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Characterisation of biodegradable pectin aerogels and their potential use as drug carriers

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ABSTRACT

The purpose of this work was to prepare stable citrus (CF) and apple (AF) pectin aerogels for potential pharmaceutical applications. Different shapes of low ester pectin aerogels were prepared by two fundamental methods of ionic cross-linking. Pectins' spherical and multi-membrane gels were first formed by the diffusion method using 0.2 M $CaCl_2$ solution as an ionic cross-linker. The highest specific surface area (593 m^2/g) that had so far been reported for pectin aerogels was achieved using this method. Monolithic pectin gels were formed by the internal setting method. Pectin gels were further converted into aerogels by supercritical drying using CO_2. As surface area/volume is one of the key parameters in controlling drug release, multi-membrane pectin aerogels were further used as drug delivery carriers. Theophylline and nicotinic acid were used as model drugs for the dissolution study. CF aerogels showed more controlled release behaviour than AF pectin aerogels. Moreover a higher release rate (100%) was observed with CF aerogels.

1. Introduction

The future of pharmacy is orienting towards a special category of therapeutics and diagnostics referred to as protein-based drugs. Currently most protein and polypeptide drugs are delivered into the body via a route other than the mouth, especially via infusion, injection or implantation, due to their high susceptibility to digestive enzymes in the gastrointestinal tract, and poor absorption. Formulations for other routes of administrations have to be developed in order to avoid constant injections. Namely, oral drug delivery is the more popular and convenient method for drug delivery. Therefore, new drug carriers for oral drug delivery could be synthesised for delivering drugs to the targeted locality (lower gastrointestinal tract). There is a wide-range of natural-origin polymeric biomaterials that can be used in oral drug delivery, such as proteins and polysaccharides. These natural biomaterials have been proved to be safe, stable, non-toxic and renewable, and have low costs because of their abundance in nature and ease of processing. Pectin as one of the naturally occurring polysaccharides that has become more and more important over recent years, as it is resistant to protease and amylase both of which are active within the upper GI tract, and is digested by micro-flora in the lower GI tract. Therefore, it could work as a drug vehicle from the mouth to the colon (Liu, Fishman, Kost, & Hicks, 2003).

Pectin by itself has applications throughout the pharmaceutical industry as it reduces cholesterol levels in blood (Sriamornsak, 2001). Pectin also acts against poisoning from toxic cations as it is namely capable of removing lead and mercury from the gastrointestinal tract and respiratory organs (Sriamornsak, 2001). When pectin is injected intravenously, it decreases the coagulation time of the blood and thus controls local bleeding. However, it also has the ability to naturally gel, thicken, and stabilise that has made pectin a very interesting carrier within the pharmaceutical and biotechnology industries.

The abilities of different pectins to form gel depend on their sources due to variations in parameters such as molecular sizes and degrees of esterification (DE) (Sriamornsak, 2003). Low ester pectins (LM) with DE of less than 50% require the presence of divalent cations for gelation by the well-known egg-box model. The obtained gel strength depends on the pectin's concentration, pH range, and the concentration of calcium ions. The resulting gels have brittle structures. LM amidated pectins, where ammonia instead of acids is used for de-esterification, require minor amounts of calcium ions for gelling. The degree of amidation is termed as DA.

Various pectin hydrogels and xerogels have already been prepared for drug delivery (Aydin & Akbuğa, 1996; Donato, Garnier, Novales, Durand, & Doublier, 2005; Itoh et al., 2007; Kubo, Konno, Miyazaki, & Attwood, 2004; Liu et al., 2003; Sriamornsak, 1998),

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but their instability under drying air conditions has led scientists to develop special coating materials for enhancing their lifetimes by several months or more instead of the current level of several hours. However, supercritical fluid technology using high-pressure and supercritical fluids is one of the more recent technologies that has been performed with the aim of preparing different biodegradable polysaccharide aerogels (Alnaief, Alzaitoun, García-González, & Smirnova, 2011; Boissière, Tourrette, Devoisselle, Di Renzo, & Quignard, 2006; Chang, Chen, & Jiao, 2008; García-González Carlos, Carenza, Zeng, Smirnova, & Roig, 2012; Mehling, Smirnova, Guenther, & Neubert, 2009; White, Budarin, & Clark, 2010). This technology is more advantageous in comparison with conventional processes. Firstly, it guarantees a better removal of organic solvents from products for medical or pharmaceutical applications without exposing drugs to high temperatures that may degrade them, especially when using protein drugs or enzymes. Dry and stable polysaccharide aerogels possess very high porosities and specific surface areas. As surface area/volume is one of the key variables in controlling drug release (Reynolds, Mitchell, & Balwinski, 2002), porous materials have attracted greater attention throughout the pharmaceutical industry. With the increasing surface areas of such nanoporous carriers, the drug could be absorbed and released in a more reproducible and predictable manner (Ahern, Crean, & Ryan, 2012; Andersson, Rosenholm, Areva, & Lindén, 2004; Charnay et al., 2004; Gren, Bjerre, Camber, & Ragnarsson, 1996; Li, Wen, Shao, & Chen, 2004; Streubel, Siepmann, & Bodmeier, 2002). Also the loading of a drug increases with increasing surface area.

Pectin aerogels by thermal and acidic gelation are already available in powder form (485 m^2/g) and as monoliths (200 m^2/g) (García-González, Alnaief, & Smirnova, 2011; White et al., 2010). In the presented research apple (AF) and citrus (CF) pectin aerogels as spheres and monoliths by cationic gelation were synthesised as a potential application for porous carriers in oral drug delivery. Amidations of galacturonic acids improves the gelation of LM-pectins, namely less calcium ions are needed and also a reduced possibility of precipitation is achieved at a high calcium concentration. Therefore, LM amidated pectins were used for the sol–gel synthesis.

2. Experimental

2.1. Materials

The pectin samples used were low-methoxyl citrus (DE = 23–28%, DA = 22–25%) and apple (DE = 27–32%, DA = 18–23%) pectins kindly provided by the Herbsteith & Fox KG company from Germany. The pectin solutions were prepared using distilled water. 0.2 M calcium chloride ($CaCl_2$) was used for ionic cross-linking by the diffusion method. Sodium hexa-metaphosphate $Na(PO_3)_6$ and dicalcium phosphate ($CaHPO_4$) were used when the internal setting method was applied for cross-linking. Glucono-δ-lactone was used for pH adjustment. Ethanol (100%) was used for solvent exchange prior to supercritical drying with CO_2. KH_2PO_4 aqueous solution was used for the phosphate buffer, 0.2 M NaOH was used for pH adjustment. HCl and NaCl were used for the preparation of simulated gastric fluid (SGF). The active substances, nicotinic acid and theophylline, were obtained from Sigma–Aldrich and Acros Organics, respectively.

2.2. Methodology

2.2.1. Preparation of pectin gel

Two methods of cationic cross-linking were used in the presented research, resulting in different gel shapes (Fig. 1). Ionically cross-linked hydrogel multi-membrane spheres were obtained by using the diffusion method. Solutions of 2% w/w pectin solutions were prepared by dispersing apple (AF) or citrus (CF) pectin into distilled water. Spheres were then produced by ionotropic gelation using a syringe pump and a needle of 0.80 mm inner diameter. The pectin solutions were dropped into a 0.2 M $CaCl_2$ solution under constant agitation. Gelled spheres were cured within the solution for 1 h, then separated by filtration and washed with distilled water. Further spheres were dropped one by one into 1% apple or citrus pectin solution filtered through a sieve and dropped into the cross-linking solution for 5 mins. Three-membranes' hydrogels were obtained by repeating the above process three times. After obtaining hydrogel, the alcogels were formed by solvent exchange. The hydrogel spheres were dehydrated by immersion within a series of successive ethanol–water baths of increasing alcohol concentrations (10, 30, 50, 70, 90 and 100%). If 100% ethanol were to be added immediately, the structure of the gel may be affected. Ethanol was later removed by supercritical drying with CO_2 (100 bar, 40 °C) which has been described elsewhere (Novak & Knez, 1997). Particle size distribution was determined by sieve analysis.

When the second ionic cross-linking by the internal setting method was applied, firstly 1% w/w or 2% w/w pectin was added to 15% EtOH solution. The prepared solutions were later mixed for 2 h at ambient temperature. Then $Na(PO_3)_6$ (1.5%w/w) was added, the mixture was stirred for 30 min and only heated up to 40 °C in the case of the used citrus pectin. Further $CaHPO_4$ was added followed by mixing for 60 min (Eq. (1)) following the addition of glucono-δ-lactone solution to reduce pH. The resulting mixture was quickly poured into cylinder moulds and left covered overnight to solidify in a refrigerator. The yielded monoliths were left in one piece or cut into smaller pieces and dehydrated by immersions in a series of successive ethanol–water baths of increasing alcohol concentrations (15, 30, 60, 90 and 100%) every hour until the equilibrium of gel shrinkage had been reached. The procedure for obtaining aerogels after gel synthesis was then the same as for spherical multi-membrane gels. By using the internal setting method the gel casting process was controlled by a chelator ($Na(PO_3)_6$) and an initiator ($C_6H_{10}O_6$). The chelator and Ca ions react with each other and form a three-dimensional network (Eq. (2)). The addition of glucono-δ-lactone slowly decomposes the complex, calcium ions are released (Eq. (3)), and the gelation process occurs (Akhondi, Taheri-Nassaj, Sarpoolaky, & Taavoni-Gilan, 2009).

$$\text{Pectin} + Ca^{+} \rightarrow \text{Ca-pectin} \quad (1)$$

$$6PO_3^{-} + 3Ca^{2+} \leftrightarrow Ca_3(PO_3)_6 \quad (2)$$

$$Ca_3(PO_3)_6 + 3C_6H_{10}O_6 \leftrightarrow 6HPO_3 + 3Ca^{2+} + 3C_6H_8O_6^{2-} \quad (3)$$

2.2.2. Preparation of drug loaded spherical aerogels

In order to test the release properties of pectin spheres, theophylline and nicotinic acid pectin hydrogels were prepared according to the same protocol as for pectin multi-membrane spheres. Model drugs, nicotinic acid and theophylline, were added to pectin solutions at the beginning of the preparation during the sol–gel process, respectively. Spheres were cross-linked with Ca^{2+} ions. The inner surfaces between the core and membrane or between two membranes were filled with the model drug. Both model drugs were chosen due to their solubility in H_2O, ethanol and supercritical CO_2. The solubility of nicotinic acid in water is 1.67 g/100 mL at 25 °C, in ethanol 0.73 g/100 mL at 25 °C and in supercritical CO_2 1.4×10^{-6} (mole fraction) at 100 bar and 40 °C. The solubility of theophylline in water is 0.76 g/100 mL at 25 °C, in ethanol 0.35 g/100 mL at 25 °C and in supercritical CO_2 0.5×10^{-6} (mole fraction) at 100 bar and 40 °C. The further process for obtaining aerogels was the same as described above, including immersion of hydrogels within a series of excessive ethanol baths and later supercritical drying.