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Preparation of Mucoadhesive Chitosan-Poly(Acrylic acid) Microspheres by Interpolymer Complexation and Solvent Evaporation Method II

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A mucoadhesive microsphere was prepared by an interpolymer complexation and solvent evaporation method, using chitosan and poly(acrylic acid) (PAA), to prolong the gastric residence time of the delivery system. The Fourier transform infrared results showed that microspheres were formed by an electrostatic interaction between the carboxyl groups of the PAA and the amine groups of the chitosan. X-ray diffraction and differential scanning calorimetry analysis showed that the enrofloxacin in the chitosan-PAA microsphere was molecularly dispersed in an amorphous state. Scanning electron microscopy of the surface and the quantity of mucin attached to the microspheres indicated that chitosan-PAA microspheres had a higher affinity for mucin than those of chitosan alone. The swelling and dissolution of the chitosan-PAA microspheres were found to be dependent on the pH of the medium. The rate of enrofloxacin released from the chitosan-PAA microspheres was slower at higher pH; therefore, based on their mucoadhesive properties and morphology, the chitosan-PAA microspheres can be used as a mucoadhesive oral drug delivery system.

Key words: Mucoadhesive microsphere, Interpolymer complex, Poly (acrylic acid), Chitosan

INTRODUCTION

The optimization of both the residence time of a system in the gastrointestinal tract and the rate of the release of the active ingredient from that system must be considered during the development of an oral drug delivery system (Chun *et al.*, 2005). Several approaches have been used to extend the gastric residence time (GRT) of systems, including the use of a passage-delaying agent (Lee *et al.*, 1999), mucoadhesive systems (Lueßen *et al.*, 1994) and floating devices (Miyazaki *et al.*, 1988). One of the most extensively studied methods for prolonging the GRT of a delivery system is the use of mucoadhesive delivery systems. Mucoadhesive delivery systems have an advantage over conventional drug delivery systems owing to their ability to increase the contact time of the drug with a biological surface, thereby increasing the level of drug absorption. Among the various synthetic and natural mucoadhesive polymers, poly (acrylic acid) (PAA) is considered one of the best, based on its excellent mucoadhesive properties, and the fact it appears to be biocompatible (Park and Robinson, 1987). Despite the excellent mucoadhesive property of PAA, its high water solubility critically limits its use as a mucoadhesive drug carrier; it may dissolve before the desired duration for delivery of the drug across the membrane (Needleman and Smales, 1995). In order to reduce the water solubility of PAA, and maintain its mucoadhesive property, chitosan was used to form a complex through intermolecular hydrogen bonding between its amino groups and the carboxyl groups of PAA (Ahn *et al.*, 2001). Chitosan is a weak cationic polysaccharide that is composed of β(14) glucosamine units, with some proportions of *N*-acetylglucosamine units. It is obtained by the extensive deacetylation of chitin, which is a polysaccharide found throughout nature. This natural polysaccharide possesses some favorable properties, such as non-toxicity, high biodegradability and biocompatibility, which are essential requirements for clinical use (Polk *et al.*, 1994). Chitosan possesses hydroxyl and amine groups

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that can form hydrogen bonds and chain flexibility due to its linear molecular structure. These properties are believed to be essential for mucoadhesion (Robinson *et al.*, 1987). Due to the cationic polyelectrolyte nature of chitosan, it is known to interact strongly with mucin and sialic acid residues (Fiebrig *et al.*, 1995), and many researchers have demonstrated these properties.

The aims of this study were to reduce the water solubility of PAA and either maintain or improve the mucoadhesive properties of PAA and chitosan for application as a transmucosal drug delivery (TMD) system. Interpolymer complexation and solvent evaporation techniques were used to reduce the water solubility of PAA and prepare mucoadhesive microspheres. The prepared chitosan-PAA interpolymer complex microspheres were characterized in terms of their spectroscopic and thermal properties, and the release rate of enrofloxacin, a model drug, was investigated.

MATERIALS AND METHODS

Materials

The chitosan (high molecular weight, degree of deacetylation: >75%, Brookfield viscosity: 800-2000 cps) and PAA (average MW: 45000) were purchased from Aldrich Chemical Co (Milwaukee, WI). The enrofloxacin was provided by LG Chemical Co. (Daejeon, Korea). The mucin (from porcine stomach, Type III) was purchased from Sigma Chemical Co. (Milwaukee, MO). The acetic acid was obtained from Junsei Chemical Co. (Tokyo, Japan) and the *n*-hexane was acquired from Duksan Chemical Co. (Kyungkido, Korea). All other chemicals were of reagent grade, and used without further purification.

Preparation of mucoadhesive microsphere

Chitosan-PAA microspheres were prepared using a solvent evaporation method. The chitosan (1.5 wt%) was dissolved in water containing 2% v/v acetic acid, and the PAA (0.3 wt%) was dissolved in water. Using a 5 mL syringe, 2 mL of the PAA solution and 5 mL of the chitosan solution were slowly dropped into 100 mL of *n*-hexane, as a continuous phase. The *n*-hexane contained 0.4% v/v span 80 (sorbitan monooleate) as a surfactant. The mixture was stirred at 500 rpm, using a magnetic bar, at room temperature for 48 h. The hardened microspheres were collected by filtration, and dried for 12 h at 80°C. In order to prepare enrofloxacin-loaded microspheres, enrofloxacin (0.2% wt) was dissolved in methanol, and 2 mL of the drug solution was dropped into the continuous phase together with the polymer solutions. Chitosan microspheres were similarly prepared, but without the use of PAA.

Morphology

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

Infra-red spectroscopy study

The Fourier transform infrared absorption spectra of the chitosan, PAA, and chitosan-PAA complex microspheres were obtained using an FT-IR spectrophotometer (FT-IR 401, Jasco, Tokyo, Japan). The samples were pressed into pellets prior to obtaining their infrared absorption spectra.

Differential scanning calorimetry

Thermal analyses were carried out using a differential scanning calorimeter (DSC 50, Shimadzu Scientific Instruments, MD). The samples (0.5 mg of enrofloxacin or 20 mg of microspheres containing 0.5 mg of enrofloxacin) were placed in an alumina pan, and heated at a scanning rate of 10°C/min from 40 to 700°C.

X-ray diffraction

The X-ray diffraction (XRD, D/MAX-3C, Riguku Co., Japan) measurements were performed at the 2θ range of 2~50°, with a step size of 0.02° and a measuring time of 4s per step.

In vitro bioadhesiveness test

The mucoadhesive property of the microspheres was examined by evaluating the interaction of the chitosan and chitosan-PAA microspheres with mucin in an aqueous solution. The aqueous mucin (Type III) solution was prepared by dissolving 10 mg of mucin in 10 mL of distilled water. Known weights (50 mg) of the dried chitosan and chitosan-PAA microspheres were dispersed in the mucin solution, vortexed, and incubated at 37°C for 2 h. The dispersions were then centrifuged at 4000 rpm for 5 min, the microspheres collected by filtration and dried at 80°C for 12 h in a drying oven. In order to compensate for the amount of microspheres dissolved during the incubation period, the amount of microspheres dissolved in the aqueous solution was determined by measuring the weight of the residual microspheres after incubating for 2 h. The interaction between the microspheres and mucin was obtained by subtracting the weight of the residual microspheres in the aqueous solution from that of the microspheres that had interacted with the mucin. The morphology and surface characteristics of the microspheres that had interacted with the mucin were also examined by FESEM.

Morphology change during dissolution study

The change in the morphology of the chitosan-PAA and

chitosan microspheres in pH 2.0 or 6.8 solution was examined using a Camscope (S/V3, Sometech, Korea) at room temperature.

Release of enrofloxacin from chitosan-PAA microspheres

The drug release test was carried out using a dissolution tester (DST 810, LABFINE, Inc, Seoul, Korea). The Chitosan-PAA or chitosan microspheres, loaded with 50 mg of enrofloxacin, were placed in 500 mL of a release medium and stirred at 100 rpm at 37°C. The pH values of the release media were 2.0 (HCl-Solution) and 6.8 (Phosphate buffer saline solution). Aliquots of the media were withdrawn at predetermined times, with equivalent amounts of fresh media added to the release medium. The collected samples were filtered through a 0.45 μm-syringe filter, analyzed using UV spectrophotometry (UV-1601, Shimadzu, Japan) at 280 nm, and the quantity of enrofloxacin released from the microspheres determined. The release of the drug was investigated at pH 2.0 for two hours, after which the microspheres were transferred to the pH 6.8 release medium. The release of the drug from the chitosan-PAA microspheres was also investigated at pH 6.8.

RESULTS AND DISCUSSION

Chitosan-PAA interpolymer complex microspheres were prepared using a solvent-diffusion and evaporation method. PAA and chitosan are known to form a complex between the carboxyl groups of the PAA and the amine groups of chitosan (Ahn *et al.*, 2002; Hu *et al.*, 2002; Chavasit and Torres, 1990) Once they form a complex, the aqueous solubility decreases remarkably, and the formed complex precipitates from the solution. The chitosan solution was first dispersed in *n*-hexane and the PAA solution then added. Although corn oil was used to prepare chitosan-PAA and poly (vinyl pyrrolidone)-PAA mucoadhesive microspheres in a previous study (Chun *et al.*, 2005; Cho and Choi, 2005), *n*-hexane was chosen as an external phase in this study to make collection and washing of the microspheres easier. The dispersed droplets of the chitosan solution collided with those of the PAA solution in *n*-hexane to form an interpolymer complex. The droplets of the chitosan-PAA complex gradually solidified, and hardened further as the water diffused from the internal phase.

The morphology of the microspheres was examined by FESEM, and they were found to have a spherical shape and smooth surface, as shown in Fig. 1. The chitosan-PAA complex microspheres prepared using corn oil as the external phase also had a spherical shape, but showed a somewhat rough surface (Cho and Choi, 2005).

The complex formation between the PAA and chitosan in *n*-hexane was examined by FT-IR. Fig. 2 shows the FT-IR spectra of the PAA, chitosan and chitosan-PAA microspheres. An amide band near 1580 cm^{-1} was observed in the chitosan and a carbonyl band of the PAA was observed near 1700 cm^{-1} due to the intramolecular hydrogen bonding of the carboxylic groups (Hu *et al.*, 2002). Once the polyelectrolyte complex between the dissociated carboxyl groups (COO^-) of the PAA and the protonated amino groups (NH_3^+) of the chitosan in aqueous solution had formed, *via* an electrostatic interaction during the preparation of the microspheres, the intramolecular hydrogen bonding between the carboxyl groups of the PAA broke, and the carbonyl band of the PAA shifted to a higher wave number, 1740 cm^{-1}. In addition, the hydroxyl band of the carboxyl group of the PAA at 3170 cm^{-1} disappeared as a result of this interaction. These results indicate that the dissociated carboxylic groups of the PAA complexed with

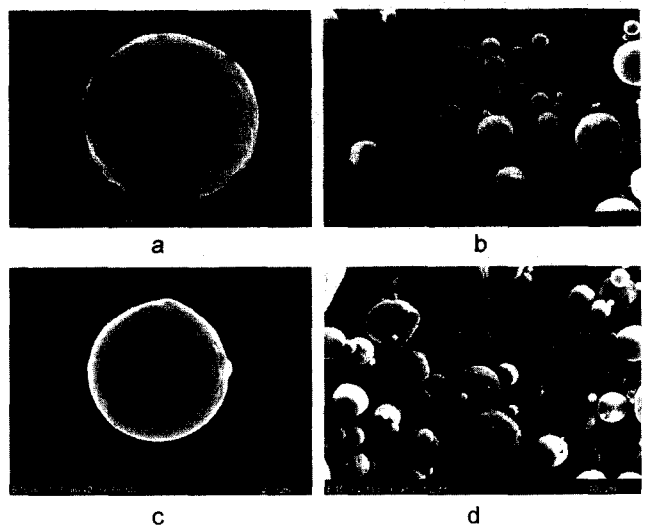

Fig. 1. Scanning electron micrographs of the microspheres: a and b; chitosan microspheres; c and d; chitosan-PAA interpolymer complex microspheres

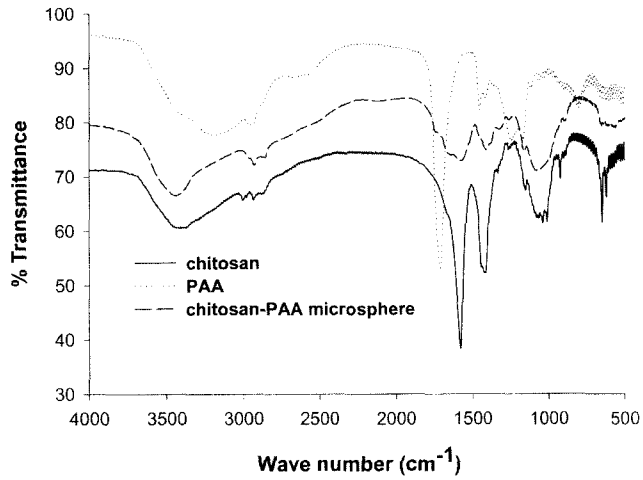

Fig. 2. FT-IR spectra of chitosan, PAA and chitosan-PAA microspheres

the protonated amino groups of the chitosan, *via* an electrostatic interaction, to form the polyelectrolyte complex during the preparation of the microspheres.

The dispersion state of enrofloxacin in the microspheres was analyzed using DSC and XRD. Figs. 3 and 4 show the XRD patterns and DSC curves of the microspheres, respectively. The XRD of the microspheres showed no crystalline peaks for enrofloxacin, indicating that enrofloxacin was molecularly dispersed in the microspheres. The DSC thermogram of the microspheres did not show a melting peak of enrofloxacin, indicating the amorphous state of the drug.

The mucoadhesive properties of the chitosan and chitosan-PAA microspheres were compared by measuring their interactions with mucin in aqueous solution. The interactions were measured by observing the surface morphology of the microspheres and the amount of mucin interacting with the microspheres. Chitosan microspheres have been reported to be promising vehicles for drug delivery in the oral cavity due to their strong mucoadhesive potential (Kockisch *et al.*, 2003). Fig. 5 shows that the surfaces of the chitosan microspheres (a and b) and chitosan-PAA microspheres (c and d) were covered with mucin. The change in the surface morphology was more significant in the chitosan-PAA than in the chitosan microspheres, indicating more mucin was attached to the former. Table I shows the amount of mucin attached on the microspheres. While 0.145 mg of mucin was attached to each mg of the chitosan microspheres, this was 0.231 mg for the chitosan-PAA microspheres. These observations confirmed that the mucoadhesive properties of the chitosan-PAA microspheres were superior to those of the chitosan microspheres. Chitosan is known to have mucoadhesive properties that are mediated by the ionic interactions between the positively charged amino groups in the polymer and the negatively charged sialic acid residues in mucus (Illum *et al.*, 1994). The mucoadhesive properties of chitosan appeared to be improved by the conjugation with the highly mucoadhesive PAA.

The swelling behavior of the chitosan and chitosan-PAA microspheres was evaluated at pH 2.0 and 6.8 by observing the morphology changes with time using optical microscopy. Fig. 6-a shows the swelling morphology of the chitosan microspheres at pH 2.0. The microspheres

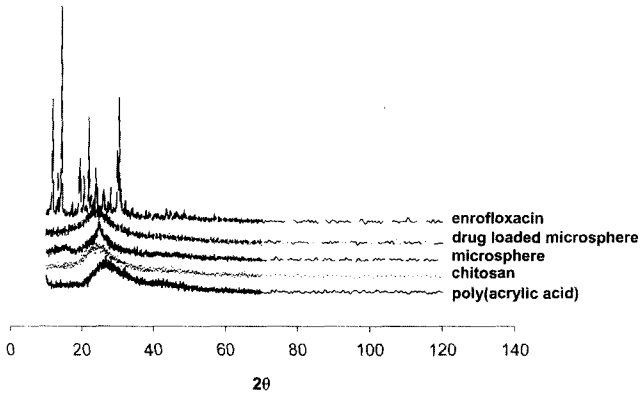

Fig. 3. Wide angle X-ray diffraction (WAXS) patterns of enrofloxacin, chitosan-PAA microspheres loaded with enrofloxacin, chitosan-PAA microspheres, chitosan and PAA

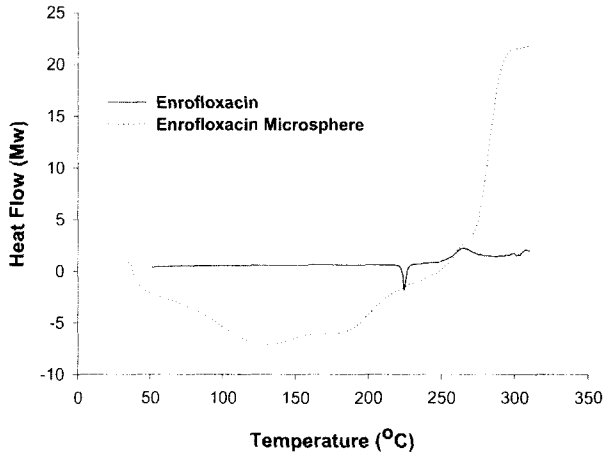

Fig. 4. Differential scanning calorimetric thermograms of enrofloxacin and chitosan-PAA microspheres loaded with enrofloxacin

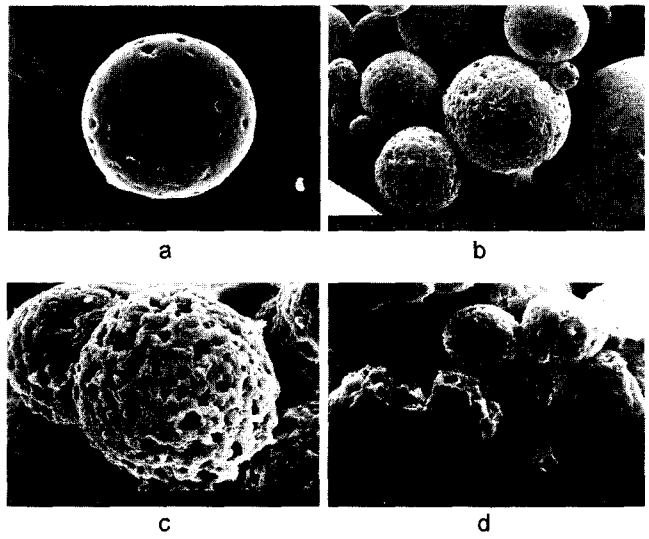

Fig. 5. Scanning electron micrographs of the microspheres covered with mucin: a and b, chitosan microspheres; c and d, chitosan-PAA microspheres

Table I. The quantity of mucin attached per unit weight of the chitosan and chitosan-PAA microspheres after incubation in a mucin solution for 2 h

Microsphere	Weight of Mucin (μg/mg microspheres; mean ± SD)
Chitosan	145 ± 7.8
Chitosan-PAA	231 ± 34.1

swelled quickly, within 5 min, and the swelling front gradually expanded to reach 2.5 times the original diameter within 60 min. Although the chitosan microspheres maintained a spherical shape, they changed into a semi-transparent loosely packed gel like structure within 5 min. Fig. 6-b shows the swelling morphology of the chitosan-PAA microspheres at pH 2.0. The chitosan-PAA interpolymer complex microspheres also swelled quickly, within 5 min, and continued to swell up to 120 min. The swelling rate during the initial 5 min was slower than that of the chitosan microspheres, but thereafter continued to swell at a faster rate than the chitosan microspheres. The swelling front diameter of the chitosan-PAA microspheres was >3.5 times that of the original diameter. While the chitosan microspheres became a semi-transparent gel in 5 min, two distinct portions were observed in the chitosan-PAA microspheres with time. The outer part of the microspheres looked like a semi-transparent gel, similar to that of the chitosan microspheres, and the inner part maintained a solid state. It was interesting to note that many air bubbles were observed escaping from the chitosan-PAA microspheres as they swelled, while no air bubbles were observed with the chitosan microspheres. The inside of the chitosan microspheres appeared to have a homogeneous structure, whereas the chitosan-PAA microspheres appeared to form a porous structure during the course of the complexation between the chitosan and PAA. The swelling behavior of the chitosan microspheres at pH 6.8 was completely different from that at pH 2.0 due to the low solubility of the chitosan at the neutral pH (Fig. 7-a). They quickly swelled, within 5 min, but no further swelling was observed. The diameter of the swelling front of the chitosan microspheres was 1.5 times that of the original diameter. The chitosan-PAA microspheres showed a similar trend, except the swelling front diameter was larger (2.4 times the original diameter) than that of the chitosan microspheres. As shown in Fig. 7-b, both types of microsphere maintained a solid core and spherical shape for 12 h at pH 6.8. This indicates that the interpolymer complex microspheres have much lower water solubility than either the chitosan or PAA microspheres at pH 2.0, and higher swelling capacities at both pH 2.0 and 6.8.

The effect of pH on the release profile of a test drug from the microspheres was examined using enrofloxacin as a model drug. Fig. 8 shows the release of enrofloxacin from the chitosan-PAA interpolymer complex microspheres and the chitosan microspheres at pH 2.0 and 6.8. Approximately 60% of the drug was released from the chitosan microspheres within 5 min at pH 2.0, with more than 90% released within 1 h. These results coincide with the dissolution behavior of the chitosan microspheres at pH 2.0, where they became a semi-transparent gel within 5 min. The release rate of the drug from the chitosan-PAA microspheres at pH 2.0 was significantly slower than that

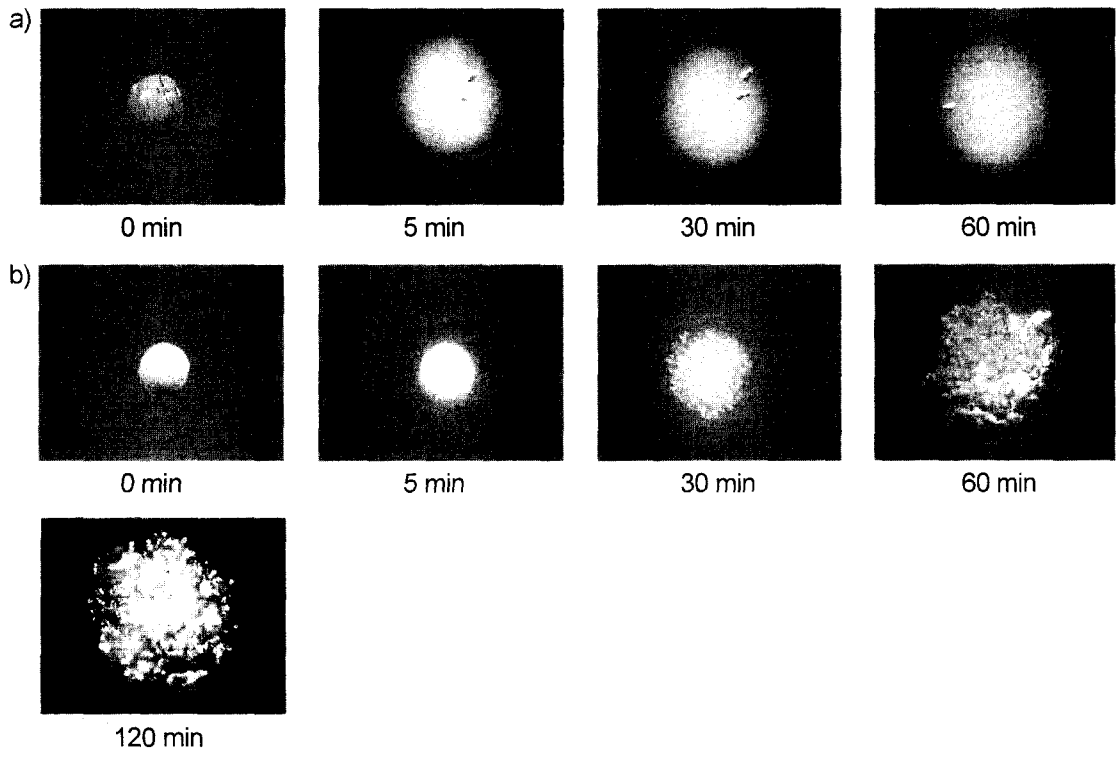

Fig. 6. Micro-photographs of the microspheres. Each microsphere was immersed in a pH 2 solution, and the pictures show the time evolution: a, chitosan microspheres; b, chitosan-PAA microspheres.

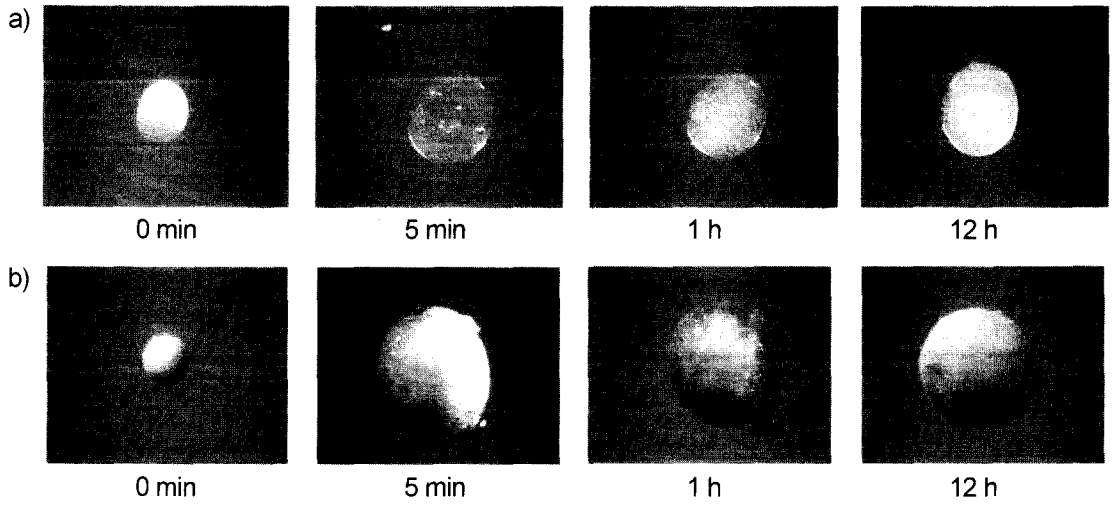

Fig. 7. Micro-photographs of microspheres. Each microsphere was immersed in pH 6.8 solution, and the pictures show the time evolution: a, chitosan microspheres; b, chitosan-PAA microspheres.

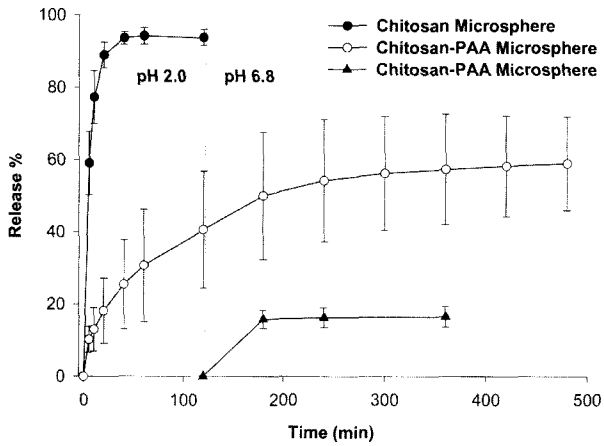

Fig. 8. Release profiles of enrofloxacin from the chitosan and chitosan-PAA microspheres. The chitosan and chitosan-PAA microspheres, in a tea bag, were placed in a pH 2.0 solution for 2 h, and thereafter transferred to a pH 6.8 solution. The chitosan-PAA microspheres were also placed in a pH 6.8 solution without exposure to the pH 2.0 solution. ●: chitosan microspheres; ○, ▲: chitosan-PAA microspheres

from the chitosan microspheres. At pH 6.8, the release rate of the enrofloxacin from the chitosan-PAA microspheres was quite slow, with < 20% of the loaded drug released within 1 h, with no further release detected afterwards. These results coincide with the results from the swelling study, which can be explained by the pKa's of the PAA (4.75) and chitosan (6.5). The majority of the carboxyl groups of the PAA, and approximately half of the amine groups of the chitosan, were ionized at pH 6.8 and the electrostatic interaction between the chitosan and PAA in the complex could be maintained, leading to a lower degree of swelling. Although the majority of the amine groups in the chitosan are in the dissociated form at pH 2.0, most of the carboxylic groups are not, leading to a weak interaction between the chitosan and PAA. In addition, it is easier for a water molecule to penetrate the polymer network. Similar release profiles were observed with chitosan-PAA nanoparticles (Hu *et al.*, 2002).

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