# Effect of Formulation Compositions on Particle Size and Zeta Potential of Diclofenac Sodium-Loaded Chitosan Nanoparticles

Rathapon Asasutjarit, Chayanid Sorrachaitawatwong, Nardauma Tipchuwong, Sirijit Pouthai

Abstract-This study was conducted to formulate diclofenac sodium-loaded chitosan nanoparticles and to study the effect of formulation compositions on particle size and zeta potential of chitosan nanoparticles (CSN) containing diclofenac sodium (DC prepared by an ionotropic gelation method. The nanoparticles consisting of chitosan, DC and tripolyphosphate (TPP) at a weight ratio of 4:1:1, respectively, were prepared at pH 5.0, 5.5 and 6.0. It was found that at pH 5.0 and 6.0, the obtained systems were turbid because of precipitation of DC and chitosan, respectively. However, the dispersed system of CSN possessing diameter of 108±1nm and zeta potential of 19±1mV could be obtained at pH 5.5. These CSN also showed spherical morphology observed via a transmission scanning electron microscope. Change in weight ratio of chitosan:DC:TPP i.e. 1:1:1, 2:1:1, 3:1:1 and 4:1:1 showed that these ratios led to precipitation of particles except for the ratio of 4:1:1 providing CSN properly. The effect of Tween 80 as a stabilizer was also determined. It was suggested that increment of Tween 80 concentration to 0.02% w/v could stabilize CSN at least 48 hours. However, increment of Tween 80 to 0.03% w/v led to quick precipitation of particles. The study of effect of TPP suggested that increment of TPP concentration increased particle size but decreased zeta potential. The excess TPP caused precipitation of CSN. Therefore, the optimized CSN was the CSN containing chitosan, DC and TPP at the ratio of 4:1:1 and 0.02% w/v Tween 80 prepared at pH 5.5. Their particle size, zeta potential and entrapment efficiency were 128±1nm, 15±1mV and 45.8±2.6%, respectively.

*Keywords*—chitosan nanoparticles, diclofenac sodium, size, zeta potential.

#### I. INTRODUCTION

CHITOSAN is a co-polymer of  $\beta(1-4)$  linked 2-acetamido-2-deoxy-  $\beta$ -D-glucopyranose and 2-amino-2-deoxy-  $\beta$ -Dglucopyranose. It is obtained from a deacetylation reaction of chitin which is natural polysaccharide from shell of shrimps, crabs and insects [1].

Chitosan could be used for mucoadhesive drug delivery due to its positively charged polymer chains forming electrostatic interaction with negatively charged mucus. Moreover, it could increase drug absorption via a paracellular route by opening tight junction of epithelial cells [2]. In ophthalmic drug delivery, the physiological constrains imposed by the effective protection mechanisms, blinking reflex and tear dilution, lead to low drug bioavailability. Therefore, frequent dosing is usually needed to compensate for the decreased precorneal drug absorption, however, leading to poor patient compliance [3]. Recently, drug-loaded chitosan nanoparticles (CSN) have been developed to overcome this problem. Because of their small size and special characteristics of chitosan consisting in the nanoparticles such as a bioadhesive property and a permeation enhancer property, these systems could sustain drug release and improve ophthalmic drug bioavailabilty significantly [4].

Diclofenac sodium (DC) is one of non-steroidal drugs. It is used to treat eye inflammatory. Today, an ophthalmic preparation of DC available in the drug market is as eye drop only. It has been used extensively for the case of eye inflammatory with 3-6 times per day [5]. Consequently, to reduce frequency of administration, a prolonged-action DC ophthalmic preparation i.e. an ophthalmic suspension of DCloaded CSN should be formulated to increase patient compliance. Although Boonsongrit et al. [6] had shown the effect of formulation composition on DC-loaded chitosan particles, the obtained DC-loaded chitosan particles were not accepted as nanoparticles because they possessed particles size in the range of micrometer. Therefore, to achieve the optimized DC-loaded CSN for using as an active ingredient of the further ophthalmic preparations, this study would formulate DC-loaded nanoparticles and investigate the effect of formulation compositions on particle size and zeta potential of CSN containing DC prepared by an ionotropic gelation technique.

#### II. EXPERIMENTAL PROCEDURE

#### A. Preparation of DC-Loaded CSN

DC-loaded CSN were prepared by ionotropic gelation technique [7]. Chitosan solutions possessing various pH (5.0, 5.5, 6.0) were prepared. Briefly, 0.04g chitosan powder (high molecular weight with 85% deacethylation) was dispersed in 0.02% v/v acetic acid solutions and stirred continuously until they were transparent. Sodium hydroxide (1 N) was added into the obtained solutions to achieve pH 5.0, 5.5 and 6.0. DC (0.01g) dissolved in 95% ethanol (10ml) with Tween 80 (at various concentrations) was dropped into each chitosan solution with a rate of 1ml/minute. The mixtures were continuously stirred by using a high speed stirrer (Ultra-Turrax T8, Germany) at a rate of 10,000rpm for 30 minutes.

R. Asasutjarit is with the Department of Pharmaceutical Sciences, Faculty of Pharmacy, Thammasat University, Pathumthani 12120 Thailand (Corresponding author to provide phone: 66 81 8312902; fax: 66 2 5041532; e-mail: rathapona@ hotmail.com).

C. Sorrachaitawatwong, N. Tipchuwong, and S. Pouthai are with the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Srinakharinwirot University, Nakhonnayok 26120 Thailand (e-mail : chayanid@swu.ac.th, dzg\_lilmonkie@hotmail.com, doraemint\_7@hotmail.com, respectively).

Then, tripolyphosphate (TPP) (0.01g) dissolved in purified water was dropped into each solution with rate of 1ml/minute. The mixtures were continuously stirred by the high speed stirrer at a rate of 10,000rpm for 30 minutes. However, apart from effect of pH on DC-loaded CSN formation, effect of ratios of chitosan:DC:TPP and Tween 80 concentration were also investigated. The formulation compositions of DC-loaded CSN were shown in Table I.

#### B. Evaluation of Size and Zeta Potential of DC-Loaded CSN

Particle size and zeta potential of DC-loaded CSN were analyzed by a photon correlation spectroscopy technique, briefly, 0.1-ml of sample was diluted with purified water to make 1ml, then they were filled into a 1-ml cuvette for measuring particle size and zeta potential using Zetasizer (Malvern Instrument NanoZS, UK). They were measured in 3 replicates and reported in a form of mean  $\pm$  standard deviation (SD).

### C. Morphology Observation

The morphological characteristic of a representative DCloaded CSN was observed by using a transmission electron microscopy (TEM) technique. One drop of diluted sample was placed on a copper grid coated with carbon film, then stained with 0.5% w/v uranyl acetate solution and allowed to dry under room temperature. The grids were imaged using a JEM-1220 (Japan) transmission electron microscope.

## D. Entrapment Efficiency of DC-Loaded CSN

Entrapment efficiency of DC-loaded CSN was evaluated in a term of %entrapment efficiency. It was calculated by using the following equation

### %entrapment efficiency

$$= \left[ \begin{array}{c} \frac{\text{total amount of drug loaded - free drug in supernatant}}{\text{total amount of drug loaded}} \right] \times 100$$

Briefly, a suspension of a representative DC-loaded CSN was centrifuged at 12,000rpm for 2 hours. The supernatant was collected for determination of DC content. It was analyzed spectrophotometrically in triplicate at wavelength of 276nm (UV-visible spectrophotometer, Shimadzu UV-1601, Japan). Calculation of DC content was performed by using a linear regression equation for the standard curve of DC in 10%v/v ethanol.

#### III. RESULTS AND DISCUSSION

## A. Effect of pH on DC-Loaded CSN Formation

DC-loaded CSN consisting of chitosan, DC, TPP at the weight ratio of 4:1:1, respectively, without Tween 80 were prepared at various pH i.e. 5.0, 6.0, 5.5 (DC-CSN 1-3, respectively). It was found that DC-loaded CSN could be achieved at pH 5.5 (DC-CSN 3) only. They possessed particle size in the range of nanometer and positive zeta potential. The TEM photograph depicted in Fig. 1 indicated that DC-loaded CSN obtained from pH 5.5 formulation possessed a solid

dense structure with almost spherical shape. For DC-CSN 1 and DC-CSN 2, the precipitation of the ingredients consisting in them was occurred. This might be that pH 5.0 of chitosan solution was close to pKa of DC which is  $4.08\pm0.04$  [8] leading to incomplete dissolving of DC. Similarly, because chitosan could be dissolved greatly in acidic solutions, but, slightly soluble in solutions possessing the pH not greater than 6.5 [9], it tended to precipitate at the pH 6.0. This suggested that pH of solvents for DC-loaded CSN preparation was a crucial variable. From this experiment, it could be concluded that pH 5.5 was appropriate for DC-loaded CSN production.



Fig. 1 Negative stain transmission electron photograph of DC-CSN 3

#### B. Effect of Weight Ratio of Chitosan: DC

Table I showed that decrease in chitosan content in the CSN formulations (formulation DC-CSN 3-6) led to a failure of DC-loaded CSN production. It was found that only DC-CSN 3 consisting of chitosan, DC and TPP at the weight ratio of 4:1:1 could form nanoparticles properly. This might be that this ratio was optimum for DC-loaded CSN formation. Due to the appropriate electrostatic interactions between positive charge of chitosan polymer chains and negative charge of DC and TPP molecules, a dense structure of chitosan gel properly entrapping DC could be obtained [10].

#### C. Effect of Tween 80 Concentration

The effect of Tween 80 concentration on particle size and zeta potential of DC-loaded CSN was investigated. For this experiment, the nanoparticles were prepared based on DC-CSN 3 with varying in Tween 80 concentration i.e. 0%, 0.01%, 0.02% and 0.03% w/v (DC-CSN 3, 7-9). The results shown in Table I indicated that increase in Tween 80 concentration led to increase in particle size but decrease in zeta potential. This might be that Tween 80 molecules acting as amphiphilic molecules deposited at the particle surface resulting in increment of particle size, moreover, they could shield surface charge of the DC-loaded CSN led to decrease in zeta potential [11]. This assumption was confirmed by sedimentation rate of DC-loaded CSN. It was found that the nanoparticles obtained from formulations containing 0.02% w/v Tween 80 (DC-CSN 8) could be suspended in solvent for 48 hours before settling at the bottom of the test tube. This might be that Tween 80 depositing on DC-loaded CSN surface provided steric repulsion effect preventing agglomeration of the DC-loaded CSN while sedimentation of DC-CSN 3 and DC-CSN 7 which contained lower Tween 80 content and possessed more zeta potential than DC-CSN 8 occurred within 24 hours. This suggested that electrostatic repulsion of DC-CSN 3 and DC-CSN 7 was not enough to stabilize DC-loaded CSN suspensions. However, DC-CSN 9 containing more Tween 80 content possessed larger particle size but less zeta potential than DC-CSN 8. It showed agglomeration and sedimentation of nanoparticles within 24 hours as well. This implied that steric repulsion effect from Tween 80 could not stabilize this system. Therefore, Tween 80 at the concentration of 0.02% w/v could be considered as a suitable concentration for stabilization of DC-loaded CSN system.

TABLE I FORMULATION COMPOSITIONS, PARTICLE SIZE AND ZETA POTENTIAL OF DC-L OADED CSN

Particle	Zeta
size*	potential*
(nm)	(mv)
n.d.	n.d.
n.d.	n.d.
$108 \pm 1$	19±1
n.d.	n.d.
n.d.	n.d.
n.d.	n.d.
110±2	17±1
128±1	15±1
148±1	13±1
594±6	11±1
n.d.	n.d.
	size* (nm) n.d. n.d. n.d. n.d. n.d. 110±2 128±1 148±1 594±6 n.d.

\*mean $\pm$ SD (n =3); n.d. = not determined

#### D.Effect of TPP Concentration

It was found that DC-CSN 10 containing more TPP content than DC-CSN 8 possessed larger particle size but less zeta potential than DC-CSN 8. This was due to electrostatic interactions between positive charge of chitosan polymer chains and negative charge of excess TPP leading to less positive surface charge of DC-loaded CSN. In addition, the high speed stirrer used in this study could not generate effective shearing force to reduce the size of DC-chitosan-TPP gel structure leading to looser structure and larger particle size of DC-loaded CSN [12]. It was found that DC-CSN 11 containing the most TPP content could not provide DC-loaded CSN. It was immediately precipitated when TPP was added completely during the production process. This suggested that the weight ratio of chitosan:DC:TPP at 4:1:3 was not appropriate for DC-loaded CSN preparation.

Due to DC-CSN 8 possessing optimum particle size, zeta potential and stability, it was selected for determination of drug entrapment efficiency. It was found that DC-CSN 8 could entrap DC with %entrapment efficiency of 45.8±2.6%.

#### IV. CONCLUSIONS

The investigation of effect of formulation compositions on particle size and zeta potential of DC-loaded CSN showed that pH of chitosan solutions was one of crucial variables for DCloaded CSN production. Increase in pH led to precipitation of chitosan, on the contrary, decrease in pH led to precipitation of DC. The results also indicated that decrease in weight ratios of chitosan:DC resulted in failure of DC-loaded CSN production. It was found that the ratio of 4:1 for chitosan:DC at pH 5.5 could provide DC-loaded CSN possessing particle size in the range of nanometer with positive surface charge. The concentration of Tween 80 and TPP also affected particle size, zeta potential and stability of DC-loaded CSN. It was found that the formulation containing more Tween 80 content gave DC-loaded CSN possessing larger particles size with less zeta potential. However, optimum Tween 80 content could stabilize DC-loaded CSN for 48 hours. For TPP, increase in TPP concentration led to increase in particle size but decrease in zeta potential. In addition, excess TPP could lead to precipitation of the system.

The results showed that DC-CSN 8 containing chitosan, DC and TPP at the ratio of 4:1:1 and 0.02% w/v Tween 80 prepared at pH 5.5 had a potential for use in further study, because it possessed optimum particle size, zeta potential and stability as well. Furthermore, it could entrap DC with %entrapment efficiency of  $45.8\pm2.6\%$ . However, to achieve acceptable ophthalmic formulations by using DC-CSN 8, process of separation of DC-loaded CSN from solvent, drying process for stabilization of DC-loaded CSN, formulations of suitable vehicles for ophthalmic use and so on should be determined in further study.

#### REFERENCES

- Dodane, V., and Vilivalam, V. D. Pharmaceutical applications of chitosan. PSTT. 1998; 6:246-253.
- [2] Ludwig, A. The use of mucoadhesive polymers in ocular drug delivery. *Adv.Drug Deliv Rev.* 2005; 571: 595-639.
- [3] Asasutjarit, R, Thanasanchokpibull, S, Fuongfuchat, A, Veeranodha, S. Optimization and evaluation of thermoresponsive diclofenac sodium ophthalmic in situ gels. Int. J. Pharm. 2011; 411: 128-135.
- [4] Mennini, N., Furlanetto, S., Maestrelli, F., Pinzauti, S., and Mura, P. Response surface methodology in the ptimization of chitosan-Ca pectinate bead formulations. Eur. J. Pharm. Sci. 2008; 35: 318-325.
- [5] Fun, L.W., MIMS. 105<sup>th</sup> edition. TIMS (Thailand) Ltd., Bangkok. 2006. p.367.
- [6] Boonsongrit, Y., Mirevej, A., and Mueller, B. W. Chitosan drug binding by ionic interaction. Eur. J. Pharm. Biopharm. 2006; 62: 267-274.
- [7] Gan, Q., Wang, T., Cochrane, C., and McCarron, C. Modulation of surface charged, particle size and morphological properties of chitosan-TPP nanoparticles intended for gene delivery. Colloids surf B: Biointerfaces. 2005; 44 : 65-73.
- [8] Llinàs, A., Burley, J. C., Box, K. J., Glen, R. C., and Goodman, J. M. Diclofenac Solubility: Independent determination of the intrinsic solubility of three crystal forms. J. Med. Chem. 2007; 50: 979–983.
- [9] Riva, R., Ragelle, H., Rieux, A., Duhem, N., Jérôme, C., and Préat, V. Chitosan and chitosan derivatives in drug delivery and tissue engineering. Adv Polym Sci. 2011; 244: 19–44.
- [10] Dudhani AR, Kosaraju SL. Bioadhesive chitosan nanoparticles: Preparation and characterization. Carbohydrate Polymers. 2010; 81:243-251.
- [11] Asasutjarit, R, Lorenzen, S, Sirivichayakul, S, Ruxrungtham, K, Ruktanonchai, U, Ritthidej, GC. Effect of solid lipid nanoparticles formulation compositions on their size, zeta potential and potential for in vitro pHIS-HIV-hugag transfection. Pharm. Res. 2007; 24: 1098-1107.
- [12] Wu Y, Yang W, Wang C, Hu J, Fu S. Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate. Int. J. Pharm. 2005; 295: 235-245.