Preparation and *in vitro* Release Mechanisms of Modified Pectin Matrix Tablets for Colon-Targeted Drug Delivery

Chen Li, Jian-Bin Li,* Cai-Yu Lei, and Pei-hua Liu

To deliver bioactive components to the colon, an oral, colon-targeted, microparticle delivery system was developed based on pectin. Pectin was modified by mechanical activation, resulting in controllable release properties, as well as dramatic decreases in solubility. Mechanically activated pectins (MAP) were characterized by Fourier transformed infrared (FTIR) spectroscopy, nuclear magnetic resonance (^{1}H-NMR) spectroscopy, differential scanning calorimetry (DSC), and scanning electron microscopy (SEM). The FTIR and ^{1}H-NMR analyses revealed that after mechanical activation, the hydrogen bonds between pectin molecules were broken, and intermolecular crosslinking was decreased. The DSC analysis indicated that the thermal stability of pectin was decreased by mechanical activation. The SEM revealed that MAP particles were smaller, more uniform, and had smoother surfaces than unmodified pectin. An *in vitro* release assay and the study of drug release kinetics demonstrated that bovine serum albumin (BSA) release from MAP-containing matrix tablets was controllable. The results demonstrated that at a suitable pectin content and hydrophobicity level, matrix tablets prepared with MAP can exhibit good colon-targeted drug release.

Keywords: Pectin; Matrix tablet; Colon; Mechanical activation; Releasing property

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INTRODUCTION

There are more than 1.1 million survivors of colorectal cancers in the United States, as estimated by the American Cancer Society. Colorectal cancer survivors currently account for 10% of total cancer survivors, and this figure is expected to increase (Lee *et al.* 2015). Colorectal cancer is a complex disease that can require care from multidisciplinary specialists who may be geographically separated. Conventional chemotherapy is not effective in its treatment, because anticancer drugs are toxic to normal tissues, which has limited the efficacy of present cancer chemotherapy (Labianca *et al*. 2010). What is more, the anticancer drugs cannot reach the target site in effective concentrations. So colon-targeted delivery of bioactive components is an effective therapeutic strategy for the treatment of colon-based diseases, and it has attracted broad scientific interest in recent years (Kosaraju *et al*. 2005; Li *et al*. 2012). Such treatments can deliver drugs more efficiently to the colon, where it can target tumor tissues more specifically (Labianca *et al*. 2010). Therefore, much attention has been given to the use of colon targeted delivery system to treat colon cancer. Colon-targeted delivery of bioactive components is an effective therapeutic strategy for the treatment of colon-based diseases, and it has attracted broad scientific interest in recent years (Kosaraju *et al*. 2005).

There have been many approaches to the development of colon-specific delivery systems. According to many scientists, the greatest challenges for colon-targeted delivery systems are the metabolic activity of our bodies and the colonic microenvironment. To overcome these issues, much of the recent work in the field has focused on novel colon-targeted delivery systems based on polysaccharides (de Vos *et al*. 2010; Rudzinski *et al*. 2016; Seeli and Prabaharan 2017). Polysaccharides may be effective carriers for colonic delivery systems because many can avoid digestion by the bioactive components in the upper gastrointestinal tract, but can then be digested by the enzymes produced by colonic microbiota or by the bioactive components of the colon (Freire *et al.* 2009; Gadalla *et al*. 2016).

A large number of polysaccharides have already been studied for their potential in colon-specific drug carrier systems, including chitosan, pectin, chondroitin sulfate, cyclodextrin, dextrans, guar gum, amylase, inulin, and locust bean gum (Elkhodairy *et al.* 2014). Among the many polysaccharides that have been investigated, pectin has proven to be the most suitable carrier material for colonic drug delivery because it forms macromolecular aggregates in the upper portions of gastrointestinal tract but is then degraded by colonic enzymes upon reaching the colon (Friend 2005; Dev *et al.* 2011). However, one major disadvantage of pectin is that it is highly soluble in acidic media, which may promote undesirable premature release of the drug (Sinha and Kumria 2001; He *et al.* 2008). Usually, in order to solve this problem, the pectin needs to be modified. Some articles have reported about various methods used for pectin modification, including alkylation, amidation, quaternization, thiolation, sulfation, oxidation, and cross-linking, but there have been no reports about mechanical activation. This method is simple and convenient, and it is an optimal method to refine pectin particles and increase specific surface area, which not only improves the compatibility of pectin, but also can achieve the aim of decreasing the solubility of pectin.

In this study, pectin was modified by mechanical activation to decrease its solubility in acidic media, and it was characterized using FTIR, ^{1}H-NMR, DSC, and SEM. The modified pectin was then used to prepare matrix tablets, which were evaluated for their solubility and swelling properties. Tablet dissolution was also analyzed to determine the potential of this material for the design of colonic drug delivery systems.

EXPERIMENTAL

Materials

Pectinex® UltraSP-L (pectinase/pectinolytic enzyme from *Aspergillus aculeatus*, activity > 9500 PG mL^{-1}) was obtained from Novozymes (Tianjin, China). High-ester pectin was purchased from the pectin company of Qu Zhou (Qu Zhou City, China). Bovine serum albumin (BSA) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

Ethylcellulose (M70), methylcellulose (M20), polyvinylpyrrolidone (K-30), sodium carboxy methylcellulose (300~800 mPa·S), methylcellulose (MC), ethyl cellulose (EC), carboxymethyl cellulose (CMC), and polyvinylpyrrolidone (PVP) were provided by Guang Yao Experimental Equipment Co., Ltd. (Nanning, China).

All reagents were of analytical grade.

Preparation of MAP

Principles of mechanical activation

In a planetary ball mill (Fig. 1), the fast planetary motion of a grinding cylinder causes high-speed rolling of abrasives and materials, producing strong impact, shear, rolling, *etc.*, and resulting in the materials being polished and smashed. A planetary ball mill has four ball-milling cylinders arranged on a horizontal rotary table. The horizontal turntable rotates clockwise, while the ball-grinding cylinder moves around the horizontal rotating axis of revolution and simultaneously spins in planetary motion around its own axis. Because of the high-speed movement of the milling drum, the abrasives polish and collide with each other, causing the materials to be milled to a size of as small as 0.1 μm.

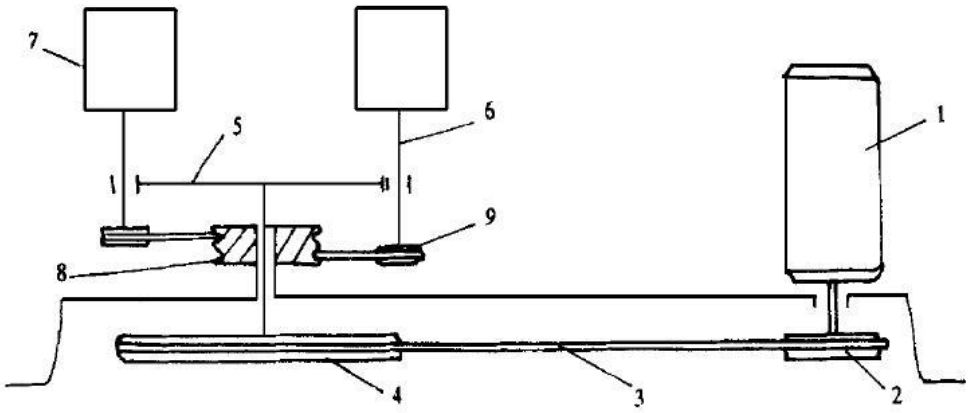

Fig. 1. Schematic view of a planetary ball mill (1-speed regulating motor, 2-small belt, 3-belt, 4-big belt pulley, 5-turntable, 6-center shaft, 7-ball mill cylinder, 8-center belt wheel, 9-planet belt wheel)

Preparation of MAP

The MAP was prepared by grinding pectin in the planetary ball mill. Pectin was first filtered with a 60-mesh sieve, then added to the planetary ball mill with the proper amount of balls. After treatment, the pectin was removed and stored for later use.

Solubility

Three 100 mL centrifugal tubes were labeled as 1, 2, and 3. Then 0.05 g of MAP (0, 4, 8 h) samples were added each centrifugal tube, and a few drops of anhydrous ethanol were also added to disperse the samples. Then 50 mL of artificial simulated gastric juice were added. The mixtures were placed in a Shaker at 37 ºC. After 2 h, they were centrifuged at 4000 rpm for 15 min. The residue was dried for 24 h at 60 ºC. Finally, the specimens were weighed.

Solubility SM was calculated as,

$$SM(\%) = \frac{W + W_1 - W_3}{W} \times 100 \quad (1)$$

where W is the mass of the test sample; W_1 is the mass of the centrifuge tube; and W_3 is the mass of wet base precipitation and centrifugation tube after drying.

FTIR Analysis

The FTIR analysis of the tablets was carried out as previously described by Newton *et al.* (2012), with slight modification. A Spectrum One spectrophotometer (Nexus 470 FTIR, Tianjin, China) equipped with a universal attenuated total reflectance device was used. The FTIR spectra were recorded in the spectral region of 4000 to 650 cm^{-1} with 64 scans at a 4-cm^{-1} resolution. Samples were prepared in KBr discs (1 mg sample in 100 mg KBr) using a hydrostatic press at a force of 5 kg/cm^2. Experiments were duplicated for reproducibility.

^{1}H-NMR Analysis

High-resolution ^{1}H-NMR spectra were recorded on a Bruker AV-600 spectrometer (Representative Office in Beijing, China). The samples were prepared as described by Heux *et al.* (2000). Native or modified pectin was dissolved in D_2O. These solutions were then frozen and thawed three times to exchange labile protons with deuterium, and their spectra were recorded three times at 330 K (Zheng *et al.* 2015).

DSC Analysis

The MAP samples were analyzed using DSC as previously described, with slight modification (Soares *et al.* 2013; Prezotti *et al.* 2014). The DSC curves were registered in a TA instrument DSC200PC (Netzsch, Representative Office in Beijing, China) at a heating rate of 10 °C/min between 20 and 350 °C under nitrogen gas (50 mL/min). Experiments were performed using a sealed aluminum pan with a central pinhole in the lid containing approximately 1 mg of the sample. A sealed aluminum pan with a central pinhole in the lid was used as a reference.

SEM Analysis

The surface morphology of the pectin was analyzed using previously reported methods, with slight modification (Varshosaz *et al.* 2012; Meneguin *et al.* 2014). The surface characteristics of the pectin and MAP were analyzed using a scanning electron microscope (Hitachi, S-3400N, Tokyo, Japan). The samples were glued on the conductive adhesive and coated with gold using a sputter coater. The shape and surface characteristics of the pellets were observed in the electron micro-analyzer, and photographs were taken at magnifications of 200× and 15,000×. All of the samples were examined using an accelerating voltage of 10 kV.

Preparation of Matrix Tablets

Matrix tablets were prepared according to the method described by Weh *et al.* (2014), with slight modification. Pectin (0.5 g), adhesives (MC,0.25 g), bovine serum albumin (0.5 g), calcium salt (0.008 g), and magnesium stearate (0.001 g) were combined with a 2% hydroxypropyl methyl cellulose (HPMC, 0.025 g) ethanol solution and sieved with sieve No. 40. The granules were dried in a conventional hot air oven at 50 °C until a constant moisture content was achieved. The dried granules were passed through sieve No. 40. Talc and magnesium stearate (1% to 2% of total mass) were added as lubricant and glidant and were mixed with the granules using a spatula before compression. Finally, the granules were weighed and compressed using a manual, single-punch tablet machine. A pair of 6-mm stainless steel round, flat punches with a break line was used. The granules were compressed with a pressure of 1.75 kg/mm^2. Each matrix tablet weighed about 0.25 g and contained 80 mg of drug.

In vitro BSA Release from Matrix Tablets

An *in vitro* release analysis was performed using a protocol modified from Ribeiro *et al*. (2014). Bovine serum albumin (0.2500 g) was dissolved in deionized water in a 250-mL flask to prepare a 1 mg/mL BSA standard solution, which was kept in a thermostatic bath at 37 °C. The release study was performed for 10 h to simulate the sequential pH changes that occur *in vivo*. First, the matrix tablets containing BSA were incubated for 2 h in deionized water (as a blank control). Second, they were incubated in a solution of HCl (pH 1.2) for 3 h to simulate the gastric tract fluid. Third, the tablets were incubated at a pH of 6.8 for 2 h (by adding 0.03 g NaOH, 0.40 g $NaH_2PO_4 \cdot H_2O$, and 0.62 g NaCl to the pH 1.2 solution) to simulate the first zone of intestinal fluid. Finally, the tablets were incubated at a pH of 7.4 for 4 h (by adding 1 M NaOH to the previous solution of pH 6.8), mimicking the colonic intestinal zone. Aliquots (5 mL) of each medium were withdrawn every hour to analyze the amount of BSA released from the drug-loaded matrix tablets by UV spectrophotometry (Unico, UV-2802S, Shanghai, China) (279 nm). The measured sample was then added back to the medium to keep the medium constant. All experiments were performed in triplicate.

Determination of *in vitro* Release Rate of BSA

The *in vitro* release rate of BSA was determined using a method published in 2010 in the Chinese Pharmacopoeia of the People's Republic of China (National Pharmacopoeia Commission. 2010). The different pectin matrix tablets were placed in 500 mL of artificial gastric tract fluid and rotated at 100 rpm at 37.5 °C ± 0.5 °C for 10 h. Samples (5 mL) were withdrawn from the dissolution apparatus every hour using a sampling cannula with filter attachments. An equal amount of fresh medium of the same temperature was replaced in the apparatus. All of the samples were filtered through a 0.8-μm filter membrane before measurement of absorbance at 279 nm using a UV-visible spectrophotometer.

The cumulative percentage of released BSA for each time point was calculated according to Eq. 2,

$$c(\%) = A_n + \frac{(A_{n-1} + \cdots\cdots + A_2 + A_1) \times V_1}{V_2} \quad (2)$$

where A_n is the drug dissolution of all sampling time points (%), V_1 is the fixed sampling volume at each time point (mL), and V_2 is the volume of the dissolution medium (mL).

RESULTS AND DISCUSSION

Effect of the Duration of Mechanical Activation on the Solubility

Pectin treated by mechanical activation for 0 h, 4 h, and 8 h were the specimens designated as 1, 2, and 3, respectively. The solubilities of the MAPs are shown in Fig. 2. At equal concentrations and temperatures, the solubility of pectins decreased after treatment by mechanical activation. Pectin solubility was dramatically decreased with the increasing of mechanical activation time, which indicates that the pectin formed self-avoiding polymer chains (Ventura and Bianco-Peled 2015). This may be due to the fact that the intramolecular hydrogen bond is destroyed after mechanical activation. From Fig. 2, it also can be seen that pectin solubility was largest in pH 6.8 buffer solution. This may be because free carboxylic acids dissociated to their corresponding salt form in the pH

6.8 buffer solution, so the MAP particles can be stretched sufficiently to increase the solubility.

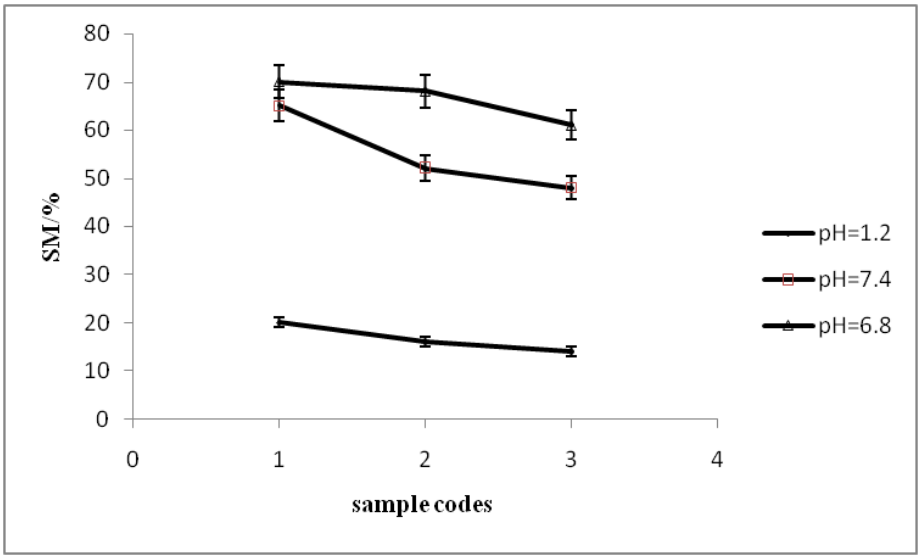

Fig. 2. Solubility of MAP at different pH values

FTIR Spectroscopy of Pectin

The FTIR spectra of MAP indicated a broad band at 3373 cm^{-1}, which is related to OH groups (Li *et al.* 2011) (Fig. 3). The peak at 2937 cm^{-1} is characteristic of C–H stretching attributed to the absorption band of $–OCH_3$ (Ma *et al.* 2009). Bands at 1726 and 1610 cm^{-1} are typical of carboxyl and carbonyl groups, respectively (Wang *et al.* 2009). Absorption at 1440 and 1235 cm^{-1} may be attributed to C–H bending vibration (Zhang *et al.* 2009). The range from 1250 to 500 cm^{-1} is the fingerprint area. Additionally, other C–O functionalities such as C–OH (1015 cm^{-1}) and C–O–C (1146 to 1100 cm^{-1}) were clearly visible (Chandra *et al.* 2010).

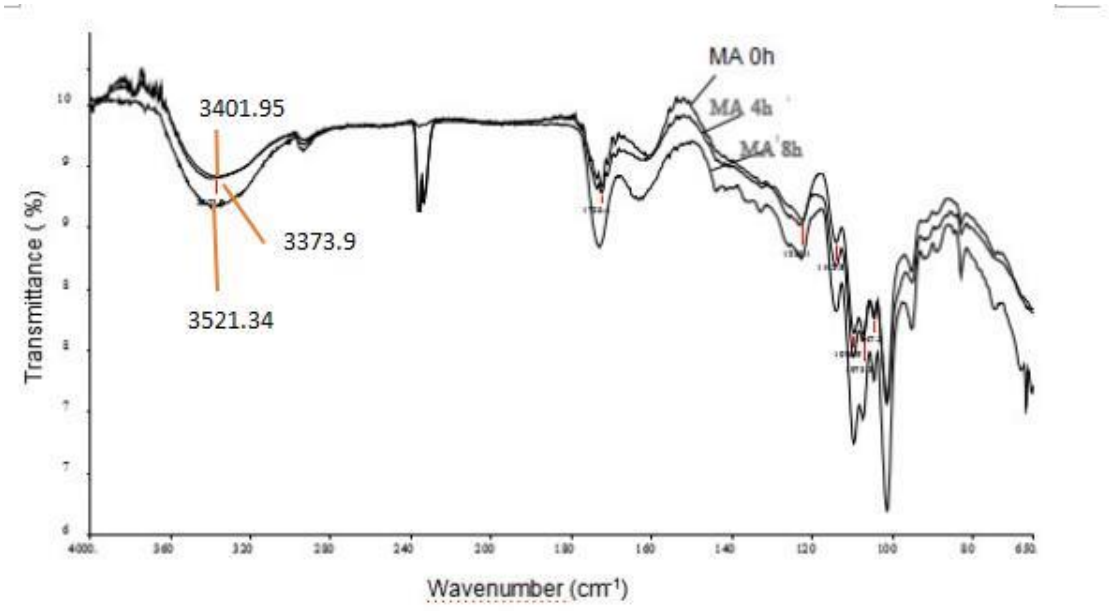

Fig. 3. FTIR spectra of mechanically activated pectin

There was a weak absorption peak for the asymmetric stretching vibration of the D-pyran ring near 923 cm^{-1}. The absorption peak at 890 cm^{-1} for vertical βC1–H vibration, which is a characteristic absorption peak of the D-pyran ring, suggests that the pectin molecules contained a β-glycosidic bond. The absorption peak at 832 cm^{-1}, which is a characteristic peak for αC1–H vibration of the D-pyran ring, suggests that the pectin molecule had large amounts of α-glycosidic bonds. The absorption peak at 750 cm^{-1} is attributed to symmetric stretching vibration of the pyran ring. These results suggest that the pectin molecule contained many α-glycosidic bonds and a smaller amount of β-glycosidic bonds.

Compared with the original pectin, the MAP for 4 h and 8 h pectin exhibited no new absorption peak, indicating after treatment with mechanical activation, no new groups had been generated and the main chemical structure of pectin had not changed. However, with the increase of mechanical activation time, the hydroxyl stretching vibration peak position was greatly offset to the high frequency direction, such that the vibration peak positions of 0, 4, and 8 h were 3373.97, 3401.95, and 3521.34 cm^{-1}, respectively. Also, the strength was enhanced obviously; this may be because of the intramolecular hydrogen bond cleavage and a corresponding increase in intermolecular hydrogen bonds.

^{1}H-NMR

The ^{1}H-NMR spectra of the pectins that were mechanically activated for 0 and 8 h are presented in Fig. 4. Mechanical activation resulted in changes in the proton vibrational peaks at 3.13, 4.66, and 5.30 ppm, which are attributed to alcohol hydroxyl groups. It is hypothesized that the mechanical activation caused the stress to reach its theoretical strength and broke the hydrogen bonds between molecules, thus increasing the electron cloud density of the nuclear outer. As a result, there was a decreased chemical shift because of the absence of the hydrogen bond proton. At the same time, the mechanical activation depolymerized the pectin and increased the number of active hydrogens in the alcohol hydroxyl groups. These molecular changes may be responsible for the appearance of new protons and vibrational peaks in the spectrum.

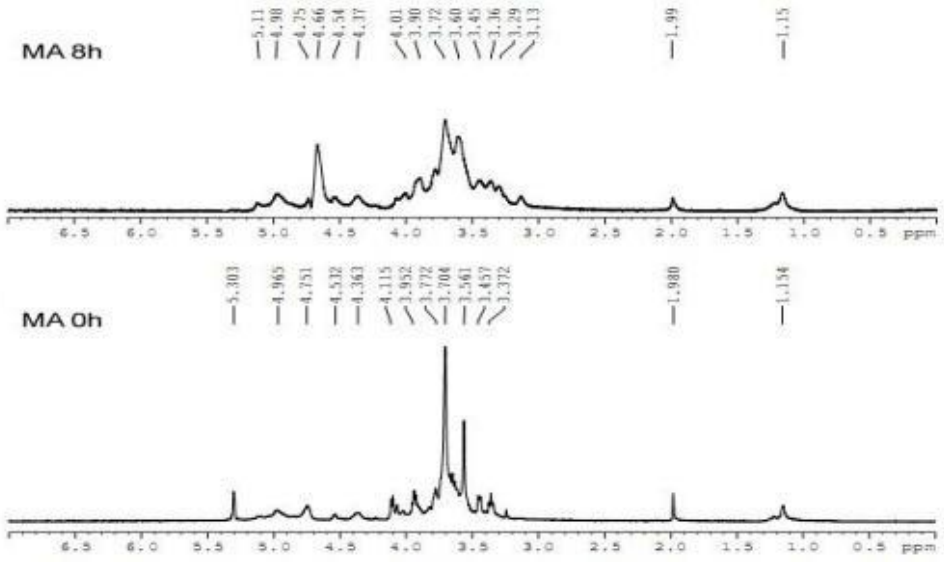

Fig. 4. ^{1}H-NMR spectra of mechanically activated pectin (8 h and 0 h)

DSC

The DSC curves for pectin are presented in Figs. 5 and 6. Unmodified pectin exhibited an absorption peak at about 30 °C. However, the absorption peak of MAP shifted to high temperature, which was attributed to the ability of the mechanical activation to destroy molecular hydrogen bonding of pectin, changing the physical and chemical properties of pectin to a certain extent (Fig. 5). In the range 200 to 300 °C, the T_{on} (onset) temperature of MAP was lower than that of the unmodified pectin, and the T_p (peak) and T_{off} (offset) temperatures decreased at the same time (Fig. 6 and Table 1). This is because that mechanical activation may have caused the mechanical energy of MAP to be converted to specific surface energy, thus changing the crystal structure and diminishing thermal resistance and thermal stability. What is more, after mechanical activation, the energy state and low thermal stability of MAP caused a decrease in the T_p and the heat enthalpy (E_{max}). Compared with unmodified pectin, MAP had more uniform particles, a longer recovery time, a lower recovery rate, and a higher T_{exoff} (extrapolated offset).

Table 1. Parameters of Thermal Analysis of Mechanically Activated Pectins

Sample	T_{exon} DSC (°C)	T_p DSC (°C)	T_{exoff} DSC (°C)	E_{max} (mW·mg^{-1})
MA 0 h	237.8	253.7	269.6	-0.2779
MA 4 h	234.0	250.6	269.6	-0.2593
MA 8 h	215.7	251.9	269.3	-0.2649

T_{exon} and T_{exoff} are the extrapolated onset and offset temperatures, and T_p is the peak temperature of the signal. E_{max} is maximum heat flow.

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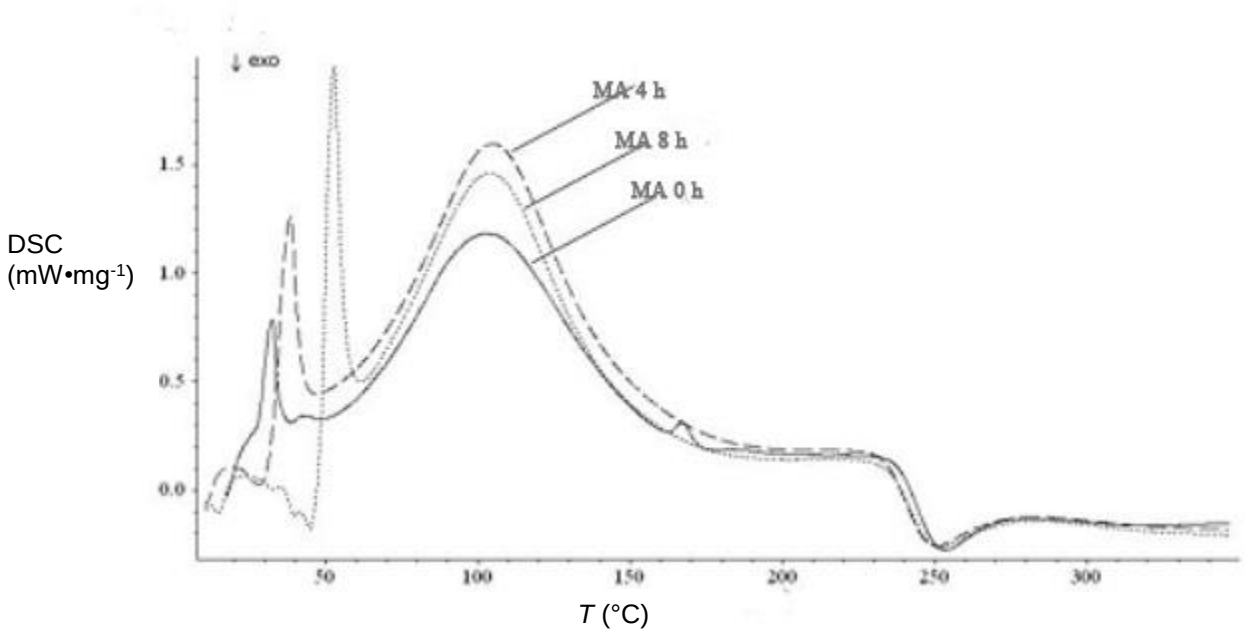

Fig. 5. DSC curves of MAP (0 to 350 °C)

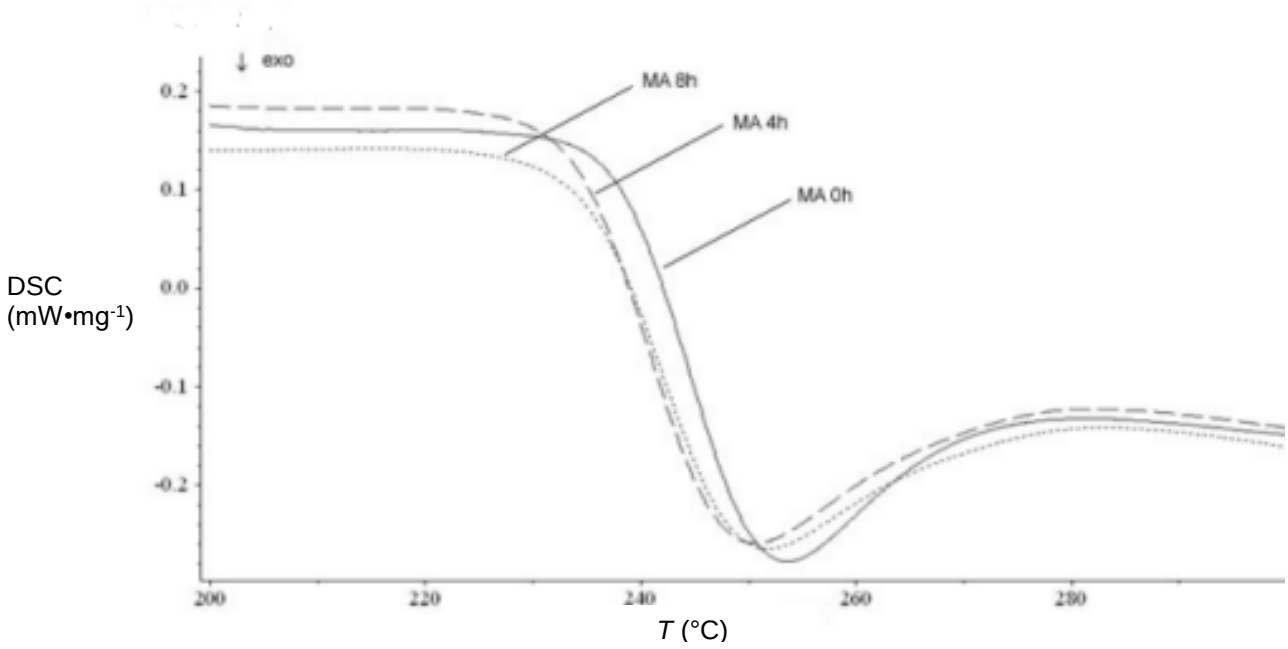

Fig. 6. DSC curves of MAP (200 to 300 °C)

SEM Micrographs of Pectin

Unmodified and mechanically activated pectins (4 or 8 h) were observed by SEM (Fig. 7).

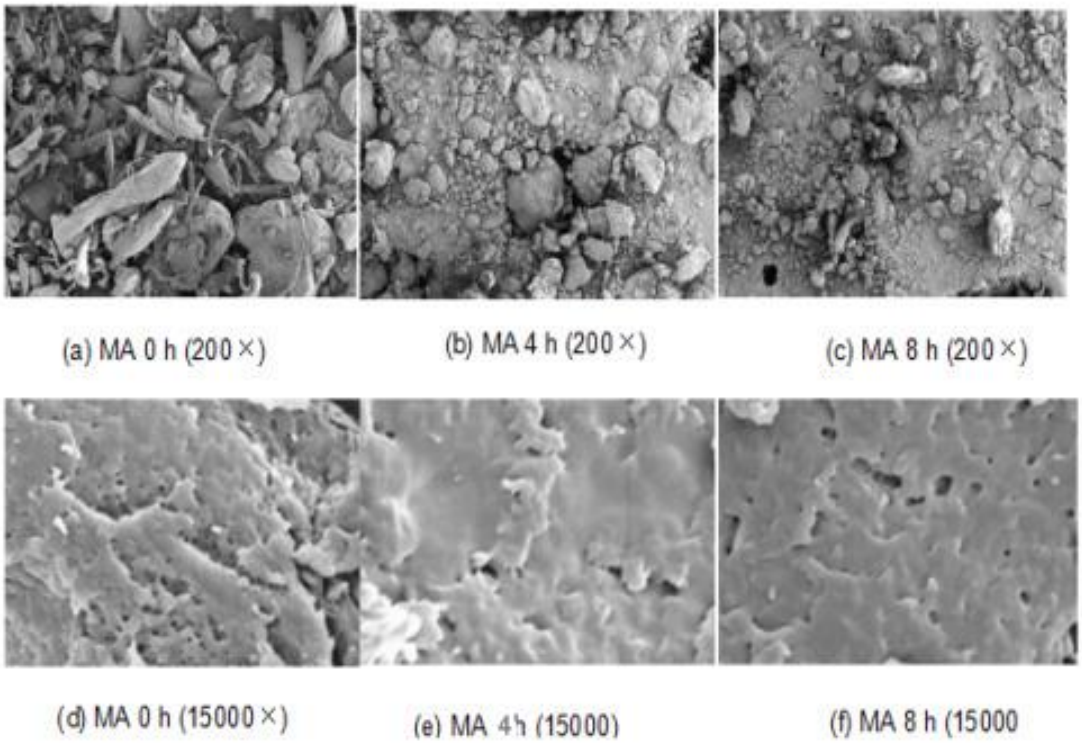

Fig. 7. Scanning electron micrographs (SEM) of MAP surfaces

Unmodified pectin exhibited a more irregular and porous surface (Fig. 7a and d). Mechanically activated pectins (Figs. 7b, c, e, and f) exhibited a smoother and more continuous surface with increasing mechanical activation time. These differences may be attributed to the impact, grind, and shear forces of mechanical activation, which decrease the intermolecular forces between pectin molecules and thus increase their motion (Vieira *et al.* 2011).

In vitro Enzymatic Digestion Assay

Mechanically activated pectin was evaluated to determine its resistance to pectinase digestion; 0.5 g of MAP was put into gastrointestinal tract (GIT), and 4 mL of pectinase and 0.008 g $CaSO_4$ were added in it. The MAP (8 h) exhibited lower susceptibility to enzymatic digestion (Fig. 8) because of its higher level of network reorganization and increased crystallization, which both restrict enzymatic access. This phenomenon is critical for preventing premature drug release in the upper segments of the gastrointestinal tract (GIT). Indeed, these results showed that the drug release time of MAP was longer than that of unmodified pectin, indicating that mechanical activation has a favorable effect on drug release.

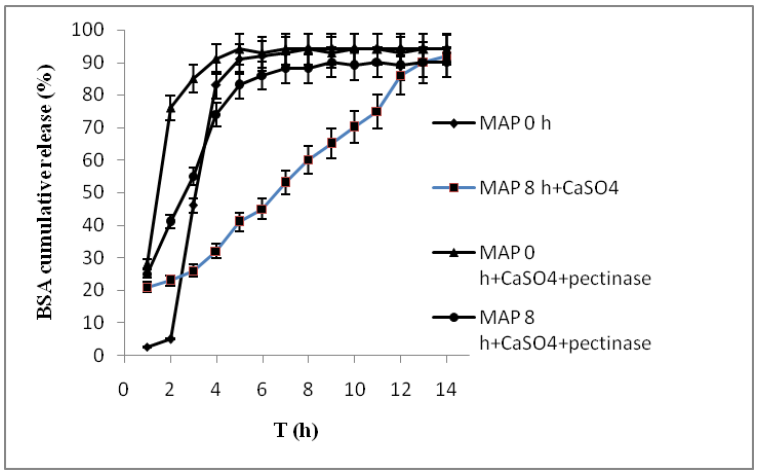

Fig. 8. Effect of pectinase on the release of BSA from MAP-based matrix tablets

Effect of Mechanical Activation Time on the Release of MAP-Based Matrix Tablets

As shown in Fig. 9, the initial release of MAP-matrix tablet was increased with the increase of mechanical activation time. The maximum drug release of MAP (0, 4, and 8 h) did not result in much difference, but the MAP (0 and 4 h) exhibited burst release phenomena in 4 h and 8 h, respectively, resulting in premature drug release, which may be because the gel layer was thinner, allowing water to enter more easily into the internal structure, leading to rapid dissolution of matrix materials and acceleration of the drug release. MAP (8 h) exhibited almost no burst release phenomena, but the drug release was increased at 18 h. This is mainly because the MAP (8 h) formed a thick gel layer in the water. Thus, water entered into the matrix of the tablet slowly, which resulted in the dissolution of tablet excipients more slowly, so drug release occurred slowly, which indicated a good sustained release effect.

When the mechanical activation was 12 h, MAP formed a thick and dense gel layer in water easily, and water was difficult to get into the framework of the matrix tablet, and the drug could not be completely released. So in this study, we choose MAP (8 h) to prepare matrix tablets.

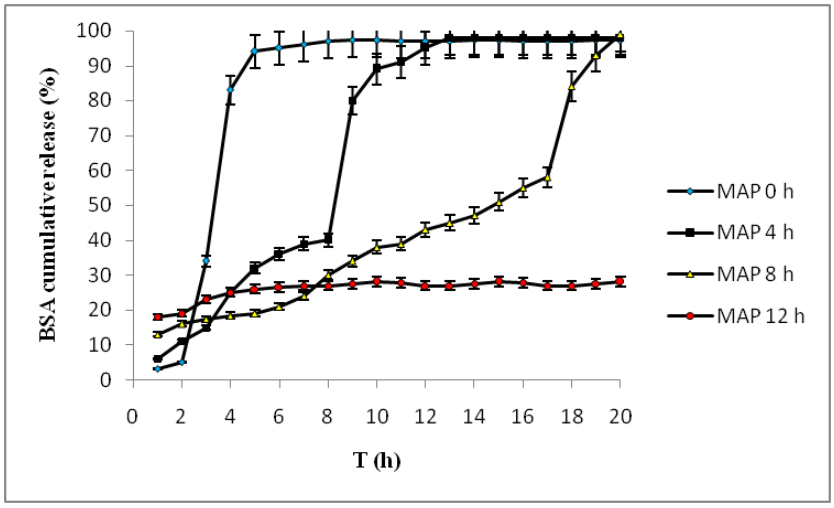

Fig. 9. Effect of mechanical activated time on the release of PT MA-based matrix tablets

Drug Release Kinetics

Dissolution dates were fitted to mathematical models such a zero order model (1), first order model (2), Higuchi release model (3), corrosion model (4), and diffusion-corrosion model (5) to study drug release kinetics and mechanisms. Regression coefficients of the drug release kinetics for all formulations are summarized in Table 1.

$$\frac{M_t}{M_\infty} = kt \quad (1)$$

$$\frac{M_t}{M_\infty} = 1 - e^{-kt} \quad (2)$$

$$\frac{M_t}{M_\infty} = kt^{\frac{1}{2}} \quad (3)$$

$$\frac{M_t}{M_\infty} = k_1 t + k_2 t^2 + k_3 t^3 \quad (4)$$

$$\frac{M_t}{M_\infty} = k_1 t^{\frac{1}{2}} + k_2 t + k_3 t^2 + k_4 t^3 \quad (5)$$

It is apparent from Table 2 that the linear regression coefficient corresponding to the diffusion dissolution model was the highest, so the mechanical activation release behavior of MAP - based matrix tablets can be fitted by dissolution diffusion model. The experimental data of MAP (8 h) -based matrix tablet was chosen to fit by diffusion dissolution model, and the fitting coefficient were K_1= 30.42, K_2=-11.85, K_3=1.639, and K_4=-0.06576. The fitting curve is shown in Fig. 10.

Table 2. Regression Coefficients of Drug Release Models from AP-based Matrix Tablets

Sample	Zero-order	First-order	Higuchi	Corrosion	Diffusion-corrosion
1	0.5597	0.9417	0.8787	0.9831	0.9886
2	0.3067	0.9604	0.8588	0.9828	0.9862
3	0.5530	0.5193	0.9080	0.9905	0.9911
4	0.5801	0.9734	0.9173	0.9928	0.9928
5	0.8384	0.9052	0.7941	0.9686	0.9793
6	-0.2122	0.9737	0.8280	0.9725	0.9849
7	0.06708	0.9357	0.8570	0.9527	0.9659

1-4：AP 20 min；AP 40 min；AP 60 min；AP 80 min；5：AP 60 min+$CaSO_4$；6：AP 60 min+pectinase；6：AP 60 min+$CaSO_4$+pectinase

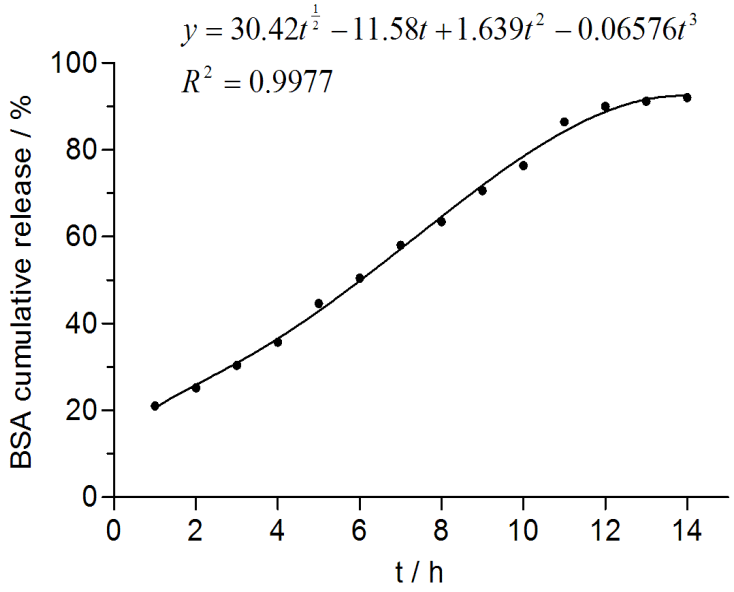

Fig. 10. Diffusion-corrosion model fit curve of MAP-based matrix tablets

CONCLUSIONS

1. Mechanical activation of pectin increases its stability and decreases its solubility. The structure and properties of pectin are significantly affected by mechanical activation.
2. Fourier transform infrared and ^{1}H-NMR analyses demonstrated that mechanical activation does not change the structure of the pectin chain, but breaks hydrogen bonds and decreases the degree of molecular association, resulting in better compatibility.
3. Differential scanning calorimetry showed that the thermal stability of pectin was decreased with mechanical activation. Scanning electron microscopy showed that

after mechanical activation, pectin particles became smaller and more uniform, with a smoother surface and decreased pores, resulting in lower solubility.

4. *In vitro* drug release studies and drug release kinetics indicated that formulations prepared with MAP exhibited sustained drug release in enzymatic medium. The prepared MAP matrix tablets demonstrated specific degradation by pectinase, effective colon-targeted drug delivery, and biological degradation. These results indicate that MAP may be a useful technology for oral dosage forms intended for colon-specific drug delivery.

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