ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

Characterization of cross-linked chitosan by ionic and covalent crosslinking agents as wall material of red ginger oleoresin microcapsules

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ABSTRACT:
The aim of this study was to determine the characterization of ionic and covalent interactions to form a crosslinking network with chitosan as a wall material of microcapsules. Red ginger oleoresin microcapsule was prepared by emulsion crosslinking method. Red ginger oleoresin was mixed and stirred with chitosan solution to form an emulsion, then added to corn oil and stirred again to form a second emulsion. Crosslinking agents of glutaraldehyde saturated toluene (GST) or sodium tripolyphosphate (TPP) solution was slowly added to the emulsion. When using a crosslinking agent of TPP solution was added glacial acetic acid to adjust pH. Red ginger oleoresin microcapsules were filtered and washed with petroleum ether followed by n-hexane and then dried in an oven. Microcapsules from chitosan that are cross-linked with GST produce higher yield and size of microcapsules compared to TPP. Whereas, the highest encapsulation efficiency produced from chitosan cross-linked with TPP was 91.63±0.02%. Meanwhile, the highest cumulative release was obtained from TPP cross-linked chitosan microcapsules of 63.71% and the lowest was 50.01% from chitosan microcapsules that cross-linked with GST. The conclusion of this study was the differences between ionic and covalent crosslinking agents produce different microcapsule characteristics. Microcapsules from chitosan cross-linked with GST generated more compact with a smoother surface than with TPP.

KEYWORDS: Chitosan, Emulsion Crosslinking, Glutaraldehyde Saturated Toluene, Red Ginger Oleoresin, Sodium Tripolyphosphate.

INTRODUCTION:
Natural materials such as biopolymers have the ability to transport bioactive compounds to different tissues, cells, and cell compartments despite having low drug binding ability and large distribution. Based on these capabilities, biomaterials are more get attention than synthetic materials because they are biodegradable, biocompatible, low immunogenicity, and antibacterial activity[1]. Biomaterials for drug delivery have been widely provided by various biopolymers such as sodium alginate, gelatin, and chitosan[2].

The drug delivery system serves to overcome unwanted drug concentration fluctuations from conventional drug dosage forms leading to the safety and efficacy of drugs. The technology of microcapsules is an innovation developed into an optimal drug delivery system by providing the desired release profile[3]. Microcapsules as drug carrier provide advantages can increase the lifespan of active constituents and control the release of drugs. Another advantage is used for controlled release of insoluble drugs because of small in size[3].

Chitosan-based microcapsules are being widely developed as drug delivery because of their biological properties such as non-toxic, biocompatibility, biodegradation and antibacterial. Chitosan-based microcapsules have also been deeply exploited for regenerative medicine in controlled delivery[4,2]. Several methods used for preparation of chitosan-based

Received on 22.05.2020 Modified on 28.06.2020
Accepted on 21.07.2020
Research J. Pharm. and Tech. 2021; 14(6):3380-3388.
DOI: 10.52711/0974-360X.2021.00588

microcapsules have been reported previously such as spray drying, coaservation, Co-crystallization, Molecular inclusion, Interfacial polymerization, crosslinking emulsion, Spray chilling/cooling, Extrusion, and Fluidized beds with various types of coating materials (microcapsule wall materials)[5]. A suitable method for comparing the characterization of microcapsules based on differences in ionic and covalent interaction used emulsion crosslinking. This method can be used for encapsulation of soluble, insoluble, solid, and liquid materials, and also can produce micro/nanocapsules[6].

Emulsion crosslinking is a method that utilizes the interaction between amine groups of chitosan and a functional group of a crosslinking agent. In this method, the droplet emulsion is prepared previously and then is cross-linked by crosslinking agent to harden the droplet[6]. Cross-linker mostly used in previous research is glutaraldehyde which is saturated in toluene or glutaraldehyde saturated toluene (GST). This cross-linker interacts with chitosan by covalent crosslinking. The crosslinking reaction between the aldehyde group (glutaraldehyde) and amine (chitosan) is a covalent interaction that form a imine bond with an amino group (chitosan) and an acetal bonds with a hydroxyl group provides efficiency in the crosslinking with chitosan[7,8]. Emulsion crosslinking methods have been used to prepare chitosan-based microcapsules such as to encapsulate diclofenac sodium[7], ibuprofen[9], mitoxantrone (novantrone)[10], theophylline, griseofulvin, and aspirin[11], phenobarbitone[12], 5-Fluorouracil[13], red ginger oleoresin[14].

The emulsion crosslinking with ionic crosslinking agents such as TPP has never been done but usually uses the ionic gelation method. This method disperses bioactive (drug) into a polymer solution (biopolymer) then the mixture is put into a crosslinking agent solution or vice versa. The ionic gelation method is carried out without emulsion while the emulsion crosslinking is previously formed an emulsion then a crosslinking agent is added. TPP is a non-toxic polyanion. Ionic interactions between TPP and chitosan occur through electrostatic forces. Ionic cross-linked networks are created because of the interaction between negative ions from TPP with protonated amine groups[15]. The difference in covalent and ionic interactions between chitosan and crosslink agents gives different properties and results as microcapsule walls. This study provides information on the effect of using different crosslinking agents are glutaraldehyde saturated toluene (GST) and sodium tripolyphosphate (TPP) on yield, encapsulation efficiency, particle size, cumulative release, and characterization of chitosan-based microcapsules filled red ginger oleoresin. Chitosan-based microcapsules are used to protect red ginger oleoresin which is sensitive to light, heat, and oxygen so that active components such as shogaol, gingerol, and zingiberene are not degraded. Another disadvantage is having low solubility and bioavailability. The carrier of red ginger oleoresin in the form of microcapsules can improve stability, solubility, and bioavailability[16]. The aim of this study was to determine the characteristics of glutaraldehyde saturated toluene (covalent crosslinking agent) and sodium tripolyphosphate (ionic crosslinking agent) cross-linked chitosan-based microcapsules containing red ginger oleoresin.

MATERIAL AND METHODS:

Material

Red ginger oleoresin was produced from Lansida group, sodium tripolyphosphate from Sigma-Aldrich, 25% (v/v) glutaraldehyde solution and 100% (v/v) glacial acetic acid obtained from Merck, toluene technical provided by CV. Tri Jaya Dynamika, chitosan with a degree of deacetylation (DD) = 87.2% produced by PT. Biotech Surindo, corn oil obtained by CV. Surya Agung, and Petroleum Ether, n-Hexane, Methanol solution is the technical grade from CV. Labora.

Preparation of glutaraldehyde saturated toluene (GST):

25% (v/v) glutaraldehyde solution and toluene solution with a volume ratio of 1:1 was mixed at 500rpm for 3 hours. After that, the mixture was kept overnight then the top layer was taken as glutaraldehyde saturated toluene (GST). This step was referred from study that was reported by Thanoo, et al[11].

Preparation of chitosan-based microcapsules filled red ginger oleoresin using GST and TPP as crosslinking agents:

The preparation of chitosan microcapsules filled with red ginger oleoresin using the emulsion crosslinking method was a modification of the study reported by Jayanudin, et al[14]; Campos, et al[17], and Jarudilokkul, et al[18]. Preparation of chitosan-based microcapsules was begun by mixing 4g of red ginger oleoresin with 40mL chitosan solution with a concentration of 4% (w/v) then mixed with the homogenizer Ika-Werk Ultra-Turrax for 30 minutes to form oil in water (O/W) emulsion. The emulsion is added to 150mL of corn oil, then mixed and stirred again for 1hour to form oil in water in oil (O/W/O) emulsion. The second step was the addition of a GST or TPP as crosslinking agents for 10mL and 20 mL. The addition of GST or TPP is done in stages. After completing the addition of GST then added 2ml of 25% (v/v) glutaraldehyde solution. Whereas for TPP was added 2% (v/v) of glacial acetic acid solution to reach pH 5. The third step was chitosan microcapsules filtered then washed using petroleum ether followed by hexane.

The fourth stage was chitosan microcapsules dried in an oven with a temperature of 75ºC then analyzed.

Determination of yield:

Yield encapsulation is the ratio of weight between microcapsules with the total of the core red ginger oleoresin and chitosan. The determination of yield was used equation (1)

$$\%\ \text{"Yield"} = \frac{\text{(Total weight of microcapsules)}}{\text{(Weight of red ginger oleoresin+weight of chitosan)}} \times 100 \quad (1)$$

Encapsulation efficiency:

The step to determine encapsulation efficiency referred to the method that reported by Tan, et al[19] and Tonon, et al[20]. Surface oil is determined by adding 1g of microcapsules to hexane then shaken for 1 minute and dried. Whereas the total oil stage is determined by 1g microcapsules added to the soxhlet extractor and extracted in 200ml of methanol solution for 6 hours then dried and weighed. Equation 2-4 was used to determine encapsulation efficiency.

Total oil = initial weight - weight after extracted by Soxhlet 2)

Surface oil = initial weight - final weight of microcapsules 3)

$$\%\ \text{"Encapsulation efficiency"} = \frac{\text{(Total oil-Surface oil)}}{\text{(Total oil) x 100}} \quad 4)$$

Scanning Electron Microscope (SEM):

The difference in morphology of microcapsules cross-linked with GST and TPP was carried out using a scanning electron microscope (SEM) with JEOL type JSM-6510LV. Resolution: high vacuum mode: 3.0nm (30 kV) and low vacuum mode: 4.0nm (30 kV), acceleration voltage: 0.5 to 30 kV. The morphology analysis of red ginger oleoresin microcapsules was coated with platinum

Thermogravimetric analysis (TGA):

Differences in the strength of cross-linked microcapsule walls with GST and TPP were carried out by TGA/DTA analysis to determine the effect on temperature. Thermal stability analysis of microcapsules on temperature changes was carried out using thermogravimetric analysis (TGA) with DTG-60 detector. Samples were heated from 30 to 300ºC at a rate of 10ºC/min with a constant nitrogen flow rate of 30mL/min.

Analysis of particle size

100 microcapsules were observed to determine the average diameter of microcapsules using a digital microscope. The equation 5 was used to determine the average diameter of microcapsules.

$$\bar{d} = \sum_{i=n}^{n} \frac{d_i}{N} \quad 5)$$

Where, d_i is each microcapsule diameter, N is total number of calculated microcapsules and $\bar{d}$ is the number of average diameter.

Release analysis of red ginger oleoresin from chitosan-based microcapsules.

The release process of red ginger oleoresin from chitosan-based microcapsules was carried out in phosphate buffer with a pH of 7.4 and 30ºC. 0.2g oleoresin microcapsules of red ginger were put into the phosphate buffer solution and stirred at 220rpm. 10ml samples were taken to be analyzed by UV-Vis spectrophotometer with a wavelength of 283nm. This method referred to study that reported by Jayanudin et al[21]. The release mechanism of red ginger oleoresin from microcapsules was used by the Korsmeyer-Peppas model which was seen in equation 6.

$$\frac{M_t}{M_\infty} = kt^n \quad 6)$$

Where, $\frac{M_t}{M_\infty}$ = the cumulative release, k = constants of Korsmeyer-Peppas, and n = the release mechanism.

The diffusion coefficient (D) uses the equation reported by Siepman and Siepman[22] which could be expressed in equation 7.

$$\frac{M_t}{M_\infty} = 1 - \exp\left[\frac{3R_0DKt}{R_i^2R_0 - R_i^3}\right] \quad 7)$$

Where, D was the diffusion coefficient; R_0 was the outer radius of microcapsules; R_i was the radius in microcapsules; K was the partition coefficient of the drug between the membrane and the reservoir.

RESULTS AND DISCUSSION:

The differences mechanism of ionic and covalent interaction of cross-linking agents with chitosan:

Chitosan has great potential as a drug carrier to provide controlled release. One method for preparing chitosan microcapsules is emulsion crosslinking. There are two types of crosslinking agents, that are ionic and covalent crosslinking agents. Both types of crosslinking agents have different characteristics. Ionic crosslinking agents form electrovalent (ionic) bonds between crosslinking agents and chitosan chains. There are two types of crosslinking agents, namely ionic and covalent crosslinking agents. Both types of crosslinking agents have different characteristics. Ionic crosslinking agents form electrovalent (ionic) bonds between crosslinking agents and chitosan chains. The electrostatic attraction of polymer chains to the ionic crosslinking agents induces ionic crosslinking effects. While covalent crosslink agents produce covalent bonds between crosslinking agents and polysaccharide chains (polymers). The commonly used crosslinking agent is glutaraldehyde[23].

The type of crosslinking agent used in this study was glutaraldehyde which was saturated in toluene or called glutaraldehyde saturated toluene (GST) and sodium tripolyphosphate (TPP). The crosslinking process between GST or TPP and chitosan has the same initial mechanism of emulsion formation. This study used a double emulsion; it was oil layer (corn oil) and chitosan layer which wrapped red ginger oleoresin. After the emulsion was formed then the GST as a crosslinking agent was added. Toluene would dissolve in the oil layer while glutaraldehyde would crosslinking with chitosan evenly to create a layer of chitosan to form a gel and with crosslinking further make chitosan denser. Uniform crosslinking process makes microcapsules have good spherical geometry[11]. Chitosan cross-linked with GST forms covalent imine bonds because of resonance generated with adjacent double ethylenic bonds via the Schiff reaction. Glutaraldehyde is a dual function crosslinking agent that is not only interacting with amino groups but also interacts with carboxyl groups and other groups of proteins[24]. Illustration of the crosslinking reaction between chitosan as a wall material with crosslinking agents of covalent (glutaraldehyde) and ionic (sodium tripolyphosphate) could be seen in (Fig. 1).

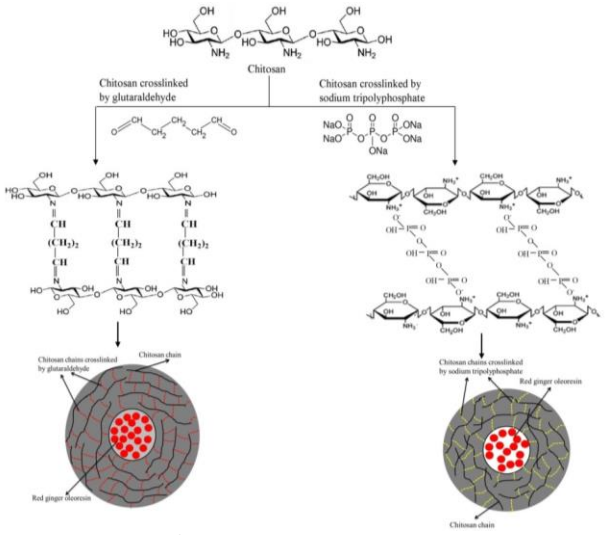

Fig. 1: Schematic of cross-linking interactions between chitosan as wall material of microcapsules with glutaraldehyde and tripolyphosphate as crosslinking agents

The crosslinking mechanism of chitosan with TPP solution was done by dropping TPP solution into the emulsion droplet then glacial acetic acid is added to adjust the pH. at lower pH, TPP would be more ionized in the form of phosphate ions ($–P_3O_{10}^{-5}$) compared to hydroxy ions ($-OH^-$). While at higher pH, TPP will be ionized in $-OH^-$, so the cross-linking of TPP could occur ionically between $–P_3O_{10}^{-5}$ with $–NH_3^+$ ions of chitosan and $-OH^-$ with $–NH_3^+$ ions from chitosan because of deprotonated[25]. Initially there was competition between $-OH^-$ and $–P_3O_{10}^{-5}$ ions to react with amino groups. After gel formation in the outer layer, $–P_3O_{10}^{-5}$ ion resistances is greater to diffuse through the gel film into a higher matrix compared to OH- ions because the molecular size is smaller than $-OH^-$, then gelation of chitosan is produced from neutralizing the protonated chitosan amino group $-OH^-$ ion. These results indicate that complex deposition is formed by deprotonation and ionic crosslinking by $-OH^-$ and $–P_3O_{10}^{-5}$ ions which dissociate from TPP solution diffuse into chitosan droplets during the solidification process[26].

Data for yield, encapsulation efficiency, and particle diameter for red ginger oleoresin microcapsules cross-linked with GST have been reported previously by Jayanudin, et al[14].

Yield

The comparison of differences in crosslinking agents and their effects on the yield and efficiency of chitosan microcapsules filled with red ginger oleoresin could be seen in (Fig. 2).

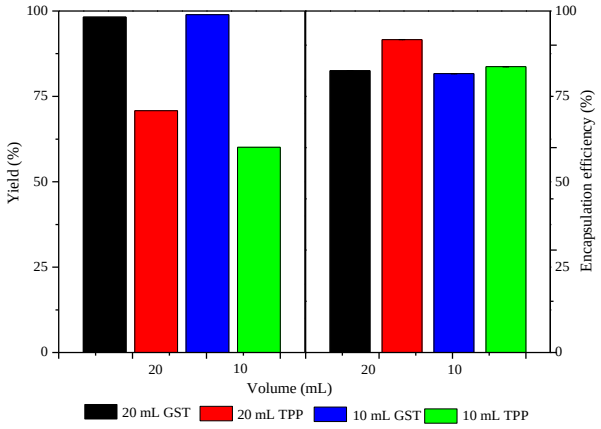

Fig. 2: Comparison of ionic and covalent cross-linkers to the yield and encapsulation efficiency effect of chitosan microcapsules filled with red ginger oleoresin. The chitosan microcapsules were prepared using 4% (w/v) concentration of chitosan solution, and volume at 20 mL and 10 mL for crosslinking agents, whereas for the preparation of chitosan microcapsules cross-linked with TPP was done pH adjustment at 5

Fig. 2 showed the comparison of yield produced from microcapsules using GST and TPP as crosslinking agents. The concentration of TPP used was 5% (w/v), while the concentration of glutaraldehyde in toluene was 3.3 $\frac{g}{100\ mL}$. This highest value of the concentration of glutaraldehyde in toluene has previously been reported by Jayanudin, et al[27] which was analyzed using hydroxylamine hydrochloride. Yield produced by cross-linked microcapsules with GST was higher than TPP in all volumes of crosslinking agents, although the concentration of glutaraldehyde solution in toluene (GST) was lower than TPP solution. The highest

encapsulation yield was 98.93% obtained from chitosan cross-linked with 10mL of GST, while the lowest yield is 60.12% from chitosan cross-linked with 10mL of TPP. The yield obtained from this study was higher than reported by Panchal, et al[28], which was 96.54% using 12 mL GST and also lower than the study reported by Ofokansi, et al[9] which was 98.53% at 10mL GST and decreased at 84.43% when using 20mL GST.

The yield of encapsulation obtained using the TPP as a crosslinking agent from this study was higher than the study reported by Patel, et al[29], which was 55.1-61.2% because in this study the concentration of TPP used was higher. Research conducted by Patil, et al[30] obtained a higher yield of 78.57-97.58%, but used a different crosslinking method. The research method carried out by Patil, et al[30], chitosan solution was added to TPP solution, while in this study a crosslinking process was carried out by dripping TPP solution into chitosan. While in this study a crosslinking process was carried out by adding TPP solution dropwise into the emulsion (emulsion of red ginger oleoresin in chitosan layer in corn oil layer or oil in water in oil (O/W/O)).

The cause of the low yield of chitosan microcapsules crossed with TPP is that the acid solution forms an unstable bond between chitosan and TPP solutions[23]. pH greatly influences the ionic activity of the phosphate group from TPP with amine from chitosan. pH adjustment was done by adding 2% (v/v) acetic acid. Therefore, the emulsion pH was lowered to pH 5 for increasing ionic interaction and provides more negative sites, but this addition causes a decrease in the concentration of chitosan solution in the emulsion. While for chitosan microcapsules cross-linked with GST did not need to adjust the pH of the solution to form microcapsules. This factor was likely to cause yield encapsulation with TPP lower than GST.

Encapsulation efficiency:

Encapsulation efficiency determines the amount of core material (bioactive) in microcapsules. Encapsulation efficiency is determined by the amount of surface oil and total oil. Surface oil is the amount of bioactive (core material) that is on the surface of the microcapsules, while total oil is the total core material (bioactive) that exists on the surface and in microcapsules. The higher bioactive that encapsulated that was the success of the encapsulation process. Figure 3 showed a comparison of chitosan microcapsules cross-linked with GST and TPP against encapsulation efficiency.

The encapsulation efficiency produced from chitosan microcapsules cross-linked with GST and TPP did not show a significant difference. The highest of encapsulation efficiency was obtained from chitosan microcapsules cross-linked with 20 mL TPP of 91.63±0.02% and the lowest was obtained from chitosan microcapsules cross-linked with 10mL GST of 81.65±0.03%. Increased GST volume makes many bonds that form between aldehyde and amino and cause the microcapsule walls to become more rigid. Denser microcapsule walls make a little red ginger oleoresin diffuse out from the core of the microcapsules during the solidification process. The same thing happened to the crosslinking agent of TPP. Increased TPP volume resulted in higher encapsulation efficiency. The encapsulation efficiency of microcapsules cross-linked with TPP was higher than GST. This was likely because chitosan microcapsules cross-linked with GST produced denser microcapsule walls compared to cross-linking with TPP so that when the microcapsules were extracted there was still red ginger oleoresin in microcapsules. This made the value of total oil was lower and caused the encapsulation efficiency of microcapsules to be lower.

Particle size

The particle size of the microcapsules cross-linked with TPP was smaller than GST. The difference in particle size produced was very significant. The particle size of the chitosan microcapsules using GST at 20ml was generated at 161.42±40.1µm. This particle size was 3.9 times larger than the particle size of chitosan microcapsules cross-linked with 20mL TPP was 41.43±8.3µm. As well as the use of 10ml crosslinking agent, the particle size of microcapsules cross-linked with GST was 167.62 ±39.01µm greater than 2.4 times compared to TPP which was 69.88±11.6µm. The particle size comparison of the microcapsules from crosslinked chitosan with GST and TPP is shown in (Fig. 3).

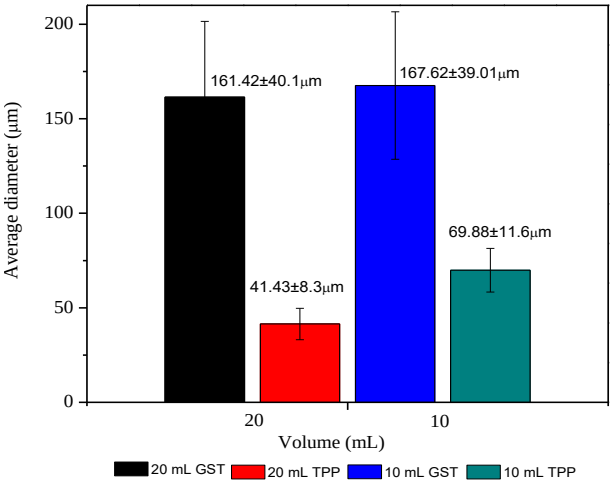

Fig. 3: Comparison of the diameter size of microcapsules filled with red ginger oleoresin cross-linked with GST and TPP. Chitosan microcapsules were prepared with 4% (w/v) concentration of chitosan solution and volume of crosslinking agents (GST and TPP) of 10mL and 20mL. For microcapsules cross-linked with TPP was added acetic acid to adjust pH 5

The preparation process of chitosan microcapsules filled with red ginger oleoresin begun with the prepare emulsions between red ginger oleoresin and chitosan then followed by the formation of the second emulsion where the first emulsion was added to corn oil and stirred to form a second emulsion. This stage was the same for both crosslinking agents (GST and TPP) and the next step was added the crosslinking agents. The effect of adding GST might not change the size of the droplet emulsion causing the size of the microcapsule to be the same as the size of the droplet emulsion but for the addition of the crosslinking agent of TPP, there is a change in the size of the droplet emulsion. Sodium tripolyphosphate (TPP) is a crosslink agent and also as a surfactant that could affect the size of the droplet emulsion. The decrease in interface tension is caused by the addition of TPP[31]. The low interface tension makes droplet emulsion size decrease[32]. The Low value of interface tension occurred because of surfactant adsorption, polyelectrolyte adsorption caused by steric barriers, and adsorption of polymer-surfactant complexes leads to high measurements of dilational elasticity which was associated with larger aggregates formation[33]. In addition, the preparation of chitosan microcapsules with TPP had the stage of adding acetic acid to adjust pH. This stage affects the interaction of phosphate ions of TPP solution and amine groups of chitosan. The side effect of adding acetic acid has decreased the concentration of chitosan solution in the emulsion. The effect of decreasing chitosan concentration made the droplet size decrease. These factors caused the use of TPP to produce microcapsule size to be lower compared to GST.

The size of cross-linked microcapsules with GST in this study was larger than reported by Ofokansi, et al[9], which were 116.72±9.16µm for 20ml GST and 100.05±8.82 µm for 10 ml GST. Whereas the chitosan cross-linked with TPP as the wall material of microcapsules in this study was larger than reported by Wu, et al[34] which was produced particle size of 172.3±5.49 - 217.2±9.67nm, but the research method was different namely the mixture of chitosan solution and drug was stirred and then TPP was dropped with a concentration of 0.1% (w/v) to the mixture for the crosslinking process. While in this study a double emulsion was formed then TPP with a concentration of 5% (w/v) was dropped on the emulsion.

Morphological analysis of red ginger oleoresin microcapsules:

Fig. 4 showed a morphological comparison of red ginger oleoresin microcapsules cross-linked with GST and TPP. Chitosan microcapsule walls cross-linked with GST shown in Fig. 4A3 were more compact than cross-linked with TPP (Fig. 4B3). Fig. 4A2 showed the chitosan microcapsules cross-linked with GST had good spherical geometry and smooth surface compared to chitosan microcapsules cross-linked with TPP (Fig. 4B2). Good spherical caused by a crosslinking reaction that is evenly distributed throughout the surface.

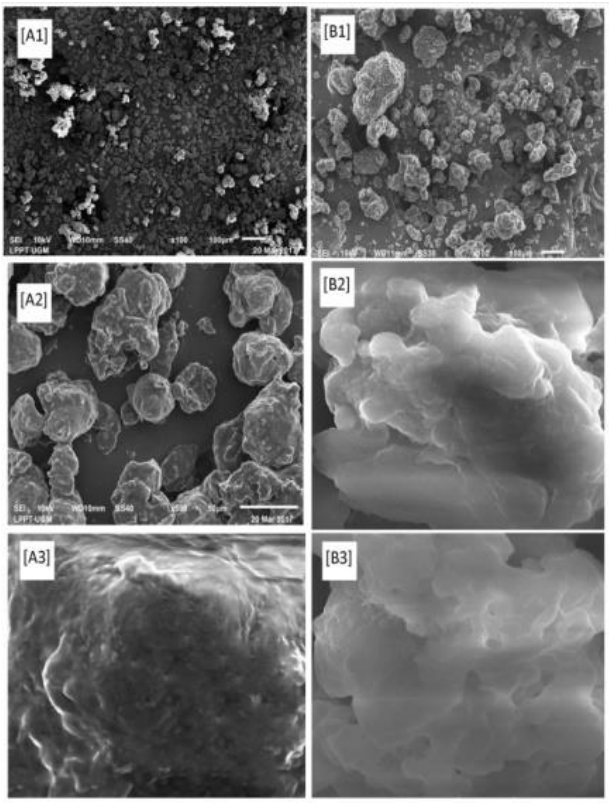

Fig. 4: Surface morphology analysis of chitosan microcapsules filled with red ginger oleoresin prepared using crosslinking agents: [A] glutaraldehyde saturated toluene (GST), [B] sodium tripoliphosphate. SEM magnification for [A1] = 100x, [A2] = 500x, and [A3] = 2000x, while for [B1] = 100x, [B2] = 1000x, and [B3] = 2000x

Glutaraldehyde saturated toluene (GST) makes the solubility in oil medium uniformly available for cross-linking on droplet surfaces[11] whereas microcapsules crosslinked with TPP did not produce microcapsules with perfectly spherical geometry because of the possible crosslinking reaction of droplet surfaces with TPP did not occur uniformly. This is related to the solubility of TPP solution in oil medium was low.

TGA/DTA Analysis:

TGA/DTA analysis is an analysis conducted to determine the effect of temperature changes on microcapsules. TGA/DTA analysis of red ginger oleoresin microcapsules can be seen in (Fig. 5).

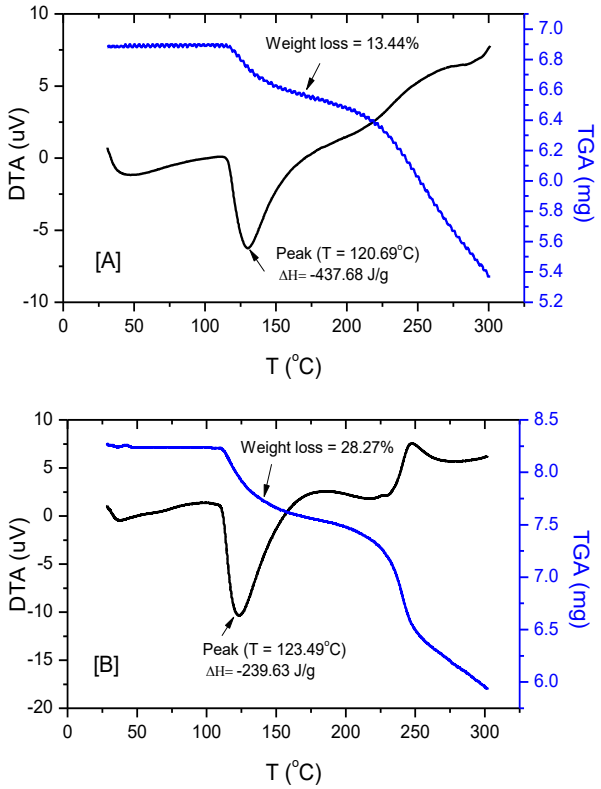

Fig. 5: Comparison of TGA and DTA analysis of chitosan microcapsules cross-linked with [A] glutaraldehyde saturated toluene (GST) and [B] Sodium tripolyphosphate (TPP). Chitosan microcapsules made from 4% (w/v) concentration of chitosan solution, 20 mL TPP solution, and for chitosan microcapsules cross-linked with TPP was used pH 5

Fig.5 showed a TGA analysis for chitosan microcapsules cross-linked with GST was begun from temperatures of 30 to 300ºC and occurred in two stages. The first stage occurred at a temperature of 30-110ºC and the second stage occurred at 120-300ºC. Chitosan microcapsules cross-linked with TPP occurred from 30-112ºC for the first stage and the second stage at 112-300ºC. The first stage was a decrease in mass because of water evaporation contained in microcapsules and the second stage was melting and decomposition of microcapsule walls. This second stage had a significant loss of microcapsule mass due to the occurrence of dehydration of saccharide rings, depolymerization and decomposition of acetylation units and deacetylation of polymers[35,36].

The mass loss of chitosan microcapsules cross-linked with GST was lower (13.44%) than TPP (28.77%). Whereas enthalpy at the melting point of chitosan microcapsules cross-linked with GST was higher, namely $\Delta\widehat{H} = -437.68\ J/g$ than TPP which was $\Delta\widehat{H} = -239.63\ J/g$. Based on the DTA analysis showed that the microcapsule walls of chitosan which were cross-linked with GST were harder than the TPP.

Release of red ginger oleoresin from chitosan microcapsules:

The interaction between crosslinking agents and chitosan solution could affect the strength of the microcapsules wall and directly influence the amount of core material (drug) released from the microcapsules. Figure 7 showed a comparison of the amount of red ginger oleoresin released from microcapsules with chitosan wall material that cross-linked ionically and covalently. This study was used glutaraldehyde saturated toluene (GST) and sodium tripolyphosphate (TPP) as crosslinking agents.

The release medium used in this study was phosphate buffer solution at pH 7.4 and temperature 37ºC. The release process begins with the diffusion of the buffer solution into the microcapsules and dissolves the red ginger oleoresin then diffuses back out through the microcapsule wall. The density of microcapsule walls could affect the release rate of red ginger oleoresin. Chitosan as a wall of microcapsules cross-linked with GST and TPP has different characteristics because it produces different densities. Figure 7 showed that the number of red ginger oleoresin released from chitosan microcapsules cross-linked with GST was lower than TPP for the volume of 20ml and 10ml. Chitosan microcapsules cross-linked with 20ml of GST produced a cumulative release at 50.01% while with 20ml TPP produced at 57.78%. For chitosan microcapsules cross-linked with 10ml of GST generated the cumulative release of 51.99% and 10ml of TPP was produced at 63.71%.

The low red ginger oleoresin released from chitosan which was cross-linked with GST was denser than TPP. Fig. 5 shows physically; the chitosan microcapsules cross-linked with GST look more rigid than TPP. As well as the results of the TGA/DTA analysis shown in Figure 6, the enthalpy value needed to melt chitosan microcapsules cross-linked with GST higher than TPP. The study reported by Gonçalves, et al[37] compared ionic and covalent crosslinking agents were epichlorohydrin and glutaraldehyde to the release of diclofenac sodium. The result was diclofenac sodium released from microspheres crossed with glutaraldehyde (covalent crosslinking) was lower compared than epichlorohydrin (ionic crosslinking) after release of 12 hours. Chitosan microcapsules cross-linked with glutaraldehyde (covalent) have high porosity and higher bioactive loading, lower the degree of polymer swelling make drug release was low. Chitosan which was cross-linked with ionic produces a lower porosity, a higher swelling rate causes access of solvent to the drug through the polymer matrix making it increase contact with it and results in greater release[37]. The results of this study had the same phenomenon reported by Gonçalves, et al[7] that the bioactive release from chitosan microcapsules cross-

Table 1: Value of release kinetics and diffusion coefficient of chitosan microcapsules prepared using crosslinking agents of GST and TPP

Parameters	Model of Korsmeyer-Peppas			Partition coefficient between membrane and reservoir (K)	Diffusion coefficient (D) cm^2/s
	k_{K-P}	n	R^2		
20 ml GST	4.122	0.37	0.69	0.955	7.962×10^{-13}
10 ml GST	4.069	0.38	0.71	1.151	9.591×10^{-13}
20 ml TPP	3.621	0.41	0.76	0.394	3.282×10^{-13}
10 ml TPP	3.642	0.42	0.77	0.733	6.112×10^{-13}

linked with ionic crosslinking agents (TPP) was a greater release compared to covalent crosslinking agents.

The release mechanism was done by determining the release kinetics with the Korsmeyer-Peppas model using equation 6. The Korsmeyer-Peppas constant (k_{K-P}) and the release mechanism (n) could be seen in Table 1.

The release mechanism that occurs in the red ginger oleoresin microcapsules prepared with the crosslinking agent GST and TPP uses the Korsmeyer-Peppas model. The value of n obtained from this study was between 0.37-0.42. The value of n obtained was then referred to the provisions made by Ritger and Peppas[37], which were released in diffusion. The determination of the diffusion coefficient uses the equation reported by Siepman and Siepman[22] shown in equation 7.

This equation was used for drugs that were molecularly dispersed in the core which was encapsulated by the membrane. After microcapsules come into contact with the liquid then penetrate into the system, dissolved the drug and diffuse out of the device through the surrounding membrane. The diffusion step of this drug was considered mathematically using Fick's law. The diffusion coefficient obtained from this study was shown in Table 1. The diffusion coefficient of microcapsules using GST was greater than TPP. This was related to the diameter of microcapsules where the diameter of microcapsules with GST was greater than that of TPP.

CONCLUSION:

The comparison between microcapsules with wall materials from chitosan cross-linked with glutaraldehyde saturated toluene (GST) and sodium tripolyphosphate (TPP) produced different characteristics based on yield, encapsulation efficiency, and particle size. The highest yield and particle size were obtained from chitosan microcapsules cross-linked with GST were 98.93% and 167.62±39.01µm while the largest encapsulation efficiency was obtained from chitosan microcapsules cross-linked with TPP was 91.63±0.02%. The highest cumulative release value was obtained from chitosan microcapsules cross-linked with 10 ml TPP was 63.71±0.7% and the lowest cumulative release was 50.01±1.2% from chitosan microcapsules cross-linked with GST. The release mechanism of red ginger oleoresin from chitosan microcapsules occurred by diffusion with the highest diffusion coefficient obtained from the microcapsules cross-linked with GST which was 9,591 x $10^{-13}$$cm^2/s$ and the lowest at 3,282 x 10^{-13} cm^2/s was obtained from chitosan microcapsules cross-linked with TPP.

ACKNOWLEDGEMENT:

The authors are grateful to the Chemical Engineering Department, Gadjah Mada University for the availability of all research facilities. We also thank to the Chemical Engineering Department of Universitas Sultan Ageng Tirtayasa for all their support so this research has been a success.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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