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# Mucoadhesive microspheres prepared by interpolymer complexation and solvent diffusion method

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**Abstract**

Mucoadhesive microspheres were prepared to increase gastric residence time using an interpolymer complexation of poly(acrylic acid) (PAA) with poly(vinyl pyrrolidone) (PVP) and a solvent diffusion method. The complexation between poly(acrylic acid) and poly(vinyl pyrrolidone) as a result of hydrogen bonding was confirmed by the shift in the carbonyl absorption bands of poly(acrylic acid) using FT-IR. A mixture of ethanol/water was used as the internal phase, corn oil was used as the external phase of emulsion, and span 80 was used as the surfactant. Spherical microspheres were prepared and the inside of the microspheres was completely filled. The optimum solvent ratio of the internal phase (ethanol/water) was 8/2 and 7/3, and the particle size increased as the content of water was increased. The mean particle size increased with the increase in polymer concentration. The adhesive force of microspheres was equivalent to that of Carbopol. The release rate of acetaminophen from the complex microspheres was slower than the PVP microspheres at pH 2.0 and 6.8.


*Keywords:* Poly(acrylic acid); Poly(vinyl pyrrolidone); Mucoadhesive microsphere; Gastric residence time

## 1. Introduction

In order to develop oral drug delivery systems, it is necessary to optimize both the residence time of the system in the gastrointestinal (GI) tract and the release rate of the active ingredient from the system. One of the most extensively studied methods for prolonging the residence time in the GI tract is using mucoadhesive polymers that adhere to the mucus layer and release the loaded drug in a sustained manner (Lueßen et al., 1994; Wang et al., 2001). The intimate contact of the mucoadhesive polymer with the mucous surface can result in an increased drug retention time and drug concentration in the GI tract (Wang et al., 2001). This should have

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an improved therapeutic effect for the gastric diseases (Wang et al., 2000; Nagahara et al., 1998). Moreover, mucoadhesive dosage forms have also been reported to improve the absorption and systemic bioavailability of the drugs that are normally poorly absorbed (Nagai and Machida, 1985). While the GI residence time of some of the mucoadhesive dosage forms was not extended in vivo (Khosla and Davis, 1987; Harris et al., 1990), various mucoadhesive microspheres have been successful in extending the GI residence time (Wang et al., 2001; Nagahara et al., 1998). When the mucoadhesive dosage form is administered in either tablet or capsule form, they may or may not adhere to the mucous surface due to the weight of the dosage form and the vigorous movement of the GI tract, resulting in a large variation. However, the mucoadhesive microspheres have some advantages. These include a light weight and a smaller dose variations due to the large number of microspheres administered.

The widely studied mucoadhesive materials include chitosan, hydroxypropyl cellulose, poly(acrylic acid) (PAA) and their derivatives. Although, PAA is considered to be one of the best mucoadhesive polymers, the high water solubility of PAA critically limits its use as a carrier for the sustained release of a drug. PAA based interpolymer complexation (Choi et al., 1999; Chun et al., 2001, 2002a,b) has been examined in order to reduce the water solubility of PAA. In those studies, it was shown that the water solubility of PAA could be reduced and the adhesive force could be maintained via the complexation of the PAA with proton accepting polymers such as poly(ethylene glycol), poly(ethylene glycol) macromer, poloxamer and poly(vinyl pyrrolidone) (PVP) (Choi et al., 1999; Chun et al., 2001, 2002a,b). It was also observed that PAA and PVP aggregate and precipitate in ethanol and water in a relatively short period of time, resulting in the formation of a PVP/PAA interpolymer complex, suggesting that the intensity of hydrogen bonding between PAA and PVP is quite strong. It was believed that this strong complexation could be utilized to prepare mucoadhesive microspheres. Each component is soluble in water. However, when they come together they form a complex and precipitate. If a PAA solution and PVP solution can be emulsified and droplets of each emulsion collides afterwards, complexation should occur, which will solidify to form microspheres.

This study investigated the effect of various preparation parameters on the formation and morphology of microspheres and optimized the preparation conditions for microspheres. In addition, the release of a drug from the prepared mucoadhesive microspheres was examined.

## 2. Materials and methods

### 2.1. Materials

The PVP (MW: 42,500) was obtained from BASF (Ludwigshafen, Germany). The PAA (MW: 450,000) was purchased from Aldrich (Milwaukee, WI). Sorbitan monooleate (span 80) was purchased from Junsei Chemical (Tokyo, Japan). Corn oil was acquired from CJ Corporation (Seoul, Korea). All other chemicals were of reagent grade available commercially.

### 2.2. Methods

#### 2.2.1. Preparation of mucoadhesive microspheres

The mucoadhesive microspheres were prepared by interpolymer complexation and solvent diffusion method. PAA (0.2 g) was dissolved in 4.8 g of ethanol/water (7/3, w/w) mixture and PVP (0.32 g) was dissolved in 1 g of ethanol/water (7/3, w/w) mixture unless otherwise specified. When two solutions were combined together, the concentration of polymer, PAA and PVP, was 8.2%. Using a syringe, the PAA solution and PVP solution were sequentially dropped into 200 ml of corn oil, which was used as the external phase. The external phase contained 0.04% v/v of span 80 (sorbitan monooleate) as a surfactant. They were stirred with a magnetic bar at 500 rpm at an ambient temperature over 36 h. The microspheres were gradually hardened and the hardened microspheres were collected by filtration. They were washed several times with *n*-hexane and dried at 80 °C over 12 h. The yield was calculated by dividing the weight of the collected microspheres by the total weight of all the non-volatile components used for preparing the microspheres.

In order to examine the effect of the amount of internal phase used, the effect of the solvent ratio of the internal phase, the surfactant concentration, and the polymer concentration on the formation of microspheres, each relevant variable was changed with other variables

fixed as previously described. The effect of the stirring speed on the formation of the microspheres was investigated using stirring speeds of 300, 400 and 500 rpm.

In order to prepare acetaminophen-loaded microspheres, acetaminophen was dissolved in PAA solution and PVP solution, respectively. The concentration of acetaminophen in each polymer solution was 5% of the polymer used.

In order to prepare PVP microspheres, PVP (8.2%) and drug (5%) were dissolved in ethanol/water mixture (7/3, w/w). PVP microspheres were prepared in the same way as described previously.

*2.2.2. IR spectroscopy*

The infrared absorption spectra of the PAA, PVP, and PAA/PVP complex microspheres were obtained using a FT-IR spectrophotometer (FT-IR 401, Jasco, Tokyo, Japan). The samples were pressed into a pellet before measuring their infrared absorption spectra. To prepare the pellets, a few milligrams of the sample were ground together in a mortar with about 100 times the quantity of KBr. The finely ground powder was introduced into a stainless steel die. The powder was then pressed in the die between polished stainless steel anvils at a pressure of about 10 t/in.$^2$.

*2.2.3. Particle size analysis*

The mean particle size of the microspheres was measured using a particle size analyzer (HELOS/BF, Sympatec GmbH, Germany).

*2.2.4. Measurement of adhesive force*

A motor driven auto peeling tester (C.K. Trading Co., Korea) was used to measure the adhesive force of the PVP/PAA interpolymer microspheres and Carbopol 971 to a plastic (polypropylene) plate. The microspheres were pressed into a tablet before measuring adhesive force. The specimens, discs with the area of 1.33 cm$^2$, were wetted with pH 2.0 HCl solution at room temperature for 15 s before testing, and then placed between two plastic plates. The plates subjected to a pressure of 1.2 N/cm$^2$ for 60 s before measurements were made. The peak force required to detach the disc from the plastic plate was measured.

*2.2.5. Morphology*

The morphology of the microspheres was examined by field emission scanning electron microscopy (S-4700, Hitachi, Japan). The sample was mounted onto an aluminum stub and sputter-coated for 120 s with platinum particles in an argon atmosphere.

*2.2.6. Release of acetaminophen from the microspheres*

The drug release test was carried out using a dissolution tester (DST 810, LABFINE, Inc., Korea). The PAA/PVP complex or PVP microspheres loaded with acetaminophen, 60 mg was placed in 500 ml of a release medium and stirred at 100 rpm at 37 °C. The release media tested were pH 2.0 HCl solution and pH 6.8 phosphate buffer solution. An aliquot of the release medium was withdrawn at predetermined time intervals and an equivalent amount of fresh medium was added to the release medium. The collected samples were filtered through a 0.45 μm-syringe filter, and analyzed by HPLC (Shimadzu Scientific Instruments, MD, USA), consisting of a UV detector (SPD-10A), a pump (LC-10AD), and an automatic injector (SIL-10A), in order to determine the amount of acetaminophen released from the microspheres. The wavelength of the UV detector was 254 nm and a reversed-phase column (Luna 5 μm C8, Phenomenox, USA) was used. The column temperature was maintained at 30 °C. The flow rate was 1 ml/min, and the mobile phase was acetonitrile/water (30/70).

## 3. Results and discussion

### *3.1. Preparation of PAA/PVP complex microspheres*

PAA and PVP are known to form a complex in an aqueous solution and in some organic solvents such as ethanol (Chun et al., 2002a). Once they form a complex, the aqueous solubility greatly decreases without losing the mucoadhesive properties of PAA and the complex formed precipitates in the solution. This principle was utilized to prepare the mucoadhesive microspheres. The PAA solution and the PVP solution were sequentially dropped and dispersed in corn oil. Corn oil was chosen as the external phase because the ethanol/water mixture as an internal phase is not miscible with corn oil and the complex is not soluble in it. As the dispersed droplets of the PAA solution collided with those of the PVP solution in corn oil, they formed an interpolymer complex. The droplets of the PAA/PVP complex grad-

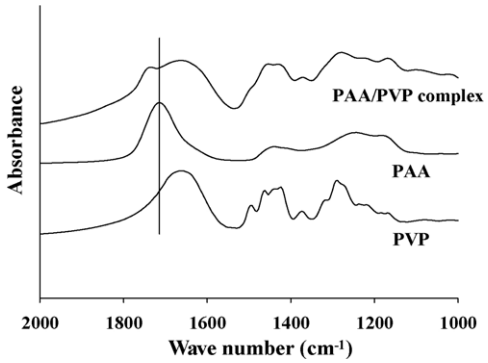


Fig. 1. FT-IR spectroscopy of the PAA/PVP complex microspheres, PAA or PVP.

ually solidified and hardened as the ethanol and water diffused out of the internal phase.

The complexation between the PAA and PVP via hydrogen bonding was confirmed by a shift in the carbonyl absorption bands of PAA using FT-IR. The PAA alone has a band at 1716.3 $cm^{-1}$ due to intramolecular hydrogen bonding of the carboxyl group of PAA. However, some of the intramolecular hydrogen bonding breaks when PAA and PVP forms an interpolymer complex, since new hydrogen bonds are formed between the carboxyl groups of PAA and the carbonyl groups of PVP. Therefore, once an interpolymer complex has formed, the carbonyl absorption band of PAA shifts to a higher wave number (Chun et al., 2002a; Baranovsky et al., 1992). The FT-IR spectrum of the prepared microspheres showed that the carbonyl absorption band of PAA at 1716.3 $cm^{-1}$ had shifted to a higher wave number at 1735.6 $cm^{-1}$ (Fig. 1). These results suggest that mucoadhesive microspheres were formed by an interpolymer complexation between PVP and PAA.

In order to identify the optimum preparation conditions, the effects of the various experimental parameters, such as the internal phase volume fraction, the solvent ratio of the internal phase, the surfactant concentration, the polymer concentration, and the stirring speed, on the formation of microspheres were investigated.

### 3.2. Effect of internal phase volume fraction

The volume fractions of the internal phase tested were 2.2, 2.9, 4.8, and 7.8% of the internal phase with the PAA and PVP concentrations in the internal phase being kept constant. The yield of the PAA/PVP complex microspheres formation is shown in Table 1. At an internal phase volume fraction of 2.2 and 2.9%, the yield was relatively good. In the case of 4.8 and 7.8%, the yields abruptly decreased and the degree of aggregation increased. As the volume fraction of the internal phase was increased, the medium became densely populated with internal droplets. Until ethanol and water inside the droplets completely diffused out and evaporated, the formed microspheres would be sticky. Due to the dense population and sticky properties of the droplets, they have a greater chance of coming in contact with each other and aggregating. Therefore, as the volume fraction of the internal phase exceeds the optimal range, larger droplets tend to aggregate and smaller

Table 1
Effect of various parameters on the formation and mean particle size of the microspheres ($n = 3$)

| Processing parameter | Mean particle size (μm) (mean ± S.D.)[a] | Yield (%) (mean ± S.D.)[a] |
|---|---|---|
| Internal phase volume fraction | | |
| 2.2% | 109.5 ± 4.8 | 73.9 ± 2.7 |
| 2.9% | 73.3 ± 13.8 | 82.9 ± 0.8 |
| 4.8% | 36.9 ± 3.4 | 28.3 ± 2.2 |
| 7.8% | – | – |
| Solvent ratio of internal phase (ethanol/water) | | |
| 9/1 | – | – |
| 8/2 | 66.1 ± 6.3 | 82.7 ± 0.7 |
| 7/3 | 73.3 ± 13.8 | 82.9 ± 0.8 |
| 6/4 | 86.5 ± 5.6 | 66.7 ± 1.8 |
| 5/5 | 61.2 ± 14.7 | 40.7 ± 0.8 |
| 4/6 | – | – |
| Surfactant (span 80) concentration | | |
| 0% | 72.4 ± 9.4 | 78.2 ± 5.6 |
| 0.04% | 73.3 ± 13.8 | 82.9 ± 0.8 |
| 0.1% | 73.8 ± 7.6 | 81.5 ± 1.6 |
| 0.2% | 78.7 ± 14.6 | 57.6 ± 21.1 |
| 0.4% | 69.4 ± 5.1 | 59.7 ± 14.9 |
| Polymer concentration | | |
| 3% | 33.1 ± 2.4 | 38.4 ± 9.8 |
| 5% | 50.8 ± 3.1 | 63.4 ± 9.8 |
| 8.2% | 73.3 ± 13.8 | 82.9 ± 0.8 |
| 10% | 93.7 ± 6.5 | 80.8 ± 1.6 |
| Stirring speed | | |
| 300 rpm | 147.9 ± 28.3 | 77.1 ± 5.9 |
| 400 rpm | 86.6 ± 31.0 | 83.6 ± 0.5 |
| 500 rpm | 73.3 ± 13.8 | 90.3 ± 0.7 |

[a] Standard deviation.

droplets form microspheres, resulting in a smaller particle size.

### 3.3. Effect of solvent ratios of internal phase

The effects of the various solvent ratios of the internal phase (ethanol/water) on the formation and the particle size of microspheres were investigated (Table 1). Over an ethanol/water ratio of 9/1 as well as below a ratio of 4/6, the microspheres hardly formed. At the ratio of 5/5, the microspheres showed irregular shapes and some of them were aggregated, as shown in Fig. 2. However, at ratios of 6/4, 7/3 and 8/2, most of the microspheres formed showed spherical shape. The content of water in the internal phase appeared to play a key role in the formation of microspheres. After ethanol preferentially diffused out into the corn oil phase, water mainly constitutes the core of the emulsion droplets. As a result, the water content directly affects the solidification time of the microspheres. When the ethanol/water ratio was below 5/5, the solidification time of the microspheres increased due to the relatively large amount of water, which increased the collision frequency between the incompletely solidified microspheres with adhesive property in the wet state. As a result, the degree of aggregation increased and the yield of microspheres decreased. As the ethanol/water ratio increased, the mean particle size of the microspheres decreased. After the ethanol preferentially diffused out into the corn oil phase, water became the major constituent of the core of the emulsion droplets. Meanwhile, a polymer complex formed between PVP and PAA solidifies within the water and the size of the water droplet determined the size of the microspheres. When pure ethanol was used as an internal phase solvent, it spread over the surface of corn oil as soon as the ethanol solution was dropped into the corn oil and formed a film as the ethanol quickly evaporated from the surface of the corn oil. At an ethanol/water ratio of 9/1, the drops temporarily submerged into the corn oil and then floated to the surface immediately after. Subsequently, they either adhered to the wall of the beaker or aggregated.

### 3.4. Effect of surfactant concentration

The effects of the surfactant (span 80) concentration in the corn oil on the formation of the microspheres and

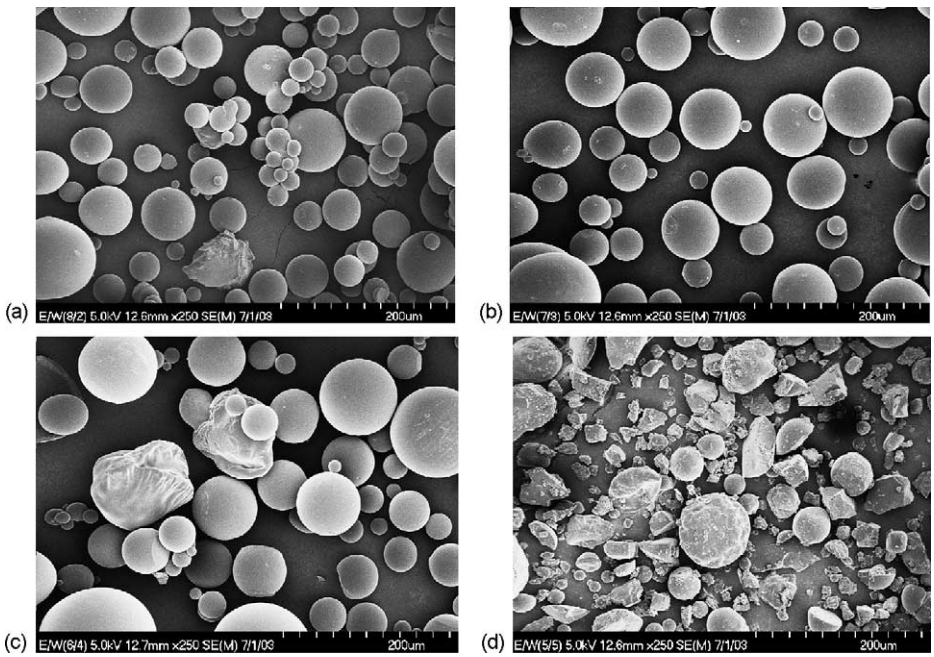


Fig. 2. Scanning electron micrographs of the microspheres as a function of the solvent ratios of the internal phase (ethanol/water): 8/2 (a), 7/3 (b), 6/4 (c) and 5/5 (d).

the particle size are shown in Table 1. No significant difference in the mean particle size was observed at different surfactant concentrations used. However, when 0.2 or 0.4% of span 80 was used, large aggregations were formed and the yield was low. As the concentration of span 80 increased to beyond 0.1%, the internal phase droplets tend to rise to the surface of the corn oil and they aggregated as soon as the ethanol had quickly evaporated from the surface. When 0 or 0.1% span 80 was used, some broken microspheres and microparticles formed, as shown in Fig. 3. Since it is difficult to separate microparticles from microspheres, the microparticles are included in the yield measurement. Therefore, the actual yield of the microspheres would be much lower than the measured value in the case where 0 or 0.1% span 80 was used. When 0.04% span 80 was used, the morphology of the microspheres was spherical and only a minimal amount of microparticles was formed.

### 3.5. Effect of polymer concentration

The effect of the polymer concentration on the formation of microspheres and the mean particle size is

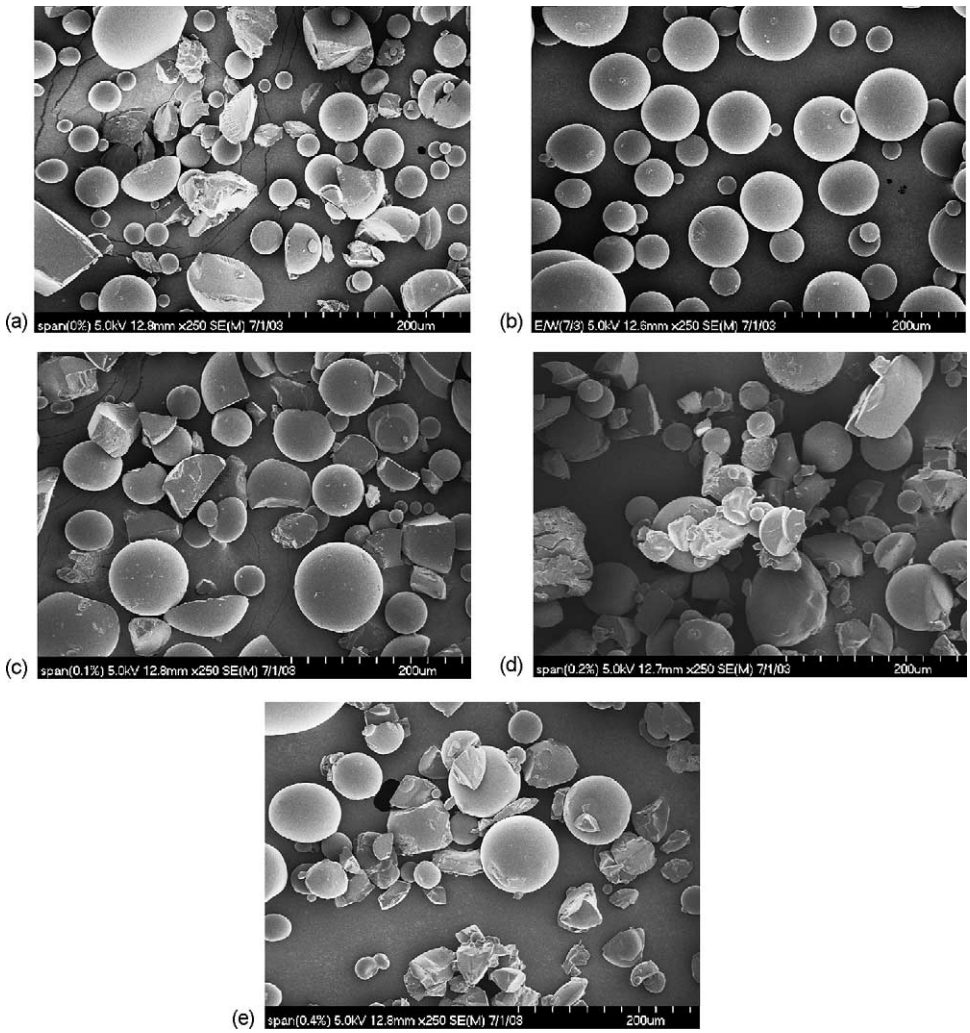


Fig. 3. Scanning electron micrographs of the microspheres as a function of the surfactant (span 80) concentration: 0% (a), 0.04% (b), 0.1% (c), 0.2% (d) and 0.4% (e).

shown in Table 1. The polymer (PAA and PVP) concentrations tested include 3, 5, 8.2 and 10%. The final internal phase volume fraction was kept constant (2.9%). The mean particle size increased with increasing polymer concentration. The higher the concentration of the polymer in the internal phase, the more polymers would be present in each droplet of the internal phase. The higher polymer content in a droplet will result in a larger particle size and a thicker wall. Similar results were observed when the microspheres were prepared using an acrylic polymer (Lee et al., 1999). Another reason for the increased diameter with the higher polymer concentration is that the viscosity of the internal phase increases with increasing polymer concentration. As the viscosity of the internal phase increases, it will be more difficult to break up the droplets to a smaller size and larger droplet will be formed. It was also suggested in the literature that the stirring efficiency was reduced due to the increased viscosity with increased polymer concentration, resulting in an increase in the particle size (Singh and Udupa, 1997). In the case of the 3 and 5% of polymer concentration, some microparticles were observed, as shown in Fig. 4. It appeared that if the amount of polymer in each droplet was not sufficient, the microspheres formed would not have insufficient strength to withstand the stirring, and some of the formed microspheres were broken down.

### 3.6. Effect of stirring speed

Table 1 shows the effect of the stirring speed on the formation of microspheres and particle size. As the stirring speed increased, the mean particle size decreased. Stirring provides the energy to break up the emulsion droplets and it is obvious that smaller droplets will be formed as the stirring speed increases. The particle size was significantly reduced as the stirring speed increased from 300 to 500 rpm. However, the stirring speed did not alter the morphology of the microspheres.

### 3.7. Adhesive force

The adhesive force of PVP/PAA complex microspheres was compared with that of Carbopol 971 by determining the force required to break the contact between the plastic plate and the PVP/PAA complex mi-

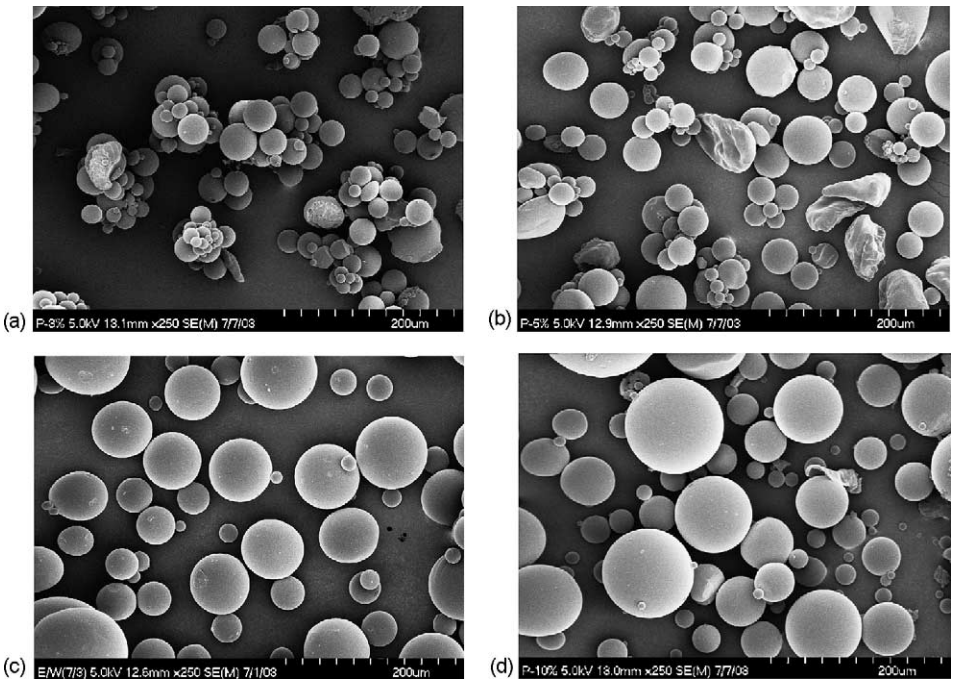


Fig. 4. Scanning electron micrographs of the microspheres as a function of the polymer concentration: 3% (a), 5% (b), 8.2% (c) and 10% (d).

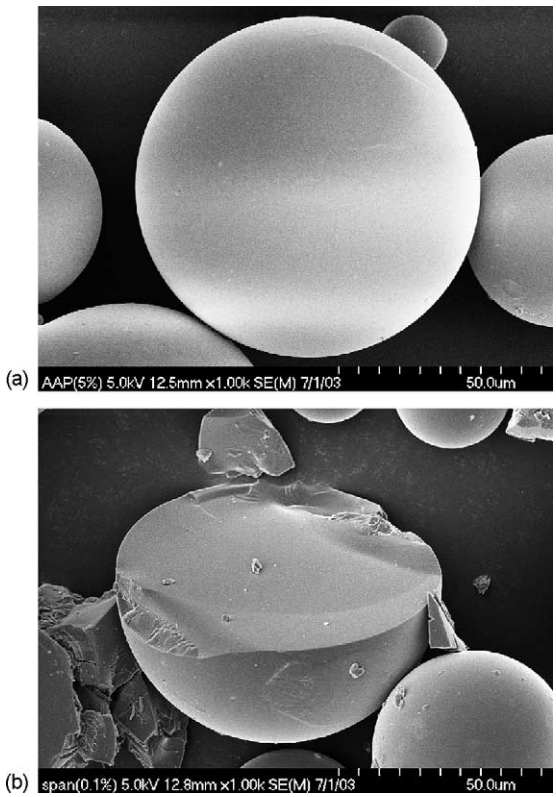


Fig. 5. Morphology of the complex microsphere: the surface (a) and the inside (b) of the microsphere. The composition of the measured microsphere was as follows: the solvent ratio of the internal phase (ethanol/water) was 7/3; the surfactant (span 80) concentration was 0.1%; the polymer concentration was 8.2%; the feeding ratio of the drug (acetaminophen) was 5%.

crospheres or Carbopol 971, respectively. The plastic plate was used instead of intestinal mucosa since there was relatively good correlation between the adhesive force of the PAA/PEG polymer complexes to pig intestinal mucosa and that of the complexes to polypropylene plate in our previous study. In order to wet the surface of each tablet, pH 2.0 solution was used to estimate the adhesive force to gastric mucosa. When wetted with pH 2.0 solution before the measurement, the adhesive force was $3.21 \pm 0.84\,N/cm^2$ (mean ± standard deviation) in the case of PVP/PAA complex microspheres and $2.74 \pm 0.92\,N/cm^2$ in the case of Carbopol 971, indicating that the prepared microspheres may provide sufficient mucoadhesive force in GI tract.

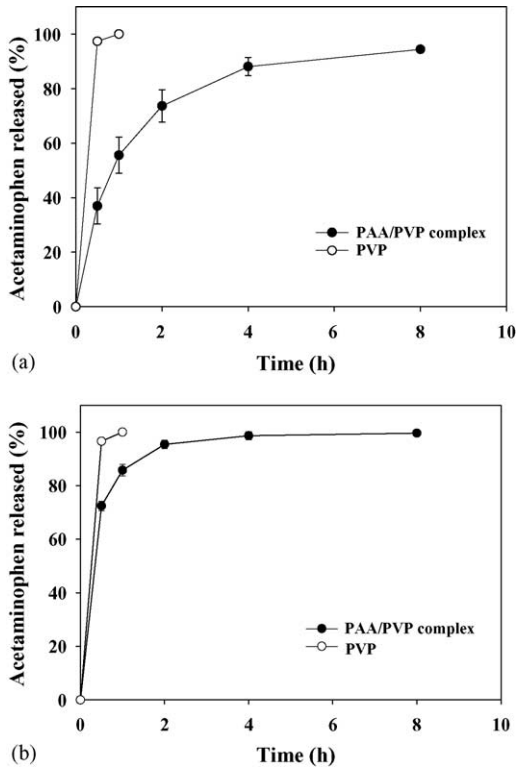


Fig. 6. In vitro release of acetaminophen from the PAA/PVP complex and the PVP microspheres at pH 2.0 (a) and 6.8 (b) ($n = 3$).

### 3.8. Morphology

The morphology of the microspheres was examined by scanning electron microscopy. The view of the microspheres showed a spherical shape with a smooth surface morphology (Fig. 5). The inside of the microspheres was completely filled, indicating that complexation had occurred everywhere within the microspheres.

### 3.9. In vitro drug release

Fig. 6 shows the in vitro release profile of a model drug, acetaminophen, from the microspheres made from PAA/PVP complex and PVP at pH 2.0 and 6.8. Microspheres were made using PVP only to compare the release rate. The release rate of acetaminophen from the PAA/PVP complex microspheres was significantly slower than the PVP microspheres at pH 2.0 and 6.8. There were no significant difference in the release rate

of acetaminophen from the PVP microspheres at pH 2.0 and 6.8. However, the release of acetaminophen from the PVP/PAA complex microspheres at pH 2.0 was much slower than at pH 6.8. Hydrogen bonding between PAA and PVP was so strong at a pH much lower than the p$K_a$ of PAA that they barely dissociated into PAA and PVP. These results suggest that the PVP/PAA microspheres can be used as a drug-delivery system for treating gastric diseases.

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