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Preparation of mucoadhesive microspheres containing antimicrobial agents for eradication of *H. pylori*

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Abstract

Mucoadhesive microspheres containing either amoxicillin or clarithromycin were prepared via the interpolymer complexation of poly(acrylic acid) (PAA) with poly(vinyl pyrrolidone) (PVP) and solvent diffusion method. The complexation between the PAA and PVP in an ethanol/water mixture was confirmed by the change in the transmittance of the solution as a function of repeating PAA and PVP unit ratio. The loading efficiency of clarithromycin in the complex microspheres was higher than that of amoxicillin due to the stronger interaction of clarithromycin with the PAA. The microspheres had a spherical shape with a smooth surface and the inside of the microspheres was completely filled. The dissolution rate of the complex microspheres was significantly slower than that of the PVP microspheres, particularly at pH 2.0. Amoxicillin and clarithromycin degraded significantly during the release study at pH 2.0. Therefore, their release rates were corrected using first order degradation rate constants. The amoxicillin release rates were similar regardless of the pH of the medium, while those of clarithromycin differed depending on the pH. The release mechanism of amoxicillin was mainly by a diffusion process and that of clarithromycin was via a dissolution process. The drug release rate from the complex microspheres was significantly lower than that from the PVP microspheres.

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

1. Introduction

Since the discovery of *Helicobacter pylori* by Marshall and Warren (Marshall and Warren, 1983, 1984), *H. pylori* is believed as a main microorganism causing gastric or peptic ulcers (Peterson, 1991). Therefore, eradicating *H. pylori* is a prerequisite for curing a gastric or peptic ulcer and preventing a recurrence (Labenz, 2001). Although the microorganism is susceptible to many antimicrobial agents in vitro, clinical trials with a single antimicrobial agent have resulted in a low eradication rate of *H. pylori* (Labenz,

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2001; Umamaheshwari et al., 2003). The antibiotic with the highest eradication rate in monotherapy in vivo is clarithromycin. In order to enhance therapeutic efficacy against *H. pylori*, a dual therapy combining two antibiotics or a proton pump inhibitor and an antibiotic has been investigated. The best clinical efficacy was obtained when a triple therapy combining two antibiotics and an anti-secretary agent was used (Labenz, 2001).

Another requirement for enhancing the eradication rate of *H. pylori* is to extend the residence time of the antibiotic agents in the stomach. The extended release of the drug can maintain a higher antibiotic concentration in the gastric region where *H. pylori* exists thereby improve the therapeutic efficacy. Mucoadhesive drug carriers may prolong the residence time in the GI tract because they can adhere to the mucus surface, resulting in an effective localized drug concentration (Wang et al., 2001; Nagahara et al., 1998). Mucoadhesive dosage forms were also reported to improve the absorption and systemic bioavailability of the drugs that were normally poorly absorbed (Nagai and Machida, 1985). Among several mucoadhesive polymers, poly(acrylic acid) (PAA) and its lightly crosslinked commercial form, Carbopol, usually have strong mucoadhesive properties and are known to be biocompatible (Park and Robinson, 1987). However, PAA has some limitations as a mucoadhesive drug carrier e.g. its high water solubility. The high water solubility critically limits its use as a mucoadhesive drug carrier because it may be dissolved long before the drug is delivered across the membrane (Needleman and Smales, 1995). Moreover, when the mucoadhesive dosage form is administered in tablet form, they may or may not adhere to the mucous surface as a result of the weight of the dosage form and the vigorous movement of the GI tract. However, mucoadhesive microspheres have some advantages. These include a lightweight and a smaller dose variation due to the large number of microspheres administered.

PAA based interpolymer complex microspheres were previously examined with the aim to reduce the high aqueous solubility of PAA (Chun et al., 2002b). In that study, the mucoadhesive microspheres were prepared by a solvent evaporation and interpolymer complexation method. The release of acetaminophen from the prepared microsphere was significantly retarded when compared with those from the PVP microspheres, particularly at pH 2.0. In the present study, the PAA/PVP complex microspheres were used to develop a drug delivery system for eradicating *H. pylori*. Amoxicillin and clarithromycin were chosen as the model drugs and were loaded into the complex microspheres. This choice was made because a combination of two antimicrobial agents such as amoxicillin, clarithromycin is known to have enhanced therapeutic efficacy (Labenz, 2001). This study further characterized the complex microspheres by examining the release rate of the active ingredient, the dissolution rate of the microspheres, their morphologies, and turbidity.

2. Materials and methods

2.1. Materials

The PVP (MW: 50,000) was provided by BASF (Ludwigshafen, Germany). The PAA (MW: 450,000) was purchased from Aldrich (Milwaukee, WI). The sorbitan monooleate (Span 80) was purchased from Junsei Chemical (Tokyo, Japan). Corn oil was purchased from CJ Corporation (Seoul, Korea). Amoxicillin was purchased from Sigma Chemicals (Milwaukee, WI). Clarithromycin was provided by Hanmi Pharmaceutical Co. (Seoul, Korea). All other chemicals were of reagent grade and used without further purification.

2.2. Methods

2.2.1. Preparation of mucoadhesive microspheres

PAA (0.2 g) was dissolved in 4.8 g of an ethanol/water (70/30% w/w) mixture and PVP (0.32 g) was dissolved in 1 g of the ethanol/water (70/30% w/w) mixture. Either clarithromycin or amoxicillin were dissolved in both the PAA and PVP solutions. The feed ratio of the antimicrobial agent was 5% of the polymer weight. When the two solutions were combined, the concentration of the polymer, PAA and PVP, was 8%. The weight ratio of the PAA and PVP was fixed at a 1:1 repeating unit ratio. Using a syringe, the PAA 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 the ambient temperature for more than 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 in a vacuum oven at the ambient temperature for 24 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 prepare the PVP microspheres, which were used as the control, PVP (8%) and an antimicrobial agent (5% of polymer weight) were dissolved in ethanol/water mixture (70/30% w/w). The PVP microsphere was prepared using the same method as described previously.

2.2.2. Turbidity measurements

The formation of the complex through hydrogen bonding between the PAA and PVP at various PAA/PVP repeating unit ratios was investigated by monitoring the transmittance of the solution at a wavelength of 500 nm using a spectrophotometer (UV-1601, Shimadzu, Japan). In order to measure the turbidity, PAA (0.2 g) and PVP (0.31 g) were dissolved in 100 ml of an ethanol/water mixture (70/30% w/w), respectively. The PAA and PVP solution were mixed at various volume ratios to obtain the appropriate repeating unit ratio (PAA/PVP), and the turbidity was then measured.

In order to compare the intensity of the interaction of PAA with the test drugs (clarithromycin and amoxicillin), PAA (0.36 g) and each drug (0.12 g) were dissolved in 60 ml of a pH 4.0 phosphate buffer solution, respectively. The same amount of the test drug solution and PAA solution were mixed and their turbidities were then measured.

2.2.3. Loading efficiency

Approximately 3 mg of the drug-loaded microspheres were dissolved in 15 ml of the NaOH solution (pH 10). In order to determine the amount of antimicrobial agents loaded in the microspheres, the solution was then filtered through a 0.45 μm syringe filter and the filtrate was 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 the case of amoxicillin, the wavelength of the UV detector was 230 nm and a reversed-phase column (Luna 5 μm C8, Phenomenex, USA) was used. The column temperature was maintained at 30 °C, the flow rate was 1 ml/min, and the mobile phase consisted of an aqueous 0.05 M phosphate buffer containing 0.1% v/v triethylamine (pH 3.0)–methanol (90/10% v/v) (Erah et al., 1997). In the case of clarithromycin, the wavelength of the UV detector was 210 nm and a reversed-phase column (Luna 5 μm C8, Phenomenex, USA) was used. The column temperature was maintained at 30 °C, the flow rate was 1 ml/min, and the mobile phase consisted of aqueous 0.07 M potassium dihydrogen phosphate-acetonitrile (65/35).

2.2.4. Particle size analysis

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

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. Dissolution rates of the microspheres

The dissolution tests were carried out using a dissolution tester (DST-810, LABFINE, Inc., Korea). One hundred micrograms of the microspheres were weighed and placed in a tea bag. The dissolution rates of either the PAA/PVP complex or PVP microspheres were determined by placing the tea bags containing the microspheres in 500 ml of a dissolution medium and stirring them at 100 rpm at 37 °C. The dissolution media tested were either a pH 2.0 HCl solution or a pH 6.8 phosphate buffer. After a predetermined time interval (1, 2 and 4 h), the tea bags were withdrawn and dried at 80 °C for more than 12 h, and their weight was measured. The degree of dissolution was calculated using $[(W_p - W_s)/W_p] \times 100$, where W_p and W_s are the dried weights of the samples before and after testing, respectively.

2.2.7. Release of antimicrobial agents from the microspheres

The release rate of amoxicillin or clarithromycin from the microsphere was measured using a dissolution

tester (DST-810, LABFINE, Inc., Korea). The PAA/PVP complex microspheres loaded with the antimicrobial agent were placed in 500 ml of the release medium and stirred at 100 rpm at 37 °C. The pH of the release medium tested was 2.0, 4.0 and 6.8. An aliquot of the release medium (3 ml) was withdrawn at the predetermined time interval 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 mixed with a NaOH solution (0.014 M) to adjust the pH of the sample to approximately 5.0 in order to prevent further degradation. In order to determine the amount of the antimicrobial agent released from the microsphere, the samples were then analyzed by HPLC (Shimadzu Scientific Instruments, MD, USA), which consisted of a UV detector (SPD-10A), a pump (LC-10AD) and an automatic injector (SIL-10A).

2.2.8. Degradation of antimicrobial agents

The degradation rate of the antimicrobial agents at pH 2.0 was examined under the same conditions as the release test. A known amount of amoxicillin or clarithromycin was added to the medium, which was preheated at 37 °C, to make a final concentration of 5.0 μg/ml. An aliquot of the medium (3 ml) was withdrawn at predetermined time intervals and neutralized with a NaOH solution before being quantified by HPLC.

3. Results and discussion

3.1. Preparation of PAA/PVP complex microspheres

It was shown in a previous study that PAA and PVP form a complex via hydrogen bonding in 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 formed complex precipitates from the solution. It was also shown that this phenomenon can be used to prepare mucoadhesive microsphere (Chun et al., 2002b). In order to determine the optimum ratio to form a complex between PAA and PVP in a water/ethanol mixture, the change in the transmittance of the solution was measured as a function of the repeating unit ratio between PAA and PVP (Fig. 1). The transmittance was expected to drop as the PAA and PVP formed a complex as a result of the low solubility of the complex. The transmittance decreased from pure PAA until the repeating unit ratio of PAA/PVP reached 1/1 and then increased up to the pure PVP. This indicates that the optimum PAA and PVP ratio needed to form a complex via hydrogen bonding is 1/1, and one of the components will remain uncomplexed at different ratios.

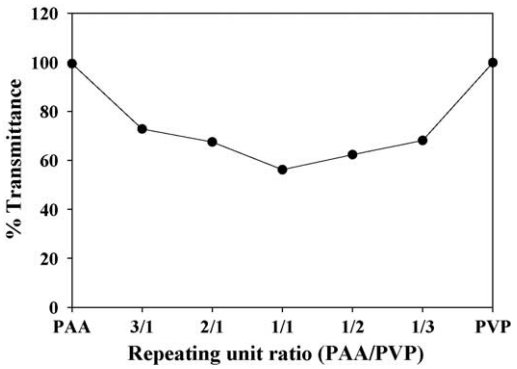

Fig. 1. Change in the transmittance of the solution as a function of the PAA to PVP ratio.

The mucoadhesive microspheres containing either amoxicillin or clarithromycin were prepared using PAA and PVP (1/1) by a method reported previously (Chun et al., 2002b). The size of the drug-loaded complex microsphere was slightly smaller than the microsphere without the drug, and the yield of the drug-loaded complex microsphere was better than the microsphere without the drug, as shown in Table 1. It is interesting to note that the loading efficiency of the clarithromycin in the complex microspheres was higher than that of amoxicillin. In order to determine the reason why clarithromycin and amoxicillin have a different loading efficiency, the extent of their interaction with PAA was investigated. The transmittance of the amoxicillin–PAA solution and the clarithromycin–PAA solution was measured. The transmittance of the amoxicillin–PAA solution was 97.2%, whereas that of the clarithromycin–PAA solution was 62.6%. More precipitate formation was expected as the intensity of the interaction between PAA and the drug increased, resulting in a lower transmittance. Therefore, the stronger interaction between PAA

Table 1
Comparison of the yields, loading efficiencies and particle sizes of the PAA/PVP complex microspheres ($n = 3$)

Loaded drug	Yield (%)	Loading efficiency (%)	Amount loaded (mg/g)	Particle size (μm)
Non	82.9 ± 0.8	–	–	73.3 ± 13.8
Amoxicillin	88.7 ± 1.8	57.5 ± 3.5	28.8 ± 1.8	62.7 ± 4.7
Clarithromycin	90.8 ± 4.5	93.5 ± 5.7	46.8 ± 2.9	65.4 ± 4.6

The value are represented as mean ± S.D., where S.D. is the standard deviation.

and clarithromycin appeared to result in a higher loading efficiency.

3.2. Morphology

The morphology of the microspheres was examined by scanning electron microscopy. The microspheres containing either the amoxicillin or clarithromycin had a spherical shape with a smooth surface (Fig. 2). The loading of the antimicrobial agent did not cause any significant change in morphology. The inside of the microspheres was completely filled, indicating that complexation had occurred everywhere within the microsphere.

3.3. Dissolution of the microspheres

Fig. 3 shows the dissolution rates of the PAA/PVP complex microsphere and PVP microsphere at pH 2.0 and 6.8 after 1, 2 and 4 h. The degree of dissolution of the PVP microsphere was 100% in 1 h at both pHs tested. However, that of the PAA/PVP complex microsphere was significantly reduced due to complex formation and did not dissolve completely after 4 h at both pHs. The dissolution rate of the complex microspheres was pH dependent, and the dissolution rate at pH 2.0 was much slower than that of the complex microspheres at pH 6.8. This can be explained by the dissolution characteristics of the PAA based complex. When the pH is

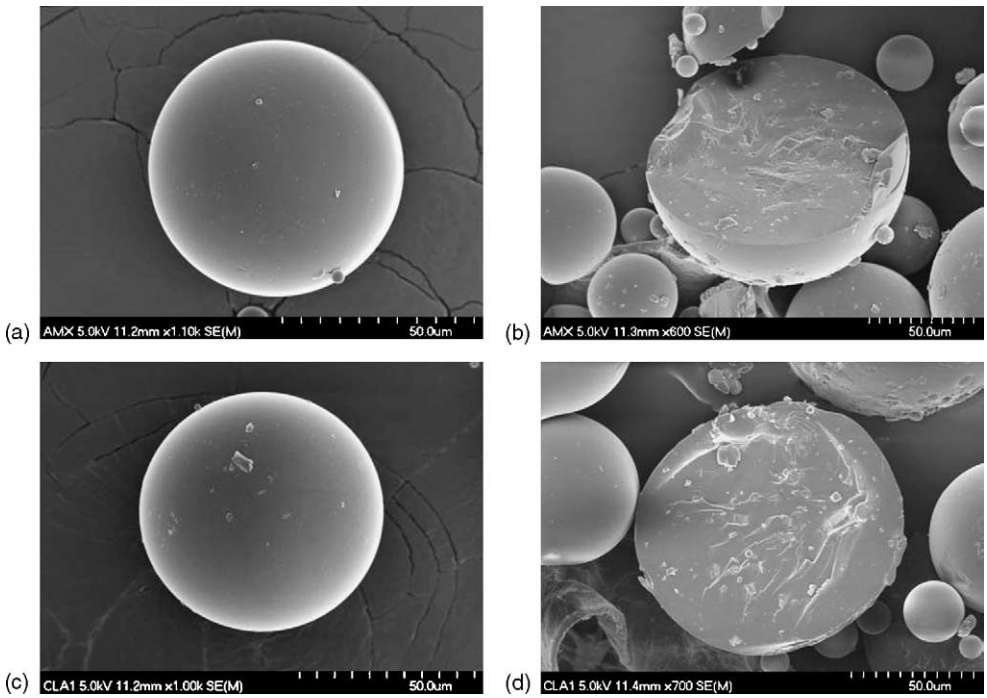

Fig. 2. Morphology of the complex microsphere: the surface (a) and the inside (b) of the amoxicillin-loaded microsphere, the surface (c) and inside (d) of the clarithromycin-loaded microsphere.

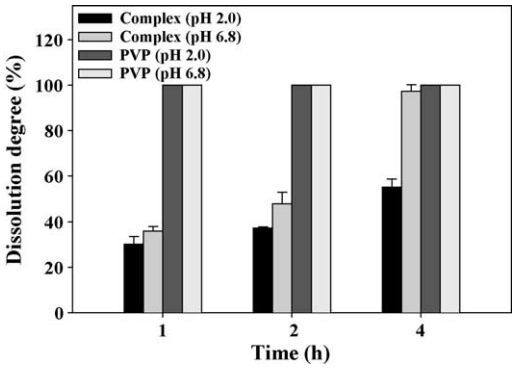

Fig. 3. Comparison of the dissolution rate of the PAA/PVP complex microspheres and PVP microspheres at pH 2.0 and 6.8 after 1, 2 and 4 h ($n = 3$).

lower than the pK_a of PAA (4.75), the majority of the carboxyl groups of PAA are not ionized, and the hydrogen bonds between PAA and PVP in the complex can be maintained, leading to a slower dissolution rate. However, when the pH is higher than the pK_a of PAA, the majority of carboxyl groups of PAA are ionized and the hydrogen bonds cannot be maintained, leading to a higher dissolution rate (Chun et al., 2002a). This shows that PAA/PVP complex microspheres can be used to delay the release of a drug, particularly in the stomach.

3.4. Stability of amoxicillin and clarithromycin

It was reported that both amoxicillin and clarithromycin are unstable in acidic solutions (Erah et al., 1997; Nakagawa et al., 1992). Therefore, the results obtained from the dissolution study will underestimate the amount of the drug released from the microspheres. Hence, in order to calculate correct amount of the drug released the degradation rate constant will need to be determined. Fig. 4 shows the degradation rates of amoxicillin and clarithromycin at pH 2.0 at 37 °C. The degradation profile of both compounds appeared to follow first order kinetics. It was also reported that the degradation of amoxicillin and clarithromycin at low concentrations follow pseudo-first-order kinetics (Nakagawa et al., 1992; Tsuji et al., 1978). The first order degradation rate constant and the half-life of the degradation were calculated, and the results are shown in Table 2. The half-lives of amoxicillin and clarithromycin were 19.1 and 1.47 h, respectively, and the

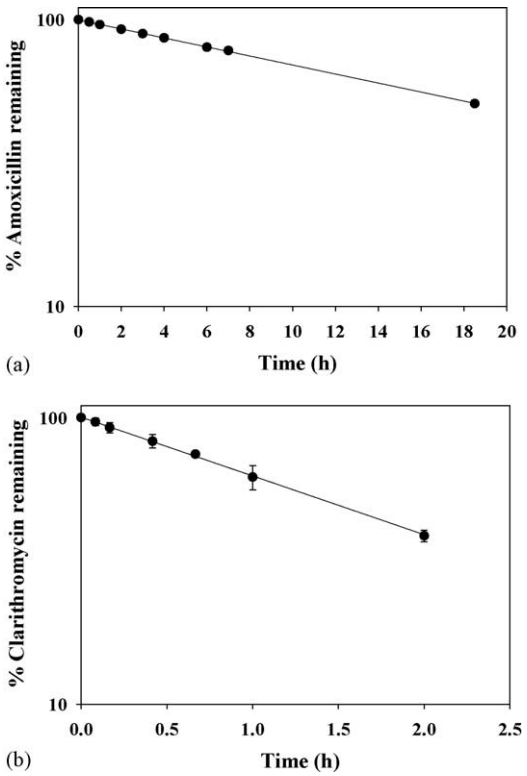

Fig. 4. Degradation of amoxicillin (a) and clarithromycin (b) in a pH 2.0 HCl solution at 37 °C ($n = 3$).

degradation rate constants were 0.0362 and 0.472 h^{-1}, respectively.

3.5. In vitro drug release

Fig. 5 shows the in vitro release profile of amoxicillin and clarithromycin from the PAA/PVP complex and PVP microspheres at pH 2.0, 4.0 and 6.8. At pH 2.0, the total amount of amoxicillin and clarithromycin released initially increased with time and then decreased. As discussed in the previous section, the decrease in the release profile is due to the degradation

Table 2
Degradation rate constants and half-lives for amoxicillin and clarithromycin at pH 2.0 at 37 °C

Drug	Degradation rate constant (h^{-1})	Half-life (h)
Amoxicillin	0.0362	19.1
Clarithromycin	0.472	1.47

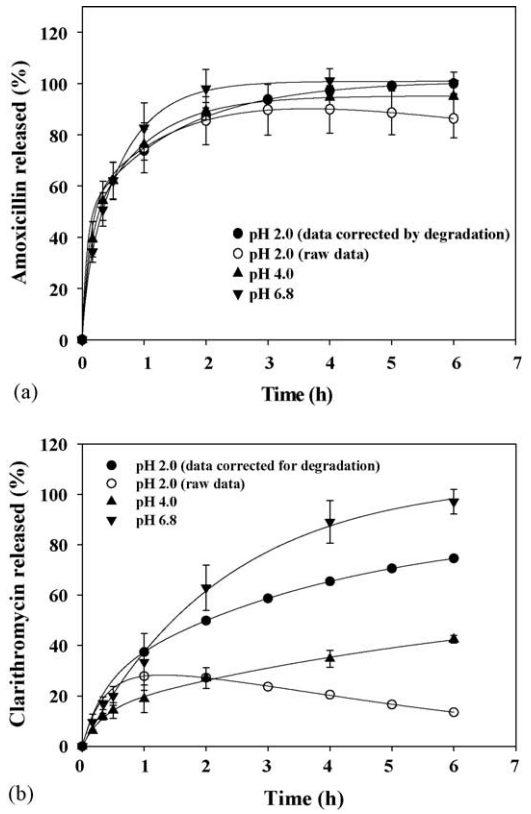

Fig. 5. In vitro release of amoxicillin (a) and clarithromycin (b) from the PAA/PVP complex and the PVP microspheres at pH 2.0, 4.0 and 6.8 at 37 °C ($n = 3$).

of both compounds at the low pH. The release rate initially dominates the degradation rate but after a certain time point, the degradation rate dominates the release rate. The release profile of clarithromycin began to decrease 1 h after the release study while that of amoxicillin began to decrease 3 h after the study as a result of the faster degradation rate of clarithromycin.

The following equation was used to estimate the amount of amoxicillin or clarithromycin released at pH 2.0:

$$\frac{dC}{dt} = \frac{dQ}{V\,dt} - kC$$

where C is the concentration of the drug at time t, Q the total amount of the drug released at time t, V the volume of the release medium, and k the first order degradation rate constant. The corrected release profiles of amoxicillin and clarithromycin are plotted in Fig. 5. The release rate of amoxicillin was faster than that of clarithromycin at all pH values examined due to the weaker interaction of amoxicillin. The release rate of amoxicillin was almost independent of the pH, while that of clarithromycin was dependent on the pH. As the pH of the medium increases, the release rate of the drug increases due to PAA/PVP dissolution. In addition, as the pH of the medium decreases, the release rate of the weak basic drug on account of the diffusion process increases due to the higher solubility at the lower pH. In the case of amoxicillin, the diffusion of the drug appeared to dominate the dissolution of the PAA/PVP matrix because the release rate of amoxicillin was much faster than the dissolution rate of PAA/PVP. Amoxicillin is an amphiphilic compound with two pK_a values (2.8 and 7.2) and the measured solubility of amoxicillin was 6.1, 4.2, and 5.1 mg/ml at pH 2.0, 4.0, and 6.8, respectively. Although the release rate of amoxicillin at pH 6.8 is expected to be slightly slower than that at pH 2.0, the faster dissolution of the PAA/PVP matrix increased the release rate, resulting in a slightly higher release rate at pH 6.8 than at pH 2.0. In the case of clarithromycin, the diffusion process could not play as much an important role in the release of the drug as amoxicillin due to the lower solubility and stronger interaction with the PAA/PVP complex polymer. The release of clarithromycin at pH 6.8 closely matched the dissolution of the PAA/PVP matrix, indicating that the release mechanism of clarithromycin at pH 6.8 is mainly a PAA/PVP dissolution process. The estimated release of clarithromycin at pH 2.0 was somewhat greater than the dissolution of the PAA/PVP matrix, indicating that some of clarithromycin was released via a diffusion process as a result of the higher solubility at pH 2.0. The slowest release rate of clarithromycin at pH 4.0 appeared to be due to the low solubility of the drug and the slow dissolution rate of the PAA/PVP matrix.

4. Conclusions

A mucoadhesive microsphere was prepared by a solvent evaporation and interpolymer complexation method. The dissolution rate of the complex microspheres was significantly retarded when compared with that of the PVP microspheres, particularly at pH 2.0. The results of this study indicate that it may be

feasible to use PAA/PVP mucoadhesive microspheres as a gastric retentive drug delivery system for eradicating *H. pylori*. The release rate of the antimicrobial agents will be retarded due to the slower dissolution rate of the complex polymer.

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References

Chun, M.-K., Cho, C.-S., Choi, H.-K., 2002a. Mucoadhesive drug carrier based on interpolymer complex of poly(vinyl pyrrolidone) and poly(acrylic acid) prepared by template polymerization. J. Contr. Rel. 81, 327–334.

Chun, M.-K., Cho, C.-S., Choi, H.-K., 2002b. Mucoadhesive microsphere prepared by interpolymer complexation of poly(acrylic acid)/poly(vinyl pyrrolidone). Proc. Int. Symp. Contr. Rel. Bioact. Mater. 29, 586–587.

Erah, P.O., Goddard, A.F., Barrett, D.A., Shaw, P.N., Spiller, R.C., 1997. The stability of amoxicillin, clarithromycin and metronidazole in gastric juice: relevance to the treatment of *Helicobacter pylori* infection. J. Antimicrob. Chemother. 39, 5–12.

Labenz, J., 2001. Current role of acid suppressants in *Helicobacter pylori* eradication therapy. Best Pract. Res. Clin. Gastroenterol. 15, 413–431.

Marshall, B.J., Warren, J.R., 1983. Unidentified curved bacilli in gastric epithelium in active chronic gastritis. Lancet 1, 1273–1275.

Marshall, B.J., Warren, J.R., 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1, 1311–1315.

Nagahara, N., Akiyama, Y., Nakao, M., Tada, M., Kitano, M., Ogawa, Y., 1998. Mucoadhesive microspheres containing amoxicillin for clearance of *Helicobacter pylori*. Antimicrob. Agents Chemother. 42, 2492–2494.

Nagai, T., Machida, Y., 1985. Mucosal adhesive dosage forms. Pharm. Int. 6, 196–200.

Nakagawa, Y., Itai, S., Yoshida, T., Nagai, T., 1992. Physicochemical properties and stability in the acidic solution of a new macrolide antibiotic, clarithromycin, in comparison with erythromycin. Chem. Pharm. Bull. 40, 725–728.

Needleman, I.G., Smales, F.C., 1995. In vitro assessment of bioadhesion for periodontal and buccal drug delivery. Biomaterials 16, 617–624.

Park, H., Robinson, J.R., 1987. Mechanism of mucoadhesion of poly(acrylic acid) hydrogels. Pharm. Res. 4, 457–464.

Peterson, W.L., 1991. Helicobacter pylori and peptic ulcer disease. N. Engl. J. Med. 324, 1043–1048.

Tsuji, A., Nakashima, E., Hamano, S., Yamana, T., 1978. Physicochemical properties of amphoteric β-lactam antibiotics. I. Stability, solubility and dissolution behavior of amino penicilins as a function of pH. J. Pharm. Sci. 67, 1059–1066.

Umamaheshwari, R.B., Jain, S., Jain, N.K., 2003. A new approach in gastroretentive drug delivery system using cholestyramine. Drug Del. 10, 151–160.

Wang, J., Tabata, Y., Bi, D., Morimoto, K., 2001. Evaluation of gastric mucoadhesive properties of aminated gelatin microspheres. J. Contr. Rel. 73, 223–231.