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Synthesis and Characterization of Chitosan-Citrate Microparticle Using Ionic Gelation Methods

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Abstract

A study has been conducted which aims to synthesize chitosan-citrate using the ionic gelation method and its characterization with FTIR, SEM, SAA, and PSA, as well as solubility and swelling tests. The results showed that chitosan reacted with sodium citrate producing chitosan-citrate particles. The product of chitosan-citrate has a rough and thickened surface morphology. The surface area of chitosan-citrate obtained is 35.233 m^2/g and the pore size is 0.027 cc/g, smaller than chitosan. Based on solubility and swelling tests in acidic, alkaline, and neutral media, chitosan-citrate has good resistance and low swelling effect.

Keywords: Chitosan, synthesis, gelation, sodium citrate, morphology.

INTRODUCTION

Biopolymers are polymers that are naturally found in the cell. The prefix 'bio' indicates that the polymer is produced by living organisms, so it is renewable and able to decompose naturally. Some examples of commonly used and environmentally friendly biopolymers are chitin, chitosan, carrageenan, alginates, starches, gelatin, and casein gluten. Increasing public awareness about environmental issues makes these biopolymers have been used as an alternative to replace petrochemical-based polymers (Dhote et al., 2019).

Chitosan is a natural polysaccharide. It is composed of ß-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine with random distribution. Chitosan is produced through the deacetylation process of chitin biopolymer, which are the main components in the shells of Crustacea animals such as crabs and shrimps (Rahayu et al., 2020; Hasanela et al., 2020). Today, chitosan has been widely applied commercially to the chemicals, foods, and pharmaceutical industries (Li et al., 2007). For example, chitosan has been an adsorbent for heavy metals (Bijang et al. 2021; Rahayu et al., 2020; Tanasale et al., 2018) and a capping agent for nanoparticles (Badi'ah, 2021). Due to its special properties such as mucoadhesive, biocompatible, biodegradable, non-toxic, and low levels of immunogenicity, chitosan is a very promising biomaterial for its use as a carrier in drug delivery systems (Mardliyati et al., 2012).

Pharmaceutical ingredients in microparticle form can offer number of advantages, for example, they can be used for controlled release dosage and can maintain the stability of the active ingredients. The process of incorporating the active ingredients can be achieved through the encapsulation process and can be used to deliver drugs with a controlled release profile over a long period of time (Sari et al., 2012). Drug delivery through microparticles can be applied in the field of tissue engineering, replace or regenerate tissue through the manufacture of tissue outside the body and then implanted into the body to replace damaged tissue. Microparticles can be used to encapsulate bioactive substances for cell growth and then release these substances in a controlled manner over a certain period of time so that cells differentiate into the desired tissue (Sukmawati et al., 2015).

Chitosan can form microparticles using a variety of methods, one of that is ionic gelation. Ionic gelation is a method that attracts a lot of attention from researchers because the process is simple, does not use organic solvents, and can be controlled easily (Mardliyati et al., 2012). This method is based on the gelation of chitosan when associated with certain polyanions due to the formation of inter and intra-molecular cross-linking mediated by polyanions (Elzatahry and Eldin, 2008). The process of ionic cross-linking can use compounds such as citrate and tripolyphosphate because it is not toxic and multivalent (Nurbaiti, 2015).

Putra and Khabibi, (2014) synthesized chitosan microparticles by ionic gelation process, showing that

chitosan microparticles from 1% and 2% chitosan solutions obtained particle sizes of 15-115 μm and 30-220 μm, respectively. Mardliyati (2012) synthesized chitosan-tripolyphosphate nanoparticles with the ionic gelation method: the effect of concentration and volume ratio to particle characteristics, the results of the study showed that under preparation conditions of 0.2% chitosan concentration, 0.1% TPP concentration and 5:1 chitosan volume ratio, chitosan nanoparticles formed were below 100 nm, fairly-uniform, and relatively stable.

Many studies have been carried out on the preparation and characterization of chitosan-tripolyphosphate. Modification of Chitosan-tripolyphosphate causes an increase in the value of the tensile strength, the percentage of elongation, and the flexibility of the film (Astriyani, 2011). Besides the modification of chitosan with tripolyphosphate, the ionic cross-linking of chitosan can also be carried out using sodium citrate as a crosslinking agent. Chitosan-citrate cross-linking films have been carried out by immersing the chitosan film into the citrate solution. The chitosan-citrate crosslinked film showed that there was a relationship between the pH of the sodium citrate solution and the swelling ability and drug release properties. A crosslinked film of chitosan with sodium citrate has also been made as a moxifloxacin drug delivery system. Chitosan-citrate cross-linked films were made using the immersion method, which was chitosan films, made from 4% chitosan solution in 4% acetic acid, being soaked with 4% sodium citrate in pH 5 producing good tensile strength and film folding resistance, and good physicochemistry (Chinta et al., 2013).

The ability of chitosan to interact with negative charges from the mucosal surface is because chitosan is a polyelectrolyte compound that can be combined with polyanions, such as sodium citrate. In addition, chitosan is a polycation polymer that can cause interactions with negatively charged (anionic) components. Ionic interactions occur between the negative charge of the crosslinking agent and the positive charge of chitosan. The ionic cross-linking method is a simple and easy procedure. The presence of ionic crosslinking allows the modified chitosan to be formed into a variety of drug delivery systems, such as microparticles and nanoparticles (Iswandana et al., 2014).

Based on the explanations above, a study about the synthesis and characterization of chitosan-citrate microparticles using the ionic gelation method has been conducted

METHODOLOGY

Materials and Instrumentals

Material: Analytical balance (OHAUS), magnetic stirrer, hot plate, Buchner filter, spatula, stir bar, test tube, sieve, FTIR, PSA, SEM-EDX, and SAA. Commercial chitosan (Sigma Aldrich), sodium citrate (Merck), acetic acid (Merck), NaOH (Merck), distilled water, Whatman filter paper no.42. This research took place for 6 months at the Chemical Physics Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon. Characterization of samples with FTIR was carried out at the Laboratory of Organic Chemistry, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon. Characterization of samples with PSA, SEM-EDX, and SAA was carried out at the Integrated Laboratory of UNDIP, Semarang.

Methods

Initial Preparation of Chitosan-Citrate

Chitosan-citrate was made using the ionic gelation method through the complexation of a polyelectrolyte positively charged chitosan and negatively charged sodium citrate. Dissolved 2 g of commercial chitosan into 100 mL of 2% acetic acid solution, then stirred using a magnetic stirrer and allowed to stand at room temperature for 24 hours. Prepared 1 g of sodium citrate and dissolved with distilled water in a 50 mL volumetric flask.

Synthesis of Chitosan-Citrate

A total of 50 mL of sodium citrate solution was added slowly into 100 mL of the prepared chitosan solution until a suspension of chitosan-citrate microparticles was formed. Stirring was continued for 1 hour so that the cross-linking process took place perfectly to produce a gel. The chitosan-citrate gel formed was slightly yellowish in color, filtered, and dried in an oven at 70 – 80 ˚C. The granules were ground and filtered using a 100-mesh sieve (Mardliyati et al., 2012). The sample was characterized using FTIR, PSA, SEM-EDX, and SAA instruments

Solubility Test of Chitosan-Citrate

Commercial chitosan and chitosan-citrate powders were tested to see their solubility in 2% (v/v) acetic acid, 1M NaOH and 100 mL of distilled water, respectively. About 0.1 g of chitosan and chitosan-citrate powders were added to the three different solutions and stirred for 24 hours (Ngah and Fatinathan, 2010).

Solubility Test of Chitosan-Citrate

A total of 0.1 g of commercial chitosan and chitosan citrate powder were each put into 3 different test tubes then the powder level of each tube was marked and measured as h_0. Then each tube was filled with 2 mL of 2% (v/v) acetic acid, 1 M NaOH and distilled water, and allowed to stand for 24 hours. After 24 hours, the height of the powder in the tube was measured as h_t. The percentage of swelling is calculated based on the following Equation 1.

$$S = \frac{h_t - h_o}{h_0} \text{x } 100 \quad (1)$$

Where S is the swelling percentage, h_t is the swelling powder height at time t, and h_0 is the initial height of chitosan and chitosan-citrate powder (Ngah and Fatinathan, 2010).

RESULTS AND DISCUSSION

Synthesis of Chitosan-Citrate

The synthesis of chitosan-citrate microparticles uses the ionic gelation method, which involves the interaction between positive ions from chitosan and negative ions from sodium citrate. Chitosan is first dissolved in the acetic acid solvent so that the chitosan undergoes protonation (Figure 1) which produces NH_3^+ ions.

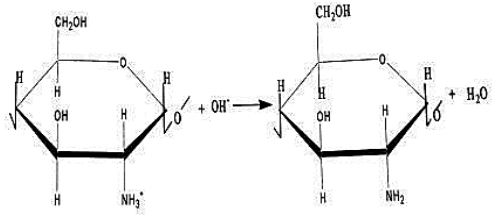

Figure 1. chitosan undergoes protonation reaction.

The protonated chitosan was reacted with sodium citrate solution. When the sodium citrate solution is added to the chitosan solution, there will be a cross-linking process between the negative charges of sodium citrate with a positive charge of chitosan to form a suspension of chitosan-citrate nanoparticles. The chitosan-citrate suspension was then filtered to produce a gel which was then dried in an oven at a temperature of 70-80 ˚C to remove the water content. The dried solid of chitosan-citrate crosslinks was ground using a mortar and pestle as soon as possible. This is because the chitosan-citrate sample formed is hygroscopic so if it is exposed to air for too long it will make the sample elastic and cannot be ground to form a powder. Then sifted using a 100-mesh sieve to obtain a chitosan-citrate powder.

Physical appearance

Sodium citrate modified chitosan showed changes in physical appearance. Chitosan powder (Figure 2a) has a white color while chitosan-citrate (Figure 2b) is yellow.

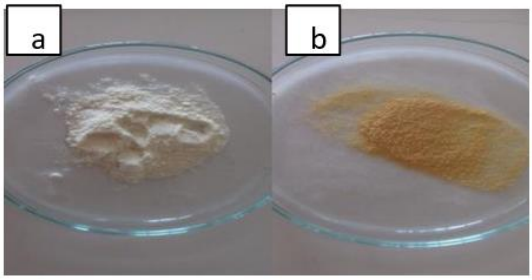

Figure 2. (a) Chitosan and (b) Chitosan-citrate powder

This color change occurs due to the drying process of the chitosan-citrate gel. Chitosan has a fine powder form while the resulting chitosan-citrate is in the form of flakes. The hygroscopic nature of Chitosan-citrate makes the grinding process difficult, so the resulting chitosan-citrate was in the form of flakes. Chitosan is also odorless, while chitosan-citric smells sour. The sour smell of chitosan-citrate comes from acetic acid which is used as a solvent for chitosan during the protonation process of chitosan before being reacted with sodium citrate.

Characterization of Chitosan-Citrate using FTIR

Characterization of functional groups using FTIR was carried out to determine whether the chitosan-citrate crosslinking process was formed or not. This can be seen by comparing the chitosan FTIR spectrum (Figure 3) which was used for the synthesis, with the chitosan-citrate spectrum (Figure 4). The absorption peaks in the chitosan and chitosan-citrate spectra did not differ much, there were only some changes in the chitosan-citrate spectrum which indicated a cross-linking process occurred. The FTIR spectrum of chitosan and chitosan-citrate has a peak at 3300-3100 cm^{-1} which indicates the presence of an –OH group. This is due to the –OH group peak covering the peak of the $–NH_2$ group. The spectrum of 1600-1500 cm^{-1} shows the presence of N-H and C=O groups in chitosan and chitosan-citrate. FTIR analysis of chitosan-citrate found that there was a sharper carbonyl group absorption compared to the chitosan spectrum which indicated the increase in the number of the carbonyl group. This is due to the interaction between the amide in chitosan and the carboxylate group of citrate so that the amide group is reduced because it turns into NH_3^+ and C=O increases due to the carboxylate group of citrate (Figure 3).

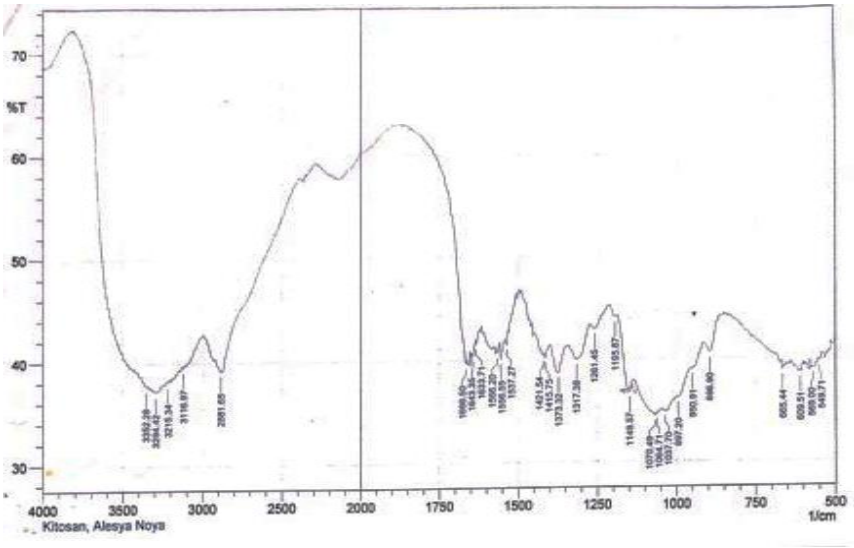

Figure 3. Chitosan FTIR spectrum.

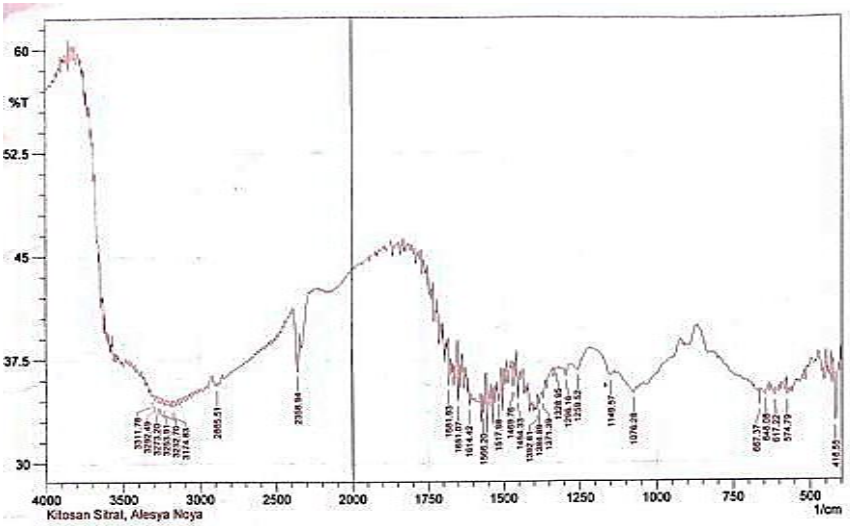

Figure 4. Chitosan-citrate FTIR spectrum.

The spectrum of chitosan and chitosan-citrate has typical absorption bands as shown in Table 1**.** The peak of 1469-1454 cm^{-1} in chitosan-citrate is a COOH group formed from cross-linking between chitosan and citrate. The chitosan-citrate spectrum (Figure 4) also showed a shift from the peak of 1392-1328 cm^{-1} to 1373-1317 cm^{-1} the chitosan spectrum. This is in accordance with the wavenumber of the C-O group on the COO- ion (Nurbaiti, 2015). The spectrum of chitosan and chitosan-citrate has typical absorption bands as shown in Table 1.

SEM-EDX Characterization of Chitosan-citrate

Chitosan has a surface morphology similar to surface chitosan which is slightly rough, but there is a change in chitosan-citrate (Figure 5a) which shows the surface morphology as clumping and denser than the surface morphology of chitosan (Figure 5b) which looks more tenuous.

Table 1. FTIR spectrum data of chitosan and chitosan citrate.

Functional group	Wave number (cm^{-1})	
	Chitosan	Chitosan-citrate
-OH stretching	3352-3116	3311-3174
-CH stretching	2881	2885
C=O	1666-1633	1681-1614
-NH (NH_2)	1566-1537	1566-1517
$-COO^-$	-	1469-1454
-C-O	1373-1317	1392-1328

The results of the EDX analysis of chitosan and chitosan-citrate showed the presence of the following

elements: C, O, Na, Cl, and Cu with the compositions presented in Table 2.

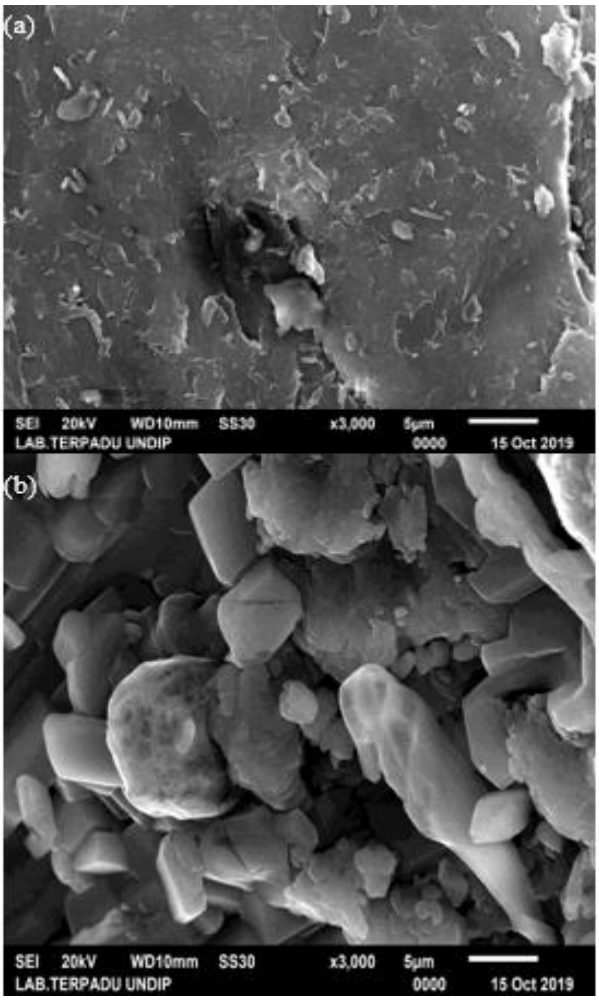

Figure 5. (a) SEM Chitosan magnification 3000x. (b) SEM Chitosan-citrate magnification 3000x.

Table 2. EDX data of chitosan and chitosan-citrate.

Element	Mass (%)	
	Chitosan	Chitosan-citrate
C	57.25	38.45
O	42.21	49.44
Na	0.13	11.24
Cl	0.15	-
Cu	0.25	0.87

The presence of Na, Cl, and Cu on the surface of chitosan detected by EDX was considered an impurity. Chitosan is a natural polymer obtained from the deacetylation of chitin from shrimp shells and crab shells which may contain metals or pollutants found in the sea (Wowor et al., 2015). The Na and Cl elements contained in chitosan are thought to come from the chitin deacetylation process using NaOH and HCl solutions. After the crosslinking process between chitosan and citrate, there was a change in the composition of all elements (Table 2). The elemental composition of O and Na increased indicating that the chitosan had been modified by citrate.

Surface Area Analyzer (SAA) Characterization of Chitosan-citrate

Based on the results of the BET analysis, the chitosan surface area obtained was 108.080 m^2/g and the chitosan-citrate surface area was 35.233 m^2/g. This change indicates that chitosan has been modified by citrate so that the surface area of chitosan-citrate is smaller than that of chitosan. In addition to surface area data, from the nitrogen adsorption analysis, pore size data from chitosan and chitosan-citrate samples were also obtained, after being calculated using the BJH method, the pore diameter data were 38.138 Å for chitosan and 30.530 Å for chitosan-citrate. This shows that the pore size of chitosan-citrate is smaller than that of chitosan because chitosan-citrate undergoes agglomeration of several particles to form larger granules and reduce the pores.

Particle Size Analyzer (PSA) Characterization of Chitosan-citrate

The particle size of chitosan-citrate was determined based on the average volume diameter. Based on the results of PSA analysis, chitosan particles have a larger average diameter than chitosan-citrate which indicates that chitosan has been modified as shown in (Table 3). However, the measurement results show micrometer-sized (μm) chitosan-citrate particles because of the chitosan-citrate agglomerates during the drying process.

Table 3. Particle size of chitosan and chitosan-citrate.

Samples	Average particle diameter (μm)
Chitosan	92.628
Chitosan-citrate	89.653

Chitosan has a larger size than chitosan-citrate because the chitosan particles are very large and dense, so that they clump together to form aggregates into larger particles making it difficult to break up into smaller particles (Mardliyati et al., 2012). The addition of citrate to chitosan can reduce the size of the microparticles and increase the strength of the chitosan matrix.

Chitosan and Chitosan-Citrate Solubility Test

The results of the quantitative solubility test (Table 4) showed that chitosan was soluble in acid, insoluble in distilled water, and partially soluble in base. In contrast to chitosan, chitosan-citrate has better resistance in all three media. The solubility of chitosan-citrate in acid decreased so that it was only partially soluble, which was because most of the amino groups in chitosan had reacted with citrate and reduced the number of primary amino groups present in chitosan. The lack of H^+ ions in basic and neutral media and the reduction of amino groups in chitosan-citrate in the protonation process causes chitosan-citrate to be insoluble in both basic and neutral media.

Table 4. Solubility Test Results of Chitosan and Chitosan-Citrate

Samples	Solubility effect in 100 mL solution		
	2% CH_3COOH	Distilled water	1 M NaOH
Chitosan	soluble	insoluble	insoluble
Chitosan-citrate	insoluble	insoluble	insoluble

Chitosan and Chitosan-Citrate Swelling Test

The swelling of the polymer chains is determined by the nature of the solvent used during immersion. In this study, the swelling power test was carried out on chitosan and chitosan-citrate in acidic, basic, and neutral solutions (Nasution et al., 2013). Based on the results of measurements and calculations presented in Table 5, the percentage effect of polymer chain swelling is different for each solution based on the nature of the solvent used. After soaking for 24 hours in each medium, the height of the chitosan and chitosan-citrate powder showed that the swelling of chitosan was much greater than that of chitosan-citrate. The swelling power of chitosan-citrate decreased because it was influenced by the density of the crosslinks. The tight bonds between chitosan-citrate polymers resulted in a small swelling ability.

Table 5. Swelling Test Results of Chitosan and Chitosan-Citrate

Samples	Swelling effect (%)		
	2% CH_3COOH	Distilled water	1 M NaOH
Chitosan	soluble	110.5	110
Chitosan-citrate	116.67	23	85

Chitosan is soluble in 2% CH_3COOH due to protonation of amino groups in chitosan. Meanwhile, chitosan-citrate can expand in an acidic medium due to protonation of the amine group to NH_3^+ so that chitosan-citrate can expand in an acidic medium due to the presence of an amine group in chitosan. Similar to the acid medium, swelling in the alkaline medium also occurs because the carboxylic group (-COOH) turns into a carboxylate ion (-COO-) (Yuliani, 2012).

CONCLUSION

Based on the research results obtained, it can be concluded that the chitosan-citrate can be synthesized through a cross-linking process using chitosan and sodium citrate. Chitosan-citrate has a rough and thickened surface morphology. Chitosan-citrate has a surface area of 35.233 m^2/g and a smaller pore size than chitosan, which is 30.53 Å.

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REFERENCES

Astriyani, F. (2011). Preparasi Kitosan Tripolifosfat sebagai Eksipien dalam Sediaan Tablet Enterik. *Undergraduated Thesis*. Universitas Indonesia.

Badi'ah, H.I. (2021). Chitosan as a capping agent of silver nanoparticles. *Indonesian Journal of Chemical Research*, 9(1), 21-25.

Bijang, C.M., Tanasale, M.F.J.D.P., Kelrey, A.G., Mansur, I.U., & Aziz, T. (2021). Preparation of Natural Ouw-Chitosan Composite and Its Application As Lead and Cadmium Metal Adsorbent. *Indonesian Journal of Chemical Research*, 9(1), 15-20.

Chinta, D. P., Katakam, P., Murthy, V. S. N., & Newton, M. J. (2013). Formulation and In-Vitro Evaluation of Moxifloxacin Loaded Crosslinked Chitosan Films for The Treatment of Periodontitis. *Journal of Pharmacy Research*, *7*(6), 483–490.

Dhote, V. K., Dhote, K., Pandey, S. P., Shukla, T., Maheshwari, R., Mishra, D. K., & Tekade, R. K. (2019). Fundamentals of Polymers Science Applied in Pharmaceutical Product Development. In R. K. Tekade (Ed.), *Basic Fundamentals of Drug Delivery* (pp. 85–112). Academic Press.

Elzatahry, A. A., & Eldin, M. S. M. (2008).

Preparation and Characterization of Metronidazole-Loaded Chitosan Nanoparticles for Drug Delivery Application. *Polymers for Advanced Technologies*, *19*(12), 1787–1791.

Iswandana, R., Jufri, M., & Effionora Anwar. (2014). Formulasi Nanopartikel Verapamil Hidroklorida dari Kitosan dan Natrium Tripolifosfat dengan Metode Gelasi Ionik. *Jurnal Farmasi Indonesia*, *6*(4), 201–210.

Hasanela, N., Tanasale, M.F.J.D.P. & Tehubijuluw, H. (2020). Karakterisasi Biopolimer Kitosan Hasil Deasetilasi Limbah Kepiting Rajungan (*Portunus sanginolentus*) Menggunakan $NaBH_4$ dalam NaOH. *Indonesian Journal of Chemical Research*, 8(1), 66-71.

Li, Y., Chen, X. G., & Liu, N. (2007). Physicochemical Characterization and Antibacterial Property of Chitosan Acetates. *Carbohydrate Polymers*, *67*.

Mardliyati, E., Muttaqien, S. El, & Setyawati, D. R. (2012). Sintesis Nanopartikel Kitosan-Trypoly Phosphate Dengan Metode Gelasi Ionik : Pengaruh Konsentrasi dan Rasio Volume Terhadap Karakteristik Partikel. *Prosiding Pertemuan Ilmiah Ilmu Pengetahuan Dan Teknologi Bahan*, 90–93.

Nasution, Z., & Harry Agusnar, Zul Alfian, B. W. (2013). Pengaruh Viskositas Kitosan Dari Berbagai Berat Molekul Terhadap Pembuatan Kitosan Nanopartikel Menggunakan Ultrasonic Bath. *Jurnal Teknologi Kimia Unimal*, *2*(2), 68–79.

Ngah, W. S. W., & Fatinathan, S. (2010). Adsorption Characterization of Pb(II) and Cu(II) Ions Onto Chitosan-Tripolyphosphate Beads: Kinetic, Equilibrium and Thermodynamic Studies. *Journal of Environmental Management*, *91*(4), 958–969.

Nurbaiti, R. (2015). Preparasi dan Karakterisasi Kitosan-Sitrat Sambung Silang sebagai Eksipien dalam Sedian Film yang Mengandung Veramil. *Undergraduated Thesis.* UIN Syarif Hidayatullah.

Putra, H. W., & Khabibi, K. (2014). Sintesis Mikropartikel Kitosan dengan Proses Gelasi Ionik sebagai Adsorben Logam Cd(II). *Jurnal Kimia Sains Dan Aplikasi*, *17*(3), 104–108.

Rahayu, R., Tanasale, M.F.J.D.P., & Bandjar, A. (2020). Isoterm adsorpsi Ion Cr (III) oleh Kitosan Hasil Isolasi Limbah Kepiting Rajungan dan Kitosan Komersil. *Indonesian Journal of Chemical Research*, 8(1), 28-34.

Sari, R., A, D. P. R., & Rijal, M. A. S. (2012). Pengaruh Perbandingan Obat-Polimer Terhadap Ketoprofen-Kitosan. *Mikropartikel* Pharma Scientia.1(2), 7-11.

Sukmawati, A., Yuliani, R., & Wahyuni, A. S. (2015). Formulasi Dan Evaluasi Mikropartikel Dexamethasone Lepas Lambat Dengan Matriks Ethyl Cellulose (EC). *University Research Colloquium*, 18–26.

Tanasale, M.F.J.D.P., Bandjar, A. & Sewit, N. (2018). Isolasi Kitosan dari Tudung Jamur Merang (*Vollariella volvaceae*) dan Aplikasinya Sebagai Adsorbent Logam Berat Timbal (Pb). *Indonesian Journal of Chemical Research*, 6(1), 44-50.

Wowor, A. R. Y., Bagau, B., Untu, I., & Liwe, H. (2015). Kandungan Protein Kasar, Kalsium, Dan Fosfor Tepung Limbah Udang Sebagai Bahan Pakan Yang Diolah Dengan Asam Asetat (CH3COOH). *Zootec*, *35*(1), 1.

Yuliani, A. A. (2012). Preparasi dan Karakterisasi Mikrosfer Kitosan Suksinat Tersambung Silang Natrium Sitrat. *Undergraduated Thesis*. Universitas Indonesia.