RESEARCH ARTICLE

The Effect of the Comparison of Sodium Alginate-Gelatin Levels on Microspheres Characteristics (Produced by Ionic Gelation Method Aerosolized Technique)

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ABSTRACT

The aim of this research is to investigate the effect of concentration ratio of sodium alginate-gelatin on the characteristics of microspheres. Microspheres were prepared with ionotropic gelation methods aerosolization technique with sodium alginate and gelatin as polymer matrixes, and calcium chloride (1.5 M) as the cross-linker, and then dried using freeze dryer. The concentration ratio of sodium alginate-gelatin that was used to make microsphere was divided into F1: 2.25:0.25%, F2: 1.75:0.75%, and F3: 1.25:1.25%. Resulting microspheres were characterized in terms of characteristics (yield, particle size, and swelling index). The result showed that there was an increase in yield and particle size, as sodium alginate concentration increased on formula. The statistical test showed the concentration ratio of sodium alginate-gelatine showed a significant meaning different from the yield and particle size. The swelling index shows that the swelling peak of microspheres became more quickly with increased sodium alginate concentration.

Keywords: Aerosolization, Characterization, Gelatin, Ionotropic gelation, Microsphere, Sodium alginate.

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INTRODUCTION

The topical drug delivery system is defined as a direct administration of drug formulation on the skin for intracutaneous or local delivery.[1,2] In topical use, the desired drug can provide long activity, and its release is slowed so as to increase its effectiveness.[3,4] The example of a drug delivery system that can meet this goal is microspheres.[5] Microspheres are small spherical particles with a diameter in the micrometer range (1-1,000 μm).[6] Topical microspheres provide constant and extended drug activity so that they reduce the frequency of use and improve patient compliance. Topical microspheres can be used to improve bioavailability and reduce the occurrence of side effects.[7]

Some methods of microspheres making include spray drying, solvent evaporation, hot melt, coaservation, and ionic gelation.[6] In this study, the method of making microspheres used was the ionic gelation method with the aerosolization technique. The ionic gelation is a cross-linking event of polymers with divalent cations, such as, Ca^{2+} forming an insoluble gel.[8] Ionic gelation method with aerosolization technique was carried out by spraying the polymer solution into a cross-connect solution to form microspheres without the involvement of organic solvents. The aerosolization technique is used because it can encapsulate drugs to be protected from the environment, the process is easy and fast, and the cost is relatively cheap.[9] The concentration of cross-linking $CaCl_2$ solution commonly used is 0.5 to 1.5 M.

Microspheres can be made from various kinds of natural or synthetic polymer materials.[6] Alginate is an anionic polymer of brown algae, and has been extensively studied used for biomedicine, which is biocompatible, low toxicity, relatively inexpensive, and has the advantage of easily forming a gel matrix. The main components of alginate constituents are L-gluronic and D-manuronate.[10] The mechanism of sodium alginate in the formation of microspheres is by forming an egg box structure, when reacted with divalent cations, such as, calcium, barium, and strontium. The divalent cation is able to bind two COO groups on adjacent L-gluronate alginates.[11] Drug release from microspheres with only alginate matrices is considered as delayed and simultaneous release so that additional polymers that are able to help to control the active ingredients are released gradually. One of the polymers that can be used is gelatin. Gelatin is a polyamoteric polymer that is naturally biodegradable, non-toxic, and has a good ability

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of swelling.[12] Under conditions of pH below the isoelectric point (pI 4.8-5), the gelatin will have an active group of NH_3^+ that expected to be electrostatically bonded with the COO manuronate group from alginate, which does not experience cross-linking so that it can cover the pores of the formed microspheres.

Factors that influence the characteristics of microspheres made by the ionic gelation method and aerosolization technique are the type and concentration of matrix polymer, as well as cross-linking solutions, cross-linking time, mixing speed, spraying air pressure, spray hole size, and spraying distance. Based on the results of the optimization carried out, the amount of polymer that can produce homogeneous microspheres and does not interfere with the spraying process is 2.5% with the spraying distance from the tip of the sprayer until the surface of the cross-connect solution is 8 cm and stirring speed is 1,000 rpm. With the control variable, which is 2.5% of total polymer, 1.5 M of the concentration of $CaCl_2$ cross-linking solution, and 90 minutes of stirring time, the difference in the ratio of the constituent polymers influences the characteristics of the microspheres.

Based on the description, it is necessary to do a research on the effect of the ratio of sodium alginate:gelatin (2.25:0.25%; 1.75:0.75%; 1.25:1.25%) to the characteristics of the microspheres (recovery, particle size, and swelling index) made by ionic gelation and aerosolization technique. Microspherical characterization carried out included organoleptic, infrared spectroscopy, shape, and surface morphology of microspheres, yields, moisture content, particle size, and size distribution, as well as, the swelling index.

MATERIALS AND METHODS

Chemicals

Pharmaceutical-grade sodium alginate, pharmaceutical-grade gelatin B, pharmaceutical-grade $CaCl_2$, food-grade maltodextrin, and aquademineralisata.

Equipment

Infrared spectrophotometer, differential thermal analysis (Mettler Toledo FP-65 DTA P-900 Thermal), pH meter SCHOTT glass Mainz type CG 842, Mettler Toledo HB43 S moisture analyzer, plate stirrer (Dragon Lab MS pro), analytic balance, aerosolization sprayer, Buchner funnel, optical microscope, scanning electron microscopy (inspect S50 Type FP 2017/12), and other glassware.

Microsphere Formula

Three microspheres formula were made with different matrix compositions, as shown in Table 1.

Table 1: Microspheres formula

Ingredients	*Functions*	*FI*	*FII*	*FIII*
Sodium alginate	Polymer	2.25%	1.75%	1.25%
Gelatin	Polymer	0.25%	0.75%	1.25%
$CaCl_2$	Cross connectors	1.5 M	1.5 M	1.5M
Malto-dextrin solution	Lyoprotectant	5%	5%	5%

Sodium alginate was weighed according to the formula, then dissolved in 40 mL of aquadem, stirred at a speed of 1,200 rpm for 15 minutes. Gelatin was also weighed according to the formula, then dissolved in 20 mL of aquadem 60ºC, stirred at a speed of 1,200 rpm for 15 minutes. Gelatin solution was put into a sodium alginate solution, then stirred at 1,200 rpm for 15 minutes. The pH of the solution was adjusted using 0.1 M HCl to obtain a pH solution of 4 + 0.05. Then, aquadem was added to 100 mL.

Calcium chloride dehydrate was weighed as many as 44.106 grams, then dissolved in 200 mL of aquadem, and stirred until being dissolved. Polymer solutions and medicinal ingredients were sprayed using nozzle aerosolization into calcium, chloride solution while stirred at a speed of 1,000 rpm, then left for 90 minutes.

The microspheres formed were separated by filtering using a Buchner funnel, while being washed with aquadem free calcium chloride. Microspheres that were free of calcium chloride were squeezed using filter paper until the amount of water was minimal, then weighed. The microspheres were then dispersed in a 5% maltodextrin solution, as many as, ten times the weight of the microspheres. The microspheres were dried using a freeze dryer for 24 hours. After that, the characterizations of the microspheres were formed.

Microspheres Characterizations

Organoleptic

The organoleptic examination was performed on shape, color, smell, and taste.

Fourier Transform Infrared (FTIR) Spectroscopy Examination

The evaluation of the occurrence of cross-linking reactions was carried out by infrared spectra examination using KBr pellet technique. The result of the examination was compared with the infrared spectrum of sodium alginate, gelatin, and sodium alginate microspheres.

Shape and Surface Morphology of the Microspheres

To see the shape and surface morphology of the resulted microsphere, it was carried out using an optical microscope, and the sighting was taken using a camera and using a scanning electronic microscope (SEM). The test using SEM was carried out by means of microspheres placed on the preparation handle with adhesive material containing metal grains, for example, Pt metal. Then, gold on the chamber was evaporated so that gold vapor coated the entire surface of the microspheres. After that, the surface of the gold-coated microspheres was observed with SEM.

Determination of Yield

Microsphere yield was determined by weighing the dry microspheres produced then compared with the weight of the ingredients of the microspheres.

$$\text{Yield} = \frac{\text{weight of resulted microspheres}}{\text{weight of microspheres constituents}} \times 100\%$$

Moisture Content Analysis

Analysis of moisture content began with preparing moisture analyzer and waiting until the screen showed the following display: 0.000 g. After the tool lid was opened, a blank pan sample was inserted into the pan sample handler on the tool, and then the tool cover was lowered and automatically directed to the "zero" condition. Then, a number of samples were inserted above the sample pan to show the minimum weight for measuring the sample; then, the tool cover was lowered. The tool would automatically start the measurement.

The time had been set for 10 minutes; after the measurement was complete, the measurement results would be printed on the screen. When finished, the tool lid was opened, the pan sample was taken and cleaned from the rest of the sample.

Size and Size Distribution of Microspheres Particle

Particle size measurement was carried out with a microscopy method using an optical microscope equipped with an ocular and objective micrometer. The ocular scale was calibrated by installing an ocular micrometer on the microscope by observing it, until the two scales were clearly visible. Then, the starting line of the ocular scale with the initial objective scale was squeezed so that the right line could be determined to coincide on both scales, then the ocular price was determined, microspheres were measured by placing microspheres on glass objects and measuring the diameter of the microspheres (300 particles). After that, the grouping of sizes from the smallest to the largest was done and divided into several intervals and classes. Then a particle size distribution curve was made.

Determination Swelling Index

A 50 mL of aquadem was prepared. Then, 50 mg of dry microspheres were weighed and put in a beaker filled with water. The swelling process was carried out at 37°C, and a change in weight at a certain time period was observed. Samples were drained each time using filter paper, then weighed as final wet weight. Swelling index price was calculated by:

$$\text{Swelling Index} = \left| \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \right| \times 100\%$$

Statistical Analysis

Statistical analysis was performed on yield, moisture content, average particle size, and swelling index. Statistical analysis was using a one-way ANOVA method, and then analyzed with the fisher LSD test using IBM SPSS Statistics 22.0 program with 95% confidence level.

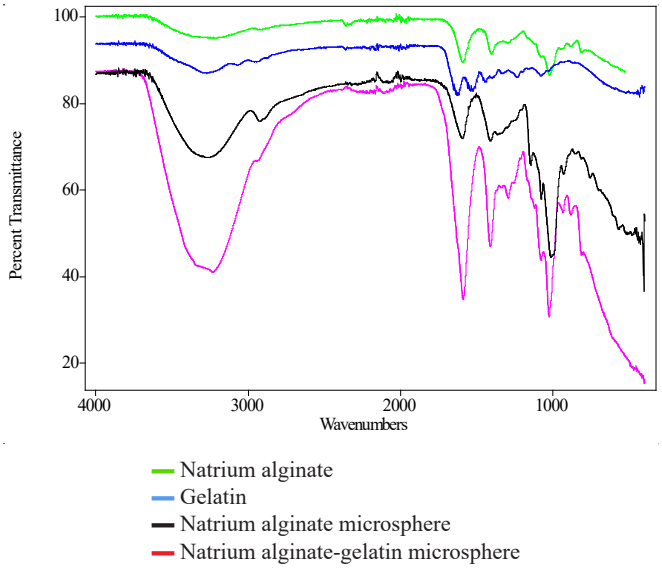

Figure 1: Microspheres infrared spectra and their constituents

RESULTS AND DISCUSSION

Organoleptic Examination Result

The result of microspheric organoleptic examination revealed that all formulas had the same organoleptic, which were powdery, white, odorless, and tasteless.

Microspheres Infrared Spectra Result

Proof of the microspheres' formation was carried out using infrared spectrophotometry. The microspheres formed were analyzed for the absorption of the groups and compared with the absorption of the raw material groups that made up the microspheres. The infrared spectra as shown in Figure 1, concluded that the microspheres formed had a wide absorption with strong intensity at 3,231.38 cm^{-1} wave number, which was estimated to be a combination of the absorption of the hydroxyl group in sodium alginate and maltodextrin (3,500-3,200 cm^{-1}) and the amide group possessed by gelatin (3,350-3,000 cm^{-1}). Absorption of carboxylic salt in sodium alginate was found in wavenumbers of 1,592.32 and 1,405.42 cm^{-1}, and the microspheres experienced a leftward shift of 1,599.76 and 1,412.69 cm^{-1}. This shift was estimated because of the substitution of the carboxylic salt constituent from Na^+ to be Ca^{2+} due to the cross-linking process experienced by two gluronic groups in alginate which were close together. At wave number of 1,024.26 cm^{-1}, the microspheres had a strong intensity of absorption, which could be a sign of CO group absorption possessed by maltodextrin (1,1501,000 cm^{-1}). Absorption at wave number of 850 to 810 cm^{-1}, which was a manuronate alginate fingerprint on the microspheres, did not show absorption. This absorption loss was due to the manuronate group in alginate reacting with NH_3^+ group from gelatin. Proof of microspheres formation through infrared spectra showed that the microspheres formed consisted of alginate, gelatin, maltodextrin, and $CaCl_2$ as the cross-linker.

Particles Shape and Surface Examination Result

The result of the examination of the shape and morphology of the particles using SEM showed that the microspheres produced were spherical with a smooth and flat surface (the result shown in Figure 2). Using nozzle with a certain size and spraying with pressure would make the polymer solution came out of nozzle in a spherical form so that the resulted microspheres would also have a spherical shape.

Yield Determination Result

From the examination result of yield microspheres, that shown in Table 2 and Figure 3, it was known that there was an increase in microsphere recovery as the concentration of sodium alginate increased in the formula. Under the conditions of $CaCl_2$ cross-linking solution, which still allowed for the occurrence of cross-linking reactions, the increasing level of sodium alginate would make the microspheres formed more units and the amount of alginate that formed one particle of the microspheres also increased so that the total weight of the microspheres increased, which caused the yield to also increase.

Based on statistical analysis, the result showed that the ratio of sodium alginate-gelatin level showed a significant meaning difference toward the microspheres yield. The existence of significant differences was indicated by sig values, which was smaller than 0.05. F1 and F2 had sig values amounting to 0.025, F1 and F3 amounted to 0.001, and F2 and F3 amounted to 0.011.

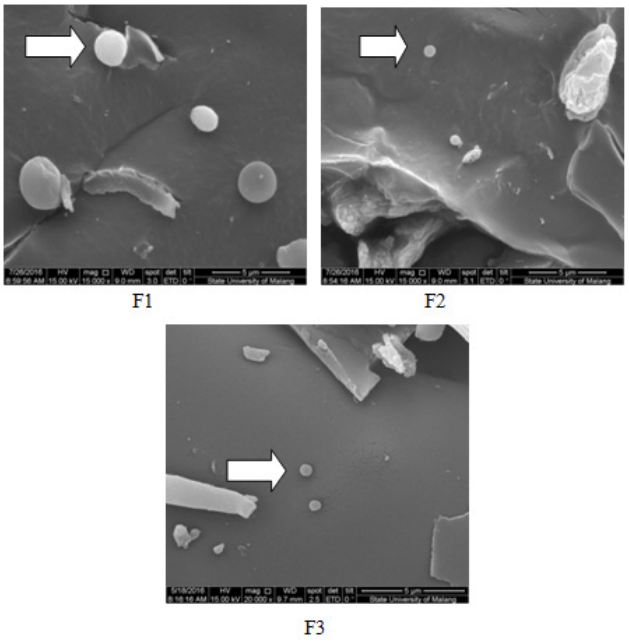

Figure 2: The results of the examination of the surface shape and morphology of the microspheres particle using a scanning electron microscope (SEM) at a magnification of 20,000x

Table 2: Result of the determination of microspheres yield

Formula	*Replication*	*Yield*	*Average ± SD (%)*	*KV*
	1	64.02		
F1	2	58.15	57.94 ± 6.2	10.7
	3	51.63		
	1	51.25		
F2	2	44.34	46.04 ± 4.61	10.01
	3	42.52		
	1	28.88		
F3	2	30.02	31.50 ± 3.60	11.42
	3	35.6		

Moisture Content Examination Result

On the examination of microspheres' moisture content, as shown in Table 3, obtained results that the microspheres were dried with the freeze-drying method for 24 hours and had the moisture content range from 8.99 ± 0.54% to 10.29 ± 0.45%. Based on statistical analysis, the result showed that the ratio of sodium alginate-gelatin level showed no significant difference in meaning for microspheres moisture content. The absence of a significant difference was indicated by greater sig value than 0.05. F1 and F2 had sig value amounted to 0.689, F1 and F3 amounted to 0.109, and F2 and F3 amounted to 0.061. Moisture content value must be in optimal condition. Moisture content, which was too high, could cause microspheres quickly overgrown with microorganisms, and because of the high humidity, it could also cause instability of active ingredients that were easily degraded due to the presence of water. Additional freeze-dry drying time, and a decrease in percent use of lyoprotectants could be done to produce lower moisture content value. Too low moisture content value could cause the physical shape of the microspheres to become brittle and the surface to easily erode.

The average particle size of microspheres that show in Figure 4, resulted (in µm) F1 = 4.50 ± 0.17, F2 = 3.76 ± 0.04, F3 = 3.20 ± 0.14. Based on statistical analysis, the result showed that the ratio of sodium alginate-gelatin level showed

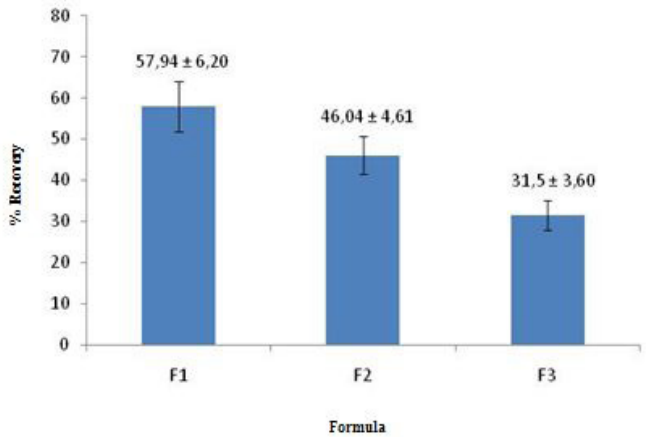

Figure 3: Graph of the average microspheres yield

Table 3: Moisture content examination result

Formula	*Replication*	*Moisture content (%)*	*Average ± SD (%)*	*KV*
	1	10.18		
F1	2	9.27	9.78 ± 0.47	4.76
	3	9.9		
	1	10.56		
F2	2	9.77	10.29 ± 0.45	4.4
	3	10.55		
	1	9.56		
F3	2	8.48	8.99 ± 0.54	6.03
	3	8.94		

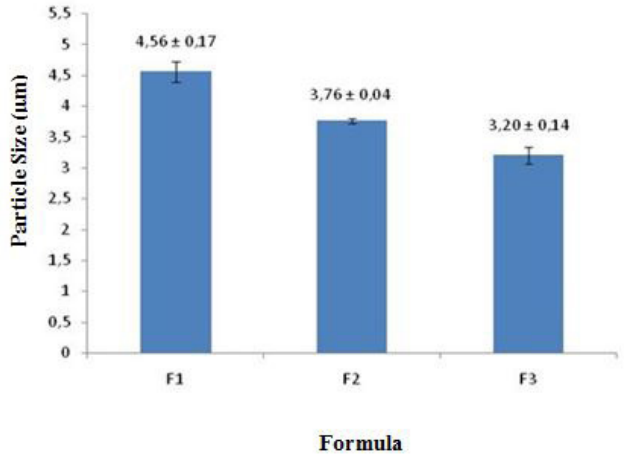

Figure 4: Graph of the average microspheres particle size

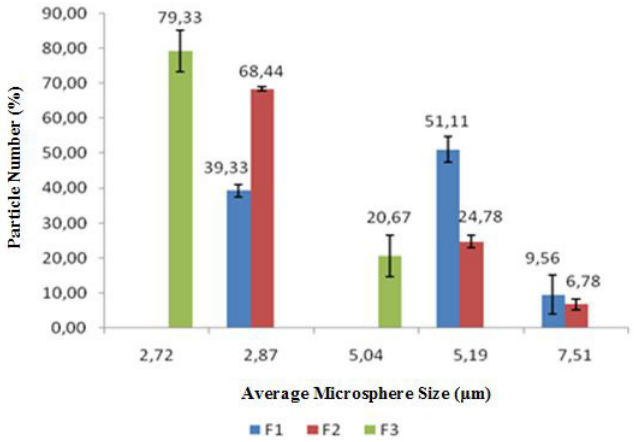

Figure 5: Graph of microspheres particle size distribution

significant differences in the particle size of the microspheres. The existence of a significant difference was indicated by sig value that was smaller than 0.05. F1 and F2, and F1 and F3 had sig values equal to 0, then F2 and F3 to 0.002. The larger level of sodium alginate caused an increase in viscosity in the polymer solution, which would be sprayed through the aerosol spray so that the formation of the particle size sprayed into the cross-linking solution was also getting bigger. In addition, with the increasing level of sodium alginate, the amount of alginate that formed one particle of the microspheres also increased; therefore, it also increased the particle size of the microspheres.

Particle Size Distribution

All formulas were predicted to produce optimal particle size (5-50 µm) for a topical delivery system with the addition of drug ingredients in the system. The result of particle size determination showed that the range of microsphere particle size distribution is 2 to 8 µm (Figure 5).

Swelling Index Examination Result

On swelling index observation, F1 data obtained (in percent) had a swelling value at the maximum of 323.33 ± 6.43 achieved at the 1st hour, F2 that was 332 ± 8 achieved at the second hour, and F3 that was 338 ± 4 achieved at the third hour. All three formulas provided different swelling index profiles, as shown in Figure 6. Based on statistical analysis, the result showed that the ratio of sodium alginate-gelatin level showed a significant meaning difference toward the microspheres swelling index profile at the time of observation of the first hour until the fourth hour. At the time of observation at 5 o'clock, only the ratio sodium alginate-gelatin levels in F1:F3 and F2:F3 showed a significant meaning difference toward the microspheres swelling index. At the time of the sixth observation, the ratio of sodium alginate-gelatin level did not show a significant meaning difference toward the microspheres swelling index. There was a significant difference indicated by value sig, which is smaller than 0.05, and there is no significant difference indicated by sig value that was greater than 0.05. The larger level of sodium alginate caused the microsphere to reach the maximum swelling maximum at a faster time but had a smaller

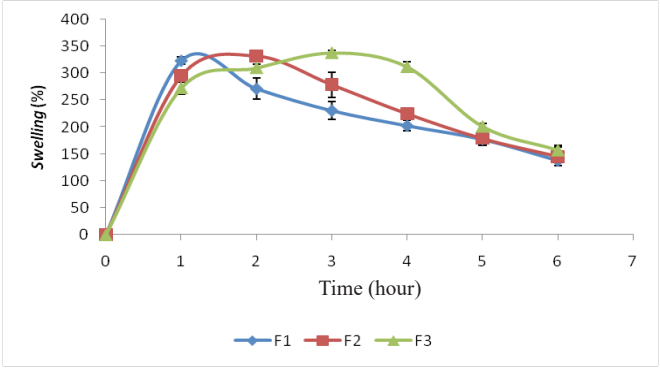

Figure 6: Swelling index profile of microspheres (%)

% swelling value. In F1, the highest level of sodium alginate and the lowest gelatin were estimated to have the largest size and number of pores on the surface compared to F2 and F3 because the amount of gelatin which bound to the manuronate group in alginate, which was not cross-linked, was also limited so the microsphere could experience maximum hydration in a shorter time. With the presence of gelatin which interacted electrostatically with the manuronic group, it was estimated that it could cover the pore on the surface, so that water would be more difficult to enter into the microsphere and make the microspheres reached the maximum swelling value at a longer time. An increase in % swelling value at F2 compared to F1 and the biggest at F3 was estimated because of the amount of gelatin found in each formula. Gelatin itself had the swelling property that was good, thus the more level of gelatin in the microspheres, the % swelling value would also increase.

CONCLUSION

Based on the description above, the results show that the ratio of sodium alginate-gelatin level affects the yield, particle size, and microspheres swelling index profile. Increased level of sodium alginate and decreased level of gelatin result in the increase of recovery, increase in particle size, and decrease in value and acceleration in microspheres maximum swelling index achievement.

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