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## Chitosan Microparticles Prepared by the Water-in-Oil Emulsion Solvent Diffusion Method for Drug Delivery

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**Abstract:** In the present study biodegradable microparticles of chitosan were prepared by the simple water-in-oil emulsion solvent diffusion method for use as drug delivery systems. Aqueous chitosan solution and ethyl acetate were used as water and oil phases, respectively. Gentamicin sulphate was used as a hydrophilic model drug. Effects of drug content and sodium tripolyphosphate cross-linker on chitosan microparticles and drug release behaviors were investigated. The both drug-free and drug-loaded chitosan microparticles were deflated microparticles and porous structures with 200-400  $\mu\text{m}$  in size. The cross-linking did not affect on microparticle shape and size. The drug release rates decreased as the drug content decreased and the amount of cross-linker increased.

**Key words:** Biodegradable polymers, emulsification-diffusion method, cross-linking, controlled release

### INTRODUCTION

Recently, interest in microparticles of biodegradable polymers has increased steadily because of their potential applications in drug delivery systems (Ravi Kumar *et al.*, 2004; Muzzarelli and Muzzarelli, 2005). Many methods have been reported for producing biodegradable microparticles. However, a suitable and simple method for preparing biodegradable microparticles remains to be identified. The oil-in-water (O/W) emulsification-diffusion method is a simple and suitable for producing particles of water-insoluble biodegradable polymers such as poly(D,L-lactide-co-glycolide) (Song *et al.*, 2006; Tsukada *et al.*, 2009). It may be expected that a W/O emulsification-diffusion method could be used to prepare particles of water-soluble biodegradable polymers such as chitosan.

Chitosan is a copolymer of 2-glucosamine and N-acetyl-2-glucosamine produced by alkaline deacetylation of chitin that have received great attention in medical, pharmaceutical and metal ion treatment applications because of their biodegradability, biocompatibility and high concentration of amine functional groups (Crini, 2005). Usually, chitosan microparticles were fabricated by precipitation, spray drying and W/O emulsification-cross-linking methods (Learoyd *et al.*, 2008; Yuan *et al.*, 2007). Herein, we firstly presented the preparing chitosan microparticles with deflated and hollow shape by the W/O emulsification-

diffusion method. The hollow and porous chitosan microparticles were also interested for controlled-release drug delivery (Peng and Zhang, 2005; Li *et al.*, 2008) and adsorbent applications (Kanai *et al.*, 2008), respectively.

In this work, an alternative method for preparing chitosan microparticles for drug delivery was reported. This method is a water-in-oil (W/O) emulsion solvent diffusion method. Influence of drug ratios and cross-linking on the chitosan microparticles was investigated and is discussed. Gentamicin sulphate was used as a water-soluble model drug for entrapment within chitosan microparticles. *In vitro* drug release from the chitosan microparticles was also determined.

### MATERIALS AND METHODS

This research was conducted during December 2008-October 2009 at Mahasarakham University, Mahasarakham, Thailand.

**Materials:** Chitosan with 85-90% degree of deacetylation and average molecular weight of 100 kDa was purchased from Seafresh Chitosan Lab Co., Ltd., (Thailand). Gentamicin sulphate, water-soluble model drug was kindly supplied by Sang Thai Co., Ltd. (Thailand). O-Phthaldialdehyde (Fluka), sodium tripolyphosphate (Fluka) and ethyl acetate (Labscan) in analytical grade were used. All chemicals were used without further purification.

Table 1: Preparatory conditions for chitosan (CS) microparticles with and without drug entrapment and their particle sizes (10 mL of 1% w/v chitosan solution was used for all conditions)

Sample No.	CS:drug ratio (w/w)	CS:cross-linker ratio (w/w)	Gentamicin sulphate <sup>a</sup> (mg)	Sodium tripolyphosphate <sup>b</sup> (mg)	Particle size <sup>c</sup> (μm)
1	10:00	10:00	-	-	239±45
2	10:01	10:00	10	-	246±52
3	10:02.5	10:00	25	-	262±47
4	10:05	10:00	50	-	295±61
5	10:02.5	10:00.5	25	5	263±68
6	10:02.5	10:01	25	10	265±74

<sup>a</sup>Hydrophilic model drug, <sup>b</sup>Cross-linking agent, <sup>c</sup>Measured from light scattering analysis. Average values and standard deviations were reported (n = 3)

## Methods

**Preparation of chitosan microparticles:** Chitosan solutions (1.0% w/v) were prepared by dissolving chitosan flakes in 4% (v/v) acetic acid solution. Chitosan microparticles were prepared by the water-in-oil emulsion solvent diffusion method in 600 mL beakers with magnetic stirring. The 10 mL of chitosan solution was added drop-wise to 400 mL of ethyl acetate with stirring speed of 600 rpm for 45 min. The beaker was tightly sealed with aluminum foil to prevent evaporating of ethyl acetate during emulsification-diffusion process. The chitosan microparticles were recovered by centrifugation and finally dried in a vacuum oven at 30°C for 24 h. The gentamicin sulphate was directly dissolved in chitosan solution before microparticle production to prepare drug-loaded microparticles. For drug-loaded cross-linked chitosan microspheres, the drug and sodium tripolyphosphate, cross-linker were directly dissolved in the chitosan solution before microparticle preparation. The conditions for preparation of the chitosan microparticles with different drug and cross-linker contents are reported in Table 1.

**Characterization of chitosan microparticles:** Fourier Transform Infrared (FTIR) spectroscopy was chosen to determine chemical structures of the chitosan microparticles. A Perkin-Elmer Spectrum GX FTIR spectrophotometer was used with air as the reference (Niamsa *et al.*, 2009). The resolution of 4 cm<sup>-1</sup> and 32 scans were chosen. FTIR spectra were obtained by KBr disk method. Morphology of the chitosan microparticles was investigated by Scanning Electron Microscopy (SEM) using a JEOL JSM-6460LV SEM. The samples were coated with gold for enhancing conductivity before scan. Particle size and size distribution of the chitosan microparticles were measured by light scattering analysis using a LS230 particle size analyzer at 25°C (Srisuwan *et al.*, 2009). The distilled water was used as a dispersed medium.

**Determination of gentamicin sulphate content:** Since gentamicin sulphate does not absorb ultraviolet nor visible lights, an indirect method was required for the spectrophotometric analysis of this drug. The

phthalaldehyde was used as a derivatizing agent. It reacts with the amino groups of gentamicin to yield chromophoric products. The reaction was carried out making 2 mL of sample gentamicin solution react with 2 mL of isopropanol (to avoid the precipitation of the product form) and 2 mL of phthalaldehyde reagent. The concentration of gentamicin sulphate complex was determined by UV-Vis spectroscopy using a Perkin-Elmer Lambda 25 UV-Vis spectrophotometer at 332 nm.

**In vitro drug release test:** An exact amount of drug-loaded chitosan microparticles (10 mg) was charged into a plastic tube containing 1 mL of Phosphate Buffer Solution (PBS) with pH 7.4. The sample tubes were incubated at 37°C under shaking at the rate of 150 rpm. At predetermined time intervals, the tubes were centrifuged at 5,000 rpm for 5 min before removing all PBS medium. Then 1 mL of fresh PBS was added into the tube for continuing the release test. The released gentamicin sulphate was measured by a UV-Vis spectrophotometer as described above. According to a predetermined gentamicin sulphate concentration-UV absorbance standard curve, gentamicin sulphate concentration of the release medium was obtained and % gentamicin sulphate release was calculated. Each average value was calculated from the three measurements.

## RESULTS

Chemical structures of chitosan microparticle matrix and entrapped drug were determined from the FTIR spectra, as shown in Fig. 1a-e. Figure 1a presents the FTIR spectrum of gentamicin sulphate, model drug. The FTIR spectrum of pure chitosan microparticles in Fig. 1b shows the absorption bands at 1.654 and 1.587 cm<sup>-1</sup>. These bands correspond to the amide carbonyl groups (amide I) and the free amino groups, respectively. The absorption intensities of drug characteristic bands in the range of 1.000-1.200 cm<sup>-1</sup> increased with the drug ratio. This suggested that the drug-loaded chitosan microparticles with drug entrapment can be prepared. Figure 2a-d shows SEM images of the chitosan microparticles with different drug loading contents. It was found that the all microparticles were deflated and hollow in shapes. The different drug ratios did not affect on the

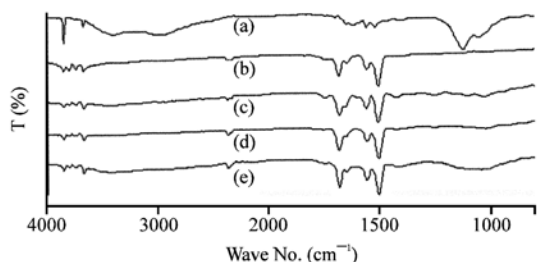


Fig. 1: FTIR spectra of (a) gentamicin sulphate powder, (b) pure chitosan microparticles (sample No. 1) and drug-loaded chitosan microparticles with chitosan:drug ratios of (c) 10:1 (sample No. 2), (d) 10:2.5 (sample No. 3) and (e) 10:5 (sample No. 4) by weight

microparticle shape. The drug-unloaded and drug-loaded microparticle matrix showed porous structure. The cross-section of microparticle matrix was shown in Fig. 3 a and b for the drug-loaded microparticles (sample No. 4). This indicates that the microparticles were light-weight particles.

The drug-loaded cross-linked chitosan microparticles can be prepared by cross-linking of chitosan solution with sodium tripolyphosphate before microparticle formation. The SEM images of cross-linked chitosan microparticles are illustrated in Fig. 4a, b. It was found that the cross-linked chitosan microparticles showed also deflated and hollow in shapes as those of uncross-linked chitosan microparticles. This suggests that cross-linking did not affect on the chitosan microparticle shape.

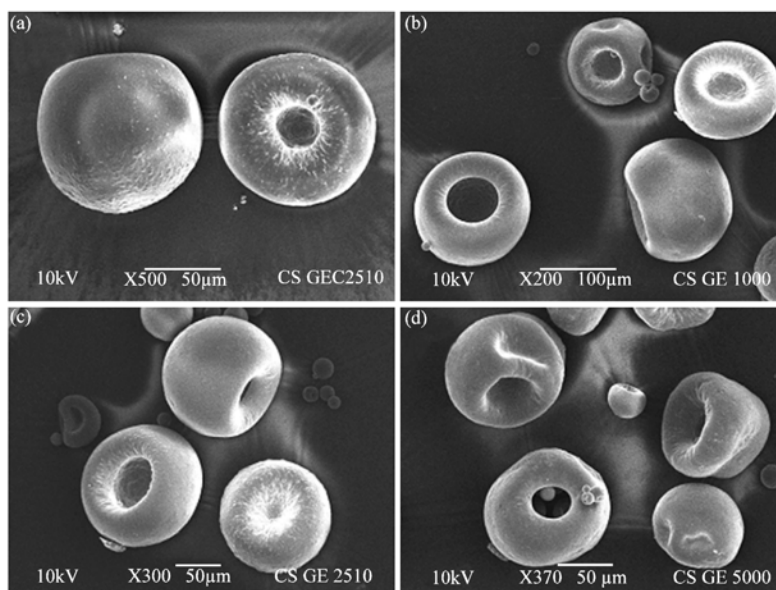


Fig. 2: SEM micrographs of (a) chitosan microparticles (sample No. 1) and drug-loaded chitosan microparticles with chitosan:drug ratios of (b) 10:1 (sample No. 2), (c) 10:2.5 (sample No. 3) and (d) 10:5 (sample No. 4) by weight. All bars = 50 µm, except (b) = 100 µm

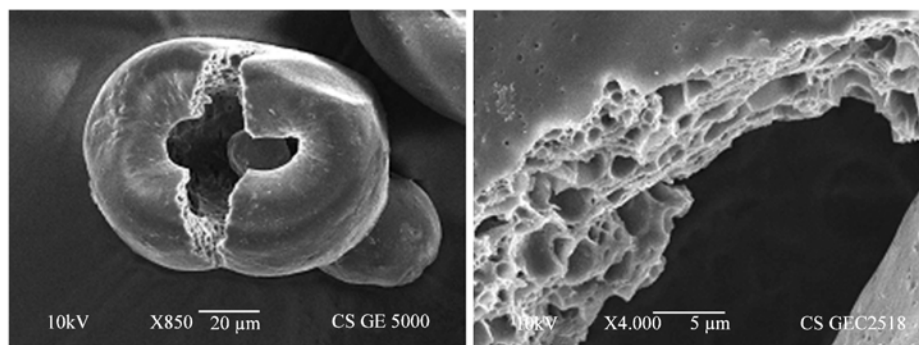


Fig. 3: SEM micrographs of drug-loaded chitosan microparticle matrices (sample No. 4) with magnifications of ×850 (left) and ×4,000 (right). Bars = 20 and 5 µm for left and right, respectively

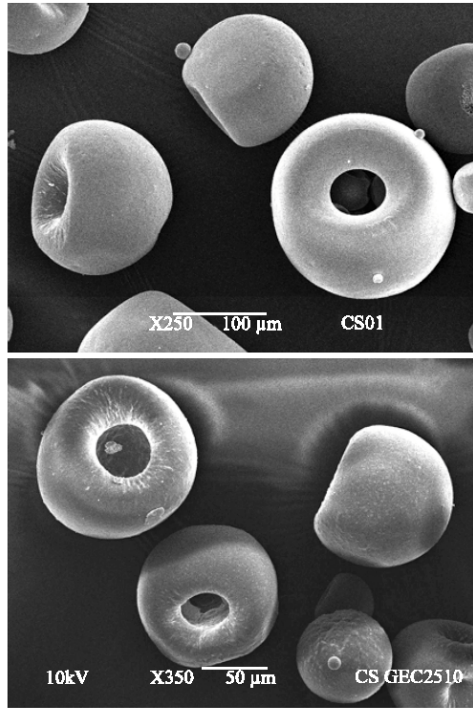


Fig. 4: SEM micrographs of drug-loaded chitosan microparticles cross-linked with chitosan: cross-linker ratios of (left) 10:0.5 (sample No. 5) and (right) 10:1.0 (sample No. 6) by weight. Bars = 100 and 50 µm for left and right, respectively

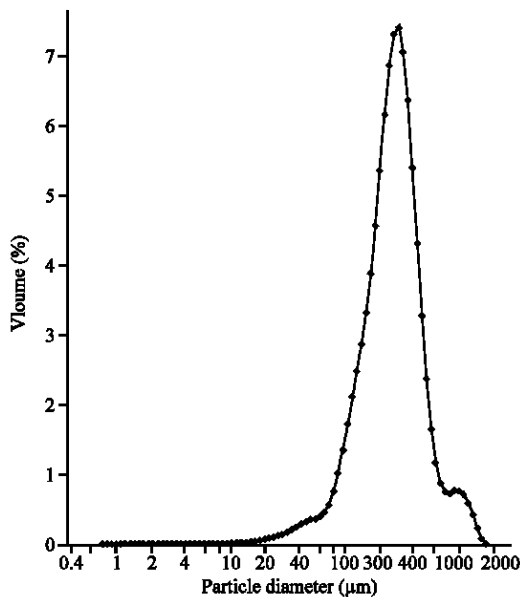


Fig. 5: Particle size curve of drug-loaded chitosan microparticles with chitosan: drug ratio of 10:5 by weight (sample No. 4)

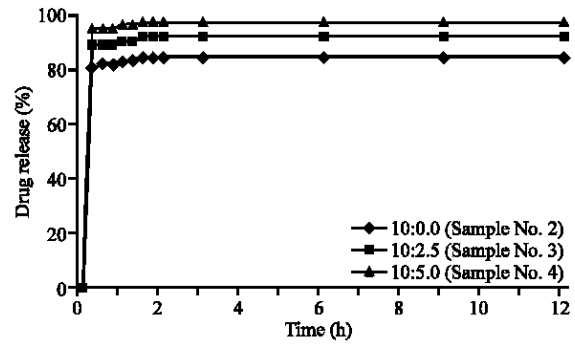


Fig. 6: *In vitro* drug release profiles of drug-loaded chitosan microparticles with chitosan

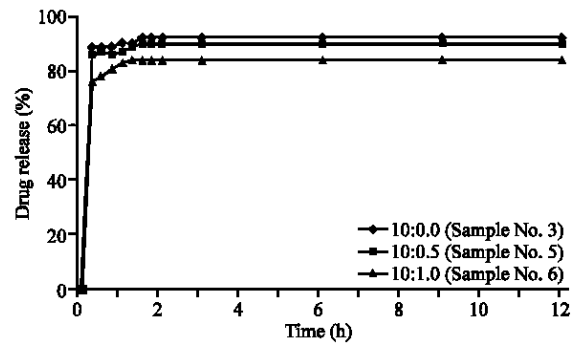


Fig. 7: *In vitro* drug release profiles of drug-loaded cross-linked chitosan microparticles with chitosan (chitosan: drug ratio of 10:2.5 by weight was used for all samples)

Particle sizes and size distributions of the chitosan microparticles with and without drug entrapment were measured by light scattering analysis. The results of chitosan microparticle sizes prepared with different drug and cross-linker contents are summarized in Table 1. They were in the range of 239-295 µm with monodisperse type. The particle size graph was shown as example in Fig. 5 for drug-loaded chitosan microparticles. The chitosan microparticles were slightly larger with the drug content.

The results of drug release tests were shown in Fig. 6 and 7 for the influences of drug content and amount of cross-linker, respectively. It was found that the all formulations show initial burst release effect following with slow drug releasing. The drug release rates and initial burst releases decreased when the drug ratio was decreased. The increasing cross-linker ratios induced slower drug release from microparticles.

## DISCUSSION

The drug-unloaded and drug-loaded chitosan microparticles were solidified and formed after the water

of emulsion droplets diffused to continuous phase, ethyl acetate. In previous study, the silk fibroin microparticles can be prepared by the same method (Baimark and Srihanam, 2009). The results suggested that the W/O emulsion solvent diffusion method is a potential method for fabricating the microparticles of water-soluble polymers such as silk fibroin and chitosan. These biodegradable microparticles may be interested in drug delivery systems, especially water-soluble drugs. The pure chitosan microparticles were deflated and hollow particles as shown in Fig. 2a. Deflated structures occurred due to dehydration during the diffusion process. The deflated particles of protein have been formed because of dehydration effect (Dibbern *et al.*, 2006). The morphological results indicate that the W/O emulsion solvent diffusion can be used for preparing chitosan microparticles.

The morphology of the drug-loaded chitosan microparticles with different drug ratios was also investigated from their SEM images, as shown in Fig. 2b-d. The drug-loaded chitosan microparticles were similar to the drug-unloaded chitosan microparticles. The results suggest that the drug entrapment did not affect chitosan microparticle shape. The porous structures of chitosan microparticles (Fig. 3) may be induced due to dehydration in the diffusion step.

The cross-linking of drug-loaded chitosan microparticles was performed with ionic cross-linking. For this purpose, the sodium tripolyphosphate was used as a cross-linker. The chitosan is a cationic polysaccharide at the  $-NH_3^+$  side groups. Therefore, the cross-linking was formed between  $-NH_3^+$  side groups and tripolyphosphate anion of the chitosan and the cross-linker, respectively. The drug-loaded cross-linked chitosan microparticles were similar to the uncross-linked chitosan microparticles. The results suggest that the cross-linking did not affect chitosan microparticle shape. It can be concluded that the preparatory conditions of chitosan microparticles used in this work are suitable for producing drug-loaded and unloaded chitosan microparticles with and without cross-linking.

Particle sizes and size distributions of the chitosan microparticles are summarized in Table 1. The size of the chitosan microparticles slightly increased when the quantity of entrapped drug was increased (Table 1). The particle size results indicate that the chitosan microparticle size is directly related to drug content. This suggests that the W/O emulsion solvent diffusion method is appropriate for preparing drug-loaded chitosan microparticles. This is due to the poor solubility of both chitosan and gentamicin sulphate model drug in the continuous ethyl acetate phase. The increasing microparticle size after drug

entrapment suggests the high drug entrapment efficiency of W/O emulsion solvent diffusion method.

*In vitro* drug release was investigated in PBS (pH 7.4) at 37°C for 12 h. Influences of drug and cross-linker contents on drug release profiles are shown in Fig. 6 and 7. All drug-loaded chitosan microparticles showed initial burst release effect. It would be expected that the burst release effect occurred due to rapid drug release from the dissolution of entrapped drug on the microsphere surfaces. However, the burst release effect and release rate of drug from the chitosan microparticles increased as the drug content increased and the cross-linker content decreased. It can be concluded that the rate of burst release is strongly dependent upon the drug and cross-linker ratios. Thus drug release rate from the chitosan microparticles can be adjusted by varying the drug and cross-linker content.

## CONCLUSION

The gentamicin sulfate-loaded cross-linked chitosan microparticles were successfully prepared by the simple and rapid W/O emulsion solvent diffusion method. The chitosan microparticles formed after diffusion of water from emulsion droplets to continuous ethyl acetate phase. The chitosan microparticles with and without drug entrapment showed deflated and hollow structures. The chitosan cross-linked with sodium tripolyphosphate before microparticle formation did not affect microparticle shape and size. The microparticle matrices were porous structures. The drug release rates decreased as the drug ratio decreased and cross-linker ratio increased. Therefore, the technique described is potentially very useful for drug delivery applications, especially water-soluble bioactive molecules.

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