PHYSICAL CHARACTERISTICS OF ERYTHROPOETIN ENCAPSULATED INTO ALGINATE POLYMER USING AEROSOLIZATION TECHNIQUE

by Noorma Rosita

Submission date: 08-Jun-2020 08:55PM (UTC+0800)
Submission ID: 1340078383
File name: Physical_Characteristics_of_Erythropoetin_Encapsulated.pdf (890.54K)
Word count: 5905
Character count: 33267

Journal of Physics: Conference Series **1445** (2020) 012012 doi:10.1088/1742-6596/1445/1/012012

PHYSICAL CHARACTERISTICS OF ERYTHROPOETIN ENCAPSULATED INTO ALGINATE POLYMER USING AEROSOLIZATION TECHNIQUE

[1]Dewi Melani Hariyadi1, [1]Noorma Rosita, [1]Kamila Amalia

[1]Pharmaceutics Department, Faculty of Pharmacy, Universitas Airlangga, Indonesia

Email: dewi-mh@ff.unair.ac.id

Abstract. The aim of this research was to evaluate physical characteristics of erythropoietin encapsulated into alginate polymer using a microparticle technology called aerosolization technique at different polymer concentrations. Erythropoetin is a model of neuroprotectant drugs. The sodium alginate concentrations used were 1%, 2%, and 3% with 1M cross-linker CaCl2. The physical characteristics in terms of morphology, particle size, swelling index, yield, and structural integrity of erythropoietin-Ca alginate microspheres were determined. Microsphere evaluation included FT-IR, DTA, moisture content, morphology using SEM, particle size distribution using optical microscopy, determination of swelling index, and yield. SDS PAGE electrophoresis was also conducted to evaluate the molecular weight of erythropoietin before and after the microencapsulation process.

The resulting microspheres had spherical form, smooth surface, and the sizes below 5 µm. The determination of swelling index was performed using two methods, namely calculation of mass differentiation and size differentiation at 24 and 30 hours. Results of examination from swelling indices in F1, F2, F3 were 0.58, 1.25, 1.43 (at 24 hours) and 0.78, 1.78, 2.16 (at 30 hours), respectively, whereas resulted of swelling index of 0.50, 1.15, 1.32 (by size method at 24 hours), and 0.65, 1.80, 1.98 (at 30 hours). The result of yield microspheres of F1, F2, F3 were 75.55% ± 0,350; 77,84% ± 0,290; and 86,65% ± 0,191. Statistical result showed that an increase of alginate concentration causes an increse in particle size, swelling indeks, and yield. From SDS PAGE profile, it was confirmed that erythropoietin maintains its structural integrity evidenced by the similar molecular weight before and after encapsulation process. This study has demonstrated the potential of erythropoietin-alginate microspheres that may be effective as a neuroprotectant drug.

Keywords: Erythropoetin, alginate microspheres, physical characteristics, swelling index, yield, integrity structure

1. INTRODUCTION

Stroke is the fourth leading cause of death in the world and causes disability in more than 15 million people every year [1]. Stroke is caused by a disruption of blood supply to the brain. Stroke is classified

to ischemic and hemorrhagic stroke. Reduced blood flow causes disruption of nutrient distribution in the brain, which in turn causes necrotic cells in the brain to die. To prevent further damage, an agent is needed to prevent it, namely a neuroprotectant [1].

A neuroprotectant is generally defined as an agent that prevents the death of neuronal cells by inhibiting one or more pathophysiological sequence processes that result in nervous system attack or ischemia [2]. There are 80 neuroprotective classifications, one of them from the cytokine category; an example of a cytokine class neuroprotective is erythropoietin [2].

Erythropoetin (EPO) is a glycoprotein hormone. This hormone is produced in the kidney and liver and functions as a regulator in the poliferation and maturation of red blood cells [3]. In healthy humans, one percent of the total red blood cells are broken every day and will be replaced by reticulocyte. The availability of oxygen in the blood is the main regulator of erythropoiesis; hypoxia induces the expression of the EPO gene in the kidneys and liver [4]. EPO has also been known to have neuroprotective and neurodegenerative effects, and EPO is also known to reduce the extent of brain damage following a stroke [1]. Erythropoetin is given by intravenous or subcutaneous injection [4].

EPO is a hormone that most of its constituent structures are proteins. This EPO has some disadvantages such as a half-life of only 6-8 hours, so to keep the EPO concentration in the therapeutic range, repeated injections are needed is required [5]. In addition, pH instability and extreme temperatures can lead to structural denaturation [6]. For parenteral preparations, a certain particle size is required (less than 5μm). To overcome the existing problems, a delivery system in the form of sustained-release or control-release can maintain the optimum concentration of the drug in the blood circulation and its release rate can be predicted and maintained for a certain duration, to increase its stability, and set the particle size of the preparation to fit the specification. Of all existing drug delivery systems, microspheres are very potential to deliver protein-based drugs [8].

Microspheres are hollow spherical materials that have a range of nano-to-micro diameter ranges, with the presence or absence of material trapped or located on its surface. Microspheres are an important application in biotechnology [8]. Microspheres have a size between 1-1000μm [9]. The mechanism of the microspheres is to trap the ingredients in them in order to protect them from environmental influences, and its drug release occur in proportion to the degradation of the matrix in biological fluids [10].

The methods used in the manufacture of microspheres include spray-drying, emulsification-solidification, phase separation (coacervation), template-assembly, and ionotropic gelation [8]. The ionotropic gelation method has the advantage of stable medicinal materials, uniform and spherical particle sizes, easy and quick manufacturing process, safer procedures, and a relatively low cost [11]. In the manufacture of microspheres by the ionotropic gelation method, there are several factors affecting the resulting microsphere, such as the ratio of drug-polymer concentrations, crosslinking solution concentrations, and crosslinking time. It will affect particle size and distribution, entrapment efficiency, and drug release profile [12]. Other factors such as pH and temperature may affect the structural integrity of the EPO and also affect the interaction between the drug ingredients and the additional ingredients present [13,14].

Many variations of both natural and synthetic polymers can be used for the manufacture of microspheres. Synthetic polymers that can be used include polyester (PLGA and polylactide (PLA)). Natural polymers that can be used in microsphere preparations include protein groups (collagen, gelatin, and albumin), as well as polysaccharide groups (starch, dextran, inulin cellulose, and alginate) [8]. One of the most commonly used natural polymers is alginate. Alginate is a natural biopolymer extracted from brown algae and has properties that may be used as a matrix [15]. One of the most commonly used alginates is sodium alginate.

Sodium alginate is a natural biopolymer that has biocompatible, biodegradable, non-toxic, and inexpensive properties [16,17]. The sodium alginate concentration used affects the characteristics of the generated microspheres. In the study of ovalbumin-alginate microspheres, increased concentrations of sodium alginate (1%, 1.5%, 2.5%) leads to an increase in particle size from the microsphere [18]. In another study, which is on bovine serum albumin-alginate microsphere with a concentration of sodium

alginate polymer of 2%, 3%, and 4% showed an increase in the sodium alginate polymer concentration above, giving no significant bovine serum albumin entrapment efficiency [19].

In the manufacture of microspheres of alginate polymers, a crosslinking solution is required to form alginate hydrogels. Some examples of crosslinking solutions that can be used include Ba2+, Sr2+, Ca2+, and others [15]. Ca2+ is more commonly used as an ionic crosslinker to reduce the dissolution of the alginate matrix for many applications. In addition, Ca2+ has lower toxicity compared with other divalent cations [16, 20]. From various sources of Ca2+, calcium chloride (CaCl2) is the most commonly used source because it is easily soluble in water and also Ca ion2+ can crosslink alginate droplet alginate because Ca ions are bound to the guluronic acid residue, which is a component of Na alginate[21, 20]. In the study of ovalbumin-alginate microspheres, a CaCl concentration of2 1.5 M provides characteristics of smooth microsphere surface and a spherical shape [18].

To improve the stability of alginate microspheres, maltodextrin may be used as a lyoprotectant. Maltodextrins can maintain the stability of alginate microspheres during the drying process with freeze dry and prevent aggregation and sedimentation. Microspheres with lyoprotectant maltodextrin produce microspheres of small size (1-5µm), spherical shape, and smooth surfaces [22].

In this research, we made the EPO-alginate microspheres by ionotropic gelation method and aerosolization technique using Na alginate concentrations of 1%, 2%, and 3% w / v and CaCl2 1 M crosslinker. This aims to determine the effect of alginate concentration on physical characteristics (particle shape, size, and surface appearance, drug loading, trapping efficiency, and yield) of EPO-alginate microspheres.

2. MATERIALS AND METHODS

2.1. Materials:

Erythropoietin Recombinant (Daewong Pharmaceutical Co.Ltd.), Pharmaceutical-grade Sodium alginate (Sigma-Aldrich Inc); pharmaceutical-grade $CaCl_2.2H_2O$ (Solvay Chemical International), pharmaceutical grade Maltodextrin (Bratachem); Demineralized water.

2.2. Method

2.2.1. Microspheres Formula

Table 1. Erythropoetin-Alginate Microspheres Formula

FORMULA	ACTIVE INGREDIENT	POLYMER	CROSSLINKING SOLUTION	LYOPROTECTANT	CROSSLINKING TIME
	Erythropoetin	Na-Alginate	$CaCl_2$	Maltodextrin	
F1	5000 IU	1.0% b/v	1 M	5%	30 minutes
Blank F1	-	1.0% b/v	1 M	5%	30 minutes
F2	5000 IU	2.0% b/v	1 M	5%	30 minutes
Blank F2	-	2.0% b/v	1 M	5%	30 minutes
F3	5000 IU	3.0% b/v	1 M	5%	30 minutes
Blank F3	-	3.0% b/v	1 M	5%	30 minutes

2.2.2. Erythropoetin - Alginate Microspheres Production

Sodium alginate (according to the formula) was dissolved in 100 ml of demineralized water. 5000 IU of Erythropoetin was dispersed into alginate solution and was stirred until the solution became

homogeneous. The resulting erytropoetin-alginate solution was sprayed using aerosol spray with a hole size of 35 µm, a constant pressure of 40 psi, and a spraying distance of 8 cm into 200 ml $CaCl_2$ and was stirred constantly for 30 minutes at the speed of 1000 rpm. Microspheres that were formed in centrifuges at a speed of 4000 rpm for 6 minutes were then washed using distilled water for 2-3 times. The washed microspheres were suspended in a 5% maltodextrin solution, then dried with freeze drying at -26^{O}C for 30 hours.

2.2.3. Characterisation of Erythropoetin-Alginate Microspheres

Microspheres Morphology Evaluation

Observations on the shape and surface of the resulting erythropoetin-alginate microspheres were performed using an optical microscope and the display was taken using a camera. In addition, observations using Scanning Electron Microscopy (SEM) were also conducted.

Measurement of Moisture content (MC)

$$\%MC = \frac{\text{Microsphere weight before analysis} - \text{Microsphere weight after analysis}}{\text{Microsphere weight after analysis}}$$

2.2.4. Infrared Spectroscopy Examination

The infrared spectrophotometry test through KBr disc method using Infra Red Spectroscopy.

2.2.5. Differential Thermal Analysis (DTA)

Identification was performed using the DTA FP 900 Thermal System.

2.2.6. Particle Size Distribution Test

The test was performed using optical microscope. 300 particles measurement was conducted. The distribution of the microsphere was presented in the form of a histogram, then the particle size distribution of each microsphere of the formula was compared, and the mean diameter was calculated using the formula:

$$\text{D average} = \frac{\sum nd^3}{\sum nd^2}$$

Notes:
n = number of microparticles observed
d = microparticle size

2.2.7. Determination of Swelling Index (SI)

The determination of swelling index was performed by weighing 100 mg microspheres and then adding 5 ml PBS pH 7.4 in vial. The swelling index determination was performed for 24 and 30 hours. After observation time had been reached, the wet microspheres were filtered using filter paper. After no PBS was dripped from the filter paper, the wet microspheres were transferred to a dry filter pad and then the microspheres were turned back to remove the adsorbed water on the microsphere surface until the filter paper was not too wet. Afterwards, the wet microspheres were ovened at a temperature of 37 °C for 2 hours or until the wet microsphere's weight became constant. After being ovened, the microsphere was weighed as the swelling weight. The swelling index (SI) value was calculated using the formula:

$$SI = \frac{Swelling\ weight - dry\ weight}{dry\ weight}$$

The swelling index observation was also performed based on microsphere size. A little microsphere were taken to be observed for microsphere size during swelling using optical microscope. The swelling index (SI) value was calculated using the formula:

$$SI = \frac{swelling\ microsphere\ size - dry\ microsphere\ size}{dry\ micrsophere\ size}$$

2.2.8. Yield Determination

The microsphere yield was calculated from the total amount of dry microspheres produced compared to the amount of Na alginate and EPO added to make EPO-alginate. The calculation resulted in a yield percentage from the erythropoetin microsphere obtained.

$$\%\ \text{Yield} = \frac{Total\ dry\ microsphere\ weight}{Erythropoeitin\ and\ Na\ alginate\ total\ weight}\ \text{x}\ 100\%$$

2.2.9. Erythropoetin structure integrity test using SDS PAGE

The procedure was to dilute the erythropoetin-alginate microsphere sample and fresh erythropoetin as the standard to the PBS (pH 7.4) then insert a 30 μl sample and standard ovalbumin to the hole of the stacking gel. The electrophoresis process was carried out at a constant voltage of 125 V with a current strength of 40 mA. The end of the electrophoresis process was when the sample boundary had reached the bottom part of the gel. Then the staining process was performed by soaking the gel into coomasie blue solution for 45 minutes; afterwards, the gel underwent destaining for 2 hours, and its profile of electrophoresis was recorded.

Data Analysis

To determine the effect of sodium alginate concentration on the physical characteristics of EPO-alginate microspheres, the data from each examination of quantitative content of EPO in alginate microspheres, EPO entrapment efficiency, and EPO-alginate microsphere yield were analyzed statistically with SPSS 20 using one-way ANOVA with a 95% confidence degree ($\alpha = 0.05$).

3. RESULTS AND DISCUSSION

Erythropoetin (EPO) is a hormone that regulates the poliferation and maturation of red blood cells. Erythropoetin has also been known to have neuroprotective and neurodegenerative effects [1]. EPO has several disadvantages, such as a half-life of only 6-8 hours, so to keep the EPO concentration in the therapeutic range, repeated injections are needed [5]. EPO is formulated in microsphere preparations to extend its effects and improve patient compliance.

This research is about the effect of the amount of polymer concentration on EPO-alginate microsphere characteristics. The microsphere observations observed in this study included the shape and appearance of microspheres, moisture content, particle size distribution, swelling index, and yield. At the beginning of the study, qualitative examinations such as organoleptic, infrared spectrum analysis, DTA thermal analysis for alginate, $CaCl_2$, and maltodextrin. This examination aims to ensure the raw materials used in ensuring the materials used in the study have met the provisions listed in the literature.

Based on the organoleptic examination, the EPO raw material is a clear, odorless, liquid solution in accordance with the organoleptic specifications of EPO listed on the product's brochure. In the IR spectrum examination, EPO gives a specific absorption peak which indicating the presence of an amine group (NH) at 3267.56 cm^{-1} and Amide I at 1636.09 cm^{-1}.

The identification of the organoleptic Na-alginate in the form of powder is odorless, tasteless, and has brownish yellow color in accordance with the literature. IR spectrum examination showed that Na-alginate gave an absorption peak which showed the presence of OH stretching, CH stretching, carboxylic salt group, C-O stretching, C-O-C stretching, and guluronate fingerprint. On the observation of DTA Na-alginate temogram, an exothermic peak was reached at 238.7 ^{o}C, corresponding to the temperature in the literature, namely 240 ^{o}C [12].

The identity of $CaCl_2$ is in the form of white, hard, and odorless flakes showing the results of observations in accordance with the literature of Pharmacopoeia Indonesia edition V (2015), namely in the form of granules or white, hard, and odorless flakes. The results of DTA CaCl temogram$_2$ shows a

melting peak at a temperature of 176.5 °C, which also corresponds to the literature value of 176°C for $CaCl_2$ dihydrate [24].

The result of organoleptic identification of lyoprotectant used is maltodextrin, in the form of white, maltodextrin-like smelling, and of non-sweet tasting maltodextrin. The maltodextrin DTA thermogram results showed its melting peak at 185.2 °C that entered in the melting range in the library of 40-185 °C. The IR spectrum results show that maltodextrin has a stretching OH group and a polysaccharide group (C-O stretching).

After all the raw materials were declared to be in accordance with the literature, the next step is making erythropoetin-alginate microsphere using ionotropic gelation method with aerosolization technique. There are 3 microsphere formulas used in this study; each of the formulas distinguished by the amount of concentration of sodium alginate polymer used, i.e. F1 using 1% sodium alginate solution, F2 using 2% sodium alginate solution, F3 using 3% sodium alginate solution. The researchers investigated the effects of the difference in the amount of alginate solution polymer concentrations to the physical characteristics of the microsphere.

Prior to the manufacture of EPO-alginate microspheres, it is necessary to check the pH and viscosity in sodium alginate solution and $CaCl_2$ solution used for the procedure. This is because the stability of EPO is influenced by pH. The results were that the pH of alginate solution and $CaCl_2$ 1.0 Molar (M) solution was still at the EPO stability pH. Furthermore, as much as 70 µL (5000IU) EPO were added to the Na-alginate solution. The EPO-alginate solution was sprayed into CaCl solution$_2$ using a nozzle, which was then stirred for 30 minutes at 1000 rpm. Before usage, the researchers had to check the state of the sprayer for whether there was a blockage on the nozzle by filling the equipment with hot water and used it to spray with pressure. After the microsphere was formed, a microsphere washing process of 2-3 times was performed by using demineralized water. Microsphere washing was performed to remove excess $CaCl_2$, which does not react with sodium alginate because excess $CaCl_2$ can reduce the entrapment efficiency [25].

The washing process was performed by using a centrifuge at 4000 rpm for 10 minutes. The purpose of the use of centrifuges at the microsphere washing stage was to facilitate the microspheres gliding process.

3.1. Characterization of Eryropoetin-Alginate Microspheres

The wet microspheres formed from each formula were observed in terms of particle size and particle size distribution by an optical microscope. From the calculation results, there was an increase in particle size along with the increase of polymer concentration. This phenomenon may be caused by the increase in polymer concentration, increasing viscosity that would form bigger microspheres [26]. However, the overall formula yielded particle sizes of less than 5 µm. This is in accordance with the particle size requirements for subcutaneous preparations that should be less than 5 µm [7].

The wet microspheres were then dried by the freeze-drying method. This method has the advantage of preventing stability disorders, both physical and chemical ones, which occur when wet microspheres are stored for long periods of time [27]. However, freeze-drying may cause stress variation during the freezing and drying process, so lyoprotectant is added to protect the microsphere from stress due to freezing and desiccation [27]. The added Lyoprotectant was maltodextrin. The nature of maltodextrin as lyoprotectant in stabilizing the microsphere during the freeze-drying process was described in the water replacement hypothesis [27].

Dry microspheres obtained from the whole formula were white and odorless powder. The third dry microsphere formula was then subjected to moisture content (MC) examination. The measurement of moisture content was performed to determine the degree of hygroscopicity of a product [27]. High water content can cause agglomeration of particles that may decrease the stability of the microspheres and active ingredients. From the measurement results, there was an increase in MC along with the increase of polymer concentration. This can be due to the more alginates binding to $CaCl_2$. Calcium chloride induces the interaction of carboxyl alginate groups to form polymer structures that can achieve water-resistance easier [28].

3.2. Morphological Observation Results

3.2.1. Optical microscope

The results of examination of the shape and surface of wet microspheres using an optical microscope can be seen in Figure 1

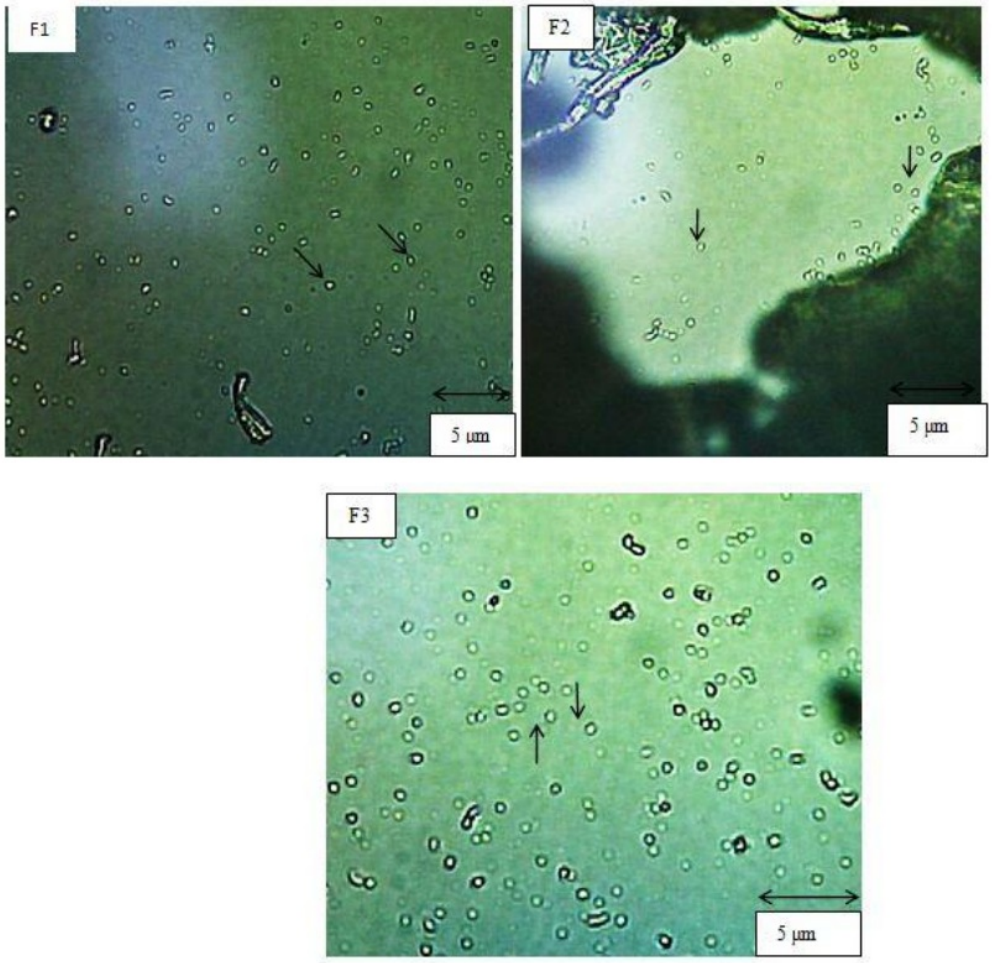

Figure 1. Observation of EPO-alginate microspheres using optical microscope with 400x magnification

3.2.2. Scanning Electron Microscope (SEM)

The results of examination of the shape and surface of wet microspheres using Scanning Electron Microscope (SEM) can be seen in Figure 2. From the observation, the whole formula was found to be having a spherical shape with a smooth surface.

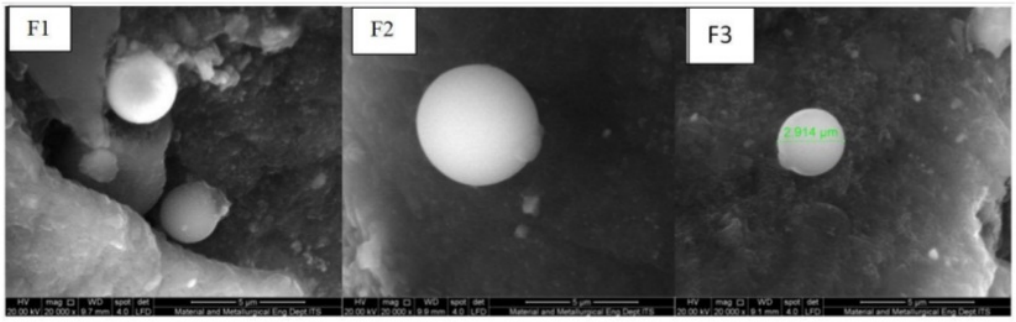

Figure 2. 2 Observation of the shape and surface of the EPO-alginate microspheres (alg. 1% b/v), F2 (alg. 2% b/v), and F3 (alg. 3% b/v) using Scanning Electron Microscope (SEM) with 20,000x magnification.

3.2.3. *Particle Size Distribution*

The results of microsphere particle size distribution examination of the three EPO-alginate formulas and microsphere blanks without EPO can be seen in table 2 and figure 3.

Table 2. Particle size distribution of EPO-alginate microspheres

Formula	Mean diameter of the microsphere size (µm)	
	BLANK	WITH EPO ± SD (% KV)
F1	2.93	2.95 ± 0.046 (1.56%)
F2	2.98	3.07 ± 0.058 (1.89%)
F3	3.10	3.13 ± 0.064 (2.04%)

Notes:
F1: EPO-alginate microspheres (1% b/v Alginate, $CaCl_2$ 1 M solution)
F2: EPO-alginate microspheres (2% b/v Alginate, $CaCl_2$ 1 M solution)
F3: EPO-alginate microspheres (3% b/v Alginate, $CaCl_2$ 1 M solution)

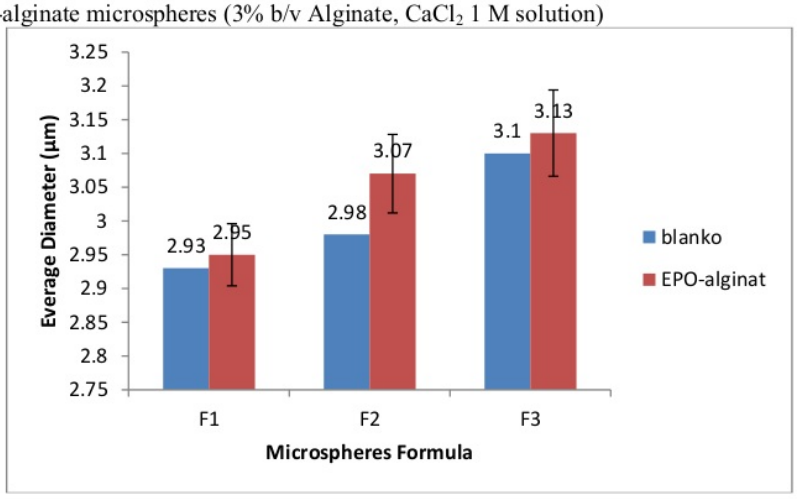

Figure 3. Comparison of blank and EPO-alginate microsphere particle sizes

The results showed that the particle size between the blanks and the EPO microspheres showed differences in the three formulas. The observations showed an increase in particle size as the polymer concentration increased.

In the analysis of the effect of increasing alginate polymer concentration on the particle size of the microspheres using One Way ANOVA statistical analysis using the IBM SPSS Statistics 23.0 program. From the results obtained, the comparison of F1 with F2, F1 with F3, and F2 with F3 obtained sig values greater than 0.05, indicating no significant difference of particle size obtained from different polymer concentrations.

3.2.4. Swelling Index

The results of swelling index examination of erythropoetin microspheres (EPO) -alginate F1, F2, and F3 based on mass and particle size can be seen in figure 4 and 5 as follows:

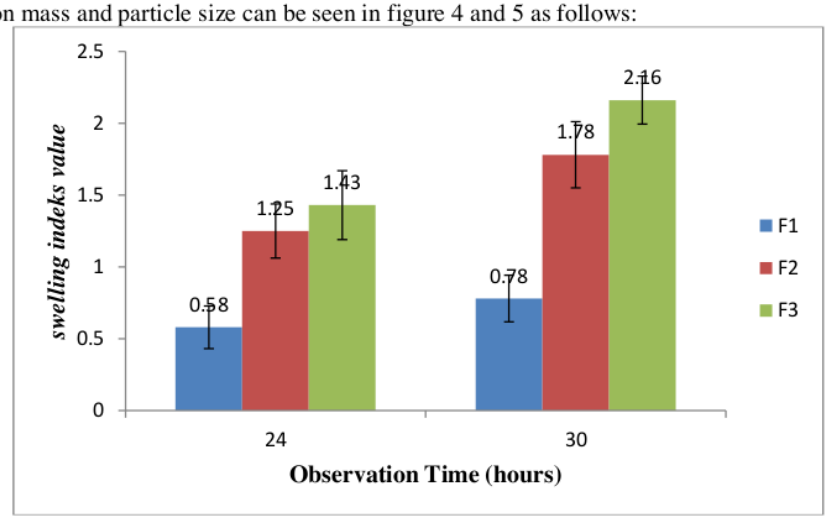

Figure 4. Swelling index by mass measurement method on EPO-alginate microspheres.

The observations of swelling indexes of microspheres with erythropoetin (EPO) F1, F2, and F3 based on particle size in figure 4 showed elevated polymer concentration of sodium alginate and erythropoetin (EPO) concentration used in the formula, causing an increase of swelling index in microspheres. An increase in index swelling was observed at 24 hours and 30 hours of observation.

Observations of swelling indices on microspheres were performed using two methods: mass change calculation method and method of particle size change method. In this analysis, statistical tests were performed using independent t-test. From the analysis results, the sig values of F1, F2, F3 are 0,505; 0.616; 0.694. The sig value of the whole formula is greater than 0.5, meaning that there is no significant difference between the mass change method and the particle size change.

The observation of the increase in swelling index from 24 to 30 hours was done in the form of statistical test using paired t-test. From result of analysis from formula F1, F2, and F3 using the mass observation method, sig values 0,106; 0.002; 0.065 were obtained. From the above results, it can be concluded that the increase of swelling index of F1 and F3 is not significantly different. Meanwhile, F2 experienced a significant difference

The analysis of the effect of increasing alginate polymer concentration on the particle size of the microspheres was performed using One Way ANOVA statistical analysis through the IBM SPSS Statistics 23.0 program. The analysis was performed on swelling index by mass method at 24 and 30

hours. The results of the analysis found the microsphere comparison of F1 with F2 and F1 with F3 with sig values smaller than 0.05 indicating a significant difference from the swelling index obtained from different polymer concentrations. Meanwhile the comparison of F2 and F3 microspheres obtained a sig value greater than 0.05, which shows no significant differences from swelling index obtained from different polymer concentrations.

.

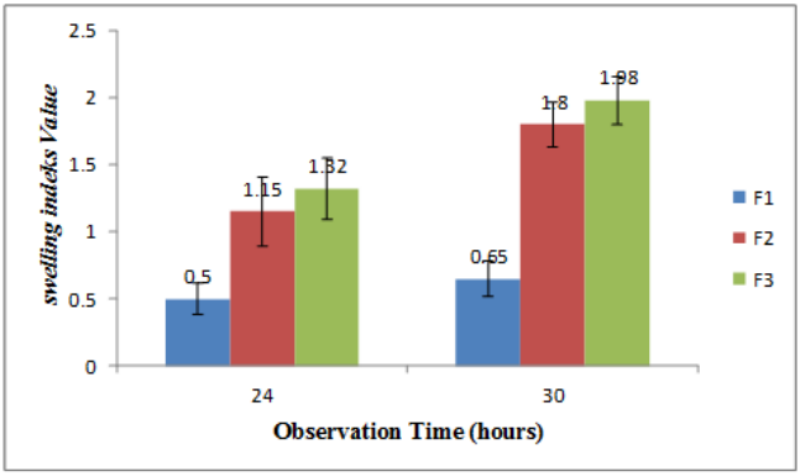

Figure 5. Swelling index using particle size measurement method on EPO-alginate microspheres.

In this research, swelling index test was conducted on all formula. The swelling index examination aims to determine the extent to which microspheres can enlarge in a given buffer [37]. This examination was performed using two methods, namely weight measurement method and addition of microsphere size diameter measurement method. The results of statistical analysis showed that the two methods used are not significantly different. The result obtained is that the more polymers are added, the higher the swelling index value. This is due to an increase in polymer concentration that can increase the concentration of water entering the microsphere, thus increasing microsphere swelling [12] (Manjanna et al., 2010). The results of statistical analysis showed that the two methods used are not significantly different.

3.2.5. Yield

The results of the microsphere yield can be seen in Table 3

Table 3. Yield of EPO-alginate microspheres

Formula	Yield (%) ± SD	KV (%)
F1	75.55 ± 0.350	0.46
F2	77.84 ± 0.290	0.37
F3	86.65 ± 0.191	0.22

Notes:

F1: EPO-alginate microspheres (1% b/v Alginate, $CaCl_2$ 1 M solution)
F2: EPO-alginate microspheres (2% b/v Alginate, $CaCl_2$ 1 M solution)
F3: EPO-alginate microspheres (3% b/v Alginate, $CaCl_2$ 1 M solution)

From the results of observation of microspheric yield with erythropoetin (EPO) F1, F2, and F3 in table 3, it can be seen that the microsphere yield increases along with the increase of polymer concentration of sodium alginate used in the formula.

In the analysis of the effect of increased alginate polymer concentration on the particle size of the obtained microsphere, a sig value of 0.000 <0.05 was obtained for comparison between F1: F2; 0.000 <0.05 for comparison between F1: F3; 0.000 <0.05 for comparison between F2: F3. Sig values smaller than 0.05 indicate a significant difference, meaning that the results of this analysis show that the increase of polymer concentration gives a significant difference for the ratio of F1 with F2, and F1 with F3, and F2 with F3 to the yield of EPO alginate microspheres.

From result of % yield measurement, it is known that there is an increase of % yield from F1, F2, and F3 formulas. This is because when the polymer concentration increases, the rate of binding of the croslinker to the droplet surface is slower, allowing increased penetration of the crosslinker into the droplet so that the polymer bond with the crosslinker progresses into the droplet. The result of statistical analysis shows that the effect of polymer concentration gives significant differences with the sig value of 0.000 <0.05 for comparison between F1: F3; 0,000 <0.05 for comparison F1: F3; and 0.000 <0.05 for comparison between F2: F3 to microspheres gain because the value is less than 0.05.

3.2.6. SDS Page Electrophoresis

The results of the SDS-PAGE examination showed that the erythropoetin used in the study is in accordance with the standard erythropoietin in the literature that has a molecular weight of 60 kDa (Figure 6).

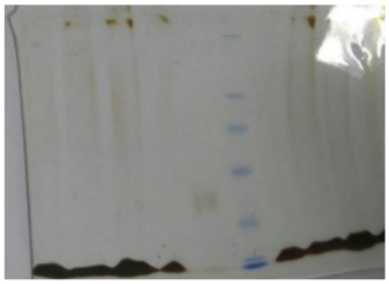

Figure 6. SDS PAGE of Erythropoetin Standard; Protein Marker; and Eryropoetin-Alginate Microspheres Formula

In microspheric formulations produced by encapsulation of processes using aerosolization, erythropoetin in the formula remains stable and capable of maintaining its structural integrity. The 60 kDa molecular weight was observed in the SDS-PAGE profile when compared to standard protein markers. This indicates that erythropoetin in the microsphere remains stable after the encapsulation process.

4. CONCLUSION

The microspheres production of Erythropoetin-Ca alginate by aerosolization technique produced microspheres with a size of <5 µm that are spherical and smooth. The increase in polymer concentrations of 1%, 2%, and 3% resulted in the increase of swelling index and yield values, but no significant increase occurred in terms of particle size distribution. The integrity of erythropoetin can be seen from the lack of changes in molecular weight of erythropoetin before and after the encapsulation process. Further research is suggested on the stability test of EPO-alginate microspheres by observing the denaturation of EPO.

ACKNOWLEDGMENTS

The authors would like to thank the Faculty of Pharmacy Universitas Airlangga for all laboratory and instrument facilities that have supported the implementation of this research.

References

[1] Wang, Yunfei., Cooke, Michael J., Morshead, Cindi M., Shoichet, Molly S., 2012. Hydrogel delivery of erythropoietin to the brain for endogenous stem cell stimulation after stroke injury. Biomaterials. 33 p.2681-2692
[2] Jain, Kewal K., 2011. The Handbook of Neuroprotection. New York: Humana Press
[3] Melli, Giorgia., Keswani, Sanjay C., Hoke, Ahmet., 2006. History and biology of erythropoietin in hematopoietic and non-neural tissues. Erythropoietin and Nervous System p.1-13
[4] Jelkmann, Wolfgang., 2013. Physiology and Pharmacology of Erythropoietin. Transfusion Medicine and Hemotherapy p.302-309
[5] Rong, Xianfang., Yang, Sixing., Miao, Huamao., Guo, Tingling., Wang, Zhenyu., Shi, Wanru., Mo, Xiaofen., Yuan, Weien., Jin, Tuo., 2012. Effects of Erythropoietin-Dextran Microparticle-Based PLGA/PLA Microspheres on RGCs. IOVS. 53(10) p.6025-6034
[6] Sola, Ricardo J. and Griebenow, Kai., 2009. Effects of Glycosylation on the Stability of Protein Pharmaceuticals. J Pharm Sci. 98(4) p.1223-1245
[7] Patel, Rajesh M., 2010. Parenteral suspension: an overview. International Journal of Current Pharmaceutical Research, 2(3), p.4-13
[8] Ma, Guanghui and Su, Zhiguo., 2013. Microspheres and Microcapsules in Biotechnology. US: Taylor & Francis Group, LLC
[9] Sahil, Kataria., Akanksha, Middha., Premjeet, Snadhu., Bilandi, Ajay., Kapoor, Bhawana., 2011. Microsphere: a review. International Journal of Research in Pharmacy and Chemistry p.1184-1198
[10] Umer, Hammad., Nigam, Hemlata., Tamboli, Asif M., Nainar, M. Sundara Moorthi., 2011. Microencapsulation: process, techniques and applications. International Journal of Research in Pharmaceutical and Biomedical Sciences.2(2) p.474-481
[11] Hariyadi, Dewi Melani., Hendradi, Esti., Piaya, Octavia Librayanti Vierdina., Ramadani,Chandra Nourmasari., 2013. Optimasi mikrosfer ovalbumin-alginat yang diproduksi dengan teknik aerosolisasi. Pharmascientia. 2(1), p.21-30
[12] Manjanna, K. M., Kumar, T. M. Pramod., Shivakumar, B., 2010. Calcium alginate cross-linked polymeric microbeads for oral sustained drug delivery in arthritis. Drug Discoveries & Therapeutics 4 (2).p.109-122
[13] Chang, S.H., Kim, H.J., Kim, C.W., 2013. Analysis of the Structure and Stability of Erythropoietin by pH and Temperature Changes using Various LC/MS. Bull. Korean Chem. Soc . 34(9), p. 2663-2670
[14] Ghosh, A.K., Bandyopadhyay, P., 2012. Polysaccharide-Protein Interactions and Their Relevance in Food Colloids. The Complex World of Polysaccharides p 395-408
[15] Gombotz, Wayne R. and Wee, Siow Fong., 2012. Protein release from alginate matrices. Advanced Drug Delivery Reviews 64. p. 194-205
[16] Baimark, Yodthong. and Srisuwan, Yaowalak., 2014. Preparation of alginate microsphere by water-in-oil emulsion method for drug delivery: Effect of Ca2+ post-cross-linking. Advanced Powder Technology 25. p. 1541-1546
[17] Patil, Poonam., Chavanke, Daksha., Wagh, Milind., 2012. A review on ionotropic gelation method: Novel approach for controlled gastroretentive gelispheres. International Journal of Pharmacy and Pharmaceutical Sciences. 4(4), p.27-32
[18] Hariyadi, DM., Hendradi, Esti., Purwanti, T., Fadil, Farah D.G.P., Ramadani, Chandra N., 2014. Effect of cross linking agent and polymer on the characteristics of ovalbumin loaded alginate microspheres. International Journal of Drug Delivery Technology. 6(4), p. 469-474
[19] Nochos, Argyrios., Douroumis, Dionysios., Bouropoulos, Nikolas., 2008. In vitro release of bovine serum albumin from alginate/HPMC hydrogel beads. Carbohydrate Polymers. 74. p. 451-457
[20] Leong, Jun-Yee., Lam, Weng-Hoong., Ho, Kiang-Wei., Voo, Wan-Ping., Lee, Micky Fu-Xiang., Lim, Hui-Peng., Lim, Swee-Lu., Tey, Beng-Ti., Poncelet, Denis., Chan, Eng-Seng.,

2015. Advances in fabricating spherical alginate hydrogels with controlled particle designs by ionotropic gelation as encapsulation systems. Particuology 24. p. 44-60.

[21] Lee, Kuen Yong and Mooney, David J., 2012. Alginate: Properties and biomedical applications. Progress in Polymer Science 37. p. 106-126

[22] Hariyadi, DM., Purwanti, T., Kusumawati, I., Nirmala, RN., Maindra, H.M.C., 2015. Physical characterization and in vivo study of ovalbumin encapsulated in alginate microspheres. International Journal of Drug Delivery Technology. p. 48-53

[23] Bajpai, S.K. and Kirar, N. 2016. Swelling and drug release behavior of calcium alginate/poly (sodium acrylate) hydrogel beads. Designed Monomers and Polymers, 19 (1), p. 89-98

[24] Rowe, Raymond C., Sheskey, Paul J., Quinn, Marian E., 2009. Handbook of Pharmaceutical Exicipients sixth edition. London: Pharmaceutical Press

[25] Balasubramaniam J., Vinay U., Rao M. Vasudha., Jeevan Babu, P.S Rajinikanth., 2007. Sodium alginate microspheres of metformin HCl Formulation and In Vitro Evaluation.

[26] Lothfipour F., Mirzaeei S., Maghsoodi M., 2012. Evaluation of the effect of CaCl2 and alginate concentrations and hardening time on the characteristics of Lactobacillus acidophilus loaded alginate beads using response surface analysis. Advanced Pharmaceutical Bulletin, 2(1), 71-78

[27] Abdelwahed, W., Degobert, G., Stainmesse, S., Fessi, H., 2006. Freese-drying of nanoparticles: Formulation, process and storage considerations. Advanced Drug Delivery Reviews 58. p. 1688-1713

[28] Rangel M.M., Moltalvo P.C., Palou E., Lopez M.A., 2016. Optimization of the moisture content, thickness, water solubility and water vapor permeability of sodium alginate edible films. Recent Advances in Chemical Engineering, Biochemistry and Computational Chemistry, p. 72-78.

[29] Pavia D.L., Lampman G.M, Kriz G.S, Vyvyan J.R., 2009. Introduction To Spectroscopy. Brooks/Cole, Cengage Learning.

[30] Daemi, H. and Barikani, M., 2012. Synthesis and characterization of calcium alginate nanoparticles, sodium homopolymannuronate salt and its calcium nanoparticles. Scientia Iranica F .19 (6), 2023–2028

[31] Parmar H., Bakliwal S., Rane B., Pawar S., 2010. Different Methods of Formulation And Evaluation of Muchoadhesive Microsphere. International Journal of Applied Biology and Pharmaceutical Technology. 1(3), p 1157-1167

PHYSICAL CHARACTERISTICS OF ERYTHROPOETIN ENCAPSULATED INTO ALGINATE POLYMER USING AEROSOLIZATION TECHNIQUE

ORIGINALITY REPORT

8%	5%	5%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	repository.unair.ac.id Internet Source	3%
2	Yuanfei Wang, Michael J. Cooke, Cindi M. Morshead, Molly S. Shoichet. "Hydrogel delivery of erythropoietin to the brain for endogenous stem cell stimulation after stroke injury", Biomaterials, 2012 Publication	1%
3	Nguyen Thi Thanh Uyen, Zuratul Ain Abdul Hamid, Nguyen Xuan Thanh Tram, Nurazreena Ahmad. "Fabrication of alginate microspheres for drug delivery: A review", International Journal of Biological Macromolecules, 2020 Publication	<1%
4	Nguyen Thi Thanh Uyen, Zuratul Ain Abdul Hamid, Le Anh Thi, Nurazreena Binti Ahmad. "Synthesis and characterization of curcumin loaded alginate microspheres for drug delivery", Journal of Drug Delivery Science and	<1%

Technology, 2020
Publication

5	D. J. Stewart. "Erythropoietin and mesenchymal stromal cells in angiogenesis and myocardial regeneration: one plus one equals three?", Cardiovascular Research, 05/02/2008 Publication	<1%
6	Ketaki D. Kanbargi, Sachin K. Sonawane, Shalini S. Arya. " Encapsulation characteristics of protein hydrolysate extracted from seed ", International Journal of Food Properties, 2017 Publication	<1%
7	www.whitehousescientific.com Internet Source	<1%
8	Farzaneh Shemirani, Seyed Reza Yousefi. "Selective extraction and preconcentration of cerium(IV) in water samples by cloud point extraction and determination by inductively coupled plasma optical emission spectrometry", Microchimica Acta, 2006 Publication	<1%
9	Yodthong Baimark, Yaowalak Srisuwan. "Preparation of alginate microspheres by water-in-oil emulsion method for drug delivery: Effect of Ca2+ post-cross-linking", Advanced Powder Technology, 2014 Publication	<1%

10	link.springer.com Internet Source	<1%
11	www.hindawi.com Internet Source	<1%
12	Joachim Fandrey. "Oxygen-dependent and tissue-specific regulation of erythropoietin gene expression", American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2004 Publication	<1%
13	journal.uad.ac.id Internet Source	<1%
14	www.wseas.us Internet Source	<1%
15	Yan Zhang, Philipp S. Schattling, Fabian Itel, Brigitte Städler. "Planar and Cell Aggregate-Like Assemblies Consisting of Microreactors and HepG2 Cells", ACS Omega, 2017 Publication	<1%
16	Shaivad Shabee Hulhasan Abadi, Afrasim Moin, Gangadharappa Hosahalli Veerabhadrappa. "Review Article: Fabricated Microparticles: An Innovative Method to Minimize the Side Effects of NSAIDs in Arthritis", Critical Reviews™ in Therapeutic Drug Carrier Systems, 2016 Publication	<1%

17	issuu.com Internet Source	<1%
18	library.wur.nl Internet Source	<1%
19	"Tuesday Poster Sessions: Biopharmaceutics", Journal of Pharmacy and Pharmacology, 07/01/2009 Publication	<1%
20	www.ijprd.com Internet Source	<1%
21	eprints.utar.edu.my Internet Source	<1%
22	"Wnt Signaling", Springer Science and Business Media LLC, 2009 Publication	<1%
23	aip.scitation.org Internet Source	<1%
24	Yu-Tsai Yang, Anthony J. Di Pasqua, Weiling He, Tsuimin Tsai, Katsuhiko Sueda, Yong Zhang, Michael Jay. "Preparation of alginate beads containing a prodrug of diethylenetriaminepentaacetic acid", Carbohydrate Polymers, 2013 Publication	<1%
25	C.A. McKay, R.J Gilbert. "Using cerebrospinal	

fluid for in situ fabrication of injectable alginate hydrogels for spinal cord repair", 2011 IEEE 37th Annual Northeast Bioengineering Conference (NEBEC), 2011
Publication

<1%

26 Jun-Yee Leong, Weng-Hoong Lam, Kiang-Wei Ho, Wan-Ping Voo et al. "Advances in fabricating spherical alginate hydrogels with controlled particle designs by ionotropic gelation as encapsulation systems", Particuology, 2016
Publication

<1%

27 P.L. Lam, R. Gambari. "Advanced progress of microencapsulation technologies: In vivo and in vitro models for studying oral and transdermal drug deliveries", Journal of Controlled Release, 2014
Publication

<1%

28 Gang Zhao, Zhijie Wang, Honghao Zhao, Junjie Yang. "Bending force enhancement of sodium alginate-based polymer gel paper actuators", Cellulose, 2019
Publication

<1%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		

PHYSICAL CHARACTERISTICS OF ERYTHROPOETIN ENCAPSULATED INTO ALGINATE POLYMER USING AEROSOLIZATION TECHNIQUE

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11

PAGE 12

PAGE 13