J. Exp. Biomed. Sci. 2011, 17(3): 211~216

Effects of Chitosan Coating for Liposomes as an Oral Carrier

Chang-Moon Lee[1,2,3], Dong-Woon Kim[4,†] and Ki-Young Lee[5,6,†]

[1]Department of Nuclear Medicine, [2]Institute for Medical Sciences, [3]Research Institute of Clinical Medicine, Chonbuk National University Medical School and Hospital, Jeonju 561-712, Korea
[4]Department of Clinical Pathology, Gwangyang Health College, Gwangyang 545-703, Korea
[5]Faculty of Applied Chemical Engineering, [6]The Research Institute for Catalysis, Chonnam National University, Gwangju 500-757, Korea

The chitosan-coated liposomes (chitosomes) were designed to improve the stability in the gastrointestinal (GI) tract and to enhance the efficacy for oral drug delivery of liposomes. The phosphatic acid (PA)-incorporated anionic liposomes were surface-coated with water soluble chitosan (WSC) by electro-ionic interaction. The shape of the chitosomes observed by transmission electron microscopy (TEM) was spherical in all the formulations and the coating layer by WSC could be founded through TEM images. The mean size and the zeta potential values of the chitosomes increased significantly with depending on the content of WSC added for coating the liposomes. The stability of the chitosomes in the GI tract was confirmed through the change of relative turbidity of the liposomal suspension. The plain liposomes (plasomes) suspension without adding WSC clearly showed the change of relatively turbidity in simulated gastric fluid (SGF), while the change degree of turbidity of the chitosomes in the SGF decreased as increasing of WSC content added for coating liposome. In the 5-CF release study from the plasomes and chitosomes, the plasomes released >90% of the initial 5-CF content at 4 h of release measurement. In contrast, the chitosomes released below 40% of initial content of 5-CF. In conclusion, these results indicate that the chitosomes can be used as a potential carrier for effective oral drug delivery.

Key Words: Oral drug delivery, Liposomes, Chitosan, Stability in SGF

INTRODUCTION

Liposomes are enclosed vesicles that are composed primarily of phospholipids. They have been extensively considered as useful carriers for delivery therapeutically active compounds due to their biocompatibility, biodegradation, and variable particle size, surface charge and membrane fluidity (Han et al., 1997; Childers et al., 1990; Gregoriadis et al., 1990). However, when they are orally administrated into the body, native state liposomes are rapidly degraded by bile salts and other components of the gastrointestinal (GI) intraluminal environment (Kokkona et al., 2000; Minato et al., 2003; Parmentier et al., 2011). Thus, many studies have been focalized on preventing the degradation of these liposomes and improving ability as an oral carrier (Zhu et al., 2005; Venkatesan et al., 2000; Takeuchi et al., 2005; Takeuchi et al., 1994). To overcome these problems many researchers have been reported a change of phospholipid compositions and modification of liposomal surface (Zhou et al., 2002; Barea et al., 2010; He et al., 2010). In the previous paper, we have investigated that the curdlan-coated liposomes were more clearly stable than non-coated liposomes in simulated gastric fluid (SGF) and bile acid and suggested that the curdlan-coated liposomes can be used as a potential carrier for oral admin-

*Received: 9 September, 2011 / Revised: 27 September, 2011
Accepted: 28 September, 2011
†Corresponding author: Dong-Woon Kim, Department of Clinical Pathology, Gwangyang Health College, Gwangyang 545-703, Korea.
Tel: +82-61-760-1469, Fax: +82-61-763-9009
e-mail: kdw0223@hanmail.net
†Corresponding author: Ki-Young Lee, Faculty of Applied Chemical Engineering, Chonnam National University, Gwangju 500-757, Korea.
Tel: +82-62-530-1843, Fax: +82-63-530-1676
e-mail: kilee@chonnam.ac.kr

istration of therapeutic drugs (Lee et al., 2005). When liposomes are modified with polysaccharides such as curdlan, chitosan, pullulan, and etc., they can be relatively stable both *in vitro* and *in vivo* (Venkatesan et al., 2000; Guo et al., 2003; Sihorkar et al., 2001; Moreia et al., 1996).

Among various polysaccharides, chitosan can form the surface layer on the negative liposomal membrane by the electrostatic interaction, improving liposomal characteristics, specific, prolonged, controlled release, and etc. (Manconi et al., 2010; Cao et al., 2009). Chitosan derived from chitin is a polysaccharide composed of glucosamine and *N*-acetylglucosamine. It has received substantial attention in the potential use as a pharmaceutical agent and a food additive because of its high biocompatibility and anti-bacterial activity (Porta et al., 2011; Illum et al., 2001). Especially, chitosan has been used as a bioadhesive drug delivery carrier because of high affinity to cell membrane and mucoadhesive properties (He et al., 1998; Dodane et al., 1999).

Herein, we anticipated that liposomal characteristics such as the colloidal stability and controlled drug release may be improved by the chitosan coating onto their surface. The chitosan-coated liposomes (chitosomes) can be formed through ionic interaction between the positively charged chitosan and negatively charged phosphate on the surface of the liposomes. In this study, we investigated the ability of chitosomes as a drug carrier for oral administration.

MATERIALS AND METHODS

Materials

Water soluble chitosan (WSC, molecular weight: 2.5 KDa, deacetylation: 87%) was supplied from Kittolife Co. (Seoul, Korea). Egg phosphatidylcholine (PC), cholesterol (Chol), phosphatic acid (PA), and 5-carboxyfluorecense (5-CF) were purchased from Sigma (St. Louis, MO). All other chemicals were analytic-grade and used directly without further purification.

Synthesis of chitosomes

Chitosomes were prepared using the electrostatic attraction between positively charged chitosan and the opposite charge on liposomes (Guo et al., 2003). Firstly, for preparation of the negative charge liposome the lipid mixture, PC (7 mol%), Chol (2 mol%), and PA (3 mol%) were dissolved in 5 ml of chloroform/methanol mixture (1:1 volume ratio). The lipid solution was evaporated with rotary evaporator under vacuum for 1 h at 60℃. The formed lipid film was hydrated with 4 ml PBS (pH 7.4). To make uniform and small vesicles, the liposomal suspension was sonicated for 1 min for 4℃. Next, to coat liposomes with chitosan, WSC was dissolved in 2 ml of PBS (pH 7.4) and then the WSC solution was added to liposomal suspension. The coating of liposomes was carried out through incubation under stirring for 1 h.

Characterization of chitosomes

The morphological shape of chitosomes was evaluated by a transmittance electron microscopy (TEM, JEM 1200, JEOL, Japan). For observation of their shape phosphotungstic acid (1%, w/v) was used as a negative stain. Carbon-coated samples were placed over a copper grid and subjected to TEM analysis. Size distribution of the liposomes was analyzed using an electrophotonetic light scattering (ELS, Photal, ELS-8000, Osaka, Japan) tuned at a wavelength of 488 nm. Chitosomes were used without a filtration and the intensity autocorrelation was measured at scattering angle of 90° at 25℃. To indirectly confirm the coating of the liposomes, zeta potential was determined by Malvern zeta-sizer (Malvern Instruments Ltd. Malvern, UK).

Stability against SGF

The stability of chitosomes in SGF was evaluated by the turbidity measurement reported by Regen et al. (1980). To investigate the stability of chitosomes in gastric fluid, the coated and non-coated liposomal suspension was respectively added into SGF under stirring. SGF was made of 0.2% (w/v) sodium chloride, 0.32% (w/v) pepsin, and 0.7% (v/v) hydrochloric acid. The final solution was adjusted about pH 1.2. The change of relatively turbidity was measured at 400 nm under continuous stirring for 5 h at 37℃.

Table 1. Various formulation codes and mean vesicle size of chitosomes

Formulation code	Weight ratio (Lipid : WSC)	Mean vesicle size (nm)
CS 1	10 : 0	186.5±23.8
CS 2	9 : 1	386.2±32.6
CS 3	8 : 2	452.8±28.6
CS 4	7 : 3	523.6±42.3
CS 5	6 : 4	749.6±69.5

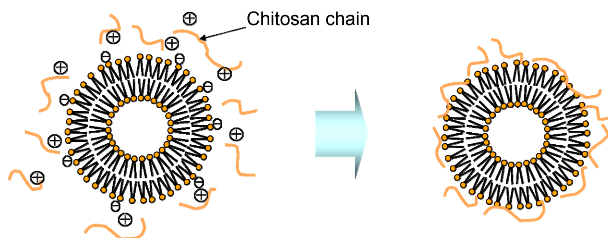

Fig. 1. Schematic illustration of the chitosan coating of the liposomal surface by the electrostatic binding. Chitosan may form an adsorptive layer by strong electrostatic attraction with the polar head groups on the surface of phospholipids bilayers.

Release study of 5-CF from chitosomes

In the preparation of liposomes, PBS containing 5-carboxyfluorescein (5-CF, 10 mM) was used for the hydration. Untrapped 5-CF was separated using a PD-10 column (Amersham, Arlington Heights, IL). The measurement of 5-CF release from chitosomes was performed at 37℃ in PBS (pH 7.4). The chitosome suspension including 5-CF was added into a dialysis tube (MW 12,000 cut off; Sigma) and the fluorescence signal of released 5-CF in the medium was then analyzed using UV spectrophotometer (UV-1201; Shimadzu, Kyoto) at 515 nm.

Statistical analysis

Statistical analyses were performed using the SPSS software package (Version 12.0, SPSS Inc., Chicago, USA). Descriptive data are presented as the mean ± standard deviation (SD). Continuous variables were compared using Student's t test. The difference was considered to be significantly if the P value was less than 5%.

RESULTS AND DISCUSSION

Characterization of chitosomes

Chitosan is a potential for oral drug delivery system due to its biocompatibility, biodegradability, low cost and ability to open intercellular tight junctions. When chitosan is conjugated on the liposomal surface, the ability of the liposome as carrier for oral drug delivery may be more improved (Henriksen et al., 1997; Zhuang et al., 2010). The anionic liposomes were prepared by incorporating PA into vesicle membrane and then WSC was coated on the surface of liposomes by ionic interaction (Fig. 1). The variation of phospholipids and WSC ratio in the preparation of liposomes was demonstrated in Table 1. The morphology of the chitosomes observe by TEM were spherical for all liposomes tested. As shown in Fig. 2, the existence of WSC layers surrounding the liposomes was well visualized on the surface of liposomes. The WSC layers were observed on the all liposomes tested. Similarly, several groups demonstrated that in the study of the polymer-coated liposomes, the presence of coating layers on the surface of liposomes was well observed by TEM (Guo et al., 2003). The size of the chitosomes was increased on increasing of the contents of WSC in the liposomal formulation (Fig. 3). The mean diameters determined for all the chitosome formulation were 192±23 nm for CS 1, 386±33 nm for CS 2, 452±29 nm for CS 3, 524±42 nm for CS 4, and 750±70 nm for CS 5, respectively. The size of the plasomes without WSC

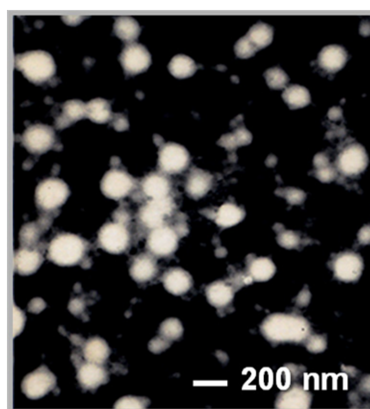

Fig. 2. TEM photograph of negatively stained the chitosome for CS 2. The shape of chitosomes is spherical and the presence of coating layer was seen on the surface of liposomes. Scale bar indicates 200 nm. The formulation CS 2 indicates that weight ratio of lipid to WSC for the chitosomes is 9 : 1.

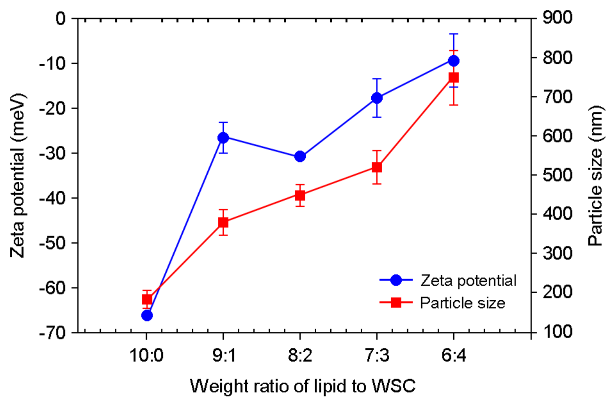

Fig. 3. The influence of the WSC content on mean sizes and zeta potential of the chitosomes. The results are the mean of three experiments. As the WSC contents increased on the liposomal formulation, the mean size and zeta potential value of liposomes increased significantly.

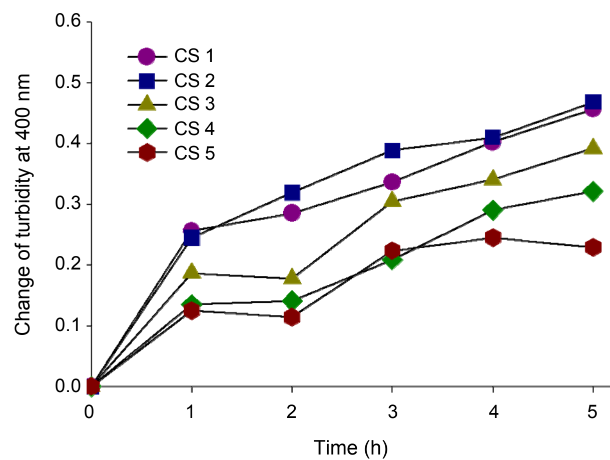

Fig. 4. Change of relative turbidity of the chitosomes in simulated gastric fluid at pH 1.2 and 37℃. The change of turbidity of the liposomal suspension was dependent on the WSC content added to the liposome solution.

coating layers was in the broad range of 100~200 nm. As the WSC contents increased on the liposomal formulations, the size of liposomes was increased significantly. The changes of the particle size are due to an increase in thickness of the coating layer by conjugation of WSC on the surface of liposomes through the electrostatic attraction between WSC chains and polar head groups of the liposomal bilayers (Henriksen et al., 1997; Demarger-Andre et al., 1993). In addition, the formation of WSC layer on the surface of the liposomes was confirmed by comparing the zeta potential of the liposomes before and after the WSC coating. The results of the zeta potential were shown in Fig. 3. The plasomes without WSC coating layer were negatively charged by PA, and the value of zeta potential was around -65.6±0.3 meV. The zeta potential values of liposomes coated with WSC increased gradually with increasing concentration of WSC solution for coating of the liposomal surface. The values were -26.5±3.4 meV for CS 2, -30.6 ±0.5 meV for CS 3, -17.7±4.2 meV for CS 4, and -9.6± 5.7 meV for CS 5, respectively. The reason that the zeta potential values are increased is due to attachment of the WSC by electrostatic interaction (Guo et al., 2003). The adsorption of WSC means that the density of positive charge is increased on the liposomal surface and the zeta potential value increases as the WSC concentration. These results suggest that the coating layer by WSC is successfully formed on the surface of the liposomes.

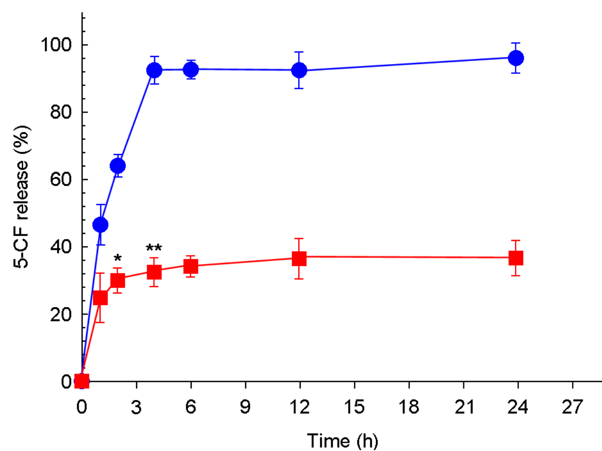

Fig. 5. Release profiles of 5-CF from the chitosomes (square) and plasomes (circle) in PBS (pH 7.4) at 37℃. At 4 h of initial release, the plasomes released >90% of their initial content of 5-CF, whereas the chitosomes released <40% of their initial content of 5-CF. These results were expressed as mean ± S.D.. *P<0.05 and **P<0.01 vs. the plasomes (Student's *t* test).

Stability of chitosomes in SGF

In the oral drug delivery system, it is important to obtain information about the stability of liposomes in the gastric fluid. Liposomes are vesicles with the problems in potential chemical and physical stability. During storage, changes in size and relative turbidity can occur due to aggregation or fusion of vesicles. To evaluate stability of chitosomes in SGF, the change of relative turbidity of the liposomal suspension was measured for 5 h. The change degree of

relative turbidity in gastric fluid was dependent on the content of WSC added into the liposomal solution (Fig. 4). The plasome suspension without adding WSC clearly showed the change of relatively turbidity, while the change degree of turbidity of the chitosomes decreased as increasing of WSC content added for coating liposome. This may be because plasomes without WSC coating layer were unstable in the gastric fluid. In contrast, the chitosomes were more stable in the fluid. This indicates that the aggregation of vesicles was prevented by attachment of WSC on the surface of liposomes, whereas the turbidity change of plasome suspension occured due to aggregation or fusion of vesicles (Prestegard et al., 1974; Larrabee, 1979; Lee et al., 2005). These results certainly indicate that chitosomes are more stable in SGF compared with the plasomes.

Release property of 5-CF from chitosomes

From the results of particle size, zeta potential, and stability studies, formulation CS 4 was considered to be at the optimum level and chosen for further studies such as drug release and mucoadhesive study. To evaluate the possibility of the delivery of drugs or antigens, the release properties of chitosomes was measured on the basis of 5-CF release. Fig. 5 shows the profiles of 5-CF release from the plasomes and chitosomes. Chitosomes exhibited significantly slower release pattern than that of the plasomes. After 4 h of release measurement, the plasomes released >90% of the initial 5-CF content. In contrast, the chitosomes released below 40% of initial content of 5-CF. This difference for 5-CF release profiles may be due to the higher bilayer curvature of the plasomes than the chitosomes with coating layer (Miyazaki et al., 2003). This result indicates that the chitosomes are stable and suitable for oral drug delivery.

Conclusion

In summary, the liposomes coated with WSC were prepared, characterized and evaluated for their potential use in oral drug delivery. Liposomes were prepared by the film method and coated with WSC using an electrostatic attraction. The shape of the chitosomes observed by TEM was spherical for all the tested formulations. The size of the chitosomes was dependent on the WSC content. As the WSC contents increased in the composition of liposomes, the size of the liposomes was increased significantly. The attachment of the WSC to the liposomal surface elevated the zeta potential values of the liposomes. These results confirmed that WSC was coated on the liposomal surface by the electrostatic attraction. The chitosomes were more clearly stable than the plasomes without chitosan-coated layers in SGF. Therefore, we concluded that chitosomes can be used as a potential carrier for effective oral drug delivery.

Acknowledgements

This study was supported by the National Research Foundation of Korea Grant funded by the Korean Government (Ministry of Education, Science and Technology) [NRF-2010-359-D00004].

REFERENCES

Barea MJ, Jenkins MJ, Gaber MH, Bridson RH. Evaluation of liposomes coated with a pH responsive polymer. Int J Pharm. 2010. 402: 89-94.

Cao J, Sun J, Wang X, Li X, Deng Y. N-trimethyl chitosan-coated multivesicular liposomes for oxymatrine oral delivery. Drug Dev Ind Pharm. 2009. 35: 1339-1347.

Childers NK, Denys FR, McGee NF, Michalek SM. Ultrastructural study of liposome uptake by M cell of rat Peyer's patch: an oral vaccine system for delivery of purified antigen. Reg Immunol. 1990. 3: 289-296.

Demarger-Andre S, Domard A. Chitosan behaviours in a dispersion of undecylenic acid. Carbohyd Polym. 1993. 22: 117-126.

Dodane V, Khan MA, Merwin JR. Effect of chitosan on epithelial permeability and structure. Int J Pharm. 1999. 182: 21-32.

Gregoriadis G. Immunological adjuvants: a role for liposomes. Immunol Today 1990. 11: 89-96.

Guo J, Ping Q, Jiang G, Huang L, Tong Y. Chitosan-coated liposomes: characterization and interaction with leuprolide. Int J Pharm. 2003. 260: 167-173.

Han M, Watarai S, Kobayashi K, Yasuda T. Application of liposomes for development of oral vaccines: Study of *in vitro* stability of liposomes and antibody response to antigen associated with liposomes after oral immunization. J Vet Med Sci. 1997. 59: 1109-1114.

Henriksen I, Vågen SR, Snade SA, Smistad G, Karlsen J. Interaction between liposomes and chitosan II: effect of selected parameters on aggregation and leakage. Int J Pharm. 1997. 146: 193-204.

He P, Davis SS, Illum L. *In vitro* evaluation of the mucoadhesive properties of chitosan microspheres. Int J Pharm. 1998. 166: 75-88.

He S, Zhu J, Xie F. Preparation and characterization of tramadol PEG-coated multivesicular liposomes for sustained release. Pharmazie. 2010. 65: 467-470.

Illum L, Jabbal-Gill I, Hinchcliffe M, Fisher AN, Davis SS. Chitosan as a novel nasal delivery system for vaccines. Adv Drug Deliv Rev. 2001. 51: 81-96.

Kokkona M, Kallinteri P, Fatiuros D, Antimisiaris SG. Stability of SUV liposomes in the presence of cholate salts and pancreatic lipase: effect of lipid composition. Eur J Pharm Sci. 2000. 9: 245-252.

Larrabee A. Time-dependent changes in the size distribution of disteroylphos-phatidylcholine vesicles. Biochemistry. 1979. 18: 3321.

Lee CM, Lee HC, Lee KY. *O*-palmitoylcurdlan sulfate (OPCurS)-coated liposomes for oral drug delivery. J Biosci Bioeng. 2005. 100: 255-259.

Manconi M, Mura S, Manca ML, Fadda AM, Dolz M, Hernandez MJ, Casanovas A, Díez-Sales O. Chitosomes as drug delivery systems for C-phycocyanin: preparation and characterization. Int J Pharm. 2010. 392: 92-100.

Minato S, Iwanaga K, Kakemi M, Yamashita S, Oku N. Application of polyethyleneglycol (PEG)-modified liposomes for oral vaccine: effect of lipid dose on systemic and mucosal immunity. J Control Release. 2003. 89: 189-197.

Miyazaki Y, Ogihara K, Yakou S, Nagai T, Takayama K. *In vitro* and vivo evaluation of mucoadhesive microspheres consisting of dextran derivatives and cellulose acetate butyrate. Int J Pharm. 2003. 258: 21-29.

Moreia JN, Almeida LM, Geraldes CF, Costa ML. Evaluation of *in vitro* stability of large unilamellar liposomes coated with a modified polysaccharide (*O*-palmitoylpullulan). J Mater Sci: Mater Med. 1996. 7: 301-303.

Parmentier J, Becker MM, Heintz U, Fricker G. Stability of liposomes containing bio-enhancers and tetraether lipids in simulated gastro-intestinal fluids. Int J Pharm. 2011. 405: 210-217.

Porta R, Mariniello L, Di Pierro P, Sorrentino A, Giosafatto CV. Transglutaminase crosslinked pectin- and chitosan-based edible films: a review. Crit Rev Food Sci Nutr. 2011. 51: 223-238.

Prestegard JH, Fellmeth B. Fusion of dimyristoyllecithins vesicles as studied by proton magnetic resonance spectroscopy. Biochemistry. 1974. 13: 1122.

Regen SL, Czech B, Singh A. Polymerized vesicles. J Am Chem Soc. 1980. 102: 6638.

Sihorkar V, Vyas SP. Potential of polysaccharide anchored liposomes in drug delivery, targeting and immunization. J Pharm Pharmaceut Sci. 2001. 4: 138-158.

Takeuchi H, Matsui Y, Sugihara H, Yamamoto H, Kawashima Y. Effectiveness of submicron-sized, chitosan-coated liposomes in oral administration of peptide drugs. Int J Pharm. 2005. 303: 160-170.

Takeuchi H, Yamamoto H, Niwa T, Hino T, Kawashima Y. Mucoadhesion of polymer-coated liposomes to rat intestine in vitro. Chem Pharm Bull. 1994. 42: 1954-1956.

Venkatesan N, Vyas SP. Polysaccharide coated liposomes for oral immunization-development and characterization. Int J Pharm. 2000. 203: 169-177.

Zhou F, Neutra MR. Antigen delivery to mucosa-associated lymphoid tissues using liposomes as a carrier. Biosci Rep. 2002. 22: 355-369.

Zhu J, Yan F, Guo Z, Marchant RE. Surface modification of liposomes by saccharides: Vesicle size and stability of lactosyl liposomes studied by photon correlation spectroscopy. J Colloid Interf Sci. 2005. 289: 542-550.

Zhuang J, Ping Q, Song Y, Qi J, Cui Z. Effects of chitosan coating on physical properties and pharmacokinetic behavior of mitoxantrone liposomes. Int J Nanomedicine. 2010. 5: 407-416.