FORMULATION AND CHARACTERIZATION OF ASCORBIC ACID NANOPARTICLE WITH CHITOSAN AS A CARRIER FOR TOPICAL ADMINISTRATION

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Abstract

The use of nanoparticle in drug delivery system have been developed rapidly to increase the physical properties of active compound. This research was aimed to find out optimal condition of chitosan-ascorbic acid nanoparticle formulation which have an opalescent condition and have nanometer in particle size. Ionic gelation method was used. Two variable were optimized, i.e. pH (4; 5; 5,5) and concentration of chitosan (0,35 mg/ml and 0,45 mg/ml) in constant concentration of ascorbic acid (0,4 mg/ml) and sodium tripolyphosphat (0,14 mg/ml). An opalescent condition was used as a respons, and the optimum formulation was measured its particle size, Polidispercibility Index (PI), and loading efficiency. The results showed that optimum condition were gained in pH 5 and chitosan concentration was 0,45 mg/ml, with 42 % in the loading efficiency. This nanoparticle has 268,93 nm in size and 0,314 in PI.

Key words : *ascorbic acid, nanoparticle, chitosan*

ISBN : 978-979-18458-5-4

INTRODUCTION

The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. In conventional dosage forms, only a small amount of administered dose reaches the target site, while the majority of the drug distributes throughout the rest of the body in accordance with its physicochemical and biochemical properties. Therefore, developing a drug delivery system that optimizes the pharmaceutical action of a drug while reducing its toxic side effects. Polymeric nanoparticles, which possess a better reproducibility and stability profiles, have been proposed as alternative drug carriers that overcome many of these problems.

The use of nanoparticles as drug delivery system is receiving significant attention. The nature of the particle, such as its size and composition, will influence the manner of nanoparticle interact with the skin and the speed and extent with which it releases an associated 'active' species into the stratum corneum (SC), the outermost and principal barrier layer of the membrane (Wu et al, 2010). Nanoparticles are solid colloidal particles ranging from 1 - 1000 nm. They consist of macromolecular materials in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached (Birrenbach and Speiser, 1976; Mohanraj and Chen, 2006; Wang, 2006). They may be prepared from a variety of materials such as proteins, polysaccharides and others polymers (Krauland and Alonso, 2007; Vila et al, 2002). Chitosan, a biodegradable polymer has shown favorable biocompatibility characteristics as well as the ability to increase membrane permibility, both in vitro and in vivo (Leelapornpisid, et al, 2010).

Ascorbic acid is a water-soluble vitamin. It is important in forming collagen, a protein that gives structure to bones, cartilage, muscle, and blood vessels. Ascorbic acid is called an antioxidant because can donating its electrons, it prevents other compounds from being oxidized. As an electron donor, ascorbic acid is a potent water-soluble antioxidant in humans. Antioxidant effects of ascorbic acid have been demonstrated in many experiments in vitro (Padayatty, et al., 2003). Ascorbic acid is an unstable molecule, so for increased the stability and percutan absorbtion, ascorbic acid was made to nanoparticle which used chitosan as a carrier.

Method which used to make ascorbic acid nanoparticle is an ionic gelation (Calvo et al, 1997a and 1997b). Water-soluble polymers offer mild and used the simple preparation methods without the use of organic solvent and high shear force. Among water-soluble polymers available, chitosan is one of the most extensively studied, because chitosan possesses some ideal properties of a polymeric carrier for nanoparticles such as biocompatibility, biodegradability, non-toxicity, and low cost (Majeti and Kumar, 2000). It possesses a positive charge and exhibits an absorption enhancing effect. This characteristic can be employed to prepare cross-linked chitosan nanoparticles. Some research showed that chitosan is efective for drug delivery system (Wang et al, 2008). Chitosan also used for vaccine delivery (Tiyaboonchai, 2003).

In this research, had been formulated a nanoparticle ascorbic acid with chitosan as a carrier and Natrium Tripolyphosphat (Na TPP) as a crosslinking agent. Optimum formula could be gotten, by using a matrix design with two variable which optimized, that were pH and the concentration of chitosan, and the respon which be observed were the opalescent condition (good formula) and then measured the particle size and loading efficiency for the good formula. Complexs can occur, because of ionik interaction between chitosan, ascorbic acid, and Na TPP. PH is the most important factor can influence the creation of this complex, so the optimal pH must be choosen to produce ascorbic acid nanoparticle (Dustgani et al., 2008) (Jang and Lee, 2008).

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EXPERIMENTAL

Material

Ascorbic acid from Merck, chitosan and sodium tripolyphosphat (Na TPP) from Sigma Aldrich, all other chemicals used were of analytical grade.

Method

1. Matrix design for optimization the formula

The concentration of chitosan on some pH (4; 5; 5,5) were optimized (table1)

Table 1. Chitosan-Ascorbic Acid Nanoparticle Formulation

		pH of chitosan-ascorbic acid nanoparticle formulation		
chitosan concentration (mg/mL)		4	5	5,5
	0,35	F1	F7	F12
		F2	F8	F13
		F3	F9	F14
	0,45	F4	F9	F15
		F5	F10	F16
		F6	F11	F17

Preparation Method

The first, mixture 1,5 mL chitosan solution (chitosan in acetate daphar 0,15 M pH 4; 5; or 5,5), it was added with 0,5 mL ascorbic acid solution (ascorbic acid in aquadest). Then, they mixed used a magnetic stirrer, with 350 rpm in 10 minutes, after that it was added with 0,5 mL Na TPP solution (the concentration of Na TPP was 0,14 mg/mL) under stirred with magnetic stirrer until 30 minutes, keep from light. Then was sonicated 10 minutes.

After that the formation of opalescent condition from the formula were observed. The formula which have an opalescent condition are good formula. And then to the good formula we measured the size of particle with the Particle Size Analyzer (PSA) and calculated the loading efficiency.

2. Calculation of *Loading efficiency Chitosan-Ascorbic Acid Nanoparticle*

The good formula which had gotten from optimation, then centrifuge with 10000 rpm in speed in 50 minutes at 5oC (Biofuge Solvatar Primo R). Supernatant had separated from the sediment. The concentration of ascorbic acid in this supernatant was determined using UV spectrofotometre with 265 nm in λ max. Loading efficiency was calculated using equation (1).

$$LE\ (\%) = \frac{\text{Amount of ascorbic acid which be entrapped into nanoparticle}}{\text{Amount of ascorbic acid in the formula}} \times 100 \quad \ldots\ldots\ldots (1)$$

3 . Caracterization the Chitosan-Ascorbic Acid Nanoparticle

The best condition which had opalescent condition was calculated the loading efficiency and the particle size were determined using Particle Size Analyzer (Beckman Coulter)

RESULT AND DISCUSSION

1. Formulation Optimation of Chitosan-Ascorbic Acid Nanoparticle

The result was shown in table II.

ISBN : 978-979-18458-5-4

Table II. Optimation of chitosan-ascorbic acid nanoparticle. The blue and red cell did not show the oppalescent dispersion

		pH of chitosan-ascorbic acid nanoparticle formulation		
chitosan concentration (mg/mL)		4	5	5,5
	0,35	F1	F7	F12
		F2	F8	F13
		F3	F9	F14
	0,45	F4	F9	F15
		F5	F10	F16
		F6	F11	F17

In table II the concentration of chitosan and pH influence the nanoparticle assembling. In pH 4 (F1-F6), the dispersion were not opalescent both in concentration 0,35 mg/mL and 0,45 mg/mL, but in this condition moleculer dispersion were created. In pH 5 (F7-F9) with 0,35 mg/mL chitosan concentration showed that the moleculer dispersion were created too. In pH 5,5 (F12-F17) showed that the condition of formula were coarse dispersion. And the good formula which had opalescent condition were achieved in pH 5 with 45 mg/mL in chitosan concentration. The opalescent condition can be seen in figure 1 (a).

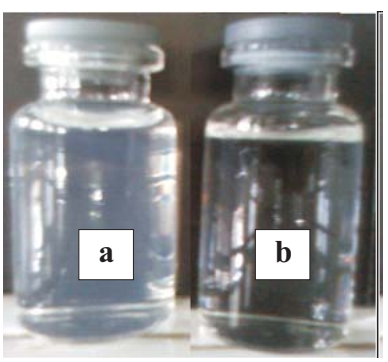

Figure 1. Formula (a) which have opalescent condition and formula (b) is a solution

Figure 1 (a) is the opalescent condition, is called the colloid system which between the solution (molecular dispersion) (in figure 1 (b)) and the coarse dispersion (suspension which have micrometre in particle size). The colloid system (nanoparticle) is more stable from precipitation than the suspension (microparticle).

Based on table II, it is shown that the best condition for nanoparticle assembling is in pH 5. In this pH, chitosan will be occur as kation because of dissosiation in amin group. Since the pKa of chitosan is 6,5 the amount NH3+ more than 99% so chitosan have big chance to interact with ascorbic acid and TPP.

Ascorbic acid is a weak acid, it has pKa 4,7, so it will be occur in anionic form more than 50% in pH 5. This condition will enforce the complex ionic interaction, and ascorbic acid will be loaded in nanoparticle chitosan. Sodium TPP dissociate to form polianion TPP and interacted with cation from chitosan in more than 1 side, more than 1 macromolecule chitosan. This condition will enhance chitosan nanoparticle loading efficiency. In another hand if the sodium TPP is too large, it can form the bigger particle, the risk is occur microparticle instead nanoparticle (Chun, et al, 2007) and decreasing of drug release from nanoparticle (Mengatto et al., 2010).

On 0,35 mg/mL concentration of chitosan, the opalescent condition didn't achieve, in pH 5,5, amount of kation from chitosan less than in pH 4 and 5, the condition formula were coarse dispersion so the microparticle were created (not nanoparticle). In pH 5 with 0,45 mg/mL in chitosan concentration had good formula

ISBN : 978-979-18458-5-4

(opalescent) compare with 0,35 mg/mL (molecular dispersion), because when the chitosan concentration too low, the nanoparticle were not created.

The loading efficiency which had been gained was 42 %, so we must optimized again to get the better formula which have higher loading efficiency. We can optimized the concentration of ascorbic acid and chitosan.

2. Caracterisation Chitosan-Ascorbic Acid Nanoparticle

The diameter of nanoparticle is 268,93 nm and it is agree with another research that nanoparticle which have been prepared with ionic gelation method using TPP as crosslinking agent has 200-400 nm in diameter. (Patel and Jivani, 2009; Calvo et al, 1997a). The diameter of nanoparticle govern their ability as a drug delivery carrier. It is claimed that nanoparticle less than 400 nm in diameter is a good carier for drug delivery (Rao et al, 2010). There are many process condition governing nanoparticle size, i.e.: method of assembling, the ratio of chitosan and TPP, the ratio of drug and chitosan, pH, and speed of stirring (Dustgani et al., 2008) (Jang and Lee, 2008).
The diagram that show the size of particle can be seen in figure 2.

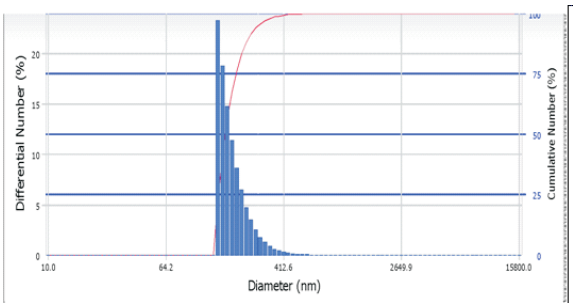

Figure 2. Size of chitosan ascorbic acid nanoparticle

Polidispersibility index from measured the particle size was 0,314. Polidispersibility index describe the distribution of particle size in the nanoparticle. The dispersion is monodisperse if the PI is low than 0,5. If the difference of particle size to much, it can influence the characteristic of the particle and the velocity of precipitation (Manmode dkk., 2009). In this research we didn't get the value of zeta potential. We measured the zeta potential from the good formula, but the formula is too dilute, so the data couldn't be read. We can increase the amount of precipited in the formula so we can measured the zeta potential.

Solution of ascorbic acid in water occurs in anionic species. This anionic must be penetrated to the skin via hydrophilic pathway using passive diffusion mechanism. This hydrophilic pathway is stratum corneum and epidermal cell contructed from keratin which is very dense so that difficult to diffused. Commonly, the penetration to the skin is via intercelluler rute which has been constructed by lipid lamella, dominant in lipoid fase. It is the reason that ascorbic acid penetration to the skin does not occur from this formula. Different with species in moleculer size (Armstrong) which is transported using passive diffusion mechanism, nanoparticle is transported using pinositosis mechanism. Since the skin is negative in charge, positive charge nanoparticle is easy to adhere in the surface of skin, and the engulfment will be happened. This research is agree with Wu et al (2010). The chitosan-losartan nanoparticle will be digested by lysozime, and the ascorbic acid release in this side.

Besides that, chitosan has ability to disturb keratin fluidity via enhance the water content in keratin, so chitosan can be used as skin permeation enhancer (He et al, 2009). Xueqin et al (2009) observes the effect of interaction between chitosan and keratin. The SEM showed that structure of keratin is changed by chitosan. The melting point (analyze using DSC) of keratin was decrease because of chitosan. From the IR Spectra (was gotten using FTIR) showed that the wavelength number of amida was shifted. He concluded that chitosan can change the conformation of keratin so that it will be easier to be acrossed (Xueqin et al, 2009).

The other research which showed the capability of chitosan nanoparticle to increased

the penetration of drug into the skin was the quercetin-loaded nanoparticles which showed higher permeation ability, and significantly increased accumulation of quercetin in the skin, especially in the epidermis. After administration indicated that the interaction between ingredients of the nanoparticles and the skin surface markedly changed the morphology of the stratum corneum and disrupted the corneocyte layers, thus facilitating the permeation and accumulation of quercetin in skin (Tan et al., 2011).

CONCLUTION

1. Chitosan ascorbic acid nanoparticle can be made with *ionic gelation* method with pH 5 with 0,45 mg/mL of chitosan concentration.
2. Nanoparticle which had gotten, has 268,93 nm in diameter with 0,314 in polidispersibility index, and the loading efficiency was 42 %.

REFERENCE

Birrenbach, G. and Speiser, P., 1976, Polymerized Micelles and Their Use as Adjuvants in Immunology, *J. Pharm. Sci*., **65**: 1763-1766.

Calvo, P., Remunan-Lopez, C., and Vila-Jato, J.L., 1997[a], Chitosan and Chitosan/Ethylene Oxide-Propylene Oxide Block Copolymer Nanoparticles as Novel Carriers for Proteins and Vaccines, *Pharm. Res.*, **14**: 1431-1436.

Calvo, P., Remunan-Lopez, C. and Vila-Jato, J.L., 1997[b], Novel Hydrophilic Chitosan-Polyethylene Oxide Nanoprticles as Protein Carriers, *J. Appl. Polymer Sci.*, **63**: 125-132.

Chun,W., Xiong,F., and LianSheng,Y., 2007, Water-Soluble Chitosan Nanoparticle as a Novel Carrier System for Protein Delivery, *Chines Sci. Bull*.,**52**(7):883-889.

Dustgani,A., Farahani,E.V., and Imani,M., 2008, Preparation of Chitosan Nanoparticles Loaded by Dexamethasone Sodium Phosphate, *Iranian J. Pharm.Sci*., **4**(2):111-114.

He, W., Guo.X., Xiao,L., and Feng,M., 2009, Study on the mechanisms of chitosan and its derivatives used as transdermal penetration enhancers, Pharmaceutical Nanotechnology, Intern. J. Pharm.,**382** (1-2): 234-243.

Jang,K., and Lee,G., 2008, Stability of Chitosan Nanoparticles for L-Ascorbic Acid during Heat Treatment in Aqueous Solution, *J.Agric.Food Chem*.,**56**:1936-1941.

Krauland, A.H. and Alonso, M.J., 2007, Chitosan Cyclodextrin Nanoparticles As Macromolecular Drug Delivery System, *Int. J. Pharm*., **340**: 134-142.

Leelapornpisid, P, Leesawat, P., Natakarnkitkul, S., And Rattanapanadda, P., 2010, Application Of Chitosan For Preparation Of Arbutin Nanoparticles As Skin Whitening, *J.of Metals, Materials and Minerals,*.**20(**3):101-105.

Majeti, N.V. and Kumar, R., 2000, A review of chitin and chitosan applications, *React. Funct. Polym.*, **46**: 1–27.

Manmode, A.S., Sakarkar, D.M., and Mahajan, N.M., 2009, Nanoparticles-Tremendous Therapeutic Potential:A Review, *Int. J. PharmTech. Res*., **1**(4) 1020-1027.

Mengatto,L., Luna,J.A., and Cabrera,M.I., 2010, Influence of Cross-lingking Density on Swelling and Estradiol Permeation of Chitosan Membranes, *J. Mater Sci*., **45**:1046-1051.

Mohanraj, V.J. and Chen Y., 2006, Nanoparticles-A Review, *Trop. J. Pharm. Res*., **5**(1) 561-573.

Padayatty, S.J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, H., Chen, S., Corpe, C., Dutta, A., Dutta, S.K., FACN, and Levine, M., 2003, Review Ascorbic acid

as an Antioxidant: Evaluation of Its Role in Disease Prevention, *J. American College of Nutrition*, **22**(1):18–35.

Patel, J.K., and Jivani,N.P., 2009, Chitosan Based Nanoparticles in Drug Delivery Review Article, *Intern. J. Pharm. Sci. Nanotech*., **2**(2):517-522.

Rao,D.P., Srivastav,S.K., Prasad,C., Saxena,R., and Asthana,S., 2010, Role of Nanoparticles in Drug Delivery**,** *Intern. J. Nanotech. and Applications*, **4**(1):45-49.

Tan, Q, Guo, C, and Zhai, G, 2011, Preparation and evaluation of quercetin-loaded lecithin-chitosan nanoparticles for topical delivery, *Int J Nanomedicine*, 6: 1621–1630.

Tiyaboonchai, W. 2003, Chitosan Nanoparticles: A Promising System for Drug Delivery, Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand, *Naresuan Univ*. *J*., **11**(3) 51-66.

Vila, A., Sanchez, A., Tobio, M., Calvo, P., and Alonso, M.J., 2002, Design of Biodegradable Particles for Protein Delivery, *J. Control. Release*., **78**: 15-24.

Wang, W., 2006, Polymer Characterization Techniques, in Uchegbu I.F., Schatzlein A.G., 2006, *Polym. Drug Deliv*., hal 23-31, Taylor and Fracis Group Academic Division of Informa plc., Boca Raton London New York.

Wang, X., Xu, W., Mohapatra, S., Kong, X., Li, X., Lockey, R.F., and Mohapatra, S.S., 2008, Prevention of Airway Inflammation with Topical Cream Containing Imiquimod and Small Interfering RNA for Natriuretic Peptide Receptor, *Genet*. *Vaccines Ther*., **6:** 7.

Wu,X., Landfester,K., Musyanovych,A., and Guy, R.H., 2010, Disposition of Charged Nanoparticles after Their Topical Application to the Skin**,** *Skin Pharmacol Physiol*., **23**:117–123.

Xueqin,Z., Guifeng,Y., Dongzhi,L., and Kangde,Y., 2009, *In Vitro* Investigation On The Interactions of Oligo-Chitosan With Keratin, *Acta Polymerica Sinica*, 8:781-785.