Trop J Nat Prod Res, November 2023; 7(11):5286-5291 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

The Effect of Polymer-Drug Ratio on Characteristics, Release and Stability of Ciprofloxacin-Alginate-Kappa Carrageenan Microspheres

Mochamad F. Deliaz[1,2], Esti Hendradi[2,3], Dewi M. Hariyadi[2,3]*

[1]*Master Program of Pharmaceutical sciences, Faculty of Pharmacy, Universitas Airlangga, Campus C Mulyorejo, Surbaya 60115, Indonesia*
[2]*Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Campus C Mulyorejo, Surabaya 60115, Indonesia*
[3]*Nanotechnology and Drug Delivery System Research Group, Faculty of Pharmacy, Universitas Airlangga, Campus C Mulyorejo, Surabaya 60115, Indonesia*

ARTICLE INFO

Article history:
Received 07 October 2023
Revised 01 November 2023
Accepted 21 November 2023
Published online 01 December 2023

ABSTRACT

Tuberculosis is the top contagious disease in the world caused by the bacterium *Mycobacterium tuberculosis*. The increasing incidence of drug resistance in *Mycobacterium tuberculosis* requires alternative therapies, one of which is fluoroquinolones such as ciprofloxacin. Pulmonary delivery systems are used to avoid problems with oral administration, such as first-pass metabolism and targeted delivery. Microsphere formulation is chosen because pulmonary delivery requires a small size (1-5µm) for deposition in the lungs. The purpose of this research is to investigate the influence of the drug-polymer ratio on the characteristics, release, and stability of the microspheres. The polymer used is a combination of sodium alginate and kappa carrageenan. Microspheres were prepared using the ionic gelation technique with aerosolization. The results showed spherical microspheres with smooth surfaces, particle sizes were ranging from 2.25 ± 0.14 µm to 2.83 ± 0.12 µm, moisture contents were below 5%, flow properties were good, yields were ranging from 91.71% ± 5.52 to 93.62% ± 3.97, drug loadings were 2.03% ± 0.12 - 3.55% ± 0.13, and entrapment effiencies were ranging from 75.27% ± 2.57 to 78.45% ± 1.89. Over a period of 720 minutes, ciprofloxacin release from the microspheres were about 48.20% - 91.35%. The release of ciprofloxacin increased with higher ratio of drug-polymer, and the release kinetics followed the Korsmeyer-Peppas model with a release mechanism following Fick's diffusion law. Microspheres remained stable over 28 days. The study suggest that this microspheres system holds significant potential as a drug delivery system to the lungs.

***Keywords*:** Ciprofloxacin, Microspheres, Sodium Alginate, Kappa Carrageenan, Characteristics, Release, Stability.

Introduction

Tuberculosis (TB) is an infectious ailment resulting from the presence of the bacterium *Mycobacterium tuberculosis*, with the lungs being the primary site of infection. TB bacteria can be transmitted through the air when an individual with TB coughs, sneezes, or expels saliva. It is responsible for causing around 1.5 million deaths annually, ranking it as the leading global infectious disease fatality.[1]

The increasing occurrence of drug resistance in *Mycobacterium tuberculosis* requires alternative therapies. Fluoroquinolones meet most of the criteria for an ideal class of antimycobacterial drugs. One type of fluoroquinolone that exhibits activity against mycobacteria is ciprofloxacin. Ciprofloxacin is bactericidal against *Mycobacterium tuberculosis* at a concentration of 2 mg/L.[2] The Minimum Inhibitory Concentration (MIC50) for ciprofloxacin and ofloxacin is 1 mg/L, and the MIC90 for ciprofloxacin is 4 mg/L.[3]

The administration of ciprofloxacin to treat common respiratory infections is given orally or intravenously.

*Corresponding author. E mail: dewi-m-h@ff.unair.ac.id
Tel: +62 81232238383

Citation: Deliaz MF, Hendradi E, Hariyadi DM. The Effect of Polymer-Drug Ratio On Characteristics, Release and Stability of Ciprofloxacin-Alginate-Kappa Carrageenan Microspheres. Trop J Nat Prod Res. 2023; 7(11):5286-5291. http://www.doi.org/10.26538/tjnpr/v7i11.38

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

However, both oral and intravenous methods come with certain disadvantages, including suboptimal pharmacokinetic characteristics within the lower respiratory system and a relatively brief half-life spanning approximately 3-5 hours. Additionally, ciprofloxacin experiences first-pass metabolism and exhibits an oral bioavailability of approximately 70%, as well as low solubility.[4] Therefore, alternative delivery routes are needed to address these issues, such as pulmonary delivery.[5]

Pulmonary delivery has several advantages, this includes smooth absorption and penetration into the systemic bloodstream, facilitated by the thin protective barrier and extensive blood supply surrounding the lungs.[6] It does not undergo first-pass metabolism, protects the drug from enzymatic degradation, delivers the drug directly to the specific location in the lungs where *Mycobacterium tuberculosis* bacteria reside within lung macrophages, minimizes adverse side effects, and maximizes therapeutic efficiency.[7]

Pulmonary delivery requires small doses and particle sizes, and therefore, microspheres are chosen as the formulation for pulmonary delivery. A microsphere is a particle dispersion system created by either adsorbing or dispersing a drug within a polymer matrix, with diameters ranging from 1 to 1000 mm.[8] Microspheres are composed of a biodegradable polymer matrix, allowing the drug to naturally break down in the body. They exhibit widespread biocompatibility (with low potential for triggering immune responses and toxicity), have a high rate of bioavailability, and can sustain the release of drugs over an extended period. The use of microspheres provides several benefits, including the reduction of toxicity, the increase of effectiveness, and an the improvement in patient adherence and comfort.[9]

The purpose of this research is to determine the influence of the drug-polymer ratio on the characteristics, release, and stability of microspheres. The encapsulation effectiveness of the drug by

microspheres is affected by the drug ratio. The drug-polymer ratio must be appropriate and precise because an excess of polymer ratio will increase the gel density, thereby increasing the particle size. Additionally, the active ingredient ratio must also be in the proper proportion with the polymer as it affects the trapping efficiency. [10] An increase in the drug-polymer ratio can lead to an increase in microsphere density, thereby reducing the release rate.[11]

In this research, a combination of polymers, namely alginate and carrageenan, was used. The combination of polymers can provide beneficial synergistic effects compared to single polymers. The combination of polymers influences the formulation's mechanical characteristics. A stronger mechanical properties resulting from the polymer combination can reduce leakage of the drug encapsulation matrix and prolong drug release from the polymer matrix. This is achieved through the reduced porosity of alginate-carrageenan microspheres, thereby preventing burst release by increasing the mechanical properties.[12]

The formation of microspheres requires cross-linking, and the attachment of cross-linking agents can enhance the synergy of polymer combinations.[24] The cross-linking agent used in this research is $CaCl_2$. Valence cations like calcium from $CaCl_2$ salt interact with the negative groups of the polymer, such as carboxyl, hydroxyl, sulfate groups through hydrogen bonding, and amino groups.[24] The strength of drug formation is reported to increase with the addition of divalent cations.[13] Ca^{2+} metal ions can form interactions with alginate and carrageenan polymers, which can enhance the synergistic effect.[12]

The ionic gelation method is chosen for use in this research because it has several advantages, such as no heating involved, which can damage the active ingredient, making it suitable for encapsulating unstable drugs. It is easy and simple to prepare, and it does not use hazardous organic solvents.[14] The use of aerosolization technique for the freeze-drying process is preferred because it eliminates the need for organic solvents and high temperatures, which helps preserve the stability of the drug compound. However, freeze-drying requires a lyoprotectant to protect the microspheres from pressure during freeze-drying. The chosen lyoprotectant is maltodextrin because it can prevent aggregation along with sedimentation, and the resulting microspheres have a spherical shape with a smoother surface.[15]

This research will produce ciprofloxacin HCl microspheres with a combination of alginate and kappa carrageenan polymers in addition to $CaCl_2$ as a cross-linker in various drug-polymer ratios made using the ionotropic gelation technique with aerosolization method to determine their effects on the characteristics, release, stability, tuberculosis activity, and toxicity testing of ciprofloxacin HCl microspheres.

Material and Methods

Materials

Ciprofloxacin (Combiphar), Sodium Alginate (Sigma-Aldrich Inc,), Kappa Carrageenan (Danisco-Cultor), $CaCl_2.2H_2O$ (Solvay Chemicals International), Maltodextrin (PT Bratachem), Phosphate Buffered Saline (Oxoid-England), Tri-Sodium Citrate Dihydrate (SAP Chemicals), Citric Acid Monohydrate Extra Pure for Analysis (CV. Chemical Indonesia Multi Sentosa). All of the chemicals use are of pharmaceutical grade.

Production of Ciprofloxacin HCl Microspheres

Microspheres are made using the ionotropic gelation technique with aerosolization method using a sprayer with 35 µm nozzle diameter. A combination solution of sodium alginate and carrageenan polymers is prepared at a concentration of 0.9%. Then, a separate solution of ciprofloxacin HCl is prepared at concentrations of 0.2%, 0.3%, and 0.4%. Next, the ciprofloxacin HCl solution is added to the combination polymer solution and stirred (Thermo Fisher Cimarec$^+$, US) at 1000 rpm centrifugation (Hettich Rotofix 32A, Germany) at a temperature of 25°C. A $CaCl_2$ solution (0.5 M) was prepared using distilled water. The prepared polymer-ciprofloxacin solution is sprayed as an aerosol into the CaCl2 cross-linking solution from a distance of 8 cm, using a constant pressure of 40 psi. Simultaneously, the mixture is continuously stirred (Thermo Fisher Cimarec$^+$, US) at 1000 rpm for up to 2 hours. The microspheres that have been created are isolated by subjecting them to centrifugation (Hettich Rotofix 32A, Germany) at a speed of 2500 rpm for 6 minutes. The microspheres were washed with distilled water twice and centrifuged again The microspheres were then filtered (Whatman filter paper 25µm), weighed (Ohaus PX224/e, US), and immersed in a 5% maltodextrin solution as lyoprotectant. Subsequently, the microspheres were dried using a freeze dryer (Buchi Lyovapor L200,Germany). at a temperature of -80°C for 120 hours.[16] The microspheres formula can be seen in Table 1.

FTIR analysis

Fourir transform infrared (Bruker Alpha II, US) was conducted on all materials and completed microspheres with a wave number range of 4000-450 cm^{-1} to determine the characteristic functional group features of each material.[16]

Particle Size Analysis

Particle size determination was conducted using an optical light microscope (Nove XSZ-1xx, China) and Miconos optiLab® (Indonesia Ver 2.0) software for measurement. The measurement was performed on 300 particles on each formula.[15]

Morphology

Surface morphology analysis of the microspheres was performed using a Scanning Electron Microscope (Thermo Fisher Phenom Pharos G2, US).[16]

Moisture Content

Moisture content determination is carried out using the Moisture Analyzer (Mettler Toledo HB43S, US).[16]

Flow Properties

Observations were made during the flow properties test include Carr's Index and Hausner ratio using a motorized tapping device (Erweka, Germany).[17]

Yield

Yield was obtained by comparing the total weight of the microspheres obtained to the weight (Ohaus PX224/e, US) of the materials used to form the microspheres.[17]

Table 1: Microspheres Formula

Components	Formula 1 (F1) Drug:Alginate-κ-carrageenan (0.2 : 1.8)	Formula 2 (F2) Drug:Alginate-κ-carrageenan (0.3 : 1.8)	Formula 3 (F3) Drug:Alginate-κ-carrageenan (0.4 : 1.8)
Ciprofloxacin HCl	0.2%	0.3%	0.4%
Sodium Alginate	0.9%	0.9%	0.9%
Carrageenan	0.9%	0.9%	0.9%
$CaCl_2$	0.5 M	0.5 M	0.5 M
Maltodextrin	5%	5%	5%

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Drug Loading

Fifty milligrams of microspheres were dissolved in a 50 mL citrate buffer with a pH of 4.4, followed by agitation with a magnetic stirrer at a speed of 1000 rpm for 3 hours. Afterward, the absorbance of ciprofloxacin was assessed using UV-Vis Spectroscopy (Shimadzu-UV 1800, Japan) at a wavelength of 275 nm.[16] The ciprofloxacin concentration was determined by inputting the absorbance value into the linear regression equation generated from the standard ciprofloxacin curve. Then, drug loading is measured using the formula:

$$\text{Drug loading} = \frac{Measured\ drug\ content}{Total\ weight\ of\ dry\ microspheres} \times 100\%^{15} \quad (1)$$

Entrapment Efficiency

Microspheres entrapment efficiency was computed by assessing the ciprofloxacin content enclosed within the microspheres using the formula :

$$\text{Entrapment Efficiency} = \frac{Measured\ drug\ content}{Theoritical\ drug\ content} \times 100\%^{16} \quad (2)$$

In-vitro release study

The drug release characteristics was studied with the aid of a thermoshaker (Wina Instrument Waterbath Shaker 605H, Indonesia) at a speed of 100 rpm and a temperature of 37°C. An equivalent amount of 400 mg of ciprofloxacin is weighed and placed in 100 mL of phosphate saline buffer at pH 7.4. The mixture was then placed into the thermoshaker, which is preheated to a temperature of 37 ± 0.5°C, and stirred at a speed of 100 rpm. Sample aliquots of 5 mL are taken at 15, 30, 60, 90, 120, 180, 240, 300, 360, 420, 480, 540, 600, 660, and 720 minutes. After each sample collection, the release medium is replaced with fresh medium. The samples were subjected to absorbance measurement utilizing a UV-Vis spectrophotometer (Shimadzu-UV 1800, Japan) set at a wavelength of 268 nm.[17] The concentration of ciprofloxacin is decided by applying the absorbance values to the standard curve equation previously (y= 0,0758x + 0,0108) established.[17]

Release kinetics

The kinetics measurement of ciprofloxacin release from microspheres were decided by mathematical models: zero-order (cumulative release of drug against time), first-order (log cumulative amount of drug remaining against time), Higuchi (cumulative percentage of drug release against the square root of time), and Korsmeyer-Peppas (log cumulative percentage of drug release against log time). The best model fitted the release data was evaluated based on the coefficient determination (R^2) obtained from the plotted graph. The models were constructed based on the model's theoretical equation.[15]

$$M_t = M_0 + K_0 t \quad (3)$$

$$\text{Log } M_t = \log M_0 + K_1 t/2{,}303 \quad (4)$$

$$Q = K_H \times t^{1/2} \quad (5)$$

$$M_t / M\square = K_{kp} t^n \quad (6)$$

where M_0 is the initial amount of ciprofloxacin HCl in dissolution media, M_t is the amount of ciprofloxacin HCl released in time t, $M\square$ is the amount of drug released after time □ , K_0, K_1, K_H, and K_{kp} are the release rate constants, Q is the cumulativeamount of kojic acid ester released in time t per unit area, raction of ciprofloxacin HCl release over time, n is the release exponent and t is the time.[15]

Stability Test

Assessment of the microspheres stability is conducted through accelerated stability testing. The microsphere powder is stored in vial bottles at temperatures of 25°C ± 2°C and 40 ± 2°C, with a relative humidity (RH) of 75 ± 5%, for 28 days at intervals of 0, 7, 15, and 28 days. Organoleptic changes of the microspheres, as well as drug loading and moisture content of the powder, are observed to assess the stability of the dry powder inhalation.[18]

Data Analysis

The data are presented as mean ± standard deviation of triplicates (n=3) and analyzed using SPSS statistical 25. One-way, two-way ANOVA and Post-Hoc multiple comparison test were performed to examine the differences among the group. The data is significatnly different if the p-value is <0.05.

Results and Discussion

The first step in this research was the observation of infrared spectra on microspheres. The results of the infrared spectrum observation indicate a shift in wavenumbers. The wavenumber shift suggests an interaction between the drug, polymer, and crosslinker. Furthermore, the wavenumber shift also signifies the formation of microspheres. The interaction of crosslinking between the crosslinker ($CaCl_2$) and the polymer is marked by a wavenumber shift in the guluronate group of sodium alginate polymer and the S=O group in kappa-carrageenan, which binds with Ca^{2+} from $CaCl_2$. In the guluronate group, there is a wavenumber shift from 884.91 cm^{-1} to 925.22 cm^{-1} (F1), 925.40 cm^{-1} (F2), and 925.86 cm^{-1} (F3). In kappa-carrageenan, there is a wavenumber shift in the S=O group from 1223.52 cm^{-1} to 1261.54 cm^{-1} (F1), 1264.53 cm^{-1} (F2), and 1265.90 cm^{-1} (F3). The interaction of crosslinking between Ca^{2+} ions and the guluronate group in sodium alginate polymer and the S=O group in kappa-carrageenan will undergo an ionic gelation reaction, forming an "egg-box" structure capable of trapping ciprofloxacin HCl.

Particle Size

The particle size measurement outcomes demonstrated that as the drug ratio in the formulation increased, there was a corresponding enlargement in particle size (Table 3). The One-Way ANOVA analysis yielded a P-value <0.05, manifesting a significant difference in particle size due to the increase in drug-polymer ratio. Post-Hoc Tukey HSD analysis further revealed significant differences between certain formulations, specifically F1 with F3 (P-value <0.05) and F2 with F3 (P-value <0.05). Increasing the drug-polymer ratio from (0.2:1:8) to (0.4:1,8) resulted in an increase in particle size from 2.25 ± 0.14 µm to 2.83 ± 0.12 µm. This is attributed to the increased viscosity of the combination of polymer and ciprofloxacin solutions with an increase in drug concentration, leading to larger droplets and consequently larger microsphere particle sizes. The measurement results showed that all formulations had sizes below 5 µm, meeting the requirements for particles to reach the alveoli, which should be within the range of 1-5 µm. Particles smaller than 1 µm may be exhaled out of the respiratory tract with the air, while particles larger than 5 µm may only reach the oropharynx.[19]

Morphology

Based on the observation of morphology and shape using SEM, all three formulations showed spherical shapes with smooth surfaces (Figure 1). These findings suggest that the microspheres have been successfully generated as planned and are suitable for inhalation purposes, facilitating their deposition in the alveoli.[20]

Moisture content

The test results were analyzed using One-Way ANOVA, yielding a P-value >0.05, indicating no significant difference in moisture content. Based on the moisture content (MC) testing, all formulations have MC less than 5% (Table 3). These results meet the requirements for pulmonary delivery,[16] which should be less than 10%.[16] Moisture content affects the formation of aggregates in the particles.

Flow properties

Observations made during the flow properties test include Carr's Index and Hausner ratio (Table 3). Based on the observations for all formulations, F1 and F2 have Carr's Index values < 15% and Hausner ratios in the range of 1.12-1.18, falling into the "Good" flow properties category. On the other hand, F3 has a Carr's Index of 15.57 (> 15%)

and a Hausner ratio of 1.19, falling into the "Fair" flow properties category. The increase in the drug-polymer ratio results in increased density. Larger density causes a greater reduction in the final volume during compaction, leading to higher Carr's Index and Hausner ratio values[17]. This indicates that higher drug-polymer ratios result in poorer flow properties. The statistical results indicate that the Carr's Index and Hausner ratio values have a significance level P-value >0.05. This suggests that the increase in the drug-polymer ratio does not result in a significant difference in the Carr's Index and Hausner ratio values.

Yield

Based on the yield data obtained, it was ranged from 91.71% ± 5.52 to 93.62% ± 3.97 (Table 3). The data were statistically analyzed using One-Way ANOVA, yielding a P-value >0.05 (not significant). The statistical results indicate that the increase in the drug-polymer ratio does not significantly affect the yield value. The yield values for each formulation indicated good yields, as they approach 100%. This suggests that the technique employed in the fabrication of microspheres yields the highest quantity of microspheres with fair efficiency.[21]

Drug Loading

The drug loading measurement results for F1, F2, and F3 were 2.03% ± 0.12, 2.77% ± 0.14, and 3.55 ± 0.13, respectively (Table 3). One-Way ANOVA analysis yielded a P-value <0.05. This value indicated that the increase in the drug-polymer ratio significantly affects the drug loading of the microspheres. Post-Hoc Tukey HSD analysis further shows significant differences between each formulation with a P-value of <0.05. The increase in drug loading with the increase in the drug-polymer ratio is due to the higher amount of ciprofloxacin in the polymer structure, which can fill more empty egg-box sites. Additionally, an increase in the amount of ciprofloxacin increased the viscosity, resulting in larger droplet sizes and, consequently, higher drug loading. These results suggest that drug loading increases along the increase in the drug-polymer ratio.

Entrapment Efficiency

Based on the entrapment efficiency results, all formulations were ranging from 75.27% ± 2.57 to 78.45% ± 1.89 (Table 3). One-Way ANOVA analysis yielded a P-value >0.05, indicating that the increase in the drug-polymer ratio does not significantly affect the entrapment efficiency of the microspheres. This is because the polymer content in each formulation is the same, therefore it cannot encapsulate more drugs.

Release Profile of Ciprofloxacin HCL from Microspheres

This test used a phosphate-buffered saline (PBS) solution with a pH of 7.4 over a 12-hour period at a temperature of 37°C. According to the release test findings, there was a correlation between an increase in both of the drug-polymer ratio and the drug release rate from the microspheres. The results of the statistical analysis show a P-value <0.05, indicating that an increase in the drug-polymer ratio results in a significant difference in the cumulative amount of released ciprofloxacin. The analysis was further carried out using Post-Hoc Tukey HSD, which showed a significant difference between F1 and F3 with a P-value <0.05. Based on the 12-hour release test results (Figure 2), F3 is the formulation with the highest cumulative drug release. This is due to the fact that F3 has the highest drug concentration, leading to a greater amount of drug being released. Additionally, the low polymer level in the formula results in a thinner polymer layer. A thin polymer layer leads to more drug being released, resulting in the formation of more channels and contributing to a faster drug release rate.[22]

Release Kinetics

The release kinetics of ciprofloxacin from the microspheres were assessed by fitting release data into several release kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models.

Table 2: FTIR Spectra Analysis of materials and microspheres

Functional group	Wave Number (cm^{-1})					
	Ciprofloxacin HCL	**Sodium Alginat**	**Kappa carrageenan**	**F1 (0.2:1.8)**	**F2 (0.3:1.8)**	**F3 (0.4:1.8)**
OH stretch	3516.74	3310.90	3352.32	3527.53	3523.16	3493.15
CH stretch	2914.76	2895.76	2892.99	2947.29	2909.82	2947.26
C=O stretch	1699.31	1592.77		1786,68	1766.19	1747.12
Quinolone N-H bending	1612.62			1615.91	1616,09	1617.80
OH bending	1263.03			1261.54	1264.53	1265.90
C-F Stretch	1272.64			1351.30	1306.13	1265.90
C-C stretch		1080.87		1074.03	1075.74	1075.16
Guluronic finger		884.91		925.22	925.40	925.86
Mannuronic finger		810.48		844.33	841.33	846.71
S=O			1223.52	1261,54	1264.53	1265.90
Galactose sulfat			841.94	844.33	841.33	846.71

Table 3: Physical characteristics of ciprofloxacin loaded alginate-carragenan microspheres

Formula	Particle Size (µm)	Moisture Content (%)	Yield (%)	Drug Loading (%)	Entrapment Efficiency (%)	Flow properties	
						Carr's Index (%)	**Housner Ratio**
F1	2.25 ± 0.14^{a}	3.38 ± 0.78	91.71 ± 5.52	2.03 ± 0.12^{a}	75.27 ± 2.57	13.29 ± 3.68	1.15 ± 0.04
F2	2.51 ± 0.09^{a}	2.98 ± 0.28	93.17 ± 3.35	2.77 ± 0.14^{b}	77.13 ± 2.96	14.93 ± 0.01	1.17 ± 0.01
F3	2.83 ± 0.12^{b}	3.6 ± 0.74	93.62 ± 3.97	3.55 ± 0.13^{c}	78.45 ± 1.89	15.57 ± 1.52	1.19 ± 0.02

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 4: Drug release kinetics of ciprofloxacin loaded alginate-carragenan microspheres

Formula	Zero order	First order	Higuchi	Korsmeyer-Peppas
F1	r = 0.7021	r = 0.7955	r = 0.8907	r = 0.9930
F2	r = 0.7292	r = 0.8574	r = 0.9020	r = 0.9882
F3	r = 0.8201	r = 0.9634	r = 0.9580	r = 0.9852

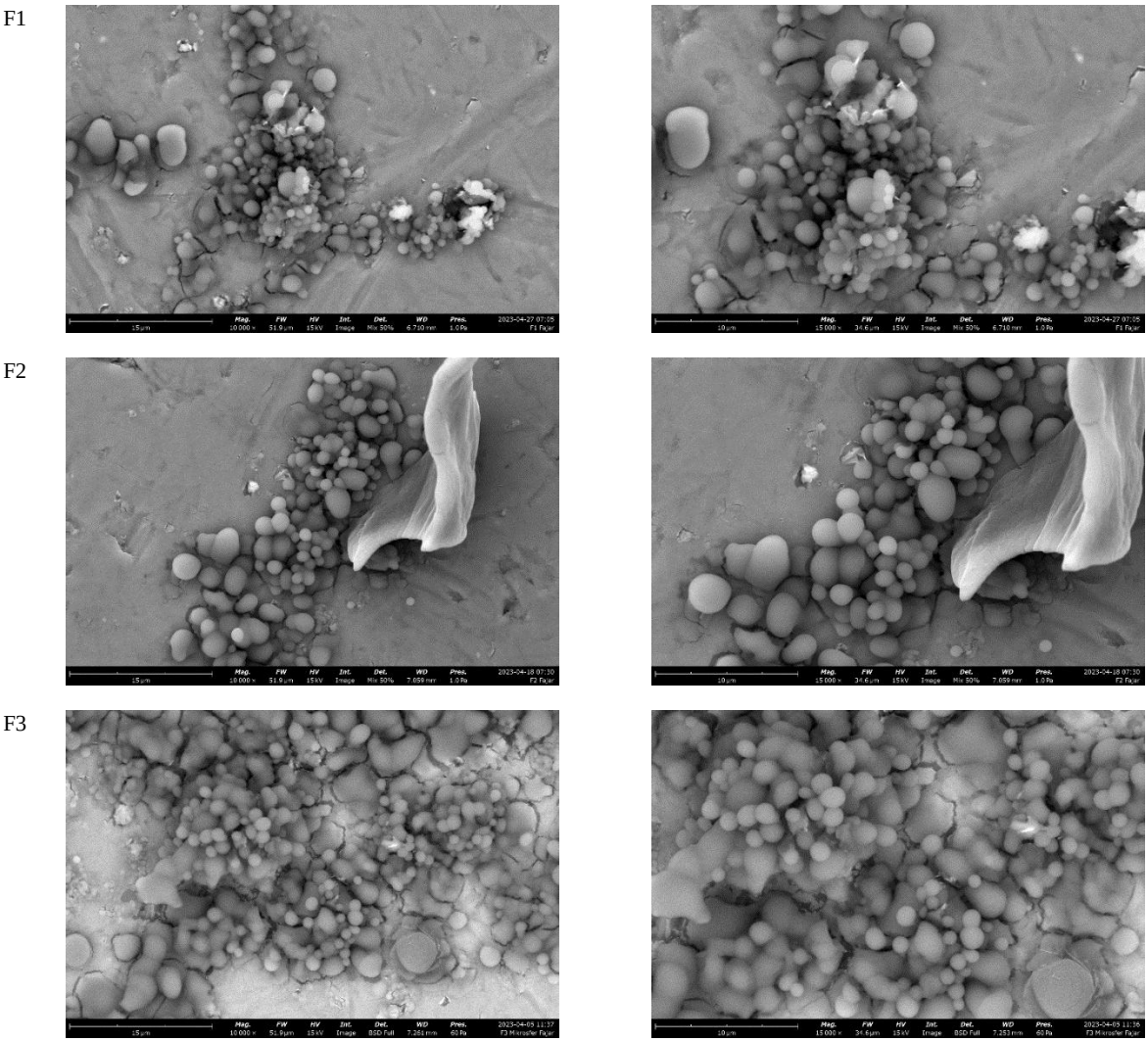

Figure 1: Morphology of ciprofloxacin loaded alginate-carrageenan microspheres in magnification a.10000x b.15000x

Next, to inspect the release kinetics, the value of the correlation coefficient (r) that fits the best was considered. The selected kinetic model for ciprofloxacin microspheres is the Korsmeyer-Peppas model due to its correlation coefficient (r) value closest to one (Table 4). The correlation coefficient (r) values for the Korsmeyer-Peppas model are F1=0.993, F2=0.9882, and F3=0.9852. The drug release mechanism using the Korsmeyer-Peppas equation is determined by the value of n (the release exponent with respect to time). If the value of n is < 0.5, the drug release follows Fick's diffusion law. If 0.5 < n < 1, it belongs to non-Fickian or anomalous transport.[23] A value of n=1 follows Case 1 transport model, and if n > 1, the release mechanism follows zero-order kinetics or the Super Case II model.[23] The release mechanism in ciprofloxacin microspheres follows Fick's diffusion law as it has an n value of < 0.5.

Stability Test

The parameters observed in the stability test are drug loading and moisture content. This test was conducted at temperatures of 25°C and 40°C with a storage duration of 28 days. Based on the data from the observation of drug loading and moisture content (Table 5), there is a slight increase in moisture content and an decrease in drug loading during storage. However, the results of the Two-Way ANOVA analysis yielded a P-value of >0.05 for both temperature, days, and temperature*days (interaction between both factors). This indicates that the temperature and 28-day storage duration do not significantly affect drug loading and moisture content in all formulations. According to these results, the microspheres are stable.[21] The decrease in drug loading is attributed to polymer degradation during the storage period, leading to the drug being released from the matrix, hence a decrease in drug loading.[21]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

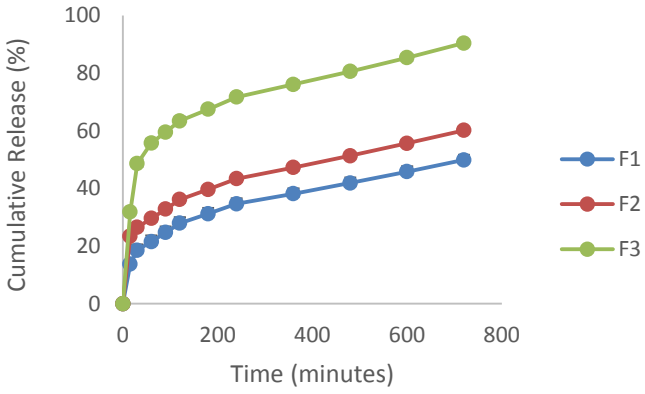

Figure 2: Drug release profile of ciprofloxacin loaded alginate-carrageenan microspheres

Conclusion

Ciprofloxacin microspheres with sodium alginate and kappa carrageenan polymer combination have been successfully fabricated using the ionic gelation method with aerosolization technique. The physical characteristics indicated spherical microspheres with smooth surfaces. The Particle size, drug loading and release of ciprofloxacin increased with higher drug-polymer ratios. Increasing the drug-polymer ratio had no effect on other characteristics and stability. The model kinetics of release followed the Korsmeyer-Peppas model. This research findings suggest that this microspheres system holds significant potential as a drug delivery system to the lungs..

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors would like to thank the Faculty of Pharmacy Universitas Airlangga, Surabaya, Indonesia for all of the support and facilities.

References

1. World Heath O. Global tuberculosis report 2022. Work Heal Saf. 2022;68.
2. Akcali S, Surucuoglu S, Cicek C, Ozbakkaloglu B. In vitro activity of ciprofloxacin, ofloxacin, and levofloxacin against Myobacterium tuberculosis. Ann Saudi Med. 2005;25(5):409–12.
3. Rodriguez JC, Ruiz P, Causse M, Vaquero M, Casal M. In Vitro Activity of Tedizolid against Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2001;63(4):229–31.
4. Katzung. Basic & Clinical Pharmacology. McGraw-Hill Education. New York. 2018 : 14.
5. Hariyadi DM, Purwanti T, Adilla S. Influence of crosslinker concentration on the characteristics of erythropoietin-alginate microspheres. 2018;6(200):250–9.
6. Paranjpe M, Müller-Goymann CC. Nanoparticle-mediated pulmonary drug delivery: A review. Int J Mol Sci. 2014;15(4):5852–73.
7. El-Sherbiny IM, El-Baz NM, Yacoub MH. Inhaled nano- and microparticles for drug delivery. Glob Cardiol Sci Pract. 2015(1):1–14.
8. Hariyadi DM, Hendradi E, Pratama HE. A Review microspheres as pulmonary delivery systems. Journal Chinese Pharm Sci 2021:30(7): 545-555
9. Yue S, Zhang B, Sun R, Liu W, Zhu Q, Zhang X, Wang R, Chen C. PLGA-based biodegradable microspheres in drug delivery: recent advances in research and application. Taylor & Francis Group 2021. 28: 1397-1418.
10. Patil JS & Sarasija S. Pulmonary drug delivery strategies: A concise, systematic review. Lung India. 2012;29(1), 44–49.
11. Rastogi R, Sultana Y, Aqil M, Ali A, Kumar S, Chuttani K, & Mishra AK. Alginate microspheres of isoniazid for oral sustained drug delivery. Int Jour of Pharm, 2017;334, 71–77.
12. Annisa V, Sulaiman TNS, Nugroho AK, Nugroho AE. Review Sinergisitas Kombinasi Polimer Alami Serta Pemanfaatan dalam Formulasi Obat. Maj Farmasetika. 2021;6(5):436.
13. Ataide J, Cefali L, Rebelo M, Spir L, Tambourgi E, Jozala A, et al. Bromelain Loading and Release from a Hydrogel Formulated Using Alginate and Arabic Gum. Planta Med. 2017;83(10):870–6.
14. Dounighi MN, Eskandari R, Avadi MR, Zolfagharian H, Mir Mohammad Sadeghi A, Rezayat M. Preparation and in vitro characterization of chitosan nanoparticles containing Mesobuthus eupeus scorpion venom as an antigen delivery system. J Venom Anim Toxins Incl Trop Dis. 2012;18(1):44–52.
15. Azhar SN, Ashari SE, Ahmad S, Salim N. In vitro kinetic release study, antimicrobial activity and in vivo toxicity profile of a kojic acid ester-based nanoemulsion for topical application. Roya Society of Chemistry. 2020. 10. 43894.
16. Hariyadi DM, Hendradi E, Kurniawan TD. Alginate Microspheres Encapsulating Ciprofloxacin HCl: Characteristics, Release, and Antibacterial Activity. Int J of Pharm Research and Health Sci, 2019;7(4), 3020–3027.
17. Hariyadi DM, Hendradi E, Sharon N. Development of Carrageenan Polymer for Encapsulation of Ciprofloxacin HCL: In Vitro Characterization. Int J Drug Deliv Technol. 2019;9(01):89–93.
18. Debnath SK, Saisivam S, Debanth M, Omri A. Development and evaluation of Chitosan nanoparticles based dry powder inhalation formulations of Prothionamide. PLoS One. 2018;13(1):1–12.
19. Chaurasiya B, Zhao Y-Y. Dry Powder for Pulmonary Delivery: A Comprehensive Review. Pharmaceutics. 2020;13(1):31.
20. Vishwa B, Moin A, Gowda D V., Rizvi SMD, Hegazy WAH, Abu Lila AS. Pulmonary Targeting of Inhalable Moxifloxacin Microspheres for Effective Management of Tuberculosis. Pharmaceutics. 2021;13(1):79.
21. Kumar BP, Chandiran IS, Bhavya B, Sindhuri M, Pharmaceutics D, Krishna G. Microparticulate Drug Delivery Systems. Handb Encapsulation Control Release. 2015;1(1):1091–134.
22. Abdullah MF, Nuge T, Andriyana A, Ang BC, Muhamad F. Core-Shell Fibers: Design, Roles, and Controllable Release Strategies in Tissue Engineering and Drug Delivery. MDPI. 2019:1-45.
23. Bruschi ML. Strategies to Modify the Drug Release from Pharmaceutical Systems. Elsevier; 2015.
Annisa V, Sulaiman TN, Nugroho AK, Nugroho A E. Review of the Synergy of Natural Polymer Combinations and Their Use in Drug Formulation. Farmaceutical 2021:6(5):436.