Using starch molecular complexes as carriers for therapeutics and nutrients

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Introduction

Starch, one of the most abundant food grade polymers, can form molecular inclusion complexes with low molecular weight ligands. Recently, we have demonstrated the potency of these inclusion complexes to serve as a molecularly controlled release system, based on their susceptibility to enzymatic digestion in the gastrointestinal tract (GIT). Thus, by controlling their structure, one can potentially tailor the release of biologically active materials and nutrients to specific loci in the GIT. Moreover, the physical properties of these semi-crystalline structures can also be manipulated t control the release by temperature and possibly by pH.

Oral delivery of naturacutiacls and pharmaceuticals can offer many advantages over parenteral delivery, in terms of convenience, cost and patient acceptability. Mammalian and bacteriall enzymes assisting delivery systems are considered to be more specific to the intestine and colon than, for example, pH or time dependent systems (McConnell et al., 2007). Amylose, a natural polymer from starch, is known for its ability to form unique inclusion complexes with different guest molecules such as iodine, fatty acids and aroma compounds (Heinemann et al., 2005; Biais et al., 2006). This polysaccharide can act as small intestine/colonic delivery system having the advantages of being readily available, cheep, non-toxic and biodegradable. Improved drug delivery systems are required for targeting the drug to a specific site in the intestine, to reduce systemic side effects, lower dose of drug, supply of the drug to the biophase only when it required and maintenance of the drug in its intact form as close as possible to the target site (Chourasia and Jain, 2003). The efforts today are focus on the use of a biodegradable polymer that will use as a general platform to carry a variety of molecules without the need to invest in efforts to design a carrier for each drug separately (Rubinstein, 2005).

Amylose is a linear homopolymer of starch that form α(1-4) linkage. When amylose molecules accommodated a guest molecule they form a single left handed helix structure, called V amylose. These complexes are based on a non covalent interaction between amylose and hydrophobic molecules which create inclusion compounds (Lalush et al., 2005; Shogren et al., 2006). The importance of such a system is the ability to deliver those important nutrients to specific sites in the digestive tract, to release it in the lower GI or in the colon, whether by human enzymes or bacteria, and to protect them from heat, oxidation and low pH in the food matrix. The binding strength, the dimensions of the amylose helix, and the location of the ligand vary depending on the properties of the guest molecules (Biais et al., 2006). The interactions between amylose and different kind of guest molecules have been studied using a variety of techniques such as amperometric iodine titration, XRD, NMR, FTIR, DSC, TEM and etc (Heinemann et al., 2005; Biais et al., 2006; Lalush et al., 2005; Cardoso et al., 2007; Helbert, and Chanzy, 1994).

In V amylose structures, depending on the complexing molecules, different types of helix with 6, 7 or 8 glucosyl units per turn, with varied pitches have been described (Biais et al., 2006). In the V_8 family, the helical cavity is larger, which may allow the inclusion of bulky molecules (Yamashit and Monobe, 1971; Uchino et al., 2002), where In the V_6 family there are three known types of

crystalline packing $V6_I V6_{II}$ and $V6_{III}$, where I, II and III define unit cells of different size in which the $V6_I$ structure has the smallest unit cell. However, in this family the pitch diameter are similar (0.8nm), but the difference is in the space that could be available between the helixes for trapping, depending on the size of the complexing molecule (Biais et al., 2006).

We produced starch complexes with a series of nutrients and other biologically active chemicals, and demonstrated their stability and controlled release under simulated GIT conditions. Furthermore, a continuous process for the formation of sub-micron and micron scale particles, based on molecular inclusion bodies, has been developed.

Material and methods

Preparation of amylose-guest complexes: The complexation is carried out by an acidification method (Lalush et al. 2005). The mixture was precipitated by adjusting the pH to 4.7 using H_3PO_4 2% (w/w), held for 24h under gentle stirring. All samples are then centrifuged (14,000 rpm, 15 min), washed twice with ethanol/water mixture (50/50 w/w), and freeze dried. ***Characterization of the complexes:*** The formation of a V-type complex was verified by XRD (X-ray diffraction). DSC (Differential Scanning Calorimeter) was used for thermal analysis. AFM (Atomic Force Microscopy) was used to image the nanoparticles. Dynamic Light Scattering (DLS), and cultter counter were used to determine the particles size. ***The guest molecule content in the complex*** was calculated on the basis of the free guest molecule released by full hydrolysis with pancreatic amylase as done by Lalush et al. (2005). The released bioactive was quantified by RP-HPLC or Gas-Chromatography. The degree of enzymatic digestion is measured by determination of the reducing hemiacetal groups. ***Continuous process for the complex formation in pilot scale:*** A process has being preformed using dual feed jet homogenaizer. This process is being preformed by initial dissolution of both the starch/amylose and the guest molecule separately in alkali solution, followed by an online homogenization at 18-23 Kpsi and mixing with phosphoric acid (H_3PO_4) to reach pH = 4.7($\pm$ 0.5).

Results and Discussion

Complex formation: XRD diffraction pattern characteristic of amylose-genistein and amylose-genistin (phytoestogens) complexes showed a characteristic diffraction pattern with main peaks at 2θ = 8.3, 10.2, 13.5, 15.4, 17.2, 20. On wnother study, complexation carried out with menthone amylose and natural starches show that amylose tends to give V7 diffraction (peaks at 18, 13 and 7), HACS gives a V6 diffraction (peaks at 20, 13 and 8), and in corn starch both diffractions can be noticed. A third study, provided AFM images show that particle produced by amylose and hydrophobic ligands (linoleic acid in the example) are indeed at the nano-scale, with typical 400nm diameter,

Release of bioactives: Complexes were subjected to stability tests under enzymatic, temperature, and pH challenges. These experiments determined the extent of protection provided by the nano-capsules and the kinetics of their degradation. When complexes were incubated in pH range of pH=3 to pH=8, regardless of the bioactive, the spontaneous release ranged from insignificant to moderate release. To test the enzymatic digestion – simulating release in the GI, pancreatic digestion was used in-vitro. As shown in Figure 3A, pancreatic digestion release was as efficient as extraction, while spontaneous release was much lower. For temperature dependent release, temperature was adjusted to 30°C, 50°C and 70°C. The effect of temperature on the release of genistein (Figure 3B) show that the spontaneous release of genistein rises significantly with temperature, starting from almost zero at 30°C to ~65% at 70°C. It should be noted that for

unsaturated fatty acids and other low molecular weight ligand, the release at elevated temperatures was negligible.

Developing continuous process for the complex formation: A dual-feed homogenizer was used for in situ complexation in accord with homogenization, to form micron and sub micron particles. Results show that pre-dissolving the starches in a hot alkali solution leads to the formation of 0.04-20µm or 0.04-3µm V-type particles from High Amylose Corn Starch or corn starch, respectively. When tested with stearic acid as a model ligand, stearic-acid-loaded particles exhibit V-type X-ray diffraction and release the stearic acid mainly upon pancreatic amylases treatment. The technology could prospectively be used in numerous applications including as a delivery system for the controlled delivery of bioactives.

Conclusions

This cumulative data provides evidence on the potential use of amylose V-type inclusion complexes as a delivery system. Evidently, the properties of the complex depend on the nature of the binding chemical. Beyond the obvious commercial advantages of the continuous process described here, it has implications on the supplementation and controlled delivery of bioactive nutraceuticals and therapeutic agents based on V-type starch micro/nano-capsules. These molecular inclusion complexes could prospectively be used in the food, pharmaceutical and biotechnology industries as a delivery system for the controlled and targeted delivery of nutraceuticals and/or drugs to the lower gastrointestinal tract.

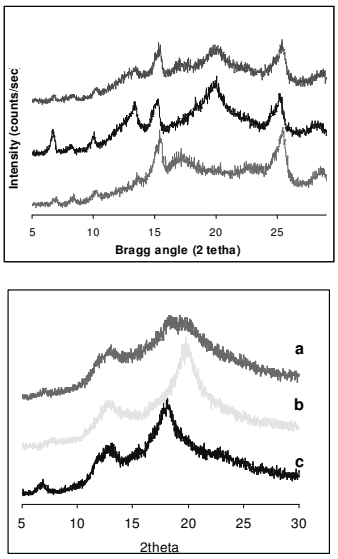

Figure 1: X-ray diffraction patterns of Top: (a) amylose-genistin complex; (b) amylose-genistein complex; (c) amylose no guest (control); Bottom: inclusion complex of menthone with (a) corn starch; (b) HACS and (c) amylose.

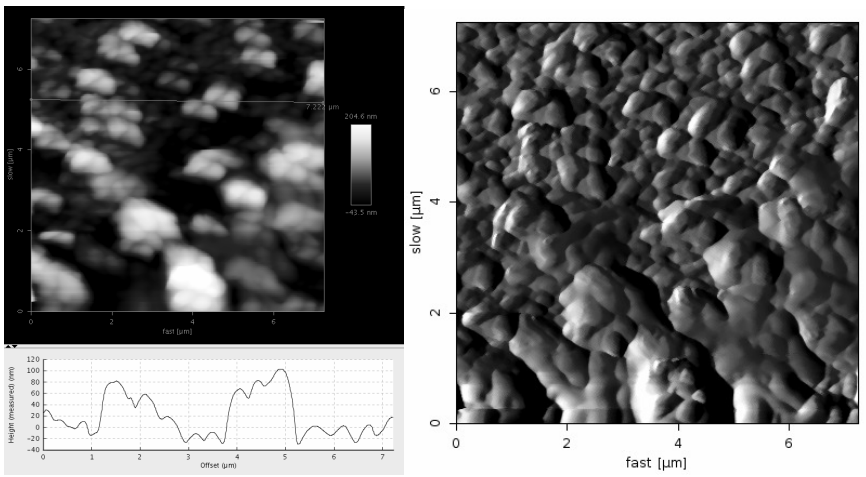

Figure 2: AFM images of amylose-linoleic acid particles. Left: height signal with cross section analysis. Right: error signal of the nanoparticles layer.

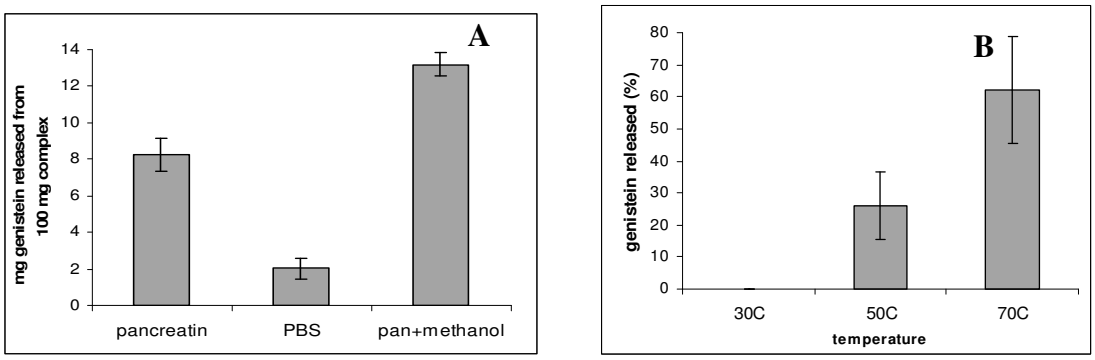

Figure 3: Amount of genistein released (mg genistein in 100 mg complex). [A] Genistein released from defatted HACS complexes by enzymatic digestion, by PBS, and the total amount of genistein in the complex measured by combining enzymatic digestion and 80% methanol extraction. [B] Genistein release in PB at different temperatures (30, 50, 70°C). The % of genistein being released was normalized to the amount released by enzymatic digestion (100%) at the same temperature.

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