Formulation of Metformin-Loaded Alginate Microspheres by Ionotropic Gelation-Aerosolization Technique

(Formulasi Mikrosfera Alginat Muatan-Metformin oleh Pengegelan Ionotropik- Kaedah Aerosol)

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INTRODUCTION

Metformin is an orally-administered anti-hyperglycemic drug commonly prescribed for the treatment of type 2 diabetes mellitus (Choudhury et al. 2009). Oral administration of metformin HCl is characterized by a short half life (1.5 - 3 h) and incomplete absorption.

ABSTRACT

Metformin hydrochloric acid (HCl)-loaded alginate microspheres prepared using aerosolization method were subsequently evaluated for their physico-chemical characteristics in terms of particle size, morphology, drug loading, entrapment efficiency, yield and in vitro release. A two factorial Design of Experiment (DoE) was used to study the influence of polymer alginate and cross-linker calcium chloride (CaCl$_2$) concentrations on microparticle characteristics. The results indicated that all microspheres were spherical in shape, while their particle size was less than 5 µm, although this increased with the intensification of alginate and CaCl$_2$ concentrations. Encapsulation efficiency, loading, and yield were all enhanced by increasing alginate concentration and, conversely, decreasing CaCl$_2$ concentration. The highest encapsulation efficiency, loading, and yield were 40, 31, and 73%, respectively, produced by a formula containing 1.75% alginate and 3% CaCl$_2$. The drug release of Metformin-loaded microparticles in HCl pH 1.2 ranged from 22 to 28% during a two-hour period, while further drug release of PBS pH 7.4 increased from 67 to 95% over ten hours. The total amount of drug released during a 12-h period increased by reducing alginate concentration. Furthermore, a kinetic study of the dissolution data confirmed the prevalence of a diffusion-controlled mechanism or Higuchi pattern of drug release.

Keywords: Aerosolization; alginate microspheres; design of experiment; metformin

ABSTRAK

Mikrosfera alginat muatan-metformin asid hidroklorik (HCl) yang disediakan menggunakan kaedah aerosol telah dinilai untuk ciri fizikal-kimia berdasarkan saiz zarah, morfologi, muatan ubat, kecekapan pemerangkapan, kadar hasil dan pelepasan in vitro. Dua reka bentuk uji kaji (DoE) faktor digunakan untuk mengkaji pengaruh alginat polimer dan kepekatan penghubung silang kalsium klorida (CaCl$_2$) pada ciri mikrozarah. Keputusan kajian menunjukkan bahawa kesemua mikrosfera mempunyai bentuk sfera, manakala saiz zarah kurang daripada 5 µm, walaupun ia meningkat dengan peningkatan kepekatan alginat dan kepekatan CaCl$_2$. Kecekapan pengkapsulan, muatan, kadar hasil kesemua dipertingkatkan dengan peningkatan kepekatan alginat dan sebaliknya penurunan kepekatan CaCl$_2$. Kecekapan pengkapsulan, muatan dan kadar hasil tertinggi masing-masing adalah 40,31 dan 73%, dihasilkan dengan formula yang mengandungi alginat 1.75% dan CaCl$_2$ 3%. Perlepasan ubat mikrozarah muatan-metformin dalam HCl pH 1.2 berjulat antara 22 sehingga 28% ketika tempoh dua jam, manakala perlepasan ubat daripada PBS pH 7.4 meningkat daripada 67 sehingga 95% dalam masa 10 jam. Jumlah keseluruhan perlepasan ubat ketika tempoh 12 jam meningkat dengan penurunan kepekatan alginat. Selain itu, kajian kinetik berkenaan data perlarutan memperakui prevalens mekanisme terkawal-penyerapan atau pola Higuchi perlepasan ubat.

Kata kunci: Aerosol; metformin; mikrosfera alginat; reka bentuk uji kaji

Following administration, metformin is primarily absorbed by the small intestine and has a relatively low bioavailability of 50 to 60% (Venkateswara et al. 2013). Commercial metformin products are generally immediate release formulations which should be administered 2 - 3 times daily in order to maintain optimum plasma
et al. (2013a, 2013b) argued that by ionotropic gelation-aerosolization technique. Matrix: < 43 nm to 5 µm) were absorbed by the small intestine and subsequently detected by transmission electron microscopy were absorbed rapidly (≤ 5 min) by the small intestine and administration. Following administration, microspheres locally to the jejunum or ileum of young male rats by oral (diameter range: 500 nm to 5 µm) which were delivered (2013) used nonbiodegradable polystyrene microspheres shown to have an advantage over nanoparticles below 500 particles larger than 5000 nm (5 microns) have been reported by Reineke et al. (2013) noted that microspheres with (microspheres vs nanospheres) may prove more effective. In another study reported by Morishita et al. (2004), insulin-loaded polymer (ILP) microparticles composed of poly(methacrylic acid) and poly(ethylene glycol) were analyzed which have pH-dependent complexation and mucoadhesive properties thought to be potential carriers of insulin via an oral route. Nevertheless, further optimization of the polymer delivery system is required to improve clinical application. Therefore, Morishita et al. (2004) studied the effect of ILP particle size (L-ILP: 180-230 µm, S-ILP: 43-89 µm, SS-ILP: < 43 µm) on insulin absorption in the in situ loop system, hypothesizing that smaller ILP particle sizes could induce greater hypoglycemic effects due to increased mucoadhesive capacity. These researchers confirmed burst effect by the smaller particles, while demonstrating the delayed release of the drug by larger particles which could adhere to the mucosa for a more protracted period and release the drug in a sustained fashion.

Déat-Lainé et al. (2013a, 2013b) argued that Whey protein and alginate hydrogel microspheres are demonstrably supportive of insulin intestinal absorption based on their evaluation of the permeability enhancement properties observed in Caco-2 cells. The same authors reported in another study that the insulin-loaded Whey protein/alginate microspheres with high quantities of entrapped drug exhibited in vitro matrix swelling and a protective effect in addition to excellent mucoadhesive properties. An improvement in intestinal delivery of insulin and increased bioavailability using microspheres was recorded.

A study by Hebrard et al. (2013, 2010) confirmed the usefulness of whey protein and alginate microspheres for successfully delivering probiotics. De et al. (2005) in their study of the effect of particle size of nanospheres and microspheres on the cellular-association and cytotoxicity of paclitaxel in 4T1 Cells, indicated that cell-association of paclitaxel increased in 4T1, Caco-2, and Cor-L23/R as particle size increased. Paclitaxel delivered by 1-µm microspheres was three times more cytotoxic to 4T1 cells compared to the drug delivered by nanospheres or in solution.

In studying the relationship between the effects of drugs and the particle size of insulin loaded bioadhesive microspheres, Pan et al. (2002) reported all the microparticles of three formulated sizes were within the desired size range, possessed an increase loading capacity, exerted an influence on the release property and produced various degrees of hypoglycemic effects after 10 h. They showed that adhesive Chitosan microparticles promoted an increase in the relative pharmacological bioavailability of insulin, while the distinct advantage of appropriate particle size enhanced the effects of drugs.

The authors prepared fine alginate microspheres (< 5 µm) by ionotropic gelation-aerosolization technique. The screening experiments conducted indicated a
relationship between the characteristics of microspheres and the amount of polymer sodium alginate and cross linker CaCl₂ contained in the formula. Previous studies had demonstrated that increasing the amount of alginate present in the formula produced microparticles possessing larger particle size and more efficient drug entrapment (Joshi et al. 2012; Rani et al. 2012; Singh et al. 2014). In contrast, augmenting the amount of CaCl₂ produced larger microparticles, but which demonstrated lower entrapment efficiency (Balasubramaniam et al. 2007). The DoE represents an effective and efficient tool with which to investigate the influence of individual formulation parameters and their interactions while conducting the minimum number of experiments. The application of DoE to pharmaceutical formulations has played a key role in the identification and optimization of critical formulation parameters.

The present study was undertaken in an attempt to understand the factors influencing the preparation of alginate microparticles by ionotropic gelation-aerosolization technique. The effect of different concentrations of alginate and crosslinker, together with the combination of these two factors on the physical characteristics and the release of metformin from the alginate microspheres prepared was systematically analyzed.

**MATERIALS AND METHODS**

**MATERIALS**

Pharmaceutical grade Metformin HCl was obtained from Combiphar, Indonesia, while sodium alginate (Alginic acid sodium salt from brown algae medium viscosity, MW 216,1212) was purchased from Sigma-Aldrich Inc; both maltodextrin and sodium citrate were acquired from Bratachem, Indonesia. All the other chemicals utilized were of analytical grade sourced from Merck, Indonesia.

**METHODS**

**MICROPARTICLE FORMULATION**

Microparticles were prepared by ionotropic gelation aerosolization technique involving different concentrations of sodium alginate (1.25 and 1.75%) and concentrations of CaCl₂ (3 and 5%) as shown in Table 1.

| TABLE 1. Formulas containing metformin-loaded alginate microparticles at various concentrations of sodium alginate and CaCl₂ |
|--------------------------------------------------|------------------|------------------|------------------|------------------|
| Formula | Formula 1 (F1) (%) | Formula 2 (F2) (%) | Formula 3 (F3) (%) | Formula 4 (F4) (%) |
| Na Alginate | 1.25 | 1.25 | 1.75 | 1.75 |
| CaCl₂ solution | 3 | 5 | 3 | 5 |

**PREPARATION OF METFORMIN-LOADED ALGINATE MICROPARTICLES**

Sodium alginate and CaCl₂ was dissolved in 100 mL of deionized water at a concentration corresponding to the formula in Table 1 and 500 mg of metformin HCl was dissolved in 100 mL of alginate solution. The co-solution was then introduced into the aerosolization nozzle and sprayed into 100 mL of CaCl₂ solution which was continuously agitated with a magnetic stirrer at a speed of 1,000 rpm. After 30 min, microspheres were collected by means of centrifugation at 2,500 rpm and washed three times with distilled water at room temperature, before being suspended in 5% of maltodextrin lyoprotectant solution and freeze-dried for a period of 29 h.

**CHARACTERIZATION OF MICROPARTICLES**

**PARTICLE SIZE AND MORPHOLOGY**

Microparticle size was determined using a 400× magnification optical microscope. 300 microparticles were measured to calculate their mean diameter, while
their shape and surface morphology were subsequently examined by means of scanning electron microscopy (SEM) at a working distance of 10 mm, with 20.0 kV beam energy, and a spot size of 5.0.

FTIR SPECTROSCOPY
FTIR spectroscopy was employed to determine the interaction between the drug and polymer within the microparticles. KBr pellet samples were taken and scanned in an IR range from 600 to 4000 cm\(^{-1}\).

MICROPARTICLE YIELD
The microparticle yield was calculated by subtracting the lyoprotectant from the total weight of dry microspheres, dividing the resulting figure by the total weight of the alginate and adding the weight of the aerosolizing drug present in the solution and can be refer from equation (1).

\[
\text{Yield (\%)} = \frac{\text{Total dry microspheres weight} - \text{maltodextrin}}{\text{Total weight of Metformin HCl+Alginate}} \times 100 \% \tag{1}
\]

DRUG LOADING AND ENTRAPMENT EFFICIENCY
150 mg of microspheres were dissolved in 50 mL of 0.5 M sodium citrate buffer (pH 8.5) and agitated at 1000 rpm for seven hours. The metformin content in the sample solution was measured using a UV spectrophotometer at an absorbance wavelength of 239 nm and calculated by comparison with a standard curve produced using a series dilution of metformin in sodium citrate.

Drug loading was calculated on the basis of equation (2):

\[
\text{Drug loading (\%)} = \frac{\text{Weight of metformin in microparticles}}{\text{Weight of dry microsphere}} \times 100 \% \tag{2}
\]

Entrapment efficiency was the calculated by means of equation (3):

\[
\text{Entrapment efficiency (\%)} = \frac{\text{Measured metformin content}}{\text{Theoretical Metformin content}} \times 100 \% \tag{3}
\]

DOE
Data relating to drug loading, encapsulation efficiency, and particle yield and size were analysed by Factorial Design to establish the effect of polymer and cross-linker content on the characteristics of the metformin loaded alginate microspheres. DoE was performed using software to determine the pareto chart, interaction plot, contour plot, range of polymer and crosslinker content in order to produce the optimal microsphere characteristics.

RESULTS AND DISCUSSION
In order to increase the stability alginate microspheres, maltodextrin was used as a lyoprotectant due to its ability to maintain the stability of alginate microspheres during freeze drying, and prevent aggregation and sedimentation (Hariyadi et al. 2015).

METFORMIN HCL IR SPECTRA
IR spectra examination results of metformin HCl-Ca alginate microsphere formulas F1, F2, F3 and F4 can be seen in Figure 1. Observations of all four formulas for IR spectra showed an interaction between the sodium alginate and CaCl\(_2\) cross linker solution. This was marked by the shifting of the wavenumber and the loss absorption of the carboxylate salt group, C-O stretching, and guluronic of alginate fingerprints. This showed that alginate was crosslinked with CaCl\(_2\). Specific groups of metformin HCl, namely; N-H stretching, C-H stretching and C-N stretching were present in all formulas. This suggests that metformin HCl did not participate in the reaction. The loss absorption of O-H and C-O stretching of maltodextrin indicated an interaction between maltodextrin and the microspheres formed due to maltodextrin acting as a lyoprotectant through a water replacement hypothesis stabilization mechanism.

PARTICLE SIZE OF MICROSPHERES
The measurement results for size were presented in a histogram of the ratio between particle size of the microspheres and a blank formula as can be seen in Figure 2.
From the calculations, the average particle size of metformin HCl-Ca alginate microspheres in each formula was 1.87 (F1), 2.54 (F2), 2.17 (F3), and 2.84 μm (F4), respectively, while that of blank microspheres was 1.73 (F1), 2.19 (F2), 1.85 (F3), and 2.21 μm (F4). These results indicate that the particle size of metformin HCl-Ca alginate microspheres in all formulas was larger than that of the blank. The observations also showed that particle size expanded with increasing concentrations of alginate and CaCl₂. Increasing concentrations of polymer can cause greater viscosity of the media with the result that the larger the dimensions of the droplets formed, the greater
the particle size of microspheres produced (Singh et al. 2014). Moreover, an increase in the concentration of CaCl\textsubscript{2} can cause both higher viscosity of the dispersion media and concentration of Ca\textsuperscript{2+} which can subsequently bind with the crosslinker. In addition, the larger guluronic acid binds more extensively to the crosslinker to form larger microsphere particles (Balasubramaniam et al. 2007).

![Figure 2. Particle size of microspheres formula and blank microspheres](image)

THE SHAPE AND SURFACE OF THE MICROSPHERES USING OPTICAL MICROSCOPE

Observation of the morphology of metformin HCl-Ca alginate microspheres by means of an optical microscope confirmed that the wet microspheres produced a spherical shape and homogeneous particle size.

DRUG LOADING, ENTRAPMENT EFFICIENCY, AND YIELD

Drug loading was measured by firstly producing the standard solution of metformin HCl and standard curve. From the measurement results obtained, the standard solution of metformin HCl showed an slope (b) of 0.0492, intercept (a) of 0.0238 resulting in the regression equation of \( y = 0.0492x + 0.0238 \) with a correlation coefficient (r) of 0.9990. The correlation coefficient obtained was greater than the \( R \) table (0.878) with a confidence level of 95% (5% significance level). This confirmed the existence of a linear relationship between metformin HCl concentrations through absorbance. The results of drug loading, entrapment efficiency, and microsphere yield can be seen in Table 2.

| Formula | Drug loading (%) | Entrapment efficiency (%) | Yield (%) |
|---------|------------------|---------------------------|-----------|
|         | Average ± SD     | CV (%)                    | Average ± SD | CV (%) | Average ± SD | CV (%) |
| F1      | 17.31 ± 0.83     | 4.77                      | 22.38 ± 1.09 | 4.88    | 72.73 ± 4.17 | 5.73    |
| F2      | 12.17 ± 0.63     | 5.18                      | 14.62 ± 1.31 | 8.99    | 70.33 ± 3.10 | 4.41    |
| F3      | 15.22 ± 0.40     | 2.65                      | 40.23 ± 1.65 | 4.10    | 74.80 ± 5.66 | 7.56    |
| F4      | 10.34 ± 0.57     | 5.54                      | 23.04 ± 1.18 | 5.11    | 76.53 ± 3.90 | 5.09    |

The results showed that increasing concentrations of alginate and CaCl\textsubscript{2} crosslinker reduced the drug loadings of metformin HCl probably due to the higher concentration of the alginate causing the number of reacting alginate-crosslink molecules to continue increasing and a larger amount of dried microspheres to be produced. Therefore, the percentage of metformin was lower. Increasing concentrations of CaCl\textsubscript{2} also reduced the loadings of metformin HCl, causing the viscosity of the internal phase to affect more profoundly the bond connecting cross linker
during the encapsulation process (Balasubramaniam et al. 2007; Hariyadi et al. 2015). Statistical analysis by factorial design produced a significance of less than 0.05 which indicated that the difference in the concentrations of alginate polymer and CaCl₂ resulted in a significant difference in the loadings of metformin HCl in the Ca-alginate microspheres.

With regard to entrapment efficiency, the results indicated that increasing concentration of alginate polymer can enhance the encapsulation efficiency of metformin HCl-Ca alginate microspheres, while greater concentration of CaCl₂ crosslinker can reduce the entrapment efficiency of microspheres. Increasing concentrations of alginate can improve efficiency due to the improved viscosity of alginate causing an increase in droplet size. Therefore, a high number of alginate molecules were also produced which reacted with crosslinkers resulting in larger microsphere particle size containing more metformin HCl which was entrapped in the microspheres (Balasubramaniam et al. 2007; Nagpal et al. 2012). Microsphere entrapment efficiency decreased the higher the concentration of CaCl₂ crosslinker. Such an increase in this concentration can induce greater viscosity during the internal phase, in turn, potentially affecting the bond which connects the crosslinker during the encapsulation process (Hariyadi et al. 2015; Nagpal et al. 2012). From the statistical analysis results, the sig. value was found to be less than 0.05 which indicated that the alginate and CaCl₂ concentration showed a significant difference in the encapsulation efficiency of Ca-alginate microspheres.

The yield results confirmed that increased concentrations of alginate polymer can enhance yield due to their potentially causing a higher amount of alginate to react with Ca²⁺ crosslinker, thereby resulting in dry microspheres. While at low concentrations of alginate (1.25%), increased concentrations of CaCl₂ reduced yield, in contrast, elevated concentrations of alginate (1.75%) and increased concentrations of CaCl₂ can improve yield. At low concentrations (1.25%), alginites that had already reacted continued to cross link with Ca²⁺. Excess Ca²⁺ can affect the bond connecting the crosslinker during encapsulation, thereby producing fewer microspheres (Balasubramaniam et al. 2007; Nagpal et al. 2012). Despite the concentration of alginate (1.75%), guluronic acid from alginate continuing to crosslink with Ca²⁺. In this case, the concentration of crosslinker was so low as to induce the formation of microspheres which were easily broken and amorphous (Nethaji et al. 2016; Suksamran et al. 2009). The statistical analysis results indicated that the differences in concentrations of alginate and CaCl₂ were not significant to the recovery of the Ca-alginate microspheres, as indicated by the sig figure of > 0.05.

The low value of metformin HCl loading in the microspheres obtained was partly due to metformin HCl being a drug possessing low molecular weight and high water solubility. Consequently, metformin HCl is readily detached from the microspheres during the formulation process. The low levels of metformin HCl in microspheres are also due to the lack of an optimum concentration ratio between the alginate and CaCl₂. A comparison of alginate concentration and CaCl₂ can be made and the optimum ratio calculated using an equal crosslink reaction between the carboxylic group of alginate with Ca²⁺ ions of CaCl₂. Each molecule of CaCl₂ reacts with two molecules of guluronic acid derived from alginate. A simulation of the reaction between guluronic acid with Ca²⁺ ions can be seen in Figure 3.

Based on the calculations performed, the Ca²⁺ of crosslinking reaction between clusters of alginate guluronic with Ca²⁺; the purity of CaCl₂ materials, and the ratio of guluronic groups to the type of alginate employed, the ratio of the optimum concentration of alginate to CaCl₂ was one of 1.6:1. Therefore, it is recommended by the authors that any subsequent study investigate the use of specific concentrations of alginate to CaCl₂ in order to obtain microspheres with a higher degree of continued crosslink resulting in greater drug entrapment.

**THE SHAPE AND SURFACE OF THE MICROSPHERES OBSERVED WITH A SEM**

Based on the drug loading and entrapment efficiency results, formulas F1 and F3 were found to be optimal and the shape and surface of freeze dry microspheres produced were subsequently observed by means of a SEM the results of which can be seen in Figure 4. Observation of the morphology of metformin HCl-Ca alginate microspheres, the results of which are contained in Figure 4, confirmed that the freeze dry microspheres of formulas F1 and F3 possessed spherical shapes and smooth surfaces.

![Figure 3: Simulation of a reaction between the alginic acid carboxylic group and Ca²⁺](image-url)
IN VITRO RELEASE STUDY

Prior to the conduct of the release test, the necessary loadings of metformin HCl in microspheres with a Na Citrate solution to break the microspheres were determined. The metformin HCl content of microspheres obtained was subsequently used to calculate the percentage of drug initially employed in this study. Release testing was conducted in two types of media; firstly, HCl pH 1.2 for 2 h and, secondly, PBS pH 7.4 for 12 h.

The results of the release of metformin HCl from the microspheres can be seen in Figure 5. The lower release of metformin HCl during the first 2 h within the HCl pH 1.2 medium, compared to the PBS pH 7.4 medium was partly due to the acidic pH of H⁺ ions replacing Ca²⁺ when binding to the carboxylate alginate alginic acid groups to form water-insoluble microspheres. In contrast, at alkaline pH, Na⁺ ions present in the PBS component replaced Ca²⁺ to form sodium alginate which was re-dissolved in water. The rate of release of metformin HCl by alginate microspheres is indicated by the value b (slope) of the line equation y = bx + a relating to the data release profile between the time (x-axis) and %age of cumulative metformin HCl released by the microspheres (y axis) under consistent conditions. The value release rate obtained was then compared with the formula and subjected to statistical testing methods by means of a Factorial Design ANOVA.

The release rate was determined by regressed steady state points (from 120 to 720 min) in the release profile of metformin HCl with time as the x-axis and the cumulative percentage of metformin HCl release as the y-axis. The regression equation slope represents the speed of the metformin HCl microspheres release system. The determination results of the release rate of metformin HCl for the fourth formula are contained in Table 3.
The results presented here indicate a trend where increasing concentrations of alginate lowered the release rate of microspheres. Based on Factorial Design ANOVA statistical results with a degree of confidence of 95%, it can be seen that an increase in the concentration of alginate to one of 5% CaCl\(_2\) can significantly reduce the release rate of metformin HCl (\(\text{sig} = 0.010 < 0.050\)), whereas the concentration of 3% CaCl\(_2\) did not differ significantly (\(\text{sig} = 0.368 > 0.050\)). This occurs because the concentration of cross linker was low, while the number of Ca\(^{2+}\) ions which can bind to the carboxylate groups in alginate was also extremely limited (Manjanna et al. 2010). If the concentration of alginate alone increased, then the amount of Ca\(^{2+}\) available was inadequate to increase the degree of cross-linking (Nayak et al. 2013) with the result that there was no release of significant barriers.

Increasing concentrations of alginate decreased the release rate by lowering the diffusion coefficient of the drug substance, thereby causing the number of alginate particles coating the surface to increase. They also reduced both the pore size particles and the rate of swelling of particles within bodily fluids resulting in a deceleration in penetration of the particle by body fluids (Suksamran et al. 2009).

Observation of the effects of increased cross linker concentration confirmed that it accelerated the release rate of metformin HCl microspheres significantly in alginate concentrations of 1.25% (\(\text{sig} = 0.001 < 0.050\)) and 1.75% (\(\text{sig} = 0.023 < 0.050\)). Similar results were obtained during the studies conducted by De et al. (2015) and Nayak et al. (2013). This phenomenon was due to, firstly, the increased cross linker concentration being too high to reach saturation point and, secondly, unbalanced binding with polymer. This indicated that increasing crosslinker concentration did not enhance the strength of the gel because it represented a repulsive force between the calcium ions which hindered the release of small molecules through the crosslinker networks (Pawar et al. 2008). Illustrations of the effect of Ca\(^{2+}\) ion concentration in crosslinking and the release rate of metformin HCl microspheres can be seen in Figure 6.

Based on the statistical results, it can also be seen that increasing concentration of alginate did not produce a significant difference (\(\text{sig} = 0.096 > 0.050\)) in relation to the release rate of metformin HCl from alginate microspheres. That was because the influences of these two factors opposed each other in as much as increased concentrations of alginate augmented the release rate, whereas increased concentrations of crosslink CaCl\(_2\) lowered it. No significant change in the release rate was found in instances of a simultaneous increase in the concentrations of both substances.

| Formula | Average slope ± SD |
|---------|-------------------|
| F1      | 7.0834×10\(^2\) ± 0.0019541 |
| F2      | 9.9050×10\(^2\) ± 0.0073595 |
| F3      | 6.3229×10\(^2\) ± 0.0045168 |
| F4      | 7.9730×10\(^2\) ± 0.0060850 |

**FIGURE 6.** Illustration of the effect of Ca\(^{2+}\) ion concentration (●) the cross linker bond (◻) and release metformin HCl (●) of microspheres.
DOE OF MICROSPHERES

The results of the DoE of multiple concentrations of alginate and cross linker on entrapment efficiency, drug loadings, yield, and particle size are presented in Figure 7.

Differences in concentrations of alginate (1.25 and 1.75%) and CaCl$_2$ (3 and 5%) did not produce significant results for yield, metformin HCl loading, entrapment efficiency, and particle size. However, in terms of yield, the effect of alginate concentrations was greater than that of CaCl$_2$ concentrations, while the loading of metformin HCl, entrapment efficiency, and particle size was more influenced by CaCl$_2$ concentration than that of alginate. The relationship or interaction between concentrations of alginate and crosslinker on yield, entrapment efficiency, drug content, and particle size can be seen in Figure 8.

The CaCl$_2$ contour plot of the results in Figure 9 showed that increased concentrations of alginate microspheres can improve yield. CaCl$_2$ can reduce microsphere yield, although an increase in yield due to the concentration of alginate was more influential than the decline in yield due to that of CaCl$_2$. Yield above 76% can be obtained from alginate at concentrations of more than 1.6% and a concentration of CaCl$_2$ about 4.5 - 5%. The efficiency of entrapment can also be enhanced by increased concentrations of alginate, but the intensified concentration CaCl$_2$ can reduce entrapment efficiency (EE). EE in excess of 40% can be obtained from alginate concentrations of more than 1.6% and concentration of CaCl$_2$ 3-3.5%.

The high loading of metformin HCl can be obtained with lower concentrations of alginate (<1.3%) and CaCl$_2$ (3-3.5%). The loading of metformin HCl concentration decreased with increasing concentrations of alginate and CaCl$_2$. In terms of size, the smaller particle size of less than 2 µm can be obtained from lower concentrations of alginate and CaCl$_2$. However, although increasing concentrations of alginate above 1.75% and CaCl$_2$ above 5% increased the size of particles, the resulting microspheres remained within the desired range for oral delivery (less than 10 µm).

FIGURE 7. The pareto chart of multiple concentrations of alginate and cross linker on drug loadings, entrapment efficiency, yield and particle size.
FIGURE 8. Interaction between concentrations of alginate and CaCl$_2$ on yield, entrapment efficiency, drug loading and particle size

FIGURE 9. Contour plot to drug loading, entrapment efficiency, yield, and particle size
CONCLUSION

Metformin HCl-Ca alginate microspheres were successfully produced using an aerosolisation technique. This research studied the design of experiments on the concentration of alginate and CaCl₂ crosslinker within the physical characteristics of metformin HCl-Ca alginate microspheres. The results of several evaluations indicated that intensifying the concentrations of alginate microspheres increased the particle size, reduced the loading of metformin HCl, and improved the entrapment efficiency of microspheres. Moreover, intensifying the concentration of CaCl₂ crosslinkers increased microsphere particle size, while reducing the loading of metformin HCl and entrapment efficiency of microspheres. The difference in concentrations of alginate within a CaCl₂ crosslinker produced no effect on the shape and appearance of the microspheres, or their yield.

Metformin alginate microspheres prepared by ionotropic gelation method and aerosolization techniques can maintain the release of metformin HCl for more than 12 h. Metformin HCl released from alginate microspheres was affected by differences in the concentrations of polymer and crosslinker. Increased concentrations of alginate (from 1.25 to 1.75%) reduced the release of metformin HCl in microspheres, while increased concentrations of CaCl₂ (from 3 to 5%) enhanced in the release of metformin HCl alginate microspheres. It was also shown that simultaneously intensifying the concentration of alginate and CaCl₂ did not affect the metformin HCl released by alginate microspheres. DoE made several recommendations for further formulation of microspheres.

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