Int J Pharma Res Health Sci. 2019; 7 (5): 3051-3055
DOI:10.21276/ijprhs.2019.05.02
Rahayu *et al.*
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Design, Optimization and Characterization of Cefixime Microspheres

Asti Rahayu [1, *], Dewi Perwito Sari [1], Tamara Gusti Ebtavanny [2]

[1]Faculty of Health Sciences, University of PGRI Adi Buana Surabaya, Indonesia.

[2] Faculty of Medicine, University of Brawijaya, Indonesia.

ARTICLE INFO

Received: 26 Sep 2019
Accepted: 20 Oct 2019

ABSTRACT

Cefixime is an orally active antibiotic that had poor bioavailability, short half-life, and restricted absorption of intestine limits the therapeutic potential. Hence, the objective of this study was prepare cefixime by using microspheres delivery system. Microspheres are multiparticulate drug delivery system which are prepared to obtain prolonged or controlled drug delivery to improve bioavailability, stability and and targeting to a specific site at a predetermined rate. Alginate and HPMC K4 have been used as carriers of microspheres for the prolonged release of active agents. Cefixime-alginate Microspheres were prepared using the ionotropic gelation method by aerosolization. A randomized full factorial design applied to four different formulations of cefixime loaded alginate microspheres. The design was applied for all formulations to study the effect of independent variables of concentration polymer on the entrapment efficiency (EE), drug loading (DL), particle size, and yield. The cefixime-alginate Microspheres had a high EE ranging from 64.77 ± 0.21%to 85.13 ± 0.14%, with small particle sizes ranging from 11.61 ± 0.24 µm to 15.42 ± 0.08 µm, drug loading ranged from 36.95 ± 0.27% to 6.23 ± 0.02%. EE, DL, and particle size variables had a significant effect on the dependent variables (p-values < 0.05), and yield variables had no significant effect on the dependent variables (p-values > 0.05).

Keywords: Cefixime microspheres, alginate, HPMC-K4, characteristics, design.

1. INTRODUCTION

Cefixime is an orally active antibiotic in the 3rd generation cephalosporin family. Cefixime against *Streptococcus pneumonia*, *Neisseria gonorrhea*, *Haemophilus influenza*, Enterobacteriaceae, *Streptococcus pyrogens*, *Moraxella*, *E.coli*, *Protease*, from multiplying by preventing bacteria wall that surround them and is resistant to many β lactamases. Cefixime is primarily absorbed from the stomach and upper part of the intestine due to its weak acid.

Corresponding author *
Asti Rahayu
Faculty of Health Sciences, University of PGRI Adi Buana Surabaya, Indonesia.
E-mail: astirahayu@unipasby.ac.id

It usually remains unionized at acidic pH, which helps in increasing the absorption in the stomach region. Cefixime is insoluble in water. It is absorbed after oral administration incompletely, which results in poor bioavailability of 40-50%. [1, 2]. The half-life of Cefixime is 3.0±0.4 hours. The dose of Cefixime is 200 mg twice a day for 7-10 days. Based on these properties of Cefixime, the aim of the present study is to prolong the gastric residence time of Cefixime containing formulation, which helps in increasing the oral bioavailability.

Oral route drug administration is the preferable route for taking medications. However, their short half-life and restricted absorption of intestine limit the therapeutic potential of many drugs. Such a pharmacokinetic limitation leads in many cases to frequent dosing of medication to achieve therapeutic effect [3,4]. Microspheres are multiparticulate drug delivery systems which are prepared to obtain prolonged or sustained drug delivery for improved bioavailability, stability, and to target the drug to a specific site at a predetermined rate [5].

Alginate microspheres have been universaly used as carriers of microspheres for the prolonged release of drugs; due to their low immunogenicity and mucoadhesive properties [6]. Alginate is a natural polysaccharides consisting of guluronic and mannuronic acid units. Sodium alginate has shown many uses in biomedical and pharmaceutical applications due to their low cost, low toxicity, biocompatibility, and biodegradability.. Divalent cations, e.g., $Ca^{2+,}$ are frequently used for the purpose of ion cross-linking to reduce the dissolution of the alginate matrix for many applications. Ca^{2+} ions are located between electronegative alginate molecules, like eggs in an egg-box. This is known as the 'egg-box' model [7]. Actually, alginate moves from the gel core towards this gelling zone. A combination polymer of HPMC was used in a few of the formulations along with alginate as the main polymer. HPMC forms water-soluble complexes with several drugs and may be useful in the release of the drug. HPMC is a biodegradable polymer and can form rigid gels by the action of calcium ions, which cross-link the galacturonic acid chains of pectin to yield hydrogels that are stable at low pH [8]. The objective of this study was to formulate and evaluate Cefixime-alginate microspheres to prolong gastric residence time and increase drug bioavailability with decreased gastrointestinal side effects.

2. MATERIALS AND METHODS

Materials

The drug Cefixime (Sigma-Aldrich Inc); Sodium alginate pharmaceutical grade (Sigma-Aldrich Inc); HPMC K4(Sigma-Aldrich Inc); $CaCl_2.2H_2O$ *pharmaceutical grade* (Solvay Chemicals International); distilled water.

Methods

Experimental design for optimization

A twenty two randomized full factorial design was used in this study. In this design, two factors were evaluated followed by optimization of formulation ingredient was done in order to determine the values of concentration of HPMC K4 (X1) and sodium alginate concentration (X2) as shown in Table 1. It was required getting an optimized formula with optimum values of drug loading (Y1), entrapment efficiency (Y2), particle size (Y3) and yield (Y4) using Minitab. A randomized full factorial design was applied to prepare four different formulations of cefixime microspheres.

Table 1: Formulation factors for the multilevel factorial design

independent factors	Low	High
X1 = Concentration of HPMC K4	0.5 % HPMC K4	1 % HPMC K4
X2 = Concentration of Polymer	1 % Sodium Alginate	2 % Sodium Alginate
Dependent Variables		**Goal**
Y1 = Entrapment Efficiency (EE%)		Maximize
Y2 = Drug Loading (DL%)		Maximize
Y3 = Particle Size		Maximize
Y4 = Yield (Y%)		Maximize

Preparation of cefixime microspheres

The alginate-cefixime solution was sprayed into a cross-linking agent solution calcium chlodide ($CaCl_2$) and stirred at 1000 rpm for two hours. Microspheres were thoroughly washed by centrifugation at 2500 rpm for 6 minutes and washed twice using distilled water. Cefixime-loaded alginate microspheres were then collected and freeze-dried at -80°C for 29 hours. Formulas of cefixime-alginate microspheres were shown in Table 2.

Table 2: Formula of cefixime-alginate microspheres

Compounds	Function	Concentration of Compound			
		I	II	III	IV
Cefixime	Active Compound	0,4 g	0,4 g	0,4 g	0,4 g
HPMC K15M	Polymer	0.5 %	0.5 %	1 %	1 %
Alginate	Polymer	1 %	2 %	1 %	2 %
$CaCl_2$ Solution	Crosslinker	1 M	1 M	1 M	1 M

Determination of entrapment efficiency

Cefiximemicrospheres of 400 mg was added into 50 mL sodium citrate 0.1 M, and then it was stirred at 1000 rpm for six h to allow the separation the entrapped drug from the un-trapped drug and was then analyzed spectrophotometrically at 407 nm using UV spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The percentage of EE of Cefixime in the microspheres was calculated by applying the following equation [9].

$$EE = \frac{\text{Amount of entrapped cefixime}}{\text{Total amount of cefixime}} \times 100$$

Determination of drug loading

Microspheres equivalent to 400 mg of the drug were accurately weighed to calculate drug loading. The dried microspheres were dissolved into 100 mL sodium citrate 0.1 M; then, it was stirred at 1000 rpm for six h. This resulting

solution was filtered through whatmann filter paper followed by 1 ml of solution was withdrawn at predetermined time and diluted to 10 ml with water. The absorbance of the resulting solution was measured using a UV spectrophotometer against water as a blank [10].

$$\text{Drug loading} = \frac{\text{Amount of entrapped cefixime}}{\text{Total amount of dried microspheres}} \times 100$$

Determination of particle size

The formulated particles were analyzed using an optical microscope (OPTILAB Viewer 2.2 by Micronos Nusantara, Indonesia). The prepared microspheres were placed in a glass slide, and the size of the mean microspheres calculated by considering 300 microspheres using a calibrated ocular micrometer [11].

Yield

The yield was calculated by the percentage of total microspheres (grams) divided by total amounts of polymer and surfactant, Cefixime (grams) [12].

$$\text{Yield} = \frac{\text{Total weight of microspheres}}{\text{Total weight of drug and polymer}} \times 100$$

3. RESULTS AND DISCUSSION

Preparation of cefixime microspheres

Cefixime microspheres were prepared and designed for a 2^2 randomized full factorial design to get an optimized formula and to study the effect of independent variables. Two different independent variables were used, which include: Concentration of HPMC K4 (X1) and Concentration of Alginate (X2) (Table 2). The independent variables were analyzed using Minitaband four different formulations were obtained, as represented in the table. All formulations were prepared using the ionotropic gelation technique and evaluated for entrapment efficiency, drug loading, particle size, yield, *in-vitro* drug release profile.

Table 3: The designed formulations of cefixime microspheres

Formula	Cefixime(mg)	The concentration of HPMC K4 (X1)	The concentration of Alginate (X2)
F1	400	-1	-1
F2	400	-1	1
F3	400	1	-1
F4	400	1	1

The entrapment efficiency of cefixime microspheres (Y1)

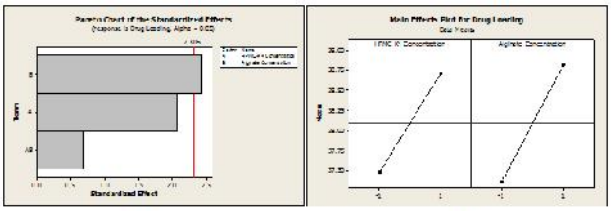
As shown in table 3, the prepared cefixime microspheres exhibited a good EE with values ranging from (64.77 ± 0.21%) for F1 to (85.13 ± 0.14%) for F4. Figure 1 illustrated the effect of X1 dan X2 on the EE of cefixime using Minitab plus software. As shown on the Pareto chart as seen in figure 1A, X1 and X2 have significant effects on the entrapment efficiency, with p values of 0.005 and 0.05, respectively. The linear regression models for the EE of cefixime microspheres are represented in Equation (1) as obtained from a randomized full factorial study design.

Y1 = 74.1 + 4.92 X1 + 5.26 X2 ……..(1)

The main effect plot for the EE in figure 1B, showed that the EE of cefixime microspheres increased with increasing in concentration of X1, and X2. The same results were obtained through the Pareto chart (Figure 1A), which illustrates the effect of two variables on EE. The EE was increased in case of concentration 1% of polymer alginate, as compared to 2 % of polymer alginate, respectively. The entrapment efficiency of F4 formulation was higher as compared to other formulation. Entrapment efficiency was increased as the concentration of the polymer increased; it was due to medium viscosity also increased with increased binding sites of calcium availability with increased cross-linking agents. Therefore it leads to the formation of droplets with higher entrapment efficiency of active agents [13].

Table 4: Characterization of cefixime microspheres

Formula	EE (%) (Y1)	Drug Loading (%) (Y2)	Particle Size (µm) (Y3)	Yield(%) (Y4)
F1	64.77 ± 0.21	36.95 ± 0.27	11.61 ± 0.24	88.36 ± 1.26
F2	73.56 ± 0.82	38.00 ± 0.48	14.98 ± 0.08	88.60 ± 2.45
F3	72.89 ± 0.34	37.78 ± 1.90	15.08 ± 0.08	89.67 ± 2.98
F4	85.13 ± 0.14	39.64 ± 0.60	15.42 ± 0.08	89.29 ± 0.74



(A) (B)

Fig 2: The effect of independent variables on the DL (Y2) including the Pareto chart (A), and main plot effect (B)

Particle size analysis (Y3)

The particle size range for microspheres found to be 11.61 ± 0.24 µm to 15.42 ± 0.08µm shown in table 3. As shown on the Pareto chart (Figure 3A), X1 and X2 have significant effects on the particle size, with p values of 0.005; and 0.05, respectively. The linear regression models for the particle size of cefixime microspheres are represented in Equation 3, as obtained from a randomized full factorial design study.

Y3= 14.3 + 0.977 X1 + 0.927 X2 …… (3)

The main effect plot for the particle size (Figure 3B) showed that the particle size of cefixime microspheres increased with increasing X1, and X2 increased. The same results were obtained through the Pareto chart (Figure 3A), which illustrated the effect of two variables on particle size. Here, keeping drug ratio constant and varied polymer ratio as the polymer concentration increases viscosity, which influenced the interaction between the disperse phase and dispersion medium that affects the size distribution of particle. If there was an increase in the amount of polymer concentration,

there was an increase in relative viscosity, so as a result, it increased in mean particle size [14].

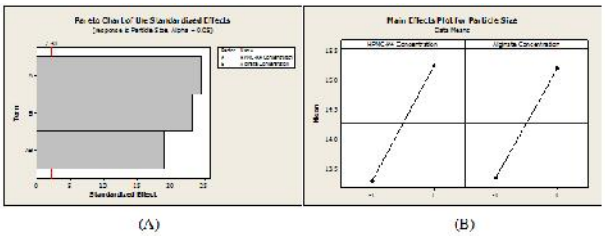


Fig 3: The effect of independent variables on the particle size (Y3) including the Pareto chart (A), and main plot effect (B)

Yield (Y4)

The percentage yield of the different formulations was found in the range of 88.36 ± 1.26% to89.29 ± 0.74%, which is depicted in Table 3. As shown on the Pareto chart (Figure 4A), X1 and X2 have no significant effects on the particle size, with p-values > 0.05, respectively. The linear regression models for the particle size of cefixime microspheres are represented in Equation 3, as obtained from a randomized full factorial design study.

Y4 = 89.0 + 0.500 X1 – 0.038 X2……...(4)

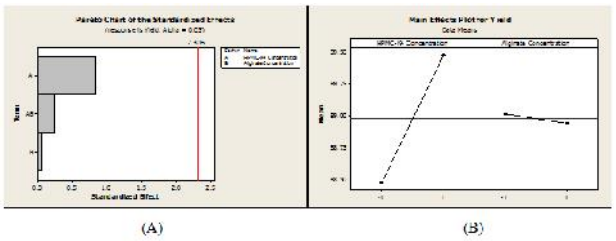


Fig 4: The effect of independent variables on the Yield (Y4) including the Pareto chart (A), and main plot effect (B)

From the analysis of the percentage of yield, loading, and encapsulation efficiency of cefixime-alginate microspheres, it was observed that as the polymer concentration in the formulation increased, the yield also increased. The low percentage of yield in some formulation could be due to microspheres lost during the washing process. The percentage yield of all formulations varied from 88.36 ± 1.26% to 89.29 ± 0.74%; the best formulation was F4, as given in Table 3. Vasam *et al*., 2017 showed that microspheres production yields, which were prepared using the Ionic Gelation method, were found between 84.65 - 88.58%, and yield was higher with a higher percentage of HPMC K15 concentration [15].

4. CONCLUSION

Cefixime microspheres were prepared successfully by using the ionotropic gelation method by aerosolization. Polymer-drug ratio influenced the particle size as well as the percentage of drug release from microspheres. All formulas produced high yield and encapsulation efficiency and small size particles. From the 2^2 randomized full factorial designs, there was showed that the combination of the use of alginate and HPMC K4 significantly affected DL, EE, and particle size but not for yield. Formula F4 using 2% alginate, HPMC K4 1%, and 1M $CaCl_2$ were selected as the optimized formula. These results indicate thatF4 could be a potential candidate for the activity test and stability test for further optimized formula as a topical drug delivery system.

5. ACKNOWLEDGEMENT

This work was supported by grant University of PGRI Adi Buana Surabaya. We would also like to thank the faculty of Pharmacy Airlangga University (UNAIR) for supporting research facilities.

6. REFERENCES

1. Sean L, Sweetman. Martindale Complete Drug Release. 33rd ed. UK: Published by Pharmaceutical Press; 2002.
2. Antony C, David MM, Osselton, Clarks BW Analysis of Drugs & Poisons. 3rd ed. Great Britain: Published by Pharmaceutical Press; 2004 .
3. Jamini M., and Rawat S., A review on microsphere. Res J Pharm Boil Chem Sci 2013; 4:1223-33.
4. Patel N R, Patel D A, Bharadia P D, Pandya V, Modi D. Microsphere as a novel drug delivery. Int J Pharm Life sci 2011;2:992-7.
5. Singh C, Purohit S, Singh M, Pandey B L. Design and evaluation of microspheres: A Review. J Drug Del Res 2013;2:18-27
6. Lee K Y, Mooney D J. Alginate : Properties and Biomedical Applications. Prog Polym Sci 2002; 37: 106 – 26.
7. Islam A, TaufiqYap Y H, Ravindra P, Moniruzzaman M, Chan E S. Developmentof a procedure for spherical alginate-boehmite particle preparation. Adv Powder Technol 2013; 24: 1119–112.
8. Leon Lachnnam, Herbert A. Liebermann. Microencapsulation polymer technique – Theory and practice of Industrial pharmacy; 2001: 412
9. Trivedi P, Verma AML, Garud N. Preparation andcharacterization of aceclofenacmicrospheres. Asian J of Pharmaceutics 2008; 1 : 110-15
10. Venkatesan P, Manavalan R, Valliappan K. Preparation and evaluation of sustained release loxoprofen loaded microspheres. J Basic Clin Pharma 2011; 2: 159
11. Sinko P J. Martin's physical pharmacy and pharmaceutical sciences. 5th ed., New Delhi: Wolters, 2008.
12. Surini S, Angriani, Anwar E. Study of mucoadhesive microspheres based on pregelatinized cassava starch as a new carrier for drug delivery. J Med Sci 2009; 9: 249-56.
13. MallikarjunV, SriHarsha S N. Preparation and Release Kinetic Studies of Cefdinir Microspheres. J Scientific Res Pharmacy 2015; 4: 98-101

14. Trivedi P, Verma AML, Garud N. Preparation and characterization of aceclofenac microspheres. Asian J Pharm Sci 2008; 110-15.
15. Mallikarjun V. Raja Sridhar Rao Ponugoti. Formulation And Pharmacokinetic (In-Vitro And In-Vivo) Evaluation Of Cefixime Microspheres.World Journal Of Pharmacy And Pharmaceutical Sciences 2017; 6 : 1476-88.

Conflict of Interest: None
Source of Funding: Nil