Biotechnology and Bioprocess Engineering **2004**, 9: 191-195

Pectin Microspheres for Oral Colon Delivery: Preparation Using Spray Drying Method and *In Vitro* Release of Indomethacin

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Abstract Drug delivery systems that are based on pectin have been studied for colon specific delivery using the specific activity of colon microflora. The aim of this study was to design a novel method of manufacturing pectin microspheres without oils and surfactants and to investigate the potential use of the pectin microspheres as an oral colon-specific drug carrier. The pectin microspheres were successfully formed using the spray drying method and crosslinking with calcium chloride. From the crosslinked pectin microspheres, indomethacin (IND) release was more suppressed than its release from non-crosslinked microspheres. In a low pH (pH 1.4) environment, the pectin microspheres released IND at an amount of about 18 ± 2% of the total loaded weight for 24 h while the release rate of IND was stimulated at neutral pH (pH 7.4). IND release from the pectin microspheres was increased by the addition of pectinase. The results clearly demonstrate that the pectin microspheres that were prepared by the spray drying and crosslinking methods are potential carriers for colon-specific drug deliveries.

Keywords: pectin, colon, indomethacin, spray drying, crosslinking

INTRODUCTION

The oral route is the most preferred route for drug administration, especially for chronic therapies where repeated administration is required [1-2]. Oral administration offers less pain than injection using a needle and syringe, and reduces side effects and risk of infections. The orally administered drugs, however, can be ineffective due to the digestive enzymes and acidic pH in the gastrointestinal (GI) tract [1,3-4]. Thus new strategies, such as coating the drug with pH-sensitive polymers, the use of polysaccharides and time dependent formulations, have been developed to overcome these disadvantages [5-6].

In particular, the first part of the lower intestine, known as the colon, is exposed to numerous pathological conditions [1,7]. In order to treat the inflammatory diseases, oral administration of anti-inflammatory drugs, such as chemotherapy agents and antibiotics, to the colon is required. Hence, several methods for colon specific delivery have been developed, and these delivery systems are based on exploiting the degradation of colonic bacterial enzymes [8].

For the most part, polysaccharides are degraded by various polysaccharidases that are produced by the intestinal microflora. The other side, the polysaccharides are stable in the upper GI tract due to the properties that make a complex network in the acidic condition. Especially because pectin is degraded by colonic bacterial enzymes and is not digested in the human stomach and small intestine, pectin based systems are suitable for use in the colon delivery system [8]. Interestingly, pectin remains as an aggregate of macromolecules in acidic conditions, and in neutral environments, it has a tendency to dissociate [9].

Pectin is a natural, water-soluble polysaccharide that can be obtained from the cell wall of most plants. It has linear chains of (1,4)-linked α-D-galacturonic acid residues (Fig. 1), and these uronic acids contains carboxyl groups that are presented as methyl esters and have reacted with ammonia. The degree of esterification (DE) and the degree of amidation (DA) are also based on the classification of pectin [1]. Because amidated low methoxy pectin (with DE < 50%) forms a rigid gel with divalent cations, it has been used for controlled-release delivery of drugs and also used as a carrier for colonic delivery system [10].

In order to make pectin carriers in many studies, cal-

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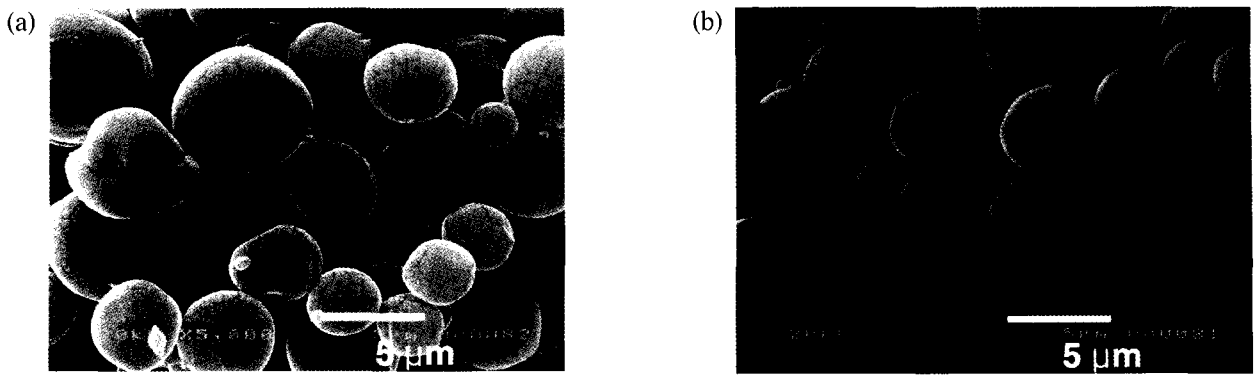

Fig. 1. Scanning electron micrographs of the pectin microspheres (a) before and (b) after crosslinking with calcium chloride.

cium chloride was employed as a crosslinker and the emulsification method is chiefly used to produce microspheres. However, this method entails a relatively complicated process due to pouring oils and adding surfactants. After a process of manufacturing microspheres, the used oil and surfactant has to be removed and the organic solvents can be used for the elimination of oil [11].

Therefore, the aim of this study was to design a novel manufacture method for pectin microspheres without oils or surfactants, and another aim was to investigate the potential use of pectin microspheres as oral colon-specific drug carriers. The pectin microsphere was formed through the spray drying method, and then, the formulation was crosslinked with calcium chloride. In general, formulation by spray drying is weak with moisture and can be dissolved without difficulty [12]. The pectin microspheres that were crosslinked by divalent cation are comparatively stable against moisture and have useful properties as drug carriers. In this study, pectin microspheres were prepared as described above and the morphological shape of these microspheres was observed by using a scanning electron microscope (SEM). Drug release from pectin microspheres was investigated *in vitro* in acidic and neutral conditions and the influence of the enzyme was also evaluated on drug release.

MATERIALS AND METHODS

Materials

Amidated low-methoxyl pectin (DE 9%), indomethacin (IND), pectinase (from *Aspergillus niger*, 25 U/mg at pH 4, and 25°C) and calcium chloride were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals were used without further purification.

Preparation of Pectin Microsphere

Pectin microspheres were prepared by spray-drying process [13]. Briefly, 9 g of pectin was dissolved in 300 mL of distilled water, and then, 90 mg of indomethacin was added under vigorous stirring. The prepared solution was spray dried to form microspheres through the nozzle (0.2 mm diameter) of the spray-dryer (SD-1000, Eyela, Tokyo, Japan). The conditions of spray-drying were as follows: inlet air temperature was 130°C, outlet air temperature ranged from 45-50°C, spray flow was 5 mL/min, and compressed spray air flow (represented as the volume of the air input) was 200 kPa. Pectin microspheres containing IND were collected from the spray-dryer cyclone, and the microspheres that were formed were crosslinked with a 1 M calcium chloride solution for 10 min. The morphological shape of the pectin microspheres was observed under SEM (JSM-6400, JEOL, Tokyo, Japan).

Differential Scanning Calorimetry (DSC) Analysis

The melting points of pectin, microspheres including IND, IND, and the physical mixture pectin and IND were measured by the method of differential scanning calorimetry (DSC) (PerkinElmer instruments, Tokyo, Japan) at a temperature range of 50 to 200°C under nitrogen and at a rate of 5°C/min.

Determination of IND Included in Pectin Microspheres

The amount of IND included in the pectin microspheres was determined by placing the microspheres in phosphate buffered saline (PBS, pH 7.4) for 48 h at 37°C with vigorous stirring. The concentration of IND was analyzed using a UV-spectrophotometer (Shimadzu UV-1201, Kyoto, Japan) at 319 nm. The percentage of loading efficiency and content was expressed as with the following equations:

Loading efficiency (%)
= (weight of loaded drug in microspheres/initial feeding weight of drug) × 100
Loading content (%)
= (weight of loaded drug in microspheres/weight of microspheres) × 100

In Vitro Drug Release Studies

The release rate measurements *in vitro* were carried out as follows: 50 mg of IND-loaded pectin microspheres

were suspended in 1 mL of phosphate buffered saline (PBS, 0.1 M, pH 7.4) and HCl buffer (0.1 M, pH 1.4) respectively. The solution was placed in a dialysis tube (MWCO=12,000 g/mol, Sigma Co., St. Louis, MO, USA), and then the tube was introduced into 50 mL of release buffer, and the medium was stirred at 100 rpm at 37°C. At predetermined times, 1 mL of the sample solution was removed and the same volume of fresh buffer was added respectively. The concentration of IND that was released into buffer was detected through a UV-spectrophotometer (Shimadzu UV-1201, Kyoto, Japan) at 319 nm.

Influence of Enzyme on Drug Release

The influence of enzyme on drug release from pectin microspheres was evaluated through the addition of pectinase. 50 mg of pectin microspheres were suspended in 50 mL of PBS (0.1 M, pH 7.4) and then 40 U/mg of pectinase (activity 25 U/mL at pH 4, Sigma Co., St. Louis, MO, USA) was added while the solution was being stirred. The medium was stirred at 100 rpm at 37°C, and samples of 1 mL were periodically taken. The absorbance of the sample containing IND was measured using a UV-spectrophotometer (Shimadzu UV-1201, Kyoto, Japan) at 319 nm.

RESULTS AND DISCUSSION

Preparation and Characterization of IND-Loaded Pectin Microspheres

The shape of the pectin microspheres that were observed by SEM was spherical, and the diameter of the microspheres was approximately 3-5 μm (Fig. 1).

Immediately after spray drying, the microspheres obtain a crack on the sphere surface and are not completely spherical in shape. On the other hand, after crosslinking with calcium chloride, the crack on the surface vanished, and the microspheres returned to its spherical shape. These results indicate that the spray dried pectin microspheres are crosslinked with divalent calcium chloride [14]. Thus, after crosslinking, the pectin microspheres may have a greater stability against moisture compared to the microspheres that are not crosslinked with divalent calcium chloride.

DSC Analysis

Fig. 2 shows that the DSC was used to confirm the physical state of IND and the polymer. The melting point of pectin was observed at 192.7°C, and the melting point of pure IND was measured to be 164.4°C. The endothermic peak of the physical mixture of pectin and IND (mixture ratio, 1:1) was observed at 187.3 and 163.5°C, and the peaks was similar to the melting points of pectin and IND. On the other hand, pectin microspheres including IND show only an endothermic peak of pectin at 192.7°C, but the IND peak was not observed. The reason that the melting temperature of IND was depressed may be due to the encapsulating by the pectin matrix. Consequently, this data suggests that the IND was included in the pectin microspheres.

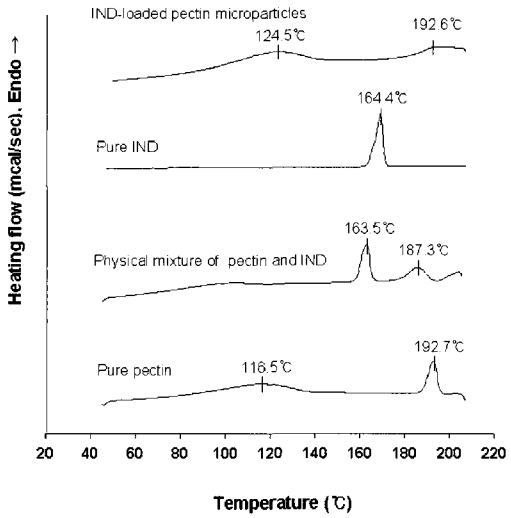

Fig. 2. DSC thermograms of pectin, IND, physical mixture, and IND loaded pectin microspheres.

Table 1. Drug loading efficiency of the pectin microspheres

Pectin microspheres	Drug content (wt.-%)	Drug loading efficiency (wt.-%)
Crosslinked microspheres	5.2 ± 0.4	13.5 ± 0.3
Non-crosslinked microspheres	5.7 ± 0.3	14.8 ± 0.3

IND Loading Efficiency and *in vitro* Release from Pectin Microspheres

The IND loading efficiency of the pectin microspheres was shown in Table 1. The amount of IND loaded in the pectin microspheres after spray drying and crosslinking was 5.7 ± 2 and 5.2 ± 4 μg of the drug per milligram of microspheres, respectively. Release studies were performed to examine the suitability of pectin mciroparticles for an oral colonic delivery system. The release pattern from the pectin microspheres was represented by plotting the amount of IND release against time.

Fig. 3 shows the release profiles of the IND-loaded pectin microspheres. The rates of the IND release at pH 1.4 were significantly slower than the release rate at pH 7.4. Moreover, the amount of IND released from the non-crosslinked pectin microspheres at pH 7.4 reached about 90% within 2 h, whereas release from the crosslinked microspheres was gradually released over period of 24 h. The cross-linking of pectin with calcium chloride inhibits the release of the included drug from the pectin microspheres by suppressing the dissolution and

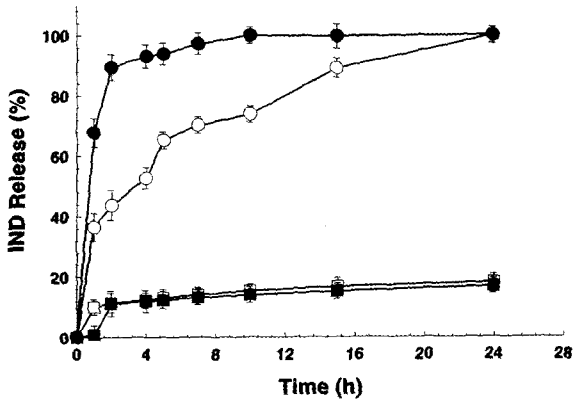

Fig. 3. Release profiles of IND from the non-crosslinked (closed) and crosslinked (open) pectin microspheres at pH 1.4 (square) and pH 7.4 (circle).

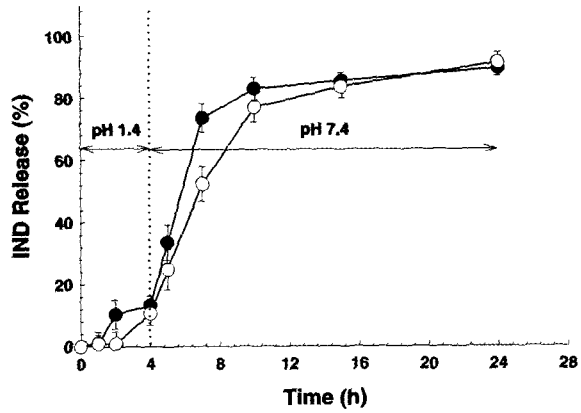

Fig. 4. Release profiles of IND from the non-crosslinked (●), and crosslinked (○) pectin microspheres associated with the change of pH condition.

by the swelling of the pectin macromolecules [15]. The release profiles of IND from the pectin microspheres against the change of pH were shown in Fig. 4. At a pH of 1.4, which is similar to the acidic conditions of a human stomach, IND release was suppressed, and after 4 h, drug release rapidly occurred at pH 7.4. These results suggest that the cross-linked pectin microspheres are stable in the acidic pH of the stomach and are gradually dissolved in small intestine and colon.

Influence of Enzyme on Release from Pectin Microspheres

The main reason for selecting pectin as drug carrier was its biodegradable property in the colon by colonic microflora. [16]. Thus, in this study, the influence of colonic bacterial enzyme on drug release from the pectin microspheres was evaluated by using pectinase. Fig. 5 shows the change of IND release rate from pectin microspheres by the addition of pectinase. After the addition of pectinase, IND release rate was significantly faster than

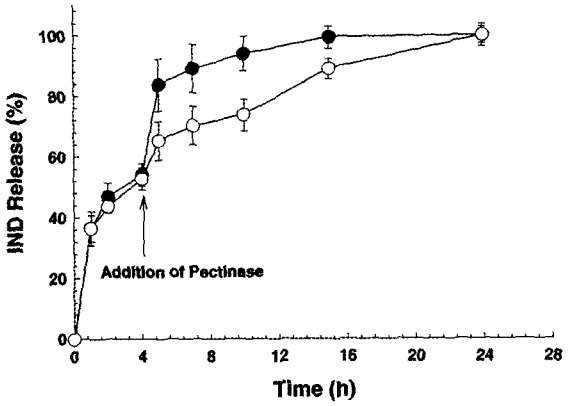

Fig. 5. The influence of pectinase on the release profiles of IND from the crosslinked pectin microspheres at pH 7.4; Profiles by the addition (●), and non-addition (○) of pectinase.

the release rate without pectinase. This result shows that pectin microshperes was degraded by pectinase, and as a result, IND release was increased. Under the influence of pectinase that is found in the colon, drug release rate from the pectin microspheres would be faster than in small intestines [17]. Therefore, the microspheres can be use as an oral drug carrier for colonic delivery.

CONCLUSION

In this study, the pectin microspheres were prepared using a novel formulation method, and its potential as an oral colonic carrier was investigated *in vitro*. The pectin microspheres were formed through the use of the spray drying method and were crosslinked with calcium chloride. The rate of drug release from the pectin microspheres was controlled and increased by the degradation of pectinase. Consequently, the pectin microspheres may be used for oral colonic delivery system.

Acknowledgement This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (03-PJ1-PG10-21200-0009).

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[Received February 20, 2004; accepted June 3, 2004]