International Journal of Biological Macromolecules 101 (2017) 852–861

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Pectin–chitosan physical hydrogels as potential drug delivery vehicles

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ARTICLE INFO

Article history:
Received 7 July 2016
Received in revised form 27 March 2017
Accepted 28 March 2017
Available online 30 March 2017

Keywords:
Chitosan
Pectin
Hydrogels
Drug delivery

ABSTRACT

Pectin–chitosan hydrogels are intriguing and relatively new type of physically crosslinked hydrogels. Here we present for the first time a study exploring the suitability of pectin–chitosan hydrogels to serve as drug carriers and the mechanism controlling the release patterns. Using drug release assays, we demonstrated sustained release of three model drugs (mesalamine, curcumin and progesterone) over a period of 24 h in physiological conditions. Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) experiments were used to characterize the interactions between the investigated drugs and the polymers. These experiments, as well as swelling analysis, support the claim that the magnitude of interactions strongly affect the release rates. These new pectin–chitosan thermoreversible hydrogels may improve the life style of many patients by reducing the daily uptake of chronic medicines.

1. Introduction

Hydrogel-based drug delivery systems are attracting considerable attention as the next generation of designed vehicles capable of releasing their content at the desired rate and location in the body. Hydrogels are three-dimensional networks of polymer chains that are crosslinked *via* either physical or chemical bonds. Physically crosslinked hydrogels are particular appealing for the construction of drug delivery vehicles since their preparation does not involve any toxic compounds.

An intriguing example of physically crosslinked hydrogel was recently described in the literature. Researchers have shown that mixed solutions of pectin and chitosan at pH values below 2 form thermoreversible gels upon lowering of the temperature [1]. Chitosan is a polysaccharide composed of glucosamine and N-acetylglucosamine linked through a β (1–4) bond. Chitosan is obtained by partial deacetylation of chitin [2], the second most common natural polysaccharide on earth and the main component of the exoskeleton of crabs and shrimps [3]. In its soluble form, below its pK_a ~6.3–7.1, chitosan carries a positive charge due to its amine groups (–NH^{+3}). Chitosan has many useful characteristics including nontoxicity, biocompatibility [4], and biodegradability [5] and also has antimicrobial [6] and antifungal properties [4,7] that offer the possibility of clinical use [8]. Chitosan is also known as a permeability enhancer [9] due to its ability to open the tight junction between epithelial cells. Accordingly, chitosan is considered to be a good candidate as a colon-specific drug delivery vehicle. Pectins are a family of anionic polysaccharides extracted from plant cell walls, which are commonly used for drug delivery systems to the colon [10–12]. The pectin backbone consists of primarily (1–4)-linked α-D-galacturonyl units occasionally interrupted by (1–2)-linked α-L-rhamnopyranosyl residues, which can carry oligosaccharides. The pK_a of pectin is around 2.9–3.2, close to the pK_a of the monomeric galacturonic acid. This polymer is biocompatible and mucoadhesive. Moreover, it is almost totally degraded by colonic bacteria and is not digested by gastric or intestinal enzymes [13,14].

The properties of pectin–chitosan hydrogels were evaluated in several studied. Rheological investigations have shown that the gelation is thermoreversible, and that the gelling temperature is dependent on the weight ratio of the polysaccharides [1]. A later study revealed that in acidic pH, only a mixture with 75% pectin gelled upon cooling [15]. The reversible gelation mechanism was shown to rely on hydrogen bond formation upon cooling and hydrogen bond breakage upon heating [16]. As oppose to the rather good understanding of the physical properties of pectin–chitosan hydrogels, only one study suggested their utilization; sponges were proved compatible with human marrow-derived stem cells [17]. It has been postulated that pectin–chitosan hydrogels may be valuable for gastric drug delivery as the gel layer could provide a protective barrier sustaining drug release in the stomach [15]. Yet, this claim was not examined experimentally.

Pectin–chitosan hydrogels may possess many potential advantages arising from combination of pectin's and chitosan's benefits and lack of toxic crosslinking compounds. Utilization of these hydrogels for drug delivery applications, however, requires them

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http://dx.doi.org/10.1016/j.ijbiomac.2017.03.167
0141-8130/

to be stable for long periods in physiological conditions and display appropriate release patterns. Thus the aim of this study was to investigate their suitability as drug carriers. Three drugs were selected as models for this study: mesalamine, curcumin and progesterone. Mesalamine, also known as 5-Aminosalicylic acid or 5-ASA, is a drug used extensively for long-term maintenance therapy in patients with mild to moderate inflammatory bowel diseases (IBDs), including Crohn's disease and ulcerative colitis [18]. 5-ASA may additionally provide protection against the development of colorectal cancer. Mesalamine is a relatively small and hydrophilic molecule with a molecular mass of 153.135 g/mol containing a carboxylic group with a pK_a value of 2.4 ± 0.09 and an amino group with a pK_b value of 5.9 ± 0.04 [19]. Curcumin is a well investigated natural pharmacological material with a molecular mass of 368.39 g/mol. Its beneficial properties include anti-inflammation, anti-human immunodeficiency virus, anti-microbial, anti-oxidant, anti-parasitic and anti-mutagenic activities [20]. It is considered safe for human use, even in high doses. Curcumin was selected as a model lipophilic compound ($\log P = 3.28$ [21], whose solubility in water is 0.0027 mg/ml [22]) and having a low oral bioavailability. Progesterone is a steroid hormone vastly released through pregnancy. Vaginally dosed progesterone is being investigated as potentially beneficial in preventing preterm birth in women at risk [23]. Its lipophilicity is similar to that of curcumin ($\log P = 4.0$ [24]) but its solubility in water is higher (0.007 mg/ml [25]). Its molecular mass is 314.46 g/mol.

In this manuscript, we study for the first time several parameters that might affect drug release kinetics from thermoreversible pectin–chitosan hydrogels. As a demonstration of the hydrogels' ability to sustain the release of several drugs, we introduced into them mesalamine, curcumin and progesterone and investigated the release behaviour from hydrogels with different pectin/chitosan fractions. We then examined the swelling ability of these hydrogels and finally, thermal analysis *via* differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) studies were used to investigate the drug–polymer interactions. To the best of our knowledge, this is the first time pectin–chitosan thermoreversible hydrogels are described as a drug delivery system.

2. Experimental methods

2.1. Materials

5-Aminosalicylic acid (95% purity) and progesterone (P3972-5G) were purchased from Sigma–Aldrich. Sodium hydroxide pearls and hydrochloric acid (32% A.R.) were purchased from Bio-Lab Ltd., Israel. $Na_2HPO_4{\cdot}12H_2O$ and $NaH_2PO_4{\cdot}H_2O$ were purchased from Merck KGaA. NaCl was purchased from Frutarom Ltd., Israel. All chemicals were of analytical grade and were used as delivered, without further purification. Double-distilled water (DDW) was used in all aqueous solutions. Curcumin ($C_{21}H_{20}O_6$, 95% purity) was purchased from Alfa Aesar, Great Britain.

Low molecular weight chitosan was purchased from Sigma–Aldrich. The degree of deacetylation of the batch used in this study was determined in our previous work using FTIR and found to be 77%. The average molecular weight as determined by static light scattering was 207,000 g/mol [26]. Low-methoxyl pectin, classic citrus pectin (CU 701) was kindly gifted from Herbstreith & Fox KG, Germany. According to the manufacturer the degree of esterification was 34% and the sugar composition of the studied pectin was: galacturonic acid 86%, rhamnose 1.8%, galactose 5.3% and arabinose 3.7%.

2.2. Preparation of physically cross-linked hydrogels

Pectin–chitosan hydrogels were prepared as described elsewhere [1,15]. Briefly, 0.5% w/v, 1% w/v or 2% w/v pectin solutions were prepared by dissolving the appropriate amount of pectin and 0.5% w/v 5-ASA or 0.5% w/v curcumin or 0.05% w/v progesterone in a hot 0.1 M HCl solution (~60 °C) and stirring for 24 h, using a magnetic stirrer. Chitosan solutions were prepared under the same conditions. Mixed solutions were prepared by mixing appropriate volumes of aqueous solutions of the respective polymers at 60 °C for 1 h. The pectin weight fraction with respect to the total polymer content, r, were defined as:

$$r = m_{\text{pectin}}/(m_{\text{pectin}} + m_{\text{chitosan}}) \quad (1)$$

where m is the mass of the component. All mixtures were prepared at a constant total polymer concentration of 0.5% w/v, 1% w/v or 2% w/v. Solutions were freshly prepared, light protected and nitrogen sealed to protect 5-ASA, curcumin and progesterone. Immediately after preparation of the mixed solution, 500 μl of hot final mixture were transferred into a polylactic acid circular mould (diameter 14 mm, height 2 mm) to form hydrogels upon cooling to room temperature.

All samples displayed a transition from a solution at 60 °C to a gel-like material at room temperature. The gels without drugs were clear, indicating that the structures formed had sizes smaller than the wavelength of light [27]. The pH of mixtures without 5-ASA were approximately 1.5 and gelation occurred spontaneously after cooling the mixture. The pH of mixtures with 5-ASA, however, were approximately 3, which led to a very fast cross-linking between chitosan and pectin; thus, the pH of mixtures with 5-ASA was adjusted to 1.5 with 1 M of HCl prior to casting. 500 μl of the final mixture were transferred into a polylactic acid circular mould (diameter 14 mm, height 2 mm) to form hydrogels at room temperature. This preparation process is illustrated in Fig. 1.

2.3. Study of drug release from the hydrogels

2.3.1. Preparation of phosphate buffer saline (PBS)

130 mM of phosphate buffer solution simulating intestinal fluids was prepared by dissolving 15.585 g $Na_2HPO_4{\cdot}12H_2O$ (di-sodium hydrogen phosphate dodeca-hydrate) and 1.166 g $NaH_2PO_4{\cdot}H_2O$ (sodium di-hydrogen phosphate) in 100 ml DDW. 20 ml of this solution were mixed with 8.9 g NaCl (sodium chloride), the volume was raised to 1 L using DDW, and the pH was adjusted to 7.4 using 5 M NaOH. A 10 mM phosphate buffer solution was prepared by dissolving a kit purchased from Sigma–Aldrich in 1 L of DDW, and the pH was adjusted to 7.4 using 5 M NaOH.

2.3.2. Preparation of the HCl solution

To simulate the stomach environment, a 32% HCl stock solution was diluted with DDW to a final concentration of 0.1 M at pH 1.2.

2.3.3. Calibration curve of the drugs in the PBS solution

Known concentrations of 5-ASA in 130 mM and 10 mM at pH 7.4 PBS solution were scanned in the range 200–400 nm by a UV–Visible spectrophotometer (aSynergyTM HTBioTek®) [28]. The measurements were carried out on a 96-well UV-plate with a multi-mode microplate reader (Winooski, VT, USA). A sharp peak was noticed at 350 nm. Unknown concentrations of 5-ASA in drug release experiments were obtained in the PBS solution by measuring the absorbance value at 350 nm. For curcumin, measurements were taken at 424 nm. For progesterone measurements were taken at 270 nm.