

## **Effect of Initial Concentration of Chitosan on The Particle Size of Chitosan Nanoparticle**

Ruzanna Ahmad Shapi'ia, Siti Hajar Othman<sup>a,b,\*</sup>, Mohd Nazli Naim<sup>a</sup>, Roseliza Kader Basha<sup>a</sup>

<sup>a</sup>*Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

<sup>b</sup>*Institute of Advanced Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

*\*Corresponding Author's Email: s.hajar@upm.edu.my*

**Abstract:** In this study, chitosan nanoparticle (CNP) was produced via ionic gelation process with varying initial concentration of chitosan (0.3, 0.6, 0.9, 1.2, 1.5, 1.8, and 2.1% w/v). The particle size of CNP was measured using dynamic light scattering (DLS) and transmission electron microscope (TEM). It was found that the particle size of CNP increased with the increase in initial concentration of chitosan. This was due to the agglomeration of the CNP that produced with high concentration of chitosan as proven from the TEM images. Fourier transmission infrared (FTIR) analysis revealed the peak of amide shifted from 1658 to 1635  $\text{cm}^{-1}$ , thus confirmed the formation of CNP. Energy dispersive X-ray elemental analysis (EDX) also revealed the presence of phosphorus in the CNP, thus confirmed the cross-linking of chitosan and sodium tripolyphosphate.

**Keywords:** Chitosan nanoparticle, dynamic light scattering, ionic gelation, particle size, TEM

### **INTRODUCTION**

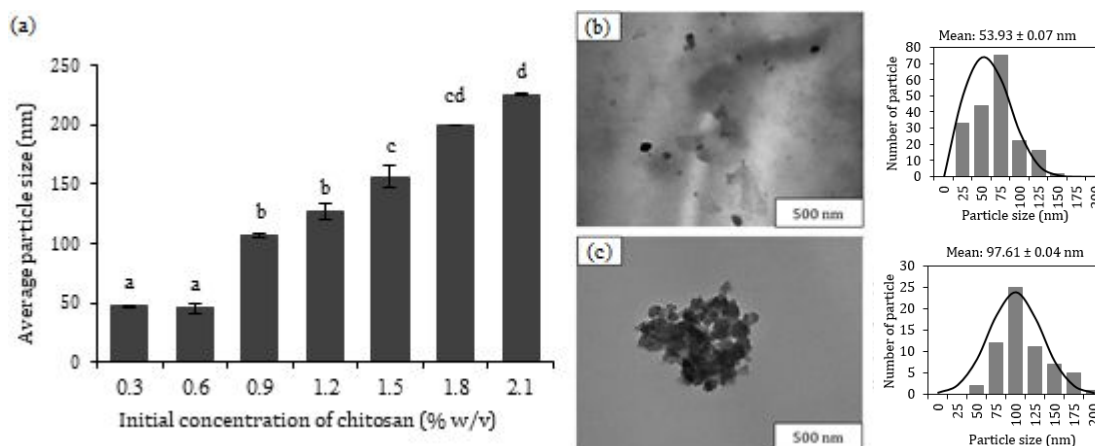
Chitosan nanoparticle (CNP) exhibits excellent properties as antimicrobial agent [1], antioxidant agent [2], reinforcing agent [3], and delivery agent [4]. Therefore, many efforts have been done to synthesize CNP which is in the size range of nanoparticle (less than 100 nm) [5]. Nevertheless, most of the previous studies produced CNP with the hydrodynamic size larger than 100 nm which was not in the range of the nanoparticle size. To the best of our knowledge, only a study by Gokce et al. [6] managed to produce CNP in the size range of 40 to 110 nm but they did not investigate the effect of initial concentration of chitosan. The initial concentration of chitosan is an imperative parameter for ionic gelation method which could affect the size of CNP [7]. Therefore, this study is directed to investigate the effect of initial concentration of chitosan on the particle size of CNP.

### **MATERIALS AND METHODS**

CNP was produced via ionic gelation method according to the optimum parameters established previously [6] with modification of initial concentration of chitosan (0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1% w/v). The particle size of CNP was measured using DLS and TEM. FTIR and EDX analysis was also done to confirm the formation of CNP.

### **RESULTS AND DISCUSSION**

From Fig 1(a), the size of CNP increased significantly (4-fold increment,  $p < 0.05$ ) from 46.76 to 225.33 nm with the increase in initial concentration of chitosan from 0.3 to 2.1% w/v. This was due to the agglomeration of CNP when high initial concentration of chitosan was used to produce CNP. As can be seen in Fig 1(b), TEM image of 0.9% w/v CNP shows the spherical shape nanoparticles with diameter range between 25 to 150 nm (average size: 53.93 nm). Meanwhile, TEM image of 1.8% w/v CNP (Fig 1(c)) shows agglomeration with the single particle size range between 50 to 200 nm (average size: 97.61 nm) and bulk particle size around 500 nm. Note that, both nominal average sizes of CNP measured using TEM was different with the size of CNP measured via DLS. This was due to the hydrated state and swollen CNP during DLS analysis, thus the size of CNP was larger than their actual size without water molecules [8]. Although DLS reported that 0.9% to 2.1% w/v of CH produced large CNP ( $\geq 106.34$  nm), it can be speculated that the actual size of CNP was smaller than the size of CNP measured by DLS.



**Fig 1. Effect of initial concentration of chitosan on the particle size of CNP (a) average particle size from DLS, (b) TEM image and corresponding particle size distribution histogram of 0.9% w/v CNP, (c) TEM image and corresponding particle size distribution histogram of 1.5% w/v CNP.**

Comparison of FTIR spectra of CNP with that of chitosan (figure not shown), demonstrated that there was a shift in the peak of amide in chitosan from  $1658\text{ cm}^{-1}$  to  $1635\text{ cm}^{-1}$ , thus confirmed the formation of cross-linkage between the chitosan and TPP [9]. The assignments of various bands and peaks for CNP in this study were consistent with those reported in the literature for similar functional groups of CNP [3, 10] thus proven the formation of CNP. EDX spectra of particles in Fig 1. (b) (figure not shown) shows the presence of nitrogen (N), sodium (Na), and phosphate (P) elements which is the elemental component of CH ( $\text{C}_6\text{H}_{12}\text{NO}_2$ ) and TPP ( $\text{Na}_5\text{P}_3\text{O}_{10}$ ), thus confirm the formation of CNP.

## CONCLUSIONS

In conclusion, an increase in initial concentration of chitosan resulted to the increase in the particle size of CNP. The size of CNP measured using TEM was smaller than the size of CNP measured using DLS due to the hydrophilic nature of CNP. Further study on the other parameter of ionic gelation methods that affect the properties of CNP should be embarked to produce the small size and stable CNP.

**ACKNOWLEDGMENT:** The authors would like to acknowledge the financial support provided by Fundamental Research Grant Scheme (Vote number: 5524980) of the Ministry of Science, Technology, and Environment, Malaysia.

## REFERENCES

- [1] Pilon L, Spricigo PC, Miranda M, Moura MR, Assis OBG, Mattoso LHC, Ferreira MD. *Int. J. Food Sci. Technol.*, 2015, **50**, 440.
- [2] Eshghi S, Hashemi M. *Food Bioprocess Technol.*, 2014.
- [3] Antoniou J, Liu F, Majeed H, Zhong F. *Food Hydrocoll.*, 2015, **44**, 309.
- [4] Qin H, Wang CM, Dong QQ, Zhang L, Zhang X, Ma ZY, Han QR. *J. Magn. Magn. Mater.*, 2015, **381**, 120.
- [5] Othman SH. *Agric. Agric. Sci. Procedia*, 2014, **2**, 296.
- [6] Gokce Y, Cengiz B, Yildiz N, Calimli A, Aktas Z. *Colloids Surfaces A Physicochem. Eng. Asp.*, 2014, **462**, 75.
- [7] Bugnicourt L, Alcouffe P, Ladaviere C. *Colloids Surfaces A Physicochem. Eng. Asp.*, 2014, **457**, 476.
- [8] Lima DR, Feitosa L, Pereira ADES, Moura MR, Aouada FA, Mattoso LHC, Fraceto LF. *Food Sci.*, 2010, **75**, 89.
- [9] Dudhani AR, Kosaraju SL. *Carbohydr. Polym.* 2010, **81**, 243.
- [10] Ashori A, Bahrami R. *Polym. Plast. Technol. Eng.*, 2014, **53**, 312.