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

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ABSTRACT – Mucoadhesive microspheres were prepared by interpolymer complexation of chitosan with poly(acrylic acid) (PAA) and solvent evaporation method to increase gastric residence time. The chitosan-PAA complex formation was confirmed by differential scanning calorimetry and swelling study. The DSC thermogram of chitosan-PAA microspheres showed two exothermic peaks for the decomposition of chitosan and PAA. The swelling ratio of the chitosan-PAA microspheres was dependent on the pH of the medium. The swelling ratio was higher at pH 2.0 than at neutral pH. The results indicated that the microspheres were formed by electrostatic interaction between the carboxyl groups of PAA and the amine groups of chitosan. The effect of various process parameters on the formation and morphology of microspheres was investigated. The best microspheres were obtained when 1.5% of the high molecular weight chitosan and 0.3% of PAA were used as an internal phase. The optimum internal phase volume was 7%. The corn oil was used as the external phase of emulsion, and span 80 was used as the surfactant. The prepared microspheres had spherical shape.

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

In the development of oral drug delivery systems, increasing the residence time of the system in the gastrointestinal (GI) tract and/or optimizing the release rate of the active ingredient from the system have been the major interests of many investigators. Mucoadhesive polymers have been widely used to prolong the residence time in the GI tract, since they can adhere to the mucus layer and release the loaded drug in a sustained manner.[1,2] The widely studied mucoadhesive polymers include chitosan, hydroxypropyl cellulose, poly(acrylic acid) (PAA) and their derivatives. PAA possesses an excellent mucoadhesive property owing to their carboxylic groups.[3] Despite excellent mucoadhesive property of PAA, it has some limitations as a mucoadhesive drug carrier due to its high water solubility at neutral pH. High water solubility critically limits its use as a mucoadhesive drug carrier, because it may be dissolved before the desired duration for the delivery of the drug across the membrane. To reduce the water solubility of PAA, chitosan was used to form a complex by intermolecular hydrogen bonding between amino groups of chitosan and carboxyl groups of PAA.[4]

Chitosan has been used in many different areas of drug delivery systems[5-8] and is obtained by extensive deacetylation of chitin, a natural polysaccharide. This natural polysaccharide possesses useful properties including biodegradability and biocompatibility. The OH and NH_2 groups of chitosan can give rise to hydrogen bonding and the linear molecular structure provides chain flexibility, the conformation of which is highly dependent on ionic strength.[3] These properties are considered essential for mucoadhesion.[9-11] Furthermore, the cationic polyelectrolyte nature of chitosan could provide a strong electrostatic interaction with mucus or a negatively charged mucosal surface.[12] These properties of chitosan have been demonstrated in earlier works by many researchers.

The objectives of this work were to reduce the water solubility of PAA, maintain mucoadhesive property, and investigate the effect of various processing parameters on the formation of microspheres. To reduce the water solubility of PAA, the interpolymer complexation with chitosan through electrostatic interaction was used.

Experimental

Materials

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). Acetic acid was purchased from the Junsei Chemical Co. (Tokyo, Japan) and corn oil was acquired from CJ Corporation (Seoul, Korea). All other chemicals were of

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reagent grade and were used without further purification.

Preparation of microspheres

Chitosan-PAA microspheres were prepared using a solvent evaporation method. Chitosan (1.0, 1.3, 1.5 wt%) was dissolved in water containing 2% v/v acetic acid and PAA (0.3, 0.6, 0.9 wt%) was dissolved in water. Using a 5 ml syringe, 2 ml of a PAA solution and 5 ml of a chitosan solution were slowly dropped into 100 ml of corn oil as a continuous phase. The corn oil contained 0.4% v/v of span 80 (sorbitan monooleate) as a surfactant. They were stirred at 500 rpm at room temperature for 48 hr using a magnetic bar. The hardened microspheres were collected by filtration. The collected microspheres were dried for 12 hr at 80°C. Chitosan microspheres as a control group were prepared in a similar manner to chitosan-PAA microspheres except that no PAA solution was dropped into the external phase.

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.

Particle size analysis

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

Differential scanning calorimetry

Thermal analyses were carried out using a differential scanning caloriometer (DSC 50, Shimadzu Scientific Instruments, MD). The Sample was placed in an alumina pan and heated at a scanning rate of 10°C/min from 40°C to 700°C.

Swelling studies

Microspheres were placed in a sealed syringe (1 ml) that was then filled with medium. The media used in this study include pH 2.0 HCl buffer, 50 mM pH 6.8 phosphate buffer, and distilled water. The swelling front was observed, and volume readings were taken from the graduation for 12 hr.

Results and Discussion

Poly(acrylic acid) (PAA) is soluble in water and chitosan is soluble in acidic aqueous solution, however, they precipitate in the solution when they form an interpolymer complex owing to electrostatic attraction between amine groups of chitosan and carboxylic groups of PAA.[8,13,14] Our previous study showed that mucoadhesive microspheres could be prepared using interpolymer complexation of poly(vinyl pyrrolidone) (PVP) and PAA.[15] While PVP and PAA are known to form a complex via hydrogen bonding between carbonyl groups of PVP and carboxyl groups of PAA, chitosan and PAA will form a complex via electrostatic force between amine groups of chitosan and carboxylic groups of PAA. In this study, chitosan and PAA were used to prepare mucoadhesive microspheres by interpolymer complexation and solvent diffusion method. The chitosan solution and the PAA solution were sequentially dropped and dispersed in corn oil. The chitosan solution was first dispersed in corn oil and PAA solution was added. The dispersed droplets of chitosan solution collided with those of PAA solution in corn oil and then formed the interpolymer complex. The droplets of the chitosan-PAA complex gradually solidified and further hardened as water diffused out of internal phase.

In order to optimize the preparation conditions, the effects of the various experimental parameters on the formation of the microspheres were investigated. In an effort to evaluate the diffusion rate of the dispersed phase, the effect of adding ethanol to the chitosan and PAA solutions on the formation of the microspheres was evaluated. It was reported that the diffusion rate of the internal phase affects the yield and morphology of the Eudragit® microspheres prepared by a solvent diffusion and evaporation method.[16] In a previous study, ethanol was used as one of the internal phase components to reduce the hardening time of the PVP-PAA mucoadhesive microspheres.[15] The diffusion rate of the ethanol from the internal phase into the external phase is expected to be faster than water. At an ethanol/water ratio of 1/9, the microspheres showed irregular shapes and some were aggregated. The extent of aggregation increased as the ethanol content in the internal phase was increased. The time required for the droplets to solidify decreased as the content of ethanol increases. If the chitosan or PAA droplets lose most of the internal phase solvent before they collide with each other, they may incompletely solidify and aggregate. Therefore, ethanol was not included in the internal phase in the subsequent experiments.

It is well known that stirring provides the energy needed to break up the emulsion droplets and it is obvious that smaller droplets will be formed as the stirring speed increases. The effect of the stirring speed on the size distribution and morphology of the microspheres were examined by changing the stirring speed from 300 to 500 rpm. As the stirring speed of the magnetic bar increased, the average particle size decreased without significantly changing their morphology. In addition, the stirring

Table I–*Effect of Various Processing Parameters on the Formation and Mean Particle Size of the Microspheres*

Processing Parameter			Particle Size Mean ± SD (μm)
Stirring	Mechanical stirrer		59.3 ± 7.5
	Magnetic stirrer		71.3 ± 1.1
Polymer Concentration	CS 1%	PAA 0.3%	53.0 ± 2.2
	CS 1.3%	PAA 0.3%	65.2 ± 4.1
	CS 1.5%	PAA 0.3%	71.3 ± 1.0
	CS 1%	PAA 0.3%	53.0 ± 2.2
	CS 1%	PAA 0.6%	180.3 ± 55.4
	CS 1%	PAA 0.9%	505.5 ± 227.0
Internal Phase Volume	5%		231.2 ± 43.7
	7%		71.3 ± 1.0
	10%		95.3 ± 16.3
Molecular Weight of Chitosan	Low		55.1 ± 2.8
	Medium		62.5 ± 3.2
	High		71.3 ± 1.0
Surfactant Concentration	0.2%		123.9 ± 1.2
	0.4%		71.3 ± 1.1
	0.6%		77.4 ± 6.9

* Unless otherwise specified, the following conditions were used for the preparation of microspheres; 1.5% of the high molecular weight chitosan and 0.3% of PAA solutions were used as an internal phase and the internal phase volume was 7%. 0.4% v/v of span 80 was used as a surfactant.

method using either a mechanical stirrer or a magnetic stirrer was compared. The average particle size of the microspheres prepared using the mechanical stirrer and magnetic stirrer was 59.3 μm and 71.3 μm, respectively (Table I). This indicates that the mechanical stirrer provided the higher energy.

Table I shows the effect of various processing parameters on the size distribution of microspheres. The effects of the polymer concentrations on the formation of microspheres were examined using 1.0, 1.3, and 1.5% chitosan and 0.3, 0.6, 0.9% PAA. The effects of the molecular weight were also evaluated using high, medium, and low molecular weight of chitosan. As the polymer concentration and the polymer molecular weight increased, the shape of the microspheres became increasingly spherical and the particle size increased. As the polymer concentration and polymer molecular weight became higher, the viscosity of the internal phase increased. It became increasingly difficult to break up the droplets into a smaller size as the viscosity of the internal phase was increased, resulting in larger microspheres. No significant aggregation was observed with increasing chitosan concentration up to 1.5%. However, the increase in the PAA concentration caused a higher level of aggregation, resulting in sharp increase in particle size. Based on the morphology, particle size, and the extent of aggregation, it was decided to use 1.5% of the high molecular weight chi-

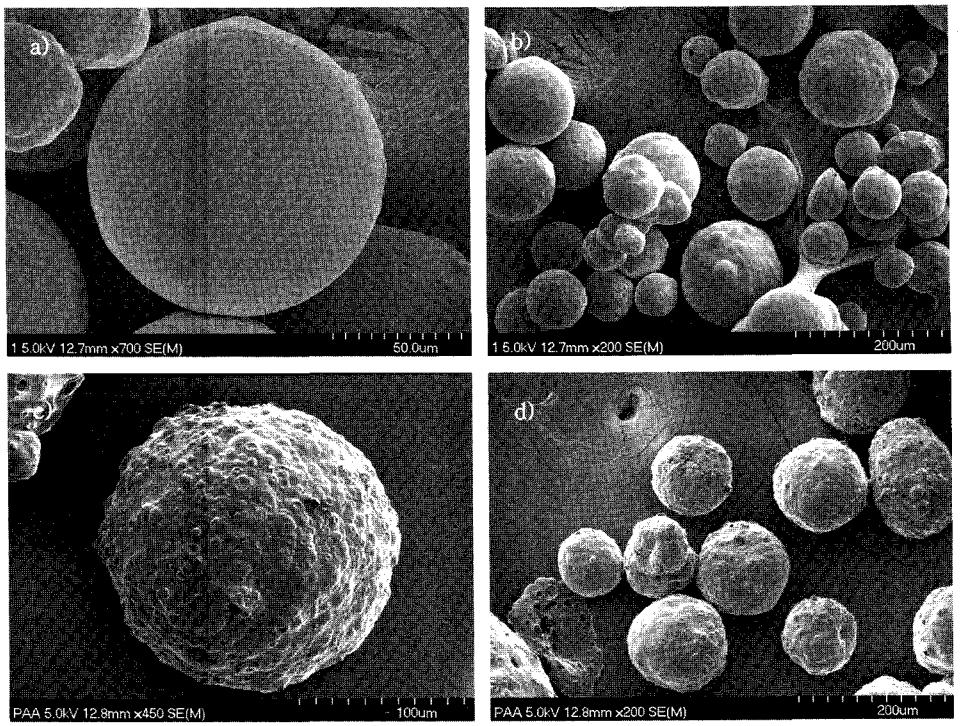

Figure 1–Scanning electron micrographs of microspheres prepared by solvent evaporation method: a), b): chitosan microspheres; c), d): chitosan-PAA interpolymer complex microspheres.

tosan and 0.3% of PAA.

The effect of internal phase volume was studied using 3, 5, 7, and 10% of the external phase. At the internal phase volume of 3 and 5%, the morphology was spherical, however, the yield was less than 50%. The optimum volume of internal phase was 7%. The yield abruptly decreased and the degree of aggregation increased at internal phase volume of 10%. A similar trend was observed in the preparation of PVP-PAA mucoadhesive microspheres. When the population of internal phase droplets is low, the droplets of one polymer have less chance to collide with the droplets of the other polymer. If a droplet does not have a chance to collide with the counter part, it may be broken into smaller particles. When the population of droplets is too high they may collide with many droplets and will be aggregated. Therefore, optimum amount of internal phase droplets should be present in the external phase to prepare spherical microspheres with appropriate yield.

The morphology of microspheres was examined by a scanning electron microscope. The view of the chitosan microspheres showed a spherical shape and smooth and dense surface, while the surface of chitosan-PAA microspheres was somewhat rough and porous as shown in Figure 1.

Figure 2 shows the differential scanning calorimetric (DSC) thermograms of chitosan, chitosan microspheres and chitosan-PAA microspheres. The DSC of chitosan shows an endothermic peak at 80°C due to the loss of water. The exothermic peak at 308°C can be attributable to the decomposition of the polysaccharide chain. The DSC thermogram of chitosan microspheres was quite different from that of chitosan itself. The solubilization of chitosan in acetic acid solution before the preparation of chitosan microspheres seemed to change the thermogram of chitosan. The DSC thermogram of acrylic acid grafted chitosan showed endothermic peak at 118°C and two exothermic transitions at 272 and 473°C.[6] The endothermic peak of chitosan-PAA microspheres at 80°C can be attributable to the loss of loose and bound water. The chitosan-PAA microspheres also showed two exothermic peaks. The exothermic peaks at 330 and 505°C can be attributable to the decom-

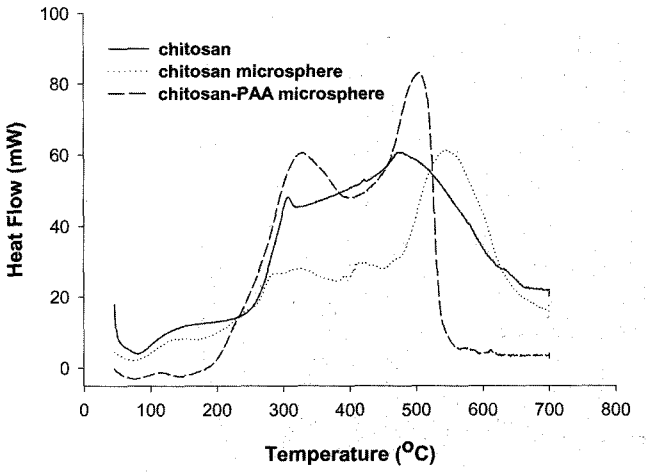

Figure 2–Differential scanning calorimetric thermogram of chitosan, chitosan microspheres, and chitosan-PAA microspheres.

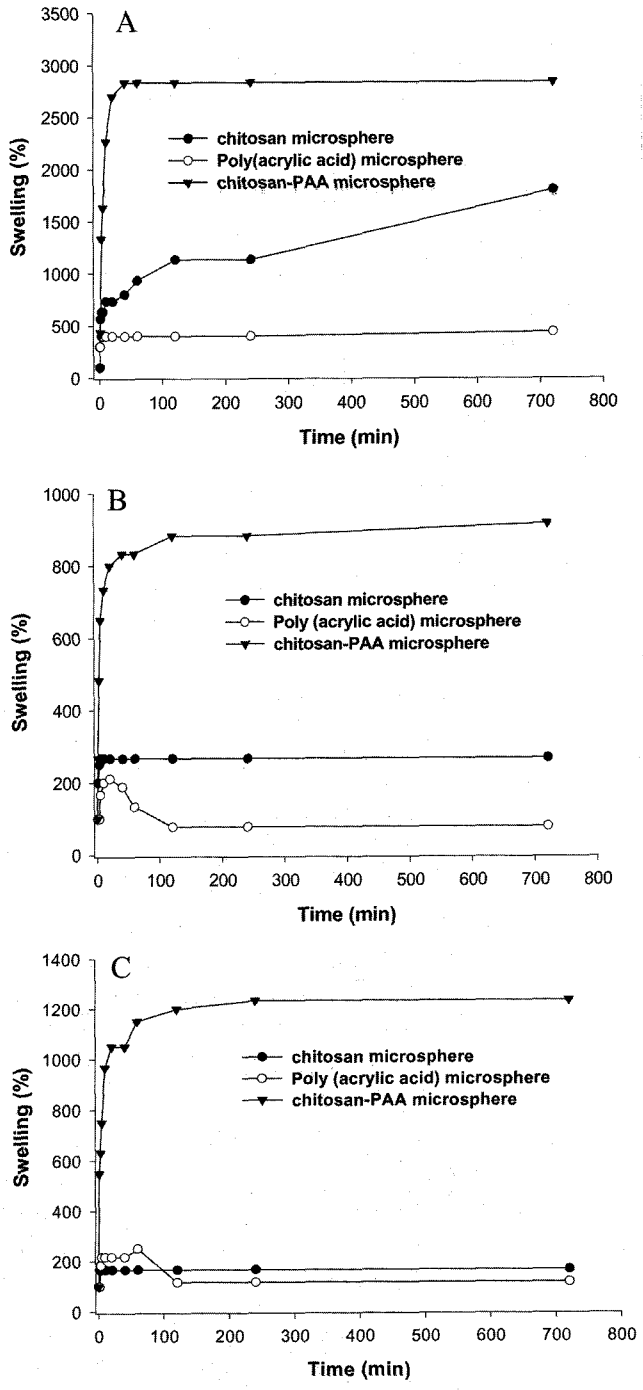

Figure 3–Effect of medium on the swelling ratio of chitosan microspheres, PAA, and chitosan-PAA microspheres. A: pH 2.0 HCl buffer; B: 50 mM pH 6.8 phosphate buffer; C: distilled water.

position of chitosan and the decomposition of PAA, respectively. The similar thermogram of chitosan-PAA microspheres to PAA grafted chitosan indicates that interpolymer complex was formed between chitosan and PAA.

Figure 3 shows the effect of the pH of the medium on the swelling ratio of the chitosan, PAA and chitosan-PAA microspheres. The 10 mg of each sample was placed in a sealed syringe followed by filling with a buffer solution at room temperature. The dynamic swelling front of microspheres was observed, and the volume readings were taken from the graduation for 12 hr. The chitosan-PAA microspheres showed rapid swelling in all medium tested. The swelling ratio of chitosan-PAA microspheres was highest at pH 2.0 due to the ionization of amine groups of chitosan. The PAA microspheres exhibited the lowest swelling ratio. The swelling ratio of PAA in pH 6.8 buffer and water decreased after certain time point since it is soluble in the test medium. The chitosan microspheres gradually swelled in pH 2.0 buffer but they did not swell significantly in pH 6.8 buffer and water since amine groups of chitosan could not be dissociated at neutral pH. It also has been shown in the literature that the swelling behavior of polyelectrolyte complex changes with pH of a medium, i. e., higher in acidic and alkaline pH and lower at neutral pH.[17,18]

Conclusion

The mucoadhesive chitosan-PAA microspheres were prepared by solvent evaporation and interpolymer complexation method. These microspheres were formed by electrostatic interaction between the carboxyl groups of PAA and the amine groups of chitosan. It was confirmed by swelling and DSC study. The microspheres had spherical shape and the surface was rough. Based on the mucoadhesive property and the reduced solubility in water of chitosan-PAA microspheres, they can be used as a carrier for oral transmucosal drug delivery system.

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