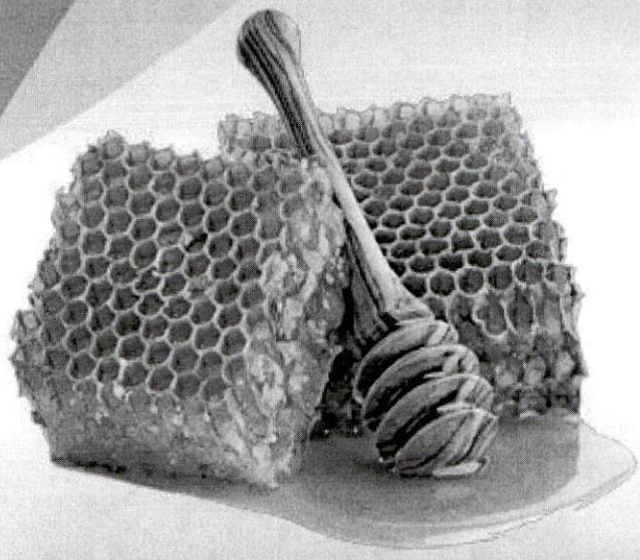
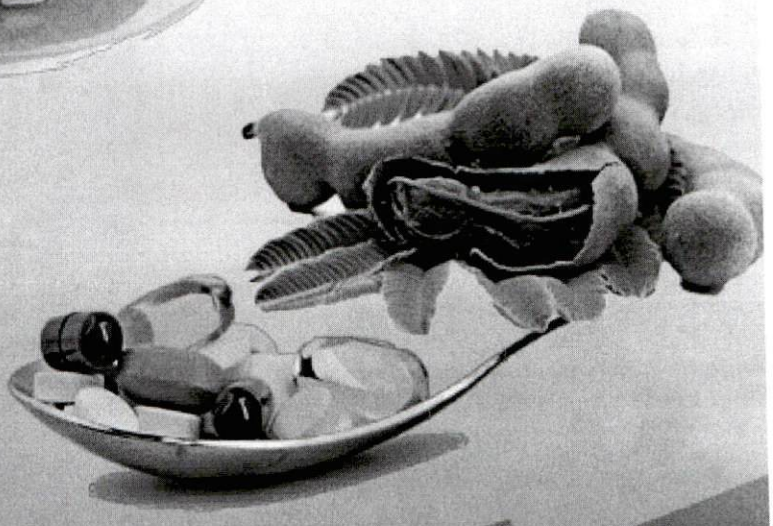
Vol. 5 No. 1 Juli 2018

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia

E-ISSN 2580-8303
P-ISSN 2406-9388





DITERBITKAN OLEH:
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VOL 5, NO 1 (2018)
JURNAL FARMASI DAN ILMU KEFARMASIAN INDONESIA

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KEYWORDS

RSUD Kota Yogyakarta, planning, consumption method, ABC analysis amilase, bakteri termofilik, enzim termostabil, Bacillus sphaericus Ak-1 antibakteri, mikrodilusi, spora; Agelas cavernosa antibiotics, bacterial meningitis, drug utilization study, ceftriaxone daun kemangi, Ocimum basilicum, fosfomolibdat, fraksi etil asetat demam berdarah, kristaloid, koloid dismenorea, kunyit, asam jawa, kedelai drug related problems, RSUD Dr M Yunus Bengkulu, stroke, SF-36 levofloxacin, MDR, Pseudomonas aeruginosa, Staphylococcus aureus madu, produk madu, Surabaya metformin HCl, alginate, microspheres, amount of drug, aerosolization metronidazol, kitosan, natrium karboksimetilselulosa, hidrogel, desain faktorial pengelolaan, puskesmas, ketersediaan obat pengetahuan, lulusan SMA, apoteker, farmasi pengetahuan, parasetamol, penggunaan obat rasional pola terapi, penyakit ginjal kronis, kardioserebrovaskular protease, bakteri termofilik, pengaruh nutrisi solid lipid nanoparticles, coenzyme Q10, kosurfaktan, ukuran partikel, efisiensi penjebakan teh hijau, kafein, HPLC, penyeduhan validation, TLC-bioautography, streptomycin vitamin B kombinasi, diabetes mellitus, neuropati diabetikum, TSS, SF-8

Effect of Total Amount of Metformin HCl on the Characteristics of Metformin-Ca Alginate Microspheres

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Abstract

***Introduction**: Metformin hydrochloride (metformin HCl) is an antidiabetic drug that is specifically used for type 2 diabetes mellitus (DM) and belongs to the biguanide antidiabetic drugs.* ***Objective**: The aim of this research was to determine the effect of total amount of* metformin *HCl on the characteristics of metformin HCl-Ca alginate microspheres using aerosolization technique.* ***Methods**: The total amount of metformin were 0.5 g (F1); 1 g (F2); 1.5 g (F3) and 2 g (F4). Drug was encapsulated into alginate and was crosslinked using $CaCl_2$.* ***Results**: The results showed that drug loadings were 5.09%; 9.61%; 13.11%; and 15.09% respectively, while the entrapment efficiencies were 48.35%; 41.99%; 38.67%; and 30.53%. The yields were 80.92%; 74.12%; 68.27%; and 59.11% respectively. Based on the statistical analysis, it was found that there were significant differences between formulas. Particles of formulas decreased as the amount of drug increased. The resulting sizes were 1.82 μm (F1); 1.96 μm (F2); 2.1 μm (F3); and 2.97 μm (F4).* ***Conclusion**: It can be concluded that amount of drug significantly affected the characteristics of metformin-alginate microspheres.*

***Keywords**: metformin HCl, alginate, microspheres, amount of drug, aerosolization*

INTRODUCTION

Metformin hydrochloride (metformin HCl) is an antidiabetic drug that is specifically used for type 2 diabetes mellitus (DM) and belongs to the biguanide antidiabetic drugs. Metformin HCl has high solubility in water (Choudhury & Mousumi, 2009). Metformin HCl has gastrointestinal side effects which include stomach discomfort, nausea, and vomiting. Metformin HCl also has short half-life, bioavailability of about 50 - 60% with no protein binding, large volume of distribution, and maximum accumulation in the small intestine (Zafar *et al.*, 2014). These side effects need to be solved by creating models of microspheres which give sustained release effect (Choudhury & Mousumi, 2009).

Microspheres are powder which is able to flow freely and ideally have a particle size of less than 200 μm (Alagusurandam *et al.*, 2009; Mujoriya, 2011). Its gradual release, sufficient to maintain the concentration of drug in the body, is one of the advantages of microsphere in the drug delivery system (Choudhury & Mousumi, 2009). On the formation of microspheres of metformin HCl with ionotropic gelation method, some polymer and cross-linking solution is needed. A natural polymer that is commonly used is alginate.

Alginate is a natural polysaccharide which is generally found in the cell walls of brown algae species (*Phaeophyceae*). Sodium alginate is a natural biopolymer that has a biocompatible, biodegradable, and non-toxic properties and has a relatively low cost (Yang *et al.*, 2007; Lee *et al.*, 2011). Na alginate is composed of (1 → 4) - β-D-acid mannuronic (M) units and (1 → 4) - α-L-guluronic acid (G) units arranged in the form of homopolymer (a block of MM = mannuronic mannuronic or GG = guluronic guluronic) and heteropolymer (a block of MG = mannuronic guluronic and GM = guluronic mannuronic) (Lee *et al.*, 2011).

Differences in the composition of mannuronic acid and guluronic acid lead to different physicochemical properties of alginate such as differences in stiffness of the yielded gel. The higher the ratio of guluronic, the harder and relatively more brittle the mass will be formed. Meanwhile, the higher the ratio of mannuronic, softer and more deformed the gel mass that is yielded (Erdinc, 2007). In this study, the type of Na alginate used is the one that has a higher guluronic ratio than mannuronic. The addition of Ca^{2+} ions or other divalent cations will form gelation through specific ionic bond and can cause a conformational change in the structure of the sodium alginate (Martin, 2002). Ca^{2+} ion is a divalent cation most commonly

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used than others (Kuo *et al.*, 2001). Calcium chloride ($CaCl_2$) can be used as cross-linker in the formation of microspheres with alginate polymer. Levels of Ca^{2+} determine the density of the gel formed (Goundanavar *et al.*, 2010).

The amount of drug affected physical characteristics of microspheres in increasing drug loading and entrapment efficiency. The method used in this study was ionotropic gelation (polyelectrolyte complexation). This method can produce spherical metformin-Ca alginate microspheres with smooth surfaces and size of 47.11 ± 16.5 µm (Balasubramaniam *et al.*, 2007). An advantage of the ionotropic gelation by aerosolization technique in this study is that all polyelectrolyte used is water-soluble therefore active agent can be encapsulated without use of organic solvents or high temperatures that can damage the active agent. In addition, this method is a simple, fast, cost-effective, and can produce small particle sizes of < 10 µm (Hariyadi, 2014).

The drying technique was aimed at maintaining the stability of active agent and microspheres. Drying using the technique of freeze drying was deemed most appropriate to anticipate the instability. In the freeze drying, lyoprotectant needs to be added, namely maltodextrin that has the ability to maintain the stability of microspheres at higher temperatures compared to the others (Laura *et al.*, 2010). Factors that may affect the characteristics of the microspheres produced are the amount of polymer, the number of crosslinker, and the amount of drug used.

This study was conducted to determine the effect of the amount of drug substance to the characteristics of metformin-Ca alginate microspheres with varying amounts of drug (0.5 g - 2 g), 2g of polymer, and 2.5 g of crosslinker, using the ionotropic gelation with aerosolization technique.

MATERIAL AND METHODS

Materials

Metformin HCl (Combiphar); sodium alginate (Sigma-Aldrich); $CaCl_2$ (Solvay Chemicals International); maltodextrin (PT BrataChem); sodium Citrate (Weifang Ensign Industry Co. Ltd) all in pharmaceutical grade; Distilled water.

Methods

Microspheres formulation

Sodium alginate (according to the formula in Table 1) was dissolved in 100 mL of aquademineralisata. Metformin HCl was dispersed into the alginate solution according to the formula and stirred until homogeneous. The resulting metformin-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 100 mL of $CaCl_2$ and was stirred constantly for 30 minutes at the speed of 1000 rpm. Microspheres that are formed in centrifuges at a speed of 2500 rpm for 6 minutes were then washed with distilled water 2 - 3 times. The microspheres that have been washed were dispersed in a 5% maltodextrin solution and dried using freeze drying at -80°C for 29 hours.

Table 1. Formulas of metformin HCl-alginate microspheres

Materials	Function	F1	F2	F3	F4
Metformin HCl	Antidiabetic drug	0.5 g	1 g	1.5 g	2 g
Na Alginate	Polymer	2 g	2 g	2 g	2 g
$CaCl_2$	Crosslinker	2.5 g	2.5 g	2.5 g	2.5 g
Maltodextrin	*Lyoprotectant*	5%	5%	5%	5%

Evaluation of metformin HCl-alginate microspheres

Following after production, metformin HCl-alginate microspheres were evaluated in terms of loadings, entrapment efficiency, yield, size and morphology.

The determination of metformin HCl content in microspheres

A standard curve of metformin HCl in a solution of 0.5 M Na Citrate pH 8.5 at a concentration of 4 - 15 ppm was produced. Then, 150 mg of microspheres were dispersed in 50 mL of 0.5 M Na Citrate pH 8.5, and the mixture were stirred using magnetic stirrer at 1000 rpm for 7 hours. Crosslinling time of microspheres was 30 minutes. The solution was then observed by a spectrophotometer at λ 239 nm as the maximum wavelength. The metformin HCl concentration was determined.

Characteristics of metformin HCl-Ca alginate microspheres

Particle size distribution

Measurement of 300 particles was conducted. The smallest and largest sizes from all samples were grouped and then divided into several intervals and class. The average diameter value was determined and a particle size distribution curve was created.

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Examination of the shape and surface of microspheres

The examination of the shape and surface of the wet metformin HCl-alginate microspheres produced was done by using an optical microscope, the appearance was taken using a camera, and an observation of freeze dried microspheres using Scanning Electron Microscope (SEM) was conducted. SEM was performed by placing microparticles on the handle of preparation with some adhesive containing grains of metal, such as Pt metal. Gold on the Chamber was evaporated so that the gold steam coated the entire surface of the microparticles. The surface of the gold-coated microparticles was observed by the SEM.

Examination of spectra infrared

The spectroscopy test was performed to determine the drug-polymer interaction during the microencapsulation process. The infrared spectroscopy method was performed using KBr pellets in which samples of each formula were crushed and then weighed for as much as 2 mg. Some pro-spectroscopy KBr powder that has been dried for as much as 300 mg was added. The mixture was crushed in a mortar until homogenous. Once homogeneous, the mixture was put in a KBr disc maker and pressed using a hydraulic press to obtain a transparent disc. The disc was placed in the *sample holder* and was recorded. Samples were observed at a wavelength of 4000 - 400 cm^{-1}. The results of the examination of the microspheres' infrared spectrums were then compared with the infrared spectrum of the microspheres without drug ingredients.

Determination of loadings, entrapment efficiency and yield

The drug loadings in the microspheres was determined by breaking the metformin microspheres that has been formed with 50 mL of sodium citrate for 7 hours. From the standard curve and the absorbance of the drug in the microspheres, calculations of entrapment efficiency, the loadings of metformin HCl, and *yield* can be performed as equation 1 and 2 (Balasubramaniam *et al.*, 2007).

$$\text{Entrapment Efficiency} = \frac{\text{metformin HCl content measured}}{\text{theoretical content of metformin HCl}} \times 100\% \ldots\ldots\ldots (1)$$

$$\text{Drug Loading} = \frac{\text{The total weight of metformin HCl}}{\text{weight of microspheres}} \times 100\% \ldots\ldots\ldots\ldots\ldots (2)$$

From data of each inspection, the percentages metformin HCl loadings in the microspheres, the *yield* of microspheres, as well as drug entrapment efficiencies were obtained. These were compared to the respective formulas. The data analysis was performed using SPSS 23 statistical program with the *One-Way ANOVA* method.

RESULTS AND DISCUSSION

The observation of spectra F1-F4 showed the interaction between the drug, polymer, and the *crosslinking* solution $CaCl_2$ (Figure 1). Such interactions were marked by shifting wave numbers, the loss of guluronic fingerprint absorption, and one absorption of the carboxylate salt group (1614 cm^{-1}) from Na alginate due to a crosslinking reaction with $CaCl_2$.

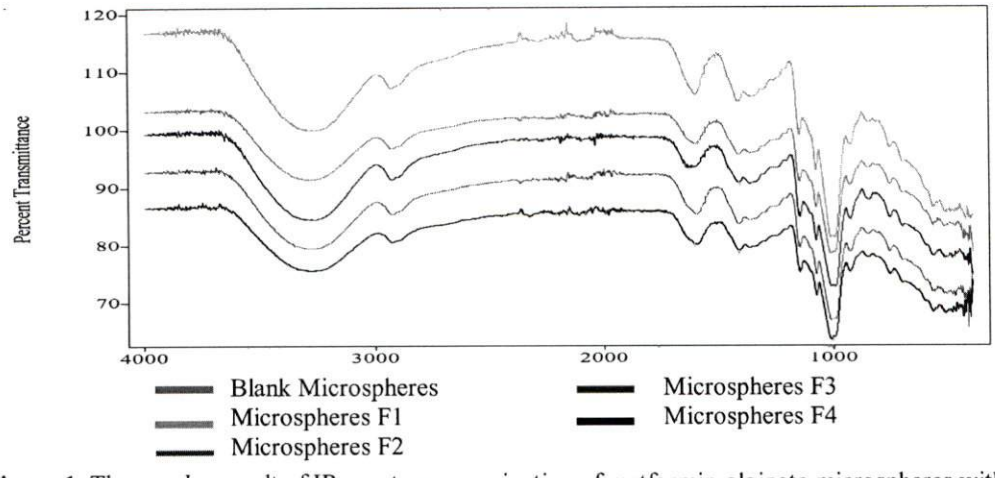

Figure 1. The *overlay* result of IR spectrum examination of metformin-alginate microspheres with the ratio of metformin amount

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Particle sizes of the all formulas showed that the particle sizes of the four formulas (Figure 2). All formulas resulted in bigger particle size than blank microspheres (0.8 – 1 μm). The observations also showed an increase in particle size with the increasing amount of metformin HCl drug from F1 to F4, which is from 0.5 g to 2 g (Figure 3).

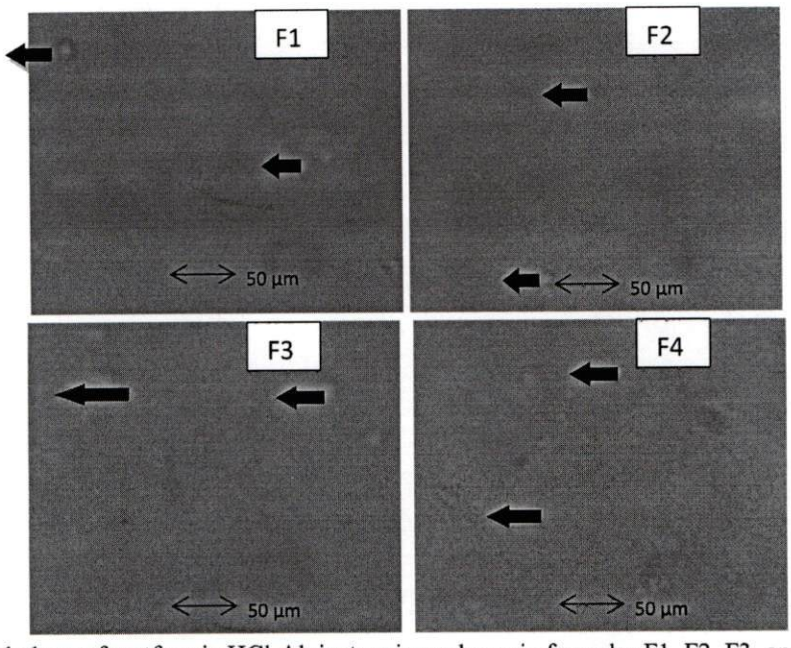

Figure 2. Morphology of metformin HCl-Alginate microspheres in formulas F1, F2, F3, and F4 using a 400x magnification optical microscope

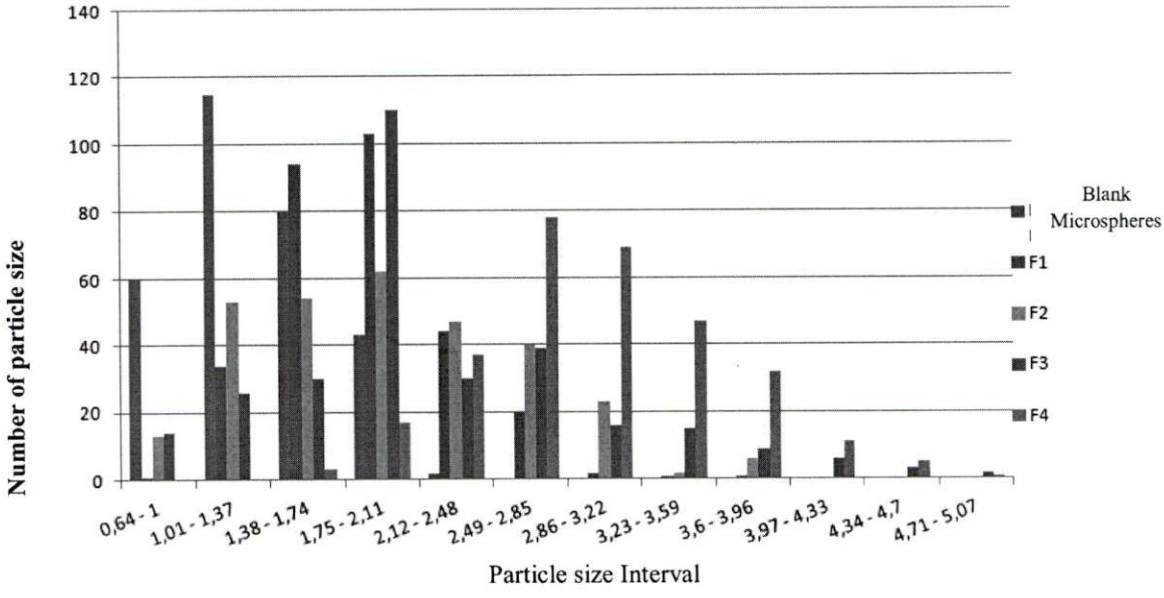

Figure 3. The particle sizes of microspheres

The results of the examination of microspheres shapes and surfaces using a Scanning Electron Microscope (SEM) showed that the microspheres of F1 to F4 were spherical and smooth in terms of surface (Figure 4). This was due to the addition of maltodextrin to make microspheres spherical, smooth, and flat in surface by covering cavities or pores on the surface of the microspheres that increased in number and size during the process of freeze-drying through the formation of hydrogen bonds with polar groups on the surface of the microspheres (Abdelwahed *et al.*, 2006).

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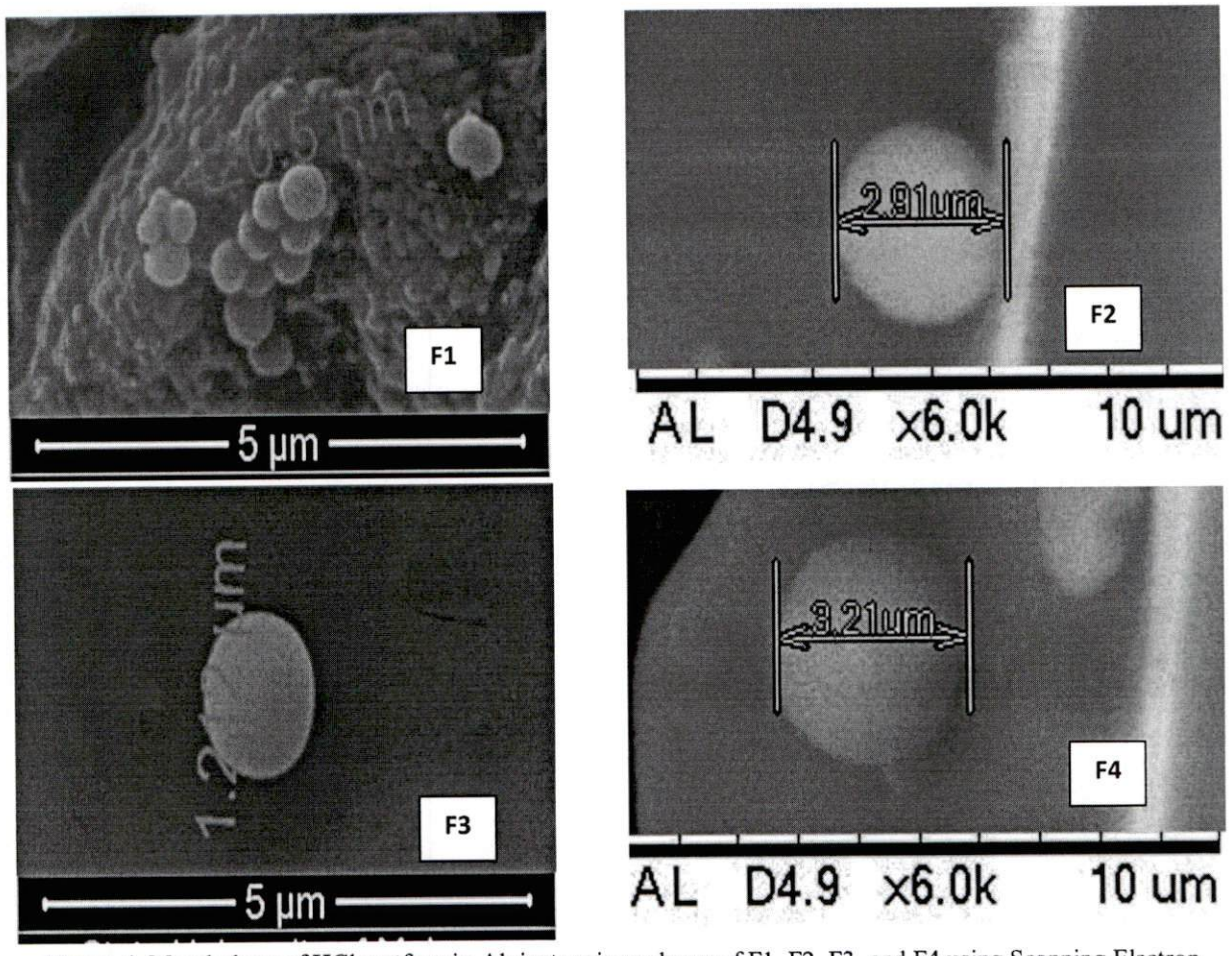

Figure 4. Morphology of HCl metformin-Alginate microspheres of F1, F2, F3, and F4 using Scanning Electron Microscope (SEM)

Entrapment efficiency, yield and drug loading

The results of the drug content, entrapment efficiency and yield of metformin HCl in microspheres can be seen in Table 2. The low drug loadings of metformin HCl in microspheres obtained was partly because metformin HCl was a drug with a low molecular weight and a high solubility in water for metformin HCl was easily detached from the microspheres during the manufacturing process and because the capacity of the microspheres was not able to adsorb the drug ingredients that are too high with amount of 2 grams of alginate (Rastogi *et al.*, 2007). Statistical analysis showed that the varying amount of metformin HCl gave a significant difference, a *sig* value of 0,001 < 0.05 for the comparison between F1: F2; 0.000 < 0.05 for the comparison between F1: F3; 0.000 < 0.05 for comparison F1: F4; 0.006 < 0.05 for the comparison between F2: F3; and 0.000 < 0.05 for the comparison between F2: F4 to metformin HCl loadings in the microspheres.

Table 2. Drug loadings, entrapment efficiency, and yield of microspheres

Formula	Drug Loadings of metformin (%)	Entrapment efficiency (%)	Yield (%)
F1	5.09 ± 0.19	48.35 ± 1.11	80.92 ± 1.78
F2	9.61 ± 0.94	41.99 ± 1.71	74.12 ± 2.24
F3	13.11 ± 1.39	38.67 ± 2.96	68.27 ± 6.78
F4	15.09 ± 0.49	30.53 ± 5.37	59.11 ± 9.77

The entrapment efficiency of the microspheres decreased by increasing in the number of metformin HCl (Table 2). The cause of the decline was because the entrapment efficiency of microspheres has a maximum capacity to adsorb drug substances at a certain alginate concentration since to produce the optimal amount of entrapped drug in the microspheres, the amount of polymer and crosslinker should be

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appropriate (Patil *et al.,* 2010). This was evidenced by the percentage of entrapment efficiency of metformin HCl in F1 that was greater than in F4. Statistical analysis showed that the influence of the amount of metformin HCl gives a significant difference with the obtained *sig* value of 0.026 < 0.05 for the comparison between F1:F3; 0.001 < 0.05 for comparison F1:F4; and 0.011 < 0.05 for the comparison between F2:F4 against entrapment efficiency of metformin HCl in microspheres since the value was less than 0.05.

The increasing number of metformin HCl decreased the yield value (Table 2). This was because the entrapment of microspheres has an adsorption capacity therefore some drug ingredients were not binding and did not get into joint microspheres and were washed during the washing of microspheres (Rastogi *et al.,* 2007). This was given that the nature of metformin HCl itself that is readily soluble in water. Statistical analysis showed that the influence of the amount of metformin HCl gave a significant difference with the obtainment of *sig* value of 0.026 < 0.05 for the comparison between F1:F3; 0.001 < 0.05 for comparison F1:F4; and 0.011 < 0.05 for the comparison between F2:F4 against entrapment efficiency of metfomin HCl in microspheres since the value was less than 0.05.

CONCLUSION

The formulation of metformin HCl-Ca alginate microspheres using the aerosolization technique was successfully produced microspheres with a size of < 10 μm, that are spherical and smooth. The increase amount of metformin HCl increased the drug loadings of metformin HCl-alginate microspheres and lowered the entrapment efficiency and yield. This results suggested the potential use for oral delivery system.

ACKNOWLEDGMENT

The author would like to thank the Faculty of Pharmacy, Airlangga University for all facilities to support this research.

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