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Fabrication of sub-micron protein-chitosan electrostatic complexes for encapsulation and pH-Modulated delivery of model hydrophilic active compounds

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ABSTRACT

Electrostatic sub-micron complexes of a protein (sodium caseinate (NaCAS) or bovine serum albumin (BSA)) and a polysaccharide (chitosan) were fabricated by associative phase separation and investigated for use in encapsulation and pH-triggered delivery applications. Various factors have been studied with respect to the extent of complexing and the size and morphology of the complexes produced, including protein type and the biopolymer mixing ratio. The effect of applying ultrasound has been considered with a view to comminuting precipitates produced under low shear to the colloidal scale to form coacervates. A simple model is suggested to explain how the biopolymer mixing ratio influences the ability for application of ultrasound to convert macroscopically phase-separated complex precipitates into coacervates. Different factors, both from a formulation and processing viewpoint, were studied with respect to encapsulation efficiency (EE) of model hydrophilic actives: fluorescein, rhodamine B, and riboflavin. Release of fluorescein and rhodamine B was measured as function of pH in order to investigate the pH-responsive molecular release capability of the fabricated structures. It is envisaged this work will add to the current tool-box of pH-responsive molecular delivery approaches, including those in the areas of foods, pharmaceuticals, and agrochemicals.

1. Introduction

Proteins and polysaccharides are polymers ubiquitous in nature. Research into how they interact, in addition to their behaviours at surfaces and interfaces, has long been undertaken (Dickinson, 2006; Rodríguez Patino & Pilosof, 2011). Due to the diverse range of chemical functionalities available, biopolymers can be assembled into supramolecular structures held together by interbiopolymer forces. Controlling the type and relative magnitude of these forces enables production of a wide range of material properties, and potentially, the prospect of physically compartmentalising compounds (i.e. functional ingredient encapsulation), a feature often considered desirable for functional food, pharmaceutical, and agrochemical formulation design (Chen, Remondetto, & Subirade, 2006; Hack et al. 2012; Madene, Jacquot, Scher, & Desobry, 2006).

In a binary biopolymer system containing a protein and polysaccharide, the net interaction between the biopolymers can be

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associative (complexing) or segregative (repulsive), depending on the precise structures of the biopolymers present and the prevailing conditions, i.e. pH, ionic strength, and mixing conditions (Dickinson, 1998; Syrbe, Bauer, & Klostermeyer, 1998; Tolstoguzov, 1991). Whilst it is recognised that such interactions impart many of the functional properties of foods, further research is required to develop uses in encapsulation and targeted delivery of active ingredients. "Active ingredients" in the broadest sense include crop protection products, pharmaceuticals, and nutrients.

One way to encapsulate functional molecules in protein-polysaccharide complexes involves 'bottom-up' self-assembly of the constituent biopolymers. If an active is included during or after complex assembly it can become entrapped—physically or chemically—within the biopolymer matrix. When the biopolymers contain ionising groups, such as those found on polypeptide side chains and polysaccharides, an electrostatic force predominates that holds the newly-formed complex together; complexes produced in this way have potential application as pH-responsive materials that can assemble and dissociate under pH control. From a green and sustainable design viewpoint, biopolymers often have good biocompatibility and toxicity profiles, in addition to being derived from under-utilised resources; transition to use of sustainable materials may reduce the dependency on petrochemical-derived synthetic materials often used in non-food applications (Doi, Clark, Macquarrie, & Milkowski, 2002).

In this study we focus on using chitosan and protein as biopolymer building blocks for complex formation. Chitosan, a polysaccharide derived from crustaceans (e.g. crab shells) has received attention for use in pharmaceutical/biomedical applications (Bugamelli, Raggi, Orienti, & Zecchi, 1998; Qin, Zhu, Chen, & Zhong, 2007), and increasingly, for food neutraceutical encapsulation (Chen et al. 2006), in addition to emulsion interfacial structure development (Ogawa, Decker, & McClements, 2003, 2004); more broadly, chitosan is regarded as a sustainable material with applications ranging from catalysis to water purification (Macquarrie & Hardy, 2005). The versatility and uniqueness of chitosan originates in its chemical structure: the preponderance of amine groups on the polysaccharide backbone makes it the only naturally-derived cationic biopolymer. Protonation of these amine groups enables chitosan to be solubilised and easily manipulated in mildly acidic conditions (e.g. dilute acetic acid). Chitosan's chemical structure is also responsible for its mucoadhesive property (Sogias, Williams, & Khutoryanskiy, 2008; Sogias, Williams, & Khutoryanskiy, 2012), which has been considered in various drug delivery contexts.

Although chitosan has not been marketed in any drug products, it has received attention for possible applications in drug delivery, muco-adhesive dosage forms, rapid release forms, improved peptide delivery, colonic drug delivery systems and for gene delivery (Baldrick, 2010). Human exposure to chitosan has occurred, though not in pharmaceutical application, through dietary supplements designed for treating obesity and hypercholesterolaemia. For foods, chitosan has been designated as: Generally Recognised as Safe (GRAS) in the USA; it is listed as a food additive in Japan, Finland, and Italy (Baldrick, 2010).

In comparison to polysaccharides it is well known that proteins differ both in structure and function. Caseins and related caseinates, which are naturally occurring milk proteins, are often used in food products as emulsifying agents for stabilisation of emulsions and foams; this contrasts with the majority of polysaccharides, including chitosan, which have minimal surface activity, and as such, are mainly used for rheology-control and/or water-holding (Dickinson, 2009). Sodium caseinate (NaCAS), which is typically produced from acid casein, is principally comprised of four water-soluble caseins (α_{s1}, α_{s2}, β, and κ) assembled together. Whilst a significant body of research has been generated to understand how NaCAS adsorbs at surfaces and interfaces, focus has recently shifted to its use as a biocompatible, non-toxic nano-carrier for encapsulation of vitamins and neutraceuticals (Semo, Kesselman, Danino, & Livney, 2007). Other research articles have demonstrated that chitosan forms complexes with a wide range of both globular and 'disordered' proteins (Anal, Tobiassen, Flanagan, & Singh, 2008; Huang, Sun, Xiao, & Yang, 2012; Lee & Hong, 2009; Yu, Hu, Pan, Yao, & Jiang, 2006). One of earliest studies investigated the precipitation of casein directly from milk for potential use in cheese making (Ausar et al. 2001). However, additional studies are required to develop complexes that enable pH-modulated delivery of functional molecules. This approach has a number of useful applications across foods, pharmaceuticals and agrochemicals.

To this end, the first part of this work investigates the complexation of chitosan and two proteins – sodium caseinate (NaCAS) or bovine serum albumin (BSA) – to evaluate how protein type influences the extent of complexing and the type of complexes produced (i.e. soluble versus insoluble complexes (precipitates or coacervates?)). BSA and NaCAS differ markedly in structure, with NaCAS being amorphous (Kontogiorgos, Ritzoulis, Biliaderis, & Kasapis, 2006) and BSA being globular crystalline (Ikeda & Nishinari, 2001). We consider how these differences in structure affect the extent of complexing and type of complexes produced under comparable processing and solution conditions.

Recently, ultrasound has been promoted as a possible route for modifying the functional properties of biopolymers (O'Sullivan, Arellano, Pichot, & Norton, 2014). The effect that ultrasound has on a molecule/polymer is related to the applied cavitation (rapid formation and collapse of bubbles) generated by localized changes in pressure and temperature. Most work in this area has been directed towards investigating ultrasound processing of single a biopolymer in solution or suspension. In particular, O'Sullivan et al. have noted that the effect of ultrasound on the functional properties of various food proteins leads to contradictory understanding, especially with regard to whether it can reduce protein molecular weight (O'Sullivan et al., 2014). It has been reported that ultrasound can reduce the molecular weight of chitosan, whilst not affecting the degree of acetylation (Wu, Zivanovic, Hayes, & Weiss, 2008). The effect of applying ultrasound to preformed mixed protein-polysaccharide complexes has received much less, if any, attention, although the effect of applying it to a kappa-carrageenan (solution) prior to complexing with a protein has previously been studied (Hosseini et al. 2013). In this work, we investigate the impact of applying ultrasound to pre-formed insoluble macroscopically phase separated protein-chitosan complexes initially formed under low shear.

Soft particles at the colloidal scale (e.g. coacervates) are potentially more useful than larger complexes/precipitates for encapsulation/controlled release application, as they are more stable to sedimentation, as well as possessing a greater surface area for molecular delivery of the encapsulated active. Furthermore, biopolymer complex colloidal suspensions (coacervates) often have similar rheological properties to oil-in-water emulsions which mean they could potentially be included within such products for controlled active or nutrient delivery.

The second part of the work goes on to demonstrate the potential for, and investigate some of the parameters that affect, the ability for various model active compounds to be encapsulated during mixed protein-chitosan complex formation. Typical formulation and processing parameters are considered in order to probe parameters that could potentially impact on encapsulation efficiency (EE). pH changes are then considered with respect to a triggering mechanism for release of the encapsulated active. It is envisaged that the information generated in this work will be

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