Optimization of Sonication Time and Surfactant Concentration for Chitosan-alginate Coated Ketoprofen Nanoencapsulation

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Abstract. Ketoprofen nanoparticles can be used to make a controlled ketoprofen delivery system. The material composition and method of making nanoparticles used had an effect on the formation of ketoprofen nanoparticles. This study aims to obtain the optimized results of each response by varying the sonication time, alginate concentration, and concentration of tween 80 and characterizing the ketoprofen encapsulation efficiency, nanoparticle size, and homogeneity level. Ketoprofen nanoparticles are made by ultrasonication method. The encapsulation efficiency of ketoprofen was evaluated using UV-Vis spectrophotometry analysis, while the size of the nanoparticles were evaluated using particle size analyzer (PSA). The optimum condition of the response was predicted at sonication time of 18.41 minutes with alginate and tween 80 concentrations of 1% (w/v) and 3% (v/v) respectively. The optimized results of each response is predicted to be around 21,412 ± 8.78% for encapsulation efficiency, particle size around 650,056 ± 284.47 nm, and homogeneity level (PdI) around 0.623 ± 0.10. The resulting equation can be used to determine the optimum value of encapsulation efficiency and particle size in the sonication time range 10 – 30 minutes, the concentration of alginate 0.2 – 1%, and the concentration of tween 80 1 – 3%.

1. Introduction
Ketoprofen is a non-steroidal anti-inflammatory drug that is commonly used for the treatment of rheumatoid arthritis, osteoarthritis, and various chronic musculoskeletal diseases. Ketoprofen has a half-life elimination in blood plasma of about 1.5 - 2.0 hours. The concentration of ketoprofen that persists in blood plasma after 24 hours is only about 0.07 mg / L. Therefore, it must be consumed frequently to maintain therapeutic levels in plasma. However, the use of high doses can cause bleeding in the stomach. To improve its release in the body, ketoprofen needs to be encapsulated [1].

Nanoencapsulation is a material coating technique which is very small in size, with an average diameter of 10 - 1000 nm [2]. Nanoencapsulation can form two types of drug coating in the nanoparticles, which are adsorbed on the surface or trapped in the cavity of the capsule matrix [3]. The advantage of using nanoparticles as a drug-controlled delivery system is that the size and surface characteristics of the nanoparticles are easy to manipulate to achieve treatment targets. Nanoparticles also regulate and prolong drug release during the transport process to the target, and drugs can be introduced into the nanoparticle system without chemical reactions. In addition, the nanoparticle...
system can be applied to various treatment targets, because the nanoparticles enter the circulatory system and are carried by the blood to the treatment target [2].

The sonochemical method using ultrasonic waves is believed to produce homogeneous micro/nano molecular weights [4]. Administration of ultrasonic waves can result in changes in the molecular weight of the liquid in the presence of viscosity degradation [5]. Changes in the molecular weight of a material are clearly influenced by the compressibility, vapor pressure, vapor stress, viscosity and the length of the sonication time [6-8].

Generally, in research using ultrasonic waves, the sonication process only varies the time and concentration of the surfactant solution. However, these variations have not actually obtained the optimum conditions from the sonication time and the concentration value of how much molecular weight or the smallest molecular size and are more homogeneous. Previous research conducted by Napthaleni [9] with samples of ketoprofen nanoparticles coated by chitosan-alginate, sonication treatment with time variations of 15, 30, and 60 minutes with variations in the concentration of surfactant tween 80 1%, 2%, and 3% showed that the sonication time of 30 minutes with a surfactant concentration of Tween 80 as much as 3% has the greatest encapsulation efficiency in the range of 43 - 73%, but the resulting particle size is still large in the range 580 - 13500 nm and the percentage of the number of particles produced is only 7% of the solution used.

Data analysis using ANOVA and RSM aims to test the adequacy of the model (lack of fit), which means that the model made is in accordance with the data. The analysis and design of the second order polynomials used when the lack of fit test on the first order polynomial model identifies that the maximum (minimum) area of the response surface has been reached and the second order polynomial model has an insignificant lack of fit test. Next, the suitable equation for the data whether linear or quadratic will be determined [10].

Based on previous research, due to the lack of scientific information regarding the relationship between sonication time, surfactant type and concentration, and alginate concentration with particle size, encapsulation efficiency, and the level of homogeneity of a chitosan-alginate coated ketoprofen nanoencapsulation, this study aims to obtain a statistical model which described the relationship between sonication time, surfactant type and concentration, and alginate concentration with particle size, coating effectiveness, and level of homogeneity.

2. Method
2.1. Time and Place of Research
This research was conducted from April to December 2018. Sample preparation and encapsulation efficiency test was carried out at the Biophysics Laboratory, Physics Department and Pilot Plant, PAU Bogor Agricultural Institute. The test of particle size and the homogeneity level of the solution was carried out at the Post-Harvest BPP2T Laboratory, Bogor.

2.2. Tools and Materials
The tools used in this study include analytical balance, Erlenmeyer flask, pippette, measuring cup, beaker glass, filter paper, 130 Watt 20 kHz Cole Parmer ultrasonic processor, magnetic stirrer, laptop with Design Expert 6.0.8 software, Particle Size Analyzer (PSA), UV-Vis Spectrophotometer, and BUChI 190 spray dryer. The materials used are distilled water, alginate, chitosan, acetic acid, TPP, ketoprofen, 96% alcohol, and tween 80.

2.3. Making of Combination Box-Behnken Formulas
The combination of formulas begins by determining the range of maximum and minimum concentration values for each component used. All concentration data were processed using the Box-Behnken model with 3 levels and 3 factorials to obtain a representative data distribution. The data processing resulted in the combination of the concentration values of the components used in the manufacture of nanoparticles.
2.4. Nanoparticles Synthesis
Chitosan solution with a concentration of 1.75% (w / v) was prepared in 1% (v / v) acetic acid. A total of 25.4 mL of chitosan solution was added each with 4.23 mL of alginate solution with various concentrations of 0.2% (w / v), 0.625% (w / v), and 1% (w / v) while stirred with a magnetic stirrer. A total of 1 mL of TPP with concentrations of 4.5% (w / v) was added to the chitosan-alginate mixture while stirred. A total of 27.78 mL of 0.8% (w / v) ketoprofen solution in 96% ethanol was mixed into chitosan-alginate-TPP while stirring until it became homogeneous. After that, 5 mL of Tween 80 with various concentrations of 1, 2, and 3% in water solvent were added to the mixture, then sonicated at a frequency of 20 kHz for 10, 20, and 30 minutes. The sonicated mixture was converted into powder form using a spray dryer with an inlet temperature of 120 °C and an outlet temperature of 65 °C.

2.5. Preparation of Standard Curves Ketoprofen
The main ketoprofen solution was prepared by dissolving 10 mg of ketoprofen in 100 mL of distilled water (100 ppm concentration). A total of 10 mL of stock solution was piped into a 100 mL volumetric flask and then added distilled water until the limit mark (10 ppm concentration). The absorbance of ketoprofen solution in a water solvent with a concentration of 10 ppm was measured at a wavelength of 200-400 nm, then the maximum absorbance value was obtained at a wavelength of 291 nm (Figure 4a). The maximum wavelength obtained was used for further analysis. Dilution was also carried out to obtain ketoprofen concentrations of 5, 15, 20, and 25 ppm. Standard curves were made from ketoprofen concentration series. The data obtained is a curve of the relationship between ketoprofen concentration and absorption.

2.6. Size and Homogeneity Level Characterization
Nanoparticles containing ketoprofen were analyzed for their particle size and homogeneity using a Particle Size Analyzer (PSA).

2.7. Encapsulation Efficiency Measurement
A total of 3 mg of nanoparticles were weighed and dissolved in 6 mL of water. The mixture was stirred for 24 hours and then filtered. The obtained filtrate was measured for its absorbance using a UV-Vis spectrophotometer at the maximum wavelength. The absorbance obtained was used to determine the ketoprofen concentration with the aid of a standard curve.

2.8. Optimization Process
The optimization process begins with the selection of criteria for each desired response. At the optimization stage each factor and response will be weighted by the importance. Weighting of importance level is a very important condition for determining a formula that will produce optimal conditions. The importance value of a response is from 1 (+) to 5 (+++++). The higher the importance value, the higher the importance of the response to be achieved. In addition to determine the level of importance, selection of target (goal) for each response is also very important to determine the limit value of the response to be selected.

3. Results and Discussion
3.1. Box-Behnken Formulation
Nanoparticles were made based on a combination of formulas obtained using the Box-Behnken experimental design (Table 1). The combination of the sonication time formula, alginate concentration, and Tween 80 concentration aims to study the effect of each of these basic components on the characteristics of the resulting nanoparticles. First, the values of each component were inputted, namely the sonication time of 10 - 30 minutes, alginate 0.2 - 1% (w / v), and tween 80 1 - 3% in the Box-Behnken program, then obtained 17 formulas as a recommended combination, with a few
repetitions of the formula. The value of each response obtained is also inputted into the table. The optimum combination of nanoparticle formulas based on the optimization results can be seen in Table 2.

3.2. Particle Size and Homogeneity Level
Ketoprofen nanoparticles were formed by breaking down molecules in the solution with the help of ultrasonic waves. Ultrasonication can break down large particles into smaller particles. The solution which has been through the ultrasonication process is then dried using a spray dryer to obtain nanoparticle powder. Spray-dried nanoparticles have the form of fine, dry, brittle granules. The ketoprofen nanoparticles in the form of a solution were analyzed using a Particle Size Analyzer (PSA) to determine the particle size and homogeneity level. The results of the analysis of particle size and homogeneity levels are shown in Table 1.

The size and homogeneity level of particles resulting from the formation of nanocapsules for each formula have varied results. The results showed that the S1 formula has the smallest average particle size of all formulas, namely 259.8 nm with a polydispersity index of 0.546, while the S16 formula has the largest average particle size of 1150.0 nm with a polydispersity index of 0.747. The size of the particles in the form of the nanoparticles determines the ease with which these particles enter the cell. The smaller the particle size, the easier it will be to enter the cells and the increased absorption in the body [11]. The measurement of particle homogeneity level showed that the polydispersity index of all samples exceeding the value of 0.5. This value indicates that the particle size distribution is in a wide range so that the particle size distribution has high heterogeneity. The smaller the polydispersity index number, the more uniform the particle size is because if the size difference between particles is greater, the result will affect the characterization of the particles [12]. The difference in size and level of homogeneity of each formula is due to differences in the composition of the nanoparticles.

| Table 1. Nanoparticle Formulations Based on the Box-Behnken Experimental Design |
|---------------------------------------------------------------|
| Code  | Sonication Time (minute) | [Alginate] (% w/v) | [Tween 80] (%v/v) | Encapsulation Efficiency (%) | Particle Size (nm) | PdI  |
|-------|--------------------------|---------------------|-------------------|----------------------------|-------------------|------|
| S1    | 10.00                    | 0.20                | 2.00              | 2.964                      | 259.8             | 0.546|
| S2    | 20.00                    | 0.20                | 3.00              | 1.218                      | 673.0             | 0.717|
| S3    | 30.00                    | 0.60                | 1.00              | 6.282                      | 685.9             | 0.601|
| S4    | 20.00                    | 1.00                | 1.00              | 15.349                     | 876.3             | 0.627|
| S5    | 10.00                    | 1.00                | 2.00              | 1.313                      | 418.3             | 0.506|
| S6    | 10.00                    | 0.60                | 3.00              | 0.236                      | 534.2             | 0.664|
| S7    | 20.00                    | 0.60                | 2.00              | 3.933                      | 1261.0            | 0.743|
| S8    | 20.00                    | 0.60                | 2.00              | 6.233                      | 887.8             | 0.697|
| S9    | 30.00                    | 1.00                | 2.00              | 3.887                      | 1125.0            | 0.756|
| S10   | 20.00                    | 1.00                | 3.00              | 29.122                     | 655.9             | 0.708|
| S11   | 30.00                    | 0.60                | 3.00              | 9.387                      | 973.3             | 0.575|
| S12   | 20.00                    | 0.60                | 2.00              | 7.961                      | 585.3             | 0.514|
| S13   | 20.00                    | 0.60                | 2.00              | 7.961                      | 585.3             | 0.514|
| S14   | 10.00                    | 0.60                | 1.00              | 10.370                     | 433.5             | 0.568|
| S15   | 20.00                    | 0.60                | 2.00              | 11.700                     | 422.1             | 0.585|
| S16   | 20.00                    | 0.20                | 1.00              | 10.386                     | 1150.0            | 0.747|
| S17   | 30.00                    | 0.20                | 2.00              | 13.960                     | 1055.0            | 0.747|
Table 2. Nanoparticle Formulations Based on Optimization Results

| Code | Sonication Time (minute) | Variable [Alginate (% w/v)] | [Tween 80 (% v/v)] | Encapsulation Efficiency (%) | Particle Size (nm) | PdI |
|------|--------------------------|-----------------------------|---------------------|-------------------------------|---------------------|-----|
| S18  | 18.41                    | 1.00                        | 3.00                | 21.412                        | 650.056             | 0.623 |

The size of the nanoparticles obtained were then entered into a combination formula in the Box-Behnken program as a response variable. The ANOVA results show that the linear model is not significant with a prob > F value of 0.0538, which means that there is a 5.38% chance of an error in the model. Furthermore, the data were analyzed in order to obtain a three-dimensional curve (Figure 3).
which can be used to see the effect of the formula combination on the size of the nanoparticles. The size of the nanoparticles increased along with the increasing sonication time. Increasing the alginate concentration tends to reduce the particle size.

\[
\text{ParticleSize} = 280.76 + 27.42A - 38.66B - 19.47C
\]

where A, B, and C were sonication time, Tween 80 concentration, and alginate concentration, respectively. Based on the above equation, factor A gives a positive value, meaning that the longer the sonication time, the larger the resulting particle size. Factors B and C give negative values, meaning that the greater the concentration of Tween 80 and the alginate used, the smaller the particle size. Based on the ANOVA results, factor A is the factor that most influences the particle size. The increased sonication time increases the particle size probably due to particle aggregation and accumulation of charges around the particles [13].

3.3. Ketoprofen Encapsulation Efficiency

Coating efficiency is a reflection of the amount of ketoprofen coated in nanoparticles. The determination of the value of efficiency is very important in the pharmaceutical field, especially for drug delivery systems in the body, because it can demonstrate the ability of ketoprofen nanoparticles to carry drugs into the body. The coating efficiency value varies in the range of 0.236% to 29.122%. These varying efficiency values are due to differences in sonication time, alginate composition, and tween 80 which affect the ease with which ketoprofen is extracted and out of the nanoparticle cavity. This efficiency value is very small when compared to the results of the 2010 Napthaleni research. This is due to the small volume of the mixture used to make the solution.
The efficiency value obtained is then inputted into the Box-Behnken formula combination as a response variable. The ANOVA result shows that the quadratic model is not significant with a prob> F value of 0.4189, which means that there is a 41.89% chance of an error in the model. The lack of fit value of the quadratic model is significant with a prob> F value of 0.0201, indicating that there is a mismatch between the research data and the regression model estimation. The data were analyzed so that a three-dimensional curve was obtained that illustrates the effect of the formula on coating efficiency (Figure 5). Figure 5a shows the effect of the sonication time and the alginate concentration on the encapsulation efficiency at a concentration of Tween 80 1% (v / v) with the highest efficiency value of 15,349%. The sonication time of up to 22 minutes increases the coating efficiency. After that the efficiency value decreased until the 30th minute, while the increase in the alginate concentration tend to decrease the coating efficiency. Figure 5b shows the effect of the sonication time and the alginate concentration on the encapsulation efficiency at a concentration of Tween 80 2% (v / v) with the highest efficiency value of 13.96%. The increase in the value of the encapsulation efficiency occurred until the 24th minute and then decreased until the 30th minute. The increase in the alginate concentration tend to increase the value of the encapsulation efficiency. Using a concentration of Tween 80 3% (v / v) (Figure 5c), the highest efficiency value was 29,122%. The sonication time up to 23 minutes increases the value of the encapsulation efficiency, then there is a decrease in the value of the encapsulation until the 30th minute. Increasing the alginate concentration tends to increase the value of the encapsulation efficiency. Based on Figure 8, it can be seen that the efficiency value fluctuates according to the optimum composition which affects the ability of the nanoparticle matrix to absorb ketoprofen.
Based on the modeling analysis that has been done, the mathematical equations for the response to encapsulation efficiency are:

\[
\text{Efficiency} = 25.81 + 1.78A - 30.52B - 31.88C - 0.05A^2 + 3.75B^2 + 16.95C^2 + 0.33AB - 0.53AC + 14.34BC
\]

where A, B, and C were sonication time, Tween 80 concentration, and alginate concentration, respectively. Based on the above equation, the value of the encapsulation efficiency will increase if the sonication time is not too long with the use of high alginate and tween 80 concentrations. As the sonication time increases, the encapsulation efficiency decreases due to the leaching of trapped drugs during the higher sonication time [13]. The increase in alginate concentration causes an increase in density in the preparation which causes the structure to become stiff and there is a reduction in free volume in the polymer matrix, so that there is a reduction in the volume of free ketoprofen that is absorbed in the polymer chitosan and sodium alginate. In addition, the high viscosity of sodium alginate causes the movement of particles in absorbing ketoprofen to be inhibited because high viscosity will inhibit the bond with chitosan to form a matrix to absorb ketoprofen [14]. Tween 80 plays a role in stabilizing the emulsion system where the higher the concentration of Tween 80 will make the dispersion medium rigid. The more rigid the dispersion medium will result in the increasing viscosity of the emulsion system, which causes the movement of particles to be inhibited [15].

### 3.4. Optimization and Verification of Chitosan-Alginate Coated Ketoprofen Nanoencapsulation

The optimization process aims to obtain process variables (formulas) and the optimal response to the manufacture of nanoparticles. Optimal data will give desirability results ranging from zero to one. The higher the desirability value, the higher the level of desire between the formula and the suggested response. Target selection in the optimization process is expected to limit the optimal value and be adjusted to the initial response conditions. This aims to reduce the error value between the response predicted by the program and the experimental response. The importance and goal components of each factor and response can be seen in Table 3.
Table 3. Optimized Response and Factor Components

| Response       | Goal          | Minimum | Maximum | Importance |
|----------------|---------------|---------|---------|-----------|
| Sonication Time| is in range   | 10.000  | 30.000  | 3         |
| Surfactant     | is in range   | 1.000   | 3.000   | 3         |
| Alginate       | is in range   | 0.200   | 1.000   | 3         |
| Efficiency     | maximize      | 0.236   | 29.122  | 5         |
| Particle Size  | is in range   | 250.000 | 650.000 | 5         |
| PDI            | minimize      | 0.506   | 0.756   | 1         |

Stage Verification is an advanced stage where the optimal data suggested by the program is then carried out experiments according to the orders in the hope that the response predicted by the program is in accordance with the experimental results that will be run. Based on the optimization results (Table 2), the optimal conditions will produce a desirability value of 0.695 or 69.5%. The desirability value shows the accuracy in the optimal solution results. The closer to the value 1, the optimum formula has a response value within the desired specifications [16]. Table 4 shows the comparison between the optimal response data predicted by the program and the experimental data that has been run.

Table 4. Comparison of Optimal Response Conditions Between Predictions and Experiments

| Response  | Prediction | SE Mean Experiment | SE Mean Prediction | Response Prediction ± Standard Deviation |
|-----------|------------|--------------------|--------------------|------------------------------------------|
| Efficiency| 21.412     | 5.770              | 8.78               | 21.412 ± 8.78                            |
| Particle Size| 650.056 | 138.720            | 284.47             | 650.056 ± 284.47                         |
| PDI       | 0.623      | 0.051              | 0.10               | 0.623 ± 0.10                             |

Based on Table 4, by using the formula at optimal conditions, the Design Expert is able to provide predictions for the results of the response to encapsulation efficiency, particle size, and polydispersity index, respectively, 21,412 ± 8.78%, 650,056 ± 284.47 nm, and 0.623 ± 0.10. The SE Mean value, which is the estimated average error, describes the distribution of the sample mean to the population mean. The smaller the SE Mean number, the less data variation value will be so that the sample estimation that represents the population is more accurate and precise. All responses had a smaller experimental SE Mean value than the predicted value. This shows that all responses generated experimentally have a better level of accuracy than predictions.

4. Conclusion
The ultrasonication method can be used in the manufacture of ketoprofen nanoparticles with a nanoparticle size of up to 259.8 nm. The coating of ketoprofen in tween 80 crosslinked chitosan-alginate produced the best ketoprofen nanoparticle formula at the composition of 1% (w / v) alginate, 3% (v / v) tween 80, and 20 minutes of sonication time with coating efficiency of 29,122%, size nanoparticles 655.9 nm with a polydispersity index of 0.708.

The optimum process conditions produced using Response Surface Methodology (RSM) predict the optimum process conditions at the sonication time of 18.41 minutes with an alginate concentration of 1% (w / v) and a concentration of tween 80 3% (v / v). Based on the prediction of sonication time and optimum concentration produced by the Response Surface Method (RSM), the optimum values of encapsulation efficiency, particle size, and polydispersity index were 21,412 ± 8.78%, 650,056 ± 284.47 nm, and 0.623 ± 0.10, respectively, with a desirability of 69.5%.

References
[1] Patil P R, Praveen S, Rani R H S and Paradkar A R 2005 AAPS Pharm. Sci. Tech. 6 E9.
[2] Mohanraj V J, Chen Y 2006 J. Pharm. Res. 5 561.
[3] Tiyaboonchai W, Ritthidej G C 2003 Songklanakarin J. Sci. Technol. 25 245.
[4] Hielscher T 2005 *Ultrasonic Production of Nano-Size Dispersion and Emulsions* Available From: https://hal.archives-ouvertes.fr/hal-00166996/document.

[5] Li J, Cai J, Fan L 2008 *J. Appl. Polym. Sci.* **109** 2417.

[6] Knapp R T, Daily J W, and Hammit F G 1970 *Cavitation* (New York: McGraw-Hill).

[7] Hammit F G 1980 *Cavitation and Multiphase Flow Phenomena* (New York: McGraw-Hill).

[8] Brennen C E 1995 *Cavitation and Bubble Dynamics* (New York: Oxford University Press).

[9] Sugita P, Napthaleni, Kurniati M and Wukirsari T 2010 *Makara, Sains.* **14** 107.

[10] Kurniati M 2014 *Optimasi kondisi proses pengempaan papan partikel dengan metode RSM dan ANN* (Bogor: Institut Pertanian Bogor)

[11] Mannuela N 2016 *Preparasi Dan Evaluasi Nanopartikel Azitromisin-Kitosan Dan Uji Aktivitas Antibakteri Terhadap Bakteri Propionibacterium acnes* (Pontianak: Universitas Tanjungpura)

[12] Manmode A S, Sakarkar D M and Mahajan N M 2009 *Int. J. Pharm. Tech. Res.* **1** 1020.

[13] Dangi R S and Shaky S 2013 *Int. J. Pharm. Life Sci.* **4** 2810.

[14] Awad R S, Abdelwahed W and Bitar Y 2015 *Int. J. Pharm. Pharm. Sci.* **7** 171.

[15] Laverius M F 2011 *Optimasi tween 80 dan span 80 sebagai emulsifying agent serta carbopol sebagai gelling agent dalam sediaan emulgel photoprotector ekstrak teh hijau (camellia sinensis l.): aplikasi desain faktorial* (Yogyakarta: Universitas Sanata Dharma)

[16] Nurmiah S, Syarief R, Peranginangin, R and Nurmata B 2013 *JPB Kelaut. Perikan.* **8** 9.