

Synthesis and Properties of Chitosan Nanoparticles Cross-Linked with Tripolyphosphate

Mostafa Yusefi ¹, Pooneh Kia ², Siti Nur Amalina Mohamad Sukri ¹, Roshafima Rasit Ali ¹, Kamyar Shameli ^{1,*}

¹ Malaysia-Japan International Institute of Technology, Universiti Teknologi Malaysia, Jalan Sultan Yahya Petra, 54100, Kuala Lumpur, Malaysia

²Institute of Bio Science, University Putra Malaysia, Serdang 43400, Malaysia

* Correspondence: E-mail: kamyarshameli@gmail.com; Tel: +603-22031200

<https://doi.org/10.37934/jrnn.3.1.4652>

ABSTRACT

Chitosan nanoparticles (ChNPs) have been extensively examined for various biomedical applications due to their advantages include large surface area, biodegradability, and biocompatibility. The purpose of this research was to synthesize ChNPs using a simple ionic gelation technique by the interaction of low molecular weight chitosan (LMWC) and sodium tripolyphosphate (TPP) as a cross-linking agent. ChNPs, TPP, and LMWC were analysed by X-ray diffraction (XRD) and Fourier transforms infrared (FTIR) spectra that indicated the formation of ChNPs, attributing to the rearrangement of the nanoparticles after adding the TPP cross-linker into the LMWC solution. XRD analysis exhibited that ChNPs were amorphous, due to the effect of TPP cross-linker. Dynamic light scattering showed the nano-dimension of ChNPs with a hydrodynamic size of 68.50 nm. Thus, the obtained results indicated that the properties of chitosan were improved through converting it into nanoparticles using TPP initiated ionic gelation procedure.

Keywords:

Chitosan Nanoparticles; Sodium Tripolyphosphate; Cross-Linking; Facile Synthesis

Received: 24 May 2021

Revised: 19 June 2021

Accepted: 23 June 2021

Published:

1. Introduction

1 Various types of nanostructures are frequently used in preclinical biomedical analysis [1].
2 However, it was proven that some nanoparticles (NPs) (such as metal NPs) have led to issues for
3 human health because they might cause risks of neurotoxicity and cytotoxicity [2]. Therefore, the use
4 of non-toxic, biodegradable, and biocompatible nanomaterials such as biopolymer can tackle the
5 above problems [2-7]. In this manner, chitosan NPs (ChNPs) are extensively popular for many
6 biomedical applications. It is the most important derivative of chitin obtained from the shells of
7

1 insects and crustacean such as prawns or crabs, as well as the cell walls of fungi [8]. Many studies
2 have used chitosan and various cross-linker to fabricate chitosan-based NPs with high
3 biocompatibility, nontoxicity, biodegradability, and colloidal stability [8, 9]. Ionic gelation, reverse
4 micellar method, emulsification solvent diffusion are the popular method for fabrication of ChNPs
5 [10]. Among them, ionic gelation is the simplest method free from using organic solvents [9]. This
6 technique is based on electrostatic interaction between positively charged amino groups of chitosan
7 and the negatively charged groups of sodium tripolyphosphate (TPP) as a cross-linking agent [11].
8 The size of ChNPs must be concerned, which is related to the factors of the synthesis process,
9 including stirring rate, temperature, and chitosan and TPP concentration [12]. Although, many
10 researchers have fabricated ChNPs with TPP cross-linking following the pioneering work of Calvo et
11 al and to evaluate the possible pharmaceutical applications, optimization of the synthesis parameters
12 and the required characteristics of the resultant ChNPs is still an ongoing vital matter in this area [9,
13 13]. These conditions not only affect the colloidal stability, drug loading, and release manner but also
14 it can domain the level biocompatibility and toxicity of the particles in the biological tissues and
15 environment. It was reported in a study [14] that the chitosan and TPP with a ratio around 1:2 could
16 cause the successful synthesis of ChNPs with desirable physicochemical and morphological
17 characteristics. The presence of hydrogen and Van der Waals forces in ChNPs could trigger
18 intermolecular interactions in the NPs. In addition, cationic groups of chitosan (NH_3^+) can react with
19 phosphoric ions in the TPP cross linker to form the deprotonation of ChNPs.

20 This current study aimed to fabricate ChNPs using the ionic gelation method through the
21 interaction of low molecular weight chitosan (LMWC) and the sodium tripolyphosphate (TPP) cross-
22 linker. The physiochemical characteristics of the fabricated ChNPs, LMWC, and TPP were evaluated
23 by X-ray powder diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and dynamic
24 light scattering (DLS) analysis.

25 **2. Materials and Methods**

26 *2.1. Materials*

27 Acetic acid glacial (CH_3COOH) (98%), chitosan (low molecular weight, 190,000–310,000 degree of
28 acetylation), Tween-80, and TPP were all purchased from Sigma Aldrich (St. Louis, MO, USA). All
29 the reagents used were of analytical grade and used without further purification. All glassware used
30 was washed with deionized water and dried before used.

31 *2.2. Synthesis of Chitosan Nanoparticles (ChNPs)*

32 ChNPs were prepared based on ionic gelation method. The ratio between LMWC to TPP cross-
33 linker was optimized as 1:2 (v/v). First, 250 mL beaker contained 80 mL mixture solution of 1.0%
34 acetic acid and 0.250 g of LMWC powder (low molecular weight). 2 drops of Tween 80 were added
35 to the solution. TPP was dissolved in deionized water at concentration of 100 mg/ml and it was added
36 dropwise to the LMWC solution under the continuously vigorous stirring of the homogenizer (9000
37 rpm) for 45 min at room temperature. The mixture solution was washed with distilled water and
38 centrifuged three times at 2500 rpm for 7 min at 25 °C. Finally, the ChNPs sample was freeze-dried
39 for 16 h and stored at 4 °C for further analysis.

40

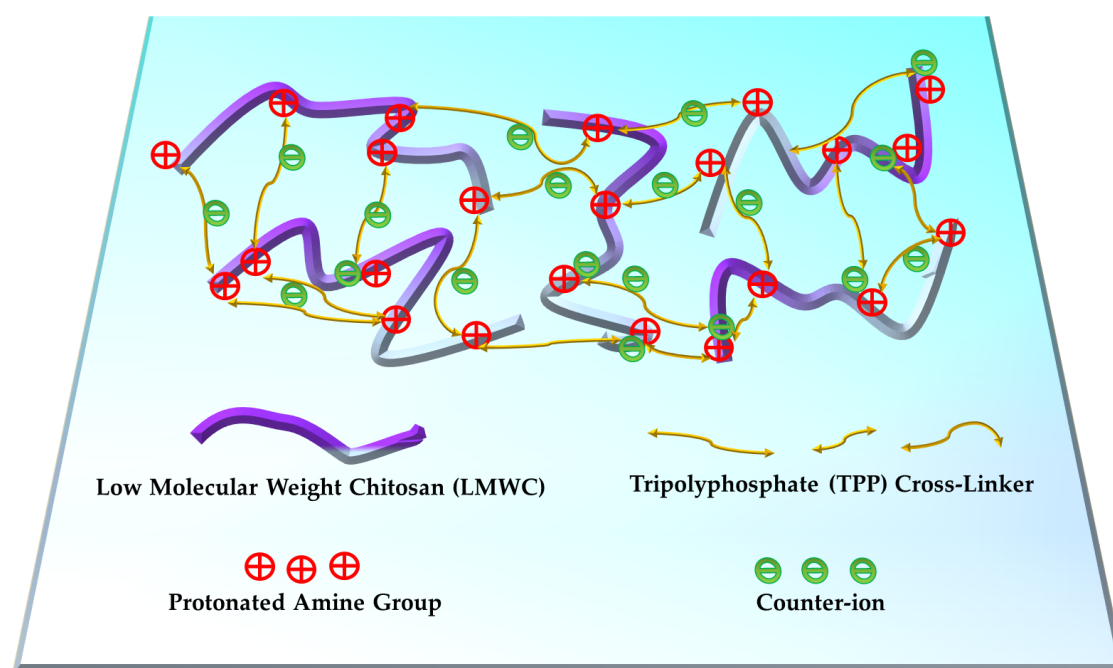
41

2.3. Characterization

XRD (Philips, X'pert, Cu Ka) at an ambient condition was used to evaluate the structure of the samples. The sample was compressed between two smooth glass films and the XRD analysis was carried out in dispersion 2 angles of 5° – 80° at a step size of 0.02° with 2 s/step as scanning rate using a voltage of 45 kV, a Ni-filtered Cu K radiation ($\lambda=1.5406 \text{ \AA}$) and a filament current of 40 mA. FTIR spectroscopy (ThermoNicolet, Waltham) determined the chemical and super-molecular structural analysis of the samples under an ambient condition. First, crushing and mixing of the sample with KBr at a ratio of 1:100 *w/w* to prepare a transparent pellet and the spectra of the plate was evaluated under a transmittance mode in a range between 4000 cm^{-1} to 400 cm^{-1} with a 4 cm^{-1} resolution and an accumulation of 128 scans. For DLS analysis, an Anton Paar instrument (Litesizer 500, Graz, Styria, Austria) was used to measure the hydrodynamic size of the synthesized samples in distilled water at 25°C .

3. Results and Discussion

Scheme 1 shows the potential interactions between active functional groups of LMWC and TPP in the fabrication of ChNPs. The ionic gelation technique and TPP is responsible for the improved degree of cross-linking and formation of ChNPs. With adjusting the crosslinking degree of TPP and LMWC to synthesize ChNPs, improved properties of the NPs can be obtained for various applications. The optimized ratio between the LMWC to TPP cross-linker was 1:2 (*v/v*) as stated in our previous study [9]. Also, a separate study [8] used this ratio between TPP and chitosan, along with 1.0% acetic acid, and 2% (*v/v*) Tween-80 in the chitosan solution to synthesize ChNPs with suitable physiochemical characteristics. During the synthesis of ChNPs, cationic groups of chitosan (NH_3^+) could react with phosphoric ions in the TPP solution to trigger intermolecular interactions and the deprotonation of LMWC.



24

25 **Scheme 1.** The potential interactions between active functional groups of LMWC and TPP in the fabrication of
26 ChNPs.

3.1. X-ray Powder Diffraction Analysis

XRD was used to assess the crystallographic structure of LMWC, TPP, and ChNPs. As seen in Figure 1, LMWC indicated peaks related to a crystalline structure approximately at $2\theta = 17^\circ$ and 20° . The XRD spectra of ChNPs does not show these peaks due to cross-linking with TPP, indicating the formation of the NPs. The crosslinking degree can act as a vital parameter for the chitosan crosslinking synthesis. The ChNPs showed a decrease in the intensity of the XRD peaks related to crystallinity because of the presence of the TPP cross-linker. ChNPs indicated a pattern related to cross-linked chitosan with peaks approximately at $2\theta = 22^\circ$ and 15° , which is in good agreement with the JCPDS card no. 04-0784. The XRD results, thus, could show the successful fabrication of ChNPs cross-linked with TPP.

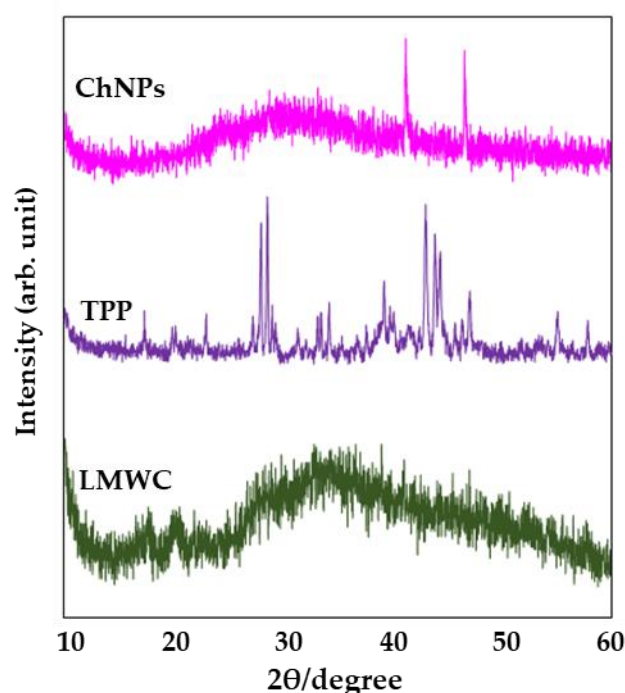


Figure 1. XRD spectra of LMWC, TPP, and ChNPs.

3.2. Fourier-Transform Infrared Spectroscopy

FTIR can indicate variations in the chemical structure of the sample by producing an infrared absorption spectrum. Figure 2 shows FTIR spectra of LMWC, TPP, and ChNPs. For the FTIR spectra of TPP, a sharp peak at 1209 cm^{-1} corresponds to the stretching vibration of $\text{P}=\text{O}$. The peak at 1157 cm^{-1} was attributed to the symmetric and asymmetric stretching vibrations in the PO_2 group. The presence of symmetric and anti-symmetric stretching vibration of $\text{O}-\text{P}=\text{O}$ was confirmed by the bands at around 1136 cm^{-1} . The peak at 889 cm^{-1} was attributed to the asymmetric stretching vibration of the $\text{P}-\text{O}-\text{P}$ bridge. ChNPs indicated the peak at 3676 cm^{-1} assigned to $-\text{OH}$ stretching vibration. The band at 3360 cm^{-1} was attributed to the stretching vibration of $\text{N}-\text{H}$ as overlapped with $\text{O}-\text{H}$ because of the amino groups of LMWC and the phosphate groups of TPP. This indicated the interactions between LMWC and TPP during synthesis of ChNPs. These results have been attributed to the cross-linking between amino groups of LMWC and phosphate groups of TPP. It is worth to mention that the freeze-drying procedure could moderate vaporization of the bound water and also offer the ice crystals over ChNPs to enhance the $-\text{OH}$ groups and synthesize white fluffy NPs [15]. Thus, the above FTIR results indicated the chemical structure related ChNPs cross-linked with triphosphate.

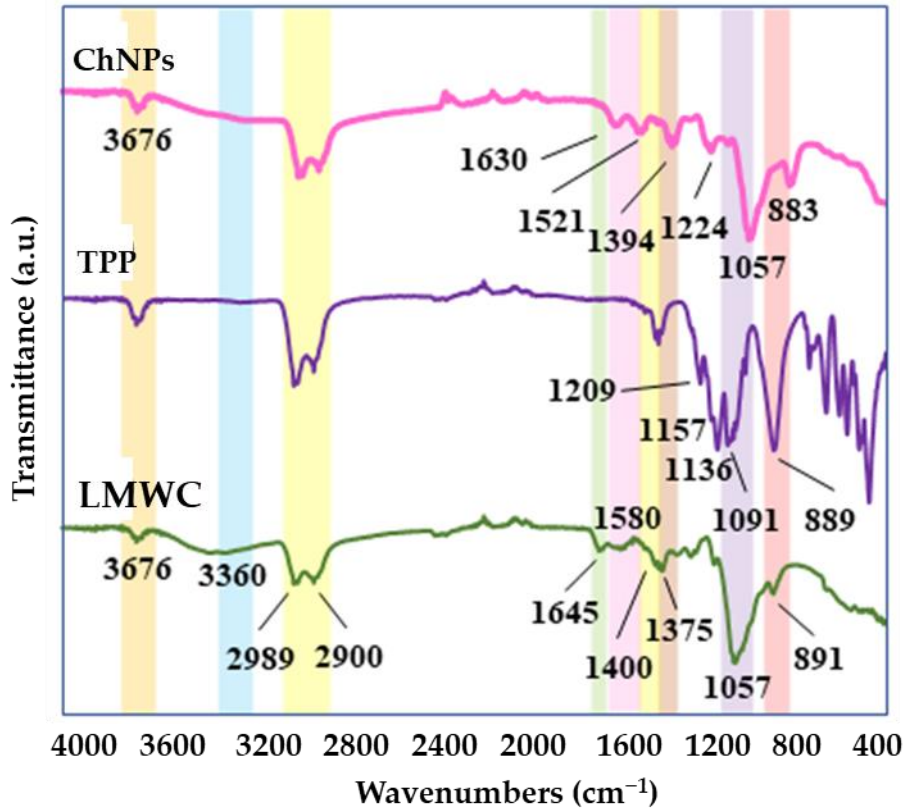


Figure 2. FTIR results of LMWC, TPP, and ChNPs.

3.3. Dynamic Light Scattering (DLS) Analysis

DLS analysis is based on the Brownian motion of particles in a dispersion. When the suspension of particles is exposed to a monochromatic wave of light, the wavelength of the light will change after hitting the particles, and a detector will detect the signal. As indicated in Figure 3, the hydrodynamic particle size of ChNP was found to be 68.50 nm. When TPP concentration reached a certain value, the sizes of NPs started to increase due to the particle aggregation. The phenomenon of the electrostatic interaction between TPP and LMWC can impact the crosslinking strength and size of ChNPs. DLS results indicated that ChNPs has a desirable nano-dimension to be used in various biomedical applications.

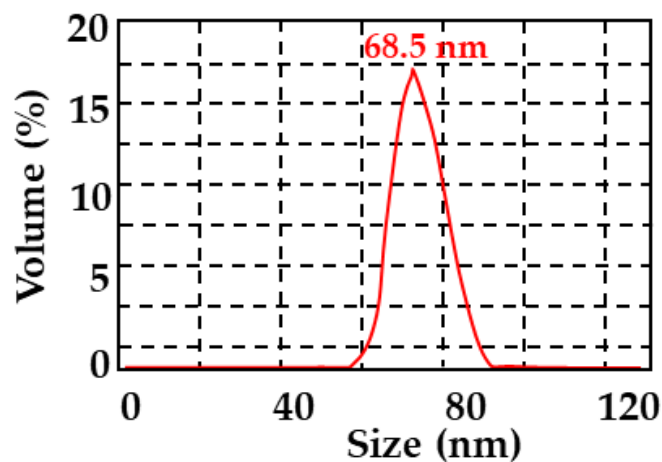


Figure 3. DLS size distribution of ChNPs.

1 4. Conclusions

2 The development in nanoscience has significantly increased the application of nano-polymers in
3 medical science. ChNPs was successfully synthesized through the ionic gelation method and TPP as
4 a cross-linking agent that Tween-80 and acetic acid were acted as a stabilizer and hydrogen ion
5 formation, respectively. XRD analysis showed that the cross-linked ChNPs were amorphous due to
6 TPP cross-linked chitosan. The FTIR results indicated the functional groups related to the formation
7 of ChNPs. From results of DLS analysis, the hydrodynamic size of the ChNPs was estimated to be
8 68.50 nm. Therefore, this study indicated the successful fabrication and characteristics of ChNPs
9 cross-linked with TPP.

10 Funding

11 This research was funded by Takasago Thermal Engineering Co. Ltd. grant (R.K.130000.7343.4B422)
12 from the research management center (RMC) of Universiti Teknologi Malaysia (UTM) and Malaysia
13 Japan International Institute of Technology (MJIIT).

14 Acknowledgement

15 Special thanks to of Universiti Teknologi Malaysia (UTM) and Malaysia-Japan International Institute
16 of Technology (MJIIT) for supports.

17 References

- 18 1. Sunasee R, Hemraz UD, and Ckless K, Cellulose nanocrystals: a versatile nanoplatform for
19 emerging biomedical applications. *Expert opin. drug deliv.* 2016;13,1243-1256.
20 <https://doi.org/10.1080/17425247.2016.1182491>.
- 21 2. Yusefi M, Shameli K, Ali R R, Pang S-W, Teow S-Y. Evaluating anticancer activity of plant-
22 mediated synthesized iron oxide nanoparticles using Punica granatum fruit peel extract. *J. Mol.*
23 *Struct.* 2020;1204,127539. <https://doi.org/10.1016/j.molstruc.2019.127539>
- 24 3. Ali A, Ahmed S. A review on chitosan and its nanocomposites in drug delivery. *Int. J. Biol.*
25 *Macromol.* 2018;109,273–286. [doi:10.1016/j.ijbiomac.2017.12.078](https://doi.org/10.1016/j.ijbiomac.2017.12.078)
- 26 4. Jahangirian H, Kalantari K, Izadiyan Z, Rafiee-Moghaddam R, Shameli K, Webster TJ. A review
27 of small molecules and drug delivery applications using gold and iron nanoparticles. *Int J*
28 *Nanomedicine* 2019;14,1633. <https://doi.org/10.2147/IJN.S184723>
- 29 5. Sukri, SNAM. Shameli K, Wong MM-T, TeowS-Y, Chew J, Ismail NA, Cytotoxicity and
30 antibacterial activities of plant-mediated synthesized zinc oxide (ZnO) nanoparticles using
31 Punica granatum (pomegranate) fruit peels extract. *J. Mol. Struct.* 2019;1189,57-65.
32 <https://doi.org/10.1016/j.molstruc.2019.04.026>
- 33 6. Izadiyan Z, Shameli K, Teow S-Y, Yusefi M, Kia P, Rasouli E, Tareq MA. Anticancer Activity
34 of 5-Fluorouracil-Loaded Nanoemulsions Containing Fe₃O₄/Au Core-Shell Nanoparticles. *J.*
35 *Mol. Struct.* 2021;131075. <https://doi.org/10.1016/j.molstruc.2021.131075>
- 36 7. Hamrayev H, Shameli K, Yusefi M. Preparation of zinc oxide nanoparticles and its cancer
37 treatment effects: A review paper. *Journal of Advanced Research in Micro and Nano Engineering*
38 2020;2,1-11.
- 39 8. Ali MEA, Aboelfadl, MMS, Selim AM, Khalil HF, Elkady GM. Chitosan nanoparticles extracted
40 from shrimp shells, application for removal of Fe (II) and Mn (II) from aqueous phases. *Sep. Sci.*
41 *Technol.* 2018;53,2870–2881. [doi:10.1080/01496395.2018.1489845](https://doi.org/10.1080/01496395.2018.1489845)

- 1 9. Yusefi M, Chan H-Y, Teow S-Y, Kia P, Lee-Kiun Soon M, Sidik NABC, Shameli K. 5-
2 Fluorouracil Encapsulated Chitosan-Cellulose Fiber Bionanocomposites: Synthesis,
3 Characterization and In Vitro Analysis towards Colorectal Cancer Cells. *Nanomaterials*
4 2021;11,1691. <https://doi.org/10.3390/nano11071691>
- 5 10. Kumar, MNR. A review of chitin and chitosan applications. *React. Funct. Polym.* 2000, 46, 1–27,
6 [doi:10.1016/S1381-5148\(00\)00038-9](https://doi.org/10.1016/S1381-5148(00)00038-9).
- 7 11. Maluin FN, Hussein MZ, Yusof NA, Fakurazi S, Idris AS, Zainol Hilmi NH, Jeffery Daim LD.
8 Preparation of chitosan–hexaconazole nanoparticles as fungicide nanodelivery system for
9 combating Ganoderma disease in oil palm. *Molecules* 2019;24,2498.
10 <https://doi.org/10.3390/molecules24132498>
- 11 12. Mohammed MA, Syeda J, Wasan KM, Wasan EK. An overview of chitosan nanoparticles and its
12 application in non-parenteral drug delivery. *Pharmaceutics* 2017;9,53.
13 [doi:10.3390/pharmaceutics9040053](https://doi.org/10.3390/pharmaceutics9040053)
- 14 13. Ribeiro, E.F., et al., *Chitosan and crosslinked chitosan nanoparticles: Synthesis, characterization and their*
15 *role as Pickering emulsifiers.* *Carbohydr. Polym.* 2020;250,116878.
16 <https://doi.org/10.1016/j.carbpol.2020.116878>
- 17 14. Maluin FN, Hussein MZ, Yusof NA, Fakurazi S, Idris AS, Zainol Hilmi NH, Jeffery Daim LD.
18 Preparation of chitosan–hexaconazole nanoparticles as fungicide nanodelivery system for
19 combating Ganoderma disease in oil palm. *Molecules* 2019;24,2498.
20 <https://doi.org/10.3390/molecules24132498>
- 21 15. Jiang F, Han S, and Hsieh Y-L. *Controlled defibrillation of rice straw cellulose and self-assembly of*
22 *cellulose nanofibrils into highly crystalline fibrous materials.* *RSC advances*, 2013;3,12366-12375.
23