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Original Article

Alginate Microspheres Encapsulating Ciprofloxacin HCl: Characteristics, Release and Antibacterial Activity

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ABSTRACT

Drug delivery system through the lungs is an alternative delivery to overcome oral and parenteral delivery problems. The drug does not experience first pass metabolism in the liver, bioavailability, low side effects, and the right dose to be delivered to the target. To efficiently deposit the lungs, particles must have a size of 1-5 µm. Ciprofloxacin HCl is an effective broad spectrum antibiotic which was formulated into microspheres. The purpose was to determine the effects of alginate polymer concentrations and $CaCl_2$ concentration on the characteristics, release, and antibacterial activity. Microspheres were produced by ionotropic gelation technique with aerosolization method. The results showed spherical characteristic microspheres, with particle size of less than 5µm,moisture content of less than 10%, yield was between 70.63% - 82.94%, drug loading and entrapment efficiency was ranged of 2.58% - 4.32% and 27.39% - 80.74%. F3 was the optimum formula. For 24 hours, the ciprofloxacin released was in the range of 80-100% at pH 7.4. Drug release kinetic showed zero order kinetics with mechanism based on non-fickian diffusion, an increased concentration of alginate and $CaCl_2$ showed a decrease in release rate. Both microspheres and released samples, antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was shown. Overall, ciprofloxacin HCl-alginate microspheres produced by ionotropic gelation technique aerosolization method were highly recommended for pulmonary drug delivery.

Keywords: Ciprofloxacin HCl, Microspheres, Alginate, Characteristics, Release, Antibacterial Activity

1. INTRODUCTION

Cystic fibrosisis a disease inherited from genetic factors that causes organ failure as in the lungs with the occurrence of thick mucus with microorganism cleansing dysfunction with the occurrence of bacterial infection and chronic inflammation [1]. Cystic fibrosis attacks the digestion and lungs of young children but becomes a multisystem disease that increases in adulthood. Disease manifests were found in

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many organs, especially in the upper and lower airways, pancreas, intestine, and reproductive tract. For most patients, lung disease is the most important problem and cause of death [2]. About 80% of adults with cystic fibrosis have chronic infections of *Pseudomonas aeruginosa* and 50% cause death in 5 years [3-5] where *Staphylococcus aureus* and *Hemophyllus influenza* as pathogen [6] .*Escherichia coli* bacterium is considered as a long-term persistent colony in the cystic fibrosis patient's respiratory tract and contributes to gastrointestinal dysfunction with nutrient malabsorption and inflammation of the intestine [7-9]. Antibiotics can be used to treat diseases of the lungs by reducing infection and controlling inflammation [6].

Antibiotics that are used inhaled either single or in combination have been widely used for cystic fibrosis treatment [8].Fluroquinolone such as ciprofloxacin has good activity against negative gram aerobic bacteria (such as *Escherichia coli*) and positive gram (such as *Staphylococcus aureus*) [10]. The form of oral and intravenous delivery of ciprofloxacin has been widely used for the treatment of lung infection.

The oral and intravenous form of ciprofloxacin has been used clinically to treat respiratory infection. However, 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 experiences first past metabolism. Ciprofloxacin HCl has an oral bioavailability of around 70%, low solubility, and low permeability [11, 12], therefore an alternative delivery to overcome this problem is highly needed.

Lung delivery route may be solved problems such as its bioavailability and first pass metabolism in the liver because it makes able to deliver the drug properly. The drug will be easily absorbed and enter the systemic circulation because of the thin barrier and high vascularity that cover the lungs [13].Targeting drugs to the lungs can be achieved by microencapsulation method that can achieve targets and control drug release [14, 15].

The dosage form with microspheres is needed for delivery of the lungs so that the drug design is encapsulated with microparticle technology. For efficient lung deposits, particles must have a diameter between 1-5 µm. Microencapsulation is the process of coating a material with a protective layer to produce microspheres measuring 1-1000 microns. Microspheres consist of a biodegradable polymer matrix where the drug can be degraded naturally in the body, general biocompatibility (low immunogenicity and toxicity) and high bioavailability, and sustained release ability for a long time [16]

Ionotropic gelation aerosolization technique is a method of producing microspheres chemically. The advantage of this method are easy, fast, relatively inexpensive to produce microspheres, does not use organic solvents, and use low temperature thereby reducing drug damage. This method is based on the ability of polyelectrolyte to cross over the ion counter to form a gel [17]. Alginate polymer is widely selected in microencapsulation since it is natural, relatively inexpensive, mucoadhesive, biocompatible, not accumulating in organs, and not toxic in its administration [14, 18]. Addition of cation divalent solution such as Ca^{2+} can selectively bind alginate polymers causing crosslinking to form a gel. Calcium chloride is widely used as a crosslinker with alginate because of its non-toxic property. Ca^{2+} is often used to form gel and form the size of holes in the gel and has a significant effect of stability [19]. The effectiveness and selectivity of the release of active ingredients on the target is influenced by the physical characteristics of the microspheres produced from the crosslinking process of the ionotropic gelation method. The characteristics of microspheres can be influenced by the concentration of alginate solution, the concentration of CaCl solution$_2$, and crosslinking times [20].

Microspheres produced with increased alginate and crosslinking concentrations resulted small particle size, entrapment efficiency, and high loading and yield [21-23]. To test the activity of microspheres to bacteria, the inhibitory parameter is measured by means of hole or well diffusion method because in this method a small number of samples are needed, easy and inexpensive, and the data results are easily interpreted. *Staphylococcus aureus* and *Escherichia coli* are widely used in the study of ciprofloxacin antibiotic activity [24].Test for ciprofloxacin HCl activity against bacteria will use nutrient agar media. The ability of inhibition of ciprofloxacin HCl is measured based on the diameter of the inhibitory zone formed [25].

This study will produce ciprofloxacin HCl-alginate microspheres with various concentrations of alginate and $CaCl_2$ which are made using *ionotropic gelation technique* by aerosolization method to determine the effect on the characteristics, release, and the antibacterial activity of the ciprofloxacin HCl microspheres against *Escherichia coli* and *Staphylococcus aureus* using the well method.

2. MATERIALS AND RESEARCH METHODS

Materials

Ciprofloxacin HCl was a donation from Interbat, Indonesia, Sodium Alginate (Sigma Aldrich), $CaCl_2.2H_2O$ (International Solvay Chemistry), Maltodextrin (Bratachem Chemistry), Sodium citrate (Merck), Na_2HPO_4 (Merck), NaH_2PO_4 (Merck), Nutrient Agar (Merck), Staphylococcus aureus 6538 and Escherichia Coli ATCC 8739, and other chemicals and reagents used were pro analysis grade.

Production of Ciprofloxacin-Alginate Microspheres

Microspheres were made with ionotropic gelation techniquey aerosolization method using a nozzle sprayer with the hole size of 35 µm, at different concentrations of sodium alginate namely 1%, 1.5%, and 2%. The solution of ciprofloxacin-alginate (containing 0.2% Ciprofloxacin HCl)

was sprayed into crossing agent solution of $CaCl_2$ (3% and 5%) at 40 psi and stirred continuously for 2 hours at a speed of 1000 rpm. The microsphere was collected by centrifugation at 2500 rpm for 6 minutes, and washed twice with distilled water. The microsphere was resuspended in a 5% maltodextrin solution and then dried with freeze dryer at -35^{o}C for 48 hours [21]. Alginate formulations can be seen in table 1.

Particle size analysis

Determination of microsphere particle size distribution was evaluated using an optical microscope (Axioscop 40-Zeiss). The particle size of 300 particles was measured using image software.

Morphology and structure of the microspheres

The morphology and structure of microspheres were characterized using Scanning Electron Microscopy (SEM) (Fei Inspect S50).

Moisture content

Moisture content was measured using the Mettler Toledo HB43S.

Yield

The yield was done by calculating the weight of the dry microspheres obtained compared to the weight of ciprofloxacin which was added with alginate and maltodextrin.

Drug Loading

100 mg of microspheres were weighed and were added by 50 mL solution of 0.05 M sodium citrate pH 7.54, and continued to be stirred using magnetic stirrer at 1000rpm for 7 hours. Absorption of ciprofloxacin HCl was measured using a UV-Vis spectrophotometer at a wavelength of 270 nm. The ciprofloxacin concentration was determined by adding the sample absorbance price into the equation of the Ciprofloxacin standard curve that had been made with the following calculation:

$$Drug\ Loading = \frac{Measured\ drug\ content}{Total\ weight\ of\ dry\ microsphere} x\ 100\%$$

Entrapment Efficiency

Entrapment efficiency was calculated from the result of ciprofloxacin HCl drug content evaluation with the following calculation:

$$Entrapment\ efficiency = \frac{Measured\ drug\ content}{Theoretical\ drug\ content} x\ 100\%$$

In vitro release study

The release test was carried out on a thermo shaker (Gerhardt) at 37^{o}C and stirring speed of 100 rpm. Microspheres (500mg) was accurately weighed. 100 ml of phosphate buffer with pH 7 and 4was inserted to the sample on a thermo shaker which had reached the temperature of 37 ± 0.5^{0}C and rotated at the speed of 100 rpm. Snippet of samples (5.0ml) was taken at the minutes of 0, 15, 30, 60, 90, 120, 180, 240, 360, 480, 600, 720, 840, 960, 1080, 1200, 1320, and 1440. In each snippet of samples, the same volume of release media was replaced, and snippet of samples was filtered using milipore filter paper 0.45 µm. The sample absorbance was observed with UV-Vis spectrophotometer (Shimadzu UV-1800) at the wavelength of 268.5 nm. The release rate of ciprofloxacin HCl from alginate microspheres was indicated by the value of b (slope) of the line equation y = bx + a from the release profile data of ciprofloxacin HCl from the alginate microsphere.

Release kinetics

To confirm the mechanism and kinetics of drug release, drug release data were analyzed using various kinetic equations, such as zero order, first order, higuchi, and Korsmeyer peppas. To be able to determine the release kinetics of a drug, it could be seen from the price of R^2 from the linear regression equation obtained from each formula. If R^2 approached one, it could be assumed that the kinetic followed the release of the regression equation from the corresponding kinetics model.

Antibacterial activity

Antibacterial activity testing was carried out with well diffusion method on nutrient media to study against *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739. The bacterial inoculum was prepared by means of bacterial culture stock that grew suspended into 0.9% NaCl. The inoculum was then measured for transmittance at the wavelength of 580 nm with a UV-Vis spectrophotometer. Transmission suspension was set to reach 25%.50 grams of microspheres were weighed and was then dissolved into 100 ml phosphate buffer solution pH 7.4 ± 0.05, after which an activity test was carried out with positive control of ciprofloxacin HCl and a negative control in blank microspheres. To make a test solution with a release treatment was by dissolving 500 mg into a buffer phosphate solution pH 7.4 ± 0.05 and in a thermoshaker with a speed of 100 rpm at 37°C, then the solution was sampled at the hours of 0, 0.25, 0.5, 1, 2, 8, 16, and 24, after that dilution was carried out until matched the microsphere formula. The test solution was then sterilized by the filtration method using membrane filter paper with the diameter of 0.22 µm.

Testing the inhibitory against bacteria was done by inserting 15µl of the bacterial inoculum on agar media in a petri dish. The test sample of 50µl was then inserted into the agar medium which had been perforated, then incubated at 37°C for 24 hours. In addition, the diameter of the clear zone formed was observed.

3. RESULTS AND DISCUSSION

Particle size

The result showed that the microspheres resulted diameters between 1.23 ± 0.07 µm and 1.43 ± 0.09 µm (Table 2). In the formula with higher alginate concentration, the size was also bigger. The increase in alginate level was followed by an increase in the diameter of the microspheres due to the increased viscosity of the alginate solution used so that big alginate droplet was formed during the addition of the alginate solution into the crosslinking solution and caused

resulting microspheres bigger.[26] Increased concentration of calcium chloride was followed by an increase in particle size. The amount of calcium chloride increased, the amount of Ca^{2+} that was available to crosslinked between guluronic acid units from sodium alginate increased, causing an increase in viscosity which led to the formation of microspheres with a larger particle size, However, in statistics there was no significant differences ($p>0.05$) both in increasing alginate and calcium chloride concentration regarding to the particle size.

Morphology and structure of microsphere examination

Observation on the morphology and shape of microspheres was carried out with SEM (figure 1). SEM result indicated that the resulting microspheres were spherical and had a smooth surface. These smooth microspheres were probably due to the viscosity of the polymer which was sufficient to form crosslinking strength.

Moisture Content

The examination of moisture content was done to determine moisture content in the microspheres after the drying process. Moisture content was less than 10% which ranged from 2.44 ± 0.48 to 3.00 ± 0.24. In the statistical analysis, there was no significant difference ($p> 0.05$) both in the increase in the concentration of alginate and calcium chloride in the moisture content result.

Yield

The yield of all formulas ranged from (75.62 ± 4.47)% to (79.40 ± 3.19)%. Increased alginate concentration and calcium chloride concentration increased the yield.[20]It was very difficult to determine the yield result related to the drying process. It was found that the drying process needed to be optimized by the formula in this method because the phenomenon of aggregation occurred at the stage of particle hydration [23]. The statistical analysis of the microspheres yield showed no significant difference in the increase in alginate and calcium chloride concentrations ($p> 0.05$).

Drug loading and entrapment efficiency

From the drug loading examination in microspheres, the obtained result ranged from (2.82 ± 0.21)% to (4.13 ± 0.30)%, from the result of the alginate concentration statistic obtained a value of $p <0.05$. This could be interpreted that there was an increase in alginate concentration and an effect of drug loading result, for the concentration of calcium chloride the value of $p <0.05$ was obtained which meant that the concentration of calicum chloride had an influence on drug loading. From the examination of entrapment efficiency in microspheres, the result obtained ranged from (30.05 ± 2.37)% to (74.53 ± 5.48)%, the statistical result at the alginate concentration obtained $p <0.05$, this showed there was a significant difference in the concentration of alginate entrapment efficiency, at the concentration of calcium chloride $p <0.05$ showed that there was a significant difference in the concentration of calcium chloride with entrapment efficiency.

The increase of the alginate concentration would increase the viscosity of the solution so that the size of the droplet became large where the particle size of the large microsphere caused the drug content to increase, resulting in increased entrapment efficiency [21, 26].The increase of the alginate concentration gave a greater number of alginate binding point for Ca^{2+} ion which formed a high viscosity resulting in a solid gel membrane formulation which in the increase of calcium chloride led to a decrease in drug loading followed by a decrease in entrapment efficiency [27]. Decrease in entrapment efficiency because calcium chloride solution binded selectively with polymer alginate forming porous egg box.[20] The high polymer concentration would inhibit the homogeneous distribution of cross linker addition which led to the formation of larger particle with reduced drug content and entrapment efficiency [28].

Release Profiles of Ciprofloxacin HCl from microspheres

The release of ciprofloxacin HCl from microspheres in all formulas in phosphate buffer media pH 7.4 ±0.05 for 24 hours can be seen in table 3 and figure 2.

The cumulative result of released ciprofloxacin was more than 50%, in which at the 720 minutes were in F1 and F4, at the 840minutes were in F2 and F5, and at the 960 minutes were in F3 and F6. Based on the data obtained, he cumulative ciprofloxacin that was released for 24 hours was between 80-100%.

The drug release from alginate depended on pH, the eroded polymer at alkaline pH and the contents were released continuously both by diffusion and slow erosion of the polymer matrix. However, swelling behavior of Ca-alginate which contained drug at a higher pH could be explained by the ionotropic effect that occured between Ca^{2+} ion from alginate and Na^{+} ion which was present in phosphate buffer and as a result, Ca^{2+} was caught by phosphate ion. Ion exchange with phosphate buffer resulted inthe swelling of alginate matrix corrosion and the formation of dissolved Ca-phosphate all had an effect on increasing the drug release rate at a higher pH. This was possible because the lower number of Na^{+} ion was present in the buffer and consequently, ion exchange rates was slower also there was polymer swelling at this pH. This result showed the rate of release of the alginate matrix drug, which was chained by the "erosion-diffusion" process [26].

A significant decrease ($p <0.05$) on the release rate was observed with an increase in alginate concentration. It could be observed that the concentration of the polymer which increased drug release from the polymer decreased. This could be due to difference in the concentration of the polymer which was high in the thickness of the gel layer around the increased microsphere that acted as if a barrier to penetrate into dissolution media. Therefore, it suppressed the diffusion of the drug from the microspheres. This could also be due to an increase in the concentration of the polymer that increased the density of the polymer matrix which resulted

in a large microsphere which also increased the length of the diffusion path where the drug molecule must be passed.

At an increase in the concentration of calcium chloride from 3% to 5%, there was a decrease in the release rate but there was no significant difference including (p> 0.05). The observed decrease in drug release with an increase in calcium chloride might be from tight junction formation between uronic acid residue from alginate and calcium ion.

Release Kinetics

The determination of ciprofloxacin release kinetics was done by entering release data in steady state condition in each release kinetic model. Release data were included in the zero order kinetic model, first order, higuchi, and korsmeyer peppas. The release profile of each formula was then entered into each kinetic model to find the correlation coefficient value that was closest to one (table 4).

The kinetics model selected from the release of the ciprofloxacin HCl microspheres followed the zero-order kinetic model with the value of R^2 of each formula F1 = 0.9919, F2 = 0.9902, F3 = 0.9896, F4 = 0.9907, F5 = 0.9913 and F6 = 0.9907. The release of drug with zero-order kinetics had a constant rate of drug release from time to time without being influenced by the drug concentration in the preparation. This model was important for several drugs such as antibiotic delivery, blood pressure guarding, pain control, and anti-depressant.[29] The release exponent (n) showed that it was at 0.45 <n <0.89 then the drug release was based on non-Fickian diffusion, which described drug release controlled by a combination of diffusion and erosion mechanisms.

Activity of Microspheres

To determine the application of microsphere to treatment, it was necessary to confirm the activity of ciprofloxacin released from the microsphere by testing the effectiveness of the active ingredient against the inhibitory power of bacterial growth carried out on *Staphyloccos aureus* and *Escherichia coli*. The result can be seen in table 5 and 6.

Based on the result of the antibacterial activity test of the ciprofloxacin-alginate formula towards *staphylococcus aureus* indicated the presence of inhibition diameters ranging from 15.05 ± 0.07 mm and 15.30 ± 0.36 mm.

The antibacterial activity of the ciprofloxacin-alginate microsphere compared with standard ciprofloxacin then in the statistical analysis showed no significant difference in antibacterial activity (p> 0.05). Furthermore, the six formulas were analyzed statistically and the alginate and calcium chloride concentrations obtained showed no significant difference in the microsphere antibacterial activity test (p> 0.05).

Based on the result of the antibacterial activity test of the ciprofloxacin-alginate formula towards *Escherichia coli* indicated the presence of inhibition diameters ranging from 15.37 ± 0.38 mm and 15.92 ± 0.28 mm.

The antibacterial activity of ciprofloxacin-alginate microspheres was then statistically analyzed between ciprofloxacin in the formula of microspheres encapsulated with ciprofloxacin HCl without encapsulation. The result showed no significant difference in antibacterial activity (p> 0.05). Furthermore, the statistic dinalysis between six formulas showed no significant difference in the microsphere antibacterial activity test (p> 0.05).

Antibacterial activity of the microspheresfrom release samples

The results showed the release of ciprofloxacin from microspheres provided inhibitory activity against *staphylococcus aureus* bacteria (Table 7). Statistical result showed no significant difference (p> 0.05) between ciprofloxacin released by standard ciprofloxacin (positive control).

The results showed the release of ciprofloxacin from microspheres could provide inhibitory activity against *Escherichia coli* bacteria (Table 8). Statistical result showed no significant difference (p> 0.05) between ciprofloxacin released sample and standard ciprofloxacin HCl (positive control).

The results showed that the activity between ciprofloxacin in the formula of fresh microspheres, ciprofloxacin release samples and standard ciprofloxacin did not have significant differences in the activity. This could mean that ciprofloxacin HCl was not degraded during the release process because it was protected by the microspheres system, thus the activity of ciprofloxacin was still good and highly recommended as drug delivery system to protect ciprofloxacin HCl from exposure at extreme temperatures and pH.

Table 1: Microspheres Formula

Components	F1	F2	F3	F4	F5	F6
Ciprofloxacin HCl	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Sodium Alginate	1%	1.5%	2%	1%	1.5%	2%
$CaCl_2$	3%	3%	3%	5%	5%	5%
Maltodextrin	5%	5%	5%	5%	5%	5%

Table 2: Ciprofloxacin HCl Microspheres Characteristics

Formula	Particle Size (µm)	Moisture Content (%)	Yield (%)	Drug Loading (%)	Entrapment Efficiency (%)
F1	1.23 ± 0.07	2.54 ± 0.34	75.89 ± 2.59	75.89 ± 2.59	35.98 ± 2.22
F2	1.30 ± 0.02	2.67 ± 0.46	77.44 ± 5.27	77.44 ± 5.27	56.79 ± 4.69
F3	1.35 ± 0.05	3.00 ± 0.24	79.40 ± 3.19	79.40 ± 3.19	74.53 ± 5.48
F4	1.32 ± 0.11	2.44 ± 0.48	79.22 ± 3.54	79.22 ± 3.54	30.05 ± 2.37
F5	1.34 ± 0.11	2.64 ± 0.32	75.62 ± 4.47	75.62 ± 4.47	38.14 ± 3.24
F6	1.43 ± 0.09	2.96 ± 0.09	77.80 ± 1.46	77.80 ± 1.46	61.04 ± 4.07

Table 3: The cumulative result of ciprofloxacin HCl that was released for 24 hours

Sampling Time (Minute)	Cumulative mean of released ciprofloxacin HCl (%) ± SD					
	F1	F2	F3	F4	F5	F6
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

15	4.07 ± 0.17	3.07 ± 0.11	3.11 ± 0.19	3.74 ± 0.39	2.96 ± 0.25	2.97 ± 0.12
30	8.41 ± 0.48	6.71 ± 0.05	6.45 ± 0.37	8,18 ± 0.96	6.54 ± 0.41	6.11 ± 0.28
60	13.42 ± 0.67	10.63 ± 0.20	10.07 ± 0.59	13.50 ± 1.06	10.84 ± 0.75	9.68 ± 0.36
120	18.45 ± 0.90	14.63 ± 0.14	13.88 ± 0.87	18,77 ± 1.09	15.37 ± 0.81	13.56 ± 0.56
240	23.84 ± 1.07	19.39 ± 0.35	17.97 ± 1.14	24.45 ± 1.62	20.53 ± 0.43	17.85 ± 0.95
360	30,33 ± 2.22	24.25 ± 0.40	22.63 ± 1.47	30.47 ± 2.22	25.68 ± 0.64	22.56 ± 1.51
480	37.27 ± 3.23	29.89 ± 0.54	27.93 ± 1.54	37.00 ± 2.38	31.18 ± 0.78	27.69 ± 1.77
600	44.80 ± 3.46	35.78 ± 0.73	33.47 ± 1.93	44.01 ± 2.45	36.81 ± 1.01	33.36 ± 2.15
720	52.81 ± 2.12	42,38 ± 1.49	39.13 ± 2.72	51,34 ± 2.36	43.02 ± 1.67	38.78 ± 2.98
840	61.04 ± 2.57	49.40 ± 1.71	45.93 ± 3.21	59.06 ± 2.23	50.28 ± 1.65	45.09 ± 3.71
960	69.68 ± 2.87	57.17 ± 2.15	52.83 ± 3.71	67,56 ± 2.15	57.97 ± 1.67	51.81 ± 4.37
1080	79.47 ± 3.16	65.50 ± 2.54	60.29 ± 4.11	77.07 ± 2.41	66.24 ± 1.89	59.36 ± 4.79
1200	90.06 ± 3.50	74.21 ± 2.72	68.18 ± 4.67	87.27 ± 2.44	75.06 ± 1.85	67.47 ± 5.67
1320	100.00 ± 3.57	83.66 ± 2.91	76.76 ± 5.25	98.06 ± 2.76	83.98 ± 1.77	76.26 ± 6.72
1440	100.00 ± 3.76	93.62 ± 3.06	87.04 ± 5.34	100.00 ± 3.26	93.06 ± 1.55	86.10 ± 7.51

Table 4: Correlation Coefficient Value (R^2) Release Kinetic Model and Release Exponent (n) of Korsmeyer Peppas model

Formula	Zero Order	First Order	Higuchi	Korsmeyer Peppas	
	R^2	R^2	R^2	R^2	n
F1	0.9919	0.8535	0.9303	0.9876	0.6355
F2	0.9902	0.8535	0.9222	0.9808	0.6231
F3	0.9896	0.8600	0.9227	0.9808	0.6101
F4	0.9907	0.8366	0.9333	0.9875	0.6344
F5	0.9913	0.8361	0.9308	0.9827	0.6274
F6	0.9907	0.8436	0.9295	0.9844	0.6243

Table 5: Inhibition zone diameter of the ciprofloxacin-alginate microspheres against *staphylococcus aureus*

Sample	Inhibited Zone Diameter (mm)			Average ± SD
	Replication 1	Replication 2	Replication 3	
Positive Control	15.50	15.80	15.6	15.63 ± 0.15
Negative Control	-	-	-	-
PB Media	-	-	-	-
Formula 1	14.90	15.10	15.30	15.10 ± 0.20
Formula 2	15.20	14.80	15.50	15,17 ± 0.35
Formula 3	15.40	14.90	15.60	15.30 ± 0.36
Formula 4	14.80	15.00	15.10	15.05 ± 0.07
Formula 5	15.20	14.95	15.20	15.12 ± 0.14
Formula 6	15.25	15.30	15.30	15.28 ± 0.03

Table 6: Inhibition zone diameter of the ciprofloxacin-alginate microspheres against *Escherichia coli*

Sample	Inhibited Zone Diameter (mm)			Average ± SD
	Replication 1	Replication 2	Replication 3	
Positive Control	16.20	15.70	15.95	15.95 ± 0.25
Negative Control	-	-	-	-
PB Media	-	-	-	-
Formula 1	15.40	15.20	15.75	15.45 ± 0.28
Formula 2	16.00	15.55	15.55	15.70 ± 0.26
Formula 3	15.60	16.05	16.10	15.92 ± 0.28
Formula 4	15.20	15.80	15.10	15.37 ± 0.38
Formula 5	15.60	15.20	15.90	15,57 ± 0.35
Formula 6	15.40	15.30	16.10	15.60 ± 0.44

Table 7: Inhibition Zone Diameter of microspheres formulas and released samples against *Staphylococcus aureus*

Sample	Average Inhibition Zone Diameter (mm)					
	F1	F2	F3	F4	F5	F6
Positive Control	20.82 ± 0.83	20.53 ± 0.31	20.47 ± 0.25	20.30 ± 0.05	20.33 ± 0.25	20.42 ± 0.26
Negative Control	-	-	-	-	-	-
Phosphate buffer	-	-	-	-	-	-
Fresh Microspheres	20.43 ± 0.35	20.27 ± 0.25	20.53 ± 0.21	20.45 ± 0.31	20.25 ± 0.13	20.48 ± 0.16
Release sample (hour 0)	-	-	-	-	-	-
Release sample (hour 0.25)	20.60 ± 0.2	21.17 ± 0.78	20.30 ± 0.3	20.58 ± 0.33	20.47 ± 0.16	20.32 ± 0.03
Release sample (hour 0.5)	20.83 ± 0.32	20.90 ± 0.61	20.67 ± 0.10	20.18 ± 0.19	20.23 ± 0.18	20,32 ± 0.13
Release sample (hour 1)	20.97 ± 0.31	20.93 ± 0.60	20.40 ± 0.18	20.52 ± 0.20	20.45 ± 0.09	20.52 ± 0.13
Release sample (hour 2)	20.63 ± 0.65	21.27 ± 0.55	20.43 ± 0.26	20.27 ± 0.06	20.42 ± 0.24	20.40 ± 0.22
Release sample (hour 8)	20.95 ± 0.40	21.48 ± 0.56	20.35 ± 0.18	20.52 ± 0.25	20.52 ± 0.10	20.50 ± 0.17
Release sample (hour 16)	20.83 ± 0.32	20.30 ± 0.44	20.38 ± 0.10	20.47 ± 0.15	20.23 ± 0.10	20.35 ± 0.15
Release sample (hour 24)	20.87 ± 0.57	20.52 ± 0.33	20.23 ± 0.15	20.42 ± 0.14	20.43 ± 0.38	20.47 ± 0.32

Table 8: Inhibition Zone Diameter of microspheres formulas and released samples against *Escherichia coli*

Sample	Average Inhibition Zone Diameter (mm)					
	F1	F2	F3	F4	F5	F6
Positive Control	20.65 ± 0.18	20.17 ± 0.21	20.45 ± 0.15	19.67 ± 0.31	18.90 ± 0.13	18.33 ± 0.15
Negative Control	-	-	-	-	-	-
Phosphate buffer	-	-	-	-	-	-
Fresh Microspheres	20.43 ± 0.32	20.30 ± 0.30	20.40 ± 0.13	19.65 ± 0.18	18.85 ± 0.26	18.35 ± 0.40
Release sample (hour 0)	-	-	-	-	-	-
Release sample (hour 0.25)	20.87 ± 0.33	20.17 ± 0.29	20.38 ± 0.08	19.97 ± 0.32	19.65 ± 1.00	20.10 ± 1.68
Release sample (hour 0.5)	20.95 ± 0.23	20.18 ± 0.10	20.63 ± 0.10	19.90 ± 0.13	19.80 ± 0.36	20.33 ± 1.94
Release sample (hour 1)	20.63 ± 0.25	20.32 ± 0.13	20.57 ± 0.13	19.85 ± 0.18	19.37 ± 1.00	20.07 ± 1.74
Release sample (hour 2)	20.88 ± 0.18	20.65 ± 0.44	20.60 ± 0.15	20.05 ± 0.23	18.95 ± 1.10	20.23 ± 1.55
Release sample (hour 8)	20.77 ± 0.21	20.48 ± 0.39	20.52 ± 0.10	20.30 ± 0.30	19.03 ± 0.56	20.08 ± 1.67
Release sample (hour 16)	20.58 ± 0.25	20.75 ± 0.15	20.60 ± 0.13	20.15 ± 0.57	18.80 ± 0.56	19.97 ± 1.61
Release sample (hour 24)	20.32 ± 0.16	20.65 ± 0.22	20.50 ± 0.13	19.95 ± 0.13	18.23 ± 0.25	19.75 ± 1.33

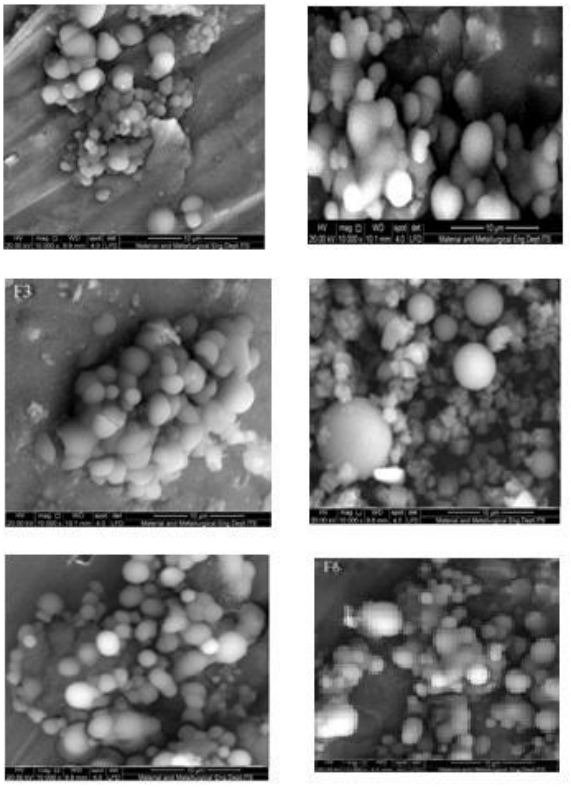

Fig 1: Morphological examination of ciprofloxacin HCl-Alginate microspheres formulas using SEM (magnification of 10000x)

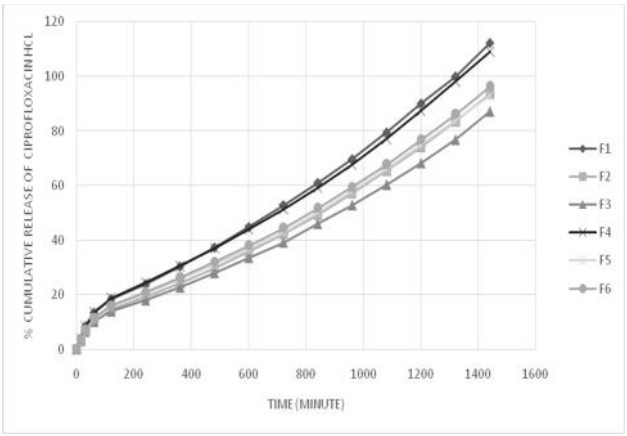

Fig 2: Release profile of ciprofloxacin HCl from microspheres

4. CONCLUSION

Microspheres has been produced using the gelation ionotropic method aerosolization technique from alginate polymer (1%, 1.5%, and 2%) with $CaCl_2$ cross linker (3% and 5%). The physical characteristics indicated spherical microspheres, with particle size of less than 5µm. Moisture content and yields showed no significant differences in the increase in alginate and calcium chloride concentrations. For drug loading and entrapment efficiency, it showed an increase in the yield of the increase in alginate and a decrease by increase in calcium chloride. Formula F3 was the optimum formula. Drug release kinetics showed zero order kinetics, with mechanism of non-fickian diffusion, increased concentration of alginate and $CaCl_2$ showed a reduced release rate. Activity against *Staphylococcus aureus* and *Escherichia coli* bacteria was shown. Overall, Ciprfloxacin HCl-alginate microspheres produced by aerosolization method can be prepared for pulmonary delivery and recommended for in vivo study.

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