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Abstract. Mangosteen pericarp extract has been reported to contain high amount of xanthones, which are secondary plant metabolites, which have high antioxidant activity and pharmacologic properties. In this research, xanthones that have poor oral bioavailability were encapsulated into chitosan alginate microparticles using ionic gelation method. The purpose of this study was to obtain a chitosan-alginate microparticle formulation with mangosteen peel extract containing mangostin which was used as an antioxidant supplement preparation and to obtain observations of the dissolution test and its hardness and hardness test. Mangosteen peel extract is obtained by maceration extraction method with ethanol solvent. The α- mangostin content in the extract was 90.04%. Mangosteen peel extract made microparticles using chitosan-alginate to protect α-mangostin which is sensitive to the environment. The α- mangostin microparticles obtained had an encapsulation efficiency of 99.925% and loading of 6.234%. The supplement formulation in tablet form by adding excipients such as diluent (mannitol and lactose), binder (Na CMC) and lubricant (magnesium stearate and talc) with two different variations. Dissolution test results show the release of mangostin occurs by *burst release* in synthetic digestive solutions.

Keywords: mangostin , α- mangostin, micro particles , antioxidant supplements, dissolution test

INTRODUCTION

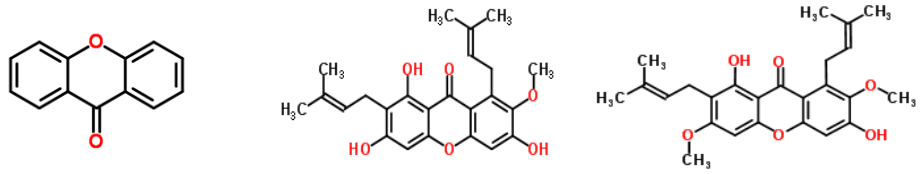
Food supplements are products that are intended to supplement the nutritional needs of food, containing one or more ingredients in the form of vitamins, minerals, amino acids or other ingredients (derived from plants or non-plants) that have nutritional value and / or physiological effects in concentrated amounts [1].

One of the benefits contained in food supplements is antioxidants. Antioxidants have the function of slowing down and preventing the oxidation of molecules in the body. The presence of antioxidant compounds reduces the incidence of chronic diseases caused by the work of free radicals in the body such as cancer, brain dysfunction, and inflammation that can cause death. The body can produce antioxidants from cell metabolism, however, with the increase in the number of free radicals, there is a need for additional antioxidant intake.

A number of studies have shown several herbal plants have high levels of antioxidants, one of which is the mangosteen (*Garcinia mangostana L.*). The xanthone compound was isolated from the pericarp (flesh) of the mangosteen fruit (*Garciana Mangostana*). Xanthone compounds derived in several studies have α-mangostin and γ-mangostin compounds. These bioactive compounds are known to have high levels of antioxidants [2,3,4]. These mangostin compounds have been known to have cytotoxic properties and can induce apoptosis of cancer cells and specifically mangostin is identified to be able to kill cancer cells selectively so that they do not affect normal cells [5,6].

Previous studies of mangosteen peel extract can be made in chitosan-alginate microparticles. As a medium for drug delivery systems, chitosan has been commonly used because of its biodegradable, non-toxic and mucoadhesive properties [7], and the addition of an alginate coating agent which is a natural polymer known to be good for the

design of controlled release drugs because of its stable pH value acid in the stomach, but can be degraded slowly at an alkaline intestinal pH [8]. The types of xanthone most contained in the mangosteen peel are α-mangostin and γ-mangostin [9]. While the structure of α-mangostin and γ-mangostin are the two types of xanton most commonly found in the skin of men shown in Figure 1 .



α-mangostin γ-mangostin β-mangostin

Figure 1. Structure of α-mangostin, γ-mangostin and β-mangostin
Source: chemspider.com

The use of the mangostin bioactive compound as a drug model has been widely studied, but it still needs to be developed to produce an optimal drug delivery and controlled release system if it is made in tablet preparations. In this study tablets will be made from mangosteen peel extract which functions as an antioxidant. Chitosan has been investigated for its use in drug-controlled release systems in the form of antibiotics, anti-defensive agents, anticancer agents, proteins, peptides and vaccines. Chitosan has been used in the manufacture of controlled release based drugs to increase the bioavailability of easily degraded substances and their use has been investigated for oral treatment [7]. Other biopolymer used for encapsulation of bioactive compounds is alginate. Alginate has been known to be a potential polymer for drug formulations related to alginate which has been used as food additives and also non-toxic properties [10]. In contrast to chitosan, alginate polymers have stable properties on the pH of the acid in the stomach, but experience swelling and begin to dissolve at pH in the intestine [8]. This makes alginate has the potential to supplement chitosan as an introduction to medicine.
For oral administration, tablet is the most common devices to deliver drug to the gastrointestinal tract. The main composition of the tablets are efficacious substances contained therein, while the fillers that are often used in making tablets are crushing agents, coatings, binders, flavoring ingredients and other additives [11]. One method of making tablets is by direct compression method. Direct compression is the technique used to manufacture tablets by directly compressing the mixture of active substances and dry excipients without prior treatment. Thus, the purpose of this research was to gain the tablet formulation that contains chitosan-alginate-mangosteen extract inside microparticles. The physicochemical characteristic of the tablet was determined as well as the release profile of mangostin in simulated gastrointestinal fluids.

MATERIALS AND METHODS

Material

Mangosteen pericarp was obtained from Solo, Central Java in January 2019 and was identified as *Garcinia mangostana* L. by Herbarium Bogoriense, Research Center for Biotechnology – Indonesian Institute of Science. Standard α-mangostin (98%) was obtained from Aktin Chemicals, China. Chitosan (medical grade; acetylation degree 93,6%; viscosity 23,3 cps) was obtained from Biotech Surindo, Indonesia. Tripolyphosphate (food grade) was obtained from Brataco Chemical, Indonesia, in sodium tripolyphosphate form, calcium chloride and alginate powder were obtained from Merck. KH2PO4, HCl, KCl, NaOH for preparation of simulated gastrointestinal fluids were purchased from Meck, Indonesia. α-amilase and β-glukosidase were purchased from Sigma, Singapore. Na CMC, Mannitol, Lactose, Magnesium Stearate, Talc were bought for tablet preparation from Merck, Indonesia.

Preparation of Mangostin Extract in Ethyl Acetate

Mangostin was obtained from mangosteen pericarp extract using method based on the procedure reported by Jung [2]. Mangosteen pericarp was washed carefully, and drained under sunlight for 5 days, then reduced its size by grinding to powder form. The maceration was done using ethanol 96% for 7 days, with ratio of mangosteen powder to ethanol was 1:3 (w/v), and stirred up periodically. Then the mixture was filtered, and ethanol was evaporated using rotary evaporator (EYEELA N-1000) to obtain a viscous extract (F001). The next step was fractionation of F001 extract by

mixture of water and ethyl acetate with ratio of 1:1 (v/v). The ethyl acetate fraction was separated, concentrated, and dried to gain a mangostin extract.

Microparticle Preparation

The microparticle preparation of chitosan-alginate was done by crosslinking and ionotropic gelation method [12]. The mangostin extract, as much as 0.1 g, was dissolved in a small amount of ethanol before it was added into 50 mL solution of acetic acid 2.5 % (v/v) that contained 1 g chitosan. The mixture was mixed using a four-blade-impeller (IKA Labortechnik) at 1000 rpm for 15 minute until a homogeneous solution formed. Meanwhile, a 100 mL solution of 1% (w/v) tripolyphosphate (TPP) was prepared. Solution of chitosan-mangostin was dropped into the TPP solution using syringe, and stirred using impeller at 600 rpm for 10 minutes. The beaker glass was covered and kept at room temperature for 30 minutes. The beads of chitosan-mangostin-TPP formed was separated, washed with water and dried using vacuum filter. Then the dry beads were grinded using mortar and sieved to gain particles with size < 100 µm.

The encapsulation of chitosan-mangostin-TPP particles with alginate was performed by addition of particles into alginate solution with various concentration: 0.25 g, 0.5 g and 2 g of alginate in 12.5 ml of water. The mixing was done by using impeller at 1000 rpm for 15 minutes, until homogeneous suspension was formed. The chitosan-alginate suspension was dropped slowly onto 6% (w/v) of $CaCl_2$ solution and was kept for 30 minutes in room temperature. After 30 minute, the beads were washed with water, separated using vacuum filter and dried using freeze dryer (EYELA FDV-1200; -49ºC; 12.2 Pa). Dry particles grinded using mortar and sieved to form particles with size <100 µm, and was stored in clear vial for further determination.

Mangostin Analysis

The presence of mangostin within ethyl acetate fraction was confirmed using High Performance Liquid Chromatography (HPLC). The Shimadzu HPLC with a reverse-phase C18 column (240mm×4.6mm, 5µm) was used at temperature of 30ºC. The mobile phase eluent was 95% acetonitrile and 5% buffer (0,1% H_3PO_4), with flow rate in 1mL/minute, and a UV detector was used at 244 nm wavelength (Aisha et al, 2012). The retention time of mangostin in ethyl acetate fraction was compared with retention time of standard α-mangostin compound. The microparticles morphology was determined using Scanning Electron Microscope (SEM; FE-SEM FEI INSPECT F50). The quantitative determination of α-mangostin in the ethyl acetate fraction as well as in the microparticles of chitosan-alginate uses UV spectrophotometry analysis (Spectroquant® Pharo 300 Merck). The absorbance data of solution of α-mangostin standards (4-20 mg/L) was obtained at the wavelength of 316 nm and it was used to form standard calibration curve.

Determination of encapsulation efficiency and loading

The encapsulation efficiency (EE) and loading were obtained by quantifying the mangostin present in supernatant during the particle preparation, using the following formulas:

$$\text{EE}\ (\%) = \frac{\text{Mass of drug present in microspheres (mg)}}{\text{mass of drugf used (mg)}} \times 100\% \quad (1)$$

$$\text{Loading}\ (\%) = \frac{\text{Mass of drug present in microspheres (mg)}}{\text{Total mass of microspheres (mg)}} \times 100\% \quad (2)$$

In-vitro Mangostin Release Study

The release profile of α-mangostin from chitosan-alginate microparticles was obtained using synthetic gastrointestinal fluids. The synthetic fluids are *simulated gastric fluid* (SGF; pH 1.2 from *buffer* 0.2 M KCl and 0.2 M HCl with ratio 1:1.7), *simulated intestinal fluid* (SIF; pH 7.4 from buffer 0.1 M KH_2PO_4 and 0.1 M NaOH with ratio 1:0.782) and *simulated colonic fluid* (SCF; pH 6.8 from *buffer* 0.1 M KH_2PO_4 and 0.1 M NaOH with ratio 1:0.448). The in-vitro release was conducted by immersing 100 mg particles in 50 mL of synthetic fluid and incubated at 37ºC

temperature. Every two hours, 4 mL of synthetic fluid was taken and concentration of α-mangostin was determined by UV-spectrophotometry analysis. The samples were analyzed until 24-hour immersed in synthetic gastrointestinal fluids. The profile release was obtained by plotting the concentration of α-mangostin as function of time immersed in synthetic fluids.

Preparation of Tablet Contains Microparticle Chitosan-Alginate-Mangostin

In the manufacture of supplement tablets in addition to the active ingredients, excipient ingredients are also added. Variations in the manufacture of these tablets are the type and concentration of the excipient used and the mass of the mangostin microparticles. The mass of the mixture to be loaded in the tablet is fixed at 500 mg.

TABLE 1. Composition of Tablets

Ingredients (mg)	Composition			
	D1 (mg)	%	D3 (mg)	%
Mangostin chi-alg microparticles	200	40%	200	40%
Na CMC	10	2%	50	10%
Manitol	230	46%	150	30%
Lactose	35	7%	75	15%
Mg Stearat	2.5	1%	2.5	1%
Talk	22.5	5%	22.5	5%
Total	500	100%	500	100%

Physical Characteristic Test on Tablet

After the tablet has been formed, a series of tests are carried out on the tablet.Evaluation is carried out to see the general appearance of the tablet with additional parameters such as shape, color, surface shape, and taste.Uniformity of tablet size is done by measuring the diameter and thickness of each tablet using calipers. According to Pharmacopoeia III, unless stated otherwise, the diameter of the tablet is not more than three times and not less than 1 1/3 of the thickness of the tablet.Tablets that have been printed from each formula are weighed one by one and the average weight is calculated. The requirement for uniformity of weights is no more than 2 tablets deviating greater than column A and none that deviate greater than column B. The tablet hardness tester used is Erweka Hardness tester. Generally the hardness of tablets ranges from 4-10 Kp (depending on the diameter and thickness of the tablet being made). The procedure is that one tablet is placed perpendicular to the instrument, then tested at what pressure until the tablet breaks [13]. The tablet hardness tester used is Erweka's Friability tester. Tablets are declared eligible as tablets if the weight loss is not more than 1% [13]. The procedure is as follows: Three tablets were cleaned of dust and weighed in total, Insert the tablet into the device and run the device at 25 rpm for 4 minutes (100 rev.). When finished, take out the tablet, clean the tablet from dust and weigh again. Calculate the percentage of tablet weight loss with the formula as follow:

$$F = \frac{a - b}{a} \times 100\% \qquad 3$$

with a is weight of the tablet before being tested and b is tablet weight after tested.

Dissolution Test

The dissolution test uses a procedure carried out using procedures as follows. A total of 1 tablet, each placed in 500 ml of the dissolution medium at a temperature of 37 ± 0 , 5 °C using a paddle type dissolution apparatus with a rotation speed of 50 rpm. Dissolution was carried out for 2 hours in a simulated gastric fluid (SGF, SIF, and SCF) solution that had been made in the previous procedure. Then the samples were taken as much as 4 ml in the 30th, 60th, 90th, and 120th minutes which were immediately replaced again with the same amount into the dissolution

flask. After completion of the solution absorption was measured with a UV-Vis spectrophotometer with a wavelength of 316 nm.

RESULTS AND DISCUSSIONS

The extraction was performed by macerating 200 grams of mangosteen skin simplicia against 600 ml of absolute ethanol analytical grade. The use of analytical grade solvents is in accordance with Good Manufacturing Practices (GMP) guidelines for Herbal Medicines. The final weight of the extract obtained is 12.17 grams, with the resukt that the yield is 6.1%

Qualitative Analysis of Extract using LCMS, HPLC and UV-Vis Spectrography

LC - MS is performed to find what components or substances contained in the extracts obtained. The operation condition using negative ion mode and collision energy range 4 – 75 V for 22 minute.

TABLE 2. Extract Composition

Compound	% Composition (LCMS)	% Content (HPLC)	% Content (UV-Vis Spec)
Garcinone C	3.57		
Alpha Mangostin	52.71	73.14	81.60
Garcinone D	5.46		
1-isomangostin hydrate	5.46		
Gamma Mangostin	3.85		
8 - Deoxygartanin	4.64		
Garcinone E	5.09		
Dulciol A	5.09		

Shown on Table 2. there are 8 derives of xanthone found in the mangosteen pericarp's extract, with the highest compound found is alpha mangostin. To validate the amount of alpha mangostin in extract, quantification method was used using alpha mangostin standard as the comparison. Analytical instrument was Uv-Vis using 316 nm [14]. For HPLC, extract was tested using acetonitrile – water (95:5%) as the mobile phase, 1 ml/minute, 244 nm for 8 minute [15]. The results were shown in Table 2. Although it was tested using the same sample, quantification using both methods yields different amounts of alpha mangostin. In UV-Vis, measured absorbance of all active compounds of chromophores resembles xanthones, so it's not specific only alpha mangostin alone, whereas in HPLC solvent is done based on differences in molecular weight and other factors such as stationary phase. Uv - Vis that only calculates the absorbance of light by the sample can not distinguish between alpha, beta or gamma, therefore, the results possessed by UV - Vis spectrometry tend to produce a higher quantification as well.

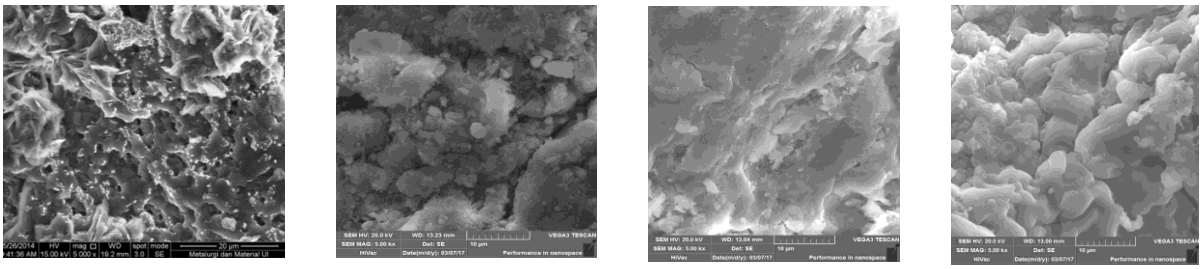
TABLE 3. Microparticle Variant

Microparticle variation			Mikropartikel
Extract (g)	Chitosan (g)	Alginate (g)	
0,1	1	0,25	A
0,2	1	0,25	B
0,4	1	0,25	C

Table 3 shows the composition of microparticles formed in this study. Each microparticle was assayd to obtain the characteristics of the microparticles used before they are blended into tablets.

Scanning Electron Microscope (SEM)

The characteristic testing by using SEM aims to determine the morphology of the microparticles. SEM morphology is performed in several variations of magnification, 100x, 500x, 1000x, and 5000x magnification at 20kV voltage. The allowable size limit is up to 1000 micrometers. Microparticle size A ranges from 67.63 micrometers to 218.93 micrometers. Microparticle B size ranges from 76.46 micrometers to 162.48 micrometers, whereas for microparticles C, the size obtained was 63.32 to 198.82 micrometers.



Chitosan-Alginate microparticle | Microparticle A | Microparticle B | Microparticle C

FIGURE 2. SEM Morphology of Microparticles in 5000 X Magnification

Figure 2. above shows the morphological comparison of the empty microparticles (chitosan-alginate microparticles), to microparticles with variations in the amount of extracts used. The outer appearance of the empty microparticles appears more prominent, branched and sharp, and looks like pores or crystals. Meanwhile, the other three microparticles have a smoother outer appearance, and are not branched but flat as if covering the compounds inside them. From figure above, it can also be observed that the more extract used, the surface of the microparticles formed will be smoother as well. In this case, the NH^{3+} branches in chitosan will split and bind to the bioactive compounds mangostin and TPP, the more bioactive compounds, the more bonds are formed, the more complex the microparticles will form. The remaining remaining NH^{3+} bonds will bind to the carboxyl group of alginates, resulting in a denser microparticles.

Physical Characteristic Tests on Tablet

This section will discuss the evaluation of tablets based on their size, weight and physical shape. Evaluation of tablets can be done by observing the shape, color, and surface of the tablet, and can also be seen whether there is a smell, taste and condition of the tablet from the presence or absence of damage. From the observations of tablets, the tablets look roundish brown in color, almost smell like medicine and have a rough texture. This brown color is derived from chitosan-mangostin microparticles coated with alginate. Table 4 is the result of evaluating the tablet size uniformity. Based on the calculation results, it appears that the uniformity of the tablets meets the requirements and the tablets are in accordance with the provisions of Pharmacopoeia III, where the diameter of the tablet is not more than three times and not less than 1 1/3 times the thickness of the tablet.

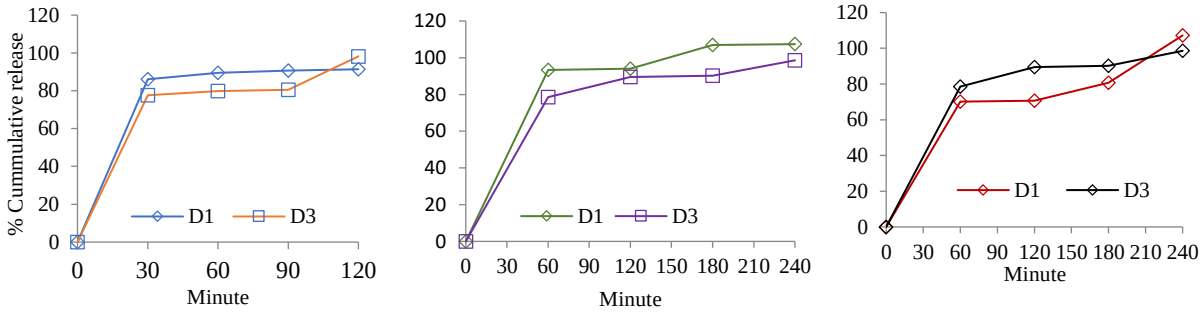
TABLE 4. Characterisctic of Tablet

Formula	Diameter (cm)	Thickness (cm)	Diameter / Thickness	Severity (%)	Weight /tablet (mg)	Deviation (%)	Hardness (kp)
D1	1.11±0.00	0.433±0.005	2.56±0.03	1.429	506.47 ± 0.58	0.09 ± 0.07	1.83 ± 0.2
D3	1.11±0.00	0.43±0.004	2.58±0.025	0.907	507.37 ± 1.84	0.25 ± 0.25	2.07 ± 0.064

Table 4 also shows the result of evaluating the uniformity of tablet weights. Based on the results of calculations, both formulations meet the weight uniformity requirements because not more than two tablets deviate greater than 5% and none deviate greater than 10% as declared by Ministry of Health of the Republic of Indonesia. Table 3 shows the result of evaluation of hardness and friability of the tablets. Hardness test aims to test the strength of the tablet at a certain pressure and also as a physical control method during the manufacturing process while the hardness test aims to determine the durability of the tablet during the manufacturing, testing and distribution process. From the calculation results, it appears that the two formulas do not meet the tablet hardness requirements because the tablet hardness requirements are 4-10 kp. From the calculation results it is also seen that only the D3 formula fulfills the specificity of rigidity, which is less than 1% [13]. Hardness and hardness in a tablet are influenced by the amount of binder concentration in the formula. The higher the concentration of the binder, the stronger the tablet and the more resistant the tablet to shocks.

Release Profile of Mangostin in Simulated Gastrointestinal Fluids

The profile of mangostin release on pH 1.2 synthetic fluid media that represents gastric conditions is shown in Figure 3 A. In dissolution testing on mangostin tablets at pH 1.2 it is known that the conditions of the release of the two variations of mangostin tablets. From the release profile of mangostin tablets on *Simulated Gastric Fluid* (SGF) pH 1.2 in Figure 3 A, it can be seen that in the first half hour, the mangostin content begins to be released in the 30th minute. This shows that the occurrence of *burst release* or release of active substances from the tablet directly. In addition, the ingredients made to make tablets are substances that dissolve easily in water, such as mannitol and lactose. This *release* also shows that the tablet also *swelled* so that the mangostin particles were released and the absorbance data were read. *Swelling* events can occur also because there are excipient materials that are soluble in water such as mannitol and lactose. From the graph above shows that in the first 30 minutes, D1 tablets experienced greater mangostin release on D3 tablets. This can be due to the greater amount of NaCMC in the composition of D3 tablets, so that the hydrogel formed can be better able to hold the active ingredient mangostin for release because when the gel layer is formed from *swelling* polymers , the speed of liquid penetration can be reduced [16]



A. in SGF (pH 2.1) B. in SIF (pH 7.4) C. in SCF (pH 6.8)

FIGURE 3. Release Profile of Mangostin in Simulated Gastrointestinal Fluids

The profile of mangostin release on synthetic fluid media pH 7.4 which represents the gastric condition is shown in Figure 3 B. It can be seen that in each variation, the mangostin content experiences a large release in the first hour. This can be caused by two types of tablets also experienced a burst It is seen that in the first 15 minutes of observation, tablet experience swelling . However, the D3 swelling tablet occurs more slowly. This is because the NaCMC content in D3 tablets is more than D1 tablets. This also causes the D1 tablet, mangostin particles have been released all before the 3rd hour. This can be seen on the graph that the release of mangostin on D1 is already 100% in minutes between 150-160. In D3 tablets, mangostin release reached 98.6%. This can be a reference that mangostin particles can be released in the small intestine. However, there needs to be further research on the release of mangostin, especially in the quantity of mangostin release from the stomach to the small intestine in the first 2 hours.

The profile of mangostin release on pH 6.8 synthetic media representing gastric conditions is shown in Figure 3 C. It shows that in each variation, the mangostin content also experiences a large release in the first hour, the same as in releases in SIF media. This is because the pH of the solution is not much different. What is interesting in this graph is that on D1 tablets, mangostin release in the first hour is lower than D3 compared to other fluid media. This can be caused by *swelling* on a slightly slower D1 tablet. In addition, there is a possibility of the interaction of chitosan with alginates which have stability in certain compositions [17], so that with different combinations of alginate concentrations, the release trends that occur will also be different even though the pH range is not much different. Thus, mangostin in D1 tablets can be released 100% at 210-215 minutes due to instability in NaCMC and chitosan-alginate concentration.

CONCLUSSION

Based on the discussion explained in the previous chapters, it can be concluded that: Tablets that have been made meet the weight and size uniformity requirements of 506.47 mg on D1 tablets and 507.37 mg on D3 tablets. Tablets that have been made in the variation of D1 do not meet the requirements of the hardness and hardness test, but the D3 tablet meets the requirements of the solidity, which is at 0.907% of the weight difference . Dissolution tests produced

in the three digestive synthetic solutions with different pH have a similarity in the release of mangostin in the first hour, which has burst release in the first 30 minutes at pH 1.2 and 60 minutes at pH 7.4 and 6.8. Tablets that have an effective composition in testing are D3 tablets, namely with a 200 mg microparticle composition, along with its exponents, namely 50 mg NaCMC, 150 mg mannitol, 75 mg lactose, 2.5 mg Mg stearate and 22.5 mg talk

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