

Facile Fabrication of Polysaccharide Nanocomposites Using Ionic Gelation Method

Mostafa Yusefi ¹, Kamyar Shameli ^{1,*}, Pooneh Kia ², Hemra Hamrayev ¹

Malaysia-Japan International Institute of Technology, Universiti Teknologi Malaysia, Jalan Sultan Yahya Petra, 54100, Kuala Lumpur, Malaysia ¹
Institute of Bio Science, University Putra Malaysia, Serdang 43400, Malaysia ²

* Correspondence: E-mail: kamyarshameli@gmail.com; Tel: +603-22031200
<https://doi.org/10.37934/jrnn.3.1.3745>

ABSTRACT

Polysaccharide-based nanomaterials with significant biocompatibility and physiochemical features have been widely analyzed in modern biomedical nanotechnology. Chitosan-coating is an advantageous procedure to provide several pharmacological characteristics of chitosan on the reinforcement. Here, we fabricated polysaccharide nanocomposites using the facile ionic gelation method and sodium tripolyphosphate (TPP) cross-linker. The polysaccharide nanocomposites comprised natural cellulose and chitosan as reinforcement and coating agents, respectively. From the image of the scanning electron microscope, the nanocomposites indicated almost spherical dimensions with sizes below 60 nm. Results from X-ray powder diffraction and Fourier-transform infrared spectroscopy showed multifunctional properties of the nanocomposites related to both cellulose and chitosan. Therefore, the ionic gelation method is potentially appropriate to synthesize the polysaccharide nanocomposites for medically-related applications.

Keywords:

Polysaccharide; Chitosan; Ionic Gelation Method; Nanocomposites

Received: 22 May 2021

Revised: 10 June 2021

Accepted: 25 June 2021

Published: 7 August 2021

1

2 1. Introduction

3 In biomedical application, the popular polymers as biocompatible coating agents are polyvinyl
4 pyrrolidone (PVP) polyvinyl alcohol (PVA), polyethylene glycol (PEG), polyethylenimine (PEI),
5 gelatin, starch, albumin, alginate, chitosan, and natural cellulose [1-3]. Chitosan is an attractive
6 polysaccharide and has a production above 100 million tons, annually [4]. It might be derived from
7 abundant natural biopolymer chitin and is a green-based amino-polysaccharide containing -(1-4)-
8 linked d-glucosamine and N-acetyld-glucosamine in deacetylated and acetylated form, respectively
9 [5]. The most abundant polymer on earth is natural cellulose, which can be distributed throughout
10 nature in plants, animals, algae, fungi, and minerals. However, the major source of cellulose is plant

1 fiber [6]. Cellulose contributes approximately 40% to the carbon fraction in plants, serving as
2 structuring element within the complex architecture of their cell walls. Natural cellulose and
3 nanocellulose based materials have been applied widely in therapeutic excipients, which
4 carboxymethyl cellulose, methyl cellulose, ethyl cellulose, and many different cellulose are also
5 analyzed for oral, topical, implantation, and injectable forms. Properties, including crystallinity,
6 surface chemical reactivity, less toxicity, proper mechanical strength, rheological and barrier
7 characteristics and proper specific surface area. Therefore, these fabulous characteristics can lead to
8 obtain structured products of “nanoenabled” as well as “nano-enhanced” with diverse applications
9 such as drug delivery vehicles for anticancer treatment, advanced composite materials, and
10 rheological modifier [7]. Of this, several studies have aimed to pinpoint synthesis and structural
11 analysis of various nanoparticles and nanocelluloses for biomedical applications [8-13].

12 In order to produce layer-by-layer chitosan-based nanocomplex and nanoparticles (NPs), ionic
13 gelation approach as an organic solvent-free solution, quick, and facile method indicated high
14 efficiency and low toxicity [14]. This approach uses the phosphate groups of sodium tripolyphosphate
15 (TPP) as a physical crosslinking agent, which has more benefits compared to other methods such as
16 emulsifying and chemical crosslinking agents. For example, this method has less toxicity to the organs
17 and no destruction to the structure of the loaded-drugs in chitosan NPs. Polysaccharide blends are
18 vital to construct advanced complexes in numerous applications [15]. The blend of degradable
19 polymers can merge the desirable properties of the polymers [16]. In addition, the crosslinking
20 procedure might considerably improve physiochemical characteristics of the polysaccharide
21 nanocomplex [17]. In biomedical applications, the most common antimicrobial coating material on
22 cellulose is currently chitosan for composite fabrication with suitable biodegradability and water-rich
23 structures [18]. Chitosan coating on natural cellulose is possibly facile since both polysaccharides
24 have a very similar structure. In addition, the complex of chitosan and natural cellulose has
25 intermolecular interactions, because of H-bonds and Van der Waals forces [19]. Most significantly,
26 chitosan-cellulose nanocomplex or composites have shown a great swelling and water absorption
27 capability. Thus, the biocompatibility, biodegradability, and physiochemical characteristics of
28 cellulose and also chitosan can be modified when cellulose as a matrix and chitosan as a coater
29 contributed in synthesis of polysaccharide nanocomposites. For biomedical application, the coating
30 agent such as polysaccharide may decrease a blockage in the blood vessels. Particularly, high colloidal
31 stability polysaccharide-based therapeutic agent can improve blood circulation to be delivered to
32 targeted tissue.

33 In this present study, polysaccharide system of chitosan-cellulose nanocomposites was fabricated
34 by the ionic gelation method and using TPP as a cross-linker. The physiochemical properties of the
35 synthesized polysaccharide nanocomposites were evaluated by X-ray powder diffraction (XRD),
36 Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM).

37 **2. Materials and Methods**

38 *2.1. Materials*

39 Acetic acid glacial (CH_3COOH) (98%), chitosan (low molecular weight, 190,000–310,000 degree of
40 acetylation), Tween-80 and TPP were all purchased from Sigma Aldrich (St. Louis, MO, USA). 5FU,
41 99%, 5-Fluoro-2,4(1H,3H)-pyrimidinedione (ACD CODE MFC D00006018) with a molecular weight
42 of 130.08 g/mol was purchased from ACROS ORGANICS part of Thermo Fisher Scientific,
43 Branchburg, NJ, USA. The chemicals were used without further purification. All glassware used was
44 washed with distilled water and dried before used.

1 2.2. Synthesis of Polysaccharide Nanocomposites of Chitosan-Cellulose (C-C NCs)

2 Fabrication of rice straw cellulose was explained earlier in our previously published studies [20,
3 21]. Ionic gelation technique was used to synthesize layer-by-layer of polysaccharide system of
4 chitosan-cellulose nanocomposites (C-C NCs) [22]. First, a 250 mL beakers contained 80 mL mixture
5 solution of 1.0% acetic acid and 0.250 g of chitosan powder (low molecular weight). Then, 2% (v/v) of
6 Tween-80 as a stabilizer was respectively added to each solution and mixed gently for 45 min to
7 obtain the chitosan solutions. After that, 0.125 g cellulose was mixed with the prepared chitosan
8 solution and homogenized vigorously at 9000 rpm for around 7 min. The 0.50 g of TPP cross-linker
9 was dissolved in 15 mL deionized water and added dropwise to the cellulose chitosan solution with
10 the continuously vigorous stirring of the homogenizer for another 45 min. The mixture solution was
11 washed with distilled water and centrifuged three times at 2500 rpm for 7 min at 25 °C. Finally, the
12 C-C NCs sample was freeze-dried for 16 h and stored at -4 °C for further analysis.

13 2.3. Characterization

14 XRD (Philips, X'pert, Cu Ka) at an ambient condition was used to evaluate the structure of the
15 samples. The sample was compressed between two smooth glass films and the XRD analysis was
16 carried out in dispersion 2 angles of 5°– 80° at a step size of 0.02° with 2 s/step as scanning rate using
17 a voltage of 45 kV, a Ni-filtered Cu K radiation (=1.5406 Å) and a filament current of 40 mA. FTIR
18 spectroscopy (ThermoNicolet, Waltham) determined the chemical and super-molecular structural
19 analysis of the samples under an ambient condition. First, crushing and mixing of the sample with
20 KBr at a ratio of 1:100 w/w to prepare a transparent pellet and the spectra of the plate was evaluated
21 under a transmittance mode in a range between 4000 cm⁻¹ to 400 cm⁻¹ with a 4 cm⁻¹ resolution and an
22 accumulation of 128 scans. The SEM images were taken via using an Electro-Scan SEM instrument
23 (model JSM 7600 F SEM). A low-acceleration voltage (10 kV) was used to prevent the degradation of
24 the sample.

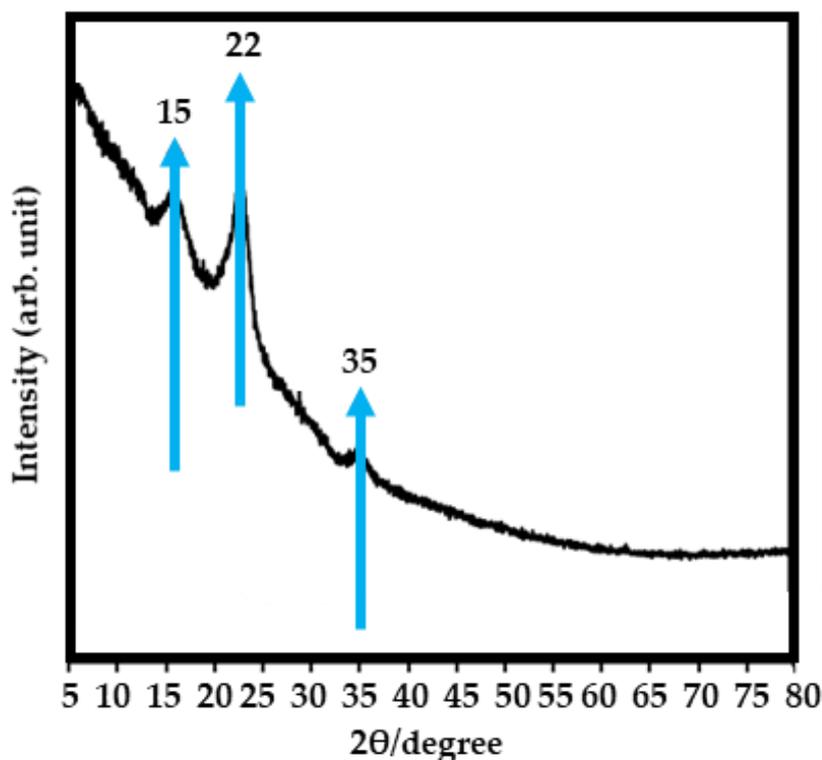
25 3. Results and Discussion

26 The ratio of the components in the polysaccharide composites is a significant concern to obtain
27 desirable size and physiochemical properties. The ratio between the chitosan powders to TPP cross-
28 linker was optimized as 1:2 (v/v). We use cellulose and chitosan with the ratio of 1:2 to fabricate the
29 polysaccharide composites. In a spate research, 2.0% (w/v) chitosan, 1.2% (w/v) carboxymethyl-
30 cellulose, and 1.0% (w/v) scleroglucan were used to synthesize a nanocomposite hydrogels that the
31 SEM images of this sample was not spherical with uniform structure[23]. Furthermore, the chitosan
32 did not display uniform coating structure on the carboxymethyl-cellulose, possibly because of
33 unsuitable ratio between the cellulose, scleroglucan, and chitosan. Investigations by Samy et al. (2020)
34 used the 1:1 ratio of chitosan and cellulose and epichlorohydrin cross-linker that this composite
35 indicated SEM size more than 100 nm [24]. In our research, thus, the effective synthesis of C-C NCs
36 with spherical size below 60 nm was due to blending the appropriate ratio of chitosan coating agent
37 and cellulose matrix with vigorous homogenizing, which dropwise adding TPP advantageously acted
38 as a crosslinking agent.

39 3.1. X-ray Powder Diffraction Analysis

40 Figure 1 shows the XRD results of C-C NCs. As the sample containing natural cellulose, it
41 exhibited the diffraction peaks approximately at $2\theta = 15^\circ$, 22° and 35° , similar to the normal cellulose-
42 I structure [25]. The highest crystalline area was shown at 22° with a sharp intensity, showing the

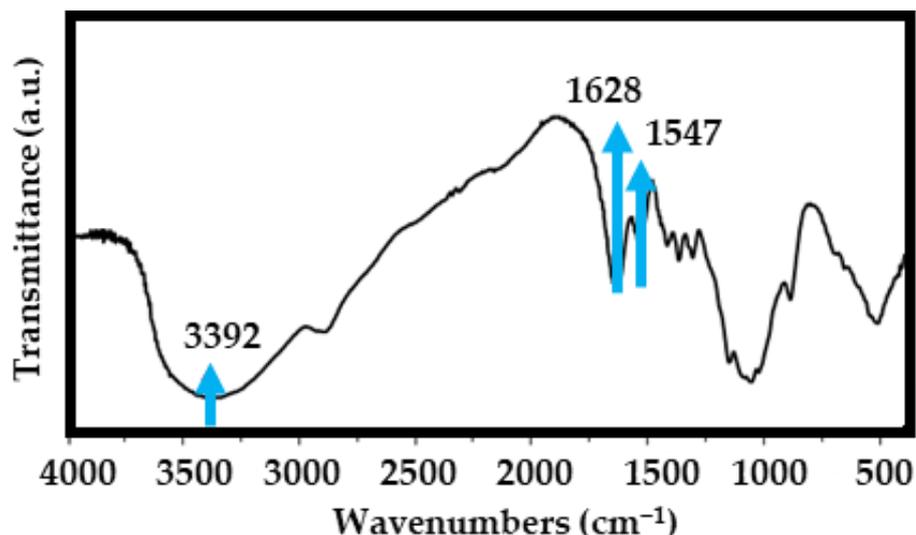
1 sufficient crystallinity of cellulose. C-C NCs indicated a pattern related to both cellulose
2 reinforcement and chitosan coater with peaks approximately at $2\theta = 22^\circ$ and 15° , which is in a good
3 agreement with the JCPDS card no. 04-0784. Noticeably, the peak at 15° is an overlapping peak
4 between cellulose and chitosan. The C-C NCs showed a decrease crystallinity peaks, due to presence
5 of the TPP cross-linker to abate the crystallinity. The XRD results could show the successful
6 fabrication of polysaccharide nanocomposites containing cellulose reinforcement and chitosan
7 coating agent.



8
9 **Figure 1.** XRD spectra of polysaccharide nanocomposites of C-C NCs.

10 3.2. Fourier-Transform Infrared Spectroscopy

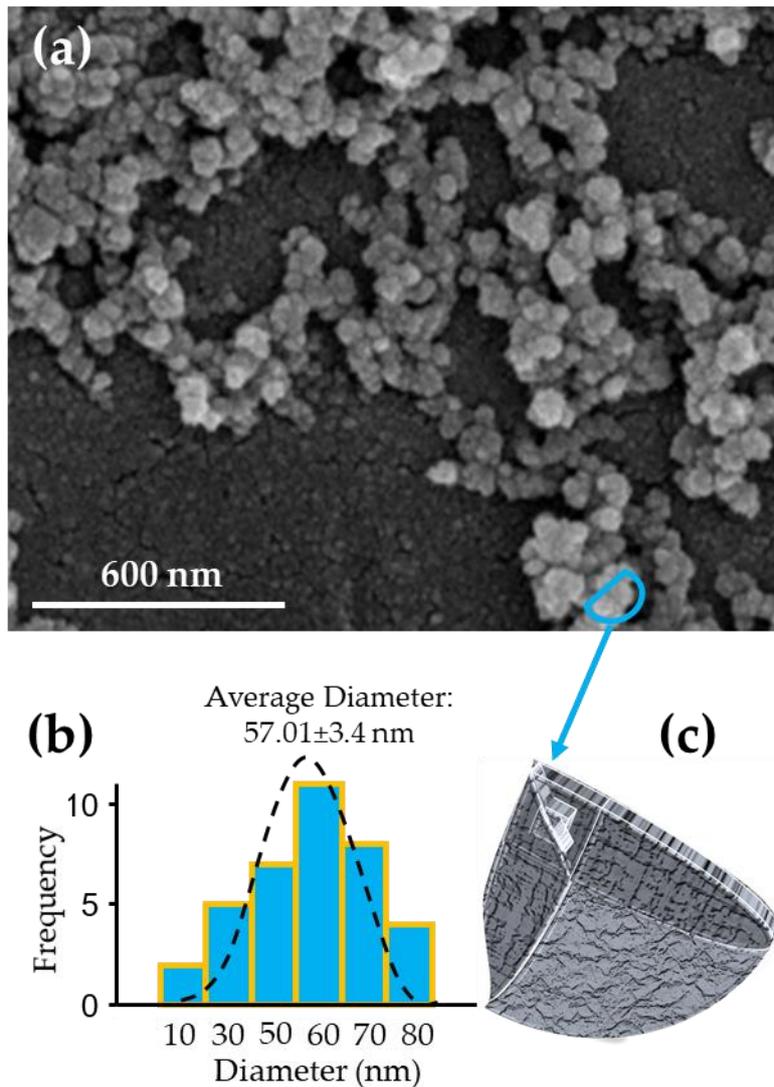
11 The FTIR findings of C-C NCs is indicated in Figure 2, showing contribution of OH or NH and
12 changes in the sugar ring, Van der Waals forces, dipole moments and hydrogen bonds [26].
13 Furthermore, the CO stretching vibration at 1628 cm^{-1} could be owed to an appropriate interaction
14 between the drug and its nano-carrier. It stated that the amine groups of chitosan with carbonyl
15 groups of natural cellulose may cause formation of functional groups of imines with carbon-nitrogen
16 double bond. Therefore, the above FTIR results identified the chemical structure of the polysaccharide
17 nanocomposites.



1
2 **Figure 2.** FTIR results of polysaccharide nanocomposites of C-C NCs.

3 *3.3. Scanning Electron Microscopy (SEM)*

4 Figure 3a-b demonstrate the SEM image, average diameter histogram, and schematic of
5 polysaccharide nanocomposites of C-C NCs, respectively. An average size of C-C NCs was estimated
6 to be 57.01 ± 3.4 nm. The size of chitosan is strongly attributed to the ratio between the TPP cross linker
7 and the chitosan. It was reported [27], that the chitosan and TPP with a ratio around 1:2 could cause
8 the formation of chitosan NPs with suitable physiochemical and morphological characteristics thus,
9 it was applied in our research. It is worth to mention that the layer-by-layer synthesis could led to
10 proper coating of cellulose reinforcement by chitosan with desirable distribution and low
11 agglomeration. This was similarly found in different investigations [28, 29]. It can be understood from
12 the SEM image that the natural cellulose was entangled within the almost spherical chitosan NPs;
13 therefore, C-C NCs mainly showed the spherical shape. The SEM analysis could prove that the ionic
14 gelation method was successful to fabricate polysaccharide nanocomposites with desirable
15 nanodimension and morphology.



1
2 **Figure 3.** (a) SEM image, (b) average diameter histogram, and (c) schematic of polysaccharide nanocomposites
3 of C-C NCs.

4 **4. Conclusions**

5 The polysaccharide system of C-C NCs was fabricated by using the ionic gelation method. Since
6 TPP can crosslink chitosan with cellulose at multiple points, C-C NCs showed a uniform and nanosize
7 structure. The ratio between the chitosan to TPP cross-linker was 1:2 (v/v) whereas, using 1.0% acetic
8 acid, and 2% (v/v) Tween-80 led to the synthesis of C-C NCs with desirable physicochemical
9 properties. Further, we use cellulose and chitosan with the ratio of 1:2 to obtain the composites with
10 a size below 60 nm, as indicated in the SEM images. The composites indicated the XRD and FTIR
11 peaks related to the chitosan cross-linked cellulose. Therefore, this study demonstrates the low cost,
12 facile, and eco-friendly polysaccharide composites for various biomedical applications.

13 **Funding**

14 This research was funded by Takasago Thermal Engineering Co. Ltd. grant (#4B422) from the
15 research management center (RMC) of Universiti Teknologi Malaysia (UTM) and Malaysia-Japan
16 International Institute of Technology (MJIIT).

1 Acknowledgement

2 Special thanks to of Universiti Teknologi Malaysia (UTM) and Malaysia-Japan International Institute
3 of Technology (MJIT) for supports.

4 References

- 5 1. Kia P, Ahmad M. B, Shameli K. Green synthesis of Sodium alginate mediated Fluorapatite
6 Nanoparticle via Sol-Gel method. *Journal of Research in Nanoscience and Nanotechnology*
7 2021;2:30-41. doi: [10.37934/jrnn.2.1.3041](https://doi.org/10.37934/jrnn.2.1.3041)
- 8 2. Hamrayev H, Shameli K. In Biopolymer-Based Green Synthesis of Zinc Oxide (Zno)
9 Nanoparticles, *IOP Conf. Ser.: Mater. Sci. Eng.* 2021;012088. doi: [10.1088/1757-899X/1051/1/012088](https://doi.org/10.1088/1757-899X/1051/1/012088)
- 10 3. Yusefi M, Shameli K, Jumaat A. F. Preparation and properties of magnetic iron oxide
11 nanoparticles for biomedical applications: A brief review. *Journal of Advanced Research in*
12 *Materials Science* 2020;75:10-18. doi: [10.37934/arms.75.1.1018](https://doi.org/10.37934/arms.75.1.1018)
- 13 4. Kumar M. N. R, A review of chitin and chitosan applications. *React. Funct. Polym.* 2000;46:1-
14 27. doi: [10.1016/S1381-5148\(00\)00038-9](https://doi.org/10.1016/S1381-5148(00)00038-9)
- 15 5. Liu S, Zhang J, Cui X, Guo Y, Zhang X, Hongyan W. Synthesis of chitosan-based
16 nanohydrogels for loading and release of 5-fluorouracil. *Colloids Surf, A Physicochem Eng Asp.*
17 2016;490:91-97. doi: [10.1016/j.colsurfa.2015.11.029](https://doi.org/10.1016/j.colsurfa.2015.11.029)
- 18 6. Yusefi M, Shameli. Nanocellulose as a Vehicle for Drug Delivery and Efficiency of Anticancer
19 Activity: A Short-Review. *Journal of Research in Nanoscience and Nanotechnology* 2021;1:30-43.
20 doi: [10.37934/jrnn.1.1.3043](https://doi.org/10.37934/jrnn.1.1.3043)
- 21 7. Österberg M, Cranston ED, Special issue on nanocellulose. *Nord Pulp Paper Res J NORD*
22 *PULP PAP RES J*;2014;29. doi: [10.3183/npprj-2014-29-01-p004-005](https://doi.org/10.3183/npprj-2014-29-01-p004-005)
- 23 8. Izadiyan Z, Shameli K, Miyake M, Teow S-Y, Peh S-C, Mohamad SE, Taib SHM. Green
24 fabrication of biologically active magnetic core-shell Fe₃O₄/Au nanoparticles and their
25 potential anticancer effect. *Mater. Sci. Eng. C* 2019;96,51-57. doi: [10.1016/j.msec.2018.11.008](https://doi.org/10.1016/j.msec.2018.11.008)
- 26 9. Jahangirian H, Kalantari K, Izadiyan Z, Rafiee-Moghaddam R, Shameli K, Webster TJ. A
27 review of small molecules and drug delivery applications using gold and iron nanoparticles.
28 *Int J Nanomedicine* 2019;14,1633. doi: [10.2147/IJN.S184723](https://doi.org/10.2147/IJN.S184723)
- 29 10. Sukri, SNAM. Shameli K, Wong MM-T, Teow S-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. doi:
32 [10.1016/j.molstruc.2019.04.026](https://doi.org/10.1016/j.molstruc.2019.04.026)
- 33 11. 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. doi: [10.1016/j.molstruc.2021.131075](https://doi.org/10.1016/j.molstruc.2021.131075)
- 36 12. 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 13. Yusefi M, Shameli K, Ali R R, Pang S-W, Teow S-Y. Evaluating anticancer activity of plant-
40 mediated synthesized iron oxide nanoparticles using Punica granatum fruit peel extract. *J.*
41 *Mol. Struct.* 2020;1204,127539. doi: [10.1016/j.molstruc.2019.127539](https://doi.org/10.1016/j.molstruc.2019.127539)
- 42 14. Ruman U, Buskaran K, Pastorin G, Masarudin M J, Fakurazi S, Hussein M Z. Synthesis and
43 Characterization of Chitosan-Based Nanodelivery Systems to Enhance the Anticancer Effect
44

- 1 of Sorafenib Drug in Hepatocellular Carcinoma and Colorectal Adenocarcinoma Cells.
2 *Nanomaterials* 2021;11,497. doi: [10.3390/nano11020497](https://doi.org/10.3390/nano11020497)
- 3 15. Schwaiger D, Lohstroh W, Müller-Buschbaum P. Investigation of Molecular Dynamics of a
4 PTB7: PCBM Polymer Blend with Quasi-Elastic Neutron Scattering. *ACS Appl. Polym. Mater.*
5 2020;2,3797-3804. doi: [10.1021/acsapm.0c00455](https://doi.org/10.1021/acsapm.0c00455)
- 6 16. Ogueri K S, Ogueri K S, Allcock HR, Laurencin CT. A Regenerative Polymer Blend
7 Composed of Glycylglycine ethyl ester-substituted Polyphosphazene and Poly (lactic-co-
8 glycolic acid). *ACS Appl. Polym. Mater.* 2020;2,1169-1179. doi: [10.1021/acsapm.9b00993](https://doi.org/10.1021/acsapm.9b00993)
- 9 17. Ignacz G, Fei F, Szekely G. Ion-stabilized membranes for demanding environments fabricated
10 from polybenzimidazole and its blends with polymers of intrinsic microporosity. *ACS Appl.*
11 *Nano Mater.* 2018;11,6349-6356. doi: [10.1021/acsanm.8b01563](https://doi.org/10.1021/acsanm.8b01563)
- 12 18. Chatterjee S, Hui PC-I, Kan C-w, Wang W. Dual-responsive (pH/temperature) Pluronic F-
13 127 hydrogel drug delivery system for textile-based transdermal therapy. *Sci. Rep.* 2019;9, 1-
14 13. doi: [10.1038/s41598-019-48254-6](https://doi.org/10.1038/s41598-019-48254-6)
- 15 19. Anirudhan TS, Christa J. Multi-polysaccharide based stimuli responsive polymeric network
16 for the in vitro release of 5-fluorouracil and levamisole hydrochloride. *New J Chem.*
17 2017;41,11979-11990. doi: [10.1039/C7NJ01745F](https://doi.org/10.1039/C7NJ01745F)
- 18 20. Yusefi M, Ali RBR, Abdullah EC, Shameli K. In Analysis on Physiochemical Properties of
19 Cellulose Fiber from Rice Straw Waste, *IOP Conf. Ser.: Mater. Sci. Eng.* 2020;012038. doi:
20 [10.1088/1757-899X/808/1/012038](https://doi.org/10.1088/1757-899X/808/1/012038)
- 21 21. Yusefi M, Shameli K, Jahangirian H, Teow S-Y, Umakoshi H, Saleh B, Rafiee-Moghaddam,
22 R, Webster TJ. The potential anticancer activity of 5-fluorouracil loaded in cellulose fibers
23 isolated from rice straw. *Int J Nanomedicine* 2020;15,5417. doi: [10.2147/IJN.S250047](https://doi.org/10.2147/IJN.S250047)
- 24 22. Yusefi M, Chan H-Y, Teow S-Y, Kia P, Lee-Kiun Soon M, Sidik NABC, Shameli K. 5-
25 Fluorouracil Encapsulated Chitosan-Cellulose Fiber Bionanocomposites: Synthesis,
26 Characterization and In Vitro Analysis towards Colorectal Cancer Cells. *Nanomaterials* 2021;
27 11,1691. doi: [10.3390/nano11071691](https://doi.org/10.3390/nano11071691)
- 28 23. Bozoğlan BK, Duman O, Tunç S. Preparation and characterization of thermosensitive
29 chitosan/carboxymethylcellulose/scleroglucan nanocomposite hydrogels. *Int. J. Biol.*
30 2020;162,781-797. doi: [10.1016/j.ijbiomac.2020.06.087](https://doi.org/10.1016/j.ijbiomac.2020.06.087)
- 31 24. Yang J, Duan J, Zhang L, Lindman B, Edlund H, Norgren M. Spherical nanocomposite
32 particles prepared from mixed cellulose–chitosan solutions. *Cellulose* 2016;23,3105-3115. doi:
33 [10.1007/s10570-016-1029-4](https://doi.org/10.1007/s10570-016-1029-4)
- 34 25. Abe K, Yano H. Comparison of the characteristics of cellulose microfibril aggregates of wood,
35 rice straw and potato tuber. *Cellulose* 2009;16,1017-1023. doi: [10.1007/s10570-009-9334-9](https://doi.org/10.1007/s10570-009-9334-9)
- 36 26. Kavaz D, Kirac F, Kirac M, Vaseashta A. Low Releasing Mitomycin C Molecule Encapsulated
37 with Chitosan Nanoparticles for Intravesical Installation. *J Biomater Nanobiotechnol* 2017;8, 203-
38 219. doi: [10.4236/jbnt.2017.84014](https://doi.org/10.4236/jbnt.2017.84014)
- 39 27. Maluin FN, Hussein MZ, Yusof NA, Fakurazi S, Idris AS, Zainol Hilmi NH, Jeffery Daim
40 LD. Preparation of chitosan–hexaconazole nanoparticles as fungicide nanodelivery system for
41 combating Ganoderma disease in oil palm. *Molecules* 2019;24,2498. doi:
42 [10.3390/molecules24132498](https://doi.org/10.3390/molecules24132498)
- 43 28. HPS AK, Saurabh CK, Adnan A, Fazita MN, Syakir M, Davoudpour Y, Rafatullah M,
44 Abdullah C, Haafiz M, Dungani R. A review on chitosan-cellulose blends and nanocellulose
45 reinforced chitosan biocomposites: Properties and their applications. *Carbohydr. Polym.*
46 2016;150,216-226. doi: [10.1016/j.carbpol.2016.05.028](https://doi.org/10.1016/j.carbpol.2016.05.028)

- 1 29. de Mesquita JP, Donnici CL, Teixeira IF, Pereira FV. Bio-based nanocomposites obtained
2 through covalent linkage between chitosan and cellulose nanocrystals. *Carbohydr. Polym.*
3 2012;90,210-217. doi: [10.1016/j.carbpol.2012.05.025](https://doi.org/10.1016/j.carbpol.2012.05.025)
4