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Original Article

Studies on the formulation optimization and controlled ionic gelation of chitosan nanoparticles using TPP-HP-β-CD inclusion complex

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ABSTRACT

Background and Aims: Ionic gelation strategy is the most common method used for the preparation of chitosan nanoparticles to obtain controlled drug delivery. Although it is a convenient and easy method, it is highly related with particle aggregation, high polidispersity index and insufficient physical/chemical stability. The aim of this study was the development of chitosan nanoparticles using tripolyphosphate-hydroxy propyl β-cyclodextrin or tripolyphosphate-sulfobutyl ether β-cyclodextrin inclusion complex as an alternative to TPP, and hence to increase physical stability, reduce polidispersity index and develop a stable nanocarrier for drug delivery purposes.

Methods: The nanoparticles were prepared with the ionic gelation technique. The effects of chitosan percent, pH, and chitosan/tripolyphosphate ratio were investigated to find out the optimum nanoparticles in terms of particle size, polidispersity index and zeta potential. After determining the conditions for the tripolyphosphate-chitosan nanoparticles, the nanoparticles were prepared using tripolyphosphate-hydroxypropyl β-cyclodextrin or tripolyphosphate-sulfobutyl ether β-cyclodextrin to make a comparison with the nanoparticles which were prepared using tripolyphosphate.

Results: The chitosan/tripolyphosphate-hydroxypropyl β-cyclodextrin nanoparticles were successfully formulated with 178 ± 84.1 nm particle size, 0.310±0.0134 PDI, 31.2±4.68 mV zeta potential. The interday changes in the measured characteristics were minimized for chitosan/tripolyphosphate-hydroxypropyl β-cyclodextrin nanoparticles as intended.

Conclusion: CS/TPP-HP-β-CD nanoparticle formulation with particle size below 200 nm, high zeta potential and increased physical stability nanoparticles would offer a promising approach especially for hydrophobic drugs to improve their stability, solubility, encapsulation efficiency and in vivo bioavailability.

Keywords: Chitosan nanoparticles, controlled ionic gelation, cyclodextrins

INTRODUCTION

In past decades, many efforts have been made to obtain a controlled and targeted release of drugs with micro and nano sized drug delivery systems. Due to their small size and large surface area, drug nanoparticles are superior to conventional drugs, with the ability to increase solubility, and hence improve bioavailability, providing a controlled release and reduced side effects. No doubt, advances in polymer science also promoted the advancement of drug-delivery technology. Among all nanoparticulate drug delivery approaches, polymeric nanoparticles have attracted significant attention since they are biodegradable, biocompatible, relatively easy to prepare and suitable for a variety of chemical drug classes and dosage forms (Crucho & Barros, 2017; Kumari, Yadav & Yadav, 2010; Rizvi & Saleh, 2018).

Polymeric nanoparticles are mainly prepared with biodegradable polymers, either synthetic or natural ones. Poly (lactide) (PLA), poly (lactide-co-glycolide) copolymers (PLGA), poly (ε-caprolactone) (PCL) and poly(amino acids) are the most common synthetic

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polymers, while chitosan (CS), alginate, gelatin, and albumin CS-based are examples of natural polymers (Elsabahy & Wooley, 2012; Mohammed, Syeda, Wasan & Wasan, 2017). CS is a very well established chitin derivative biodegradable polymer, which is composed of β-1, 4 linked 2 amino-2-deoxy-glucopyranose and 2-acetamido-2-deoxy-B-d-glucopyranose residues. It is cationic, highly basic, mucoadhesive biocompatible and approved by the FDA for tissue engineering and drug delivery purposes (Ahmed & Aljaeid, 2016; Mohammed et al., 2017). Since the CS nanoparticles were first introduced in 1994 by Ohya and colleagues, as a carrier for 5-FU, there has been tremendous research on CS nanoparticles for various drugs/proteins, application routes and preparation techniques (Grenha, 2012, Ohya, Shiratani, Kobayashi & Ouchi, 1994) Emulsion cross-linking, coacervation/precipitation, spray-drying, emulsion-droplet coalescence, the ionic gelation method, and the reverse micellar method are the methods used for CS nanoparticle prepration (Agnihotri, Mallikarjuna & Aminabhavi, 2004).

The ionic gelation strategy is one of the methods that has been extensively used for the preparation of CS nanoparticles to obtain controlled drug delivery. Nanoparticle formation occurs with the interactions between the positively charged CS chains and polyanions of cross-linkers employed such as tripolyphosphate (TPP). Despite its convenience and abstinence of organic solvents, it is generally known that the method is conducted with particle aggregation, a high polidispersity index and insufficient pysical/chemical stability. The main reason for this problem could be undesired intra and intermolecular cross-linking between TPP and CS (Agarwal, Shrivastav, Pandey, Das & Gaur, 2018; Calvo et al., 1997, Pant & Negi, 2018).

Cyclodextrins (CDs) are cyclic oligosaccharides with a hyrophobic central cavity and hydrophilic outer surface. They are widely utilized for improving solubility by forming inclusion or non-inclusion complexes and enhancing oral absorption/bioavailability of hyrophobic drugs (Agardan et al., 2016; Loftsson & Duchene, 2007; Mutlu Ağardan et al., 2014).

In this study, it was considered to use CD complex with TPP and thus controlling ionic gelation to obstruct undesired ionic interactions between cationic CS and anionic TPP. For this reason, two CD derivatives: 2-Hydroxypropyl-β-CD (HP-β-CD) and sulfobutyl ether β-CD (SBE-β-CD) were chosen to prepare complexes with TPP. HP-β-CD is a neutral derivative, SBE-β-CD is an anionic CD derivative. In addition, the effects of pH and CS/TPP ratio were also investigated to optimise nanoparticle formation. The stability of nanoparticles following preparation was compared with CS-TPP nanoparticles, and optimum formulation parameters were determined for further drug delivery studies.

MATERIALS AND METHODS

Low molecular weight CS (LWCS) (20–30 cps and ≥75% deacetylation) and TPP were purchased from Sigma Aldrich Ltd (USA). HP-β-CD and SBE-β-CD were purchased from Cylolab, Hungary.

Preparation of TPP- HP-β-CD and TPP-SBE-β-CD inclusion complexes

TPP-HP-β-CD or TPP-SBE-β-CD complexes were prepared with a solution-ultrasonic method (Pant & Negi, 2018). TPP and HP-β-CD or SBE-β-CD were dissolved in distilled water in 1:1 molar ratio and the solution was kept in an ultrasonic bath at room temperature for 1h. Then, the solutions were filtered through 0.45 µm cellulose acetate membrane filter and the filtrates were frozen at -80°C for 30 min prior to lyophilization. The frozen samples were lyophilized for 48 h at -55°C (Christ Alpha 1–2 LD plus, Osterode am Harz, Germany) in order to obtain TPP-HP-β-CD or TPP-SBE-β-CD complex powders (Miecznik and Kaczmarek, 2007; Pant and Negi, 2018). The complexes were kept in tightly sealed glass vials at 4°C for further use.

Characterization of TPP-HP-β-CD and TPP-SBE-β-CD inclusion complexes

TPP-HP-β-CD or TPP-SBE-β-CD complexes were characterized by Fourier-Transform Infrared Spectroscopy (FT-IR), X-ray Powder Diffraction (XRD) and Differential Scanning Calorimetry (DSC) measurements, comparatively with physical mixtures.

FT-IR analysis studies were performed using a PerkinElmer Spectrum 100 FT-IR spectrometer (4000–550 cm^{-1}), comparatively with, TPP-HP-β-CD physical mixture, TPP-HP-β-CD complex, TPP-SBE-β-CD physical mixture and TPP-SBE-β-CD complex in the range of 550–4000 cm^{-1}.

The thermal profiles of the physical mixture and inclusion complexes were obtained with a Rigaku Ultima-IV X-ray diffractometer. The samples were scanned over a range of 5°–95° for XRD measurements.

DSC measurements were carried out with a Shimadzu DSC-DTA 60 at 10°C/min scan speed. The accurately weighed 2 mg of samples were sealed in an aluminium pan and equilibrated at 25°C. The samples were subjected to a heating run over the temperature range of 25-350°C.

The preparation of the CS nanoparticles using TPP, TPP-HP-β-CD and TPP-SBE-β-CD inclusion complexes

CS nanoparticles was carried out using the traditional ionic gelation method using LWCS and TPP (Calvo et al., 1997; Pant & Negi, 2018). In brief, the CS solution (0.125%) in 0.5% v/v acetic acid was prepared by overnight stirring at 500 rpm, room temperature. The CS solution was filtered through a 5 µm pore sized mixed cellulose esters membrane filter to remove coarse CS residues. The TPP solutions were prepared at a concentration range between 1 mg/mL-2.67 mg/mL to obtain the CS/TPP ratios as given in Table 1. Nanoparticles were formed with a dropwise addition of 2 mL of TPP solution in different con-

Table 1. CS-TPP nanoparticle formulations.

F. code	TPP amount (mg)	CS amount (mg)	CS/TPP ratio
F1	2.00	8	4
F2	2.29	8	3.5
F3	2.67	8	3
F4	3.20	8	2.5
F5	4.00	8	2
F6	5.33	8	1.5

centrations to 8 mL of prepared CS solution under continous stirring in a magnetic stirrer at 1000 rpm, room temperature for an hour. The preparation of nanoparticles is schematized in Figure 1.

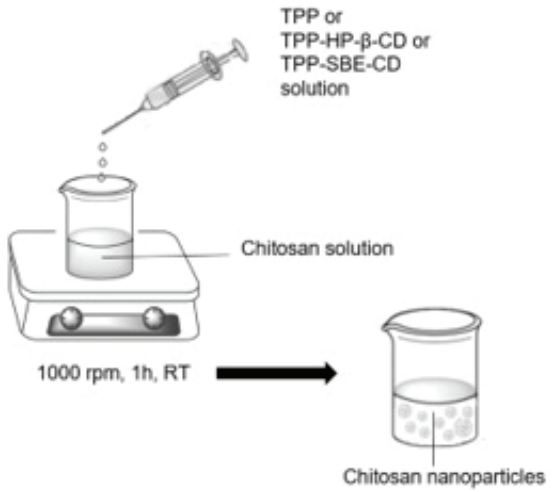

Figure 1. Preparation of CS nanoparticles.

To determine the effect of pH on nanoparticle formation, CS solutions (0.125%) were prepared in 0.5%, 0.25%, and 0.125% v/v acetic acid and used for CS-TPP nanoparticle formulation studies.

The concentrations of TPP were changed while the CS amount and the total volume were kept constant - 10 mL (Table 1). For the preparation of nanoparticles with TPP-HP-β-CD and TPP-SBE-β-CD inclusion complexes, the same procedure was followed and the equivalent TPP amounts of the complexes were calculated. According to the results of the CS-TPP nanoparticle preformulation studies, the optimum acetic acid ratio, CS/TPP ratio was chosen and used for preparing TPP-HP-β-CD or TPP-SBE-β-CD nanoparticles.

Particle size analysis and zeta potential measurements

The particle size (PS), poly dispersity index (PDI) and Zeta potential (ZP) of the CS nanoparticles were determined using a Malvern Zetasizer (Nano ZS90, Malvern instrument Ltd., UK). PS measurements were carried out in disposable polystyrene cuvettes while ZP measurements were carried out with folded capillary cells.

To confirm the effects of cyclodextrins on ionic gelation, the nanoparticles were prepared as mentioned previously and kept at room temperature. PS, PDI and ZP of the optimum CS-TPP and TPP-HP-β-CD nanoparticles were measured on the 0th, 1st, 3rd and 5th days following preparation.

Statistical analysis

All experiments were performed in triplicate and the results were presented as means ± standard deviation (SD). All results were analyzed by one-way ANOVA, and $p<0.05$ set as the minimal level of significance using GraphPad Prism 7.

RESULTS AND DISCUSSION

The TPP-HP-β-CD or TPP-SBE-β-CD complexes were prepared and characterized by FT-IR, XRD and DSC measurements comparatively with physical mixtures. Figure 2 represents the FTIR spectra of TPP-HP-β-CD, TPP-SBE-β-CD complexes and their physical mixtures. The spectrum of physical mixtures were different from the complexes as shown on the spectra. Changes at the absorption peaks at 1150 cm^{-1} and 863 cm^{-1} for both HP-β-CD/SBE-β-CD physical mixtures and complexes indicated the formation of the complexes.

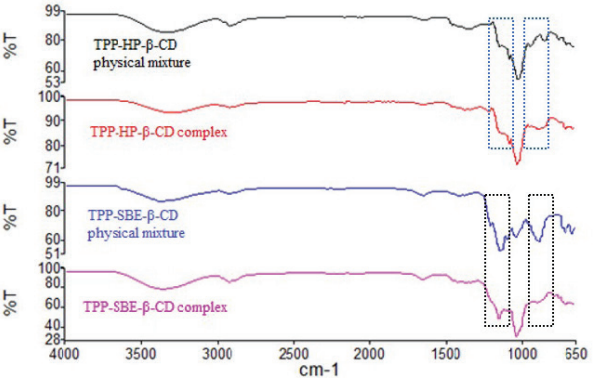

Figure 2. FT-IR spectra of TPP-HP-β-CD physical mixture (black line), TPP-HP-β-CD complex (red line), TPP-SBE-β-CD physical mixture (blue line) and TPP-SBE-β-CD complex (pink line).

As shown in Figure 3, for both TPP-HP-β-CD or TPP-SBE-β-CD complexes, the intensity of crystalline peaks in physical mixtures were significantly reduced. This result indicates a reduction of crystallinity and formation of an amorphous complex. However, neither TPP or HP-β-CD/ SBE-β-CD are water soluble, and the formed complexes are much more soluble as a result of the reduction in crystallinity (Kapor, 2008).

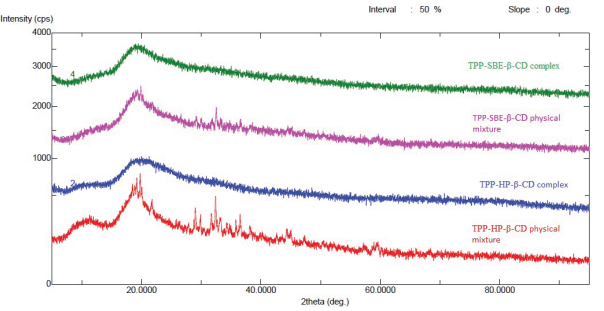

Figure 3. XRD diffractograms of TPP-HP-β-CD physical mixture (red line), TPP-HP-β-CD complex (blue line), TPP-SBE-β-CD physical mixture (pink line) and TPP-SBE-β-CD complex (green line).

The results of DSC analysis also supported the XRD results. According to the literature, TPP has a slight peak at around 200˚C. Although these slight peaks of TPP can be seen in Figure 4, at around 180˚C for physical mixtures, the peaks disappeared from the thermograms of the complexes, indicating the forma-

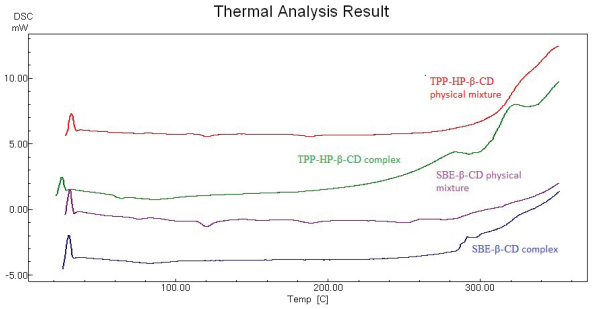

Figure 4. DSC thermograms of TPP-HP-β-CD physical mixture (red line), TPP-HP-β-CD complex (green line), TPP-SBE-β-CD physical mixture (purple line) and TPP-SBE-β-CD complex (blue line).

tion of the complexes (Nallamuthu et al., 2015). In addition, the peaks at around 280°C belonging to the cyclodextrins were much clearer for the complexes than the physical mixtures which also refers to the formation of the complexes.

The research of Calvo and his group, is one of the first studies that enlightens the conditions of CS nanoparticles prepared with TPP using the ionic gelation method (Calvo et al., 1997). Since then, there have been so many studies in literature on optimising CS nanoparticles. As a recent example, Sreekuomar and colleagues, investigated the effects of DA (degree of acetylation), DP (degree of polymerisation), NH_2/PO_4 molar ratio, and the concentration of the CS solution. It was reported that the optimum CS/TPP ratio, which this ratio denotes as the molar ratio of NH_2 groups of CS and PO_4 groups of TPP, was about 3 (Sreekumar et al., 2018).

Following the complex preparation, the CS nanoparticles were prepared with the traditional ionic gelation method using TPP, with varying CS/TPP ratios as shown in Table 1. It was considered to choose the optimum CS/TPP ratio and pH then use these parameters to prepare the CS-TPP-HP-β-CD and CS-TPP-SBE-β-CD nanoparticles.

Although the data is not shown, the CS ratios 0.5% and 0.25% were also tested but the particle sizes were found to be in micron sizes and the particle size increased proportionally with the CS concentration. Hence it was decided to use CS at 0.125%, to formulate the nanoparticles. It was clearly seen

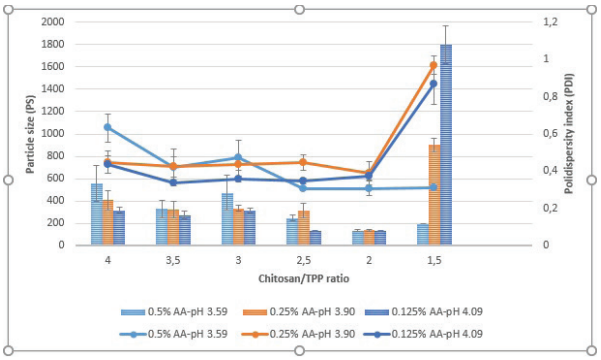

Figure 5. Effects of pH and CS/TPP ratio on PS and PDI (The bars represent changes in PS and the lines represent changes in PDI values).

that the CS/TPP ratio is the main factor affecting the nanoparticle size. The ratio 4 to 2, allows the formation of nanoparticles with varying sizes and PDIs values while the aggragates formed for the CS/TPP ratio 1.5 or lower at higher pHs, 3.90 and 4.09 (Figure 5).

On the other hand, the ZP values were found to be directly proportional to CS/TPP ratio, as the CS/TPP ratio decreases, the ZP values decreases (Figure 6). While the ZP value of the CS/TPP ratio 4 (0.5% AA) formulation was 35.9±1.42 mV, the CS/TPP ratio 1.5 (0.5% AA) formulation was found to be 12.4±0.305 mV. As the CS/TPP ratio decreased below 3, the changes in ZP values were found to be significant ($p<0.05$).

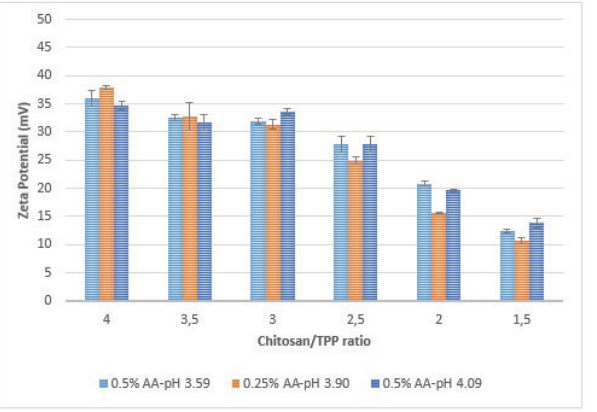

Figure 6. Relation of ZP values of formulations with varying CS/TPP ratios at different AA ratios.

According to the findings, the optimum conditions for the preparation of CS/TPP were determined as given in Table 2. Using these conditions the nanoparticles were obtained with 144 nm PS, 0.317 PDI, 21.2 mV ZP.

Table 2. Parameters for optimum formulation.

Parameters	Optimum conditions
CS concentration	0.125%
Asetic acid (AA) ratio	0.5%
pH	3.59
CS/TPP ratio	2
Stirring rate	1000 rpm

The nanoparticles were prepared with TPP-HP-β-CD and TPP-SBE-β-CD using the conditions given in Table 2. The CS/TPP-HP-β-CD nanoparticles were found to be preferable compared to the CS/TPP-SBE-β-CD nanoparticles due to their lower PS and PDI and higher ZP values (Table 3). CS-TPP-SBE-β-CD nanoparticles had also a tendency towards aggregation, such that aggregates with particle size over 2 µm could be easily seen a few hours after preparation. As both SBE-β-CD and TPP were anionic, complexing to CS caused a kind of neutralization and so aggregates could be

Table 3. Characteristics of final nanoparticles.

Nanoparticle type	PS (nm) ± SD	PDI ± SD	ZP (mV) ± SD
CS/TPP	144±22.9	0.317±0.0378	21.2±1.435
CS/TPP-HP-β-CD	178±84.1	0.310±0.0134	31.2±4.68
CS/TPP-SBE-β-CD	821.2±54.4	0.849±0.140	17.5±0.709

formed. There are studies in the literature about using SBE-β-CD instead of TPP as an anionic linker (Fulop et al., 2014; Mahmoud et al., 2011) suggesting its use as a linker itself rather than complexing with TPP.

Following the characterization of nanoparticles, the interday changes in PS, PDI and ZP of CS/TPP and CS/TPP-HP-β-CD nanoparticles were observed as a mini-stability study. As it is clearly seen in Figure 7, while the PS and PDI values of CS/TPP were increasing day by day ($p<0.05$), the values of CS/TPP-HP-β-CD did not significantly change ($p>0.1$). In addition, the ZP of CS/TPP nanoparticles dropped to 14.9 mV from 21.2 mV in 5 days, as an expected result because the initial ZP of TPP-HP-β-CD being significantly higher than the ZP of CS/TPP. The positive effect of TPP-HP-β-CD could be clearly seen to protect nanoparticle characteristics.

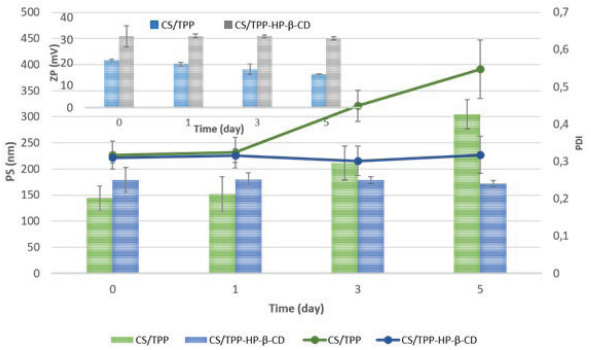

Figure 7. Interday changes in PS, PDI and ZP of CS/TPP and CS/TPP-HP-β-CD nanoparticles (The bars represent changes in PS and the lines represent changes in PDI values for PS-PDI time graph).

In a study by Pant and Negi in 2018, TPP-β-CD complexes were prepared for the first time in the literaure and used for the preparation of CS nanoparticles by ionic gelation method. The changes in the parameters PS, PDI and ZP were determined for 2 days and according to their results, the parameters changed significantly for the CS/TPP nanoparticles and β-CD enhanced interday stability (Pant and Negi, 2018). The results of this study also supported their findings, except their CS/TPP-β-CD nanoparticles were smaller than the CS/TPP nanoparticles. The MW of β-CD is lower than HP-β-CD, so this may be the reason for larger particle sizes of CS/TPP-HP-β-CD nanoparticles prepared in this study.

CONCLUSION

The ionic gelation method has been the most common and simple method for CS nanoparticle preparation since Calvo et al. first described this method for synthesis of CS nanoparticles using TPP. The method is basically related with the ionic interactions between CSs' amino groups and the phosphate groups of TPP. In the case of ionic interactions, undesirable complexations are also highly likely to occur. In this study, it was aimed to control those undesirable interactions using TPP-HP-β-CD complex instead of TPP and develop a nanocarrier for drug delivery studies. According to the results of the optimization study, optimum CS/TPP-HP-β-CD were obtained using a CS concentration 0.125% with 0.5% AA at a ratio of CS/TPP 2. CS/TPP-HP-β-CD nanoparticles were found to have a higher physical stability compared to CS/TPP nanoparticles as aimed. In conclusion, since cylodextrins are very well established molecules for enhancing solubility of hydrophobic drugs, CS/TPP-HP-β-CD nanoparticles with 178±84.1 PS and 31.2±4.68 mV would be a good candidate especially for hydrophobic drugs to improve their stability, solubility, encapsulation efficiency and in vivo bioavailability.

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Conflict of Interest: The authors have no conflict of interest to declare.

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REFERENCES

- Ağardan, N. B., Değim, Z., Yılmaz, Ş., Altıntaş, L., & Topal, T. (2016). The Effectiveness of raloxifene-loaded liposomes and cochleates in breast cancer therapy. *AAPS PharmSciTech, 17*(4), 968– 977.
- Agarwal M, A. M., Shrivastav, N., Pandey, S., Das, R., & Gaur, P. (2018). Preparation of Chitosan Nanoparticles and their In-vitro Characterization. *International Journal of Life-Sciences Scientific Research, 4*(2), 1713–1720.
- Agnihotri, S. A., Mallikarjuna, N. N., & Aminabhavi, T. M. (2004). Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *Journal of Controlled Release, 100*(1), 5–28.
- Ahmed, T. A., & Aljaeid, B. M. (2016). Preparation, characterization, and potential application of chitosan, chitosan derivatives, and chitosan metal nanoparticles in pharmaceutical drug delivery. *Drug Design, Development and Therapy, 10*, 483–507.
- Calvo, P., Remuñan-López, C., Vila-Jato, J. L., & Alonso, M. J. (1997). Chitosan and Chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. *Pharmaceutical Research, 14*(10), 1431–1436.
- Crucho, C. I. C., & Barros, M. T. (2017). Polymeric nanoparticles: A study on the preparation variables and characterization methods. *Materials Science and Engineering: C, 80*, 771–784.
- Elsabahy, M., & Wooley, K. L. (2012). Design of polymeric nanoparticles for biomedical delivery applications. *Chemical Society Reviews, 41*(7), 2545–2561.

- Fulop, Z., Saokham, P., & Loftsson, T. (2014). Sulfobutylether-beta-cyclodextrin/chitosan nano- and microparticles and their physicochemical characteristics. *International Journal of Pharmaceutics, 472*(1-2), 282–287.
- Grenha, A. (2012). Chitosan nanoparticles: a survey of preparation methods. *Journal of Drug Targeting, 20*(4), 291–300.
- Kapor A, S. S., Nikolic, V., Cvezic, Z., & Rakic, S. (2008). DSC and XRD analysis of inclusion complexes of inclusion complexes of pharmacologically active compounds. *Journal of Reseach in Physics, 32*(1), 25–31.
- Kumari, A., Yadav, S. K., & Yadav, S. C. (2010). Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids and Surfaces B: Biointerfaces, 75*(1), 1–18.
- Loftsson, T., Duchene, D. (2007). Cyclodextrins and their pharmaceutical applications. *International Journal of Pharmaceutics, 329*(1–2), 1–11.
- Mahmoud, A. A., El-Feky, G. S., Kamel, R., & Awad, G. E. A. (2011). Chitosan/sulfobutylether-β-cyclodextrin nanoparticles as a potential approach for ocular drug delivery. *International Journal of Pharmaceutics, 413*(1), 229–236.
- Miecznik, P., & Kaczmarek, M. (2007). Ultrasonic investigations of inclusion complexation of α-cyclodextrin by iodide ions in pseudo-binary aqueous system. *Journal of Molecular Liquids, 133*(1), 120–124.
- Mohammed, M. A., Syeda, J. T. M., Wasan, K. M., Wasan, E. K. (2017). An Overview of Chitosan Nanoparticles and Its Application in Non-Parenteral Drug Delivery. *Pharmaceutics, 9*(4), 53.
- Mutlu Ağardan, N. B., Değim, Z., & Yilmaz, Ş. (2014). Antitumoral and MMP-2 inhibition activity of raloxifene or tamoxifen loaded nanoparticles containing dimethyl-β-cyclodextrin. *Journal of Inclusion Phenomena and Macrocyclic Chemistry, 80*(1), 31–36.
- Nallamuthu, I., Devi, A., & Khanum, F. (2015). Chlorogenic acid loaded chitosan nanoparticles with sustained release property, retained antioxidant activity and enhanced bioavailability. *Asian Journal of Pharmaceutical Sciences, 10*(3), 203–211.
- Ohya, Y., Shiratani, M., Kobayashi, H., & Ouchi, T. (1994). Release behavior of 5-Fluorouracil from Chitosan-Gel Nanospheres Immobilizing 5-Fluorouracil Coated with Polysaccharides and Their Cell Specific Cytotoxicity. *Journal of Macromolecular Science, Part A, 31*(5), 629–642.
- Pant, A., & Negi, J. S. (2018). Novel controlled ionic gelation strategy for chitosan nanoparticles preparation using TPP-beta-CD inclusion complex. *European Journal of Pharmaceutical Sciences, 112*, 180–185.
- Rizvi, S. A. A., & Saleh, A. M. (2018). Applications of nanoparticle systems in drug delivery technology. *Saudi Pharmaceutical Journal : SPJ : the Official Publication of The Saudi Pharmaceutical Society, 26*(1), 64–70.
- Sreekumar, S., Goycoolea, F. M., Moerschbacher, B. M., & Rivera-Rodriguez, G. R. (2018). Parameters influencing the size of chitosan-TPP nano- and microparticles. *Scientific Reports, 8*(1), 4695.