RESEARCH PAPER

Fabrication of calcium pectinate microparticles from pomelo pectin by ionotropic gelation

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Abstract

The aim of this study was to fabricate calcium pectinate from pomelo into microparticles by ionotropic gelation. The effect of concentrations of pectin extracted from pomelo peels on size and drug dissolution from calcium pectinate microparticles was investigated and characterized by powder X-ray diffractometry. Ibuprofen was used as a model drug. Smooth surface and spherical microparticles of calcium pectinate was observed by scanning electron microscopy. Average particle size of calcium pectinate microparticles was about 2-5 μm. An increase in pomelo pectin increased the particle size of the microparticles. An amorphous form of the microparticles with a crystalline form of the drug was obtained. Drug dissolution from the microparticles differed from intact ibuprofen. It leads to conclude that ibuprofen could be encapsulated in the microparticles fabricated from calcium pectinate from pomelo pectin using ionotropic gelation.

Introduction

In the past decades, polymeric microparticles have received attentions because of their potential site-specific drug delivery to optimize drug therapy (Coombes et al, 1997). Microparticles play a vital role in drug delivery systems which aim to improve bioavailability of conventional drugs and/or minimizing side effects. They are characteristically free flowing powders consisting of natural or synthetic polymers which are preferably biodegradable and ideally having particle size less than 1000 μm (Christina et al, 2013). Several methods and materials have been investigated for the production of microparticles, including ionotropic gelation using natural polysaccharides and calcium ions. Ionotropic gelation technology does not require high temperatures or involve organic solvents, and can encapsulate emulsions containing hydrophilic or hydrophobic compounds; however, the gel matrix is porous (Sriamornsak et al, 1998, Sriamornsak et al, 2003).

Pectin, a biodegradable polymer, was often used as a wall component of microparticles. Pectin is extracted from plant cell walls. Fruit peels of citrus such as orange, lemon and lime, are well recognized as conventional sources of commercial pectin. Our previous study showed that pomelo, *Citrus maxima* (Family Rutaceae), one kind of Thai popular fruits, could be used as a source of pectin (Chaidedgumjorn et al, 2009, Sotanaphun et al, 2012). Its fruit is fairly large in size with considerable portion of the peel as biological wastes. Pectin has commonly been used as a gelling agent, a thickening agent and a colloidal stabilizer in food industry. The characteristic structure of pectin is a linear chain of α-(1-4)-linked D-galacturonic acid that forms the pectin backbone (May, 1990). Classification of pectin depends on degree of esterification (DE) that influenced pectin properties. Pectin can disperse in pure water and shows negative charges. Pectin with low DE can form water insoluble gel through the interaction between carboxylic group of pectin and divalent cation (Rollin, 1993) while pectin with high DE can form gel with sugar in acidic environment. The application of pectin in the pharmaceutical industry was increased in the last decade (Sriamornsak, 2011). Currently, pectin has been used in many drug delivery systems, for instance, controlled release drug delivery (Aydin and Akbuga, 1996), colonic drug delivery (Wakerly et al, 1997) and mucoadhesive system (Thirawong et al, 2007).

Ibuprofen, a non-steroidal anti- inflammatory drug (NSAID), is used in the treatment of mild to moderated pain and fever. However, the bioavailability of ibuprofen is relatively low after oral administration. Its oral absorption is often controlled by the dissolution rate in the gastrointestinal tract. In this study, the ibuprofen-loaded calcium pectinate microparticles were fabricated from pomelo pectin by ionotropic gelation technique. The effect of concentration of extracted pectin on size, drug dissolution and physicochemical properties from calcium pectinate microparticles was investigated.

Materials and methods

Pectin extracted from pomelo peel was prepared according to the previous report (Chaidedgumjorn et al, 2009). Briefly, dried peel of *C. maxima* (100 g) was extracted with water (2 L x 2 times) for 3 h. The extract was concentrated under reduced pressure to the final volume of 200 ml. It was further dialyzed (D9527, Sigma-Aldrich, USA) for 1 h, repeated 8 times, precipitated by adjusting pH to 3, and adding double volume of 95% ethanol. After centrifugation at 3,500 rpm for 8 min and washing with 95% ethanol, pectin was collected and dried at 50°C. Ibuprofen was purchased from P.C. Drug Center Co., Ltd. (Thailand) and used as a model drug. All other chemicals were of analytical grade and used as received without further purification.

Preparation of microparticles: The ibuprofen-loaded calcium pectinate microparticles were prepared by ionotropic gelation technique. Various concentrations (0.1-1.0% w/w) of pectin in water were prepared. Ibuprofen was dispersed into the pectin solution. Calcium chloride (0.01 M) was dropped, using a nozzle of 0.80 mm inner diameter, into pectin solution and homogenized (9,500 rpm, 20 minutes). The different amounts (i.e. 0.1, 0.25, 0.5, 0.75, 1.0% w/w) of dispersion were dropped using a nozzle of 0.80 mm inner diameter into distilled water and stirred gently with magnetic stirrer. The calcium pectinate microparticles formed were allowed to stand in the solution for 30 minutes.

Morphology: The optical images of microparticles were investigated by an inverted microscope (model Eclipse TE2000-s Nikon, Japan). The micro-

particles were dropped on a glass slide and covered afterward with a coverslip. The images of microparticles were then taken and investigated by the Motic Image Plus 2.0 program.

The surface morphology and internal structure of the microparticles were carried out using a scanning electron microscope (model Maxim 2000S, CamScan Analytical, U.K.) at the accelerating voltage of 15 kV. The internal structure of the beads was examined by cutting them in half with a steel blade.

Measurement of the particle size: The particle size of calcium pectinate microparticles was determined by a static laser light scattering (Laser scattering particle size distribution analyzer, Horiba, model LA-950, Japan). The particles were dispersed in deionized water with gentle stirring. The median particle size was measured under continuous stirring and obtained from the measurements of at least three batches of samples.

Zeta potential measurement: The zeta potential of calcium pectinate microparticles was measured by zeta potential analyzer (model ZetaPlus, Brookhaven, U.S.A.). The particles were dispersed in deionized water at the ratio of 1:50 (v/v) and the electric field applied was 1 V. The average and standard deviation of the measurement of three batches of samples were reported.

Powder X-ray diffractometry: The crystallinity of drug in microparticles was analyzed by powder X-ray diffractometry (model Miniflex II, Rigaku Co., Japan) at 30 kV, 15 mA over the range of 5-45° 2θ by a scanning speed of 4 degree/min using CuKα radiation wavelength of 1.5406 Å.

Dissolution test: The dissolution of ibuprofen from microparticles was done in pH 7.4 phosphate buffer using an orbital shaker at 37±0.5°C and a speed of 50 rpm. The amount of the drug dissolved was measured at the suitable time interval and was then determined spectrophotometrically at 221 nm (Model T60, PG Instruments Ltd., UK). The experiments were conducted in triplicate.

Results and discussion

The aqueous solution of pectin was dropped into calcium chloride solution and gelled particles were formed instantaneously by ionotropic gelation, in which intermolecular cross-links were formed between the divalent calcium ion and the negatively charged carboxyl groups of the pectin molecules. The microparticles were easily manufactured without any sophisticated equipment.

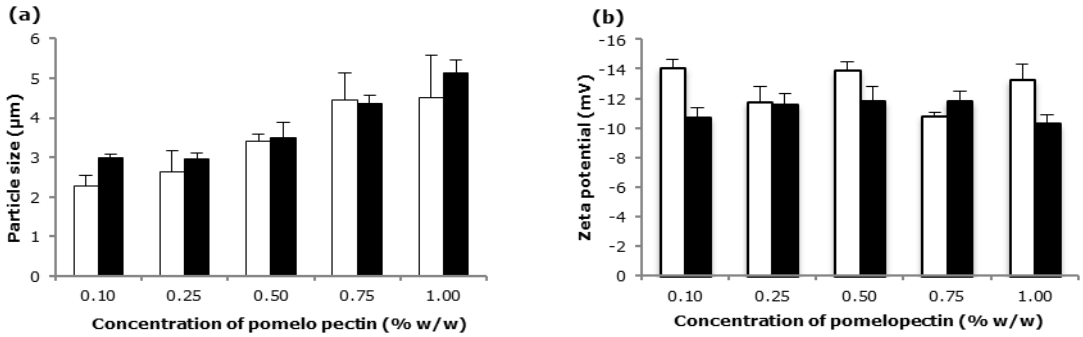

Figure 1 Effect of concentration of pomelo pectin on (a) particle sizes and (b) zeta potentials of calcium pectinate microparticles; white columns – without ibuprofen, black columns – with ibuprofen.

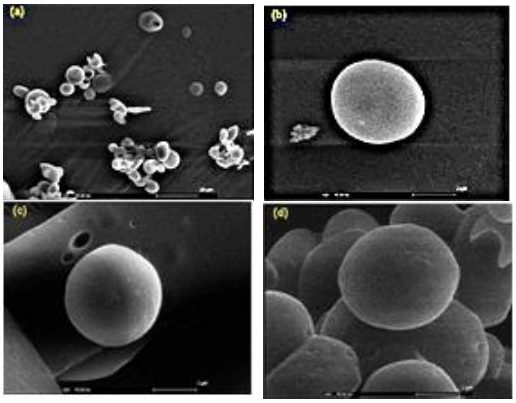

Figure 2 Scanning electron micrographs of (a) and (b) blank calcium pectinate microparticles using 0.5% w/w pectin,(c) ibuprofen-loaded calcium pectinate microparticles using 0.5% w/w pectin and (d) ibuprofen-loaded calcium pectinate microparticles using 1.0% w/w pectin. Scale bars are shown on the individual photographs.

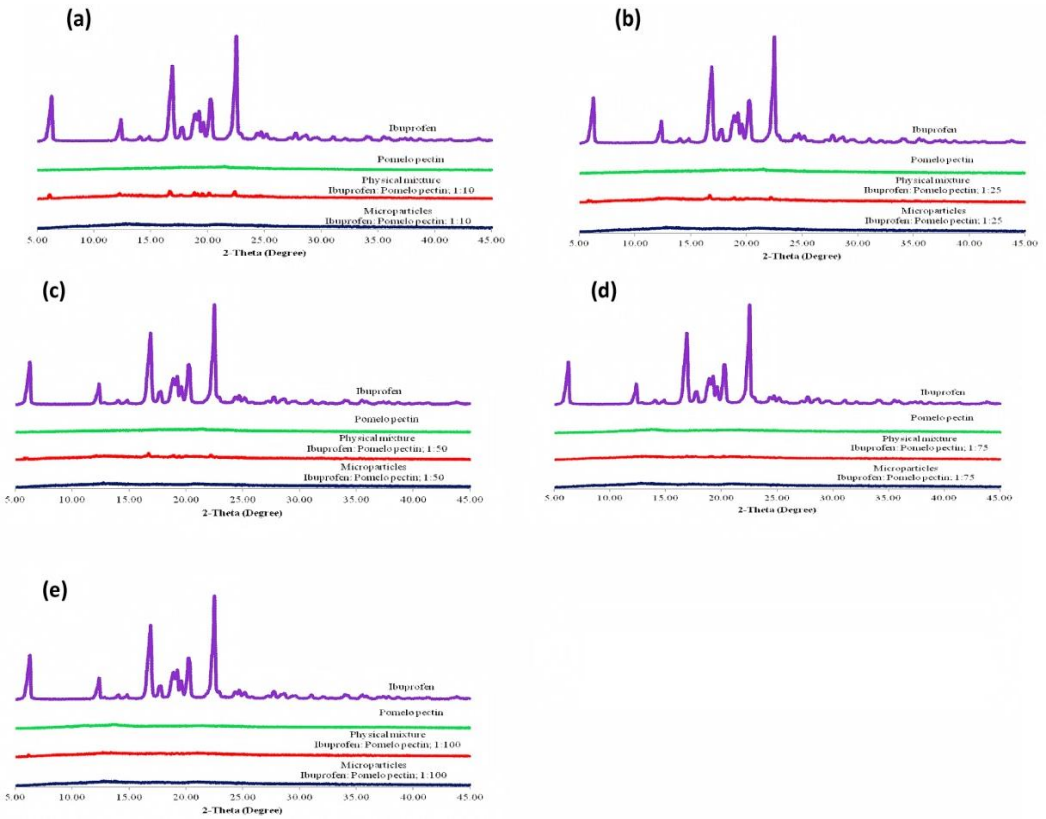

Figure 3 Powder X-ray diffractograms of microparticles prepared from various concentrations of pomelo pectin; (a) 0.1% w/w, (b) 0.25% w/w, (c) 0.5% w/w, (d) 0.75% w/w and (e) 1.0% w/w.

Fig. 1(a) shows the particle size of calcium pectinate microparticles using various concentrations of pomelo pectin. The result showed that the particle size depended on the concentration of pomelo pectin. The microparticles obtained from pomelo pectin demonstrated an increase in particle size with an increase in the concentration of pectin. The particle size of calcium pectinate microparticles was about 2-5 μm. The calcium pectinate microparticles with ibuprofen showed that the particle size was slightly larger than microparticles without ibuprofen.

As shown in Fig. 1(b), the zeta potential of calcium pectinate microparticles tended to increase when concentration of pectin was increased. The pectin contains amount of carboxyl groups that dissociate to negative charge, it could interact and form complex with the positive charged calcium chloride.

Freshly-prepared calcium pectinate microparticles developed at various concentrations of pomelo pectin, without and with ibuprofen were morphologically observed and found to be spherical with small sizes and evenly distributed in the water. Some typical scanning electron micrographs are shown in Fig. 2, to illustrate the morphology of the calcium pectinate microparticles.

After air-drying, the microparticles of all formulations become smaller and dense. This resulted from an increase in rigidity of the microparticles due to the network formation of cross-links between calcium pectinate chains. The calcium pectinate microparticles presented spherical shape with smooth surface. No drug crystal was observed on the surface of all microparticles. The appearance of blank and drug-loaded calcium pectinate microparticles was similar (Fig. 2).

Powder X-ray diffractograms analysis examined the crystalline form of various concentrations of ibuprofen in calcium pectinate microparticles. The diffractograms (Fig. 3) showed that ibuprofen powder was highly crystalline. The physical mixture of ibuprofen and pectin still showed characteristic diffraction peaks of ibuprofen. The intensity of diffraction peaks attributed to ibuprofen considerably decreased, compared with that of intact ibuprofen, demonstrating lower crystallinity of ibuprofen in the physical mixture. The microparticles prepared from different concentrations of pomelo pectin showed no characteristic peaks of ibuprofen. This indicates that transformation of ibuprofen from crystalline state to the amorphous state by loading in calcium pectinate prepared by ionotropic gelation.

This is in agreement with the SEM results (Fig. 2), in which no drug crystal was observed on the surface of microparticles. Transformation from a crystalline state into an amorphous state, the substance is in high energy and high disorder state, and thus, enhancing solubility and rate of dissolution (Won et al, 2005). In this light, the amorphous state after loading in calcium pectinate should improve the dissolution rate of ibuprofen noticeably.

The dissolution of ibuprofen from calcium pectinate microparticles was carried out in pH 7.4 phosphate buffer. The dissolution profiles are characterized by a biphasic behavior, fast dissolution period and declining rate phase (Fig. 4). The first phase includes two apparent mechanisms, swelling and diffusion, which govern the dissolution of the drug from the microparticles. During the second phase, the swelling of the microparticles becomes steady and hence, the drug dissolution is not affected (Siegel and Rathbone, 2012).

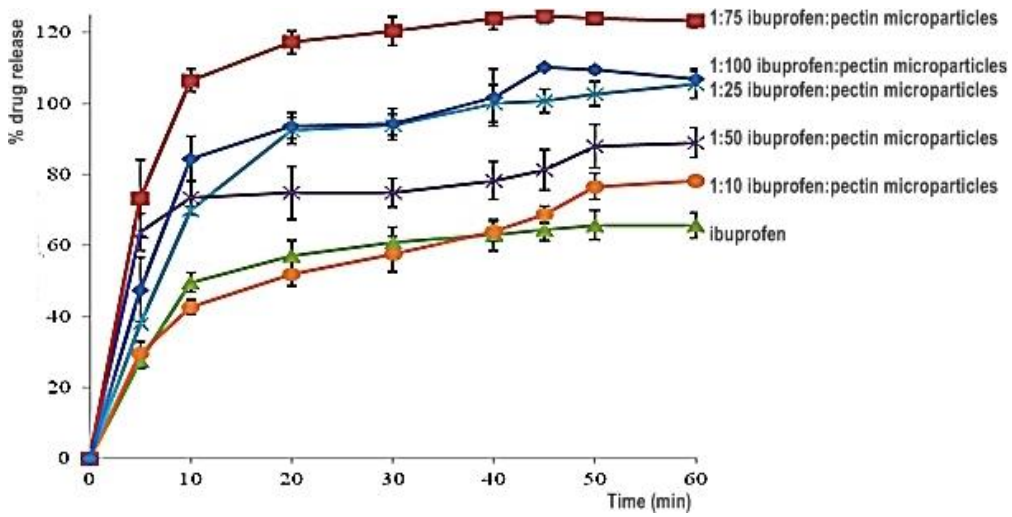

Figure 4 Dissolution profiles of ibuprofen from calcium pectinate microparticles prepared from various concentrations of pomelo pectin.

The rate of dissolution, at 10 min, of ibuprofen was slower than those of the microparticles. From Fig. 4, the highest drug dissolution was obtained from a 1:75 ratio between ibuprofen and pomelo pectin microparticles. The 10-min dissolution of drug from the microparticles with the drug-to-pectin ratios of 1:50, 1:25 and 1:10 were 74, 70 and 43%, respectively. This indicates that the dissolution of the drug from the microparticles was different from ibuprofen. An increase in dissolution from the microparticles was probably due to the wetting and solubilizing effect of pomelo pectin, which could reduce the interfacial tension between the ibuprofen and the dissolution medium (Burapapadh et al, 2010), thus leading to a higher dissolution rate than ibuprofen alone. Moreover, an amorphous form of ibuprofen in microparticles may also cause higher drug dissolution. This is also the case with low crystalline forms.

Conclusions

The results showed that ibuprofen could be encapsulated in the calcium pectinate microparticles fabricated by ionotropic gelation technique without organic solvent. The powder X-ray diffractograms showed that the drug in microparticles was in an amorphous form. Drug dissolution from calcium pectinate microparticles increased compared to the ibuprofen alone.

Acknowledgments

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