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ORIGINAL ARTICLE

Characterization of muco- and bioadhesive properties of chitosan, PVP, and chitosan/PVP blends and release of amoxicillin from alginate beads coated with chitosan/PVP

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Abstract

Purpose: To investigate the muco/bioadhesive properties of chitosan, polyvinylpyrrolidone (PVP), and chitosan/PVP blends and the release of amoxicillin (AMX) contained in AMX-alginate beads coated with these materials. *Method*: Chitosan, PVP, and chitosan/PVP blends at various volume ratios were coated onto calcium alginate beads containing AMX. The muco/bioadhesive properties of all materials and the AMX-alginate beads coated with these materials were characterized. *Results*: Measurements of their viscosity, texture, and adhesion to HT29 cells demonstrated that chitosan/PVP at a volume ratio of 5/5 had the best muco/bioadhesive properties when compared with chitosan, PVP, and blends of other ratios. Wash-off tests indicated that the mucoadhesive property of the coated AMX-alginate beads was significantly higher than that of the uncoated beads. Diffuse reflectance infrared Fourier transform spectroscopy showed that there were interactions between chitosan–PVP, chitosan–mucin, PVP–mucin, and chitosan/PVP blend–mucin. Scanning electron microscopy revealed that the surfaces of the coated beads were smoother than those of the uncoated beads. All coated AMX-alginate beads were able to provide a controlled release of AMX with Super Case II transport properties, at a pH of 4. This was probably a result of the rapid and extensive swelling of the alginate beads. The more rapid release of AMX at pH 1 was probably because of the rapid dissolution of the drug at this pH. *Conclusions*: From the controlled drug release and muco/bioahesive properties of these coated AMX-alginate beads, we suggest that the alginate-coated beads might be a promising drug delivery system to assist with the eradication of *Helicobacter pylori* infections.

Key words: Amoxicillin, bioadhesion, chitosan, coated alginate bead, mucoadhesion, polyvinylpyrrolidone

Introduction

Helicobacter pylori, a gram-negative bacterium, is associated with chronic gastritis, peptic ulcer disease, and gastric cancer[1]. The short residence time of antibiotics in the stomach is the main reason for the ineffectiveness of antibiotics for the eradication of *H. pylori*[2]. It has been recently demonstrated that *H. pylori* infections can be successfully treated if antibiotics are retained in the stomach for 1 hour[3].

Because of its mucoadhesive properties, chitosan can improve the bioavailability of drugs when used in mucoadhesive dosage forms[4]. Polyvinylpyrrolidone (PVP), a common drug excipient, has also been used in mucoadhesive formulations. The mucoadhesion capability of PVP is enhanced when it is blended with other polymers[5,6]. In recent years, polymer blends have attracted considerable attention, because blending is a simple and effective method to develop new materials with specific properties. Blends of chitosan/PVP may

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have enhanced mucoadhesive properties as compared with the individual polymers.

To date, several approaches have been investigated to increase the gastroretentive time of drugs for clearance of *H. pylori*. These included the use of beads[7] and floating mucoadhesive material-coated beads[8–10], muco/bioadhesive microspheres[11–17], and mucoadhesive nanoparticles[18]. Previous studies have demonstrated that mucoadhesive material-coated beads exhibited high mucoadhesive properties[19,20] and could control the release of the drug[20]. In this study, a new mucoadhesive bead was prepared using an amoxicillin (AMX)-loaded alginate core that was subsequently coated with chitosan/PVP blends. Beads coated with chitosan and PVP separately were also prepared for comparison. Alginate beads can be produced simply in the presence of calcium ions[20–22]. AMX, which is typically used for the treatment of *H. pylori* infections, was selected as a model drug. Alginate-AMX-coated beads could increase the residence time of AMX in the stomach, thereby increasing its effectiveness in the treatment of *H. pylori* infections. In addition, such coated beads may be useful for gastroretentive delivery of various other drugs that either act locally or are predominantly absorbed from this site. The muco- or bioadhesive properties of chitosan, PVP, and chitosan/PVP blends were evaluated using appropriate techniques including viscosity measurement, texture analysis, and an HT29 cell adhesion assay. Wash-off tests were performed to investigate the mucoadhesion of uncoated and coated AMX-alginate beads. The interactions between chitosan–PVP, chitosan–mucin, PVP–mucin, and chitosan/PVP blends–mucin were examined using the diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) technique. The surface morphologies of AMX-alginate beads were investigated using scanning electron microscopy (SEM). The AMX-loaded beads were also subjected to a swelling study and an in vitro dissolution study and the mechanism of transport determined.

Materials and methods

Materials

Chitosan was obtained from Fluka (GmbH, Buchs, Switzerland). Its degree of deacetylation was 75–85% and average molecular weight was 300,000–500,000 Da. Alginic acid sodium salt from brown algae (viscosity 250 cps, 2% solution at 25°C), mucin type 2 from porcine stomach, and AMX were purchased from Sigma (St. Louis, MO, USA). PVP K-90 (Kollidon 90), with an average molecular weight of 1,100,000, was kindly supplied by BASF, Bangkok, Thailand. All other reagents were of analytical grade.

Polymer blend and mucin preparation

The chitosan solution (2%, w/v) was prepared by dissolving chitosan (2.0 g) in 0.05 M hydrochloric acid (100 mL). PVP solution (2%, w/v) was prepared by dissolving 2.0 g of PVP in water (100 mL). Mucin solutions at concentrations of 2% (w/v) and 15% (w/v) were prepared by dispersing the appropriate amount of mucin in water. Polymer blends of chitosan and PVP were prepared by mixing the 2% (w/v) solutions of the two polymers at the volume ratios of 1/9, 3/7, 5/5, 7/3, and 9/1. All polymer blends were mixed gently until homogeneous using a reciprocating shaker.

Viscosity measurements

For viscosity measurements, 5 mL of mucin solution (15%, w/v) was mixed with 2 mL of (2%, w/v) polymer or blend solutions (chitosan, PVP, and chitosan/PVP blends). The final volume of the mixtures was adjusted to 8 mL with water, and the mixtures were mixed using a reciprocating shaker until homogeneous. The final concentrations of polymer and mucin solutions were 0.50% (w/v) and 9.38% (w/v), respectively. These mixtures of mucin–polymers and mucin–polymer blends were equilibrated at 25.0 ± 0.1°C for 1 hour before viscosity measurements.

All viscosity measurements were performed using a Brookfield model DV-III Ultra programmable viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA), equipped with a SC4-18 spindle and a small sample adaptor at 25.0 ± 0.1°C. Samples were allowed to equilibrate for 1 minute before testing. The apparent viscosity at a shear rate of 15.84 per second was selected for analyses. All viscosity measurements were performed in triplicate, and data reported as a mean ± SD.

Texture analysis

Chitosan, PVP, and chitosan/PVP blend films were prepared by casting the polymer or the polymer blend solutions on polystyrene plates and dried in an oven at 60°C for 8 hours. These film samples were used for texture analyses.

A texture analyzer, TA-XT2 (Stable Micro System, Haslemere, UK) equipped with a 5 kg load cell and mucoadhesive rig, was used for all tensile strength measurements. All polymer blend films were cut in circular shapes (diameter 1 cm) and fixed on the upper probe of the instrument using double-sided adhesive tapes. Porcine stomach was obtained from the animal immediately after slaughter at the local slaughterhouse (Faculty of Natural Resource, Prince of Songkla University, Hat-Yai, Thailand). The tissue was thoroughly washed with deionized water to remove nondigest food, kept at 4°C, and used within 6 hours. Stomach tissue was cut into square shapes (3 × 3 cm), and one square was fixed on a mucoadhesive rig on the stage of the instrument with the mucus layer upward. The film previously attached on the upper probe was wetted with deionized water (50 μL) and then moved lower at a constant speed of 1 mm/s to make contact with the tissue. The contact of the film and the tissue was maintained for 2 minutes with a 0.08 N force.

The film was then slowly moved upward at a constant speed of 1 mm/s until the film was detached from the mucus layer. Force versus distance curves, during the upward movement of the film, were obtained directly from Texture Expert software. The areas under these curves were calculated as the work of mucoadhesion. These analyses were replicated 6–10 times and data reported as a mean ± SD.

In vitro cell adhesion assay

HT29 cells (passage 121–128) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were grown and subcultured in Dulbecco's Modified Eagle's Medium containing 10% fetal calf serum, 1% nonessential amino acids, and 1% L-glutamine. Incubation was at 37°C in an atmosphere of 5% CO_2, 95% O_2.

Chitosan, PVP, and chitosan/PVP blends (100 µL) were cast on an ultra low attachment 96-well plate (Corning Costar Cat. #3474, Lowell, MA, USA). The plate was then dried in an oven at 40°C for 8 hours. Subsequently, 100 µL of a trypsinized HT29 cell suspension was added into each well. The plate was then incubated for 3 hours to allow attachment of the cell to the polymer. The cells were then washed with phosphate buffer saline three times to remove nonattached cells. Cells attached onto the polymer were quantified using the MTT test [3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide]. In brief, the MTT solution (100 µL) was added into each well, and the plate incubated for 3 hours. Formation of the purple formazan from MTT (catalyzed by the mitochondrial reductase in living cell) was dissolved with DMSO (100 µL) and quantified using a DTX-880 multimode detection microplate reader (Beckman Coulter, Fullerton, CA, USA) at 570 nm. The percentage relative cell attachment was calculated by subtracting the experimental value for polymer-coated cells from that of the corresponding value for non-polymer-coated cells. This was then compared with the data for non-washed cells; cell attachment with the non-washed cells was considered to be 100%.

Uncoated and coated calcium alginate beads preparation

Alginate beads were prepared as previously described[20] with some modifications. The alginate solution was prepared by stirring the mixture of alginate (2.0 g) and water (100 mL) for 4 hours. AMX (5.0 g) was then dispersed in the alginate solution and the mixture continually stirred for 20 minutes. Subsequently, the mixture was added dropwise using a syringe with 23-gauge needle into a gently agitated solution of calcium chloride (2%, w/v). After continuous stirring for 10 more minutes, the beads were separated by filtering through a sieve (mesh size ~250 µm), washed with water, and then dried in an oven at 40°C for 8 hours. The dried alginate beads were then coated with 1.5% (w/v) of chitosan, PVP, or chitosan/PVP blend solutions by immersing the dried beads into these solutions for 10 minutes. The coated beads were then dried in an oven at 40°C for 8 hours.

Wash-off test for evaluation of the mucoadhesive property of alginate beads

The in vitro evaluation of the mucoadhesive properties of uncoated and coated AMX-alginate beads was carried out using the wash-off test from a porcine stomach, as previously described[23,24]. The freshly slaughtered porcine stomach was washed with normal saline, cut into rectangular shapes (~2.5 × 4 cm), and attached onto a microscopic slide. Thirty uncoated or coated beads were brought into direct contact with the mucus layer of the stomach tissues using a pressure of 25 g on the glass slide for 2 minutes. The mucoadhesiveness of the beads was determined by connecting the slide with the arm of QC-21 disintegration test system (Hanson Research, Chatsworth, GA, USA). The wash-off of the beads was induced by the reciprocating motion of the disintegration apparatus in 8000 mL phosphate buffer (pH 4.0) at 37 ± 0.5°C. The remaining beads were counted every 30 minutes for 3 hours, and this data were used to calculate the percentage of bead attachment. These tests were performed in triplicate, and data reported as a mean ± SD.

DRIFTS measurement

To study the interactions between mucin and polymer as well as the mucin and polymer blends, the solutions of chitosan, PVP, or chitosan/PVP blends (2%, w/v) were mixed with mucin solution (2%, w/v) in a 1/1 volume ratio. These samples were then dried in an oven at 60°C for 8 hours before DRIFTS measurement.

DRIFTS spectra were measured using a PerkinElmer Spectrum One FTIR spectrometer (Perkin-Elmer, Waltham, MA, USA) and a PerkinElmer DRIFTS accessory. The samples were finely ground in an agate mortar and placed in the microsample cup of the DRIFTS accessory using the supplied sample cup holder. The spectra were recorded from 4400 to 450 cm^{-1} by averaging 64 scans at a 4 cm^{-1} resolution. The reflectance spectra were converted to Kubelka–Munk units using a PerkinElmer Spectrum for Windows version 5.02 software package.

Scanning electron microscopy

The morphologies of uncoated and coated AMX-alginate beads were observed using a JSM-6400 scanning electron microscope (Jeol, Tokyo, Japan) at an accelerated voltage of 15 kV. All samples were coated with gold using a direct current sputter technique.

Drug loading

Drug loading was evaluated by transferring about 15 mg of the AMX-alginate dried beads into a volumetric flask (100 mL). Phosphate buffer (80 mL, 0.1 M, pH 4) was added and the solution then stirred using a magnetic stirrer for 5 hours. The magnetic bar was removed and

thoroughly rinsed with phosphate buffer into the volumetric flask, and the phosphate solution was then added to the scale. The solution was filtered and the filtrate analyzed using an 8452A HP Diode Array spectrophotometer (Hewlett Packard, Palo Alto, CA, USA) at 230 nm. All the experiments were carried out in triplicate. The percentage of drug loading was calculated using Equation (1):

$$\% \text{ drug loading} = \frac{W_a}{W_b} \times 100, \quad (1)$$

where W_a is the analyzed AMX content and W_b is the theoretical content of AMX in the beads.

Swelling study of dried beads

Swelling studies of uncoated and coated AMX-alginate dried beads were performed in 0.1 M phosphate buffer (pH 4) at 37.0 ± 0.5°C. Accurately weighed beads (15.2–16.40 g) were immersed in the buffer solution with slight agitation with a shaker. The beads were removed periodically from the buffer solution, blotted to remove excess liquid, and weighed on an electronic balance. The swelling ratio or percentage of weight change was determined using Equation (2) as previously described[22]:

$$\% \text{ weight change} = \frac{W_t - W_d}{W_t} \times 100, \quad (2)$$

where W_t is the weight of the swollen beads at time t and W_d is the weight of the dried beads.

Dissolution study

The release of AMX from uncoated and coated beads was determined using a VK 7000 dissolution tester (Vankel Industries, Edison, NJ, USA) with a USP27 apparatus 2 at pH 4 and also at a pH of 1.2. Phosphate buffer (pH 4), consisting of 0.1 M KH_2PO_4, using either KOH or phosphoric acid to adjust the pH to 4.0, was prepared as the dissolution medium. The dissolution medium at pH 1.2 was prepared by dissolving 2 g NaCl in 7 mL HCl and adding water to 1000 mL. This medium (200 mL) was maintained at 37.0 ± 0.5°C during the test. Approximately 20 mg of beads were used in each experiment. Samples (5 mL) were taken at 6, 12, 18, 24, 30, 40, 60, 90, 120, and 180 minutes and replaced with 5 mL of fresh medium. The amount of AMX in collected samples at pH 4 was determined using an 8452A HP Diode Array spectrophotometer (Hewlett Packard) at 230 nm. High-performance liquid chromatography (HPLC) was also performed to check the possibility of degraded products of AMX, which might interfere with the analysis of AMX using the simple UV assay. However, no degradation peaks were detected for samples at the pH of 4. Thus, at this pH the more simple UV spectrometric method was used for the analysis of all dissolution samples.

Degraded products were observed when using the dissolution medium pH 1.2 and the HPLC method was used for analysis of all samples at this pH. The HPLC analyses were performed according to the conditions specified by USP 30, using a Jasco PU-2080 plus an intelligent HPLC pump, Jasco UV-1575 intelligent UV/Vis detector setting at 230 nm, and a Waters 746 Data Module. The mobile phase involved a mixture of phosphate buffer pH 5 and acetonitrile (95:5, v/v), pumped at a flow rate of 1.5 mL/min through the column, a Phenomenex C18 (250 × 4.6 mm).

The amount of AMX in each type of coated beads was determined after extracting the drug from the individual type of beads using sonication. The AMX concentration in each dissolution sample was determined as the percentage of the drug load for each type of sample and expressed as a cumulative percentage of the released AMX. These dissolution tests were performed in triplicate.

Statistical analysis

One-way ANOVA with LSD test using SPSS 10.0 was applied to investigate the difference of the mean values for data obtained from the measurements.

Results and discussion

The mucoadhesion of chitosan, PVP, and chitosan/PVP blends was evaluated using various techniques. Mucoadhesion is a complex phenomenon, and its mechanisms are not clearly understood[25]. The proposed mechanisms for mucoadhesion probably involve one or more of the following: electronic, adsorption, diffusion, and wetting theories[26]. The electronic theory explains that electron transfer between the mucus and the mucoadhesive leads to the formation of a double electric layer at the interface of the mucus and the mucoadhesive, resulting in an attraction between these two components. The adsorption theory proposes that intermolecular bonding, such as hydrogen and van der Waals bonds, causes the attraction forces between the mucus and the mucoadhesive, these forces being higher than those involved in the electronic theory. The diffusion theory envisages the interpenetration and physical entanglement of the mucus and the mucoadhesive. The wetting theory deals with interfacial energy and correlates the surface tension of the mucus and the mucoadhesive with the capability of the mucoadhesive to swell and spread on the mucus layer. In fact, not a singular mechanism, but a combination of these theories is usually applied to describe mucoadhesion.

Viscosity synergism of polymers and mucin

Determination of mucoadhesion by viscosity measurements was performed as previously described by Hassan and Gallo[27]. The interactions between polymers (chitosan, PVP, and chitosan/PVP blends) and mucin resulted in viscosity changes; these changes can be converted into

mechanical energy or work. The viscosity component caused by mucoadhesion (η_{ad}) can be calculated according to Equation (3):

$$\eta_{ad} = \eta_t - \eta_m - \eta_p, \quad (3)$$

where η_t is the viscosity of the system, η_m and η_p are the viscosity of pure mucin and polymer, respectively. All viscosity values were measured at the same temperature and rate of shear. Subsequently, the force of mucoadhesion (F) was determined using Equation (4):

$$F = \eta_{ad}\sigma, \quad (4)$$

where σ is shear rate (per second) and F is the force of mucoadhesion.

The viscosities and mucoadhesion forces of chitosan, PVP, and chitosan/PVP blends are listed in Table 1. The η_{ad} values and forces of mucoadhesion for chitosan/PVP at volume ratio of 5/5 and 3/7 are significantly higher than for the other blends, and for the single polymers ($P < 0.05$). This indicated that the chitosan/PVP blends at these ratios (5/5 and 3/7) are able to interact more strongly with mucin.

In addition, the flow behavior of chitosan, PVP, and chitosan/PVP blends in the presence or the absence of mucin was calculated using the Ostwald–de Waele rheological model or power law model (Equation 5):

$$\tau = K_c\gamma^n, \quad (5)$$

where τ is shear stress, γ shear rate, K_c consistency index, and n the power law index or flow behavior index. The values of K_c and n of all samples were determined using Rheocalc for the Windows version 3.1 software package. The n value describes the deviation from Newtonian behavior. When the value of n is lower than 1, the type of flow is shear thinning (pseudoplastic behavior). The type of flow is shear thickening (dilatant behavior) when n is greater than 1. As n approaches 1, the flow becomes less shear dependent, and the n value equal to 1 indicates Newtonian flow. The values of n and K_c of all samples are listed in Table 2. The n values of all samples are less than 1 indicating that these samples exhibit a non-Newtonian shear thinning behavior. In addition, greater K_c values were observed for chitosan, PVP, and chitosan/PVP blends with mucin, demonstrating good viscosity synergism and interactions between these polymers and mucin.

Texture analysis

Viscosity measurement is a relatively simple method for evaluation of mucoadhesion. However, it does not give any direct information on events occurring at the interface. Texture analysis is based on measuring the force or work required to detach the polymers from the mucus layer on the tissue. Measurement of such force is therefore more useful because texture analysis more closely simulated the in vivo situation[28]. Texture analysis was therefore performed to confirm the mucoadhesion properties of the polymers. For this analysis, the mucoadhesion force of polymers on the porcine stomach tissue was measured, and the work of adhesion was subsequently evaluated. The work of adhesions of chitosan, PVP, and chitosan/PVP blends is shown in Figure 1. The high mucoadhesion of polymer and polymer blends may arise from the entanglement of polymer chains and the mucin molecules, or the formation of non-covalent bonds between the polymers and mucin. The work of adhesion of chitosan/PVP blend at the 5/5 volume ratio is significantly higher than chitosan, PVP, and the other blends ($P < 0.05$). PVP shows the lowest mucoadhesion ($P < 0.05$), which is consistent with the results obtained from viscosity evaluation.

Wash-off test of uncoated and coated AMX-alginate beads

The data for the percentage attachment of uncoated and coated AMX-alginate beads to the mucosa at pH 4.0 are presented in Figure 2. Even though not statistically significant, it was found that the beads coated with chitosan/PVP at a 5/5 volume ratio exhibited the slowest wash-off after 3 hours. The percentage of coated beads still attached to the mucosa is higher than for the uncoated beads ($P < 0.05$). The results of the wash-off test indicated that beads coated with chitosan, PVP, and

Table 1. Viscosity of a 9.38% (w/v) mucin solution (η_m) plus 0.50% (w/v) polymer solution (η_p), the system (η_t), and viscosity due to mucoadhesion (η_{ad}) as well as the force of mucoadhesion (F) of chitosan (C), PVP (P), and C/P blends at various volume ratios at 25°C using a shear rate of 15.84 per second (mean ± SD, $n = 3$).

	Viscosity			F
Mucin + polymer	$\eta_m + \eta_p$	η_t	η_{ad}	(dyne/cm)
C	120.78 ± 10.42	136.19 ± 9.72	15.41 ± 0.41	2.44 ± 0.11
C9P1	131.39 ± 0.14	145.40 ± 0.05	13.98 ± 0.05	2.21 ± 0.08
C7P3	129.14 ± 0.29	143.78 ± 0.03	14.66 ± 0.03	2.32 ± 0.04
C5P5	127.39 ± 0.29	154.82 ± 0.07	27.46 ± 0.07	4.35 ± 0.11
C3P7	126.30 ± 0.14	151.42 ± 0.19	25.12 ± 0.19	3.98 ± 0.30
C1P9	124.22 ± 0.25	140.24 ± 0.30	16.06 ± 0.30	2.54 ± 0.48
P	122.72 ± 0.02	130.58 ± 0.09	7.82 ± 0.09	1.23 ± 0.14

Table 2. Power law index (n) and consistency index (K_c) derived from Equation (5) of chitosan (C), PVP (P), and C/P blends at various volume ratios with and without mucin (mean ± SD, $n = 3$).

	Flow behavior index, n		Consistency index (K_c)	
Sample	Polymer	Polymer + Mucin	Polymer	Polymer + Mucin
Mucin	–	0.70 ± 0.04	–	251.26 ± 32.94
C	0.68 ± 0.01	0.58 ± 0.05	35.32 ± 1.29	419.19 ± 81.90
C9P1	0.59 ± 0.01	0.58 ± 0.08	44.61 ± 0.98	423.28 ± 120.80
C7P3	0.60 ± 0.01	0.64 ± 0.01	36.44 ± 1.62	335.65 ± 7.29
C5P5	0.38 ± 0.01	0.61 ± 0.01	66.86 ± 3.18	376.68 ± 8.30
C3P7	0.51 ± 0.02	0.57 ± 0.05	36.57 ± 3.17	438.38 ± 86.77
C1P9	0.33 ± 0.02	0.62 ± 0.07	57.82 ± 4.04	366.78 ± 91.74
P	0.63 ± 0.02	0.65 ± 0.05	13.95 ± 0.69	316.44 ± 63.34

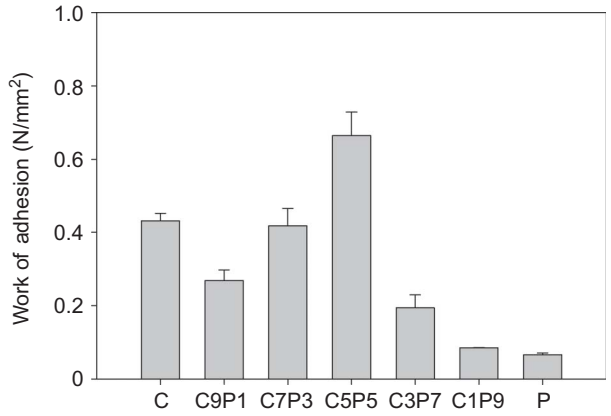

Figure 1. The work of adhesion for chitosan (C), PVP (P), and C/P blends at various volume ratios on porcine stomach tissue (mean ± SD, $n = 6$–10).

their blends have relatively strong mucoadhesive properties. Therefore, beads coated with these single polymers and their blends may be able to provide the necessary gastroretention to facilitate local drug availability in the stomach for a sufficient length of time.

Cell adhesion study

Because HT29 cells are not covered with a mucus layer, the adherence of polymers to this monolayer may be considered as bioadhesion[29]. Attachment of HT29 cells to chitosan, PVP, and chitosan/PVP blends is shown in Figure 3. The single polymers or the blends exhibit higher bioadhesion than the controls (without polymer). The chitosan/PVP blend at 5/5 volume ratio showed the highest cell attachment ($P < 0.05$). Thus, this blend demonstrates both highest mucoadhesion (adherence to mucus) and highest bioadhesion (adherence to epithelium), compared with the single polymers or other blends. This blend is therefore likely to be in intimate contact with biological tissue for an extended period of time and should be able to increase its gastrointestinal residence time even in the presence of mucus turnover.

Molecular interaction study

DRIFTS was used to investigate the interactions between chitosan and PVP in the blend, and between chitosan, PVP, or chitosan/PVP blends and mucin. In view of the large numbers of samples and spectra, only FTIR analyses of chitosan, PVP, and chitosan/PVP blend at 5/5 volume

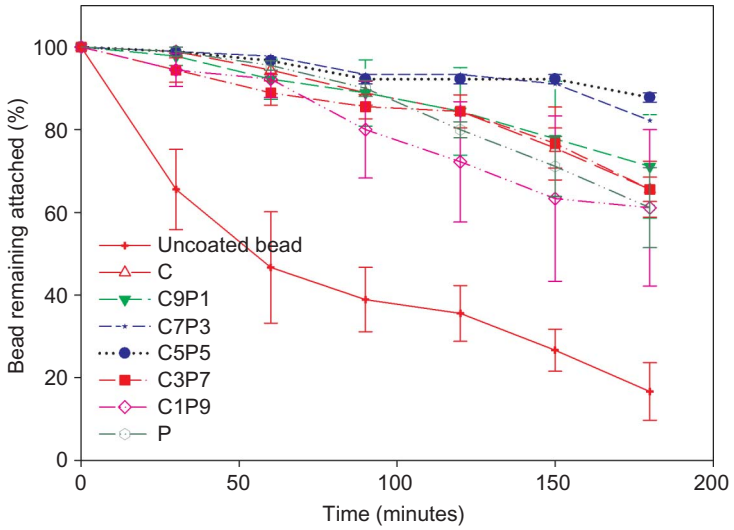

Figure 2. In vitro wash-off test for uncoated beads and coated beads with chitosan (C), PVP (P), and C/P blends at various volume ratios (mean ± SD, $n = 3$).

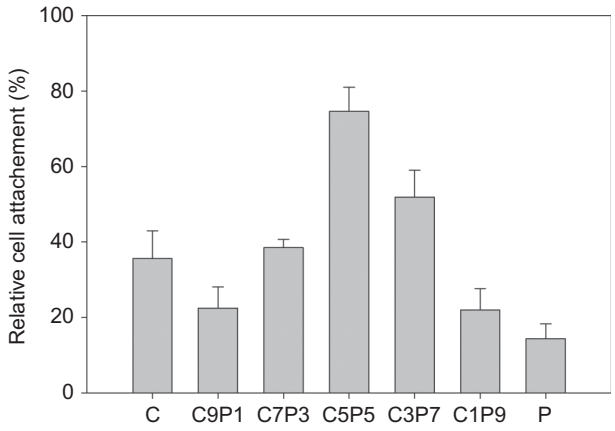

Figure 3. Percentage relative HT29 cell attachment of chitosan (C), PVP (P), and C/P blends at various volume ratios (mean ± SD, $n = 3$).

ratio (the blend that has the strongest interaction with mucin) with and without mucin are reported here. DRIFTS spectra of these samples are shown in Figure 4a. DRIFTS spectra for the other blends with and without mucin showed the same trends as for the blend at 5/5 volume ratio (data not shown). As shown in Figure 4a, the O–H and N–H stretching vibrations of mucin and chitosan show broad bands at 3500–3100 cm^{-1}; this makes spectral interpretation at this region difficult. However, prominent peaks observed in the region of 1600–1700 cm^{-1} can be used for assessing possible interactions. Mucin is comprised of a peptide backbone, consisting of alternating oligosaccharides that terminate with sialic acid and sulfate groups[30]. The DRIFTS spectrum of mucin showed a broad C=O stretching band of an amide band at 1692.5 cm^{-1}. The spectra of chitosan and PVP show an amino band and a C=O peak at 1650.8 and 1697.8 cm^{-1}, respectively (Figure 4b). The blend of chitosan/PVP at 5/5 volume ratio displayed a peak at 1685.7 cm^{-1} (Figure 4b). This peak is due to the interaction between C=O of PVP and H–O or N–H of chitosan, as previously reported[31]. Because of different methods of sample preparation, the positions of these peaks are slightly different. The band at 1685.7 cm^{-1} was shifted to 1696.1 cm^{-1} (Figure 4c) when this blend was mixed with mucin. This indicates that there are interactions between the chitosan/PVP blend and mucin. The mixture of PVP and mucin displayed a new peak at 1696.4 cm^{-1} (Figure 4d); this peak is relatively broad compared with that of PVP. The mixture of chitosan and mucin also displays a new peak at 1689.3 cm^{-1} (Figure 4e). These peaks are not the combined spectra of PVP and mucin, or chitosan and mucin. This indicates that there are interactions between the PVP–mucin and the chitosan–mucin. Mucoadhesive interactions may be due to hydrogen bonding between mucin and chitosan, PVP, or the blends through their various hydrogen donor and acceptor groups. The shift of the C=O band of PVP indicates that this group is involved in an intermolecular hydrogen

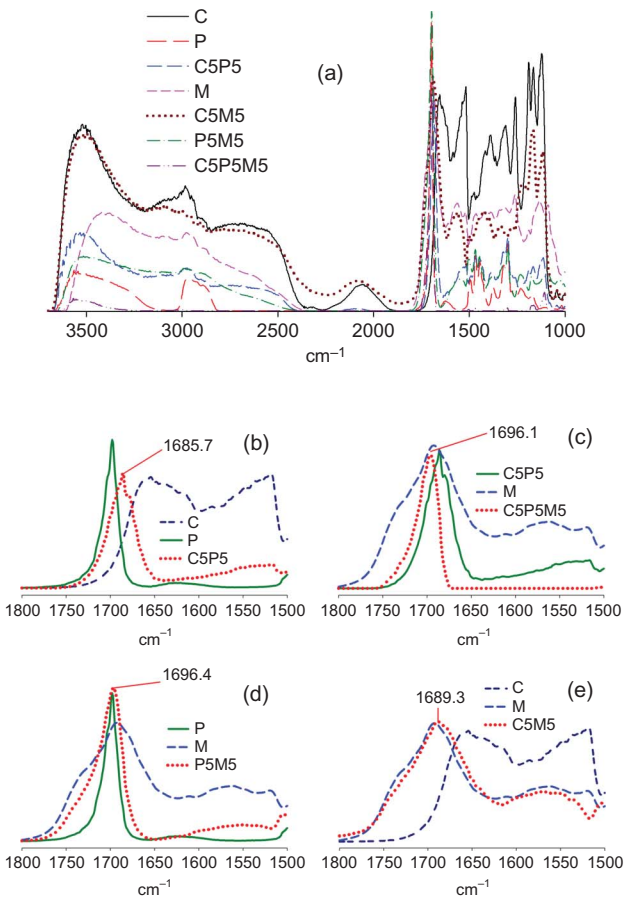

Figure 4. DRIFTS spectra of (a) chitosan (C), PVP (P), mucin (M), C5P5, C5M5 P5M5, C5P5M5; and the enlarged spectra of (b) C5P5; (c) C5P5M5; (d) P5M5, and (e) C5M5.

bond with mucin. In case of chitosan, the electrostatic interaction between the amine function of chitosan and sialic acid or sulfonated residues of mucin may be possible. Nevertheless, due to the fact that most functional groups of these molecules cannot be observed on DRIFTS spectra, it is not possible to indicate the other specific key sites for these intermolecular interactions. Undoubtedly, DRIFTS can indicate that there are interactions between polymers, or polymer blends and mucin and these interactions may produce mucoadhesion as demonstrated in the above-mentioned analyses.

Uncoated and coated bead morphology

The diameter of uncoated AMX-alginate beads measured with a Beckman Coulter LS230 (Vankel Industries, Edison, NJ, USA) equipped with a Small-Volume Module Plus and Beckman Coulter Particle Characterization software version 3.29 (USA) was 1.23 ± 0.25 mm. SEM micrographs of uncoated and coated AMX-alginate beads are shown in Figure 5. The uncoated beads had a rough surface (Figure 5a and b). The surfaces of beads coated with chitosan/PVP blend, chitosan, and PVP were smoother than the uncoated beads (Figure 5c–i).

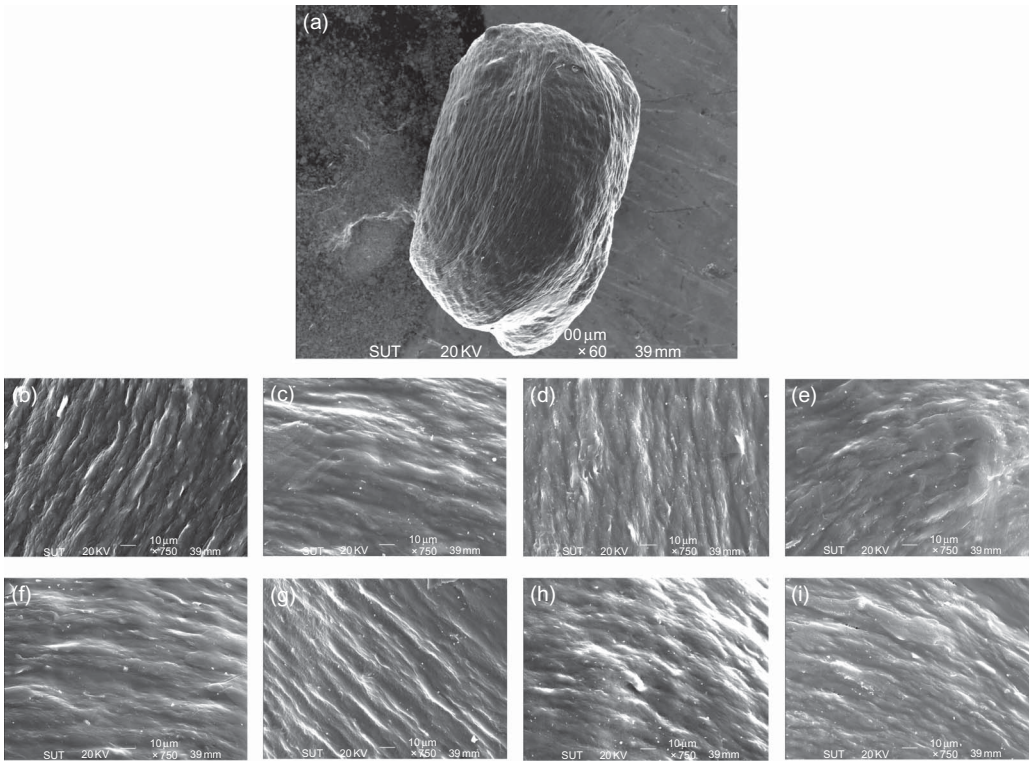

Figure 5. SEM micrographs of AMX beads: uncoated bead (a, b); chitosan/PVP-coated beads at the volume ratios of 1/9 (c); 3/7 (d); 5/5 (e); 7/3 (f); 9/1 (g); chitosan-coated bead (h); and PVP-coated bead (i).

There were no visible porous characteristics for uncoated and coated beads (Figure 5a–i).

Swelling studies

The swelling behavior of the uncoated and coated AMX-alginate beads in phosphate buffer (pH 4) (Figure 6) shows that all beads demonstrate significant swelling. The swelling of coated beads was higher than that of uncoated beads ($P < 0.05$). The swelling of beads coated with chitosan, PVP, or chitosan/PVP blends was similar.

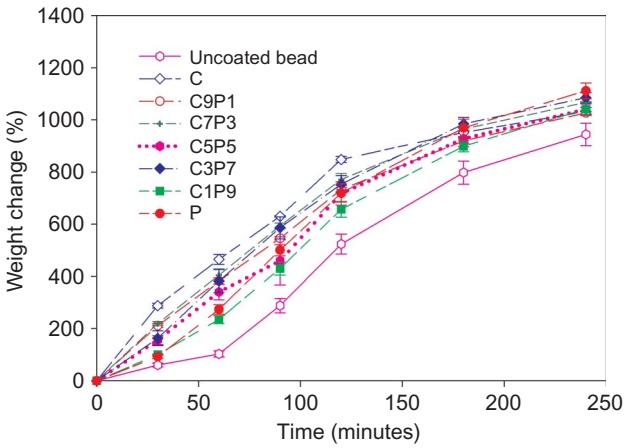

Figure 6. Swelling profiles of uncoated dried beads and dried beads coated with chitosan (C), PVP (P), and C/P blends at various volume ratios at pH 4 (mean ± SD, $n = 3$).

The hydration of the hydrophilic groups of chitosan and PVP may induce higher swelling of the coated beads compared with uncoated beads, as has been previously observed for chitosan-coated dried beads[22].

Drug release study

The drug loading for the AMX bead preparation, calculated for the uncoated beads, was 76.49%. Commonly, proton pump inhibitors or H_2-receptor antagonists such as lansoprazole, omeprazole, or ranitidine are used in combination with antibiotics for eradication of *H. pylori*[32,33]. These agents can raise the gastric pH to 3–5[34–36]. Hence, dissolution studies were investigated at pH 4. AMX release from uncoated and coated beads was compared with that from an AMX powder as shown in Figure 7a. AMX release profiles from beads showed sustained release characteristics when compared with the release from the AMX powder. The AMX release profiles for uncoated and coated beads were not statistically different ($P > 0.05$). The release of AMX from these beads was complete within 2.5 hours. The release kinetics of AMX from the beads were evaluated using the Korsmeyer–Peppas equation or power law[37]:

$$\frac{M_t}{M_\infty} = \mathrm{k}t^n \qquad (5)$$

where M_t and M_∞ are the cumulative amounts of drug released at time t and infinite time, respectively, k is the

release constant, and n is the release exponent characterizing the release mechanism. This equation is used for examining the first 60% of a release curve. The n and k values were determined from the slope and intercept, respectively, of the fitting straight line of log (M_t/M_∞) versus log (t) plots. Based on the n values, the drug transport may be classified as Fickian diffusion (Case I), Case II transport (zero order), non-Fickian (anomalous) transport, and Super Case II transport[38]. As listed in Table 3, the n values were higher than 1 indicating that the release process is Super Case II transport. This type of transport has been observed for alginate beads[39]. This transport mechanism may be caused by rapid swelling of the beads. This was in agreement with the swelling studies that showed high swelling occurring in the beads. As a result of the high swelling of these uncoated or coated dried alginate beads, the coated materials have not much influence on the release of AMX from the beads. Thus for these dried beads, the release profiles of uncoated or coated beads were similar. These phenomena were previously observed for coated dried beads[20,22]. According to the wash-off test and the cell adhesion study, these coated beads have a reasonably strong muco/bioadhesion. A high percentage of coated beads, 84.4 ± 7.17, 78.25 ± 10.36, and 70.63 ± 10.50, still remained adhering to the mucosa at 2, 2.5, and 3 hours, respectively (Figure 2). For uncoated beads only 46.67%, 38.89%, 35.56%, 26.67%, and 16.67% remained adhering to the mucosa at 1, 1.5, 2, 2.5, and 3 hours, respectively. Thus, although AMX release from uncoated and coated beads was somewhat similar, the coated beads are able to be retained longer in the stomach than the uncoated beads. Consequently, the longer presence of the drug released from these coated beads may be able to eradicate *H. pylori* infections.

To investigate the effect of pH on the release of AMX from the beads, the dissolution test was also performed at pH 1.2. The dissolution of AMX was actually rapid at pH 1.2, at the first sampling time point (6 minutes), 99 ± 2.41% of AMX powder was detected. The release of AMX from the uncoated and coated beads is faster at pH 1.2 compared with that at pH 4 (Figure 7a and b). In 30 minutes, about 90% and 82% of AMX was released from uncoated and coated beads, respectively, at pH 1.2; but only 32% and 26% was released from uncoated and coated beads, respectively, at pH 4. Interestingly, at pH 1.2, AMX release from all coated beads was significantly less than that observed for the uncoated beads in the first 18 minutes ($P < 0.05$); this is probably due to the lower swelling of the beads at this pH. According to the measurement of the bead sizes at the end of the dissolution tests, the size of the swollen beads was about 1.58 ± 0.25 and 3.24 ± 0.21 mm at pH 1.2 and 4, respectively. As a result of the lower swelling of the beads at pH 1.2, the coated layer may not be destroyed during the early stage of the test, and these coatings could control and retard the release of AMX from the beads. The rapid release of AMX with the change in medium pH from 4 to 1.2 is probably due to the high solubility of AMX at in the lower pH of the medium.

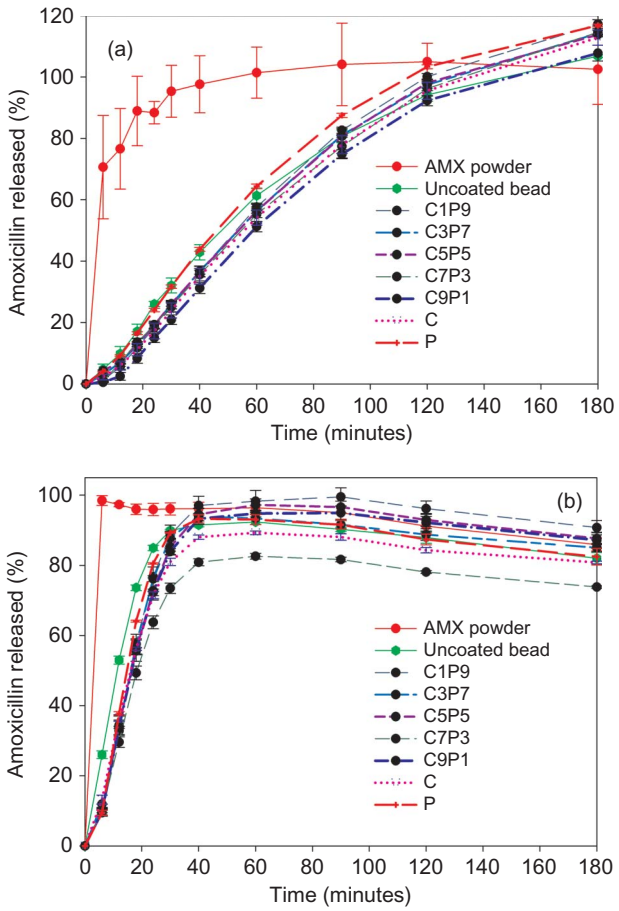

Figure 7. Dissolution profiles at pH 4 (a) and pH 1.2 (b) of AMX from powder, uncoated alginate beads, and beads coated with chitosan (C), PVP (P), and C/P blends at various volume ratios each loaded with AMX (mean ± SD, $n = 3$).

Table 3. Kinetic analysis of the release data of amoxicillin derived from Equation (6) for coated amoxicillin-alginate beads with chitosan (C), PVP (P), and C/P blends at various volume ratios.

Coating materials	n	k (minute^{-1})	r^2
C	1.4762	0.0016	0.9996
C9P1	1.6393	0.0008	0.9905
C7P3	1.3478	0.0026	0.9927
C5P5	1.1640	0.0047	0.9835
C3P7	1.3500	0.0026	0.9990
C1P9	1.5562	0.0012	0.9939
P	1.2774	0.0040	0.9990

Conclusion

This study has demonstrated that chitosan, PVP, and chitosan/PVP blends exhibit muco/bioadhesive properties. However, chitosan/PVP at a 5/5 volume ratio produced the best muco/bioadhesion compared with the

other blends. These polymers and their blends when coated onto alginate beads resulted in significantly high mucoadhesion compared with the uncoated beads. Therefore, the alginate beads coated with these materials might have potential for being utilized to increase the gastroretentive time of various drugs through muco- and bioadhesion. Coated AMX-alginate beads may be useful for more effective eradication of *H. pylori* infections, especially because it has previously been demonstrated that a 1-hour contact with antibiotics can effectively remove these microorganisms.

Declaration of interest

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