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RESEARCH ARTICLE

Optimization Performance and Physical Stability of Ciprofloxacin HCL-Ca Alginate Microspheres: Effect of Different Concentration of Alginate and $CaCl_2$

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ABSTRACT

Inhalation treatment using antibiotics is an alternative for lung delivery. However, the therapeutic efficacy of inhaled drugs is limited by their rapid clearance in the lungs. Sustained release systems in the lungs can improve therapeutic outcomes of drugs because they can retain the drug load within the lungs and progressively release the drug locally at therapeutic levels. This study presents the formulation strategies to control drug release in the lungs using an alginate polymer-based microspheres system. The microsphere's composition can be adjusted to modulate release and can encapsulate compounds with high loading. The pulmonary route is commonly used and has been well accepted as a portal for non-invasive drug delivery for many lung diseases. It is explored for decades as an alternative for systemic as well as local drug delivery. The present study explored the *in vitro* benefits of ciprofloxacin encapsulated in alginate microspheres. The studies included size, morphology, yield, drug loading, and encapsulation efficiency as well as stability.

Current results showed small, smooth, and spherical ciprofloxacin-alginate microspheres were produced using aerosolization techniques. Small particles of less than 5µm were formed, which suitable for inhalation particles for lung delivery. High entrapment efficiency up to 95%, loadings of 80%, and a yield of 89% were also showed from microspheres. It was confirmed that all microspheres were stably indicated by no significant changes in morphology, organoleptic, and drug content after 30 days of storage. The recent promising characteristics of microspheres for pulmonary delivery will need further evaluation of the potency against microorganisms in lung disease.

Keywords: Alginate, Characteristics, Ciprofloxacin HCl, Lung delivery, Microspheres, Stability.

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INTRODUCTION

The increase in lung disease treatment has gained attention in the last decade.[1] Drug delivery for lung treatment in the form of microspheres offers an alternative to delivering high drug concentration directly to the site of action to improve the therapeutic effect and minimize the side effect.[1] Active agent to be used as a model for lung delivery is an antibiotic group with microspheres delivery system as a promising approach for antibiotic inhalation.

Long-term issues include difficulty breathing and coughing up mucus as a result of frequent lung infections.[2] Other signs and symptoms include sinus infections, poor growth, fatty stool, clubbing of the fingers and toes, and infertility in males, among others. Different people may have different degrees of symptoms. Lung infections are treated with antibiotics, which may be given intravenously, inhaled, or by mouth. Usually azithromycin antibiotic was chosen for long term uses. Lung transplantation may be an option if lung function continues to worsen.

About 80% of adults have chronic infections of *Pseudomonas aeruginosa* and 50% caused death within 5 years,[3,4,5] where *Staphylococcus aureus* and *Haemophyllus influenza* are primarily pathogenic in children.[5] In isolation of saliva cultures, some patients can be infected with *Haemophilus influenza*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Escherichia coli*, and *Klebsiella pneumonia*.[6] Treatment of lung disease using antibiotics is to reduce infection and control inflammation to enhance drug efficacy and reduce the dose and side effects; microspheres are alternative to lung delivery system.[7] Chemical properties of forming polymer may provide potential efficacy for the antibiotic drug. The microsphere's composition can be adjusted

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to modulate drug release and can encapsulate compounds with high drug loading.

Alginate-based microspheres provide sustained release properties with several advantages such as minimum usage of toxic organic solvents and reduced reticuloendothelial system uptake due to the stealth nature of alginate. The present study explores the therapeutic benefits of antibiotic encapsulated alginate microspheres when administered by the pulmonary route animal model as well as in vitro physical evaluation. The encapsulation method is by aerosolization technique considered as simple, easy, and produces small and uniform particle size.

The microspheres are spherical monolithic or agent therapeutic distributed in the matrix either as a dispersion of molecular or particle or can be defined as a structure consisting of a continuous phase of one or more soluble polymer-dispersed at the molecular level or macroscopic.[8] Microspheres have a particle size of 1-1,000 nm.[9] In the field of pharmaceuticals, drug delivery systems with technology microspheres used for the preparation of slow-release and controlled, reduce and even eliminate the irritation of the gastrointestinal tract, protect the drug from the ravages and support the spread of drugs distributed in the gastrointestinal tract resulting in absorption of the drug is more reproducible.[10] The smaller the drug particle size, the greater the absorption in the gastrointestinal tract.[11]

Some of the methods used for the preparation of microspheres are emulsion solvent evaporation, continued cross-emulsion, coacervation thermal changes, spray drying, solvent diffusion emulsion, and gelation ionotropic. Ionotropic gelation technique is a method of preparation of microspheres by adding the drug solution into the polymer solution and the solution of the crosslinking agent, and then the process gellification for 24 hours.[10] The advantage of using the ionotropic gelation method in the preparation of the microspheres can maintain drug integrity so that the drug can be encapsulated without the use of organic solvents or elevated temperatures; this causes the drug to remain stable. In addition, the gelation ionotropic method was quite simple, fast, and cost-effective.[12]

The advantages of using Na-alginate are biocompatible, biodegradable, non-toxic, and have been recognized for its safety by the Food and Drug Association since 1982. The higher levels of the polymer used, the density of the polymer matrix will be increased so as to make the release rate decreases.[13] Crosslinking agent that can be used in the gelation method ionotropic is divalent and trivalent, but the divalent ions used more often. Some of the divalent ion is Ba^{2+}, Sr^{2+}, Pb^{2+}, Ca^{2+}, but is commonly used is Ca^{2+} in the form of Calcium Chloride ($CaCl_2$).[14] Divalent cations induce gelation with glucuronic binding. Calcium ions diffuse into the alginate droplet form a three-dimensional structure of ionic crosslinker.[15] There are several factors that affect the manufacture of microspheres by the method of gelation ionotropic include a comparison of the ratio of drug-polymer, the effect of concentration of crosslinker, and polymer on the entrapment efficiency, size and distribution of particles, as well as the release profile of the drug.[15]

Inhaled antibiotic drug delivery systems, either singly or in combination, are widely used for lung infection treatment.[12] Fluoroquinolone such as ciprofloxacin has good activity in gram-negative aerobic bacteria (such as Escherichia coli) and gram-positive (such as Staphylococcus aureus),[16] therefore, this study used that model. The oral and intravenous form of ciprofloxacin HCl has been used clinically to treat respiratory infections, but intravenous or oral administration has a relatively unfavorable pharmacokinetic profile in the lower respiratory tract, including a short half-life of about 3-5 hours. Ciprofloxacin undergoes first past metabolism. Ciprofloxacin HCl has an oral bioavailability of about 70% and is classified into BCS class IV because of low solubility and low permeability.[15] The lung delivery system is one of the alternative deliveries if there are problems with other routes. Bioavailability is high and does not experience first cross metabolism in the liver to deliver the drug. The drug is readily absorbed and enters the systemic circulation because of the thin barrier and high vascularization that envelopes the lungs.[17] The pharmacological benefits of lung administration include low systemic exposure, reduced side effects, appropriate doses delivered to specific targets and no need to add doses.[17] Delivery of antibiotic drugs through the lungs increases the local concentration of the drug in the lung.[18]

Preparations of alginate-based microspheres for lung delivery can be evaluated physical characteristics include shape, particle size, surface appearance, content of drug, the water content of the microspheres, and *in vitro* release from the microspheres. Based on the above, this study was to develop and evaluate a natural polymer-based inhalable drug delivery system using sodium alginate and ciprofloxacin HCl as a model.

MATERIALS AND METHODS

Materials

The materials used in this study is antibiotic aminoglycoside model Ciprofloxacin HCl (pharmaceutical grade); Sodium Alginate pharmaceutical grade (Wako Pure Chemical Industry Ltd.); $CaCl_2.2H_2O$ pharmaceutical grade; Sodium Citrate pharmaceutical grade; Phosphate Buffer Saline (PBS); and Distilled water (BRATACO).

Methods

Preparation of Antibiotic-loaded Alginate Microspheres

Preparation of antibiotic-loaded alginate microspheres made with ionotropic gelation method using aerosolization techniques, with a concentration of antibiotics from 2.0 to 3.5%. The drug was dissolved in a solution of alginate polymer and used a crosslinker $CaCl_2$ at a concentration of 0.5-1.5 M. Each formula is crosslinked for 120 minutes, and stirring is carried out at a speed of 1,000 rpm. Formula antibiotic-alginate microspheres are washed by centrifugation and drying techniques using a freeze dryer at -80°C for 29 hours with the addition of 5% maltodextrin lyoprotectant as a stabilizer. Furthermore, antibiotic-loaded alginate microspheres that form will be evaluated.

The formula which will be used for physical characterization of antibiotic-loaded alginate microspheres were shown in Table 1.

Physical Characterization of Ciprofloxacin HCl-loaded Alginate Microspheres Particle Size Distribution

This study was performed by using an optical microscope of about 300 particles. Average diameter of particle size was determined.

Morphology and Shape Evaluation

To evaluate the shape and surface of the wet microspheres was done using optical microscopy and photos are taken using the camera. Moreover, it can also be observed using Scanning Electron Microscopy (SEM).

Drug Loading

The procedure to measure drug loading was as follows: 10 mg samples of microspheres were prepared. Sodium citrate 5 mL at pH 6.0 was added in the sample microspheres and was stirred for 24 hours at a speed of 1,000 rpm. The resulting clear solution had an absorbance was measured by UV-Vis spectrophotometer at the maximum wavelength of ciprofloxacin HCl.

Encapsulation Efficiency and Yield

Encapsulation efficiency and yield was measured using the below equation:

$$\text{Encapsulation efficiency}\,(\%) = \frac{\text{Measured drug concentration}}{\text{Theoretical weight of the drug}} \times 100$$

$$\text{Yield}\,(\%) = \frac{\text{Measured drug concentration}}{\text{Total weight of drug and polymer}} \times 100$$

Moisture Content Test

Moisture content of the microspheres were analyzed using Moisture content Analyzer after the drying process.

Stability test

The accelerated stability test was carried out on the ciprofloxacin-alginate microspheres. The dried microspheres were stored in a room with a temperature of 25 ± 2°C and in an oven at 40 ± 2°C, RH 75 ± 5% for 28 days at intervals of 0, 7, 14, 21, and 30 days. Organoleptic, particle morphology, drug loading, and encapsulation efficiency were observed to check the stability of dry powder inhalation.

Data Analysis

Data parameter calculation results were analyzed by using the statistical method of one-way ANOVA using SPSS 20 for Windows evaluation version with a degree of confidence of 95% ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Morphology of Microspheres

Morphology examination of all microspheres were shown in Figure 1. SEM examination demonstrated the morphology of the Ciprofloxacin HCl-alginate microspheres surface produced smooth and spherical small particles. By increasing the concentration of alginate and crosslinker, the more spherical and smooth particles were produced.

Particle Size of Microspheres

The average diameter of particle microspheres of all formulas resulted size of less than 3 μm (Table 2). Optical microscopy of wet microspheres showed the particle size of the Ciprofloxacin HCl-alginate microspheres of all formulas were small of less than 3 μm, which was suitable for lung or pulmonary delivery (Table 2). From the results, we can see that increasing concentration of crosslinker $CaCl_2$ from 0.5 to 1.5 M reduced the particle size of ciprofloxacin-loaded alginate microspheres between all formulas. In addition, similar trends of the effect of concentration of alginate polymer on the particle size also occurred when using a low concentration of $CaCl_2$ at 0.5 M. It can be seen that microspheres size decreased by increasing the concentration of alginate polymer from 2 to 3.5% if using 0.5 M $CaCl_2$. However, no significant differences were found in terms of size when using a high concentration of $CaCl_2$ at 1.5 M at addition of alginate polymer. A sufficient amount may explain this between the availability of alginate and crosslinker $CaCl_2$ at both concentrations 0.5 M and 1.5 M produced smaller particles.

Moisture Content (MC) of Microspheres

The result of the moisture content of ciprofloxacin HCl–alginate microspheres were shown in Table 3. For moisture content, all formulas produced dry microspheres with MC content of less than 10%.

Table 1: Formula of ciprofloxacin HCl-loaded alginate microspheres

Material	*F1*	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*	*F8*
Ciprofloxacin HCl	0,1%	0,1%	0,1 %	0,1%	0,1%	0,1%	0,1%	0,1%
Alginate	2,0%	2,0%	2,5%	2,5%	3,0%	3,0%	3,5%	3,5%
$CaCl_2$	1,5M	0,5M	1,5M	0,5M	1,5M	0,5M	1,5M	0,5M
Maltodextrin	5%	5%	5%	5%	5%	5%	5%	5%

Table 2: Average diameter particle of microspheres

Formula	*Average diameter of particle (μm)*
F1	2.93 ± 0.05
F2	2.88 ± 0.02
F3	2.93 ± 0.11
F4	2.86 ± 0.05
F5	2.93 ± 0.10
F6	2.86 ± 0.20
F7	2.93 ± 0.12
F8	2.63 ± 0.04

Table 3: MC of ciprofloxacin HCl-alginate microspheres

Formula	*MC (%)*
F1	5.71
F2	6.30
F3	5.34
F4	7.44
F5	2.66
F6	3.08
F7	2.83
F8	3.71

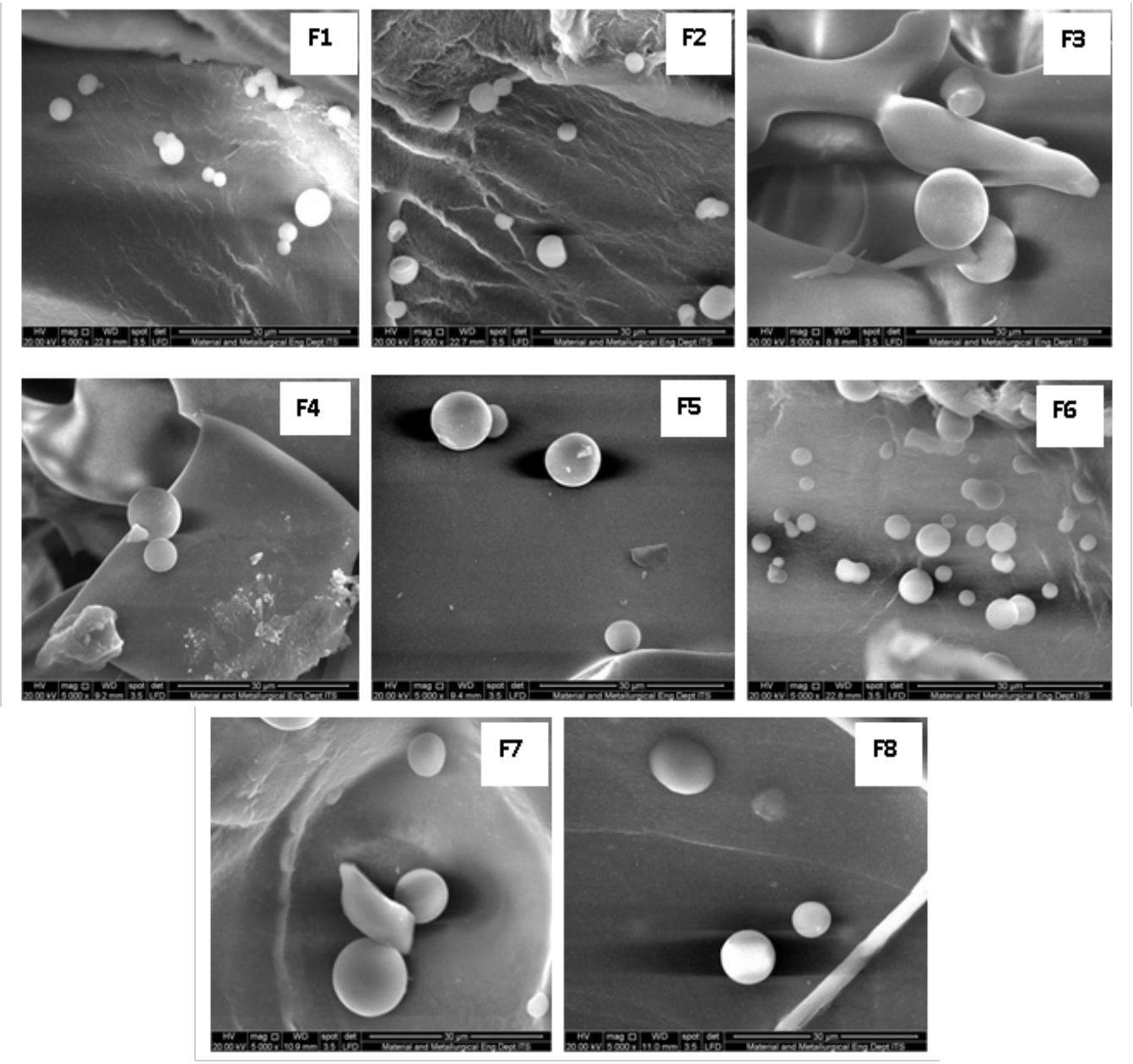

Figure 1: Scanning Electron Microscope (SEM) of freeze-dried microspheres of formulas

Yield, Drug Loading and Encapsulation Efficiency of Ciprofloxacin–Alginate Microspheres

The result of yield, drug loading and encapsulation efficiency of ciprofloxacin HCl-alginate microspheres were shown in Table 4.

For encapsulation efficiency, drug loadings and yield, consistent results of them by demonstrating similar patterns of an increasing percentage of drug loadings, encapsulation efficiency, and yield by increasing concentration of alginate from 2 to 3.5% when using crosslinker concentration of $CaCl_2$ 0.5 M and 1.5 M.

Drug loadings increased significantly from 15 to 79% by increasing alginate concentration. Similar results of encapsulation efficiency were also found at sharp increased from 14% to significantly 95% by increasing alginate of 2 to 3.5%. However, yields of microspheres of all formulas were same high level of above 71% at all concentrations of 2–3.5% alginate.

Furthermore, we can suggest that using a minimum of 2.5% alginate concentration and a low concentration of 0.5 M $CaCl_2$ were highly recommended to produce high yield, high loadings and high encapsulation efficiency of ciprofloxacin HCl-alginate microspheres. Additionally, using alginate polymer concentration of 2.5 to 3.5% and 0.5 M $CaCl_2$ were able to produce high yield, loadings, and efficiency of microspheres of all above 50 to 95%. This could be again explained by a sufficient amount between the availability of alginate (2.5–3.5%) and crosslinker $CaCl_2$ at 0.5 M to crosslink at a significant amount between polymer chains to form optimum ciprofloxacin HCl-alginate microspheres.

Table 4: Physical characterization of ciprofloxacin HCl-alginate microspheres

	Physical characteristics		
Formula	*Yield (%)*	*Drug loading (%)*	*Encapsulation efficiency (%)*
F1	71.35 ± 2.05	15.37 ± 2.55	14.81 ± 2.30
F2	80.88 ± 1.44	26.08 ± 2.05	22.45 ± 0.03
F3	78.96 ± 2.24	22.50 ± 3.03	23.39 ± 0.35
F4	82.95 ± 0.59	49.46 ± 1.22	56.43 ± 1.80
F5	79.59 ± 1.05	24.61 ± 2.10	25.51 ± 0.05
F6	84.01 ± 0.06	72.80 ± 2.08	94.40 ± 0.03
F7	87.26 ± 0.14	25.18 ± 1.20	39.64 ± 1.75
F8	88.68 ± 1.03	79.61 ± 1.05	95.29 ± 2.02

Table 5: Drug loading and encapsulation efficiency at room and accelerated temperature at interval days

		Drug loading (%)		*Encapsulation efficiency (%)*	
Sample	*Day*	*Room (25°C)*	*Oven (40°C)*	*Room (25°C)*	*Oven (40°C)*
F1	0	15.37 ± 2.55		14.81 ± 2.30	
	7	15.37 ± 2.55	15.37 ± 2.48	14.81 ± 2.23	14.81 ± 2.25
	30	15.37 ± 2.50	15.37 ± 2.48	14.81 ± 2.20	14.81± 2.25
F2	0	26.08 ± 2.05		22.45 ± 0.03	
	7	26.08 ± 2.05	26.08 ± 2.00	22.45 ± 0.03	22.45 ± 0.01
	30	26.08 ± 2.00	26.08 ± 2.00	22.45 ± 0.01	22.45 ± 0.01
F3	0	22.50 ± 3.03		23.39 ± 0.35	
	7	22.50 ± 3.03	22.50 ± 3.03	23.39 ± 0.35	23.39 ± 0.35
	30	22.50 ± 3.00	22.50 ± 3.00	23.39 ± 0.29	23.39 ± 0.30
F4	0	49.46 ± 1.22		56.43 ± 1.80	
	7	49.46 ± 1.22	49.46 ± 1.22	56.43 ± 1.80	56.43 ± 1.80
	30	49.46 ± 1.20	49.46 ± 1.20	56.43 ± 1.76	56.43 ± 1.71
F5	0	24.61 ± 2.10		25.51 ± 0.05	
	7	24.61 ± 2.10	24.61 ± 2.10	25.51 ± 0.05	25.51 ± 0.03
	30	24.61 ± 2.10	24.61 ± 2.10	25.51 ± 0.03	25.51 ± 0.03
F6	0	72.80 ± 2.08		94.40 ± 0.03	
	7	72.80 ± 2.06	72.80 ± 2.06	94.40 ± 0.03	94.40 ± 0.03
	30	72.80 ± 2.06	72.80 ± 2.06	94.40 ± 0.03	94.40 ± 0.01
F7	0	25.18 ± 1.20		39.64 ± 1.75	
	7	25.18 ± 1.20	25.18 ± 1.20	39.64 ± 1.74	39.64 ± 1.74
	30	25.18 ± 1.20	25.18 ± 1.20	39.64 ± 1.74	39.64 ± 1.70
F8	0	79.61 ± 1.05		95.29 ± 2.02	
	7	79.61 ± 1.05	79.61 ± 1.05	95.29 ± 2.02	95.29 ± 2.02
	30	79.61 ± 1.04	79.61 ± 1.04	95.29 ± 1.80	95.29 ± 1.89

Stability of Ciprofloxacin HCl-Alginate Microspheres

Result of stability test at 25°C and 40°C after storage in 30 days can be seen in Table 5. After stability study, again, it was confirmed that all formulas were stably indicated by no significant changes in morphology or organoleptic (Table 5 and Figure 2). For organoleptic, there were no physical or color changes of the dried powder microspheres. Moreover, smooth and spherical forms were still observed. In terms of drug loading and encapsulation efficiency compared to 0-day, after 30 days of storage, there was no reduction.

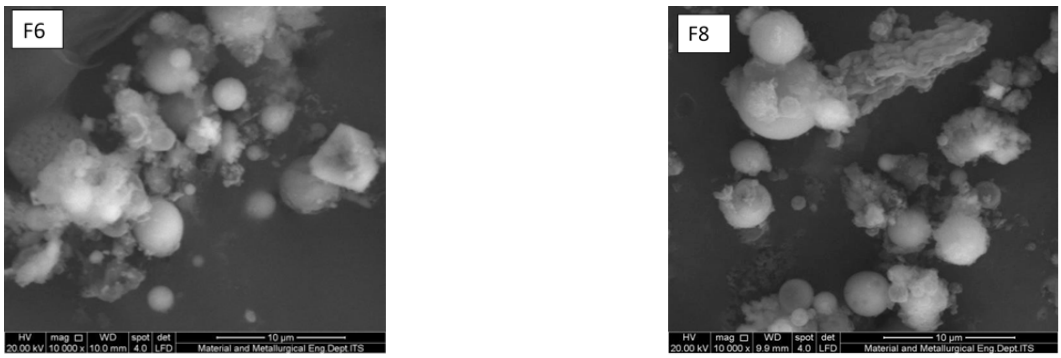

Figure 2: Formula F6 and F8 after 30 days of storage at room temperature

Morphology of Microspheres After 30 Days

Morphology of microspheres of selected best formula based on highest drug loading and efficiency, named F6 and F8, were observed after 30 days of storage, as shown in Figure 2.

These *in vitro* physical characteristics will perhaps lead to produce sustained release for lung delivery as well as optimum potency of ciprofloxacin HCl as antibiotic released from alginate microspheres against microorganism for lung diseases.

CONCLUSION

Ciprofloxacin HCl-loaded alginate microspheres were successfully formed using the ionotropic gelation aerosolization technique. Small, smooth, and regular microspheres were produced from system using 2.5% alginate polymer and 0.5 M $CaCl_2$ with high encapsulation efficiency, loading, and yield with higher stability.

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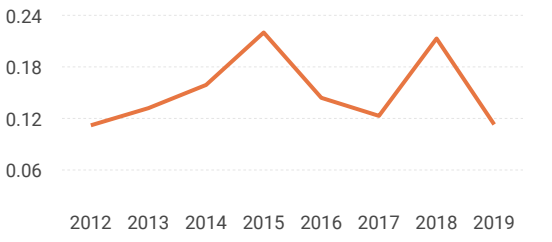

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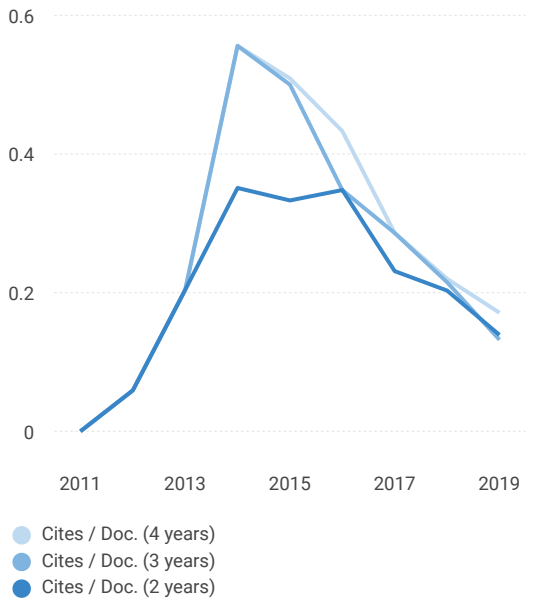

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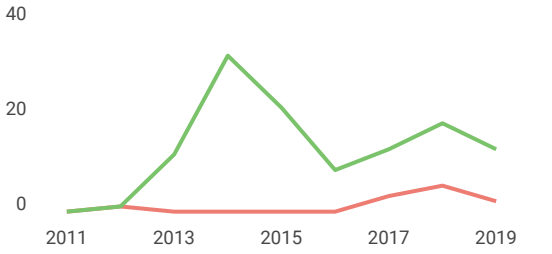

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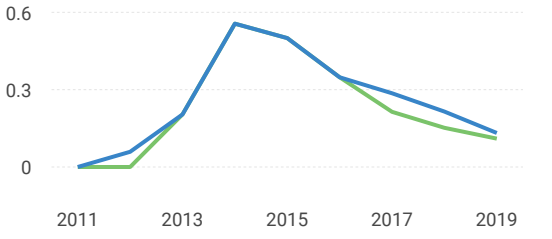

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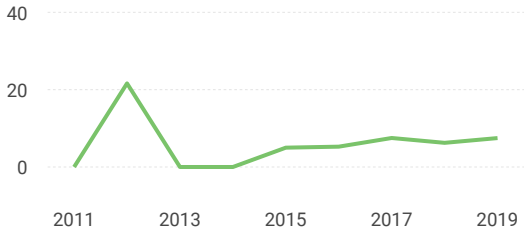

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