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Original Article

FORMULATION AND EVALUATION OF CIPROFLOXACIN MICROSPHERES DESIGNED BY USING NATURAL POLYMERS BY IONIC GELATION TECHNIQUE

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ABSTRACT

Objective: A definitive objective for supporting drug discharge is to expand the remedial movement of the medication while limiting its incidental effects. Microspheres have become a unique medicine delivery mechanism for several disorders in this area. The popular fluoroquinolone antibiotic, Ciprofloxacin, is used to treat a variety of bacterial illnesses. This research aims to create Ciprofloxacin microspheres with sustained drug delivery using natural gum polymers.

Methods: To choose and assess the ideal formulation, a variety of formulations (F1–F8) were developed. This work was completed using an innovative technology, the Ionic Gelation method. Central Composite Design (CCD) used the quadratic forward regression approach to carry out the optimization. The evaluation tests include Particle size, Scanning Electron Microscopy (SEM), FTIR, Percentage yield, Drug content, Drug Entrapment effectiveness and *in vitro* dissolution studies.

Results: It was discovered that the best formulation was F4. From optimization, the ANOVA was found to be significant. The uneven, spherical structure of microspheres with a rough outer surface is confirmed by SEM investigation. The absence of drug-polymer interaction is confirmed by the FTIR. The formulation F4 was deemed ideal due to its high drug entrapment efficiency, drug content and maximal drug release (89.25% in 12 h).

Conclusion: Due to the least plasma half-life, this drug is designed as microspheres thus maximizing the therapeutic activity and minimizing the negative effects. In this regard, microspheres have emerged as novel drug-delivery systems for various diseases. It maintains effective dose concentration, eliminates night-time dosage and decreases side effects, thus optimizing drug therapy.

Keywords: Ciprofloxacin, Ionic gelation, Microspheres, Optimization, Central Composite Design

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INTRODUCTION

A well-known antibiotic from the fluoroquinolone family is Ciprofloxacin (CPFX). It is a wide-spectrum antibiotic that works well against both many Gram-negative and Gram-positive bacteria. It mostly treats bacterial infections brought on by *Plasmodium aeruginosa*. It prevents Topoisomerase II (DNA gyrase) and IV from separating bacterial DNA, preventing cell division. The enzymes are suppressed, which leads to the fragmentation of bacterial DNA. According to the BCS classification, CPFX is in class IV due to its poor permeability and minimal solubility. Because of its short half-life (3.5–4 h) frequent dosage is necessary. As a result, it is used as the study's model drug [1]. One of the techniques selected is the use of microspheres as medication carriers for prolonged release. Microspheres are defined as homogeneous, monolithic particles in the size range of about 1-1000 μm and are widely used as drug carriers for sustained drug delivery. This formulation is used for the protection of core material, reduction of gastric irritation, decrease in volatility, conversion of liquid to pseudo-solid, microencapsulation and for designing pulsatile drug delivery systems. Administration of drugs in the form of microspheres improves the treatment by providing localized action and prolonging the drug release. Solvent evaporation, emulsion, phase separation, ionic gelation, ultrasonication and other preparation techniques are available [2, 3].

The objective goal of the current study is to use natural polymers and ionic gelation technology to extend medication release and decrease the dosing frequency. This work aims to deliver the drug to treat Urinary Tract Infection (UTI). By formulating it as microspheres, there is an extension of drug release thus making it useful to treat the infection beneficially with limited dosing frequency.

MATERIALS AND METHODS

Materials

Ciprofloxacin (CPFX) was obtained from Medopharm Pvt. Ltd. as a gift sample (Chennai). Xanthan gum, Guar gum and Sodium alginate was bought from Nice Chemicals Pvt. Ltd (Kerala). Calcium chloride was purchased from Merck Specialties Pvt. Ltd (Mumbai).

Methods

Preparation of microspheres

Separately dissolved in hot water, the necessary quantity of polymers was then allowed to cool and combine. To that, 100 mg of the medication was dissolved while being constantly stirred, resulting in a homogenous dispersion. It was incorporated dropwise into a 50 ml solution of 5% w/v calcium chloride (CaCl2) using a #20gauge hypodermic needle equipped with a 10 ml syringe. Upon coming into contact with the cross-linking agent, this was permitted to operate in a matrix. After the $CaCl_2$ had expired, it was decanted and the resulting microspheres were cleaned and dried [4, 5]. Similar to this, several formulations (F1–F8) were created and the desired formulation F4 among them, was assessed. Table 1 below lists the medication and polymer compositions utilized in 8 formulations.

Table 1: Composition of various batches of CPFX microspheres

Materials	F1(mg)	F2(mg)	F3(mg)	F4(mg)	F5(mg)	F6(mg)	F7(mg)	F8(mg)
Ciprofloxacin	100	100	100	100	100	100	100	100
Sodium Alginate	500	500	500	1500	1500	2500	2500	2500
Xanthan gum	250	1000	1000	500	500	250	250	1000
Guar gum	1000	250	1000	500	1000	250	1000	250
$CaCl_2$	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015

Evaluation parameters of microspheres

Particle size analysis

The molecule size was estimated utilizing optical microscopy. The dry microspheres were suspended in light liquid paraffin and the smear was made on a glass slide. The typical size was resolved to utilize the joined micrometer [6]. Thus, the mean molecule size of the formulations was found.

Percent yield

The percentage yield of microspheres was calculated using the formula [7]

$$\% \text{ yield} = \frac{\text{Total wt. of microspheres}}{\text{Total wt. of substance}} \times 100$$

Drug content

The microspheres were suspended with 7.4 phosphate buffer and kept aside for 24 h. Afterwards, the absorbance was estimated and the medication content was determined by [8]

$$\% \text{ content} = \frac{\text{Drug content}}{\text{Label claim}} \times 100$$

Drug entrapment efficiency

100 mg equivalent microspheres and filtrate were dissolved in 7.4 phosphate buffer and filtered. The absorbance was analysed after suitable dilutions [9]

$$\% \text{ Entrapment} = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

***In vitro* dissolution study**

It was carried out in USP II (paddle type) apparatus under sink conditions. Initially, the buffer was 0.1N HCl later replaced by 7.4 phosphate buffer to depict the stomach and intestinal conditions. 1 ml sample solution was taken at predefined intervals for up to 720 min. The withdrawn volume was replaced with fresh media in equal volume. The collected samples were diluted to sufficient concentration with the same dissolution media and absorbance was measured at 277 nm [10]. The percentage drug release was found using the formula

$$\% \text{ drug release} = \frac{\text{Amount of drug release}}{\text{Label claim}} \times 100$$

Characterization of optimised CPFX microspheres

FTIR analysis

The FT-IR of the pure drug and optimised formulation was carried by Shimadzu, Japan at the range of 4000-400 cm^{-1}. The drug-excipient interaction study was carried out using IR i.e., by the KBr pellet method [11].

Scanning electron microscopy (SEM)

SEM analysis was carried out to view the surface morphological characteristics of the microspheres. In this, the sample is sprinkled on the adhesive stub and coated with gold [12]. It is carried out by Carl ZEISS EVO-18, Germany. The microsphere was viewed at an accelerating voltage of 10-20 kV.

Optimization

It was done utilizing Design-Expert software (version 8.0.7.1). CCD was an as often as possible involved strategy as it takes care of multifaceted issues with ideal exploratory runs. In CCD, the examinations were completed in a randomized way. The desirability approach was utilized to choose the optimized formulation. ANOVA investigates the information statistically and acquires the interaction between response and process variables [13]. Given the fundamental paths, a 2^3 full factorial plan was utilized. Response Surface methodology assesses the impact of different independent variables (i.e., Sodium alginate [A], Xanthan gum [B], Guar gum [C]) on dependent variables like disintegration, drug content etc. [14].

RESULTS AND DISCUSSION

Calibration curve of CPFX

100 mg of Ciprofloxacin was dissolved in 100 ml of 0.1N HCl and a stock solution was obtained at 1000 µg/ml concentration. Serial dilutions were carried out and the final concentration ranged from 2-10 µg/ml. Absorbance was measured at 277 nm [15]. The calibration curve produces linearity and the regression value (r^2) was found to be 0.9996. A plot of concentration vs. absorbance is shown in fig. 1.

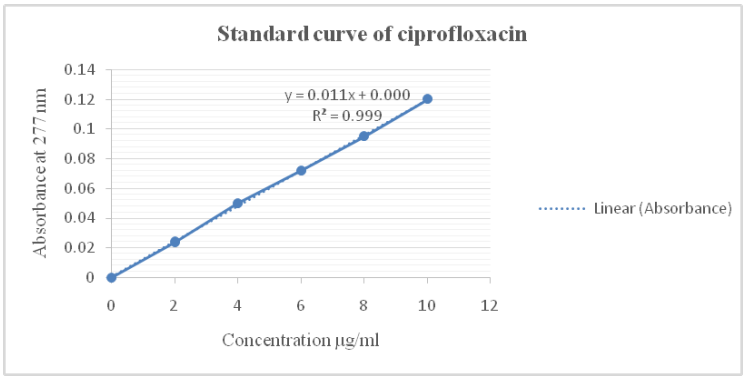


Fig. 1: Standard calibration curve of ciprofloxacin

Particle size analysis

The mean molecule size of the formulations ranges from 636.36±5.38 to 918.20±2.35 µm and is given in table 2. The particle size of the optimized formulation F4 was found as 636.36±5.38 µm.

Percentage yield

The percentage yield of optimized formulation F4 was found to be 96.15% and the percent yield of all the prepared microspheres is displayed in table 2.

Drug content

The percentage drug content values of prepared microspheres are given in table 2. The optimized formulation F4 has a drug content of 56%, indicating that it is the best formulation method for the preparation of microspheres with high content uniformity.

Drug entrapment efficiency

Depending on the drug-to-polymer ratio, the entrapment efficiency of the drug varied to a large extent, as evident from table 2. The drug entrapment efficiency of the optimized batch F4 was denoted as 44%.

Table 2: Characteristic evaluation of prepared batches of microspheres (F1-F8)

Formulations	Particle size analysis (µm)	Percentage yield (%)	Drug content (%)	Drug entrapment efficiency (%)
F1	697.43±1.20	95.90±1.20	53.00±0.72	40.50±1.19
F2	711.51±1.90	95.50±1.72	51.90±0.23	38.60±2.36
F3	833.14±0.98	95.12±1.26	49.68±1.38	35.50±0.43
F4	**636.36±0.53**	**96.15±0.84**	**56.00±1.40**	**44.00±1.13**
F5	863.53±0.86	93.94±0.89	45.90±1.23	34.90±2.78
F6	885.21±1.38	94.00±1.09	44.00±2.40	32.40±1.23
F7	914.18±2.53	88.60±1.03	42.50±1.36	30.96±2.66
F8	918.20±2.35	89.80±2.23	41.80±0.58	30.00±1.32

Each value is expressed as mean±SD (n=3)

Desirability, ANOVA and 3D surface methodology

Before initiating an optimization procedure, it is important to analyze the curvature term by factorial designs. ANOVA generated 2^k factorial design showed that curvature was significant for all responses (Drug content and *In vitro* drug release) since the p-value was less than 0.05. This implied that the quadratic method should be considered to model the formulation process**.** The insignificant term (p>0.05) was eliminated by a backward elimination process and thus, a simple and realistic model was obtained. For all the reduced models p<0.05 was obtained, implying these models were significant.

The desirability of the total design was found. The two responses in the study should be evaluated in the optimization of Ciprofloxacin microspheres. However, it is almost impossible to optimize all the objectives simultaneously because they do not coincide with each other and conflict may occur between them. The optimum condition reached in one response may have an opposite influence on another response. To find the best compromising formulation of both responses, the multicriteria problem can be treated as a single criterion problem using the desirability function approach. The desirability and 3D surface curve of the total design are shown in fig. 2.

Prediction = 0.975

Here, the red zone indicates the highly desired formulation, followed by yellow, green and blue showing the least desirability.

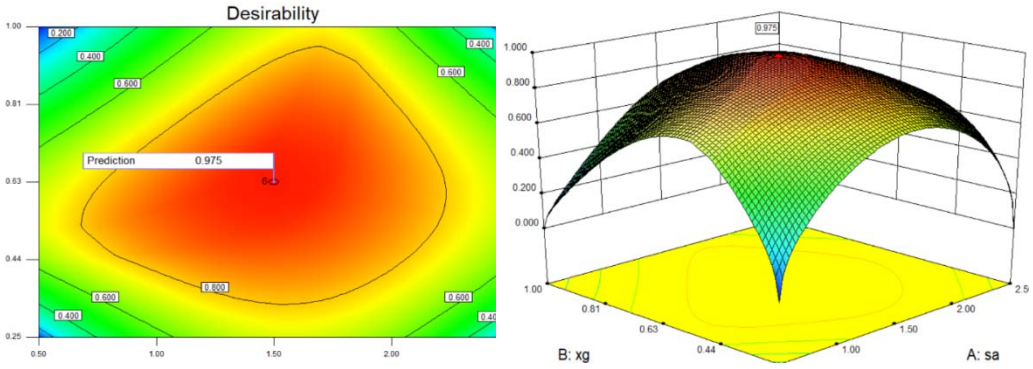
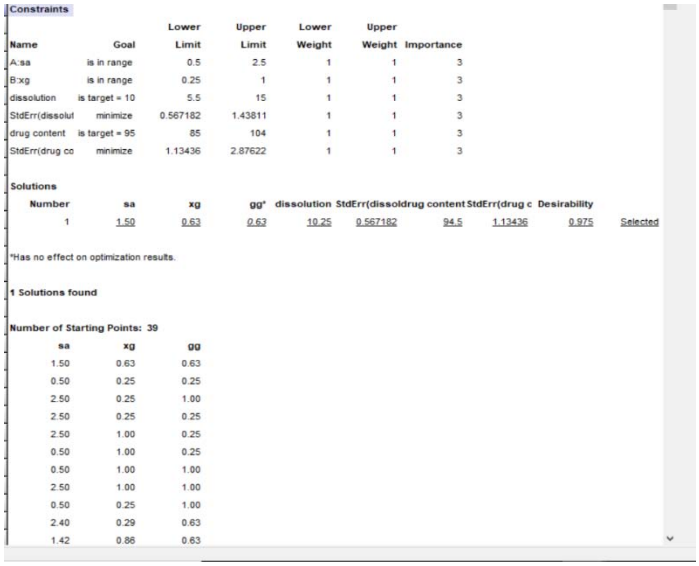


Fig. 2: Desirability and 3D response curve of the total design considering all the prepared formulations



Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:sa	is in range	0.5	2.5	1	1	3
B:xg	is in range	0.25	1	1	1	3
dissolution	is target = 10	5.5	15	1	1	3
StdErr(dissolut	minimize	0.567182	1.43811	1	1	3
drug content	is target = 95	85	104	1	1	3
StdErr(drug co	minimize	1.13436	2.87622	1	1	3

Solutions

Number	sa	xg	gg*	dissolution	StdErr(dissol	drug content	StdErr(drug c	Desirability	
1	1.50	0.63	0.63	10.25	0.567182	94.5	1.13436	0.975	Selected

*Has no effect on optimization results.

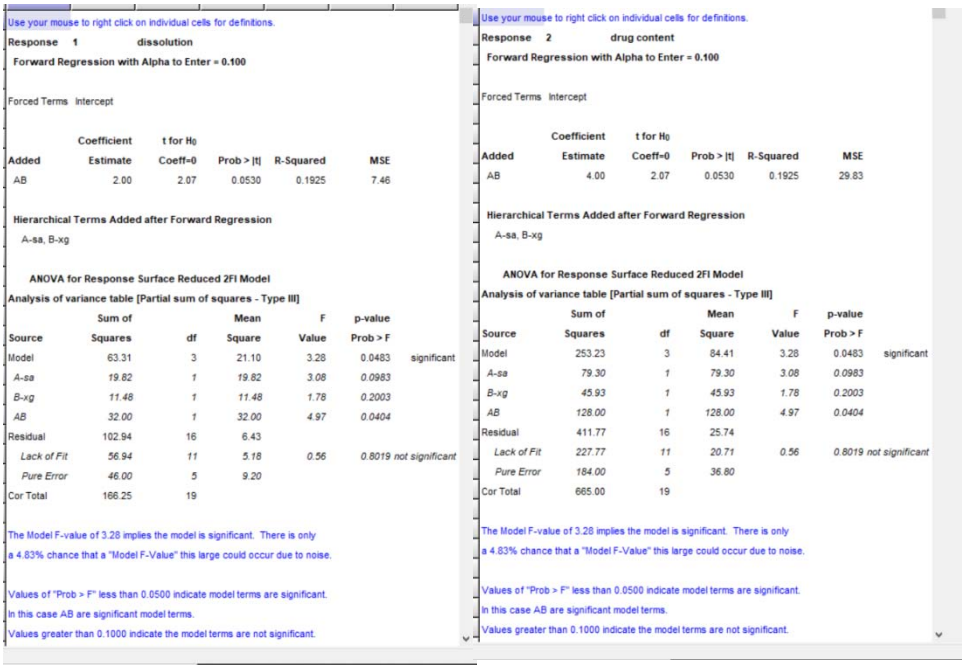
1 Solutions found

Number of Starting Points: 39

sa	xg	gg
1.50	0.63	0.63
0.50	0.25	0.25
2.50	0.25	1.00
2.50	0.25	0.25
2.50	1.00	0.25
0.50	1.00	0.25
0.50	1.00	1.00
2.50	1.00	1.00
0.50	0.25	1.00
2.40	0.29	0.63
1.42	0.86	0.63

Fig. 3: Selection of the desired formulation from the prepared 8 formulation

Thus, the optimized formulation was found to be F4 using this Central Composite Design (CCD) approach. From this design, the concentration of the polymers to formulate 8 different formulations were also selected.



Use your mouse to right click on individual cells for definitions.

Response 1 dissolution

Forward Regression with Alpha to Enter = 0.100

Forced Terms Intercept

Added	Coefficient Estimate	t for H0 Coeff=0	Prob > |t|	R-Squared	MSE
AB	2.00	2.07	0.0530	0.1925	7.46

Hierarchical Terms Added after Forward Regression

A-sa, B-xg

ANOVA for Response Surface Reduced 2FI Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	63.31	3	21.10	3.28	0.0483	significant
A-sa	*19.82*	*1*	*19.82*	*3.08*	*0.0983*	
B-xg	*11.48*	*1*	*11.48*	*1.78*	*0.2003*	
AB	*32.00*	*1*	*32.00*	*4.97*	*0.0404*	
Residual	102.94	16	6.43			
Lack of Fit	*56.94*	*11*	*5.18*	*0.56*	*0.8019*	*not significant*
Pure Error	*46.00*	*5*	*9.20*			
Cor Total	166.25	19				

The Model F-value of 3.28 implies the model is significant. There is only a 4.83% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case AB are significant model terms.

Values greater than 0.1000 indicate the model terms are not significant.

Use your mouse to right click on individual cells for definitions.

Response 2 drug content

Forward Regression with Alpha to Enter = 0.100

Forced Terms Intercept

Added	Coefficient Estimate	t for H0 Coeff=0	Prob > |t|	R-Squared	MSE
AB	4.00	2.07	0.0530	0.1925	29.83

Hierarchical Terms Added after Forward Regression

A-sa, B-xg

ANOVA for Response Surface Reduced 2FI Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	253.23	3	84.41	3.28	0.0483	significant
A-sa	*79.30*	*1*	*79.30*	*3.08*	*0.0983*	
B-xg	*45.93*	*1*	*45.93*	*1.78*	*0.2003*	
AB	*128.00*	*1*	*128.00*	*4.97*	*0.0404*	
Residual	411.77	16	25.74			
Lack of Fit	*227.77*	*11*	*20.71*	*0.56*	*0.8019*	*not significant*
Pure Error	*184.00*	*5*	*36.80*			
Cor Total	665.00	19				

The Model F-value of 3.28 implies the model is significant. There is only a 4.83% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case AB are significant model terms.

Values greater than 0.1000 indicate the model terms are not significant.

Fig. 4: ANOVA of 2 responses (Dissolution and Drug content)

The ANOVA helps to obtain the interaction between responses and process variables. From fig. 4, the *p-value* was found to be less than 0.05 (significant) and *lack of fit* was found to be not significant.

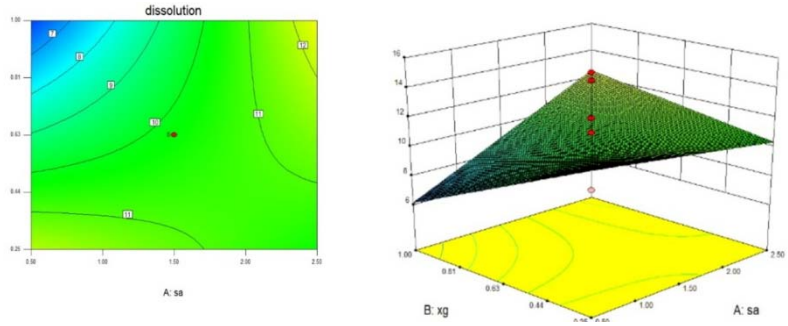


Fig. 5: Desirability and 3D surface plot by taking dissolution as the response

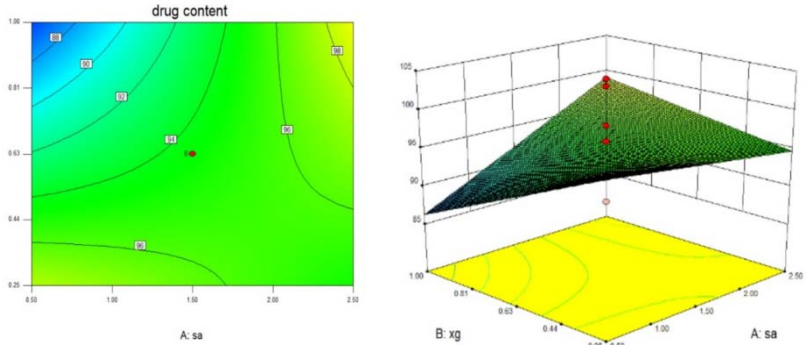


Fig. 6: Desirability and 3D surface plot by taking drug content as the response

FTIR analysis

The FT-IR was carried by Shimadzu, Japan at the range of 4000-400 cm^{-1}. The drug-excipient interaction study was carried out using IR i.e., by the KBr pellet method. In FTIR spectra of CPFX, one prominent characteristic peak was found between 3500-3350 cm^{-1}, which has been assigned to the stretching vibration of the OH group and intramolecular hydrogen bonding. Another band at 3000-2850 represents the alkene and aromatic C-H stretching and is assigned to the cyclopropyl group. The band at 1750-1700 cm^{-1} represented the carbonyl C=O stretching. The peak at 1650-1600 cm^{-1} was depicted as quinolones. The band at 1300-1250 cm^{-1} of O-H bending proved the presence of carboxylic acid. The band at 3150-3050 cm^{-1} showed the presence of aromatic compounds. A strong absorption peak between 1050-1000 cm^{-1} was assigned to C-F stretching. The FTIR of the drug and optimized formulation of the microsphere was shown in fig. 7.

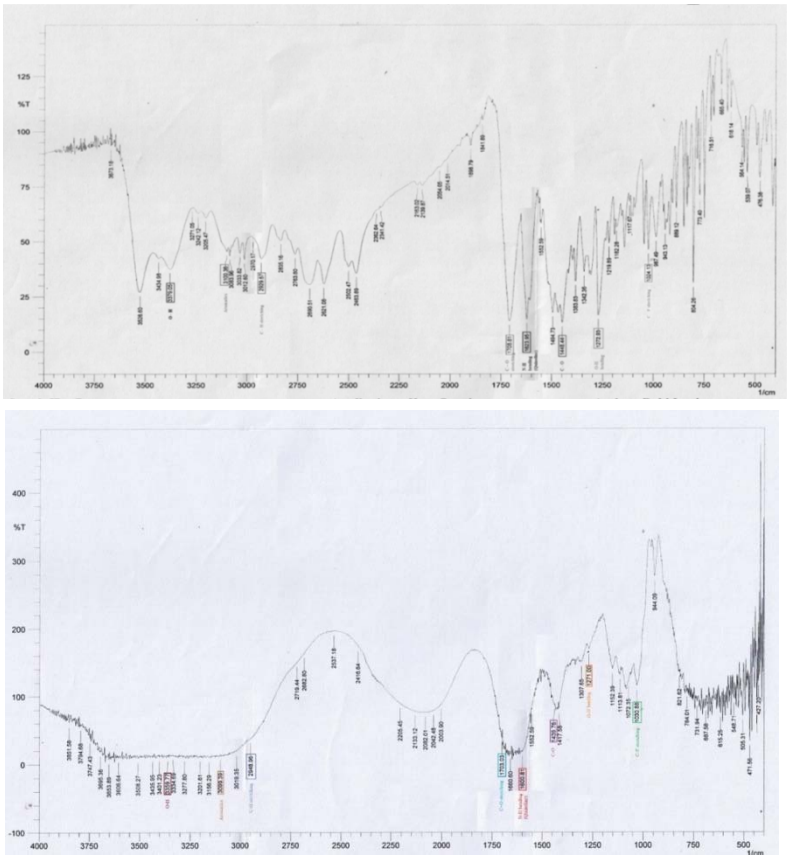


Fig. 7: FTIR of ciprofloxacin drug and optimized microsphere formulation F4

Scanning electron microscopy (SEM)

SEM analysis was carried out to view the surface morphological characteristics of the microspheres. In this, the sample is sprinkled on the adhesive stub and coated with gold. It is carried out by Carl ZEISS EVO-18, Germany. The microsphere was viewed at an accelerating voltage of 10-20 kV. The SEM image of the optimized formulation at various magnifications is shown in fig. 8

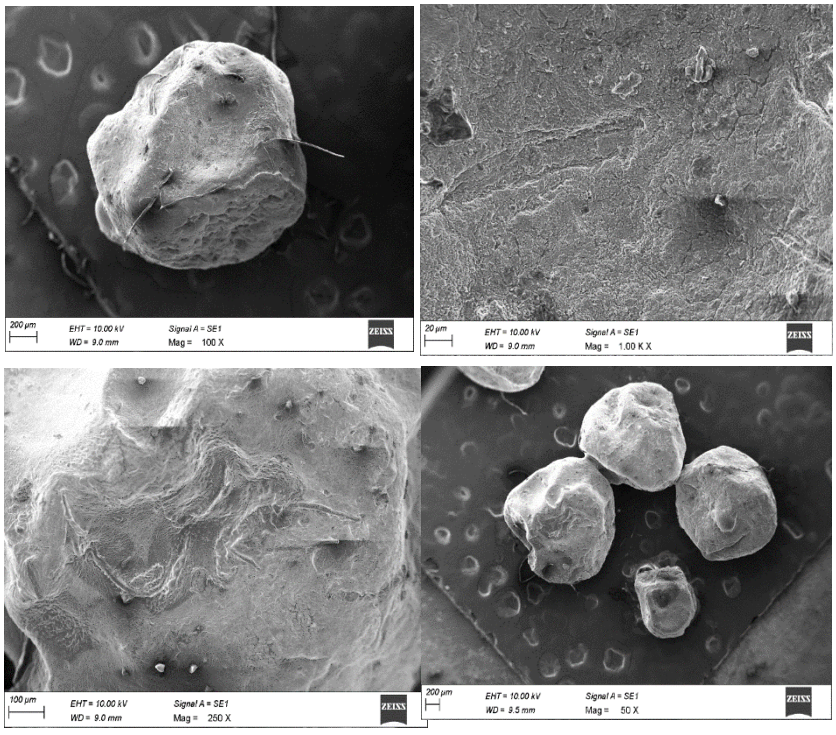


Fig. 8: Surface characteristics of F4 microsphere at various size ranges and magnification

The microspheres of selected optimized batch F4 were found to be discrete, irregular and non-uniform in size. No lumps were found with the distribution of particles.

In vitro dissolution studies

It was carried out in USP II (paddle type) apparatus under sink conditions. Initially, the buffer was 0.1N HCl later replaced by 7.4 phosphate buffer to depict the stomach and intestinal conditions. The percentage drug release was found and the optimized formulation showed a prolonged sustained release effect for nearly 8 h. The percentage drug release of the optimized batch F4 was found to be 89.25% at the end of 12 h. The graph plotted between time and percentage of drug release was depicted in fig. 9.

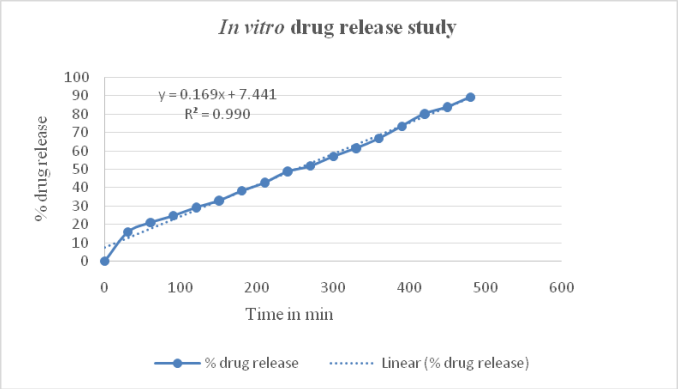


Fig. 9: *In vitro* drug release study of F4

CONCLUSION

Due to the least plasma half-life, this drug is designed as microspheres thus maximizing the therapeutic activity and minimizing the negative effects. In this regard, microspheres have emerged as novel drug-delivery systems for various diseases. It maintains effective dose concentration eliminates night-time dosage and decreases side effects thus optimizing drug therapy. F1–F8 formulation of Ciprofloxacin microspheres was prepared by using various polymer ratios. From that, F4 was found to be the optimized formulation as it showed high entrapment efficiency, drug content and maximum drug release (89.25% in 12 h). The method used to prepare the microsphere in this study is relatively simple and safe because of the absence of specialized equipment and organic solvents. So, Sustained-release microspheres of Ciprofloxacin may provide a convenient dosage form for achieving the best performance and release and show good bioavailability.

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Nil

AUTHORS CONTRIBUTIONS

Nikila confirms sole responsibility for data collection, formulation and analysis of results and manuscript preparation. Dr. S. Allimalarkodi carried out the conclusions and manuscript revisions and guided throughout the work.

CONFLICT OF INTERESTS

Declared none

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