

The Effect of Tri Poly Phosphate Concentration towards Chitosan-Alginate Micro-particle Characterization and Dissolution Process in Acidic Medium

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Abstract— Chitosan-Alginate micro-particles can serve as coating to the drug through the encapsulation process for controlled release. The addition of Tri Poly Phosphate would change the characterization of Chitosan-Alginate micro-particles because of the process of cross linking. This study aims to determine the characterization of micro-particles of Chitosan-Alginate based on particle size distribution, particle surface morphology and dissolution process in acid medium. The method used to make micro-particles of Chitosan-Alginate is using spray drying, thus the micro-particles can be made by mixing Chitosan-Alginate with a weight ratio of 1: 1, 1: 2, 2: 1 then mixed the crosslinking compound Tri Poly Phosphate (TPP) with a concentration of 0.1%, 0.3%, 0.5%, and added emulsifier Tween 80 3% v / v, stirred at a speed of 20,000 rpm for 10 minutes then inserted into the spray dryer to form micro-particles. The powder of micro-particles then analysed by Scanning Electron Microscopy (SEM) and performed in vitro of stomach acid solution using acidic medium for the test of dissolution. Based on the research that has been done, 0.1% TPP concentration of Chitosan-Alginate ratio of 2: 1 can produce the most uniform size distribution with a ratio of 40.54%. The biggest dissolution of micro-particles obtained from the samples with Chitosan-Alginate ratio of 2: 1 with a concentration of 0.1% TPP was 80.8%.

Keywords— micro-particle; chitosan-alginate; tri poly phosphate; spray dryer

I. INTRODUCTION

Encapsulation process has been widely used in drugs, cosmetics, food, and drinks industries. Encapsulation process needs non-toxic coating agent and saves to be consumed. Natural polymer can be used as the safe coating agent due to the biodegradability in human digestive system and is now widely used for medical treatment especially as drug delivery system. The Natural polymer is used to replace the synthetic polymer as the coating agent because the production of synthetic polymer uses heat and organic solvent that can affect the bioactive component structure and bio-stability. The production process of natural polymer that used a simple technique and low operation condition, supported by the solubility in water, bio-degradability, bio-compatibility, and non-toxicity, makes the natural polymer, such as Chitosan, Alginate, gelatine, and maltodextrin, the right coating agent in drug delivery system [1].

Recent studies have used a combination of two coating agent, such as Chitosan and Alginate. The advantage to combine this biopolymer of coating agents is the availability to form a polyelectrolyte complex. [2], [3].

Chitosan is a cationic bio-poly-amine-saccharide as the result of chitin deacetylation process from Anthropode and sea Crustacea. Chitosan is the largest poly saccharide after cellulose [4]. The positive charge of Chitosan that reacts to the negative charge of a surface affects the retention time and charge availability [5]. Chitosan is biopolyaminosaccharide of the process of deacetylation of chitin, which is the largest source of polysaccharide after cellulose, derived from animal's arthropods and marine crustaceans and properties are biocompatible, biodegradable and non-toxic [6].

Alginate, another natural polymer that has widely used as coating agent, can form a simple matrix structures, bio-compatible, non-toxic, and can protect the bioactive component from heat and moist that raised the stability and

bio-availability [7]. Alginate can also be used as fiber sources to reduce cholesterol level in blood [8].

Chitosan has positive charge due to the presence of amino group while Alginate has negative charge due to the carboxyl group. The coating process that only used one of these natural polymers will not be stable [9], but if the natural polymer with different charges bound to form non-charge molecule, the coating will be stable in acid condition to slower the drugs degradation in stomach and dissolve in intestine pH condition [10].

Controlled release is a condition when the coating of an active substance has to be modified to be able to pass through various factors in human body such as pH, enzyme, light, magnetic field, temperature, ultra sonic, osmotic or time [11]. Thus, the effectiveness of the active substances in the place and time of releasing of the active substances can be controlled. Encapsulation is a technology that can control the rate dissolution of the microcapsule layer as the coating agents. Encapsulation has been applied in many industries such as Pharmaceutical industry, cosmetic, food and drinks, and also Agro industry.

Release characteristic of the micro-particle can be identified by the cross link agent used as the stabilizer. Substances that can be used as cross link agent are formaldehyde, glutaraldehyde, and Tri Poly Phosphate [12]. Tri Poly Phosphate is chosen to be the cross link agents because it is safe and non-toxic [12]. The mixing of Chitosan and Alginate with the addition of Tri Poly Phosphate as coating agent will change the character of the mixture as drug delivery system. The aim of this research is to determine the effect of Tri Poly Phosphate concentration towards the characters of Chitosan-Alginate micro-particle in the dissolution process.

The method to formulate the Chitosan-Alginate micro-particle is Spray Drying because Spray drying is a process of changing from a liquid into a dry powder by contacting medium heat (hot air). The feed can be suspension, emulsions and pastes in which the powder properties depend on physical and chemical properties [13].

Spray drying is the oldest method of microencapsulation in food industry and was discovered in 1930 [14]. Spray drying is the process of spraying the material through the atomizer and contacted with hot air to produce powder with 10-50 μm size and the largest size of the particle is 2-3 mm [15]; [16]. A crosslink agent is needed to make the density of the Chitosan-Alginate micro-particle. The crosslink agent used in this experiment is Tri Poly Phosphate, as the best crosslink agent used in the previous research by Shu dan Zhu (2002) [17], due to the fact that Tri Poly Phosphate has a high negative charge density that increase the interaction with poly-cationic Chitosan. Yongmei dan Yumin (2003) [18], stated that the formation of micro-particle could only happen on a certain concentration ratio of Chitosan and Tri Poly Phosphate. Tri poly phosphate increase the mechanical strength of the matrix of Chitosan-Alginate micro-particle, thus the Chitosan will be hard and not easily be broken into small pieces [19].

Study of modified chitosan micro-particles using an emulsifier which is a crosslinking compound and a surfactant. Based on research Silva et al. [20] note that the addition of surfactant can reduce the particle size of the

chitosan-alginate. Crosslinking agent that is often used is glutaraldehyde, while a widely used surfactant is a non-ionic surfactant because it has a non-ionic hydrophilic group such as Tween 80 and Span 80. Tween 80 and Span 80 nontoxic commonly used as an emulsifier and stabilizer in food and pharmaceutical fields. Tarirai [21] has been conducting research on gelling chitosan as a carrier of ibuprofen by using crosslinking compound Tri Poly Phosphate and surfactant compound that also functions as a crosslinking, i.e., oleic acid, sodium lauryl sulphate (SLS) and Tween 80.

The surface of the microcapsule produced will analysed with SEM/ SEM is a scanning electron microscope which is able to produce high-resolution images of a sample surface. The image produced by the SEM has the characteristic of three-dimensional appearance, and can be used to determine the surface structure of the sample. The result of the SEM image is displayed only in black and white. SEM apply the principle of electron diffraction in which the same measurement as an optical microscope. The principle is fired electron will be deflected by the electromagnetic lens in SEM. Scanning Electron Microscope (SEM) is a type of electron microscope that uses an electron beam to draw the object surface profile. The working principle of SEM is firing a surface with high-energy electron beam. Surfaces that are subject to the file will reflect that file or generate secondary electrons in all directions. But there is one way in which the reflected beam with the highest intensity. Detectors in SEM detect reflected electrons and determine the location of the reflected beam with the highest intensity. Directions provides information profile surfaces such as how linear and where the direction of the slope. Terms that SEM can produce sharp images are reflective surfaces must be able to release electrons or secondary electrons when shot with an electron beam.

Releasing the active substance from the polymer coating can be happen through three mechanism; direct diffusion, degradation and swelling followed by diffusion. Diffusion happened when the active substances release through the pores of the polymer matrix that has uniform size and certain thickness of the matrix, thus the release of active substances will be constant in a certain period. The process starts when the polymer chains swelled due to interaction with pH, temperature, enzyme, or other stimulus. After the polymer chain swelled, thus the active substances will be diffused constantly, until no more active substances left. Afterwards, the polymer will be degraded as the result of hydrolysis process to be small molecules that can be absorbed by human body's system. Micro-particle of chitosan-alginate that has been produced is evaluated for the degradation by solubility testing with in vitro dissolution method means that experiments were conducted in a controlled environment.

The test is performed to determine the resistance of the microcapsules produced at the time of passing through the digestive process in the body. Micro-particles of chitosan-alginate coating is applied as a remedy which can serve to protect the drug in the process of passing through the digestive system of the stomach, because stomach acid that has a pH of dissolution media, it can be assumed that resembles the gastric pH controlled at pH 1.2 and a healthy person's body temperature is 37°C.

Media acids selected because at acidic pH will be positively charged chitosan while above pH 9 (alkaline) chitosan particles will lose its cargo from loss of H⁺ ions excess NH₃⁺ + bound by OH⁻. The Particles of chitosan that has a pH sensitivity can be used for drug delivery in the stomach cavity acid so that the particle size is maintained not undergo aggregation. [22].

Determination of the solubility profile of chitosan-alginate micro-particles is a prior measurement of the maximum wavelength of chitosan by using UV-Vis spectrophotometer. The dissolution was carried out for 1 hour by sampling as much as 6 times at specified time intervals.

The aim of this research is to determine the effect of Tri Poly Phosphate concentration and Chitosan-Alginate ratio towards pore size distribution and to determine the characters of the micro-particle using SEM.

II. MATERIAL AND METHOD

A. Material

Sodium Alginate and Food grade Chitosan was obtained from CV. Bio Chitosan Indonesia. Acetic Acid Glacial 100%, Tween 80, KCl, HCl and Sodium Tri Poly Phosphate was analytical grade from MERCKTM.

B. Method

1) *Preparation of Chitosan-Alginate microparticle:* Chitosan solution 2% 1000 mL was made by solubilizing Chitosan with acid acetic glacial 1% (v/v). Solution of sodium Alginate 1% were then added to Chitosan solution with weight ratio of Chitosan:Alginate were 1:2, 2:2 and 2:1, while continuously stirred. Afterwards, the Tri Poly Phosphate were added with various concentration 0,1%, 0,3% and 0,5% gradually while keep on stirring. The emulsion process were started by addition of 5 mL Tween 80 then stirred vigorously with homogenizer at 20000 rpm for 10 minutes.

2) *Spray drying (Figure 1):* The emulsion was then put into spray dryer with inlet temperature 120°C and outlet 85°C to form micro-particle.

3) *SEM analysis:* Micro-particle powder was analysed with SEM Hitachi type SU3500 to determine the size and the morphology of the particle surface.

4) *Dissolution Test of the Chitosan-Alginate-TPP micro-particle:* The test was done in the acidic medium in accordance with the Indonesian Farmacopeia. As much as 500 mg of the Chitosan-Alginate micro-particle were placed into a chamber filled with KCl-HCl (pH 1.2) for 60 minutes at 37 ± 0.5°C and 150 rpm. Sample was taken every 10 minutes 10 ml each, and the concentration was then analysed using Spectrometer UV-Vis at 258 nm. Every 10 ml sample taken was replaced by the same volume of medium solution.



Fig. 1 Spray Dryer

III. RESULT AND DISCUSSION

A. The effect of chitosan-alginate ratio and concentration of Tri Poly Phosphate towards the particle size distribution

The process of making Chitosan-Alginate micro-particle begins with making the emulsion of Chitosan-Alginate micro-particles, by mixing the Alginate solution with solution of Chitosan. This mixing of the Alginate solution with solution of Chitosan will form the coordinate covalent bonding interactions. Chitosan has -NH₂ group that will push the carboxyl group of Alginate by giving electrons to C atom of the carboxyl group, thus the C atom will have excess of electrons and releasing OH⁻ groups. With the loss of the amine group in Chitosan, Chitosan characteristics as soluble substance in acidic medium can be eliminated so that the matrix of chitosan will be more stable in acid. Alginate as a compound which binds to the Chitosan is a hydrophilic compound.

Chitosan-Alginate is an attempt to be used as a coating in the encapsulation process to release the drug. The addition of Tri Poly Phosphate (TPP) acts as a cross-connecting material that serves as a hardener and maintain the form of the micro-particles, so that the active components in the microcapsules releases slowly and able to achieve the desired target.

TPP concentration is varied, 0.1%, 0.3% and 0.5%. The variation is used to determine the effect of the TPP concentration towards particle size and characterization of the resulting particle. The addition of TPP has the function as the cross-linking, where the protonated amine (NH₃⁺) would bind with O⁻ group of TPP. Layer formed in the result of cross linking between Chitosan-Alginate with TPP will trap the drug / active substance that bind to the Chitosan-Alginate.

The addition of tween 80 in the emulsion process of Chitosan-Alginate micro-particles at a concentration of 3% (v/v) serves as emulsion stabilizer/surfactant. The presence of surfactant will make the Chitosan particles be covered and stabilized from one another so that the process of solving the particles will be more effective and prevent clotting (agglomeration) between the particles. Stirring is carried out

at room temperature with a speed of 20,000 rpm. Stirring aims to break large molecules into smaller portions. Then the emulsion of Chitosan-Alginate solution with diverse formulations will be produced.

The solution / emulsions that have been made will then be atomized in a stream of hot air to the formation of tiny droplets in the spray dryer apparatus. From this process, the solvent evaporates and produces particles that move freely. Then the results of Chitosan-Alginate micro-particles powder will be analysed using SEM to see the surface morphology, shape, micro-particles size and size uniformity. The dissolution analysis is carried out to analyse the solubility in acidic media

Particle size distribution was analysed by SEM (Scanning Electron Microscope). The variety of the material used in this experiment can be seen at Table 1. The Chitosan-Alginate microparticle formed using the spray dryer have relatively low percentage distribution (below 50%), this could happen because the rate flow to the spray dryer is not stable.

The pressure that apply to the nozzle part of the spray dryer effected the average particle size, because the particle size is the function of rate per nozzle and spray pressure. Fig. 2. shows the percentage of Chitosan-Alginate particle size distribution.

TABLE I
VARIETY OF CHITOSAN-ALGINATE MICROPARTICLE RATIO

Sample	Variation	
	Ratio of Chitosan/Alginate	Concentration of Tri Poly Phosphate
A	1:1	0.1%
B	1:2	
C	2:1	
D	1:1	0.3%
E	1:2	
F	2:1	
G	1:1	0.5%
H	1:2	
I	2:1	

Generally, the whole formula of micro-particles have no uniform particle size, that is proved by the percentage of micro-particles less than 50%, this is presumably because in the process of making the emulsion, the stirring of micro-particles using a stirrer with a speed of 20,000 rpm, makes the energy deployment is not the same, so that the reflected energy to the emulsion molecules in the solution will be different. Thus, the different reflection causes molecules having different characteristic, some will break faster, the other have a longer rupture resulting in larger particle size and non-homogeneous particle size [23]. Particles that have a highest uniform size are in the formula C with the largest percentage of 40.54% the content of Chitosan-Alginate 2: 1 with a concentration of 0.1% TPP.

The particle size distribution in the sample is obtained by counting the number of particles in each size range of 0-10 µm to the total number of micro-particles. Profile particle

size distribution of particles to form a peak where there are peaks and shaped like a parabola. The narrow area and the highest peak indicated the particle size distribution more homogeneous / uniform otherwise the greater area shows the particle size distribution is not homogeneous. In figure 3 shows the profile of particle size on particle size distribution of varying concentrations of TPP and the volume ratio of Chitosan-Alginate

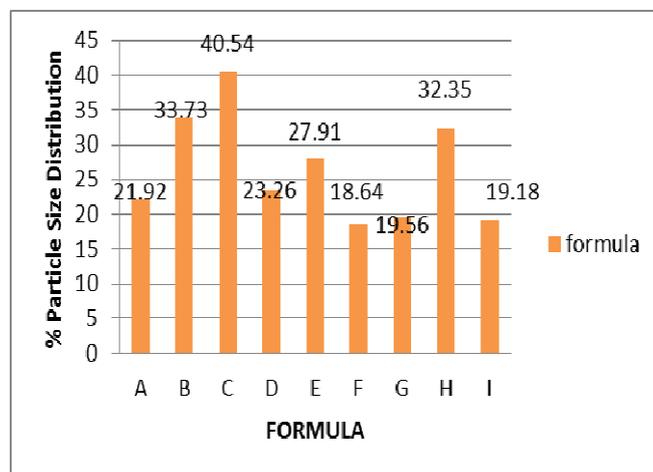


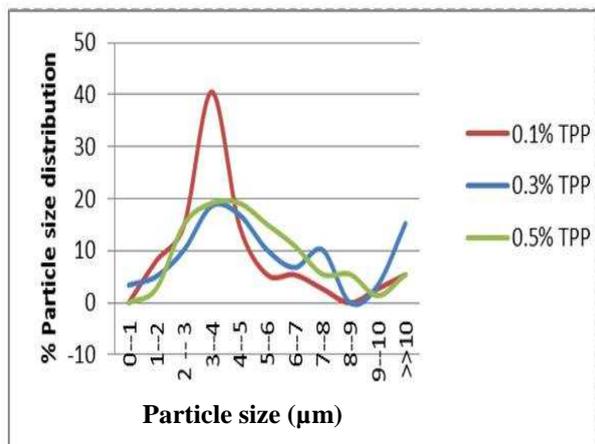
Fig.2. Percentage of particle size distribution of Chitosan-Alginate microparticle

Based on the figure 3, the ratio of the volume of Chitosan-Alginate 1: 2 shows particle size more uniform at each concentration of TPP indicated an area that is narrow and has only one peak, where the highest peak is achieved at a concentration of TPP 0.1% with a percentage of 33.7 % and the lowest at 0.3% TPP concentration of 27.9% in the size range of 3-4 µm, but at a concentration of 0.5% TPP getting smaller particle sizes in the range of 1-2 µm with a high percentage of the size uniformity at 32.3%. While the volume ratio of 2: 1 has only one peak with a narrow area, the peak of the volume ratio of 1: 1 area resulting in a fairly wide variety TPP concentration and tend to have more than one peak. This means that the particles produced in various TPP concentration result non uniform in size. So it can be concluded that a great volume ratio of Alginate will produce a more uniform distribution and will be essential in the formation of particle size with high uniformity of size.

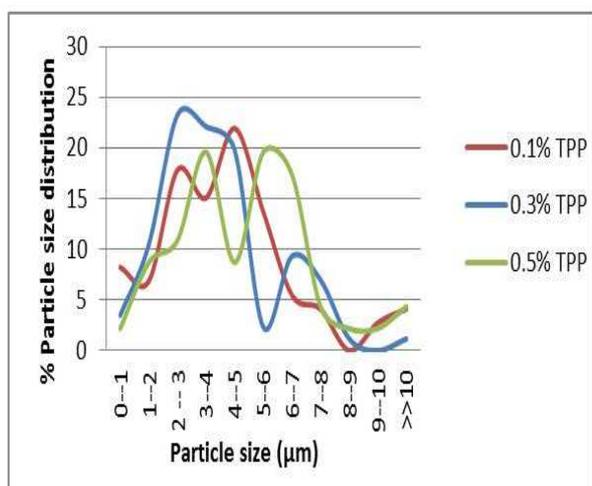
TPP role in the formation of micro-particles Chitosan-Alginate is a crosslinking agent that will strengthen the matrix of Chitosan micro-particles. Chitosan Gel occurs due to the formation of three-dimensional network between the molecules of Chitosan, tissue properties and molecular interactions, that bind the whole gel, determine the strength, stability and texture of the gel. another molecule that acts as crosslinking agent is usually used to strengthen the network in the gel [24].

The increasing amount of TPP will reduce the number of Chitosan micro-particles, means that the increasing concentration of TPP will make smallest size micro-particles difficult to form. This is proved from the results of experiments with varying concentrations of TPP shown in Figure 3. TPP concentration of 0.1% produces the most uniform size distribution indicated by the narrow area of the

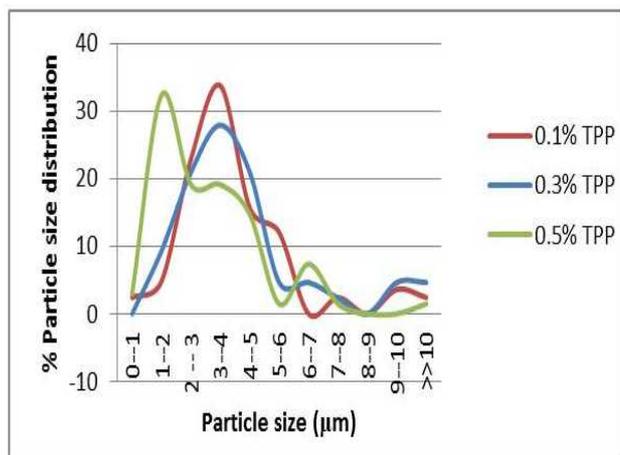
peak and peak altitude, thus obtained the size range of 3-4 μm micro-particles at each ratio of certain volume of Alginate Chitosan. Whereas, on the TPP concentration of 0.3% and 0.5% more likely to have a low size distribution.



(a)



(b)



(c)

Fig. 3 Particle size distribution with variety of Tri Poly Phosphate concentration (a) Ratio Chitosan-Alginate 2:1; (b) Ratio Chitosan-Alginate 1:1 and (c) Ratio Chitosan-Alginate 1:2

The use of crosslinking with high concentrations will cause stronger and harder gel Chitosan, so it will be increasingly difficult to split into small parts. The greater the concentration of TPP will also cause clots (agglomeration) molecules. But in comparison Chitosan-Alginate volume ratio of 1: 2 with a concentration of 0.5% TPP get a better size distribution is in the range of 1-2 μm with a high enough percentage

The raised of Tri Poly Phosphate concentration will lead to the formation of aggregate, thus will raised the particle size. Aggregation happened when the opposite charge electrostatic force attract each other. Fig.3. shows the particle size distribution of Chitosan-Alginate with variety of Tri Poly Phosphate concentration.

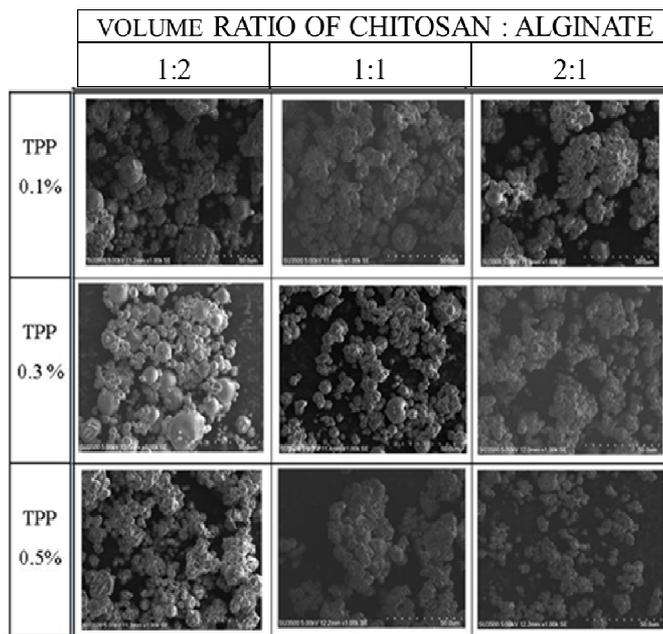


Fig. 4 The particle size distribution from SEM

SEM analysis result in Figure 4 also shows the uniformity of Chitosan-Alginate micro-particles. At a ratio of 2:1 particles formed tend to have non-uniform size and have a large particle diameter. It is suspected that it is due to the high viscosity of the emulsion. Chitosan in a solution has a high viscosity, so that the excess volume of Chitosan will cause a high viscosity of the solution, therefore a ratio of 2:1 produce the form of micro-particles which tend to have a larger size. The greater the volume of Chitosan means that the concentration of Chitosan in the mixture of emulsion increases. The concentration of Chitosan in the mixture will affect the viscosity of the solution which will affect the particle size. In general, the average size of the droplets that formed on atomization process of spray drying directly proportional to the viscosity of the solution, thus the particle size will be larger due to the increase in the viscosity. This is due to the viscosity of the emulsion is high if contacted with hot air during the process of spray drying and will produce larger droplets than the solution, which has a low viscosity.

While the ratio of 1: 1, tend to show better spread of the particles and do not form aggregates. However, this does not indicate uniformity of a pretty good size when seen from the graph in Figure 3. According to Zohri [22].

Chitosan-Alginate is a comparable ionic interaction between the molecules of chitosan to bind to Alginate overall and no free polymer in the media to crumple Chitosan-Alginate. That makes the mixture will produce more stable micro-particles.

It is suspected that the influence of stirring in a short time, causing a cross between the junction with Alginate-Chitosan moment that would cause the breakdown of particles is not effective result not only would increase the size of the micro-particles but also the number of micro-particles produced less and less uniform.

From SEM results also show Chitosan Alginate volume ratio of 1: 2 has a high uniformity of size at each concentration TPP, although particles produced aggregate. The resulting aggregate allegedly caused by the accumulation of ions (O-) of Alginate and ions (O-) of the TPP is not bound to Chitosan so that the ions to be sipped on the sidelines of Chitosan-Alginate bond that causes the bond is getting stronger and will increasingly difficult to split into smaller molecules again.

B. Test of Chitosan-Alginate micro-particles Solubility in Acid Media

Based on the research that has been done, 0.1% TPP concentration of Chitosan-Alginate ratio of 2: 1 can produce the most uniform size distribution with a ratio of 40.54%. The dissolution method is used to evaluate the degradation of Chitosan-Alginate micro-particle in a controlled condition. Chitosan-Alginate micro-particle as coating agent has to be not easily dissolved in acidic condition in stomach, thus the dissolution media in this experiment is adjusted to pH 1.2 and 37⁰C (normal human body temperature). Chitosan will bear positive charge in acidic condition and as the medium gets basic (pH above 9), the H⁺ ion bonded to NH³⁺ will be decreased. The sensitivity of Chitosan particle is a benefit to deliver drugs through stomach without easily gets aggregated [23].

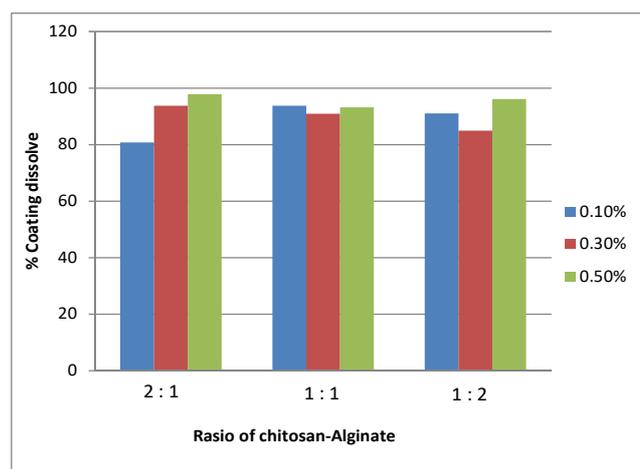


Fig. 5 The Percentage of Dissolving the mixture of Chitosan:Alginate and TPP in Acidic Medium

Based on Fig.5, the highest dissolution percentage (97.8%) in acidic medium was Chitosan-Alginate 2:1 with 0.5% Tri-Poly Phosphate, while the lowest one (80.8%) is Chitosan-Alginate 2:1 with 0.1% Tri Poly Phosphate.

TPP affects the concentration on the solubility of the coating inversely, i.e., the greater the concentration of coating the lower the solubility. TPP reaction with chitosan via crosslinking makes more tightly so that the thin layer of water molecules in acidic media is hard to diffuse into the structure of Chitosan-Tri Poly Phosphate.

The Formation of crosslinking makes the coating decreasing the solubility of chitosan in a wide pH range and also increasing the resistance to degrade the chemical or biological degradation in a long time. This is proved in Chitosan-Alginate volume ratio of 1: 1, the percentage solubility at a concentration of 0.1% TPP concentration higher than 0.5%, amounting to 87.59%, while the TPP concentration of 0.5% at 85.7%.

However, the volume ratio of Chitosan Alginate 2: 1 shows that there is a deviation where the percentage of TPP solubility at a concentration of 0.1% was lower than 0.5%. These irregularities allegedly because not all TPP with Chitosan binds perfectly that may cause weak bonds in the crosslink and not so easy dissolution process occurs.

Chitosan-Alginate volume ratio also affects the solubility of the coating percentage. The greater the percentage solubility showed that the weaker coating produced. On Chitosan-Alginate micro-particles, Alginate serves to repair mechanical properties of chitosan gel.

Characteristic of Chitosan is soluble in acid that can be stripped away so that the matrix of chitosan will be more stable in acid. So that the effect of Chitosan-Alginate volume ratio on the solubility of the coating inversely, i.e., the greater the volume of Alginate it will be the stronger of the coating produced so that the solubility percentage would be lower.

This is proved in Figure 4 Chitosan-Alginate volume ratio of 2: 1 has a greater percentage, 97.8%, compared with Chitosan-Alginate volume ratio of 1: 2 amounted to 96.2% at a concentration of 0.5% TPP. However, the TPP concentration of 0.1% and 0.3% at a ratio of 2: 1 and 1: 2 increase. It deviates in theory, this is thought to occur because of the existence of Chitosan that does not bind with Alginate and only binds to TPP. Bond between cross-chitosan with TPP is ionic bond that reversible and weak. In artificial gastric fluid medium (pH 1.2), the bond is broken and separated. Another factor is the nature of Chitosan itself is soluble in acidic pH media cause Chitosan to deteriorate [24].

IV. CONCLUSIONS

Based on the research that has been done, 0.1% TPP concentration of Chitosan-Alginate ratio of 2: 1 can produce the most uniform size distribution with a ratio of 40.54%. The biggest dissolution of micro-particle, obtained from the samples with Chitosan-Alginate ratio of 2:1 and the concentration of 0.1% TPP, was 80.8%.

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