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The Effect of Concentration on Particle Size of *Solanum muricatum* Aiton Microcapsule.

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ABSTRACT

In this study, the influence of encapsulation process conditions on the particle size of *Solanum muricatum* Aiton Microcapsule was investigated. This study was purposed to prepare microcapsules of *Solanum muricatum* Aiton by coacervation methods and to characterize the resulting microcapsules. The microcapsules were prepared using sodium alginate as a coating material. The obtained microcapsules were characterized, including its recovery andparticle size distribution. Microcapsules prepared by coacervation method had an irregular shape and size particle between 21-378 µm. SEM results show that the use of 0.625% sodium alginate and 4.5% glutaraldehide yields the smallest particle size of the microcapsule, measuring between 21.5 and 60 µm.In conclusion, the addition of sodium alginate concentration causes an increase in the size of the microcapsule particle.

Keyword: *Solanum muricatum* Aiton, encapsulation

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INTRODUCTION

Pepino (*Solanum muricatum*) also known as pepino dulce or pear melon is a plant that is originally grown in South America, mainly for its juicy fruits. The fruits of pepino are preferred not only due to their attractiveness because of colorful appearance, taste and juiciness but also due to their medicinal significance. The DPPH free radical scavenging activity of the pepino fruits were compared with that of butylated hydroxytoluene (BHT), ascorbic acid, and α-tocopherol. The % inhibition level of the methanolic extract (96.46±0.53) was found to be similar to that of BHT, ascorbic acid and α-tocopherol, while the hexane extract of the pepino fruits (74.46±0.38) exhibited significantly lower % inhibition levels [1].

Microencapsulation is a process by which solids, liquids or even gases may be enclosed in microscopic particles formation of thin coatings of wall material around the substances [2].The reasons for microencapsulation arecountless. In some cases, the core must be isolated from its surroundings, as in isolating vitamins from the deteriorating effects of oxygen, retarding evaporation of a volatile core, improving the handling properties of a sticky material, or isolating a reactive core from chemical attack. In other cases, the objective is not to isolate the core completely but to control the rate at which it leaves the microcapsule, as inthe controlled release of drugs or pesticides. The problem may be as simple as masking the taste or odor of the core, or as complex as increasing the selectivity of an adsorption or extraction process [3].

MATERIALS AND METHODS

The raw pepino fruits was obtained from a farm in Kabupaten Rejang Lebong Bengkulu Indonesia.The fruits were carefully selected in order to obtain a uniform batch in relation to size and degree of maturity.Acetic acidP.a, Tween-80 P.a, sodium alginate, glutaraldehide, ethanol P.a, were purchased from Merck and chitosan from Sigma-Aldrich. Destilled water, phosphate buffer pH 7, are available at Chemistry Laboratory, Faculty of Mathematics and Natural Science, University of Bengkulu, Indonesia.

Instrumental used

Scanning electron microscopy

Prior to scanning electron microscopy (SEM) analysis, thesamples were sprinkled on one side of double-side adhesive stuckon the stub and then was coated with gold. The SEM analysis of themicrospheres was carried out by using S3700 scanning electronicmicroscope. The microspheres were observed at anaccelerating voltage of 10 kV.

Experimental

Preparation of samples for chitosan isolation

The shells are washed with water until clean, then dried in the sun. Cleaned shells smoothed and sieved to get a size of 50 mesh.

Deproteination

At this stage the proteins from shrimp and other shrimp waste are separated by introducing 100 grams of cleaned and smoothed sample into 1000 mL of 3.5% NaOH solution (b/v) and heated at 65°C for 2 hours while continuing stirred using a magnetic stirrer. Furthermore this mixture is cooled and filtered by a filter paper. The filtered residue is washed with water until neutral then rinsed with distilled water. The neutral residue, which is a coarse chitin, is dried in an oven at 65°C for 24 hours and weighed. The residue was analyzed with FTIR.

Demineralization

Deproteinationresidue was inserted intoHCl 1 N solution slowly at room temperature with a ratio of 1 gram of sample: 15 mL of HCl 1 N solution for 1 hour. The stirring process is carried out using magnetic

stirring. The reaction product was filtered using whatman filter paper 42. The filtered residue was washed with water until neutral then rinsed with aquadest. The residue was dried in 65°C for 24 hours and weighed.

Deacetylation

The demineralization precipitate was introduced into 10% (w/v) NaOH solution for 4 hours at 100°C at a ratio of 1:10 (w/v). The mixture is stirred using a magnetic stirrer. The result is filtered using whatman filter paper 42. The residue is chitosan, washed with water until neutral and rinsed with aquadest. Chitosan is dried in an oven with a temperature of 65 ° C for 24 hours. The dried residue was analyzed with FTIR.

Making Microcapsules [13]

First chitosan solution 1.75% (w/v) was prepared with 1% acetic acid (v/v) solvent, 228.6 mL of solution. Then, 38.1 mL of alginate solution was added with a concentration range of 0.5, 0.625, and 0.750% (w/v) while stirring at a rate of 700 rpm to homogeneous. Thereafter, 7.62 mL of glutaraldehyde was added to the mixture with a concentration of 4, 4.5, and 5% (v/v) concentrations. The addition is done dropwise while continuously stirring for 1 hour for uniformity

The chitosan-alginate-glutaraldehyde mixture was mixed with 4 grams of extract dissolved in 250 mL of 96% ethanol to make a suspension of chitosan-alginate-glutaraldehyde solution and extract with 2:1 chitosan-extract ratio, then added 5 mL Tween-80 2%. This final mixture is stirred at a rate of 700 rpm for 2 hours at room temperature and converted to microcapsule by a spray dryer.

The spray dryer used has a diameter of 1.5 mm hole and with an inlet temperature of 150^{o}C, the outlet temperature is 70^{o}C, the flow rate is 60 rpm and the spray pressure is on the 2 bar scale. Each microcapsule is done 3 times andmicrocapsule surface analyzed with SEM.

RESULTS AND DISCUSSION

Isolation of chitosan

In this study isolation of chitosan taken from blood clams (*Anadaragranosa*), these shellfish obtained from restaurant waste from the area around the city of Bengkulu. Called blood clams because these clams have red blood / hemoglobin pigments called bloody cockles, so they can live on relatively low oxygen levels, even after they are harvested even without water [9].

Figure 1: Blood shells (*Anadaragranosa*)

The shell is obtained in a dirty and smelly condition, then the cleanser is cleaned from the dirt, washed with water until clean, then grown until smooth and sieve with 100 µm sieve. After the powder is obtained then the next processed is deproteination.

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Figure 2: Smoothing blood shell (*Anadaragranosa*)

In general, the process of making chitosan includes 3 stages, namely deproteination, demineralization, and deasetylation. The process of deproteination aims to reduce protein levels by using dilute alkaline solutions and sufficient heating. The demineralization process is intended to reduce the mineral content ($CaCO_3$) by using low concentration acids to obtain chitin, while the deacetylation process aims to remove acetyl groups from chitin by heating in strongly alkaline solutions with high concentrations. Figure 3 shows the process of removing acetyl groups (deacetylation) on chitin with strong alkali NaOH.

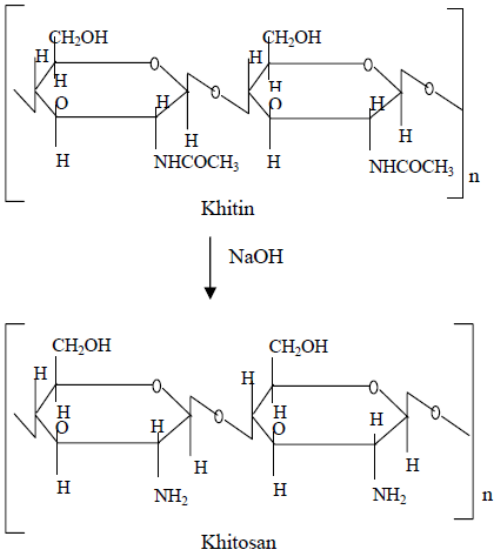

Figure 3:Deacetylation of chitin into chitosan

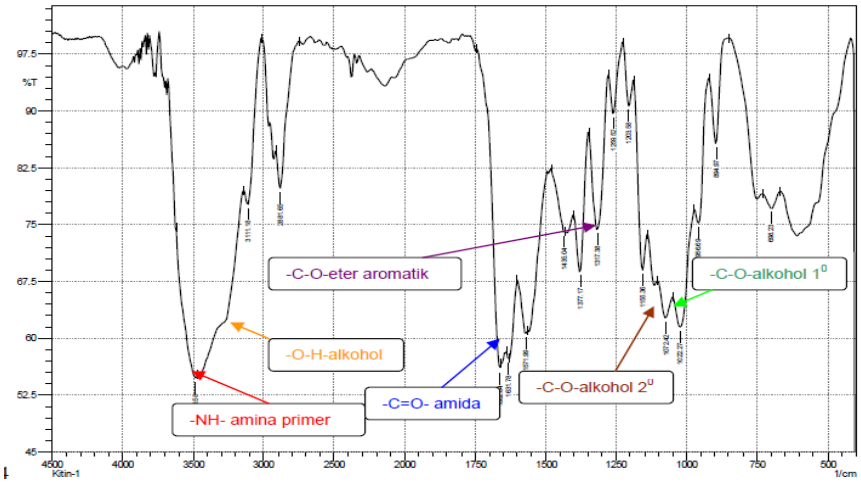

Figure 4: Chitin spectrum

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Chitin is naturally often incomplete in acetylation, whereas chitosan usually also contains acetyl groups of varying degrees. Therefore, chitin or chitosan is essentially a co-polymer of N-acetyl-D-glucosamine and D-glucosamine. Chitin usually has a deacetylation degree of less than 10%. In general the deacetylation degree for chitosan is about 60% and about 90-100% for fully deacetylated chitosan. This price depends on the chitin raw materials used and the process being run [11].

Figure 5: Deproteination

The deproteination stage is performed by reacting the chitin of demineralization with a strong base of NaOH in the extractor, protein dissolving in the NaOH solution. The deproteination reaction aims to break the bond between protein and chitin by adding sodium hydroxide. In the deproteinization reaction occurs, little bubbles form on the surface of the solution and the solution in the extractor becomes slightly thickened and reddish. The thickening of the solution in the extractor is due to the protein content of the crude chitin which detaches and binds to Na^+ ions in solution, forming sodium proteinate. The yield after deproteination is 30%. This yield is the rendement of chitin. At the deproteination stage, the extracted protein is in the form of the Na-proteinate bond, in which the Na^+ ion binds the end of the negatively charged protein chain to settle.

Figure 6: Demineralization process

The process of insulating chitin compounds from blood shell (*Anadaragranosa*)waste is done using the Hong method [10], which includes deproteination, demineralization and decolorization. Demineralization process is mixing of blood shell (*Anadaragranosa*)waste with 1 N HCl solution in extractor, there is a significant reaction. A large amount of foam and air bubbles are formed, and this lasts for about 5-10 minutes. This is due to the formation of CO_2 and H_2O gases on the surface of the solution based on the demineralization reaction shown by equations (1) and (2).

(1) $2HCl\ (s) + CaCO\ 2(g) \rightarrow CaCl_2\ (l) + CO_2\ (g) + O_2(g)$ ……(1)

(2) $Ca_3(PO_4)_2 + 4HCl_2(s) \rightarrow CaCl_2\ (l) + Ca(H_2PO_4)_2\ (l)$……(2)

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Crude chitin from the demineralization stage is rinsed with excess water to remove any residual HCl still in the presence of chitin, so that chitin is not damaged when it is reacted with a strong base of NaOH at deproteination stage, resulting from a fairly extreme pH change. The formation of CO_2 gas is an indicator the ongoing reaction of hydrochloric acid with mineral salts contained in blood shell waste. During the demineralization process, the calcium compound will react with water-soluble hydrochloric acid [6]. Proteins, fats, phosphorus, magnesium and iron are also wasted in this process.According to Marganov that the demineralization process aims to eliminate inorganic salts or mineral deposits that exist on the shrimp skin. The main mineral content is $CaCO_3$ and $Ca_3(PO_4)_2$ in small amounts, the minerals contained in blood shell are more easily separated compared to proteins because they are only physically bound [4,7].

Figure 7: Washing up to neutral pH

Isolation of chitosan compounds was obtained by conducting a deacetylation reaction process in chitin. Deacetylation is the process of converting an acetyl group (-$NHCOCH_3$) to chitin into an amine group (-NH_2) by addition of a strong base such as NaOH. The deacetylation reaction of chitin is essentially an amide hydrolysis reaction of α-(1-4) -2-acetamide-2-deoxy-D-glucose. OHion concentration greatly affects the process of removing acetyl groups from chitin acetamide groups. The stronger a base the greater the OH- concentration in the solution which can increase the strength of base affects the deacetylation process of the acetyl group from the chitin acetamide group [5].

Chitosan is a biopolymer of D-glucosamine produced from the process of chitin deacetylation using strong alkali. It is a water-insoluble cationic polymer, and an alkaline solution with a pH above 6.5. Chitosan is easily soluble in organic acids such as formic acid, acetic acid, and citric acid [8]

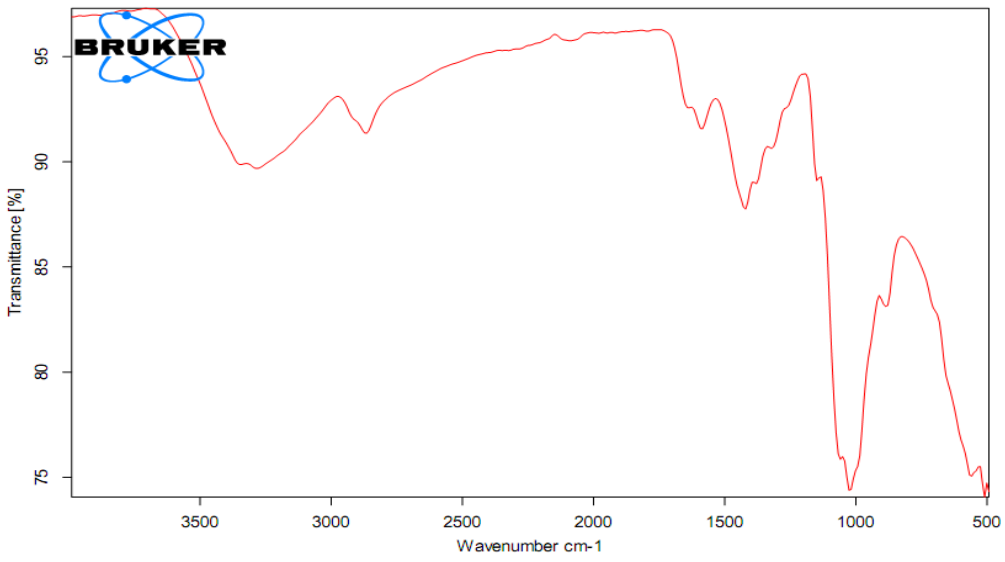

Figure 8: Spectrum of Chitosan FTIR

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Chitosan deacetylation is the process of removing acetyl groups from chitin into free chitosan amino groups using strong bases. One method that can be used to determine the degree of deacetylation of chitosan is infrared spectroscopy (IR). Figure 7shows the results of FTIR spectrum chitosan. Determination of degree of deacetylation of chitosan from FTIR spectrum result using baseline method influenced by hydroxy group and amide group. The hydroxy group absorption is present in the wave number 3700-3000 cm-1, while the C = O group of amides lies in the wave number 1680-1630 cm^{-1}.

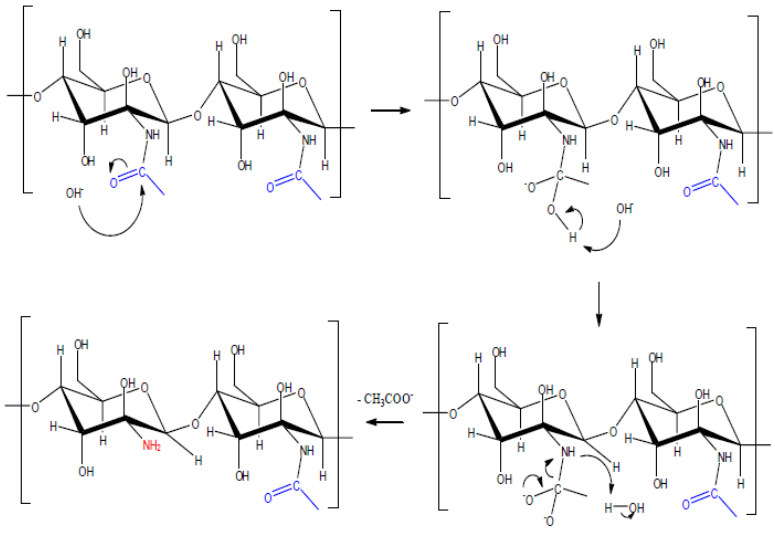

Figure 9: The mechanism of converting chitin into chitosan

Microencapsulation

Microencapsulation is a technique used to confine a compound by using a coating material of a very small size with an average diameter of 15-20 microns or less than half the diameter of a human hair. There are more than 400 billion small capsules in every gallon of thermopropsule material (Sutriyo et al., 2004). The usefulness of using this technique is to control the release of the compound, making the active compound easier and safer to hold, protecting the sensitive material from the environment, and transforming the material from liquid to solid [13].

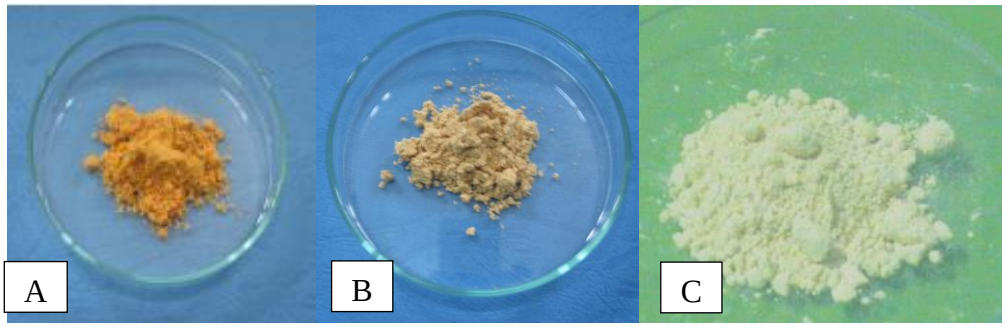

Figure 10: Microcapsules of *Solanum muricatum* Aiton fruit extract (A) and (B) without addition of glutaraldehide and Tween-80, (C) empty microcapsules

Microcapsules with the addition of glutaraldehyde and tween-80 are produced in the form of a fine brownish yellow powder (Figure 9 A). Likewise with the empty microcapsules are brownish yellow (Figure 9 B). This color is produced due to the yellow glutaraldehyde solution, consequently after addition of glutaraldehyde into the chitosan solution, the mixed color becomes aging, thus affecting the color of the solution before and after it is dried.

The following is the SEM result of some of the microcapsules that have been created.

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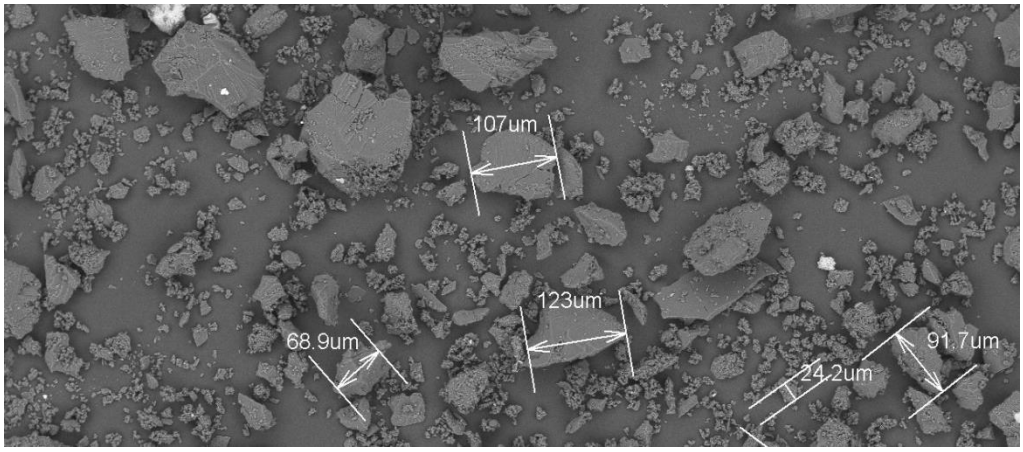

Figure 11: Surface structure of 0.5% sodium alginate and 4% glutaraldehide microstructure

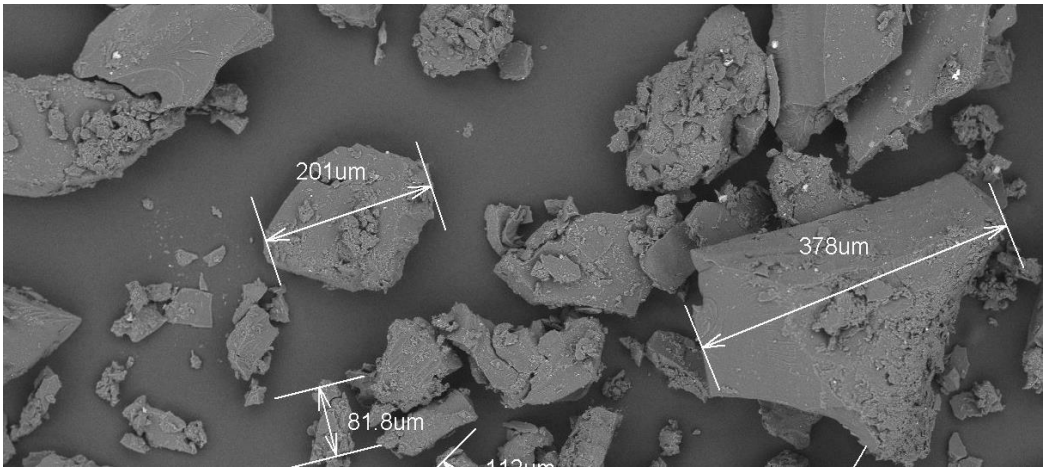

Figure 12: The surface structure of the 0.5% and 0.5% glomeraldehide sodium alginate microcapsules

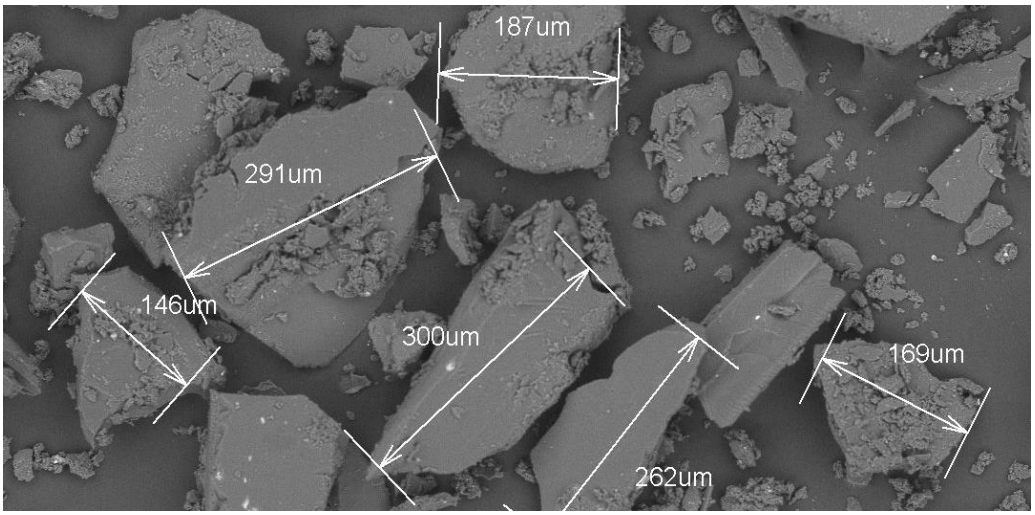

Figure13: The surface structure of 0.5% sodium alginate and 5% glutaraldehide microstructure

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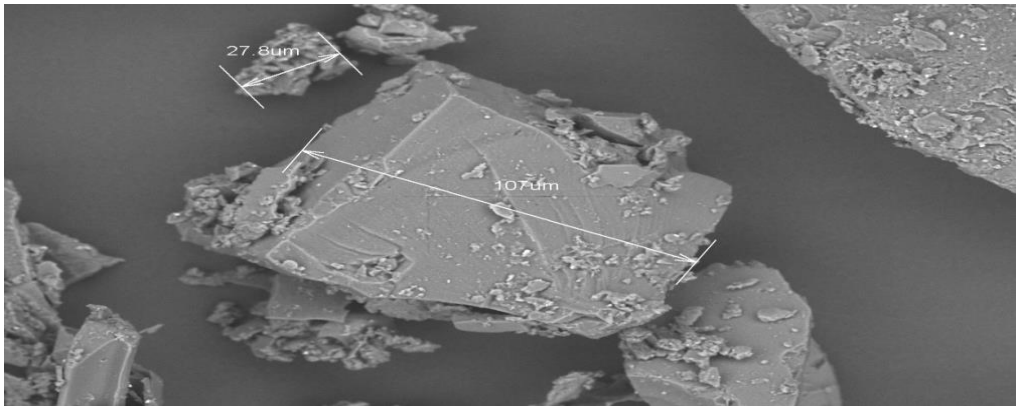

Figure 14: Surface structure of 0.625% sodium alginate and 5% glutaraldehide

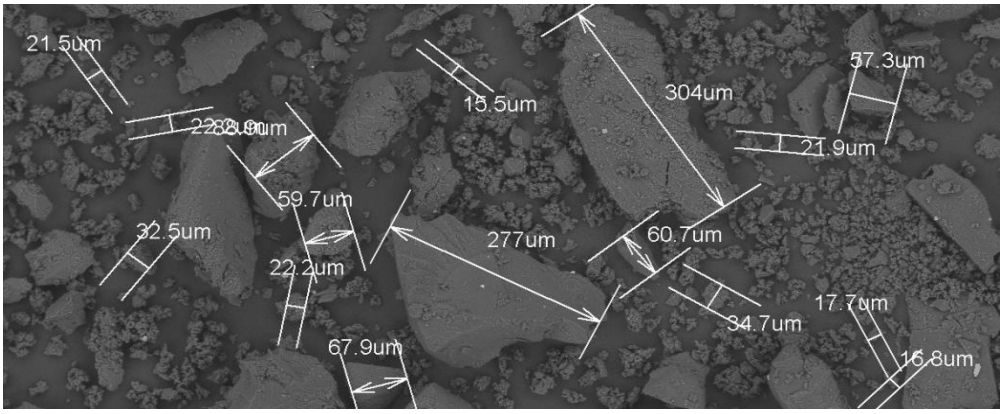

Figure 15: Surface structure of 0.625% sodium alginate and 4.5% glutaraldehide

From the above five SEM results (Fig 11, 12, 13,14,15) it is seen that the addition of glutaraldehide concentration causes an increase in the size of the microcapsule particle.Glutaraldehyde is a dwifungsi compound commonly used for protein and polymer modification. Glutaraldehyde has molecular formula $C_5H_8O_2$ with molecular weight of 100.1 g mol^{-1}, boiling point of 100°C, melting point -15°C, pH between 3.2-4.2, yellow, water soluble, alcohol and benzene (BASF 1999). Glutaraldehyde can be used as a crosslinking agent in polypeptides and crosslinking of proteins. This is due to the activity of high aldehyde groups in the form of Schiff bases with amino groups of proteins. Glutaraldehyde serves as a crosslinking intermediate for PVA and some polysaccharides [12]

CONCLUSIONS

- The addition of glutaraldehide concentration causes an increase in the size of the microcapsule particle.
- SEM results show that the use of 0.625% sodium alginate and 4.5% glutaraldehide yields the smallest particle size of the microcapsule, measuring between 21.5 and 60 µm.

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