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*Short communication*

# Screening the effects of process and formulation factors upon the physical properties of Chitosan-TPP nanoparticles as drug delivery carriers for Gentamycin sulphate

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## Introduction

Microbial infections are one of the prime causes of morbidity and mortality and have become a significant issue within the medical field. A considerable number of infections are currently linked to antibiotic-resistant bacteria, primarily due to the inappropriate and excessive utilization of antibiotics.

Nanotechnology, employing materials at the nanoscale, is progressively finding applications in clinical settings, particularly as a novel approach for addressing infectious diseases. Among these nanoscale materials, nanoparticles (NPs) have exhibited extensive antibacterial capabilities, effectively targeting both Gram-positive and Gram-negative bacteria. The main rationale behind considering NPs as an alternative to antibiotics lies in their ability to combat microbial drug resistance, presenting a promising solution in certain cases.
Over the last twenty years, researchers have been drawn to the beneficial characteristics of chitosan (CS). This compound is non-toxic, biocompatible, and edible, making it highly appealing for various applications. Moreover, CS exhibits antimicrobial properties. In particular, nanoparticles based on CS have displayed favorable antibacterial effectiveness and their full potential is not yet achieved (Al-Zahrani et al., 2021).

The aim of our study was to prepare Gentamycin sulphate loaded CS nanoparticles and screen the main effects of the formulation and process parameters on their physical properties (particle size, zeta potential), in a One Factor At a Time (OFAT) study.

## Materials and methods

CS (medium molecular weight), tripolyphosphate (TPP) and gentamycine sulphate (GS) were obtained from MerckMilipore. All other reagents used were of analytical grade.

*Preparation procedure for blank NPs:* 10 mL of the TPP solution in water was added dropwise to 25 mL of the CS solution in 1% acetic acid for a period of 4 min under different types of agitation (Table 1). The concentration of CS and TPP solutions were also varied accordingly (Table 1).

*Preparation procedure for loaded NPs:* F7 formulation was employed to prepare the GS loaded nanoparticles. A varying amount of GS (12.5 mg, 25 mg and 100 mg) was dissolved in the CS solution and prepared as described in the previous section.

*Particle size and zeta potential*: The NP dispersion was diluted 10-fold in purified water and transferred to a capillary fold cell. Particle size (z-average), polydispersity

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index (PDI) and zeta potential were measured on Zetasizer Nano ZS (MalvernPanalytical, UK).

## Results and discussion

The results from the physical characterization of the prepared formulations are reported in Table 1. The effects of the formulation and process variables on the physical characteristics of the prepared NPs are evident. When magnetic stirring is used during the preparation procedure, the particle size is larger, probably due to differences in the energy and modus of dispersion in contrast to the ultrasonication method.

Table 1. Z-average diameter and PDI of the prepared formulation using different agitation methods and varying CS and TPP concentrations

| Formulation code | Aggi-tation | CS (mg/mL) | TPP % (mL) | Z-average (nm±SD) (PDI) |
|---|---|---|---|---|
| F1 | I | 2 | 0.1 | 230.3±7 (0.52) |
| F2 | II | 2 | 0.1 | 282±102 (0.51) |
| F3 | III | 2 | 0.1 | 192±2 (0.43) |
| F4 | IV | 2 | 0.1 | 303.5±5.6 (0.84) |
| F5 | III | 2 | 0.05 | 267.6±25.2 (0.61) |
| F6 | III | 2 | 0.25 | 195.7±7.7 (0.63) |
| F7 | III | 0.5 | 0.025 | 90.2±0.36 (0.19) |
| F8 | III | 0.5 | 0.0625 | 277.5±9.6 (0.64) |
| F9 | III | 0.5 | 0.125 | 81.5±3.75 (0.36) |

*I - magnetic stirring 400 rpm, II - magnetic stirring 700 rpm, III – 4 min ultrasonic probe (50% intensity), two cycles IV – magnetic stirring 700 rpm + 4 min ultrasonic probe (50% intensity).

The CS and TPP concentration also affect the particle size, as the rate of crosslinking and the density of the crosslinked CS chains play a crucial role in the colloidal stability and surface properties of the particles. When the CS concentration is higher, the particle size increases due to the aggregation and overcharging phenomenon and possible partial phase separation. However, in contrast to CS, the effects of TPP concentration are inversely related to the size of the NP, probably due to the polycomplex stoichiometry (Sawtarie et al., 2017). All formulations were characterized by a positive zeta potential higher than 30 mV, thus favoring the stability of prepared NP.

The F7 formulation was used as an optimal carrier for loading of GS. The addition of GS didn't affect the physical characteristics and stability of the prepared NPs.

## Conclusion

In this study, a screening of the effects of different agitation methods as well as the concentration of CS and TPP, upon the physical characteristics of the polyelectrolyte NPs was performed. A distinct pattern of the effects of CS and TPP was revealed, and the most optimal mixing conditions for the preparation procedures were elucidated. In addition, GS was encapsulated in the formulation exhibiting the most appropriate properties. Further studies are needed to optimize the GS encapsulation efficiency and drug release.

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## References

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