

Formulation and Characterization of Chitosan-PEG Nanoparticles based on Cork fish (*Channa striata*) Protein Hydrolysate as a Baseline Research Solution for Treatment of Type 2 Diabetes Mellitus

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ABSTRACT

In nanoparticle technology, particles or globules on the nanometer scale have unique physical properties compared to particles at larger sizes, especially in improving the quality of delivery of drug compounds. The use of cork fish (*Channa striata*) in this research is because cork fish is a freshwater fish easily found in Indonesia and has economic value. In this case, cork fish protein hydrolysate found in meat proved to be an α -glucosidase inhibiting agent. The study aims to find the formulation and describe Chitosan-PEG nanoparticles' characterization based on Cork fish (*Channa striata*) Protein Hydrolysate. Chitosan was synthesized by the ionic gelation method, in which Chitosan was added with 1% acetic acid—then stirred for approximately 3 hours. Then added STPP and the chitosan nanoparticles above are entered with PEG 4000, and PEG 6000 compared to PEG and chitosan nanoparticles is 1:3. The encapsulation of hydrolysate with chitosan nanoparticles was carried out using the inclusion complexation method by combining fish hydrolysate and chitosan nanoparticles of 1:2 (v/v). The chemical bond between PEG and Chitosan was evaluated by Fourier-transform infrared (FTIR) spectroscopy within the wavenumber range of 450-4000 cm⁻¹. The sample was attached to 10 mm-diameter SEM stubs using two-sided adhesive tape. Gold-palladium was used to coat the specimens via the plasma deposition method, and SEM was operated at 20 kV accelerating voltage. Images were analyzed and recorded at 3,000-10,000 x magnification. The structure and particle size of the microencapsulated phycocyanin were obtained after freeze-drying. The use of nano chitosan in membrane technology emphasizes the novelty of the present work. Thus, this research results in a novel combination of membranes prepared using nano chitosan as the significant component, polyethylene glycol as the pore former in various ratios. The formation of the composite membranes was characterized using FT-IR. The studies determined the thermal stability, and SEM and TEM analysis showed its morphology. It could be concluded that the membranes prepared using PEG in different ratios have been blended well, which was confirmed by FT-IR spectroscopy. The study revealed that all the membrane ratios showed good thermal stability. In particular, the membranes e) exhibited high thermal stability. Also, protein hydrolysate of all the prepared membrane ratios indicated good miscibility and good compatibility between the polymers. From SEM analysis, it was observed that the two specific membranes were porous and showed uniformity and also found to be uneven and rough. From SEM analysis, it was observed that Nanoparticle chitosan-PEG showed that the irregularly shaped round nanoparticle vesicles. Thus, the membranes were very well supported by analytical studies such as FT-IR, SEM, and TEM.

Keywords: *Channa striata*, Chitosan-PEG Nanoparticles, Protein Hydrolysate

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INTRODUCTION

The prevalence of DM sufferers is 2.1 (1). The mechanism of DM drugs in reducing blood glucose levels is by inhibiting glucose absorption or α -glucosidase inhibitors. However, this drug has side effects if consumed for an extended period (2). Besides, the administration of oral antidiabetic drugs can cause side effects. On the other hand, taking too many drugs is bad for the body, especially the organs of excretion such as the liver and kidneys, because they contain many chemicals (3). Therefore the need for drugs with new technology to expand the surface area so that the maximum absorption of the drug.

In nanoparticle technology, particles or globules on the nanometer scale have unique physical properties compared to particles at larger sizes, especially in improving the quality of delivery of drug compounds (4). Nanocapsule antidiabetic drugs with nanotechnology have

the advantage of having a tiny size of 10⁻⁹ so that the resulting surface area is more extensive. This size causes the drug to have a larger distribution to increase bioavailability, which shows the ability of medicine to be adopted and to the target. So it will be more effective in reducing glucose levels in the blood.

The ability of nanoparticles to increase the bioavailability of drugs with low solubility in systemic circulation has been demonstrated to apply to delivery applications, including oral (5–7). Increasing the number of drugs in the blood in systemic delivery will increase the risk of side effects and adverse effects, to the chance of achieving toxic levels (8). Increased levels of drugs in the blood are essential for prescriptions to be able to cause pharmacological effects. Therefore, nanoparticles provide a suitable solution because they can give pharmacological effects at smaller (efficient) doses (6,9).

The flexibility of nanoparticles combined with other technologies makes it easier to do formulations by combining with biopolymers. The biopolymer used is chitosan-PEG. The use of chitosan-PEG biopolymers is because the amount of Chitosan in Indonesia is abundant with an economical selling price. In addition, chitosan-PEG has a high absorption efficiency, can open inter-cell latches (tight junctions) on the intestinal membrane temporarily (10) through the mechanism of Claudin-4 protein translocation (Cldn4), Zonula occludens-1 (ZO-1), and Occludin from the cell membrane to the cytosol (11,12), so it is very potential for oral application.

Chitosan-PEG nanoparticle technology used fish protein hydrolysate found in cork fish meat (*Channa striata*). The use of cork fish in this medicine is because cork fish is a freshwater fish easily found in Indonesia and has economic value. In this case, cork fish protein hydrolysate found in meat proved to be an α -glucosidase inhibiting agent. The α -glucosidase inhibiting agent is an oral antidiabetic therapy agent that can help maintain blood glucose levels in the normal range, especially after eating (13). In research testing, amino acid composition and protein potential of cork fish related to inhibition of α -glucosidase activity in cork fish meat, cork fish hydrolysate used as an antidiabetic drug have the most excellent inhibitory power of the α -glucosidase enzyme at 74%. This amount is far greater than the value of inhibiting the activity of α -glucosidase from fermented isolates and cork fish fermentation isolates. Therefore, "Utilization of Technology Nanoparticles-PEG Based cork fish Hydrolysate Solution for Diabetes Mellitus Treatment Solution Type 2" studies to produce antidiabetic drugs from nanoparticle technology based on hydrolyzed cork fish protein (*Channa striata*), where nanocapsules encapsulate the compound from chitosan-PEG as a treatment for patients with Type 2 Diabetes Mellitus in the future.

Experimental

Materials and procedure

Chitosan, acetic acid (CH_3COOH), sodium tripolyphosphate (STPP), aquades, PEG 4000, and PEG 6000.

Research tools used are glass that is commonly used in the laboratory, stirrer, centrifuge, freeze-drying, analytical balance, FTIR (Shimadzu-Fourier Transform Infrared), SEM (Scanning electron microscopy), and TEM (Transmission electron microscopy).

Active components extraction of cork fish hydrolysate

The active component of pineapple with ethanol 85% to make the bromelain enzyme. And then, 10% bromelain enzyme mixed with 50-gram cork fish flour into 100 mL aquades. And then, the mixed solution was incubated in the oven at 55°C for 6 hours.

Chitosan-PEG nanoparticles

Chitosan was synthesized by the ionic gelation method, in which Chitosan was added with 1% acetic acid-then stirred for approximately 3 hours. Then added STPP and the chitosan nanoparticles above are entered with PEG 4000, and PEG 6000 compared to PEG and chitosan nanoparticles is 1:3.

Encapsulation of cork fish hydrolysate in Chitosan-PEG nanoparticles

The encapsulation of hydrolysate with chitosan nanoparticles was carried out using the inclusion complexation method by combining fish hydrolysate and chitosan nanoparticles of 1:2 (v/v).

Fourier-transform infrared spectroscopic analysis

The chemical bond between PEG and Chitosan was evaluated by Fourier-transform infrared (FTIR) spectroscopy within the wavenumber range of 450-4000 cm^{-1} .

Scanning electron microscopic analysis

The sample was attached to 10 mm-diameter SEM stubs using two-sided adhesive tape. Gold-palladium was used to coat the specimens via the plasma deposition method, and SEM was operated at 20 kV accelerating voltage. Images were analyzed and recorded at 3,000-10,000 x magnification.

Transform electron microscopic analysis

The structure and particle size of the microencapsulated phycocyanin were obtained after freeze-drying.

RESULTS AND DISCUSSION

FTIR spectra analysis of Chitosan

IR spectroscopy is an excellent method used extensively for the qualitative analysis in which the chemical nature of a substance such as chemical bonds, molecular orientations, molecular energy levels, and molecular interactions (14) can be assessed by identifying specific absorption peaks for particular groups (15). Hence each blend has been analyzed using FT-IR, and the possible interactions between the functional groups in a polymer blend were studied.

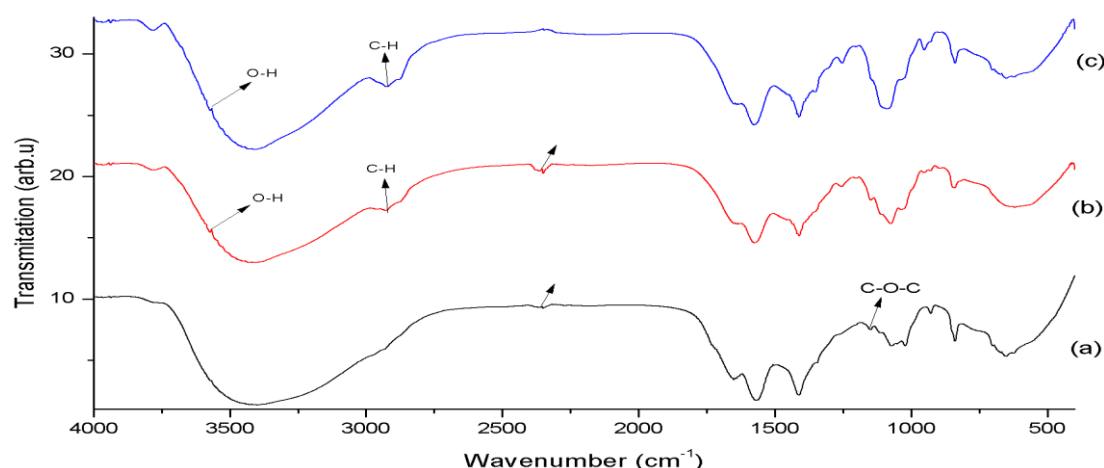


Fig 1. FTIR Spectra of nanochitosan, nanochitosan PEG 4000 3%, and nanochitosan 6000 3%

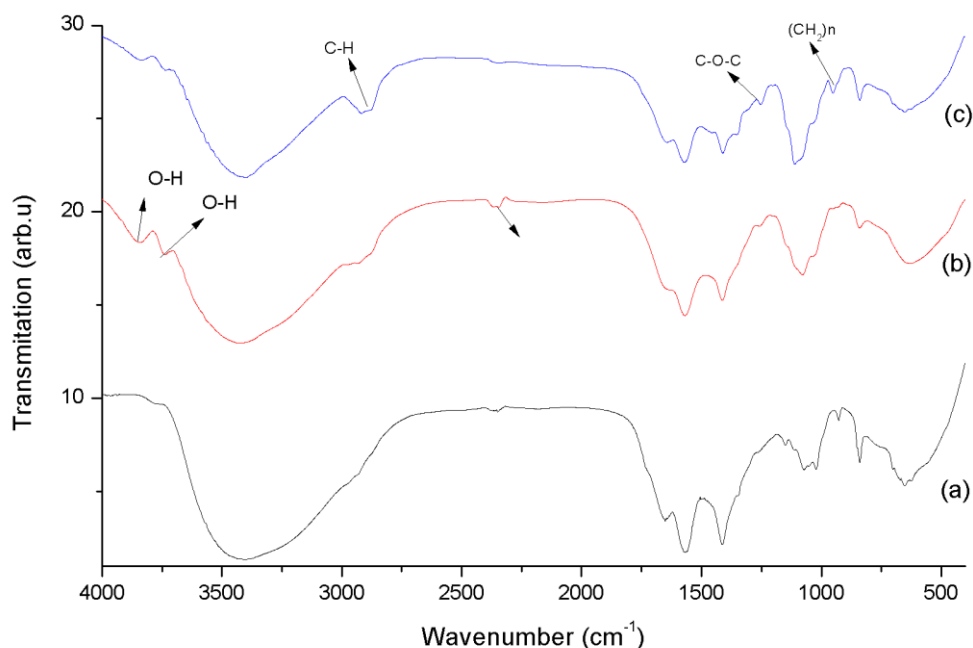


Fig 2. FTIR Spectra of nanochitosan, nanochitosan PEG 4000 1%, and nanochitosan 6000 1%

FTIR spectra of chitosan residues showed a broad absorption band at 3399 cm^{-1} indicating the OH stretching vibration band. The differences occur after the deacetylation step, in which there are changes in the absorption spectrum at 1651.2 cm^{-1} from stretching of C=O. The ranges (Figure 1) showed a shift of C=O absorption from 1412.48 cm^{-1} to 1567.8 cm^{-1} and decreased N-H absorption band in the CONH group at 1560 cm^{-1} in Chitosan. It also showed a new appearance of a weak absorption at 1555 cm^{-1} .

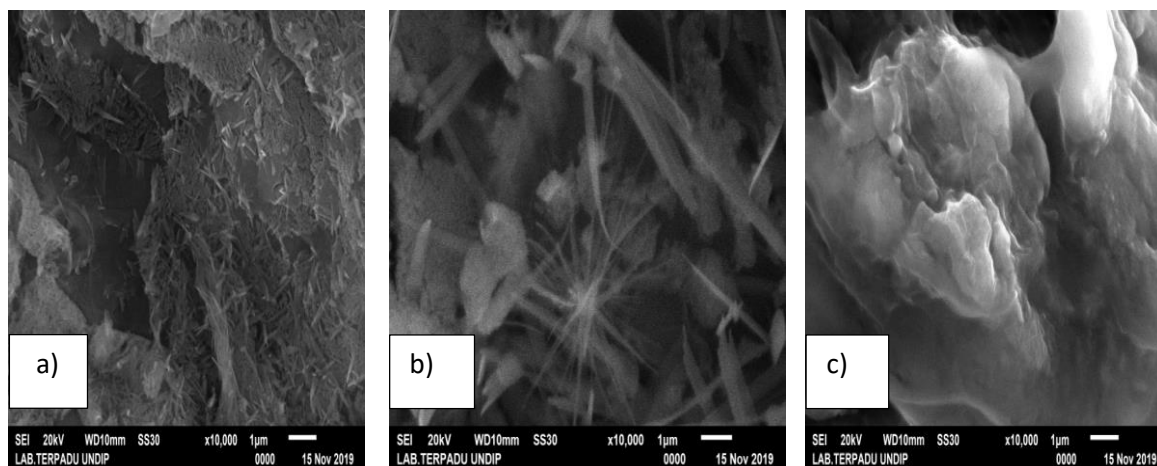
The FTIR characterization results showed a broad absorption of 3500 cm^{-1} , centered at 3400 cm^{-1} , indicates the absence of OH OH bonds free of intra and intermolecular hydrogen -OH bonds caused by the CH₂OH hydroxyl group relationship. Still, in nano chitosan-PEG, there are OH OH bonds. A new bond was found in the nano chitosan bond, namely the C-O-C group, whereas in the

PEG 4000 1% sample, the same group was located at 1230-1270 cm^{-1} .

FTIR results also show the role of acid in Chitosan. The FTIR profile showing absorbance similar to each other shows the part of acids in chitosan nanoparticles' reaction preparation. Intra- and intramolecular non-binding acids as in the 2005 Bodnar study with di- and tri-carboxylic acids. The FTIR graph does not show the formation of new polymers as in the 2008 Moura study. The absence of new polymers means that Chitosan does not polymerize with acids. It can be concluded that acids only act as proton donors, which dissolve Chitosan.

SEM analysis

A scanning electron microscopy is a handy tool to characterize the membrane's surface morphology (16) and investigate the structure of polymeric integrally skinned membrane (17).



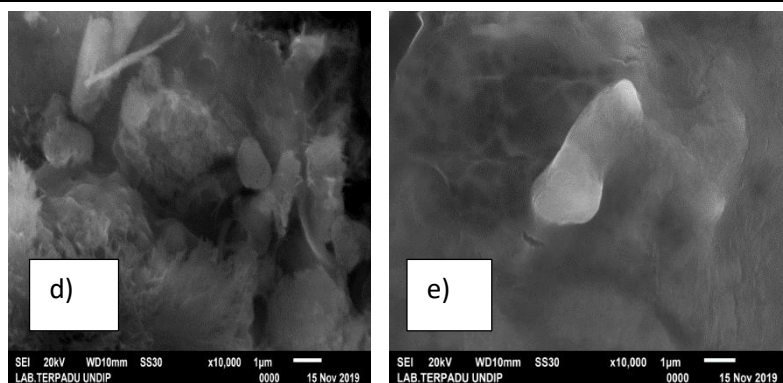


Figure 3. Surface morphology of a) nano chitosan, b) nano chitosan-PEG 4000 1%, c) nano chitosan-PEG 4000 based on protein hydrolysate of *Channa striata*, d) nano chitosan-PEG 6000 1%, e) nano chitosan-PEG 6000 based on protein hydrolysate of *Channa striata*

Figure 3a) shows the surface morphology of nano chitosan. The cross-sectional morphology of the blend membranes was observed using SEM and shown in Figures a)-e). The membranes' surface helps to identify the membrane's significance in the mechanism of selectivity and permeability. The surface of all the prepared membranes clearly showed the asymmetric nature and roughness, and porous structure. The shape and structure were related to the additive concentration and composition in the casting solution (18). Thus, the sample b) and d) were more porous with more PEG content showing small multiple uniform voids than other samples. The result was similar to other studies mentioned (19) the increasing PEG concentration causes the formation of greater macrovoids and more porous structures due to the intensification of thermodynamic instability of the casting solution by PEG.

Moreover, the number of pores and pore sizes depends on the pore-forming agent PEG, which leaches out of the gelation bath's surface during the phase separation process (20). However, the pore size of membranes was reduced with high PEG content in the blend solution due to the reduced leachability of PEG during gelation because of strong interactions of PEG with protein hydrolysate (21). Hence the membranes can facilitate the filtration process efficiently.

TEM analysis

TEM (Transmission Electron Microscopy) is a microscope technique where electrons are transmitted through ultra-thin specimens, interacting with passed samples. TEM analysis can see magnifications with high resolution above 500000 times to crystals or columns of atoms or molecules.

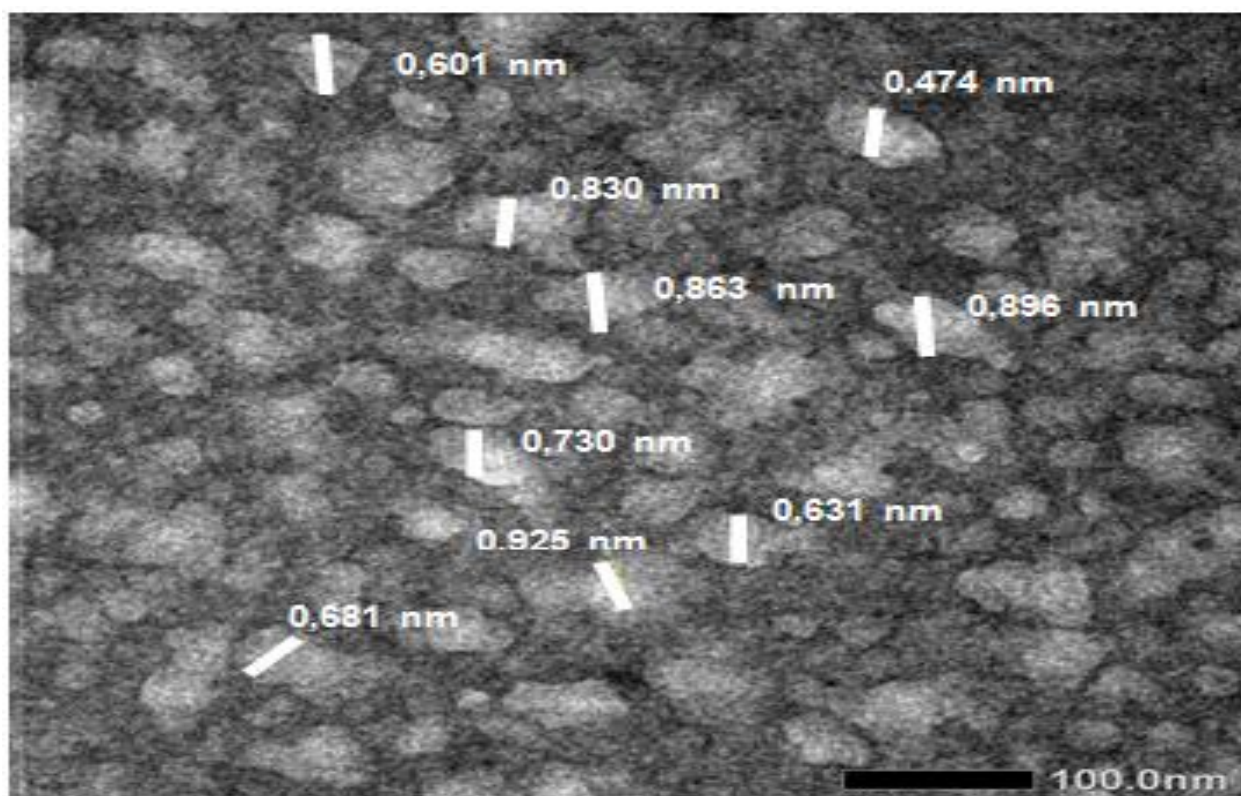


Figure 4. surface morphology of nano chitosan

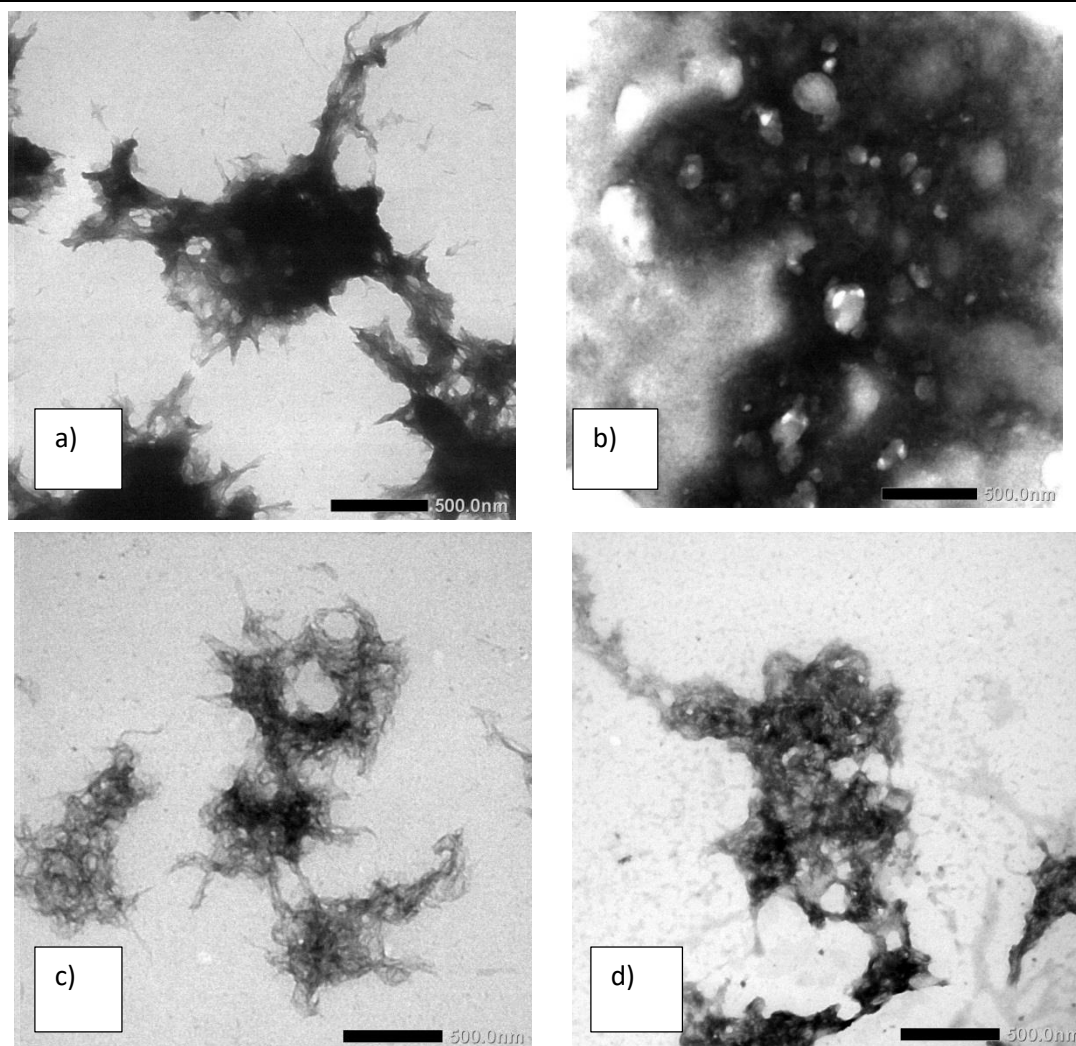


Figure 5. surface morphology of a) nano chitosan-PEG 4000 1%, b) nano chitosan-PEG 4000 based on protein hydrolysate of *Channa striata*, c) nano chitosan-PEG 6000 1%, d) nano chitosan-PEG 6000 based on protein hydrolysate of *Channa striata*

Polymers are chain molecules with recurrent monomer combined molecules. The repetition of these monomers makes polymers have robust and unique chemical properties. Biopolymers are described as polymer molecules that have biocompatibility in biological systems. Biopolymers have been widely used as biomaterials in biomedical products, primarily as a drug delivery system material. The use of biopolymers as ingredients in this drug formulation in general for several reasons, which are inert to active ingredients but compatible to be combined and have unique characteristics, for example in the use of various sugar polymer derivatives (22), can form tissues so that they can develop as a carrier system in the form of particle matrices (23,24), beads (25,26), or patches (27) for example HPMC, PLGA, pectin, alginate, and Chitosan, as well as having a sizeable functional group that allows binding of drug molecules in sufficient quantities to the system as a whole or can be said to have high absorption efficiency. This analysis shows that the nano chitosan particles produced have an almost uniform size and are relatively small. The particle size of nanoparticles obtained from Chitosan is in the range of 0.6-0.9 nm, with an average length of 0.8 nm, as shown in Figure 4. The results of observations with the Transmission Electron Microscope (TEM) nanoparticle-PEG showed that the irregularly shaped round nanoparticle vesicles. The

micrograph of the TEM results can be seen in Figure 4. The vesicles' polar portion is shown in black, while the transparent or colorless amount indicates a non-polar compound. The manufacturing method influences the shape of vesicles; in some previous studies, the shape of particles produced was spherical and uniform. However, in this study, the form of the resulting vesicles was irregularly rounded. This result needs to be developed in further research to find other manufacturing methods to produce better vesicle shapes.

As a future study, we need to perform Thermogravimetry and Differential scanning calorimetry (TG-DSC) studies for further characterization of these nanoparticles and also need more research to apply these formula of Chitosan-PEG Nanoparticles based on Cork fish (*Channa striata*) Protein Hydrolysate to expand the surface area so that the maximum absorption of the Type 2 diabetes drugs.

CONCLUSION

A well-known and versatile material, nano chitosan was prepared from Chitosan by the most popular ionotropic gelation method using sodium tripolyphosphate as a crosslinker. Nanotechnology, which encompasses science, engineering, and applications of submicron materials, has been implemented in the present study to improve the properties of Chitosan via enhancing its surface area. The

use of nano chitosan in membrane technology emphasizes the novelty of the current work. Thus a novel combination of membranes was prepared using nano chitosan as the significant component, polyethylene glycol as the pore former in various ratios. The formation of the composite membranes was characterized using FT-IR. The studies determined the thermal stability, and SEM and TEM analysis showed its morphology. It could be concluded that the membranes prepared using PEG in different ratios have been blended well, which was confirmed by FT-IR spectroscopy. The study revealed that all the membrane ratios showed good thermal stability. In particular, the membranes e) exhibited high thermal stability. Also, protein hydrolysate of all the prepared membrane ratios indicated good miscibility and good compatibility between the polymers. From SEM analysis, it was observed that the membranes, especially c) and e), were porous and showed uniformity and also found to be uneven and rough. From SEM analysis, it was observed that Nanopartikel chitosan-PEG showed that the irregularly shaped round nanoparticle vesicles. Thus the membranes c) and e) were very well supported by the analytical studies such as FT-IR, SEM, and TEM. There will be a need for further research to apply these formulas and characterization to type 2 diabetes patients.

REFERENCES

1. Ministry of Health Republic of Indonesia. Basic Health Research (Riset Kesehatan Dasar). Jakarta; 2013.
2. Buchanan DR, Collier A, Rodrigues E, Millar AM, Gray RS, Clarke BF. Effectiveness of acarbose, an alpha-glucosidase inhibitor, in uncontrolled non-obese non-insulin dependent diabetes. Eur J Clin Pharmacol [Internet]. 1988 Jan [cited 2021 Jan 6];34(1):51-3. Available from: <https://pubmed.ncbi.nlm.nih.gov/3282895/>
3. Henriksen EJ. Exercise training and the antioxidant α -lipoic acid in the treatment of insulin resistance and type 2 diabetes [Internet]. Vol. 40, Free Radical Biology and Medicine. Free Radic Biol Med; 2006 [cited 2021 Jan 6]. p. 3-12. Available from: <https://pubmed.ncbi.nlm.nih.gov/16337874/>
4. Martien R, K Irianto ID, Farida V, Purwita Sari D. Technology Developments Nanoparticles As Drug Delivery Systems [Internet]. Vol. 8, Majalah Farmaseutik. 2012 May [cited 2021 Jan 6]. Available from: <https://jurnal.ugm.ac.id/majalahfarmaseutik/article/view/24067>
5. Bhatia A, Bhatia A, Shard P, Chopra D, Mishra T. Chitosan nanoparticles as Carrier of Immunorestoratory plant extract: synthesis, characterization and Immunorestoratory efficacy. Int J Drug Deliv [Internet]. 2011 May 10 [cited 2021 Jan 6];3(2):381-5. Available from: <https://www.arjournals.org/index.php/ijdd/article/view/234>
6. Wu Y, Yang W, Wang C, Hu J, Fu S. Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate. Int J Pharm [Internet]. 2005 May 13 [cited 2021 Jan 6];295(1-2):235-45. Available from: <https://pubmed.ncbi.nlm.nih.gov/15848008/>
7. Martien R, Loretz B, Schnüren AB. Oral gene delivery: Design of polymeric carrier systems shielding toward intestinal enzymatic attack. Biopolymers [Internet]. 2006 Nov [cited 2021 Jan 6];83(4):327-36. Available from: <https://pubmed.ncbi.nlm.nih.gov/16609969/>
8. Poelstra K, Prakash J, Beljaars L. Drug targeting to the diseased liver [Internet]. Vol. 161, Journal of Controlled Release. J Control Release; 2012 [cited 2021 Jan 6]. p. 188-97. Available from: <https://pubmed.ncbi.nlm.nih.gov/22370583/>
9. Giacco F, Brownlee M. Oxidative stress and diabetic complications [Internet]. Schmidt AM, editor. Vol. 107, Circulation Research. Lippincott Williams & WilkinsHagerstown, MD; 2010 [cited 2021 Jan 6]. p. 1058-70. Available from: <https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.110.223545>
10. Martien R, Loretz B, Sandbichler AM, Schnürch AB. Thiolated chitosan nanoparticles: Transfection study in the Caco-2 differentiated cell culture. Nanotechnology [Internet]. 2008 Jan 30 [cited 2021 Jan 6];19(4). Available from: <https://pubmed.ncbi.nlm.nih.gov/21817495/>
11. Smith J, Wood E, Dornish M. Effect of Chitosan on Epithelial Cell Tight Junctions. Pharm Res [Internet]. 2004 Jan [cited 2021 Jan 6];21(1):43-9. Available from: <https://link.springer.com/article/10.1023/B:PHAM.0000012150.60180.e3>
12. Yeh TH, Hsu LW, Tseng MT, Lee PL, Sonjae K, Ho YC, et al. Mechanism and consequence of chitosan-mediated reversible epithelial tight junction opening. Biomaterials [Internet]. 2011 Sep [cited 2021 Jan 6];32(26):6164-73. Available from: <https://pubmed.ncbi.nlm.nih.gov/21641031/>
13. Chisholm-Burns MA, Wells BG, Schwinghammer TL, Malone PM, Kolesa JM. Pharmacotherapy Principles and Practice [Internet]. 4th ed. United States of America: The McGraw-Hill Companies; 2008 [cited 2021 Jan 6]. Available from: <https://epdf.pub/pharmacotherapy-principles-and-practice.html>
14. Collen B, McRae L, Deinet S, De Palma A, Carranza T, Cooper N, et al. Predicting how populations decline to extinction. Philos Trans R Soc B Biol Sci [Internet]. 2011 Sep 12 [cited 2021 Jan 6];366(1577):2577-86. Available from: <https://royalsocietypublishing.org/doi/10.1098/rstb.2011.0015>
15. Zhang H, Tang D, Knize RJ, Zhao L, Bao Q, Loh KP. Graphene mode locked, wavelength-tunable, dissipative soliton fiber laser. Appl Phys Lett [Internet]. 2010 Mar 15 [cited 2021 Jan 6];96(11):111112. Available from: <http://aip.scitation.org/doi/10.1063/1.3367743>
16. Moropoulou A, Zendri E, Ortiz P, Delegou ET, Ntoutsis I, Balliana E, et al. Scanning microscopy techniques as an assessment tool of materials and interventions for the protection of built cultural heritage. Vol. 2019, Scanning. Hindawi Limited; 2019.
17. Kesting RE. Synthetic Polymeric Membranes: A Structural Perspective .: 9780471807179: Amazon.com: Books [Internet]. 2nd ed. Wiley-Interscience; 1985 [cited 2021 Jan 6]. Available from: <https://www.amazon.com/Synthetic-Polymeric-Membranes-Structural-Perspective/dp/0471807176>
18. Sivakumar V, Doran IG. Yielding characteristics of unsaturated compacted soils. Mech Cohesive-frictional Mater [Internet]. 2000 May 1 [cited 2021 Jan 6];5(4):291-303. Available from: [https://onlinelibrary.wiley.com/doi/10.1002/\(SICI](https://onlinelibrary.wiley.com/doi/10.1002/(SICI)

- 1099-1484(200005)5:4%3C291::AID-CFM95%3E3.0.CO;2-D
19. Saljoughi E, Mohammadi T. Cellulose acetate (CA)/polyvinylpyrrolidone (PVP) blend asymmetric membranes: Preparation, morphology and performance. *Desalination*. 2009 Dec 15;249(2):850–4.
20. Ananth C V. In Praise of Our Unsung Heroes. *Paediatr Perinat Epidemiol* [Internet]. 2012 Nov 1 [cited 2021 Jan 6];26(6):596–9. Available from: <http://doi.wiley.com/10.1111/ppe.12007>
21. Mansuripur M, Erwin JK, Bletscher W, Khulbe P, Sadeghi K, Xun X, et al. Static tester for characterization of phase-change, dye-polymer, and magneto-optical media for optical data storage. *Appl Opt* [Internet]. 1999 Dec 1 [cited 2021 Jan 6];38(34):7095. Available from: <https://www.osapublishing.org/viewmedia.cfm?uri=ao-38-34-7095&seq=0&html=true>
22. Schellekens H, Finger BC, Dinan TG, Cryan JF. Ghrelin signalling and obesity: At the interface of stress, mood and food reward [Internet]. Vol. 135, *Pharmacology and Therapeutics*. Elsevier Inc.; 2012 [cited 2021 Jan 6]. p. 316–26. Available from: <https://pubmed.ncbi.nlm.nih.gov/22749794/>
23. Rafeeq E MP, krishnan PN SR. Development and characterization of chitosan nanoparticles loaded with isoniazid for the treatment of Tuberculosis. *Res J Pharm Biol Chem Sci* [Internet]. 2010 [cited 2021 Jan 7];4(1):384–90. Available from: [https://www.rjpbcs.com/pdf/2010_1\(4\)/\[42\].pdf](https://www.rjpbcs.com/pdf/2010_1(4)/[42].pdf)
24. Bisht S, Feldmann G, Soni S, Ravi R, Karikar C, Maitra A, et al. Polymeric nanoparticle-encapsulated curcumin (“nanocurcumin”): A novel strategy for human cancer therapy. *J Nanobiotechnology* [Internet]. 2007 Apr 17 [cited 2021 Jan 6];5. Available from: <https://pubmed.ncbi.nlm.nih.gov/17439648/>
25. Avadi MR, Sadeghi AMM, Tahzibi A, Bayati K, Pouladzadeh M, Zohuriaan-Mehr MJ, et al. Diethylmethyl chitosan as an antimicrobial agent: Synthesis, characterization and antibacterial effects. *Eur Polym J*. 2004 Jul 1;40(7):1355–61.
26. Mi FL, Sung HW, Shyu SS. Drug release from chitosan-alginate complex beads reinforced by a naturally occurring cross-linking agent. *Carbohydr Polym* [Internet]. 2002 Apr 1 [cited 2021 Jan 7];48(1):61–72. Available from: <https://tmu.pure.elsevier.com/en/publications/drug-release-from-chitosan-alginate-complex-beads-reinforced-by-a>
27. Ravichandran R. Nanoparticles in drug delivery: Potential green nanobiomedicine applications [Internet]. Vol. 1, *International Journal of Green Nanotechnology: Biomedicine*. 2009 [cited 2021 Jan 6]. p. 108–30. Available from: <https://www.tandfonline.com/action/journalInformation?journalCode=ugnj20>