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Research Paper

Characteristics, Release, and Stability (Kinetics and Shelf-life) of Ciprofloxacin HCl-Alginate-Carrageenan Microspheres: Effects of Drug Concentration and Type of Lyoprotectant

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Abstract
Tuberculosis, an infectious disease caused by the *Mycobacterium tuberculosis* bacteria, is one of the main causes of death worldwide. Alternative treatments are necessary due to the rising prevalence of medication resistance in *Mycobacterium tuberculosis*. Fluoroquinolones, such as ciprofloxacin HCl, are among these alternatives and are generally administered orally, but they have limitations. Therefore, pulmonary targeted inhalation delivery systems have been developed. Inhalation of microspheres enables deposition in the lungs at appropriate particle sizes. This study formulates ciprofloxacin HCl microspheres with an optimal ratio and concentration of polymer combination and crosslinker, aiming to determine the effect of drug concentration and lyoprotectant type on characteristics, release, and stability, including degradation kinetics and shelf life. The results showed that the ciprofloxacin HCl-alginate-carrageenan microsphere powder was yellowish-white, with smooth morphology, a yield percentage of 96.08% ± 0.84 – 97.00% ± 0.19, particle sizes below 5 μm, drug loading between 4.57% ± 0.13 – 6.76% ± 0.06, and entrapment efficiency ranging from 79.45% ± 2.53 – 90.80% ± 0.77. The powder had moisture content below 5% and excellent flow properties. Ciprofloxacin HCl release from microspheres at pH 7.4 for 30 hours was 84.55% ± 0.89 – 90.74% ± 0.22, following Korsmeyer-Peppas kinetics based on the Fickian diffusion mechanism. Ciprofloxacin HCl-alginate-carrageenan microspheres were stable and exhibited good shelf life. This study concluded that particle size, drug loading, entrapment efficiency, and drug release are all influenced by drug concentration, while moisture content and flow properties, with adequate shelf life, are influenced by the type of lyoprotectant.

Keywords
Ciprofloxacin HCl, Alginate-Carrageenan Microspheres, Drug Concentration, Lyoprotectant, Release, Stability

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1. INTRODUCTION

Mycobacterium tuberculosis can affect several organs, particularly the lungs (DiPiro et al., 2023). Tuberculosis kills more than twice as many people as HIV/AIDS, with more than 10 million deaths each year, and the number has continued to increase since 2021 (World Health Organization, 2024). Alternative treatments are necessary due to the growing prevalence of medication resistance in *Mycobacterium tuberculosis*. Most of the requirements for an optimal antimycobacterial medication class are fulfilled by fluoroquinolones. One of the broad-spectrum fluoroquinolone antibacterials, Ciprofloxacin HCl, stops bacteria from replicating their DNA by inhibiting the DNA-gyrase and topoisomerase IV enzymes (Thai et al., 2022).

According to Heifets and Lindholm-Levy (1987), ciprofloxacin HCl is one of the fluoroquinolone antibiotics with the lowest minimum inhibitory concentration (MIC), ranging from 0.125 to 2.0 μg/mL, against *Mycobacterium tuberculosis*. Although ciprofloxacin HCl is usually used orally, it has a number of disadvantages, such as limited permeability, first-pass metabolism, a 70% oral bioavailability, and a brief half-life of 3 to 5 hours (Bayer Health Care Pharmaceuticals Inc., 2021).

Therefore, an alternative delivery system is needed to overcome these problems, such as pulmonary targeted inhalation delivery systems (Mehta et al., 2018).

Pulmonary targeted inhalation delivery systems offer several advantages: they are non-invasive, can target tuberculosis bacilli located in alveolar macrophages, maintain high drug levels in lung tissue, avoid first-pass metabolism, require fewer doses but with a rapid onset, and reduce systemic side effects compared to oral and intravenous administration (Mehta et al., 2018). Particles that are only 1 to 5 microns in size are required for lung deposition in inhalation delivery techniques that target the lungs. Microencapsulation techniques like microspheres can do this (Lengyel et al., 2019).

The drug core or the active substance and polymer that make up microspheres allows for compatibility and natural degradation in the body, especially when natural polymers are used to enhance bioavailability (Parameswari et al., 2024). Utilizing microspheres has various advantages, such as decreased toxicity and increased effectiveness, providing prolonged drug release and increased patient compliance (El-Sherbiny et al., 2015). The efficacy of microspheres as therapeutic delivery systems is influenced by the selection of polymer for the matrix and crosslinker as the crosslinking agent (Lee and Mooney, 2012).

The natural polymer used as a matrix in this research is a blend of sodium alginate with kappa-carrageenan because alginate can swell at lung pH and has strong mucoadhesive properties (Abka-Khajouei et al., 2022), but its porous structure limits sustained release. Hence it must be combined with carrageenan to provide sustained release (Abdelghany et al., 2017). Sodium alginate contains a lot of carboxyl groups and is very attracted to bivalent cations like Ca^{2+} ions. On the other hand, kappa carrageenan and Ca^{2+} create crosslinks by electrostatic attraction. Simultaneously, the adjacent Ca^{2+} and OSO_3^- produce a crosslinking network between the macromolecular networks. Therefore, $CaCl_2$ crosslinker is employed in the combination of alginate along with carrageenan polymers, and $CaCl_2$ is safest for the body because it is non-toxic (Kurniawan et al., 2024; Yu et al., 2019).

Research on ciprofloxacin HCl microspheres combining alginate-carrageenan polymers with $CaCl_2$ crosslinkers has been widely developed, such as the effect of alginate-carrageenan polymer concentration, but still has limitations in producing low yields and burst release in microspheres (Hariyadi et al., 2023). Using a polymer ratio of 1:1 with a concentration of each polymer of 0.9% and with $CaCl_2$ crosslinkers, the best physical characteristics of ciprofloxacin HCl microspheres were demonstrated (Kolesnyk and Burban, 2015; Wijaksana, 2022). Ciprofloxacin HCl microspheres demonstrated antituberculosis activity that was not substantially different from standard ciprofloxacin HCl, with a concentration of 2.0 μg/mL as the MIC when used with concentrations of 0.3% and 0.4%. Despite this, the microspheres maintained a high release rate and low drug loading and entrapment efficiency (Deliaz, 2023). The most effective crosslinker for ciprofloxacin HCl microspheres was 1.5M $CaCl_2$, used at a 1:1 ratio with 0.9% of each alginate-carrageenan polymer. The highest drug loading and entrapment efficiency were obtained from the study, as well as the best flow characteristics, stability over 28 days, and sustained release. However, to ascertain the degradation kinetics and shelf life of microspheres, a stability test is still advised (Amiruddin et al., 2023).

Various methods of microspheres preparation have been widely developed. This study made use of the ionotropic gelation method in conjunction with aerosolization, a chemical technique that leverages the ability of polyelectrolytes to interact with counter ions to form a gel. This approach has the benefits of being simple, quick, reasonably priced, and utilizing low temperatures to minimize drug damage. It also avoids using organic solvents. Small and consistent particle sizes can be produced using aerosolization techniques (Hariyadi et al., 2018; Sacco et al., 2021).

During the microsphere-making process, drying is required while maintaining the stability of the drug. The freeze-drying method is used for drying, and it is essential to add a lyoprotectant. Maltodextrin 5% and lactose 5% were used as lyoprotectants in this research because lyoprotectants at these concentrations showed that ovalbumin microspheres remained stable during 60 days of storage (Hariyadi et al., 2016). Several studies of ciprofloxacin HCl microspheres used only 5% maltodextrin as the lyoprotectant (Hariyadi et al., 2022, 2019), but due to the hygroscopic nature of maltodextrin (Zhang et al., 2024), the use of lactose 5% as the lyoprotectant is necessary.

In this study used ionotropic gelation method, combined with aerosolization technique to formulate ciprofloxacin HCl microspheres with optimal ratio and concentration of alginate-carrageenan polymer combination and calcium chloride crosslinker to determine the effect of drug concentration (0.3% and 0.4%) and type of lyoprotectant (maltodextrin 5% and lactose 5%) on the characteristics, release, and stability, including degradation kinetics and shelf life of microspheres, so that they can overcome the limitations of previous studies.

2. EXPERIMENTAL SECTION

2.1 Materials

Ciprofloxacin HCl (Zhejiang Ltd., China), sodium alginate (Sigma Aldrich, USA), kappa-carrageenan (KCN, Indonesia), $CaCl_2$ (PT. Sumber Utama Kimia Murni, Indonesia), lactose (HiMedia Laboratories Pvt. Ltd., India), distilled water (PT. Sumber Utama Kimia Murni, Indonesia), trisodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$) (SAP Chemicals), citric acid monohydrate ($C_6H_8O_7.H_2O$) (CV. Chemical Indonesia Multi Sentosa, Indonesia), and phosphate buffered saline (Biocomma, China) were the materials used in this study. All chemicals were pharmaceutical-grade.

The equipment used in the study included a Mettler Toledo XPE 26 analytical balance (Swiss), FT-IR spectrophotometer (Bruker alpha II, Germany), differential scanning calorimeter (Linseis DSC 1000, Germany), spray aerosol, Dragon Lab MSPro stirring plate (China), freeze dryer (Biobase BK-FD12P,

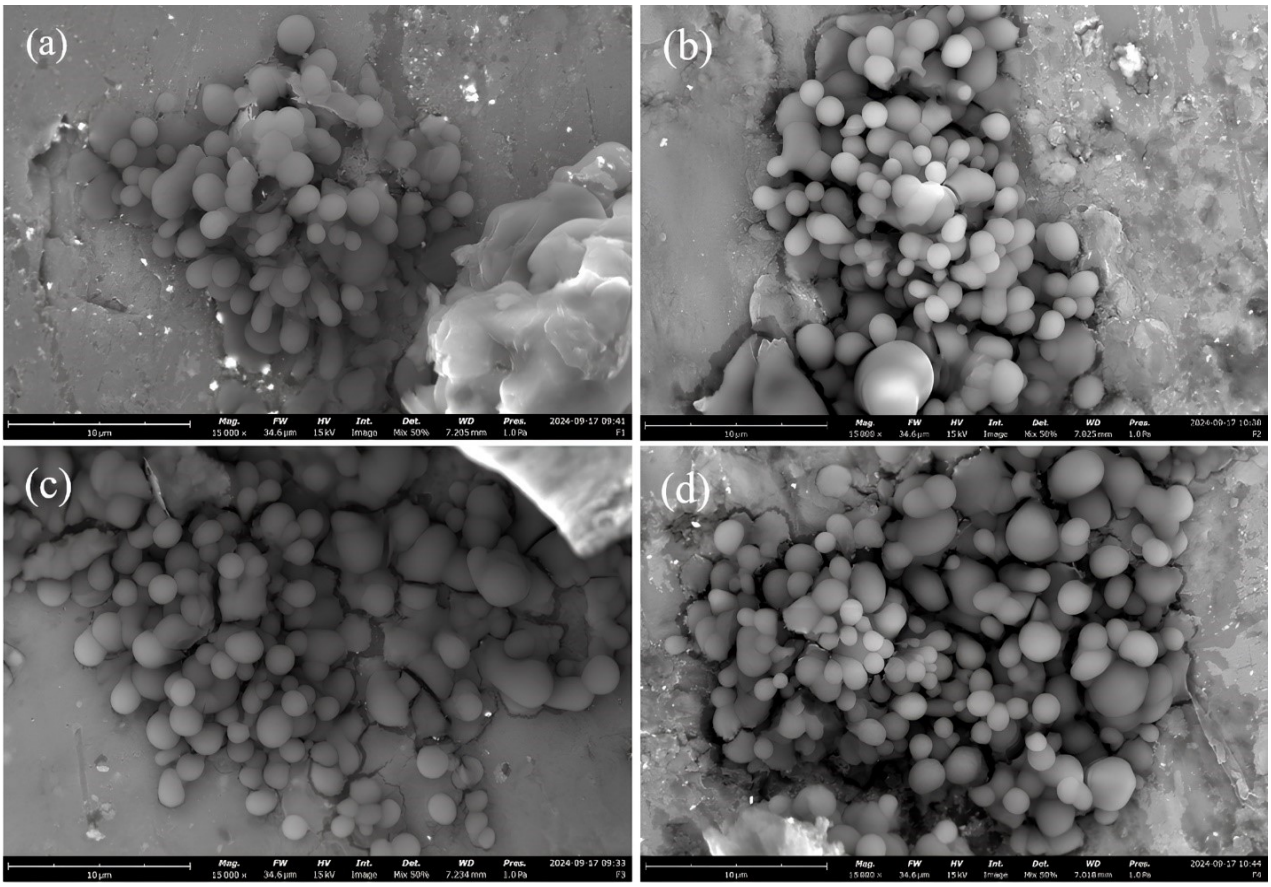

Figure 1. SEM Image of the Ciprofloxacin HCl-Alginate-Carrageenan Microspheres at a Magnification of 15000x: (a) F1, (b) F2, (c) F3, (d) F4

China), Rotofix 32 centrifuge (Germany), Shimadzu UV-1800 UV-Vis spectrophotometer (Japan), particle analyzer (Mastersizer 3000, Malvern, USA), Thermo fisher Phenom™Pro X scanning electronic microscope (USA), Mettler Toledo HB43-S moisture analyzer (USA), motorized tapping device (Erweka AR 400, France), thermoshakers (WINA Instrument Waterbath shaker 605H, Indonesia) and climatic chamber (MMM Medcenter Einrichtugen, Germany).

2.2 Ciprofloxacin HCl-Alginate-Carrageenan Microspheres Preparation

Ionotropic gelation with aerosolization technique was used to formulate the microspheres. Ciprofloxacin HCl drug at concentrations of 0.3% and 0.4% was dissolved in a 1:1 (w/w) combination solution of the alginate-carrageenan polymer at a concentration of 0.9% for each polymer. At 40 psi of pressure, the polymer-drug solution mixture was sprayed into a 1.5M $CaCl_2$ crosslinker and continuously stirred for 2 hours at 1000 rpm. To collect wet microspheres, centrifugation at 2500 rpm for 6 minutes was performed, and they were then rinsed three times with demineralized water. Dry microspheres were produced by resuspending wet microspheres in a 5% maltodextrin solution or 5% lactose lyoprotectant solution. Then, at -80 °C, the freeze dryer was set for drying until the required moisture level was reached (Amiruddin et al., 2023). The microspheres that form ciprofloxacin HCl, alginate, and carrageenan were also assessed. The microspheres formulation is shown in Table 1.

2.3 Examination of FTIR Spectroscopy

Examination of FTIR spectroscopy is a essential analytical technique for identifying certain functional groups in molecules with a Bruker Alpha II FTIR spectrophotometer. The FTIR band spectra were analyzed at wave numbers between 4000 and 500 cm^{-1} to assess structural integrity in various formulations (Amiruddin et al., 2023; Deliaz et al., 2023).

2.4 DSC Analysis

To determine the thermal characteristics of the microsphere formulation, a Differential Scanning Calorimeter (Linseis DSC 1000, Germany) was employed. Samples weighing roughly 3 mg of each formula were heated from 30 °C to 300 °C at a rate of 10 °C per minute. The prominent DSC peaks were identified as endothermic peaks that are influenced by temperature (Fitriani et al., 2025).

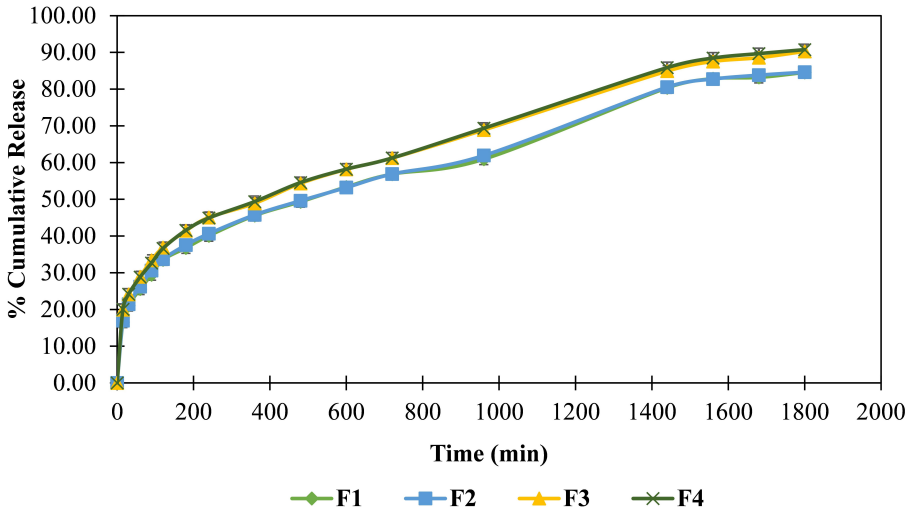

Figure 2. Release Profile of Ciprofloxacin HCl-Alginate-Carrageenan Microspheres

Table 1. Ciprofloxacin HCl-Alginate-Carrageenan Microspheres Formulation

Components	Uses	F1	F2	F3	F4
Ciprofloxacin HCl	Active Ingredients	0.3 %	0.3 %	0.4 %	0.4 %
Sodium Alginate	Natural Polymer	0.9 %	0.9 %	0.9 %	0.9 %
Kappa Carrageenan	Natural Polymer	0.9 %	0.9 %	0.9 %	0.9 %
Calcium Chloride	Crosslinker	1.5 M	1.5 M	1.5 M	1.5 M
Maltodextrin	Lyoprotectant	5 %	-	5 %	-
Lactose	Lyoprotectant	-	5 %	-	5 %

2.5 Physical Characterization of Microspheres

2.5.1 Organoleptic

The shape and colour of each ciprofloxacin HCl-alginate-carrageenan microsphere formulation were examined organoleptically (Amiruddin et al., 2023).

2.5.2 Morphology

The microspheres' surface morphology was investigated after being mounted for 120 seconds with gold coating using a Thermo Fisher PhenomTM Pro X scanning electron microscope (USA) at a distance of 5–10 mm, a magnification of 15000x, and a beam energy of 15 kV (Amiruddin et al., 2023; Hariyadi et al., 2023).

2.5.3 Yield

The results were calculated to evaluate the efficiency of producing microspheres based on the final weight of the total recoverable microparticles (Amiruddin et al., 2023; Hariyadi et al., 2023).

2.5.4 Particle Size

The dry dispersion technique and the laser diffraction principle using the Mastersizer 3000 particle size analyzer (Malvern, USA) were used to measure particle size (Horiba, 2016).

2.5.5 Drug Loading and Entrapment Efficiency

50 mg of microspheres were added to Citrate Buffer (pH 4.4 ± 0.05), and the mixture was agitated for seven hours at 1000 rpm. A Shimadzu UV-1800 UV-Vis spectrophotometer was used to assess the absorbance at 275 nm following solution filtration (results from internal validation). The validated internal calibrated standard plot was used to calculate the amount of drug, and the Equation 1 and Equation 2 were used to assess drug loading and entrapment efficiency (Amiruddin et al., 2023; Hariyadi et al., 2023):

$$\text{Drug loading} = \frac{\text{Measured drug weight}}{\text{Total microsphere dry weight}} \times 100\% \quad (1)$$

$$\text{EE} = \frac{\text{Measured drug weight}}{\text{Theoretical drug weight in formula}} \times 100\% \quad (2)$$

2.5.6 Moisture Content

To determine the moisture content, the moisture analyzer (Mettler Toledo HB43-S) was utilized. The microspheres were burned at a temperature of 100–140 °C after being evenly distributed in the pan and weighed to a minimum of 500 mg, and then they waited until the moisture content was successfully measured (Amiruddin et al., 2023; Hariyadi et al., 2023).

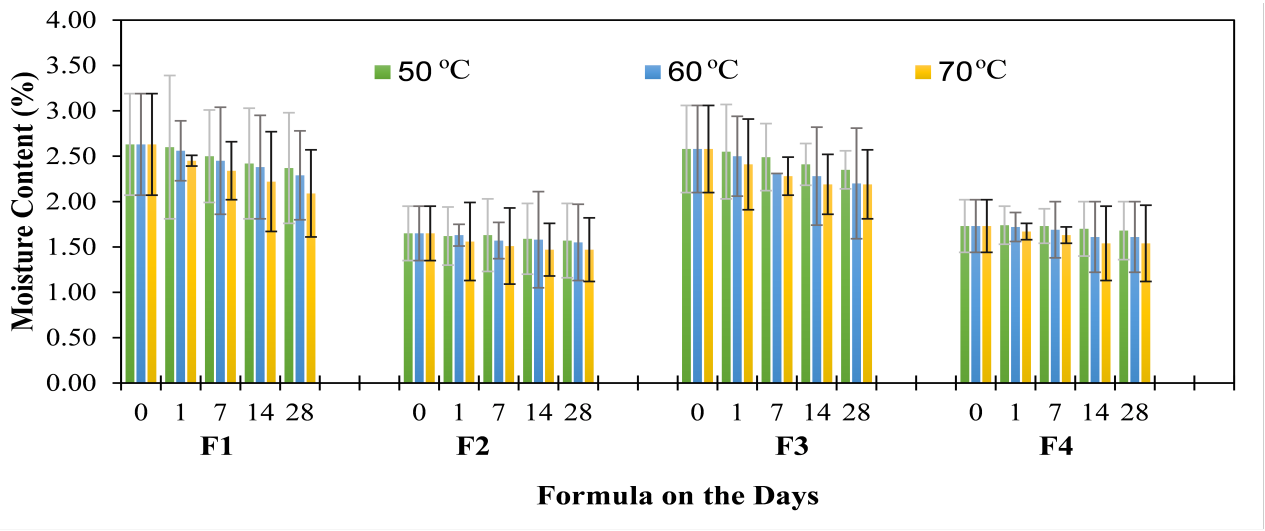

Figure 3. Moisture Content of Microspheres in Stability Test

Table 2. FTIR Formulation for All Microspheres

Functional Group	Wavenumber (cm^{-1})						
	Ciprofloxacin HCl	Sodium Alginate	Kappa Carragean	F1	F2	F3	F4
OH stretch	3524.95	3237.11	3326.70	3581.68	3568.51	3580.80	3558.02
CH stretch	2906.94	2908.50	2935.16	2948.15	2937.44	2944.16	2953.95
C=O stretch	1699.79	1595.93		1758.89	1743.79	1759.53	1727.65
Quinoline N-H bending	1620.03			1601.56	1609.08	1603.99	1603.15
OH bending	1264.63			1225.59	1243.97	1221.57	1223.26
C-F stretch	1044.16			1149.07	1020.10	1148.10	1025.55
C-C stretch		1026.52		1008.86	1020.10	1010.74	1025.55
Guluronic finger		940.38		925.99	926.22	926.81	926.42
Mannuronic finger		813.58		849.07	851.25	842.78	887.98
S=O			1223.40	1225.59	1243.97	1221.57	1223.26
Galactose sulfate			842.17	849.07	851.25	842.78	887.98

2.5.7 Flow Properties

A 25 mL graduated cylinder was used to measure the flow parameters by looking at the Carr's index and Hausner ratio readings. The density of the microsphere particles was computed after the cylinder holding the microsphere sample was mechanically tapped 500 times, and the volume drop was noted (Lane, 2016).

2.5.7.1 Bulk Density

The bulk density was computed as the weight of the microsphere powder divided by the initial volume of the container, as shown in the Equation 3 (Lane, 2016):

$$\text{Bulk density} = \frac{\text{Microsphere powder weight (g)}}{\text{Initial volume of microsphere powder (mL)}} \quad (3)$$

2.5.7.2 Tapped Density

The ratio of microsphere powder weight to tapped powder final volume was used to calculate the tapped density, as shown in the Equation 4. Carr's Index and Hausner Ratio as shown in the Equation 5 and Equation 6 (Lane, 2016):

$$\text{Tapped density} = \frac{\text{Microsphere powder weight (g)}}{\text{Final volume of tapped microsphere powder (mL)}} \quad (4)$$

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Bulk density}} \times 100\% \quad (5)$$

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad (6)$$

2.6 In Vitro Drug Release of Microspheres

2.6.1 In Vitro Release Study

Microsphere drug release was accomplished by weighing multiple microspheres equal to 15 mg of Ciprofloxacin HCl, adding

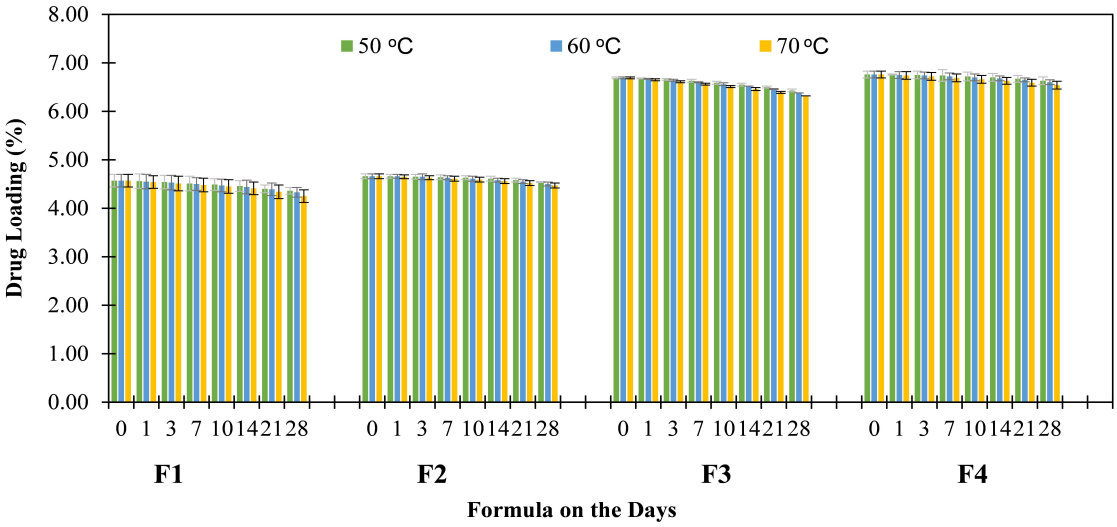

Figure 4. Drug Loading of Microspheres in Stability Test

Table 3. Physical Attributes of Ciprofloxacin HCl–Alginate–Carrageenan Microspheres

Formula	Yield (%)	Particle Size (μm)	Drug Loading (%)	Entrapment Efficiency (%)	Moisture Content (%)	Carr's Index (%)	Hausner Ratio
F1	96.08 ± 0.84	2.58 ± 0.16	4.57 ± 0.13	79.45 ± 2.53	2.63 ± 0.56	10.29 ± 1.40	1.10 ± 0.01
F2	96.14 ± 0.93	2.49 ± 0.28	4.66 ± 0.05	81.19 ± 0.42	1.65 ± 0.30	7.94 ± 1.34	1.08 ± 0.01
F3	96.89 ± 0.50	3.20 ± 0.40	6.69 ± 0.02	89.71 ± 0.24	2.58 ± 0.48	10.31 ± 1.38	1.10 ± 0.01
F4	97.00 ± 0.19	3.08 ± 0.23	6.76 ± 0.06	90.80 ± 0.77	1.73 ± 0.29	7.94 ± 1.34	1.08 ± 0.01

them to a phosphate buffer solution (PBS) at a pH of 7.4 based on lung pH, and then placing them in a thermoshaker (WINA Instrument Waterbath shaker 605H) set to 37 °C and 100 rpm. At the sampling points of minutes 0, 15, 30, 60, 90, 120, 180, 240, 360, 480, 600, 720, 960, 1440, 1560, 1680, and 1800, several samples (5 mL) were withdrawn and the medium was changed out with a suitable volume. After filtering the sample through a 0.45 μm Millipore filter paper, the absorbance was measured using a Shimadzu UV-1800 spectrophotometer set to 267.80 nm (Amiruddin et al., 2023; Hariyadi et al., 2023). By using the absorbance value to enter the previously created standard curve equation (y = 0.0839x - 0.0133), the concentration of ciprofloxacin HCl was ascertained.

2.6.2 Drug Release Kinetics

The pattern of drug release was described using drug release kinetics. Bruschi (2015) state that the drug release mechanism is determined by a variety of kinetic equation models, including zero-order kinetics, first-order kinetics, Higuchi, and Korsmeyer-Peppas. The best model was determined based on the coefficient of determination (r) that approached one from the regression equation of each kinetic model.

2.7 Stability of Microspheres

2.7.1 Stability Test

To investigate the stability of microspheres, the accelerated predictive stability (APS) approach was employed. A vial bottle was filled with the microsphere powder. The vial bottle was stored in a climatic chamber at various extreme temperatures of 50 °C ± 22 °C, 60 °C ± 22 °C, 70 °C ± 2 °C with relative humidity, RH 75 ± 5% for 1 month with intervals of 0, 1, 3, 7, 10, 14, 21, 28 days. To assess the stability of ciprofloxacin-alginate-carrageenan microspheres, dry powder inhalation was examined for organoleptic changes, moisture content, and drug loading (González-González et al., 2022; Scrivens et al., 2018).

2.7.2 Degradation Kinetics

The degradation kinetics of microspheres at different extreme temperatures of 50 °C ± 2 °C, 60 °C ± 2 °C, 70 °C ± 2 °C with relative humidity, RH 75±5% were determined by entering data on the results of drug loading reduction against time in the zero and first-order degradation kinetics models, which were then determined by the correlation coefficient (r) value closest to 1 (Scrivens et al., 2018).

2.7.3 Shelf-life

The amount of time a pharmaceutical product should remain stable and safe for its primary purpose under particular storage

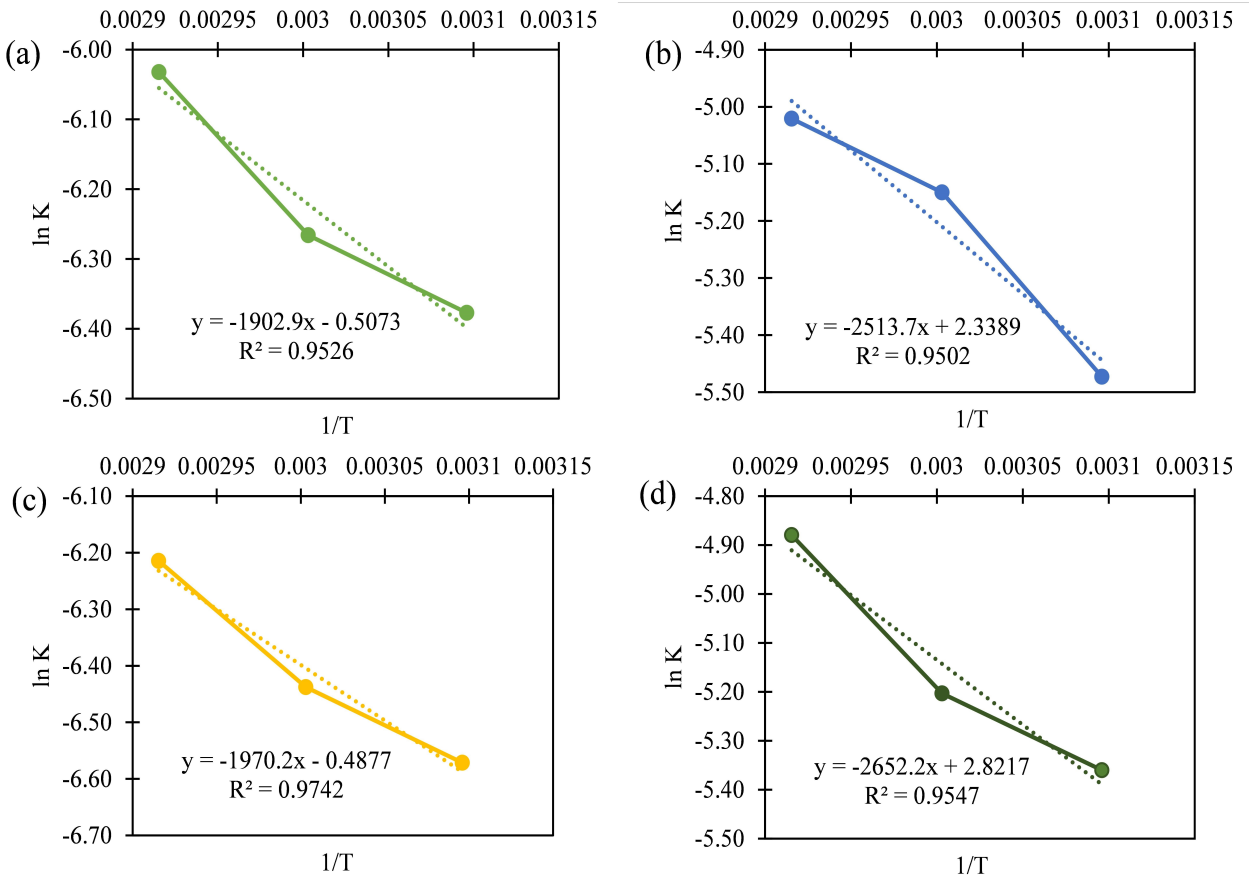

Figure 5. Stability Extrapolation Graph of 1/T Relationship to ln K in Formulations: (a) F1, (b) F2, (c) F3, (d) F4

circumstances is known as its shelf life, and the mean kinetic temperature is used to calculate a drug's shelf life (Bhangare et al., 2022). The Arrhenius regression equation can be utilized in accelerated predictive stability (APS) to ascertain how temperature affects the stability of the preparation (Scrivens et al., 2018). The most often used mathematical model for evaluating medication stability in a variety of stability investigations is the Arrhenius regression equation. This approach helps establish the relationship between temperature and rate constant to forecast the shelf life of drugs and drug products throughout the development phase (Bhangare et al., 2022).

2.8 Data Analysis

The mean ± SD was used to display all data. To analyze the statistical data, a two-way analysis of variance was performed with a 95% confidence level (α = 0.05).

3. RESULTS AND DISCUSSION

3.1 Microspheres Characterization

The formation and characterization of microspheres, which were created using the ideal ratio and concentration of alginate-carragenan polymer combination and crosslinker using ionotropic gelation with aerosolization techniques, were examined in this study in relation to the effect of drug concentration and type of lyoprotectant. The FTIR spectra confirmed the presence of functional groups in formulations F1 to F4, validating successful microsphere synthesis and indicating potential wavenumbers shifts in the raw materials, including ciprofloxacin HCl, sodium alginate, and kappa-carrageenan. The results are interpreted in Table 2. All microsphere formulations contained quinolone, as indicated by the FTIR spectra of ciprofloxacin HCl, which had distinctive stretching vibrations of OH, C=O, C-H, and C-F groups. These were accompanied by the N-H bending peak. Wavenumber shifts associated with ciprofloxacin's functional groups were observed in the N-H bending of quinolone, shifting from 1620.03 cm^{-1} to 1601.56 and 1609.08 cm^{-1}. Additionally, the C-F group shifted from 1044.16 cm^{-1} to between 1020.10 cm^{-1} and 1149.07 cm^{-1}. The guluronate and mannuronate group shifts in sodium alginate were observed at 940.38 cm^{-1} to 925.99–926.81 cm^{-1}, and at 813.58 cm^{-1} to 842.78–887.98 cm^{-1}, respectively. Changes in the kappa-carrageenan spectrum were confirmed by shifts in the sulfate galactose group from 842.17 cm^{-1} to 842.78 and 887.98 cm^{-1}, and the S=O group shifted from 1223.40 cm^{-1} to between 1221.57 and 1243.97 cm^{-1}, along with the disappearance of the C-O-C group.

These findings validate that microsphere formation occurred through chemical interactions between the polymers

Table 4. Release Kinetics with Release Exponent (n) of Ciprofloxacin HCl-Alginate-Carrageenan Microspheres

Formula	Zero-order	First-order	Higuchi	Korsmeyer-Peppas	
F1	r = 0.8995	r = 0.9802	r = 0.9831	r = 0.9921	n = 0.3382
F2	r = 0.8976	r = 0.9806	r = 0.9821	r = 0.9919	n = 0.3327
F3	r = 0.8868	r = 0.9847	r = 0.9802	r = 0.9925	n = 0.3173
F4	r = 0.8905	r = 0.9845	r = 0.9815	r = 0.9917	n = 0.3203

and cross-linkers. Based on the predicted interactions, carboxyl groups from opposing G-chains in the alginate polymer and sulfate groups from the kappa-carrageenan polymer structure were involved (Yu et al., 2019). Through their interaction with the $CaCl_2$ cross-linker, sodium alginate–kappa-carrageenan polymers created a network or matrix that bound to Ca^{2+} ions and entrapped the drug (Wathoniyyah, 2016).

3.2 Microspheres Organoleptic

The results showed that all ciprofloxacin HCl-alginate-carrageenan microsphere formulations (F1–F4) produced a yellowish-white powder and no clumping.

3.3 Microspheres Morphology

F1–F4 microspheres produced a smooth, uniformly spherical surface. The surface morphology of microspheres, as assessed by SEM, is displayed in Figure 1.

3.4 Yield

The percentage yield ranged from 96.08% ± 0.84 and 97.00% ± 0.19, as shown in Table 3. These results indicate very good yields (>90%) (Wee et al., 2023). In addition, the yield approaching 100% suggests that the preparation method was viable for creating ciprofloxacin HCl-alginate-carrageenan microspheres, in this instance by combining the ionic gelation method with the aerosolization technique (Kalalo et al., 2022; Liempepas et al., 2025). The microsphere yield is not significantly affected by the drug concentration, the type of lyoprotectant, or the combination of the two, according to statistical analysis (p>0.05).

3.5 Particle size

The particle size of ciprofloxacin HCl-alginate-carrageenan are displayed in Table 3. The microspheres ranged from 2.49 μm ± 0.28 to 3.20 μm ± 0.40. All microsphere formulation produced particle sizes below 5 μm, which meets the particle size requirements for the inhalation delivery route of 1–5 μm, allowing deposition in the alveoli (Gaber et al., 2021). In contrast, particle larger 5 μm are deposited in the oropharynx, while those smaller than 1 μm are ejected with the expired air (Karhale et al., 2012). In addition, the resulting polydispersity index was less than 0.7, suggesting a uniform microsphere size distribution and homogeneous dispersion of the microsphere (Chotchindakun et al., 2021; Wulandari et al., 2022).

Microsphere particle size was strongly impacted by drug concentration, according to statistical analysis (p<0.05). However, neither the lyoprotectant type nor the combination of the two showed any significant differences (p>0.05). Higher drug concentrations caused the polymer combination-drug solution to become more viscous, which led to larger droplets and larger microsphere particle sizes (Jelvehgari et al., 2010; Deliaz, 2023). Microsphere particle size is a crucial characteristic, particularly in lung-targeted inhalation preparations (Chaurasiya and Zhao, 2021).

3.6 Drug Loading

The drug loading percentage in the ciprofloxacin HCl-alginate-carrageenan microspheres ranged from 4.57% ± 0.13 to 6.76% ± 0.06, as shown in Table 3. The outcomes of drug loading of microspheres were significantly influenced by the drug concentration, according to statistical analysis (p<0.05). Nevertheless, neither the lyoprotectant type nor the combination of the two had a significant impact (p>0.05). Increasing drug concentration directly increases drug loading, according to previously reported studies (Ebrahimi et al., 2017; Huang et al., 2023). The larger amount of ciprofloxacin HCl in the polymer structure causes the drug loading of microspheres to rise because it may fill more empty egg-box space (Deliaz, 2023). Increased therapeutic efficacy and fewer negative effects during drug delivery are two benefits of high drug loading (Li et al., 2022).

3.7 Entrapment Efficiency

According to Table 3. The entrapment efficiency of the resultant microspheres varied between 79.45% ± 2.53 and 90.80% ± 0.77. Drug concentration had a substantial impact on the entrapment efficiency of microspheres, according to statistical analysis (p<0.05). However, neither the lyoprotectant type nor the combination of the two substantially affects the entrapment efficiency (p>0.05). In line with previously published research that indicates a positive correlation between drug concentration and entrapment efficiency, increasing the drug concentration has an impact on the entrapment efficiency of microspheres (Abbas and Alhamdany, 2020; Huang et al., 2023). High drug efficiency indicates good formulation efficiency, minimal burst release and better stability (Yadav et al., 2018).

The increasing drug concentration causes the dispersed phase to become more viscous, which improves the efficiency of microsphere trapping. Furthermore, entrapment efficiency is influenced by the drug's solubility in both the solvent and continuous phase, indicating that the drug concentration deter-

Table 5. Degradation Kinetics of Microsphere in Stability Test

Formula	Degradation Kinetics at 50°C/RH 75%	
	Zero-Order	First-Order
F1	y = -0.0076x + 4.5656	y = -0.0017x + 1.5187
F2	y = -0.0042x + 4.6637	y = -0.0009x + 1.5409
F3	y = -0.0092x + 6.6884	y = -0.0014x + 1.9004
F4	y = -0.0047x + 6.7654	y = -0.0007x + 1.9118
Formula	Degradation Kinetics at 60°C/RH 75%	
	Zero-Order	First-Order
F1	y = -0.0083x + 4.5591	y = -0.0019x + 1.5173
F2	y = -0.0058x + 4.6656	y = -0.0013x + 1.5403
F3	y = -0.0107x + 6.6777	y = -0.0016x + 1.8989
F4	y = -0.0055x + 6.7575	y = -0.0008x + 1.9107
Formula	Degradation Kinetics at 70°C/RH 75%	
	Zero-Order	First-Order
F1	y = -0.0107x + 4.5560	y = -0.0024x + 1.5167
F2	y = -0.0066x + 4.5557	y = -0.0014x + 1.5382
F3	y = -0.0128x + 6.6577	y = -0.0020x + 1.8959
F4	y = -0.0076x + 6.7461	y = -0.0011x + 1.9090

mines the entrapment efficiency (Abbas and Alhamdany, 2020; Jelvehgari et al., 2010).

3.8 Moisture Content

The produced ciprofloxacin HCl-alginate-carrageenan microspheres exhibited a moisture content of less than 5%, as displayed in Table 3. This result meets the requirements for microparticles because it is better for aerosolization (Saha et al., 2022). Statistical analysis revealed that the type of lyoprotectant had a substantial impact on the microspheres' moisture content ($p<0.05$), but drug concentration and the combination of the two had no discernible effect ($p>0.05$). Depending on the type of lyoprotectant utilized, the microspheres' moisture content changes. This is in line with previous studies that show the moisture content percentage increases with the ratio of maltodextrin to lactose in a microparticle formulation (Yang et al., 2024). This occurs as a result of the distinct characteristics of the various lyoprotectant classes. In other words, lactose is not hygroscopic, whereas maltodextrin is a little bit (Rowe et al., 2009; Zhang et al., 2024). Elevated moisture content in microsphere particles can lead to microsphere deterioration and decrease stability of microspheres (Varela-Fernández et al., 2022; Shan et al., 2016).

3.9 Flow Properties

Flow properties were assessed using Carr's index and Hausner ratio by calculating microsphere density. The particle density values for all microsphere formulations were favorable for lung delivery system, especially for aerosol performance, so that particles can be deposited in the inner lungs with a particle density value of <0.4 g/cm^3 (Chaurasiya and Zhao, 2021). The ranges of Carr's index and Hausner ratio were 7.94% ± 1.34 to 10.31% ± 1.40 and 1.08 ± 0.01 to 1.10 ± 0.01, respectively (Table 3). All microsphere formulations exhibit excellent flow quality, making them appropriate for inhalation routes that target the lungs (Lane, 2016). According to statistical analysis, the type of lyoprotectant significantly alters the flow properties ($p<0.05$), but the drug concentration and drug concentration with the type of lyoprotectant do not significantly alter these parameters ($p>0.05$). The type of lyoprotectant affects the flow properties because of the characteristics of lactose, which are able to produce particles with good aerodynamics (Dominici et al., 2022; Du et al., 2014). Variations in moisture content also influence the flow properties of microspheres. An increase in moisture content reduces the flow ability of the microspheres, which is reflected by higher Carr's index and Hausner ratio values (Kalman, 2021).

3.10 In Vitro Release Study

The release profile of ciprofloxacin HCl from microspheres is shown in Figure 2. In the release test results of all microsphere formulations, there is a burst release at the beginning of the 15th minute, which occurs because during the manufacture of microspheres, there are drug molecules attached to the surface of the polymer matrix due to van der Waals forces that diffuse out faster, then followed by a sustained release phase that occurs because the drug molecules encapsulated in the matrix are released when the polymer matrix degrades. For 30 hours, the cumulative drug release in phosphate buffer (pH 7.4 ± 0.05)

Table 6. Correlation Coefficient (r) Value of Microsphere Degradation Kinetic Model

Formula	50°C/RH 75%		60°C/RH 75%		70°C/RH 75%	
	Zero-Order	First-Order	Zero-Order	First-Order	Zero-Order	First-Order
F1	r = 0.9969	r = 0.9973	r = 0.9954	r = 0.9961	r = 0.9932	r = 0.9930
F2	r = 0.9860	r = 0.9852	r = 0.9943	r = 0.9940	r = 0.9976	r = 0.9977
F3	r = 0.9968	r = 0.9970	r = 0.9947	r = 0.9954	r = 0.9795	r = 0.9817
F4	r = 0.9925	r = 0.9922	r = 0.9955	r = 0.9954	r = 0.9885	r = 0.9894

reached 84.55% ± 0.89 for F1, 84.63% ± 0.45 for F2, 90.27% ± 0.18 for F3 , and 90.74% ± 0.22 for F4. Based on statistical analysis, it showed that the drug concentration gave a significant difference to the total ciprofloxacin HCl released from the microspheres (p<0.05), but is not significantly different on lyoprotectant type or the concentration of the drug combined with the lyoprotectant type (p>0.05). Previous studies have found that higher drug concentrations are associated with higher drug release, which is governed by the law of diffusion. As a result, higher drug concentrations cause more intense diffusion and result in higher release rates (Abdullah et al., 2019). In addition, the same polymer content in the formulation with high drug concentration will produce a thinner matrix layer so that a short diffusion layer is formed. Therefore, more drug release occurs and creates more channels that contribute to a faster drug release rate from the microsphere matrix (Abdullah et al., 2019; Deliaz, 2023).

3.11 Drug Release Kinetics

The release kinetics of all ciprofloxacin HCl-alginate-carrageenan microsphere formulations selected was the Korsmeyer-Peppas model because it had the coefficient of determination (r) value closest to 1, namely F1 = 0.9921, F2 = 0.9919, F3 = 0.9925 and F4 = 0.9917 (displayed in Table 4). Ciprofloxacin HCl-alginate-carrageenan microspheres for all formulations obtained exponent values (n <0.43), which indicated that the release mechanism is governed by Fickian diffusion (Bruschi, 2015). In diffusion-controleed systems, polymer chains form pores that allow drug molecules to diffuse and be released into the medium. This can occur due to swelling or inherent semipermeability (Jerome et al., 2020).

3.12 Stability Test

After 28 days of storage at various extreme temperatures of 50oC±2oC, 60 °C±2 °C, and 70 °C±2 °C with relative humidity (RH) of 75±5%, was displayed in Figure 3 for moisture content, while Figure 4 for drug loading. Organoleptic analyses of all ciprofloxacin HCl-alginate-carrageenan microsphere formulations at all temperatures revealed no alterations. They remained yellowish-white in color and did not aggregate. The stability test results for the moisture content and drug loading of ciprofloxacin HCl-alginate-carrageenan microspheres demonstrated that the microspheres were stable. This is in line with the statistical study, which found no discernible variation in the impact of temperature and storage duration on the moisture content of microspheres (p>0.05). Microspheres encapsulate the drug so that its stability is maintained from degradation (Verma et al., 2019).

3.13 Degradation Kinetics in Stability Test

The degradation kinetics of ciprofloxacin HCl-alginate-carrageenan microspheres are presented in Table 5 and Table 6. The degradation kinetic models for F1 and F3 follow first-order while F2 and F4 follow zero-order. The first-order drug degradation reaction rates are related to the reduction in the starting drug concentration over time, while the zero-order drug degradation reaction rates are independent of reactant concentration. The average kinetic temperature, shelf life, activation energy, and rate constant are some of the primary characteristics that are assessed in degradation kinetics (Bhangare et al., 2022). The stability of microspheres is impacted by temperature because rising temperatures can accelerate molecular motion and intensify collisions, which increases the possibility of bond breakdown and reorganization. When the temperature rises, drug degradation results from chemical reactions caused by an increase in molecules with energies equivalent to or greater than the activation energy (González-González et al., 2022). Furthermore, studies have shown that the reaction rate can be quadrupled with the same activation energy by raising the temperature by 10 °C. An increase in the number of major molecule collisions with orientations slightly over the activation energy level is the cause of this rise in reaction rate (Bhangare et al., 2022).

3.14 Shelf-Life of Microspheres

The Stability extrapolation graph of the relationship between 1/T and ln K with the Arrhenius regression equation is displayed in Figure 5, while the degradation kinetics and shelf-life of microspheres at a temperature of 25 °C/RH 75% is displayed in Table 7.

These result indicated that lower activation energy leads to a faster reaction rate, which in turn causes a shorter shelf life of the microspheres (Bhangare et al., 2022). Based on the stability test, it can be determined that the ciprofloxacin HCl-alginate-carrageenan microsphere formulation that has a longer shelf life is the microsphere using 5% lactose compared to 5% maltodextrin as a lyoprotectant. Degradation reactions proceed more slowly when lyoprotectants promote the forma-

Table 7. Shelf-Life of Microsphere

Parameter	Formula			
	F1	F2	F3	F4
Activation Energy (J/mol)	1902.0 J/mol	2513.7 J/mol	1970.2 J/mol	2652.2 J/mol
Degradation Constant (K/Day)	0.00086/Day	0.00051/Day	0.00066/Day	0.00039/Day
Shelf-life (Month)	4 Months, 3 Days	6 Months, 26 Days	5 Months, 10 Days	9 Months, 2 Days

tion of a stable, amorphous glassy state that inhibits structural collapse. Lactose, a humectant sugar as a lyoprotectant, has a higher glass transition temperature (Tg) and enough flexibility to replace hydrogen bonds, thereby improving heat stability and prolonging shelf life (Karunnanithy et al., 2024). These results are in accordance with the DSC test which shows that the Tg of lactose 139.6 °C is higher than the Tg of maltodextrin 126.0 °C. Futhermore, formulations F2 and F4 (with lactose lyoprotectant), exhibited the longest shelf life, and the highest Tg 196.0 °C (F2) and Tg 205.4 °C (F4), compared to F1 and F3 (with maltodextrin lyoprotectant), which had Tg values of 188.0 °C and 191.2 °C.

4. CONCLUSIONS

According to the study's findings, ciprofloxacin-alginate-carrageenan microspheres were effectively applied to generate inhalation utilizing the ionotropic gelation method with the aerosolization technique, with the ratio and concentration of polymer and crosslinker combinations being optimal. Microsphere powder was yellowish-white and had smooth morphology. The characteristics, drug release, and stability of microspheres were also examined in relation to the effect of drug concentration and type of lyoprotectant. The drug concentration had an impact on particle size, drug loading, entrapment efficiency, and drug release, while the type of lyoprotectant had an impact on moisture content and flow properties with good shelf life.

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