ORIGINAL ARTICLE

Effect of Polymer Concentration on Micromeritics, Kinetics, and Activity of Ciprofloxacin HCl-Alginate Microspheres

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Abstract

Purpose: This research was to design a sustain release system for ciprofloxacin HCl for inhalation delivery. Ciprofloxacin HCl was encapsulated with alginate polymer by aerosolization ionotropic gelation. **Materials and Methods:** Ciprofloxacin HCl-alginate microspheres formula consisted of 0.5–1.35% alginate polymer. Micromeritic properties, *in vitro* release, and kinetics of the formulations were characterized. Micromeritics properties were studied in terms of bulk and tapped density, Carr index, and Hausner ratio. *In vitro* drug release was studied in phosphate-buffered saline media pH 7.2 for 12 h. This research also studied the activity of ciprofloxacin HCl-alginate microspheres. Antibacterial activity test was carried out using *in vitro* diffusion technique using *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 bacteria. **Results:** Release mechanism followed Higuchi model kinetics. The obtained freeze-dried microspheres showed good flow properties. From all formulas against *S. aureus* ATCC 25923, it was indicated that activity of all formula microspheres had higher activity compared to ciprofloxacin HCl and more stable. However, for formula against *P. aeruginosa* ATCC 27853, results showed that ciprofloxacin HCl was only effective at above 0.75% of alginate polymer. Furthermore, the encapsulation process and pH exposure did not affect in activity of ciprofloxacin HCl. **Conclusion:** These concluded that ciprofloxacin HCl-alginate microspheres were stable and potential for antibacterial activity.

Key words: Activity, alginate microspheres, ciprofloxacin HCl, kinetics, micromeritics, release

INTRODUCTION

For the management of some lung or pulmonary diseases, selected pharmaceutical formulations were highly considered. Dry powder inhaler (DPI) is one of the preferable delivery systems to provide uniform particles, smooth and can achieve site target.[1,2] The pulmonary delivery system as microspheres has been recommended as a delivery system which can produce small particles, good flow properties, and able to improve the drug release mechanism.[3] Important factors include particle size and distributions, shape, and surface properties may affect the adhesion/cohesion, drug detachment, and ultimately affecting the therapeutic performance.

Ionotropic gelation using aerosolization technique has been successfully produced small microparticles of drug and protein model, due to ease of use, safe, and no organic solvents contribution and currently chosen in this study to encapsulate ciprofloxacin HCl antibiotic for lung delivery.[4,5] The physical characterization of microspheres has been intensively investigated to controlling the size, shape, and surface roughness of the particles.

Alginate biodegradable polymer is an attractive polymer for use as carriers in DPIs to control the particle size, shape, and smoothness of microspheres as well as to sustain the release of drugs. Several advantages such as avoid the use of toxic organic solvents and reduced reticuloendothelial system uptake due to stealth nature of alginate.[6] Some cross-linking agent that can be used in gelation method is Ba^{2+}, Sr^{2+}, Pb^{2+}, and Ca^{2+}, but Ca^{2+}

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in calcium chloride ($CaCl_2$) is commonly used due to safe and strong cross-linked with polymer.[7] The present study explored the benefits of ciprofloxacin HCL antibiotic encapsulated in alginate microspheres in terms of release study, kinetics micromeritics properties, and *in vitro* activity against bacteria.

Microspheres are spherical monolithic or agent therapeutic distributed in the matrix which have a particle size of 1–1000 μm.[8] In drug delivery technology, microspheres are used for the preparation of slow release and controlled release drug and to reduce irritation of the gastrointestinal tract.[9] Some methods used for the preparation of microspheres are emulsification, coacervation, spray drying, and ionotropic gelation. Ionotropic gelation technique is a simple and safe method of the preparation of microspheres by adding the polymer solution into the cross-linking agent and system was gelled for several incubation times.[10] Drug release and kinetics are some factors which influenced effectivity of microspheres due to delivery of antibiotic drugs through the lungs increased the drug concentration in the lung.[11]

Ciprofloxacin HCl has an oral bioavailability of about 70% and is classified into Biopharmaceutics Classification System Class IV due to low solubility and low permeability.[12] The lung delivery system is one of the alternative deliveries if there are problems with other routes. Bioavailability is high and does not experience first cross-metabolism in the liver so as to deliver the drug. The drug is readily absorbed and enters the systemic circulation due to the thin barrier and high vascularization that envelopes the lungs.[12] The pharmacological benefits of lung administration include systemic low exposure, reduced side effects, appropriate doses delivered to specific targets, and no need to add.[13] Delivery of antibiotic drugs through the lungs increases the local concentration of the drug in the lung.[13]

Micromeritics properties evaluation of microspheres was important to estimate the flow properties, packing properties, and porosity of powder, to determine their suitability for product formation.[14] The antibacterial activity is important to test because this parameter is directly proportional to concentration at the target site.[15]

The authors were aimed to study the development of ciprofloxacin HCl-loaded alginate microspheres for direct delivery into lungs. In this study, we characterized ciprofloxacin HCl-loaded alginate microspheres for the development of DPI formulations for achieving efficient drug delivery which was targeted into lung.

MATERIALS AND METHODS

Materials

Ciprofloxacin HCl pharmaceutical grade (Shangyu Jingxin Pharmaceutical Co. Ltd.) from Zhejiang, Na_2HPO_4 pharmaceutical grade (Merck, Germany), KH_2PO_4 pharmaceutical grade (Merck, Germany), Nutrient agar (Oxoid, Thermo Scientific), Aquadestilata pharmaceutical grade (PD. Surabaya Aqua), and 70% alcohol grade pharmaceutical (One Med Health Care, Indonesia) were used. *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 bacteria are from Laboratorium BBLK Surabaya, Indonesia.

Preparation of ciprofloxacin HCl-loaded alginate microspheres

Preparation of ciprofloxacin HCl-loaded alginate microspheres applied ionotropic gelation method using aerosolization techniques, with the concentration of drug of 0.1%. Drug was dissolved in a solution of alginate polymer and used a cross-linker $CaCl_2$ at concentration of 0.5%. Each formula was cross-linked for 120 min and stirring was carried out at a speed of 1000 rpm. Formula microspheres were washed by centrifugation and drying techniques using a freeze dryer at −80°C for 29 h with the addition of 5% maltodextrin lyoprotectant as a stabilizer. Furthermore, ciprofloxacin HCl-loaded alginate microspheres that form were evaluated. Formula of ciprofloxacin HCl-loaded alginate microspheres is as follows in Table 1.

In vitro release study

About 600 mg microspheres were weighed and were added into Erlenmeyer containing 100 ml of phosphate-buffered saline (PBS) pH 7.2 and were stirred at 37 ± 0.5°C at 100 rpm. Sampling medium of a 5 ml at regular intervals and medium was replaced with same volume of new medium solution; samples were measured at the maximum wavelength of ciprofloxacin HCl. Amount of drug release was calculated at interval time. To calculate the concentration of drug, correction factor using Wurster equation was used by considering dilution factors as follows:

$$Cn = C`n + \frac{a}{b}\sum_{s=1}^{N-1} Cs$$

Drug release kinetics

To describe the drug release pattern, the drug release kinetics was investigated. Mechanism of drug release from microspheres was examined using zero-order kinetic,

Table 1: Formula of ciprofloxacin HCl-loaded alginate microspheres

Formula	F1 (%)	F2 (%)	F3 (%)	F4 (%)
Ciprofloxacin HCl	0.10	0.10	0.10	0.10
Alginate	0.50	0.75	0.90	1.35
$CaCl_2$	0.50	0.50	0.50	0.50
Maltodextrin	5	5	5	5

first-order kinetic, Higuchi kinetic, and Korsmeyer–Peppas model.

Micromeritics studies of microspheres

Bulk density and tapped density

Graduated cylinder of 10 ml was used to measure bulk and tapped densities. The sample was poured into cylinder and was tapped mechanically for 100 times; then, tapped volume was noted down, and bulk density and tapped density were calculated.[16] Each experiment for micromeritics properties was performed in triplicates.

Carr's index compressibility index (Ci) or Carr's index value of microspheres was inserted according to the following equation:

$$\text{CarrsIndex}(\%) = 100 \times \frac{(\text{Tappeddensity-Bulkdensity})}{\text{Tappeddensity}}$$

Hausner's ratio

Hausner's ratio of microspheres was determined by comparing the tapped density to the bulk density using the equation:

$$\text{Haussner Ratio} = \frac{\text{Tappeddensity}}{\text{Bulkdensity}}$$

Results of Hausner ratio and Car's index are shown in Table 2.

Particle size analysis

The sizes of the microspheres were determined using an optical microscope. A total of 300 microspheres particles were evaluated and the mean diameter was counted. The average particle size for each formulation was reported.

Antimicrobial activity

The inoculum of *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 bacteria was prepared. Transmission of bacterial inoculum was measured by spectrophotometer at a wavelength of 580 nm until it was received 25% transmittance. The media base layer (30 mL) and seat layer (20 mL) were made with nutrient agar and then were sterilized at 121°C for 30 min. The media base layer was poured in a Petri dish. It was then cooled and solidified. After being cold and compacted, the seat layer media were added to a 20 µL inoculum solution followed by cooling and solidified. The number of samples, positive control and negative control, was prepared. The standard ciprofloxacin HCl antibiotic 0.1% was diluted 5 times dilution to 0.02% and the sample was treated with same treatment and was put into the well each as much as 20 µL. The dish was incubated for 24 h at a temperature of 32.5°C. After 24 h, the size of the zone diameter formed was measured using the caliper.

Table 2: Carr's index and Hausner ratio of powder flow

Flow character	Hausner ratio	Carr's index (%)
Excellent/very free flow	1.00–1.11	≤10
Good/free flow	1.12–1.18	11–15
Fair	1.19–1.25	16–20
Passable	1.26–1.34	21–25
Poor/cohesive	1.35–1.45	26–31
Very poor/very cohesive	1.46–1.59	32–37
Approx. non-flow	>1.60	>38

Statistical analysis

A one-way analysis of variance (ANOVA) statistical analysis using the SPSS 21 program with 95% confidence degree ($\alpha = 0.05$) was performed on particle sizes, drug release, and antimicrobial activity.

RESULTS

Ciprofloxacin HCl was used in this study to learn its release *in vitro* from alginate microspheres and to optimize the performance of dried microspheres by evaluating the micromeritics properties and study effectivity. An inhalation route could be potentially chosen as selected delivery route to enhance effectivity.

In vitro release study

Ciprofloxacin HCl release from alginate microspheres was studied for 12 h in PBS pH 7.2 [Figure 1].

Release kinetics

The release mechanism of ciprofloxacin HCL from various microspheres formulations was determined by comparing their respective correlation coefficient from release equation [Table 3] and all kinetics models are shown in Table 4.

Micromeritics properties of ciprofloxacin HCL-alginate microspheres

Micromeritics properties were shown by bulk density and tapped density parameter. Bulk and tapped densities showed good packability of the microspheres shown in Table 5.

Particle size of microspheres

Based on Table 5, it can be seen that all microsphere formulas produced small particles with a size of <2 µm and had good flow properties.

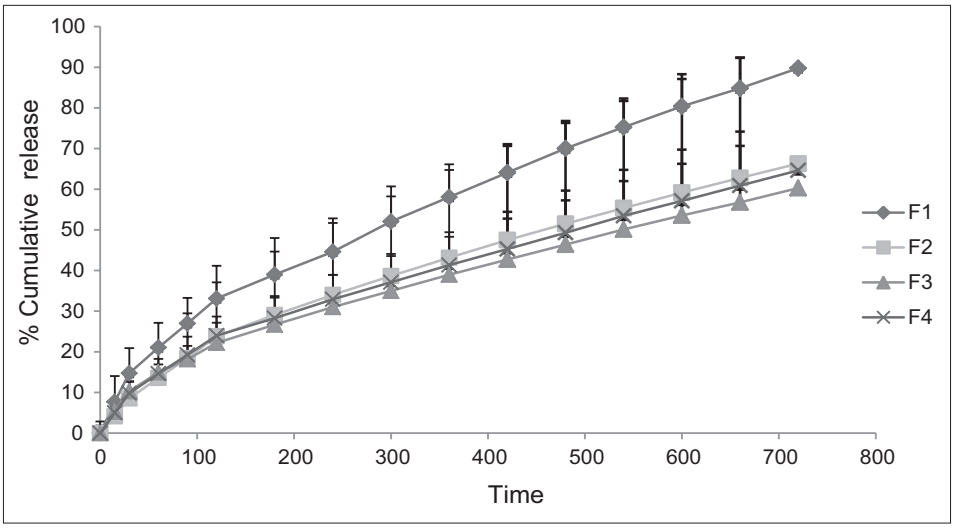

Figure 1: Release profile of ciprofloxacin HCl-alginate microspheres from all formulas

Table 3: Release equation of ciprofloxacin HCl-loaded alginate microspheres

Formula	Zero order	First order	Higuchi	Korsmeyer–Peppas
F1	y=0.111x+14.04	y=0.001x+1.187	y=3.587x–8.899	y=0.681x+0.025
F2	y=0.124x+15.53	y=0.001x+1.233	y=4.009x–10.06	y=0.681x+0.072
F3	y=0.101x+9.848	y=0.001x+1.082	y=3.239x–10.53	y=0.712x–0.125
F4	y=0.087x+12.67	y=0.001x+1.145	y=2.799x–5.143	y=0.621x+0.090

Table 4: Release kinetics of ciprofloxacin HCl-loaded alginate microspheres

Formula	Zero order	First order	Higuchi	Korsmeyer–Peppas
			R^2	
F1	0.975±0.0122	0.795±0.0440	0.993±0.0085	0.990±0.0036
F2	0.971±0.0030	0.759±0.0038	0.998±0.0010	0.990±0.0006
F3	0.973±0.0078	0.789±0.0085	0.995±0.0015	0.990±0.0061
F4	0.976±0.0045	0.780±0.0188	0.995±0.0029	0.988±0.0036

Table 5: Micromeritics properties of ciprofloxacin-alginate microspheres

Formulation code	Bulk density (g/mL)	Tapped density	Carr index	Hausner ratio	Particle size (μm)
F1	0.0333±0.01	0.0436±0.01	23.6238	1.3093	1.3148±0.1182
F2	0.0383±0.03	0.0449±0.02	14.6993	1.1723	1.4616±0.0685
F3	0.0314±0.01	0.0378±0.01	16.9312	1.2038	1.4948±0.1379
F4	0.0401±0.02	0.0472±0.01	15.0424	1.1770	1.6699±0.2913

Antimicrobial activity

Determination of antimicrobial activity of ciprofloxacin HCl-loaded alginate microspheres is shown in Table 6.

Statistical analysis

Table 7 showed ANOVA results of the released drug between formulas and it can be seen that all formulas produced significant results (sig. <0.05). Table 8 showed ANOVA summary of particle size at sig. >0.05. For the significance of antibacterial activity against *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853, Tables 9 and 10 represented the results.

DISCUSSION

For *in vitro* drug release study, it was found that all the preparation showed slower drug release and no burst release. All formulas showed good release properties. As the amount

Table 6: Antimicrobial activity of ciprofloxacin HCl-loaded alginate microspheres

Sample code	*Staphylococcus aureus* ATCC 25923	*Pseudomonas aeruginosa* ATCC 27853
	Zone diameter (mm)	Zone diameter (mm)
Microspheres F1	19.59±0.75	8.03±0.15
Microspheres F2	20.12±0.75	10.05±0.33
Microspheres F3	23.29±0.65	19.26±0.78
Microspheres F4	24.74±2.22	23.42±1.64
Control (−)	8.05±0.10	8.05±0.20
Control (+)	11.15±0.11	16.35±0.15

Table 7: The analysis of variance summary of drug release

Parameters	Sum of squares	df	Mean square	F	Sig.
Between groups	2172.136	3	543.034	5.832	0.040
Within groups	465.543	8	93.109		
Total	2637.680	11			

Table 8: The analysis of variance summary of particle size

Parameters	Sum of squares	df	Mean square	F	Sig.
Between groups	0.289	3	0.072	2.495	0.110
Within groups	0.289	8	0.029		
Total	0.578	11			

Table 9: The analysis of variance summary of antibacterial activity against *Staphylococcus aureus*

Parameters	Sum of squares	df	Mean square	F	Sig.
Between groups	42.315	3	21.158	10.655	0.011
Within groups	11.915	8	1.986		
Total	54.230	11			

Table 10: The analysis of variance summary of antibacterial activity against *Pseudomonas aeruginosa*

Parameters	Sum of squares	df	Mean square	F	Sig.
Between groups	379.963	3	189.981	170.312	0.000
Within groups	6.693	8	1.115		
Total	386.656	11			

of polymer increased, drug release was decreased. This was because surface area which was available for drug release was larger forming pore and channels, and swelling mechanism was also occurred.[17]

The kinetics release measurement was based on the R^2 value. The R^2 described the amount of the overall observed data variability/change that was explained by the model. These results showed that the Higuchi model to have more linear regression (R^2 value was higher and closer to value of 1) compared to other kinetics model. This kinetic showed that initially very less release of drug (ciprofloxacin HCl) from microspheres was found, but at longer time, all formulations showed more amount of drug release and then drug was continued to release at constant rate. It was found that the mechanism of drug release from microspheres was diffusion controlled by the Matrix-Higuchi model [Table 4]. Higuchi model has been widely used to predict the mechanism of swellable polymer systems.[18,19]

Carr's index (Ci) was ranged from 14.7% to 23.6%. F2 had lowest index, indicated excellent compressibility. For Hausner's ratio, it was ranging from 1.17 to 1.30, it means that F2, F3, and F4 microspheres formula showed that they had good flow properties and F1 had passable flow.

For inhalation purpose, microspheres must meet particle size target of lung region, this present particle size showed potential small particle of <2 µm along with micromeritics properties in line with the result of DPI microparticles which enables particles to target specific lung location.[20] The particle size between all formulas was found to have no significant differences. The current aerosolization technique allowed maintaining particle sizes approximated to the desired particle size (<2 µm). The release profiles between formulas, however, were not affected by particle size; this trend may be due to the influence of drug loading, the density of alginate matrix instead of particle size. These results were similar to other study conducted by that more concentration of alginate produce more sustained release due to increase of the polymer density inside matrix and the diffusional path length.[19,21]

Ciprofloxacin HCl-loaded alginate microspheres presented the highest antibacterial activity against *S. aureus* ATCC 25923, due to their lowest MIC values and larger inhibition diameter. Dizaj *et al*.[22] found that ciprofloxacin nanoparticles to be active against *S. aureus* and in a study by Isa *et al*.,[23] they found that ciprofloxacin nanoparticles enhanced the antibacterial efficacy of the antibiotic on *Salmonella* Typhimurium.

This present result showed that encapsulation of ciprofloxacin HCl antibiotic into alginate microspheres increased drug efficacy and activity against *S. aureus* ATCC 25923. All formulas showed significant different activities. However, regarding activity against *P. aeruginosa* ATCC 27853, results of the current study have shown that ciprofloxacin HCl was only effective against *P. aeruginosa* ATCC 27853 bacteria at higher concentration of alginate polymer. High alginate concentration of above 0.75% in the microspheres production was highly recommended to produce antimicrobial activity. Higher concentration of ciprofloxacin HCl against *P. aeruginosa* was also recommended by Grillon *et al*. in his study.[24]

CONCLUSION

Ciprofloxacin HCl-alginate microspheres were prepared successfully using ionotropic gelation method. Polymer concentration influenced drug release pattern of microspheres but not the particle size. All formulas produced small size particles which fulfilled lung region. The assessment of release kinetics showed that drug release from ciprofloxacin HCl-alginate microspheres followed the Matrix-Higuchi model (diffusion-controlled drug release mechanism). Considering the micromeritics properties and antimicrobial activity, formula microspheres which had highest concentration of alginate polymer (more than 0.75%) had the best flow property and demonstrated good free flow property. The selected best ciprofloxacin HCl-alginate microspheres provided significant inhibition of microbial against *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853. This formulation can be potentially recommended for stability test to further optimize as pulmonary drug delivery system.

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