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Controlled Release of Paracetamol from Alginate/Xanthangum Films for Oral Drug Delivery

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Abstract

The *in vitro* drug release profiles of pH-sensitive composed of sodium alginate (A) and xanthan gum (X) were investigated. The weight ratios of A/X were varied at 95:5, 90:10, 80:20. The prepared films were soaked into calcium chloride 0.5 M to obtain the crosslinked hydrogel films. The prepared films were characterized by scanning electron microscopy (SEM). Analysis of morphology showed the compatibility and well-dispersed of the Ca2+ ion. The influence of the content of A/X in the films on the properties including swelling and drug release behavior was evaluated in simulated gastric fluid, SGF, (pH 1.2) and simulated intestinal fluid, SIF, (pH 7.4) at 37 ºC. The A/X films showed lower swelling at pH 1.2 and higher swelling at pH 7.4. Paracetamol release profile of the A/X films showed relatively low (~11%) in SGF and essentially increase up to 100% in SIF. The A/X films can potentially be used in oral drug delivery application.

Keywords: : pH sensitive; Sodium alginate; Xanthan Gum; Oral drug delivery

Introduction

Drug delivery systems are devices that enable to distribute a therapeutic substance in the body[1]. The development of drug delivery systems have been designed to release the drug and maintain its concentration levels in the blood plasma for long periods without reaching a toxic level or dropping below the minimum effective level[2]. In addition, drug delivery can protect the drug from the hydrolysis or other degradation changes in the gastrointestinal tract[3]. In recent year, hydrogels have been used extensively in the development of the smart drug delivery system because it can protect the drug from unfriendly environments, e.g. the presence of enzymes and low pH in the stomach. Moreover, hydrogels can control drug release by changing the gel structure in response to environmental stimuli[4]. Hydrogels from biopolymers such as alginate, chitosan, and carrageenan are widely used in oral drug delivery due to their biocompatibility and biodegradability[5].

Alginate (A) is a natural biopolymer, which is derived from brown algae and composed of β–L-guluronic acid (G) and β-d-mannuronic acid (M) monomers forming regions of M-blocks (MM), G-blocks (GG) or alternating structures (MG)[6]. Alginate has diverse properties such as non-toxicity, biodegradability, biocompatibility, and pH sensitive, making its encouraging biopolymer for various applications in drug delivery system [7-8]. Moreover, alginate is easily gelled in the presence of divalent cations, such as Ca2+[9]. The calcium-crosslinked alginate films could protect acid-sensitive drugs from gastric juice and the drug was consequently released from the films in the intestine [10]. However, alginate is soluble at high pH leading to rapid control drug release (burst effect), which restricted its drug delivery applications [11-12].

Xanthan gum (X), a natural polysaccharide, has good water-solubility, excellent biocompatibility, and high molecular weight having branched polymeric chains derived from the bacterium Xanthomonas campestris[13]. It consists of 1,4-linked β-d-glucose residues, and its anionic nature is attributable to the pyruvic acid and glucuronic acid groups in the side chains[14]. Xanthan gum is used as a gelling agent in aqueous systems for drug delivery [15].

In this work, we intended to prepare the composite films consisting of A and X containing paracetamol as a model drug indifference ratios and using calcium ion as a crosslinking agent. The obtained A/X composite films

were investigated to evaluate their pH-responsive. The effects of crosslinkers on in vitro drug release were also studied

Materials and methods

Materials

Alginate (average molecular weight of 965 kDa) was purchased from Acros Organics) and Xanthan gum was purchased from UNION CHEMICAL 1986 CO, LTD. (Thailand). The simulated gastric fluid (SGF, pH 1.2) composed of 7 mL concentrated HCl, 2 g NaCl and 1000 mL distilled water was prepared. The simulated intestinal fluid (SIF, pH 7.4) was purchased from Merck Millipore that prepared by dissolving phosphate buffered saline tablets in 1 L distilled water. All other reagents used in this study were of analytical grade.

Methods

2.1 Preparation of A/X hydrogels films and drug loading

Alginate (1% w/w, A) and xanthan gum (1% w/w, X) were prepared and mixed with the weight ratios of (A:X) 95:5, 90:10, 80:20 under continuous stirring at 60◦C for 3 h. The mixture solutions were poured into the Petri dish and then dried at 60 ◦C for 48 h to obtain the A/X films. The crosslinked A/X films were prepared using two different methods. For the method I, the A/X films were soaked into 50 mL of 0.5 M CaCl2 for 30 min and the resulting films are denoted as A95X5Ca5(I), A90X10Ca5(I), A80X20Ca5(I). For method II, 20 mL of CaCl2 (0.2 M) was mixed directly into film-forming solutions and followed by the same as the method I which denoted as A95X5Ca5(II), A90X10Ca5(II), A80X20Ca5(II).

2.2 Characterization

The distribution pattern of metal ions within the hydrogel films was studied by scanning electron microscope-energy dispersive spectrometer (SEM–EDS, LEO, LEO1455VP, USA).

2.3 *In Vitro* Cytotoxicity Study

The cytotoxicity of hydrogel films was assessed by African green monkey kidney fibroblast (Vero) using MTT assay. The hydrogel films were soaked in phosphate buffered saline (PBS) at 37◦C for 24 h. After that, each liquor stock was obtained by filtering and then exposed in DMEM (Dulbecco's Modified Eagle Medium). Vero culture was cultured in a medium containing DMEM supplemented with 10% FBS (Fetal Bovine Serum) and seeded in a 96-well plate at 100mL/well with subsequently incubated at 37◦C for 24 h. The 100 mL of diluted liquor stock in DMEM was added to each plate and incubated at 37 ◦C for 24 h. Then, 10 mL of MTT solution (5 mg/mL) was added in each well and further incubated at 37 ◦C. After 4 h of incubation, the 9:1 of DMSO: 10% SDS was added in each well plate at 150 mL/well to dissolve the formazan crystals. The absorbance was measured at 570 nm using a microtiter plate reader. The % cytotoxicity was calculated as follows:

$$\%cytotoxicity = \frac{\mathrm{A} - \mathrm{B}}{A}\, x\, 100\%$$

Where A is the absorbance of the control and B is absorbance of the sample

2.4 Swelling behavior

The swelling of A/X hydrogel films were determined at 37 ± 0.5 ºC by immersing dried samples into 50 mL of distilled water, pH 1.2 (simulated gastric fluid, SGF) and pH 7.4 (simulated intestinal fluid, SIF) for 8 h. After an interval time, the swollen sample was removed and the weight was measured. The ratio of swelling was calculated as follows:

$$\mathrm{W} = \frac{Ws - Wd}{\mathrm{Wd}}$$

Where Ws is the weight of the swollen samples at a given time during swelling, Wd is the weight of the dried samples, respectively

2.5 *In vitro* drug release studies

Paracetamol (0.15 g) as a model drug was filled into the 2×2 cm2 sealed bag A/X films. The sealed bags were then immersed in 100 mL of the SGF for 2 h followed by SIF solution 6 h. The experiment condition was conducted under the shaker at 37 ºC. At the selected time interval, 0.2 mL of solution was taken out. Meantime, 0.2 mL of fresh SGF or SIF, as appropriate was added to keep the total volume constant. This solution was measured by using UV-Vis spectrophotometer at 242 nm. Then, the content of released drug was obtained by comparison with a standard curve.

3. Results and discussion

3.1 Characterization

Figure 1 shows the distribution pattern of Ca^{2+} within the films examined by SEM-EDS. It was obviously seen that the distribution patterns of Ca^{2+} ions were uniform. Additionally, the amount of Ca^{2+} ions in A95X5Ca5(II) film is higher than that of A95X5Ca5(I). It is because, in the preparation of A95X5Ca5Ca(II) film, there are two steps of adding Ca^{2+} ions comparing with one step in the preparation of A95X5Ca5(I) film. The relatively high amount of Ca^{2+} ions in A95X5Ca5Ca(II) film implied the higher density of crosslinking points than in A95X5Ca5(I) film.

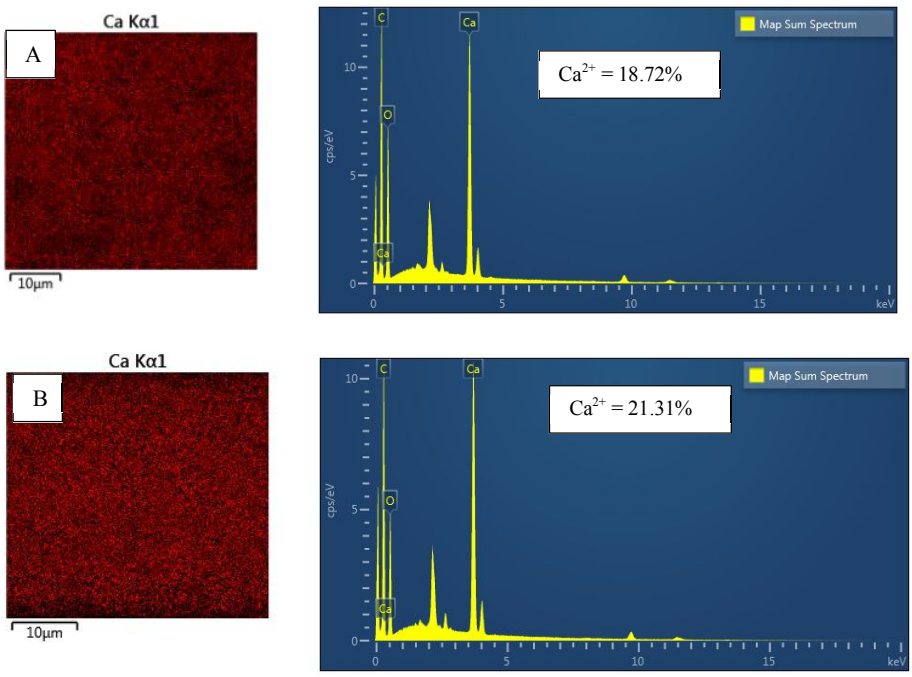

Figure.1 Distribution of Ca^{2+} ion on the cross-section of hydrogel films: (a) A95XCa5(I); (b) A95X5Ca5(II)

3.2 Cytotoxicity

Table.1 %cytotoxicity of the hydrogel films

Sample	Initial concentration [µg/ml]	%cytotoxicity
A95X5Ca5(I)	1000	5.360
A90X10Ca5(I)	1000	0.244
A80X20Ca5(I)	1000	-5.189

A95X5Ca5(II)	1000	3.423
A90X10Ca5(II)	1000	0.197
A80X20Ca5(II)	1000	5.577

Cell viability of the synthesized hydrogels was determined following the MTT assay. The results showed in Tale 1. The average percentages of cytotoxicity of A95X5Ca5(I), A90X10Ca5(I), A80X20Ca5(I), A95X5Ca5(II), A90X10Ca5(II) and A80X20Ca5(II) hydrogel films are 5.360, 0.244, -5.189, 3.423, 0.197 and 5.577, respectively. The results showed less value of %cytotoxicity (less than 50% [16]). It can be concluded that the hydrogel films are not toxic to human cells. From the results, it is suggested that these hydrogel films can be potentially used as biomaterials.

3.3 Swelling Behavior

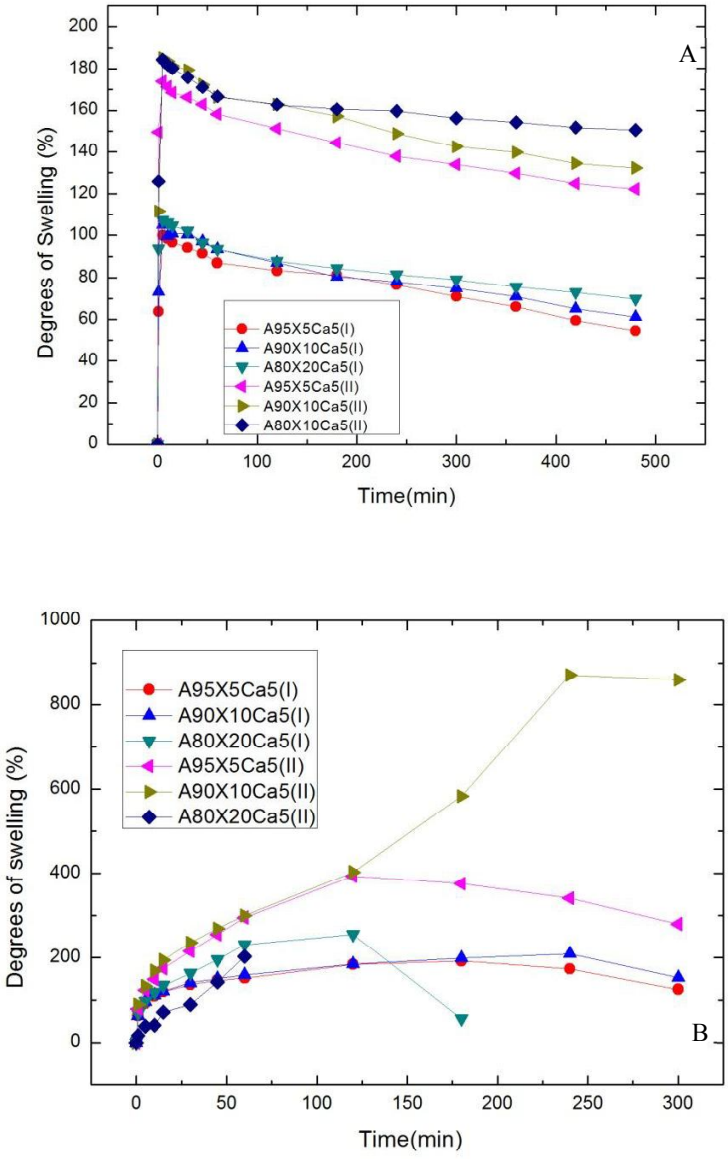

Figure.2 Degree of swelling of A/X hydrogel films : (a) in SGF at 37 ºC and (b) in SIF at 37 ºC

The degrees of swelling of A95X5Ca5, A90X10Ca5, A80X20Ca5, A95X5Ca5Ca220, A90X10Ca5Ca220, A80X20Ca5Ca220 in SGF at 37 °C (pH 1.2) and SIF 37 °C (pH 7.4) are shown in Fig.2. The swelling degrees of A/X films in SGF were much lower than those of A/X films in SIF. This was attributed to the transition between calcium carboxylate and carboxylic acid of alginate and xanthan gum. At pH 1.2, calcium carboxylate (COO^-) transferred to carboxylic acid form (–COOH) and some crosslinking points were destroyed. The polymer-polymer interaction force gradually originated from the hydrogen bond, which existed in –COOH and –OH groups of alginate and xanthan gum. The polymer-polymer interaction force was still dominant over the polymer-water interactions force. As a result of above reasons, the film hardly swelled. When the film were immersed in to SIF (pH 7.4), the ion exchanges among the Ca^{2+} inside the films and other metal ions from the SIF solution were occurred. At this point, the crosslinking points started to destroy and leave the $–COO^-$ form. The electrostatic repulsion between $–COO^-$ brought about the predominant of the polymer-water interactions force. Hence, the hydrogen bond between H_2O and $–COO^-$ was formed simultaneously, subsequently the film swelled and dissolved.

In term of the ratio of A:X, the higher content of xanthan gum in the film gained the higher degree of swelling especially in SGF, i.e. A80X20Ca5 > A90X10Ca5 > A95X5Ca5, and A80X20Ca5Ca220 > A90X10Ca5Ca220 > A95X5Ca5Ca220. It could be explained that xanthan gum is a branched polysaccharide containing 3 units of monosaccharides as side chain groups in every 2 units of mannose of the backbone chain. These side chain groups impeded the initiation of hydrogen bonding among carboxylic acid and hydroxyl groups belonging to polymer chains, thus, water can easily penetrate to the structure of xanthan gum. As comparing with alginate, a linear polysaccharide, the A/X film containing relatively high xanthan gum content could allow the penetration of water easier than the one with lower xanthan gum content.

3.4 *In vitro* drug release

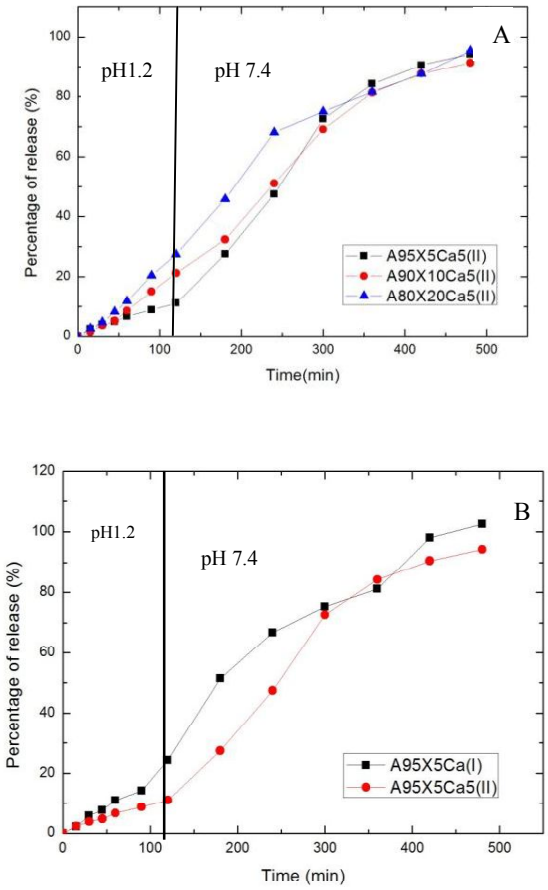

Figure.3 The release profiles of paracetamol in SGF followed by SIF at 37 °C

The drug release profiles of paracetamol as a soluble model drug from the A/X films were investigated. Fig 3 showed the release profiles of paracetamol at 37 ^{o}C for 2 h in SGF followed by 6 h in SIF. As varying the ratio of A:X in Fig.3a, the film with relatively high xanthan gum content gained more releasing rate than the lower one at the first 2 hours in SGF, i.e. A80X20Ca5(II) (~28%) > A90X10Ca5(II) (~21%) > A95X5Ca5(II) (~11%). After transferring the film into SIF, the releasing rate was raised and almost all of the paracetamol were released within 6 hour in SIF. The reason is the difference in chemical structures of alginate and xanthan gum. The former is linear polysaccharide while the latter is branched polysaccharide as discussed in swelling behavior section. The method of crosslinking also affected to the releasing rate in SGF. The films crosslinked by method II (A95X5Ca5Ca(II) film, ~11%) showed slower releasing rate than that crosslinked by method I (A95X5Ca5(I) film, ~24%) after 2 h in SGF (See Fig.3b). This is because A95X5Ca5Ca(II) film contained higher density of crosslinking points than A95X5Ca5(I) film, corresponding to SEM-EDS result.

Conclusion

Drug delivery hydrogel films based on the combination of alginate (A) and Xanthan gum (X) were developed using different methods of crosslinker. The result of SEM-EDS shows the uniform distribution patterns of Ca^{2+} ion. The swelling behavior at 37 °C in SGF and SIF showed that the increasing of xanthan gum content in the film affected the increasing of swelling degrees. *In vitro* drug release profiles in SGF demonstrated that the paracetamol released from the A95X5Ca5(II) film was relatively low (~11%), while almost all of the paracetamol were released in SIF. The crosslinking method is also affected to the releasing rate in SGF. The crosslinked films using method II gained slower releasing rate than the crosslinked film using method I. This formulation could be a potential candidate for drug delivery in the intestinal.

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