOH solution, with a maximum equilibrium swelling ratio (defined as [*Equilibrium diameter/Initial diameter*]^3) at pH = 10. In the presence of NaCl, maximum swelling can be achieved at pH = 8 since the presence of ions weakens the ionic interactions and increases the osmotic pressure difference between the ambient solution and inside of the gel (Chu et al., 1995). The ionizable groups of xanthan gum and chitosan, which are a carboxyl group and an amino group respectively, are oppositely charged and are electrostatically attracted to each other during gel formation. When the pH of the solution increases, the electrostatic linkages between the two polymers start to disappear, since amino groups deionize while carboxyl groups hold the negative charges. Meanwhile, the carboxyl groups attract the Na^+ ions and water diffuses into the complex. Both of these phenomena increase the osmotic pressure of the gel and consequently, the complex swells. However, the equilibrium swelling ratio decreases in NaOH solution at pH values higher than 10, since too much increase in the Na^+ concentration decreases the difference of the osmotic pressure between the gel and the ambient solution. In acidic solutions (HCl), swelling occurs at pH = 0. Similarly, the swelling of the complex can be explained by the neutralization of the negative charges of xanthan while the amino groups of chitosan hold their positive charge, resulting in the swelling of the gel complex (Chu et al., 1995).

Xanthan–chitosan PEC gels have been studied as microcarriers mostly for the encapsulation of enzymes (Dumitriu & Chornet, 1997; Dumitriu, Magny, Montane, Vidal, & Chornet, 1994). The studies on the applicability of the system for bacterial cells are scarce (Chu et al., 1996). In this study, probiotic bacteria were encapsulated in the xanthan–chitosan PEC gels to evaluate this hydrogel system as a targetted delivery vehicle for probiotics. Probitic bacteria are live microbial food supplements that beneficially affect the host by improving its intestinal microbial balance (Fuller, 1989). The biggest challenge in the development of the probiotic products is to maintain the adequate number of viable cells during the shelf life of the product as well as during the gastrointestinal (GI)-tract transit after consumption, so that the claimed health promoting effects can be delivered to the consumer (Hood & Zottola, 1988; Hughes & Hoover, 1991; Klaver, Kingma, & Weerkamp, 1993; Shah & Lankaputhra, 1997). Consequently, there has been a growing interest in developing techniques to enhance the survival of probiotic bacteria particularly during the GI-tract transit of the cells. Microencapsulation of the probiotic bacteria in polymeric systems is one of the techniques studied to improve the viability and activity of the cells under unfavorable conditions (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2012; Krasaekoopt, Bhandari, & Deeth, 2003; O'Riordan, Andrews, Buckle, & Conway, 2001). It is important that the encapsulation system developed is capable of keeping the cell release at the minimum until the capsules reach the intestines, where the rapid release of the cells is desirable for their colonization. For this reason, the kinetics of cell release under different conditions needs to be studied in order to understand the response of the system to environmental changes, particularly with considerable variations in ambient pH. The main goal of this study was to characterize the swelling and the release behaviors of the xanthan–chitosan hydrogel system under the simulated GI-tract conditions to be able to assess its potential as an enteric delivery system for the probiotic bacteria.

2. Materials and methods

2.1. Preparation of chitosan and xanthan solutions

Chitosan from crab shells with a minimum deacetylation degree of 85% and a molecular weight of 370 000 (reported by the supplier) was purchased from Sigma–Aldrich Chemicals (St. Louis, MO). A known amount of chitosan (Ch) was dissolved in 1 N HCl by heating and agitating. The desired solution pH (4.5 or 6.2) was adjusted by using 1 M NaOH, and deionized (DI) water was added to bring it to the final volume. Xanthan gum (X) with a molecular weight of 1.02 million (TICAXAN®) was kindly supplied by TIC Gums (Belcamp, MD). A predetermined amount of xanthan gum was dissolved in the DI water under heating and agitation. The concentrations of the polymer solutions used and the pH values of the chitosan solution were selected according to the results of the swelling study and the MDSC analysis of xanthan–chitosan capsules by Argin-Soysal et al. (2009). In order to form highly crosslinked capsule networks, four different combinations of chitosan and xanthan solutions were used for capsule formation. These combinations are: 0.7% (w/v) chitosan at pH = 6.2 with 0.7% (w/v) xanthan, 0.7% (w/v) chitosan at pH = 6.2 with 1.0% (w/v) xanthan, 0.7% (w/v) chitosan at pH = 4.5 with 0.7% (w/v) xanthan, and 0.7% (w/v) chitosan at pH = 4.5 with 1.0% (w/v) xanthan (Argin-Soysal et al., 2009). All solutions were autoclaved before use.

2.2. Microencapsulation of Pediococcus acidilactici

In this study, direct extrusion (complex coacervation) method was used for encapsulation. *P. acidilactici* (MA18/5M, National Collection of Microorganism Culture, Pasteur-France) was kindly provided by Imagilin Technology LLC (Potomac, MD). One milliliter of hydrated *P. acidilactici* cells was inoculated into 99 mL MRS broth and incubated at 35 °C for 24 h. Actively growing cells were recovered from the MRS broth by centrifuging at 10 000 rpm for 10 min, and then were washed twice with sterile phosphate buffered saline (PBS) solution under the same centrifugation conditions. The DI water was added to the cell pellet and vortexed.

P. acidilactici cells were mixed with xanthan gum solution (9:1 v/v) and encapsulation was achieved by dropwise addition of this mixture (10 mL) into the chitosan solution (60 mL) by using a manually operated syringe with 0.7-mm cannula (Becton–Dickinson, Franklin Lakes, NJ). The chitosan solution was agitated continuously for 40 min to allow crosslinking and to avoid coalescence of the capsules. The capsules were filtered through a 160-μm Millipore nylon filter, washed twice with the DI water, and then freeze-dried for 24 h. The size of the freeze dried capsules was around 1 mm.

2.3. Calibration curve of P. acidilactici

One gram of *P. acidilactici* (MA18/5M, National Collection of Microorganism Culture, Pasteur-France) powder was hydrated in 9 mL of DI water for 30 min by shaking at 260 rpm. One mL