Towards fast and cost-effective up-scaling of Nano-encapsulations by Ionic-gelation method using model drug for the treatment of atopic dermatitis

Muhammad Irfan Siddique[1,2]*, Saima Tufail[1], Ze Heng Ker[2], Tahir Mehmood Khan[1,4], Fatima Rasool[5], Muhammad Farhan Sohail[6] and Nabeel Shahid[1]

[1]Institute of Pharmaceutical Sciences, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan
[2]Centre for Drug Delivery Research, Faculty of Pharmacy, University Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, Kuala Lumpur, Malaysia
[4]School of Pharmacy, Monash University, Malaysia
[5]Punjab University College of Pharmacy, University of the Punjab, Lahore, Pakistan
[6]Riphah Institute of Pharmaceutical Sciences, Riphah International University, Lahore, Pakistan

Abstract: Chitosan nanoparticles (CSNPs) have proven their excellent drug delivery potential through various routes of administration and therefore, the need for large scale production of CSNPs for the commercialization is paramount. Their particle size and surface charge, drug loading capacity, and morphology were characterized in this study. Finally, drug release studies of both continuous and scalable modes were undertaken to ascertain suitability of CSNPs as a carrier for HC. The particle size of the large and small scale of HC-CSNPs was 253.3±16.4 nm and 225.4 ±9.6 nm, respectively. Besides, the surface charge of the large and small scale of HC-CSNPs was +35.3±0.3 mV and +32.6±2.5 mV, respectively. The size and surface charge of both HC-CSNPs were not proven to be statistically different. Drug loading capacity of large and small scale production of HC-CSNPs was high with 89%, and 83% of HC was loaded into CSNPs, respectively. Moreover, the morphology of both large and small scale production of HC-CSNPs had a similar shape and particle size. The drug release profile of CSNPs prepared by both methods showed a significantly (p<0.05) higher percentage release as compared to the free form. It is expected that positively charged nano-sized HC-CSNPs with high drug loading capacity could enhance the efficiency of drug delivery system to carry and diffuse into the target cells. The results obtained also suggested that the modified method applied could be further developed for large scale production of HC-CSNPs.

Keywords: Up-scaling, nano-encapsulation, chitosan, ionic-gelation, drug delivery.

INTRODUCTION

The success of therapy is directly associated with the carrier system which successfully delivers the therapeutic moiety to its target site, producing the desired pharmacological response. The drug delivery system indeed plays a pivotal role in controlling the desired pharmacological and unwanted toxic effects of the drugs. An optimal drug delivery system ensures that the active drug is available at the site of action for the correct time and duration (Liu *et al*., 2016).

The significant efforts have been made in the field of nanotechnology, where many nanoforms have been cracked as drug delivery system, like liposomes, micelles, polymeric nanoparticles, dendrimers and nanocrystals. These NPs can successfully be administered through invasive and noninvasive routes. Polymeric nanoparticle have been used frequently as carrier for drug delivery due to its excellent bioavailability, better encapsulation effenciency, control release and less toxic properties. Chitosan nanoparticles (CSNPs) are one of such polymeric nanoparticles developed from a biocompatible and biodegradable natural polymer and are widely used in delivering genes, proteins, anticancer drugs, and antibiotics (Bugnicourt and Ladaviere, 2016).

CSNPs can be fabricated through ionic gelation, micro emulsion, emulsification solvent diffusion, polyelectrolyte complex, emulsification cross-linking, complex coacervation, solvent evaporation, reverse micellar and self-assembling techniques, each showing their merits and demerits (Ma *et al*., 2017, Li *et al*., 2015). However, most of these fabrication methods of nanoparticles are costly, time-consuming and require several steps and corrosive solvents as an ingredient of synthesis (Yang *et al*., 2012). Ionic gelation is the most commonly employed technique to produce CSNPs. Although a huge number of studies have reported procedures of ionic gelation method, yet none has reported the suitability of their method for the bulk production of CSNPs at a commercial scale (McClements, 2018). The formulation features like physical characteristics of CSNPs and their morphology are highly affected by the method of preparation and related formulation variables which can directly compromise the therapeutic outcomes (El-Feky *et al*., 2017).

**Corresponding author:* e-mail: irfan.siddique@uvas.edu.pk

Therefore, the development of a method that produces the CSNPs with proper control over features at small and large scale would be of great interest to facilitate the facile synthesis of CSNPs. Herein, we report the optimized conditions of ionic gelation method to synthesize CSNPs for small and bulk manufacturing. The synthesized CSNPs from both batches were subjected to physical characterization to see the changes in features. The study is expected to bring benefits and good impacts to formulation development and optimization of drug delivery systems. Data generated from this study will be used for further improvement and optimization of the method to produce large batches of CSNPs with the desired physical attributes.

MATERIALS AND METHODS

Materials

Analytical grade HC (base), low molecular weight CS (specifications: MW, 70 kDa; deacetylation degree, 85%; viscosity 20–300 cP for 1%w/v solution in 1% v/v acetic acid at 25°C), was purchased from Sigma-Aldrich Chemicals Corp., Ltd., (St. Louis, MO, USA). The pentasodium tripolyphosphate (TPP) was obtained from Merck KGaA Co., Ltd., (Darmstadt Germany). All other solvents used were of analytical grade.

Methods

For large scale production of CSNPs, ionic gelation method with some modifications was used (Hussain *et al*., 2013). Briefly, 100 mg of TPP was dissolved in 100mL of distilled water to produce 0.1% (m/v) solution. 100mL of hydrocortisone (HC) solution (1mg/mL) was added to a beaker containing 1000mL of CS solution (0.01 mg/mL) at pH 5 using a dropper. The mixture was mixed by a propeller at 1000 rpm. The TPP solution was added dropwise, under continuous stirring, to the CS solution to form HC-loaded nanoparticles (HC-CSNPs). Small scale production of HC-CSNPs was achieved following the same method. However, the volume of TPP and CS solution was 100-fold reduced compared to that of the large scale.

Particle size and surface charge

The resulting CSNPs from both batches, were characterized using a Zetasizer (Malvern Instruments Ltd., Worchester, UK) for their particle size and surface charge (zeta potential). The samples were 10-fold diluted before analysis.

Loading Efficiency (% LE)

To determine drug loading efficiency of HC in CSNPs, the suspension of HC-CSNPs was centrifuged at 4℃ temperature for 30 min. The amount of free HC recovered in the supernatant was analyzed spectrophotometrically using UV spectrophotometer (Shimadzu UV-1601, Shimadzu Co. Ltd., Japan) at 595 nm. The loading efficiency was calculated according to the equation below:

$$LC = \frac{\text{The Total amount of HC} - \text{Free amount of HC}}{\text{Nanoparticles weight}} \times 100\%$$

Morphology of nanoparticles

The morphology of HC-CSNPs was determined by transmission electron microscopy (TEM). A drop of HC-CSNPs suspension was placed on a carbon film 300 mesh copper grid using a syringe (Nagarajan *et al*., 2015) and left it to air-dry. The samples were stained with 1-Muranyl acetate solution for 5 s at temperature 7^{o}C before viewing under a TEM (Hitachi H-7000 TEM, Japan).

In vitro drug release studies

In vitro HC release studies from HC-CSNPs of both small and large scale were performed to investigate the amount of drug release by dialysis bag diffusion method reported previously (Sotelo-Boyás *et al*., 2017). Briefly, 50 µg of previously lyophilized HC-CSNPs of both formulations were separately re-dispersed in 5mL of phosphate buffer solution (PBS, pH=7.4) and were sealed in dialysis membrane bag (Spectra/Por 7, 50,000 MW cut-off) and incubated in a bottle containing 50mL of PBS. Whole assembly was thermo regulated by shaking water bath with a speed of 50 rpm at temperature 37±0.5ºC. At pre-defined interval, 2mL of aliquots were with-drawn, and replaced by fresh PBS (pH=7.4) into the system to keep the volume constant. Amount of diffused HC through the membrane was analysed using RP-HPLC at 248 nm (λ_{max}) (Siddique *et al*., 2016). The remained un-diffused HC, inside the dialysis tube, at the end of the study was quantified as well.

STATISTICAL ANALYSIS

IBM SPSS Statistic 20 was used to analyze the data obtained. Descriptive statistics were applied to estimate the means and standard deviations. Independent sample t-test was used to estimate the significant difference between nanoparticles. P-value <0.05 was considered statistically significant.

RESULTS

The particle size and Morphology of HC-CSNPs

The method is based on electrostatic interaction between the amine group of chitosan and negatively charged group of polyanionic TPP. Table 1 shows the particle size analysis of both large scale and small scale HC-CSNPs. The morphology of large and small scale production of HC-loaded chitosan nanoparticles prepared in chitosan solution was visualized under a transmission electron microscope (TEM) fig. 1.

Table 1: Comparative analysis of various features of small and large scale production of HC-CSNPs.

	Mean Particle Size (nm)	Mean Surface Charge (mV)	Drug Loading Capacity (%)
Large scale HC-CSNPs	253.3 ±16.0	+32.6 ± 2.5	89.17
Small scale HC-CSNPs	225.4 ± 9.5	+35.30.3	83.00

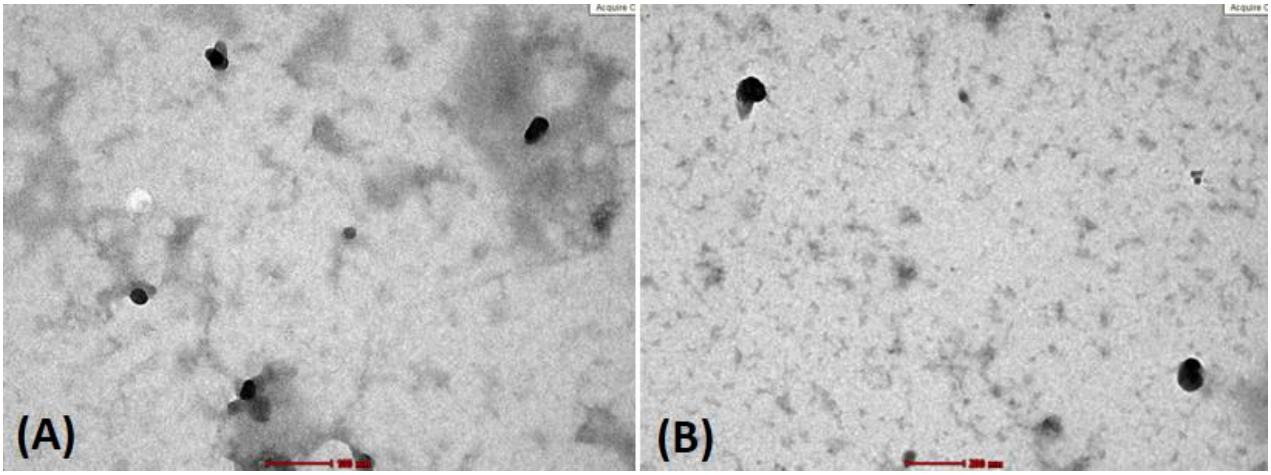

Fig. 1: Morphology of HC-loaded chitosan nanoparticles, where A is small scale and B is large scale nanoparticles.

Drug loading capacity of HC-loaded chitosan nanoparticles

Drug loading capacity is calculated with the help of in determining the free amount of HC in the supernatant (Hussein‐Al‐Ali *et al*., 2018). The drug loading capacity of small scale production of HC-loaded chitosan nanoparticles is 83.00%. The drug loading capacity of large scale production of HC-loaded chitosan nanoparticles is 89.14 %. Both small and large scale HC-CSNPs shows more than 80% drug loading effenciency. The high drug loading capacity of chitosan nanoparticles becomes one of the advantages as a good drug carrier to target cells. The higher amount of drugs can be loaded in chitosan nanoparticles, and the chitosan nanoparticles are delivered in the human circulatory system to the target cells for treatment(He *et al*., 2017). Hence, this helps to increase the effectiveness of the drug in medical treatment and drug will be more reliable in the aspect of medical treatment. Thus, it can be concluded chitosan nanoparticles is an undeniable good drug carrier and worth to be commercialized.

In-vitro drug release studies

Dialysis bag technique is one of the well-established and most widely used methods to investigate the release behavior of the drug molecule from NPs (Lee *et al*., 2016, Dudhani and Kosaraju, 2010). Figure 2 showed that drug release at each interval of time was non-significant ($p>0.05$).

DISCUSSION

The particle size of the nanoparticles plays a significant role in epithelial uptake and intracellular transport/ trafficking (Anandhakumar *et al*., 2017). It has been reported that the arterial uptake of smaller size nanoparticles is more than 3-fold than larger sized nanoparticles (Rivas *et al*., 2017). From the current study, it was seen that the average hydrodynamic particle size of small and large scale production of HC-CSNPs was found to be 225.4 ±9.5 nm and 253.3±16 nm respectively. However, the differences between these were statistically non-significant (p-value =0.164). Thus, it is concluded that there is no significant difference between both large and small scale production of HC- loaded chitosan nanoparticles. The average particle sizes of the large and small scale of HC-CSNPs remains in the standard range of particle size. The chitosan nanoparticles as the drug carrier can easily enter the cells and increase the efficiency of the effect of the drug on treatment for certain diseases like inflammation, cancer, eczema (Rivas *et al*., 2017, Auwal *et al*., 2017). Therefore, chitosan nanoparticles will have commercial value to produce nanoparticles at a larger scale.

It has been reported that the important index for the stability of nanoparticles suspension is potential zeta(Dai *et al*., 2017). Zeta potential increases when the drug encapsulation increases and thus it is said to be the stability of drug also increases. Zeta potential of chitosan nanoparticles determines the stability of the drug in the human body. Optimal encapsulation of drug is at pH 5.0 (Hussain *et al*., 2013). Above and below pH 5.0, the encapsulation of drug starts to decrease, although the zeta potential of chitosan nanoparticles increases when the pH value decreases. It has been stated that nanoparticles with

higher zeta potential bound strongly to the cell membrane and showed a higher cellular uptake(Yu *et al*., 2018). Surface charge of HC-loaded chitosan nanoparticles has been determined by Zetasizer. The average surface charge of the large and small scale of HC-loaded chitosan nanoparticles remain in the suitable range of surface charge. Both large and small scale of HC-loaded chitosan nanoparticles is positively charged. As the zeta potential is higher, the drug is more stable (Gomathi *et al*., 2017). Therefore, regarding particle size of chitosan nanoparticles, it can be concluded that chitosan nanoparticles are good drug carriers. The TEM revealed that the morphology of large and small scale production of HC-loaded chitosan nanoparticle is in a smooth spherical shape. The mean particles size of large and small scale production of HC-loaded chitosan nanoparticle is almost the same. Thus, HC-loaded chitosan nanoparticles are small enough in size and able to diffuse into the human cell as a potential drug carrier. There is no significant difference between the morphology of large and small scale production of HC-loaded chitosan nanoparticle. Therefore, it can be concluded that the chitosan nanoparticles can be largely commercialized as a good drug carrier in loading drug for treatment.

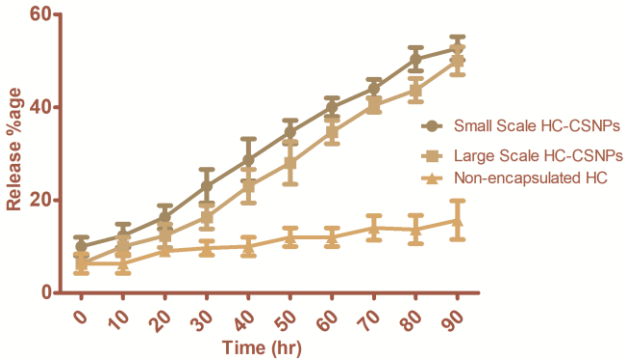

Fig. 2: *In vitro* drug, release profiles of HC from small scale and large scale HC-CSNPs performed for 60 h in duration in PBS, pH=7.4.

Drug loading capacity is calculated with the help of in determining the free amount of HC in the supernatant (Hussein‐Al‐Ali *et al*., 2018). Both small and large scale HC-CSNPs shows more than 80% drug loading efficiency. The high drug loading capacity of chitosan nanoparticles becomes one of the advantages as a good drug carrier to target cells. The higher amount of drugs can be loaded in chitosan nanoparticles, and the chitosan nanoparticles are delivered in the human circulatory system to the target cells for treatment(He *et al*., 2017). Hence, this helps to increase the effectiveness of the drug in medical treatment and drug will be more reliable in the aspect of medical treatment. Thus, it can be concluded chitosan nanoparticles is an undeniable good drug carrier and worth to be commercialized.

In dialysis bag technique, the dialysis membrane physically separate the drug-loaded nano-carrier from the bulk media and drug release over time is generally evaluated in outer media (Zhou *et al*., 2018, Wu *et al*., 2017). Phosphate buffer was chosen as a release medium to simulate with body fluids as release properties of HC may be affected by ionic strength in the release medium (Qian *et al*., 2006). During *in-vitro* drug release study, HC release from small and large scale NPs was in a control manner. It can be observed that drug release from both of these formulations with the same drug loading had the same drug release as compared to the free form. The drug release is controlled by the erosion method in which penetration of water molecule into the matrix causes swelling therefore polymer subjected to degradation (Ko *et al*., 2014). This ease of estimating drug release through dialysis bag makes it easy and straight forward to measure drug release from other nanocarriers such as liposome, nano-emulsion and nanospheres (Kamaly *et al*., 2016, Masood, 2016).

Successful delivery to the skin depends on drug release through the skin in adequate quantity to attain desired therapeutic effects(Sala *et al*., 2018). Moreover, drug release depends on physicochemical properties of the drug molecule as well as effect of the vehicle which can alter its penetration profile (Dhakar, 2012). Positively charged NPs were found to be more effective in terms of its adherence to the negatively charged skin and release the drug in an efficient interval of time, with minimizing toxicity (Wang *et al*., 2016, Sukhanova *et al*., 2018).

CONCLUSION

The study concluded that the bulk and small scale production of HC-loaded CSNPs, through ionic gelation method can be achieved, with some modifications in the protocol. From the data obtained, the particle size and zeta potential of both large and small scale production of HC-loaded CSNPs were non-significant, indicating the up scaling method was successfully designed to produce the same characteristics of nanoparticles as in small/laboratory scale. Moreover, the loading efficiency with both productions was above 80% with good release profile Thus, the simplest production method of chitosan nanoparticles could be of great interest as the chitosan nanoparticles are deniably a potential drug carrier and worth to be commercialized.

REFERENCES

Anandhakumar S, Krishnamoorthy G, Ramkumar K and Raichur A (2017). Preparation of collagen peptide functionalized chitosan nanoparticles by ionic gelation method: An effective carrier system for encapsulation and release of doxorubicin for cancer drug delivery. *Mater. Sci. Eng. C Mater. Biol. Appl.*, **70**(1): 378-385.

Auwal S, Zarei M, Tan C, Basri M and Saari N (2017). Improved *in vivo* efficacy of anti-hypertensive biopeptides encapsulated in chitosan nanoparticles fabricated by ionotropic gelation on spontaneously hypertensive rats. *Nanomaterials*, **7**(12): 421.

Bugnicourt L and Ladaviere C (2016). Interests of chitosan nanoparticles ionically cross-linked with tripolyphosphate for biomedical applications. *Prog. Polym. Sci.*, **60**: 1-17.

Dai L, Sun C, Li R, Mao L, Liu F and Gao Y (2017). Structural characterization, formation mechanism and stability of curcumin in zein-lecithin composite nanoparticles fabricated by antisolvent co-precipitation. *Food Chem.*, **237**: 1163-1171.

Dhakar RC (2012). From formulation variables to drug entrapment efficiency of microspheres: A technical review. *J. Drug Deliv. Sci. Technol.*, **2**(6): 128-133.

Dudhani AR and Kosaraju SL (2010). Bioadhesive chitosan nanoparticles: Preparation and characterization. *Carbohydr. Polym.*, **81**(2): 243-251.

El-Feky GS, Sharaf SS, El Shafei A and Hegazy AA (2017). Using chitosan nanoparticles as drug carriers for the development of a silver sulfadiazine wound dressing. *Carbohydr. Polym.*, **158**: 11-19.

Gomathi T, Sudha P, Florence JAK, Venkatesan J and Anil S (2017). Fabrication of letrozole formulation using chitosan nanoparticles through ionic gelation method. *Int. J. Biol. Macromol.*, **104**(Pt B): 1820-1832.

He B, Ge J, Yue P, Yue X, Fu R, Liang J and Gao X (2017). Loading of anthocyanins on chitosan nanoparticles influences anthocyanin degradation in gastrointestinal fluids and stability in a beverage. *Food Chem.*, **221**: 1671-1677.

Hussain Z, Katas H, Amin MCIM, Kumolosasi E, Buang F and Sahudin S (2013). Self-assembled polymeric nanoparticles for percutaneous co-delivery of hydrocortisone/hydroxytyrosol: An *ex vivo* and *in vivo* study using a NC/Nga mouse model. *Int. J. Pharm.*, **444**(1-2): 109-119.

Hussein Al Ali SH, Kura A, Hussein MZ and Fakurazi S (2018). Preparation of chitosan nanoparticles as a drug delivery system for perindopril erbumine. *Polym. Compos.*, **39**(2): 544-552.

Kamaly N, Yameen B, Wu J and Farokhzad OC (2016). Degradable controlled-release polymers and polymeric nanoparticles: Mechanisms of controlling drug release. *Chem. Rev.*, **116**(4): 2602-2663.

Ko HH, Chiang YC, Tsai MH, Liang CJ, Hsu LF, Li SY, Wang MC, Yen FL and Lee CW (2014). Eupafolin: A skin whitening flavonoid isolated from *Phyla nodiflora*, downregulated melanogenesis: Role of MAPK and Akt pathways. *J Ethnopharmacol.*, **15**(1): 386-393.

Lee E, Park SJ, Lee JH, Kim MS and Kim CH (2016). Preparation of chitosan TPP nanoparticles and their physical and biological properties. *Asian Journal of Pharmaceutical Sciences*, **11**: 166-167.

Li Z, Jiang H, Xu C and Gu L (2015). A review: Using nanoparticles to enhance absorption and bioavailability of phenolic phytochemicals. *Food Hydrocolloids*, **43**: 153-164.

Liu D, Yang F, Xiong F and Gu N (2016). The smart drug delivery system and its clinical potential. *Theranostics*, **6**(9): 1306-1323.

Ma Z, Garrido-Maestu A and Jeong KC (2017). Application, mode of action and *in vivo* activity of chitosan and its micro and nanoparticles as antimicrobial agents: A review. *Carbohydr. Polym.*, **176**: 257-265.

Masood F (2016). Polymeric nanoparticles for targeted drug delivery system for cancer therapy. *Materials Science and Engineering: C*, **60**: 569-578.

Mcclements DJ (2018). Encapsulation, protection and delivery of bioactive proteins and peptides using nanoparticle and microparticle systems: A review. *Adv. Colloid. Interface Sci.*, **253**: 1-22.

Nagarajan E, Shanmugasundaram P, Ravichandiran V, Vijayalakshmi A, Senthilnathan B and Masilamani K (2015). Development and evaluation of chitosan based polymeric nanoparticles of an antiulcer drug lansoprazole. *J. Appl. Pharm. Sci.*, **5**(4): 020-025.

Qian F, Cui F, Ding J, Tang C and Yin C (2006). Chitosan graft copolymer nanoparticles for oral protein drug delivery: Preparation and characterization. *Biomacromolecules*, **7**: 2722-2727.

Rivas CJM, Tarhini M, Badri W, Miladi K, Greige Gerges H, Nazari QA, Rodríguez SAG, Roman RA, Fessi H and Elaissari A (2017). Nanoprecipitation process: From encapsulation to drug delivery. *Int. J. Pharm.*, **532**(1): 66-81.

Sala M, Diab R, Elaissari A and Fessi H (2018). Lipid nanocarriers as skin drug delivery systems: Properties, mechanisms of skin interactions and medical applications. *Int. J. Pharm.*, **535**(1-2): 1-17.

Siddique MI, Katas H, Amin MCIM, Ng SF, Zulfakar M H and Jamil A (2016). *In-vivo* dermal pharmacokinetics, efficacy and safety of skin targeting nanoparticles for corticosteroid treatment of atopic dermatitis. *Int. J. Pharm.*, **507**(1-2): 72-82.

Sotelo-Boyas M, Correa-Pacheco Z, Bautista-Banos S and Gomez YG (2017). Release study and inhibitory activity of thyme essential oil-loaded chitosan nanoparticles and nanocapsules against foodborne bacteria. *Int. J. Biol. Macromol.*, **103**: 409-414.

Sukhanova A, Bozrova S, Sokolov P, Berestovoy M, Karaulov A and Nabiev I (2018). Dependence of nanoparticle toxicity on their physical and chemical properties. *Nanoscale Res. Lett.*, **13**: 44.

Wang HX, Zuo ZQ, Du JZ, Wang YC, Sun R, Cao ZT, Ye XD, Wang JL, Leong KW and Wang J (2016). Surface charge critically affects tumor penetration and therapeutic efficacy of cancer nanomedicines. *Nano. Today*, **11**(2): 133-144.

Wu J, Wang Y, Yang H, Liu X and Lu Z (2017). Preparation and biological activity studies of resveratrol loaded ionically cross-linked chitosan-TPP nanoparticles. *Carbohydr. Polym.,* **175**(1): 170-177.

Yang Y, Cui J, Zheng M, Hu C, Tan S, Xiao Y, Yang Q and Liu Y (2012). One-step synthesis of amino-functionalized fluorescent carbon nanoparticles by hydrothermal carbonization of chitosan. *Chem. Commun. (Camb.),* **48**(3): 380-382.

Yu H, Nguyen MH and Hadinoto K (2018). Effects of chitosan molecular weight on the physical and dissolution characteristics of amorphous curcumin chitosan nanoparticle complex. *Drug Dev. Ind. Pharm.*, **44**(1): 82-88.

Zhou F, Xu T, Zhao Y, Song H, Zhang L, Wu X and Lu B (2018). Chitosan-coated liposomes as delivery systems for improving the stability and oral bioavailability of acteoside. *Food Hydrocolloids,* **83**: 17-24.