

The Characterization and Effectiveness Penetration of Caffeine Trapped and Coated Chitosan Nanoparticles as Anti-Cellulite

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Abstract

Chitosan used as drug carrier because of is natural polycationic and easily modified in chemical and physical properties. In this research chitosan was chemically modified by coating and entrapping anti-cellulite active substance of caffeine and physically modified by minimizing chitosan particle size into nanoparticles size. The purpose of this research were to characterize the chitosan nanoparticles from its morphology, particle size, function of group, the value of adsorption efficiency, and effectiveness of chitosan nanoparticles against In Vitro penetration of caffeine as anti-cellulite using Franz diffusion cell. The morphology characterization test of caffeine trapped in chitosan nanoparticles and caffeine coated by chitosan nanoparticles resulting a smooth surface, slight convex shape, and agglomerated particles; average size of particles are 232.74 nm and 226.62 nm respectively; the function group showed a shift of wave number of amide III groups (-CN) and hydroxyl groups (-OH); the caffeine adsorption efficiency are 51.35% and 64.63% respectively. The result of effective penetration were $1.089.65 \pm 10.7 \mu\text{g}/\text{cm}^2$ and $2.170.03 \pm 6.85 \mu\text{g}/\text{cm}^2$ respectively.

Keywords

Anti-Cellulite, Caffeine, Chitosan, Chitosan Nanoparticles

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1. Introduction

Chitosan is a polymer which can be obtained from the deacetylation of chitin; non-toxic, easily degradable biologically, has a polycationic nature in acidic conditions due to the protonation of an amino group, and form a gel. Chitosan structure similar to cellulose that has properties similar matrix in drug delivery systems orally and topically (Sutriyo et al. 2005). Chitosan is widely used as a conductive matrix polycationic drug because it is a natural, biodegradable, biocompatible, mucoadhesiveness, and easy to modify the chemical and physical properties (Lee et al. 2006).

Chitosan chemical modification results in improved stability of the chitosan through the activity of existing functional groups, improvement of chitosan pore size using porogen compound, and can increase the adsorption capacity of chitosan chitosan when combined with other polymers. One chemical modification can be done through the formation of crosslinked structures produce chitosan chitosan gel (Wang et al. 2004). Physical modification leads to form nanoparticles. According to Mohanraj and Chen (2006) nanoparticles having a size range of 1-1000 nm. Manufacture nanoparticles of chitosan is affected by several factors, including the composition of the materials and methods used. The composition of materials used in the manufacture nanoparticles of chitosan is chitosan, STPP and surfactant

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(Tween 80). Many methods of making nanoparticles of chitosan nanoparticles and developed to produce a uniform morphology (Wahyono 2010).

Chitosan nanoparticles act as a carrier (carrier) by dissolving, trapping, encapsulating, or attach the drug in the matrix and deliver drugs orally and topically (Tiyaboonchai 2003). In this research, a method of making nanoparticles of chitosan by means of trapping and menyalutkan active substance to deliver them topically. One of the topical treatment of the skin increased demand due to an issue of aesthetics skin anti-cellulite preparations, namely adult women. According to the WHO survey (2012) of approximately 90% of adult women in the world have cellulite disorders. Cellulite (Gynoid limphodystrophy) is a form of condition-grated grater Uneven skin that looks like orange peel, occur in women and usually appears on certain body parts, namely the thigh, abdomen, and bokong. Selulit occur due to damage to the blood vessels and lymph causing changes in the structure of the layer of fat and collagen matrix that surrounds (Rona et al. 2006).

Cellulite skin care is done by interfering with the function of the stratum corneum barrier system using chitosan nanoparticles carrying active substances such as caffeine. Caffeine has no effect on topical lipolysis via phosphodiesterase enzyme inhibition and increases the amount of cyclic monophosphate (Rossi and Vergranini 2000). Carrier material in the form of chitosan nanoparticles combined with caffeine in the preparation of anti-cellulite can affect the penetration of caffeine on the skin. If the barrier function of the skin may be bothered by the active ingredients are absorbed by chitosan nanoparticles topically, then inhibiting fat synthesis may also be included in the formulation of anti-cellulite (Murray et al 2003).

2. Material and Methods

Materials used in the manufacture of nanokitosan antiselulit is chitosan (degree of deacetylation min 70%) (CV. Bio Chitosan, Indonesia), acetic acid 1% (Merk, Germany), tween 80 (BRATACO, Indonesia), sodium tripolyphosphate (Aditya Birla, Thailand), caffeine anhydrous (BRATACO, Indonesia), aquademineralisata (BRATACO, Indonesia), potassium dihydrogen phosphate (Merck, Germany), sodium hydroxide (BRATACO, Indonesia), and the strain Sprague Dawley female rats aged 2-3 months, weighing $\pm$ 150 grams (Bogor Agricultural University, Indonesia).

Equipment used in the manufacture of nanokitosan is ultrasonikasi (As One 110 volt, Coda JBIC Loan), a homogenizer (Ultra Turrax T8, Wika), analytical balance (EB-330 type, Shimadzu), and tools laboratory glassware.

The tools used for the analysis is the Franz diffusion cell receptor compartment with a volume of 14.0 mL (Glass Workshop ITB, Bandung), UV-VIS spectrophotometer (Type 1600, Shimadzu), Electron Microscopy Microscope (JSM-5310 LV, JEOL Ltd.), Particle Size Analyzer (TM Delsa Nano, Cordouan), Fourier transform Infrared (Type MB3000, ABB Group), digital cameras (SEL 1855, Sony), and surgical instruments.

This study covers the stages of manufacture nanoparticles of chitosan, chitosan nanoparticles characteristics testing, calibration of caffeine, the entrapment efficiency of nanoparticles of chitosan to caffeine, and penetration testing by Franz diffusion cells in vitro. This research was conducted with the two treatments, namely caffeine coated nanoparticles of chitosan and chitosan nanoparticles trapped caffeine. Chitosan is made with a concentration of 2.5%. A total of 2.5 grams of chitosan dissolved using a homogenizer for 30 minutes in 100 mL of 1% acetic acid in order to obtain chitosan concentration of 2.5% (w / v). Then the solubility of chitosan added 50 mL of STPP as much as 0.84 mg / mL soluble chitosan solution akuademineralisata with STPP were then divided into control and two treatments. Control with caffeine unallocated caffeine, chitosan nanoparticles trapped first treatment, and a second treatment with caffeine coated chitosan nanoparticles.

Control treatment with a solution of chitosan-STPP added 20 mL Tween 80 as much as 0.1 mg / mL dissolved in akuademineralisata. The first treatment with caffeine added 40 mL of 0.8 mg / mL dissolved in akuademineralisata first, and then added 20 mL of Tween 80 as much as 0.1 mg / mL dissolved in akuademineralisata. The second treatment solution of chitosan-STPP first, then added 40 mL of caffeine as much as 0.8 mg/mL dissolved in akuademineralisata.

Furthermore, the two treatments in ultrasonikasi with a frequency of 20 kHz for 1 hour. Nanoparticles of chitosan-caffeine solution that has been broken then divided into two parts. The first part in the form of a liquid solution and the second part is dried by spray dryer (spray dryer) at a temperature of 173°C so that the results obtained in powder form. Tests were conducted to nanoparticles of chitosan in this study is to determine the morphology of nanoparticles of chitosan and testing Microscopy Electron Microscopy (SEM), the analysis of particle measurement of nanoparticles of chitosan produced by testing Particle Size Analyzer (PSA), the analysis of functional groups nanoparticles of chitosan generated by testing Fourier Transform Infrared (FTIR), testing the efficiency of the adsorption of caffeine on chitosan nanoparticles with UV-VIS spectrophotometer, and testing the effectiveness of chitosan nanoparticles on the penetration of caffeine in vitro using Franz diffusion cell vertical type.

3. Results and Discussion

3.1. Characteristics of Chitosan Nanoparticles

Characteristics of caffeine stuck and coated chitosan nanoparticles were conducted in this research include morphological analysis, particle measurement analysis, analysis of functional groups, and the efficiency of the adsorption of caffeine on chitosan nanoparticles.

3.2. Morphology

Unallocated chitosan nanoparticles (control) and filled with

caffeine (stuck and coated) can be distinguished visually using Scanning Electron Microscopy (SEM). SEM analysis serves to identify the morphology of the surface and shape of nanoparticles of chitosan that is displayed through an image. Based on the characterization by SEM at 1000x magnification showed that the nanoparticles of chitosan unallocated and treatment caffeine or caffeine stuck chitosan nanoparticles coated chitosan nanoparticles produced has a surface and a different shape can be seen in Figure 1.

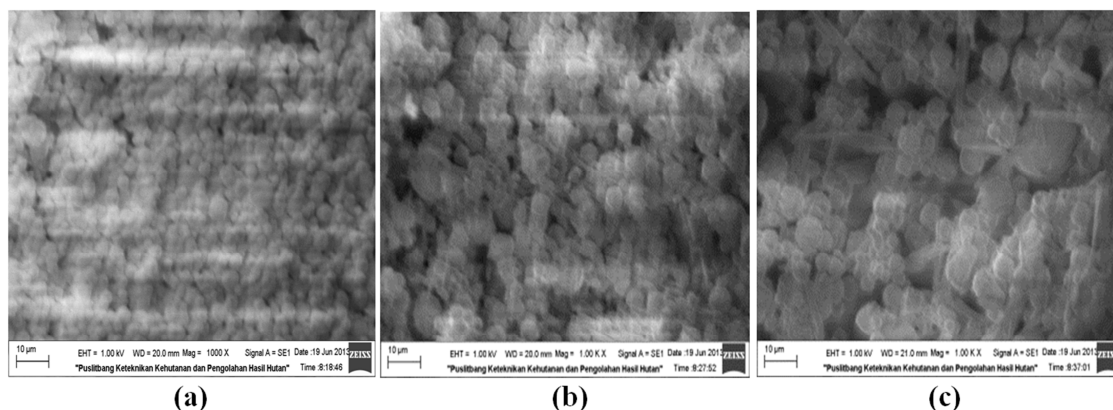


Figure 1. (a) Chitosan nanoparticles that are not filled, (b) Caffeine stuck chitosan nanoparticles, (c) chitosan nanoparticles coated Caffeine.

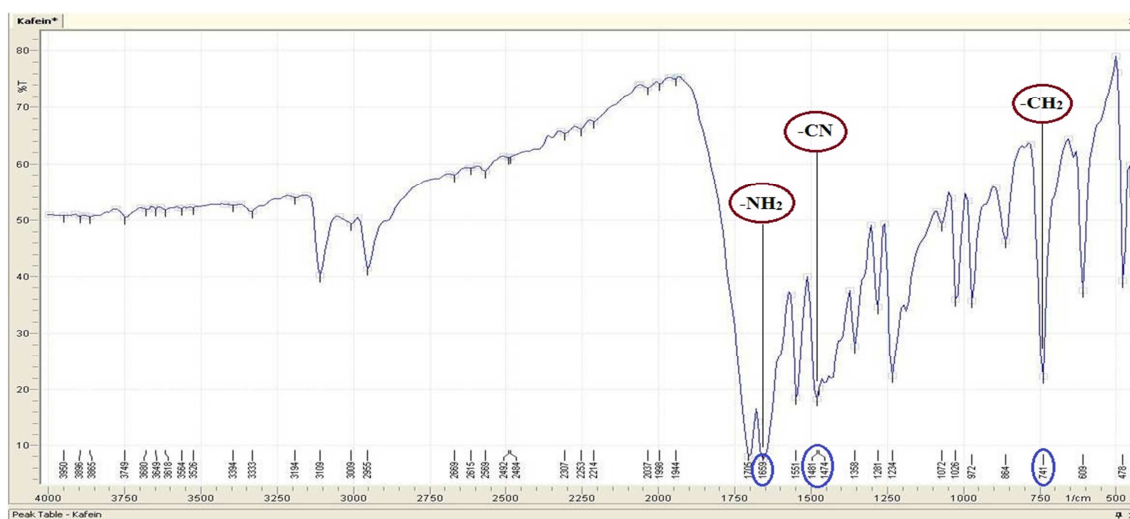
Results morphology of nanoparticles of chitosan unallocated have a rough surface and a concave but very agglomerate which can be seen in Figure 1a, while the results of morphological caffeine trapped nanoparticles of chitosan and caffeine coated nanoparticles of chitosan has a surface that is smooth, slightly convex, and clotting which can be seen in Figure 1b-c. Chitosan nanoparticles which have been filled will be shaped like a ball that is smooth and convex, whereas unallocated chitosan has a concave surface and coarse (Desai

and Park 2005)

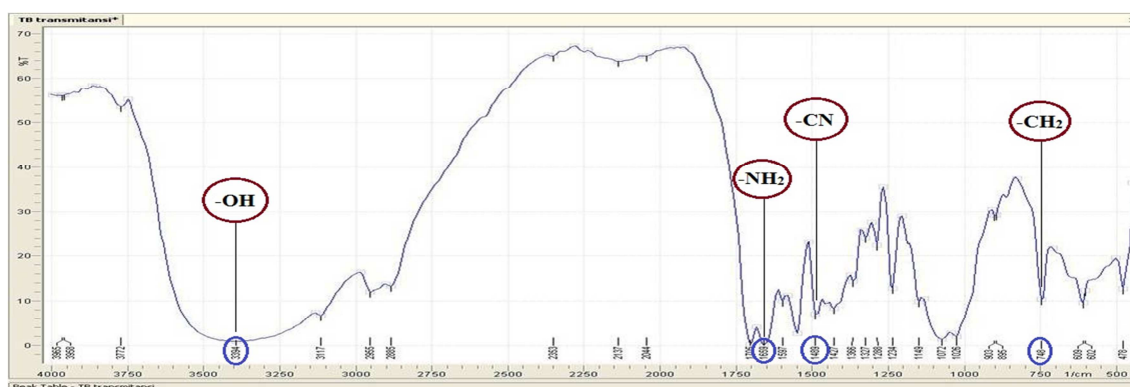
This clumping occurs because the time between mixing with a homogenizer and ultrasonikasi with spray drying for too long so that it can lead to clotting. Clotting will occur in a given chitosan STPP more. Clots can be reduced by shortening the time during the making of chitosan nanoparticles by spray drying (Yongmei and Yumm 2003). Moreover, the addition of the surfactant is too little (Benerjee et al. 2002).



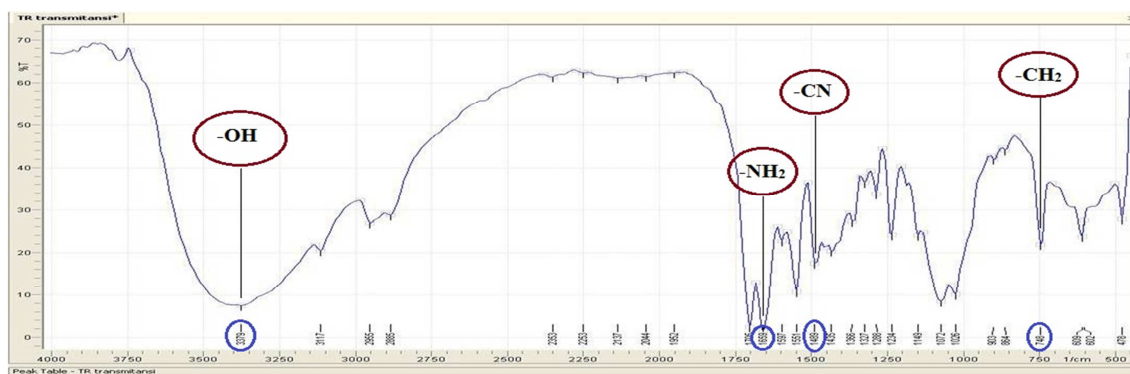
(a)



(b)

Figure 2. FTIR graph (a) nanoparticles of chitosan and (b) caffeine.

(a)



(b)

Figure 3. Graph FTIR (a) caffeine stuck chitosan nanoparticles, and (b) chitosan nanoparticles coated caffeine.

Based on Figure 2 shows the difference in caffeine position of each treatment. The differences are shown in treatment of caffeine coated nanoparticles have a lot more needle shape compared to chitosan nanoparticles trapped caffeine treatment. According to Ansel et. al (1999) of caffeine in the form of white powder and white shiny needle-shaped, usually clot. It can be seen in Figure 2b in the treatment of chitosan nanoparticles trapped caffeine, caffeine inside the cavity of

chitosan thus less visible, while Figure 3 shows the caffeine visible on the surface of the cavity of the matrix of nanoparticles in chitosan nanoparticles coated caffeine treatment.

3.3. The Particle Size

The success of a sample into nanoparticles known by looking at the size distribution, average size and polydispersity index

of the sample using a PSA test (Particles Size Analyzer). The test results of chitosan nanoparticles with chitosan nanoparticles trapped treatment of caffeine and caffeine treatment coated with chitosan nanoparticles using PSA testing (Particles Size Analyzer) can be seen in Table 1.

Based on test results of particle size using PSA testing (Particles Size Analyzer) in the treatment of caffeine stuck chitosan nanoparticles size distribution obtained from 134.93 to 489.91 nm smaller than the size distribution of nanoparticles coated chitosan caffeine treatment from 141.29

to 446.80 nm, While the average size of the chitosan nanoparticles trapped caffeine treatment 232.74 nm greater than the chitosan nanoparticles coated caffeine treatment 226.62 nm. Results of the two treatments were obtained in accordance with the category of nanoparticles. According to Mohanraj and Chen (2006) nanoparticles are defined as solid particles with a size of about 10-1000 nm. The most influential manufacturing technology is the nanoparticle formulations and methods it uses.

Table 1. Results of the analysis of particle measurement.

Treatment	Size Distribution (nm)	Average size (nm)	Polydispersity Index
Caffeine stuck Chitosan Nanoparticles	134.93 – 489.91	232.74	0.22
Caffeine coated nanoparticles of chitosan	141.29 – 446.80	226.62	0.18

The results of size distribution and average size of the two different treatment because it is influenced by the value of a polydisperse index. Polydispersity index values treated chitosan nanoparticles trapped caffeine 0.22 greater than the caffeine treatment coated nanoparticles of chitosan 0.18. Kehomogenisasi polydispersity index value determines a particle. The smaller the polydispersity indexes value the more homogeneous. Results of all treatments have shown that a homogeneous dispersion in which the value of polydispersity index has shown results below 0.5. Polydispersity index has a range of values from 0 to 1. Where a value close to 0 indicates a homogeneous dispersion, while a value greater than 0.5 indicates a high heterogeneous (Avadi et al. 2009)

3.4. Functional Groups

Analysis FTIR (Fourier Transform InfraRed) can be used to detect changes in functional groups in an organic compound or polymeric compound at wave number 400-4000 cm^{-1} . Determination of wave numbers is due in accordance with the determination of functional groups of organic compounds. The results of FTIR transmittance in the form of graphs.

Based on Figure 4 can be seen in the FTIR spectrum of nanoparticles of chitosan have specific peaks, namely the amine group ($-\text{NH}_2$) is the wave number 1643 cm^{-1} and the hydroxyl group (OH) is the wave number 3410 cm^{-1} . Uptake wave number amine group ($-\text{NH}_2$) and hydroxyl (OH) in the commercial chitosan is at wave number 1655 cm^{-1} and 3441 cm^{-1} (Pebriani et al. 2012). FTIR spectra of chitosan nanoparticles shifting transmittance intensity in the region of the spectrum. Transmittance change shows the interaction between chitosan, STPP, and Tween 80 are used in the manufacture of chitosan nanoparticles.

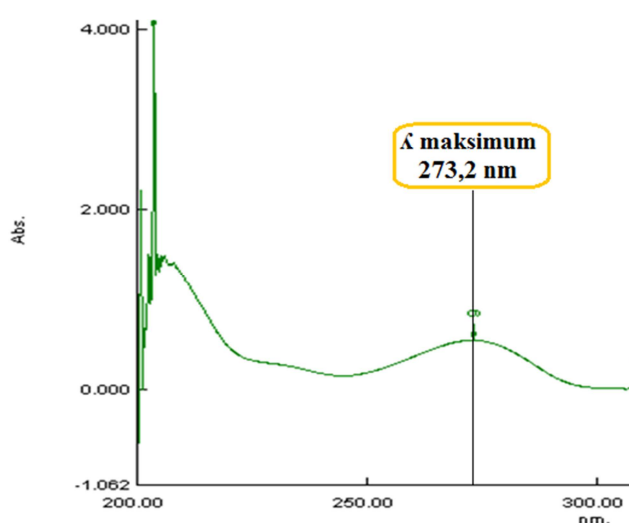


Figure 4. Curves maximum wave caffeine 10 ppm phosphate buffer pH 7.4.

There is another wave numbers on the graph FTIR chitosan nanoparticles with wave number 1412 cm^{-1} , 1257 cm^{-1} and 1149 cm^{-1} region of the spectrum that indicates an acetyl group ($-\text{CH}_3\text{CO}-$), metal ($-\text{CH}_3$), and range ($-\text{CO}-$). Chitosan has a metal group showed that the deacetylation process is done in less optimum purity is low, it still contains many impurities and the presence of water which may be absorbed thereby affecting the bond between molecules that cause differences in absorption area (Pebriani et al. 2012).

Based on Figure 3b FTIR spectrum peaks caffeine has specific, the alkyl group ($-\text{CH}_2$) located at wave number 741 cm^{-1} , amide group III ($-\text{CN}$) located at wave number 1481 cm^{-1} , and the amine group I ($-\text{NH}$) is at wave number 1659 cm^{-1} . According to Silverstein et al. (2005) spanning an alkyl group ($-\text{CH}_2$) are in wave numbers more than 722 cm^{-1} , amide group III ($-\text{CN}$) are in wave numbers more than 1400 cm^{-1} , and an amine group ($-\text{NH}_2$) are in wave numbers 1630 cm^{-1} to 1670 cm^{-1} .

FTIR results graph in Figure 5 shows that caffeine and

caffeine stuck chitosan nanoparticles coated chitosan nanoparticles have a combined force of chitosan nanoparticles with caffeine. The treatment group contained caffeine contained chitosan nanoparticles trapped alkyl group ($-\text{CH}_2$) located at wave number 748 cm^{-1} , amide group III ($-\text{CN}$) located at wave number 1489 cm^{-1} , the amine group ($-\text{NH}_2$) is at number waves of 1659 cm^{-1} , and the hydroxyl group (OH) is the wave number 3394 cm^{-1} . In the treatment of caffeine coated chitosan nanoparticles have the same group with chitosan nanoparticles trapped caffeine treatment but the shift wave number that is the wave number 3379 cm^{-1} hydroxyl (OH) and 1481 cm^{-1} amide group III ($-\text{CN}$).

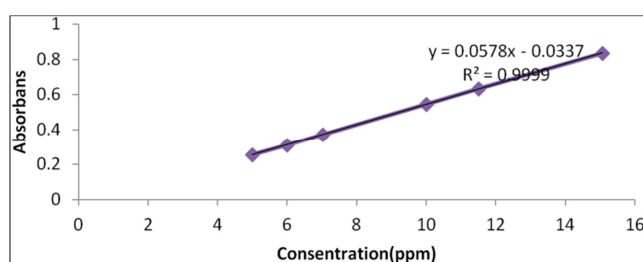


Figure 5. The relationship between the absorbance and the concentration of caffeine.

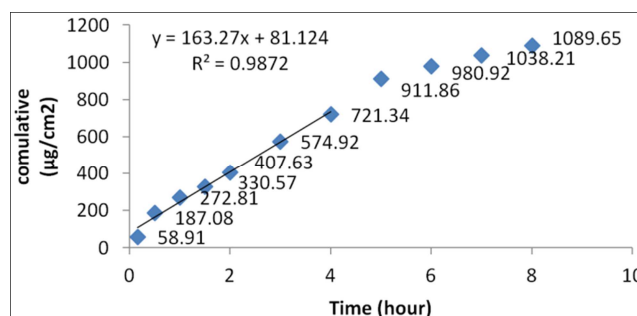
3.5. The Entrapment Efficiency

The entrapment efficiency caffeine is done by measuring the amount of caffeine stuck and coated in nanoparticles of chitosan. The amount of caffeine stuck and coated can be seen from the absorbance values measured by UV-VIS spektrofotometer at maximum wavelength with the aid of a standard curve. Therefore, the first stage before determining the efficiency of the adsorption of caffeine is to determine the maximum wavelength and make a standard curve. The maximum wavelength of caffeine solution can be seen in Figure 6.

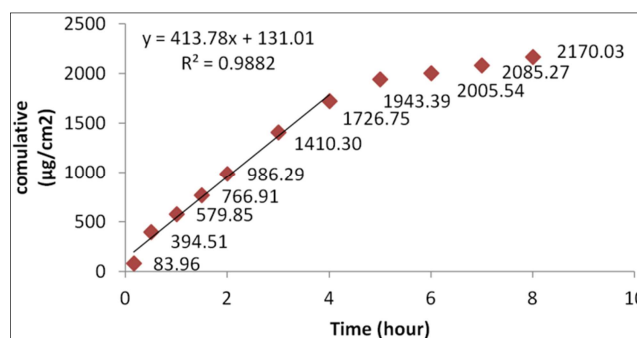
Determination of the maximum wavelength caffeine solution done on caffeine concentration of 10 ppm in the phosphate buffer at $\text{pH } 7.4$. PH value 7.4 been approached human skin pH conditions. Based on the absorbance values obtained caffeine solution with maximum absorption wavelength for the compound caffeine is 273.2 nm . Caffeine solution standard curve in Figure 6 has a high linearity shown by $r^2 = 0.9999\%$ in line equation is $y = 0.0578x - 0.0337$. This standard curve equation used to determine the amount of caffeine that is stuck and coated in nanoparticles of chitosan.

The entrapment efficiency describe the amount of caffeine

that is stuck and coated in nanoparticles of chitosan. Performed by extracting caffeine loaded chitosan nanoparticles in phosphate buffer $\text{pH } 7.4$ for 24 hours, then measure absorbansnya with UV-VIS spectrophotometer at a wavelength of 273.2 nm . Knowing the value of efficiency is particularly important in the pharmaceutical field, especially for the system of drug penetration into the skin due to the presence of the efficiency it can be seen the ability of chitosan nanoparticles in bringing caffeine into the skin.



(a)



(b)

Figure 6. Total cumulative (a) caffeine stuck chitosan nanoparticles and (b) chitosan nanoparticles coated caffeine.

The study produced different efficiency values for each treatment is shown in Table 2. The difference between the efficiency of nanoparticles of chitosan allegedly caused by the treatment method of manufacture is not the same so that caffeine is stuck and coated into each particle is not the same. The entrapment efficiency values are the highest caffeine caffeine owned treatment of the coated nanoparticles of chitosan, which is 64.63% while the caffeine treatment of chitosan nanoparticles stuck 51.35% .

Table 2. Value of caffeine on the entrapment efficiency of nanoparticles of chitosan.

Treatment	Value (y)	Value (x)	Weight a (mg)	Weight b (mg)	Efficiency (%)
Stuck	0.16	3.3	40570	3410	51.35
Coated	0.28	5.4	41430	3420	64.63

The entrapment efficiency factor is not one aspect of which is reviewed to determine the feasibility of chitosan nanoparticles as systems of drug penetration into the skin (Silva 2006). The higher the value, the better the expected efficiency of the formulation because the amount of caffeine that is entrapped in the chitosan nanoparticles more. The high value of the efficiency of treatment of caffeine coated nanoparticles of chitosan may be caused by participating terekstraksinya whole caffeine coated in nanoparticles of chitosan either on the surface or in the cavity of the matrix of nanoparticles then caffeine would be more easily extracted out, while the treatment of caffeine trapped nanoparticles of chitosan inside the cavity chitosan will require a long time to extracted out (Wahyono 2010).

3.6. Penetration Effectiveness Stuck Caffeine and Chitosan Nanoparticles Coated

In vitro penetration test is performed using Franz diffusion cells. Penetration testing was conducted to determine the amount of caffeine that can penetrate the membrane during a certain time interval of chitosan nanoparticles. The membranes used are skin abdominal strain Sprague Dawley female rats aged 2-3 months, weighing ± 150 grams. Rat skin membrane is used because it has a permeability which is almost equal to the permeability of human skin (Rawlings 2006).

Franz diffusion cell penetration test performed for 8 hours with intervals of 10, 30, 60, 90, 120, 180, 240, 300, 360, and 480 minutes. Each time interval of 0.5 mL of fluid samples taken from the receptor compartment and diluted to 5 mL with phosphate buffer pH 7.4 (Franz 2005). Each fluid samples taken from the receptor compartment must always be replaced with the same amount of volume of liquid that is picked to maintain the receptor fluid volume remains constant. 5 mL sample dilution is measured by uv-vis spectrophotometer at a wavelength of 273.2 nm to determine the absorbance.

The test results of penetration through the skin membrane of mice in Figure 7 shows the time interval 8 hours to chitosan nanoparticles trapped caffeine $1,089,65 \pm 10.7$ pg / cm² and caffeine coated nanoparticles of chitosan $2,170,03 \pm 6.85$ mg / cm². The results of treatment of caffeine coated chitosan nanoparticles have a penetration rate that is faster than caffeine stuck chitosan nanoparticles. This is influenced by the characteristics of the nanoparticles and the adsorption efficiency. Based on the results of the study, characterization of nanoparticles have different each treatment. Treatment of caffeine coated nanoparticles have an average of 226.62 nm particle size is smaller than the

caffeine treatment 232.74 nm nanoparticles trapped. The particle size affects the drug, the drug release, and the stability of the nanoparticles (Mohanraj and Chen 2006). Nanoparticles can improve the penetration of drugs through the skin because of their small size so that the larger the surface area and the smaller size of nanoparticles will lead to more active substances penetrate the skin (Inayat and Mallikarjuna 2009).

Factors influencing the adsorption efficiency of drug penetration into the skin. Based on the results of research on the treatment efficiency of entrapment of caffeine coated nanoparticles of chitosan have 64.63% more than the caffeine treatment trapped chitosan nanoparticles have 51.35%. The higher the value the efficiency of entrapment of the active substance inside the cavity chitosan nanoparticles much apart (Wahyono 2010). So the method of making nanoparticles of chitosan with menyulutkan drug into a more easily dislodged in drug delivery systems. While the method of making nanoparticles of chitosan by trapping the drug into a solution of chitosan-STTP with the addition of Tween 80 can decrease the value of the adsorption efficiency. Entrapping the drug with the addition of Tween 80 to form an emulsion of particles in solution will be stabilized so that the drug will be difficult to remove from the cavity of the chitosan nanoparticles (Silva 2006). Then the flux values obtained at steady state following the rule of law Fick.

Based on the results of the flux of caffeine penetrated per hour Figure 7 shows the caffeine treatment trapped nanoparticles of chitosan and chitosan nanoparticles coated caffeine treatment seen rising curve in the 30th minute and then the curve continued to decline in the next minute. According Ozguney et al. (2006) rising gradient curve is influenced by a large concentration in the donor compartment and the receptor while decreasing the curve due to the concentration of the active substance in the donor compartment started to decrease.

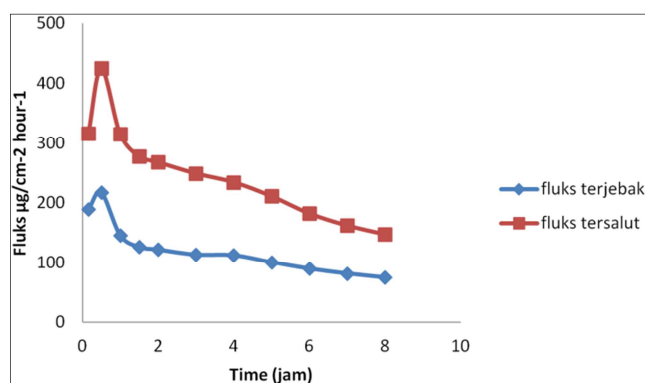


Figure 7. Graph flux penetrated caffeine.

4. Conclusion

Caffeine chitosan nanoparticles were made using two different treatments namely caffeine stuck and coated chitosan nanoparticles. Characteristics of nanoparticles of chitosan caffeine generated in this study is based on the morphology of the caffeine treatment trapped nanoparticles of chitosan and chitosan nanoparticles coated caffeine has a smooth surface and slightly convex but slightly lumpy, the average size of a row 232.74 nm and 226.62 nm. Analysis of functional groups that showed a shift wavenumber wavenumber amide group III (-CN) and wave number of the hydroxyl group (OH), as well as the efficiency of entrapment of the highest caffeine owned treatment of the coated nanoparticles of chitosan 64.63% while the caffeine treatment trapped nanoparticles 51.35% chitosan. Caffeine chitosan nanoparticle characterization results demonstrate the effectiveness of the penetration value in the treatment of caffeine coated chitosan nanoparticles have a penetration rate that is faster $2170.03 \pm 6.85 \text{ mg / cm}^2$ compared to chitosan nanoparticles trapped caffeine treatment $1089.65 \pm 10.7 \text{ mg / cm}^2$.

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