Effect of $CaCl_2$ Crosslink Solution Concentration on the Characteristics of Gelatin Alginate Microspheres

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Abstract

Alginate as a constituent of microspheres matrix has a weakness, namely the lack of ability to slow the release of drugs from the system, causing the effects of drug therapy to the body to be less than optimal. To obtain preparations with slowed release, sodium alginate needs to be combined with other polymers such as gelatin. This study aims to determine the effect of the concentration of CaCl 2 connection solution on the characteristics of alginate-gelatin microspheres. In this study, alginate-gelatin microspheres were prepared using ionotropic gelation aerosolization techniques. Three formulas were made, each of which was replicated three times. F1 with CaCl concentration2 0.5 M, F2 with CaCl concentration2 1.0 M, F3 with a concentration of 1.5 M. The three formulas were made in the ratio of alginate-gelatin (1.5: 1)% and a crosslinking time of 1.5 hours. Evaluation of the characteristics of microspheres includes yield percentage, organoleptic, moisture content, morphology of microspheres, size and size distribution of microspheres, and swelling index. The data of this study were analyzed using one way ANNOVA. The results reveals a loss of specific uptake of the COO-manuronate alginate group and the occurrence of wave number shifts as well as an increase in the absorption intensity of the C = O group. Organoleptic examination results of alginate-gelatinin microspheres showed that microspheres of the three formulas had the same organoleptic, and formed spherical microspheres. On the inspection of swelling index, it was found that F1 and F2 reached the peak of swelling at 2 o'clock, while F3 reaches the peak of swelling at 3 o'clock. The greater the level of CaCl2, then the average diameter of microspheres will also increase, while the swelling index will decrease with increasing levels of CaCl2. In the results of moisture content examination, there is no difference in different CaCl 2.

Keywords: *alginate, gelatin, microspheres, $CaCl_2$, ionotrophic gelation, aerosolization.*

Introduction

Drug delivery system is a system designed to deliver active ingredients and provide therapeutic effects on the body [1]. The drug delivery system aims to minimize the release of drugs in unwanted areas, avoid harmful side effects, and increase effectiveness [2]. Delivery systems for topical preparations can be aimed to provide local or systemic effects. Some delivery systems used for topical preparations include liposomes, niosomes, solid lipid nanoparticles, and microspheres (Castro *et al*., 2008). Microsphere is a particle that has micro size and *spherical shape.* The particle size of microspheres ranges from 1 μm to 1000 μm [3]

Microspheres can be made using a variety of methods including emulsification, coaservation and *ionotropicgelation* [4]. On the method of *ionotropic gelation,* the formation of microspheres is based on the ability of the polymer to form a hydrogel in the presence of divalent or polyvalent cations from a cross-linking solution. Based on the literature review, $CaCl_2$ concentration that is commonly used as a cross-linking solution is 0.5 M, 1 M and 1.5 M with a cross-

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connect time of 1.5 hours [5]. Alginate as a constituent of microspheres matrix has a weakness: it lacks the capability to slow the release of drugs from the system. To obtain preparations with slowed release, sodium alginate needs to be combined with other polymers, for example gelatin [6].

Increasing the concentration of the crosslinking solution will increase the number of bonds between the polymer and the crosslinking solution, so that the matrix will be more compact and lower the *swelling index,* thus the drug release from the system will be slower. However, increasing the concentration of the crosslinking solution will result in a microsphere with large particle size. This large particle size is undesirable in topical preparations since it can cause a sandy appearance when used. In addition, a small particle size is more desirable because it will produce a wider surface area when applied since the drug release will be more optimal [7].

Based on the description above, this study was conducted to determine the effect of $CaCl_2$ crosslinking solution (0.5; 1; 1.5) M on the characteristics of microspheres with sodium alginate-gelatin matrix (1.5: 1)% which includes percent recovery, moisture content, shape and surface, particle size, and *swelling index*. Microspheres production is done using the *ionotropic gelation* aerosolization techniques with a cross-connect time of 1.5 hours. The aim of this research is that the results are expected to be used as consideration in developing microspheres as delivery systems for topical routes with local effects.

Research Method

Research Material

This research uses Low Molecular Sodium Alginate (*Pharmaceutical grade*), Gelatin B (*Pharmaceutical grade*), Calcium Chloride (*Pharmaceutical grade*), Maltodextrin *(Food grade)*, HCl 0.1 N, Aquadem. microspheres production is done using *ionotropic gelation* aerosolization techniques. *Ionotropic gelation* is a simple method, does not use high temperatures, does not use organic solvents and the use of aerosolization techniques can produce smaller and more uniform particle sizes [8].

Alginate-gelatin solution is sprayed into the $CaCl_2$ solution which have been made using atomizer with *nozzle* size of 200 μm, under constant pressure at a distance of 8 cm from the surface of the crosslinking solution and rotated at a speed of 1000 rpm for 1.5 hours. The process will produce a suspension of microspheres which are then filtered using a Buchner funnel and washed with aquadem until it is free of $CaCl_2$ [7].

Research Tools

The tools used in this study are *stirer plate* (Dragon Lab MS pro)*,* pH meter SCHOTT glass mainz type CG 842, FTIR spectrophotometer (Jasco FT-IR / 5300), aerosolization spray (*nozzle* size 200 μm and 40 Psi pressure), analytical scales, ovens, petri dishes, optical microscopes, *moisture analyzer* and other glassware.

Variable Research

This study aims to determine the characteristics of alginate-gelatin microspheres with a ratio of 1.5%: 1% and $CaCl_2$ crosslinking solution with concentrations of 0.5 M, 1 M, 1.5 M, made using the *ionotrophic gelation* aerosolization technique with a cross-connect time of 1.5 hours. The independent variable is the concentration of $CaCl_2$ crosslinking solution, and the dependent variable is the microspheres' characteristics. While the control variables are the viscosity of the solution, the pH of the solution, the volume of $CaCl_2$ and polymer solution, concentration of polymer solution, ratio of alginate-gelatin concentration, diameter of *nozzle,* spray pressure, and the distance of the $CaCl_2$ solution with *nozzle*.

Analysis of Data

Examination of *Moisture Content*

Moisture content value is expressed in percent. This value is obtained from the numbers listed on the digital screen of *moisture analyzer*. Replication was done three times and the results are averaged. The moisture content obtained is compared with the conditions of *moisture content* from the references [5].

Morphological Examination of Microspheres

The shape and surface of the microspheres were observed based on the results obtained from *Scanning Electro Microscope* (SEM). This results in the form of three-dimensional appearance of the microspheres. This three-dimensional picture can be compared in terms of surface flatness and the spherical form of the microspheres produced from the three formulas made [9].

Distribution and Size of Microspheres

From the determined size of 300 particles, they are grouped into several intervals and classes. Then the particle diameter and percentage are determined. After that, the calculation results are presented in the form of a histogram with particle diameter as abscissa, and percent of particles as ordinate.

The average diameter of microspheres of each formula is calculated by the following formula:

$$D \text{ average} = \frac{\Sigma nd}{\Sigma n}$$

Note: n = number of microspheres observed d = microsphere size

From the calculation results, average diameter of microspheres was obtained, and the results were compared between formulas. Data analysis was done using *One way* ANOVA [10].

Examination of *Swelling index*

Swelling *index* is expressed as *percent swelling* (Ps) in the form of a graph with the x-axis in the form of time (hours) and the y-axis in the form of % *swelling* (weight change) at every hour. After obtaining the graph, the parameters to be compared between formulas are time and swelling profile from the microspheres produced.

Result

Table 1 Average particle size of alginate-gelatin microspheres

Formula	Replication	Particle size (µm)	Average ± SD	%KV
1	1	2.84	2.82 ± 0.02	0.75 %
	2	2.80		
	3	2.83		
2	1	3.60	3.51 ± 0.14	3.96%
	2	3.58		
	3	3.35		
3	1	4.23	3.90 ± 0.29	7.34%
	2	3.72		
	3	3.75		

From table 2 it can be seen that the particle size obtained already meets the recommended size as a microsphere intended for topical use, which is less than 300µm. Y*ield* percent of the microspheres produced were analyzed statistically using the*one way ANOVA* method and tested post hoc with Fisher LSD using the IBM SPSS Statistics 22.0 program with a 95% confidence level. The particle size of microspheres in each formula are F1 = F1 = 2.82 ± 0.02; F2 = 3.51 ± 0.14; F3 =3.90 ± 0.29 respectively.

Based on the results of statistical analysis, a value is obtained; *sig* = 0.001. Value of *sig* <0.05 indicates a significant difference in the yield percent from microspheres produced by all three formulas. After *post hoc* testing it can be seen that the difference is in F1 and F2 with values of *sig* = 0.004; F2 and F3 with values of *sig*= 0.041 and F1 and F3 with values of *sig*= 0.000. This means that the difference in $CaCl_2$ concentration has an effect on yield percent of microspheres. Examination of *swelling index* was done with three replications using the weighing method.

Table 2: Results of Alginate-Gelatin Microspheres Swelling (1.5: 1.0)%

Formula	% Swelling Average ± SD					
	1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour	6th Hour
F1	378.67 ±11.37	469.33 ±15.14	348 .00 ±27.78	158.00 ±9.17	141.33 ±6.43	134 .00 ±17.44
F2	254.67 ±12.22	410.67 ±55.08	304.67 ±45.62	232.67 ±65.86	180.67 ±18.90	152.67 ±11.01
F3	171.33 ±41.68	326.00 ±14.42	346.00 ±16.37	290.67 ±28.59	227.33 ±18.15	158.67 ±19.01

From the table above it can be seen that the high levels of $CaCl_2$ (1.5 M) takes longer to reach the *swelling*. peak. Based on the results of data processing of*swelling index* every hour, statistical analysis was carried out using *one way ANOVA* with a 95% confidence interval and the LSD Hoc post test.

Table 3 Data Statistics Test Results *of* Alginate-Gelatin Microspheres Swelling (1.5: 1.0)%

Swelling Percent/ Hour	Significant Value		
	F1 to F2	F2 to F3	F3 to F1
1	0.001*	0.008*	0.000*
2	0.790	0.230*	0.002*
3	0.151	0.168	0.942
4	0.710	0.140	0.008*
5	0.021*	0.010*	0.001*
6	0.208	0.666	0.111

*: there are significant differences in the *swelling* value between the two formulas.

Discussion

Based on *post hoc* test result, it can be seen that there are differences in F1 and F2 with values of *sig* = 0.001; F2 and F3 with values of *sig*= 0.031 and F1 and F3 with values of *sig*= 0.021. According to a literature study, percent recovery should increase with an increase in $CaCl_2$ level, as long as polymer is still available to form bonds. However, in this study the highest percentage of recovery was obtained in F2 with $CaCl_2$ levels of 1.0 billion, and decreased in $CaCl_2$ levels of 1.5 M [11]. This can be caused by several factors including the calculation of percent recovery which is influenced by the addition of maltodextrin and the drying process using *freeze drying* which cannot be controlled by researchers [5].

Tables above showsthat the morphology of alginate-gelatin microspheres using SEM, it was found that the resulting microspheres were spherical in shape and had a flat surface. This can be seen in Figure 5.4. Whereas the particle size examination obtained particle size for F1 = 2.82 ± 0.02; F2 = 3.51 ± 0.14; F3 =.3.90 ± 0.29 respectively. Based on the results of statistical analysis, a value is obtained; *sig* = 0.001. Value of *sig* <0.05 showed a significant difference in particle size of alginate-gelatin microspheres. Based on the *post hoc* test result it can be seen that the difference is in F1 and F2 with values of *sig* = 0.004; F2 and F3 with values of *sig*= 0.041 and F1 and F3 with values of *sig*= 0,000 (Liu & Wang, 2011).

The results of the qualitative analysis of sodium alginate organoleptically shows data in accordance with the research hypothesis. In the analysis with FTIR there are some specific uptake, namely uptake at 1599.76 cm^{-1} and 1405.42 cm^{-1} which shows the presence of carboxylic groups and uptake at 815.11 cm^{-1} which is the fingerprint area of mannuronates. In addition, the results of observations of thermal analysis using DTA found a*peak* in the region of 233° C, this is in accordance with the data in the literature which states that on the sodium alginate thermogram peak will occur in the area of 191.15-254.68^{0}° C. From these results it can be seen that the difference in $CaCl_2$ level has no effect on organoleptic alginate-gelatin microspheres.

The results of organoleptic examination of maltodextrin showed that the maltodexin used was in accordance with the data in the references, which is in the form of fine white powder, has a typical odor, and not sweet. The results of the analysis with FTIR show that there are some specific uptake that is consistent with the data in the references. Based on the references, maltodextrin will provide absorption at wave numbers between 980-1200 cm^{-1} which shows the presence of CO groups and absorption at 3400 cm wave $number^{-1}$ which indicates the presence of C = O carboxylic groups. In the results of the examination of gelatin used, an uptake was found at wave value of approximately 1021.5 and 1080 cm^{-1} which is a CO group. Uptake at around 1157.7 cm^{-1} and 3429.6 cm^{-1} which is the C=O group.

According to Mi et al. in his research (2019), microspheres as a delivery system for topical routes are expected to have a small size and homogeneous and can release the drug slowly [12]. This is related to the acceptability and effectiveness of topical preparations. Preparations with slow release can be obtained from microspheres with alginate-gelatin combination matrices using *ionotropic gelation* method with a $CaCl_2$ crosslinking solution. *Sustained release* in alginate-gelatin microspheres occur due to the interaction between Ca $ions^{2+}$ with the $COO\ group^{-}$ guluronate in alginates and NH $groups^{3+}$ gelatin with the $COO\ group^{-}$ manuronate in alginates, causing the microspheres formed to have a more compact structure [13].

The achievement of topical preparations with the desired criteria is influenced by the characteristics of alginate-gelatin microspheres which include morphology, size and size distribution and *swelling index*. The characteristics of the microspheres are influenced by several factors, including the concentration of the polymer, the cross-linking time and the concentration of the cross-linking solution [4]. The focus of this research is to investigate the effect of different levels of $CaCl_2$ crosslinking on the characteristics of alginate-gelatin microspheres prepared using the *ionotropic gelation* aerosolization techniques [14]. The characteristics of microspheres compared in this case include, percent recovery, size and size distribution and swelling profile [15].

The efficiency of the microsphering process can be seen from the recovery obtained in each formula. The results of the calculation of percent recovery are F1 = 39.77 ± 3.30; F2 = 64.69 ± 7.72; and F3 = 52.85 ± 2.72. From these results a statistical analysis was conducted using *one way ANOVA* and obtained the value of *sig* = 0.003. A value of sig <0.05 indicates that there is a significant difference in the percent recovery results with a difference in $CaCl_2$ levels.

Observation on *swelling index* was done to seek the profile or pattern of *swelling* from each formula to estimate the pattern of drug release from the microsphere formula. Based on the observations it is obtained that each formula has a different *swelling profile.* F1 and F2 reached the *swelling* peak at the same hour, which is the second hour, but both have different peak *swelling* values. F1 reaches greater top*swelling* compared to F2. The peak *swelling* value of the two formulas in sequence are F1 = 469.33 ± 15.14, F2 = 410.67 ± 55.08.

Conclusion

Based on the results of the study, it can be concluded that an increase in CaCl2 (0.5; 1.0; 1.5)M levels in alginate-gelatin microspheres (1.5: 1) had no effect on organoleptic and morphological microspheres, yet it increases microsphere particle size. Based on the observations it is obtained that each formula has a different *swelling profile.* F1 and F2 reached the *swelling* peak at the same hour, which is the second hour, but both have different peak *swelling* values

Statement of Informed Consent

This study involves participants in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study is carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

Conflict of Interest: There is no report about conflict of interest since thsi study has been conducted until now.

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