ejpmr, 2020,7(5), 371-380

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

FORMULATION DEVELOPMENT OF SUSTAINED RELEASE MUCOADHESIVE MICROSPHERES OF ANTIBIOTIC

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Article Received on 23/02/2020 **Article Revised on 15/03/2020** **Article Accepted on 05/04/2020**

ABSTRACT

The present investigation concerns the development of a new oral drug delivery system utilizing the concepts of controlled release and mucoadhesion, which would remain in stomach and extend the drug release for longer period of time. Levofloxacin is used to treat a variety of bacterial infections. It belongs to a class of drugs known as quinolone antibiotics. It works by stopping the growth of bacteria. Levofloxacin mucoadhesive microspheres were prepared by solvent evaporation technique using carbopol 934P and hydroxypropyl methyl cellulose K4M (HPMC K4M). The prepared microspheres were subjected to evaluation of particle size, entrapment efficiency, drug content, *in vitro* wash off test and *in vitro* drug release studies. Absence of drug-polymers interaction was confirmed by fourier transform infrared spectrophotometry. The particle sizes of batches ranged between 304.5 µm to 456.6 µm. The drug entrapment of formulations was about 70 to 88.3%. The prepared microspheres showed a strong mucoadhesive property i.e., 79.3% to 91.1%. The polymer concentration influenced the *in vitro* drug release significantly in 0.1N HCl which suggested sustained drug release from formulations and was found to be in the range of 78.9 to 87.4%. The prepared factorial batches have shown a nearly spherical shape with rough surface. F7 factorial batch was selected as optimized batch because it has shown maximum mucoadhesion and sustained the release of drug from formulation up to 8 hours. Regression analysis revealed that the drug release from the optimized batch followed zero order kinetics. From the above results, it was concluded that the mucoadhesive microspheres of levofloxacin has feasibility for eradicating *Helicobacter pylori* from the stomach more effectively because of the prolonged gastrointestinal residence time and controlled release of drug from the formulation.

KEYWORDS: Levofloxacin, carbopol 934P, hydroxypropyl methyl cellulose K4M, *Helicobacter pylori*, mucoadhesion.

INTRODUCTION

Helicobacter pylori is a spiral-shaped bacterium that is found in the gastric mucous layer or adherent to the epithelial lining of the stomach. It causes more than 90% of duodenal ulcers and up to 80% of gastric ulcers. Humans are the sole host for *H. Pylori*, that is found in stomach, duodenum, esophagus and rectum on areas of metaplastic gastric epithelial tissue. In prevalence of *H. Pylori* infection varies from 31-84% in asymptomatic persons. *H. Pylori* infection is chronic and once acquired remains life long, unless eradicated by antibiotics given for some other conditions. Human feaco-oral route and vital risk factors are socio-economic status and age. Overcrowding, poor socio-economic status and poor hygiene are related with high infection rate. Reinfection rate after eradication is quite high in developing countries due to above mentioned risk factor.[1,2]

The objective of *H. pylori* treatment is the complete elimination of the organism from the GI of the patients and once this has been achieved then the rate of reinfection is low. Development of a successful treatment for *H. pylori* infection has been fraught with difficulty. The survival capabilities of the *H. pylori* organism over a wide pH spectrum within the stomach make the task of eradication difficult. It was rapidly recognized that the therapy with a single antibiotic led to a poor cure rate and various antimicrobial mixtures were tried resulting in several effective combinations of antibiotics, bismuth and antisecretory drugs. It is vital that the infection can be treated optimally with clinically relevant *H. pylori* eradication regimens that has an acceptably high eradication rate and without major side effects and with minimal induction of bacterial resistance, in order to achieve the desirable eradication rate, the antibiotics are combined with proton pump inhibitors or ranitidine bismuth citrate. So, called triple therapies, combinations of one anti secretory agent with two antimicrobial agents for 7 to 14 days, have been extensively evaluated and several regimens have been approved by FDA. The most widely used antimicrobials in these regimens are Amoxicillin, Clarithromycin, Metronidazole, Tetracycline and Bismuth. Resistance of *H. pylori* to the limited range of antibiotics that have

efficacy in its treatment can severely affect attempts to eradicate the bacteria. Resistance to tetracycline or amoxicillin is extremely rare.[3]

Mucoadhesion is a practical method of drug immobilization or localization and an important new aspect of controlled drug delivery. The motivation for controlled drug release is the necessity to maintain a constant effective drug concentration in the body for an extended time period. For optimal performance, drug concentration in the body should be maintained above the effective level and below the toxic level. A mucoadhesive controlled release device can improve the effectiveness of a treatment by helping to maintain the drug concentration between the effective and toxic levels, inhibiting the dilution in the body fluids, and allowing targeting and localization of a drug at a specific site.[4]

Microspheres constitute an important part of these particulate drug delivery systems by virtue of their small size and efficient carrier capacity. Microspheres are the carrier linked drug delivery system in which particle size ranges from 1-1000 µm in diameter having a core of drug and entirely outer layers of polymers as coating material.[5]

Levofloxacin is a broad spectrum antibiotic of the fluoroquinolone drug class and the levo isomer of its predecessor ofloxacin. Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal and abdominal infections.[6]

MATERIALS AND METHODS

Levofloxacin and HPMC K4M were obtained as a gift sample from Mylan Laboratories, Hyderabad. Carbopol 934P, Light liquid paraffin, Span 80, Ethanol, Dichloromethane and Concentrated Hydrochloric acid was purchased from Sd fine chem, Mumbai.

Preparation of mucoadhesive microspheres

The factorial batches were formulated by design expert software using 2^3 factorial design. The design consists of selection of 3 independent variables at 2 levels (low and high). The selected variables are:

- Carbopol 934P concentration
- HPMC K4M concentration
- Stirring speed

Weighed amounts of Carbopol 934P and HPMC K4M were taken and added to the mixture of ethanol and dichloromethane (1:1 ratio) and mixed well. This solution was kept under sonication for 20 mins. Levofloxacin was added to the solution and mixed well. The solution was then extruded through the syringe in the beaker containing 100 ml of light liquid paraffin and 1% Span 80. Stirring was done by mechanical stirrer at 1000 rpm for 60 minutes. Finally, the formed microspheres were separated by filtration, washed with petroleum ether and dried at 60°C for 24 hours.

Table 1: Composition of mucoadhesive microspheres by using 2^3 factorial design.

Formulation variables	Batch code							
	F1	F2	F3	F4	F5	F6	F7	F8
Carbopol 934P (mg)	150	200	150	200	150	200	**150**	200
HPMC K4M (mg)	100	100	150	150	100	100	**150**	150
Span 80 (%)	1	1	1	1	1	1	**1**	1
Light liquid paraffin (ml)	100	100	100	100	100	100	**100**	100
Ethanol (ml)	10	10	10	10	10	10	**10**	10
Dichloromethane (ml)	10	10	10	10	10	10	**10**	10
Levofloxacin (mg)	200	200	200	200	200	200	**200**	200
Stirring speed (rpm)	1000	1000	1000	1000	1500	1500	**1500**	1500

Evaluation of mucoadhesive microspheres

Particle size analysis

It was performed with the help of an optical microscope. The eye piece micrometer was calibrated with the help of a stage micrometer. 100 particles were counted for each batch and the particle diameters of microspheres were measured randomly.[7]

The average particle size:

$$D_{mean} = \frac{\sum nd}{\sum n}$$

Where, n = Number of microspheres checked,
d = Mean size range

Surface morphology

The surface morphology was visualized by Scanning Electron Microscopy (SEM). The samples for SEM were prepared by lightly sparkling the microsphere powder on a double adhesive tape, which stuck to an aluminum stab. The stabs were then coated with gold of thickness of about 300Å using a sputter coater. The samples were then randomly scanned and photographed.

Percentage yield

Dried microspheres were weighed and the percentage yield of microspheres was calculated by using formula:[7]

Percentage yield = [Practical Yield / Theoretical Yield] x 100

Drug content and entrapment efficiency

50mg of microspheres was crushed in a glass mortar and suspended in 10 ml of 0.1 N Hydrochloric acid (pH = 1.2). After 24h, the solution was filtered and analyzed. The drug content and entrapment efficiency were calculated using the following formulas:[8]

$$\text{Drug content in microspheres} = \frac{\text{weight of drug in microspheres}}{\text{weight of microspheres}} \times 100$$

$$\%\text{Entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

***In vitro* wash off test for mucoadhesion**

2×2 cm specimen of goat stomach mucosa was mounted onto glass slides using thread. 50mg of microspheres were spread onto each wet rinse tissue specimen. Immediately thereafter the support was hung onto the arm of USP Disintegration test machine. By operating the disintegrating test machine, the tissue specimen was given a regular up and down movement in 0.1N Hydrochloric acid at 37˚C. At the end of one hour the machine was stopped and the microspheres in the 0.1N Hydrochloric acid were centrifuged, dried and weighed. The percent mucoadhesion was calculated by the following formula:[9]

$$\textit{Percent Mucoadhesion} = \left(\frac{Wo - Wt}{Wo}\right) \times 100$$

Where, Wo = weight of microspheres applied
Wt = weight of microspheres washed out

***In vitro* drug release studies**

The drug release study was performed using USP paddle apparatus at 37°C±0.5°C and 100 rpm using 900 ml of 0.1N Hydrochloric acid as a dissolution medium. Microspheres equivalent to 200 mg of levofloxacin was filled in "0" size hard gelatin capsules and placed in dissolution medium. 5 ml of sample solution was withdrawn at predetermined time intervals of 1 hour up to 8 hours and sink conditions were maintained by adding the same amount of 0.1N HCl. The absorbance of the sample was recorded using UV spectrophotometry at 293.6nm. The same procedure was conducted for pure drug levofloxacin (200 mg).[9]

Drug release kinetics

Drug release from the dosage forms follow the different kinetic rules. The release of drug from different dosage forms depends on the various factors like concentration, temperature, etc. There are several mathematical models for release kinetics which includes:[10]

- Zero-order model
- First order model
- Higuchi Model
- Hixson-Crowell model
- Korsmeyer–Peppas model

Statistical analysis of data by design expert software

A 2^3 full factorial design was selected and the 3 factors were evaluated at 2 levels, respectively. The Carbopol 934P concentration (X_1), HPMC K4M concentration (X_2) and stirring speed (X_3) were selected as independent variables and the dependent variables were percent mucoadhesion and drug release.

Table 2: Design summary of formulations.

Factor	Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded
A	Carbopol 934P	mg	Numerical	150	200	-1	+1
B	HPMC K4M	mg	Numerical	100	150	-1	+1
C	Stirring speed	rpm	Numerical	1000	1500	-1	+1

Table 3: Response summary for the factorial batches.

Response	Name	Units	Observations	Analysis	Min.	Max.
R1	Mucoadhesion	%	8	Factorial	77.5	91.1
R2	Drug Release	%	8	Factorial	78.9	87.4

The data obtained were treated using Stat-Ease Design-Expert 11.1.1 software and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to 3D response surface methodology to study the effect of Carbopol 934P concentration (X_1), HPMC K4M concentration (X_2) and Stirring speed (X_3) on dependent variables.

Stability studies

The optimized batch F7 was subjected to accelerated stability studies for 3 months in stability chamber at 40 ± 2°C, 75 ± 5% RH under ICH guidelines Q1A (R2). At the end of specified day's period, sample was withdrawn and analyzed for the drug content, mucoadhesion and drug release.[11]

RESULTS AND DISCUSSION

Particle size analysis

The size and size distribution of microspheres were determined by optical microscopy. The average particle size of factorial batches was tabulated in the table 4.

Table 4: Average particle size of factorial batches.

Formulation	Average particle size (µm) (AM*± S.D.)
F1	321.7±0.305
F2	407.6±0.425
F3	384.5±0.378
F4	456.6±0.384
F5	304.5±0.488
F6	363.8±0.490
F7	**348.6±0.405**
F8	438.4±0.444

**Each value is average of three determinations*

Surface morphology

The surface morphology was visualized by scanning electron microscopy (SEM). The SEM photograph of mucoadhesive microspheres of optimized batch F7 was shown in figures 2. The microspheres observed were nearly spherical with rough surface.

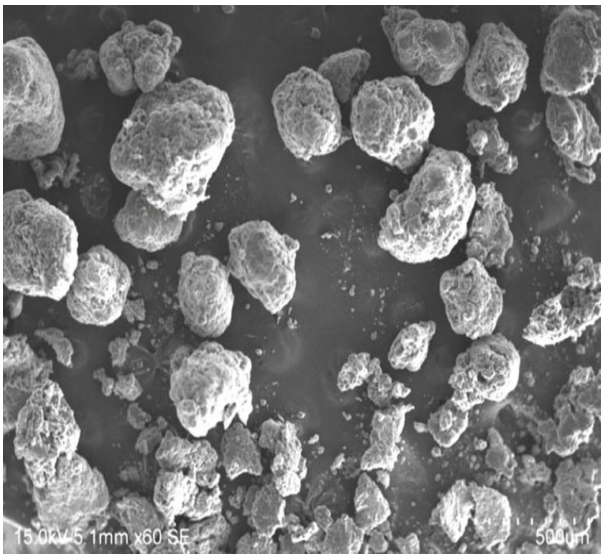

Fig. 1: SEM analysis photograph of an optimized batch (F7).

Percentage yield

The percentage yield of various formulations of levofloxacin factorial batches was found to be in the range of 91.3 to 95.6%. The results are reported in the table 5.

Table 5: Percentage yield of factorial batches.

Formulation code	Percentage yield (%)
F1	94.7
F2	92.3
F3	94.9
F4	91.3
F5	95.6
F6	92.8
F7	**93.8**
F8	94.6

Drug entrapment efficiency

The drug entrapment efficiency for Levofloxacin factorial batches was found to be higher with more concentration of Carbopol 934P. The results were in the range of 70 to 88.3% and are reported in the table 6.

Table 6: Drug entrapment efficiency of factorial batches.

Formulation code	% Drug entrapment efficiency (AM*± S.D)
F1	70.0±0.321
F2	83.9±0.450
F3	70.2±0.896
F4	84.7±0.351
F5	76.8±0.556
F6	86.0±0.611
F7	**78.2±0.850**
F8	88.3±0.416

**Each value is average of three determinations*

Drug content

The drug content of the various factorial batches was found to be in the range of 30.27 to 37.72 mg/100mg of microspheres and reported in the table 7.

Table 7: Drug content of factorial batches.

Formulation code	Drug content (mg/100mg)
F1	30.28
F2	32.24
F3	32.46
F4	36.50
F5	30.56
F6	36.06
F7	**35.90**
F8	37.72

***In-vitro* wash off test**

The percentage mucoadhesivity of levofloxacin factorial batches after 1 hour was found to be in the range of 79.36 to 91.14% and given in the table 8.

Table 8: Percent mucoadhesivity of factorial batches.

Formulation code	Percentage mucoadhesivity (%)
F1	79.36
F2	77.5
F3	82.98
F4	82.08
F5	80.94
F6	80.58
F7	**91.14**
F8	84.86

The percentage mucoadhesion for factorial batches was found to be higher with more concentrations of selected polymers. Increase in stirring speed further enhanced the

mucoadhesion of microspheres. F7 and F8 showed maximum mucoadhesion of 91.1 and 84.8% respectively.

***In-vitro* drug release**

The *in vitro* percentage drug release profile of levofloxacin mucoadhesive microspheres and pure drug (levofloxacin) in 0.1N Hydrochloric acid was tabulated in the tables 9, 10 and represented in the figures 2 and 3.

The *in vitro* drug release for factorial batches F1-F8 for 8 hours suggested sustained drug release from formulations and was found to be in the range of 78.9 to 87.4% on complete of 8 hours study. Increase in HPMC K4M concentration in batches F3, F4, F7 and F8 sustained the release of drug from formulations. Higher concentration of Carbopol 934P in batches F2, F4, F6 and F8 further extend the release of drug to some extent. Smaller microspheres obtained with increase in stirring speed for batches F5-F8, resulted in increased surface area and thus the drug release.

Table 9: *In vitro* drug release profile of factorial batches and pure drug (levofloxacin) in 0.1N HCl.

Time (hrs)	Percent drug release (AM*± S.D) (%)				
	Pure drug	F1	F2	F3	F4
1	70.8±0.74	13.1±0.96	16.0±0.24	17.0±1.34	13.9±1.21
2	89.2±0.73	28.9±0.74	27.4±0.91	25.6±1.07	26.8±1.47
3	99.9±0.001	40.2±0.91	47.03±0.73	43.1±0.43	36.2±0.98
4	-	52.7±0.96	58.0±0.48	58.9±0.73	44.5±1.18
5	-	63.9±1.69	68.3±1.21	62.3±1.18	56.5±1.06
6	-	71.6±1.04	71.5±1.44	69.1±1.11	65.3±0.84
7	-	79.5±0.83	77.9±1.19	74.0±0.66	71.5±1.20
8	-	84.4±0.97	82.9±0.68	79.8±1.23	78.9±0.73

**Each value is average of three determinations*

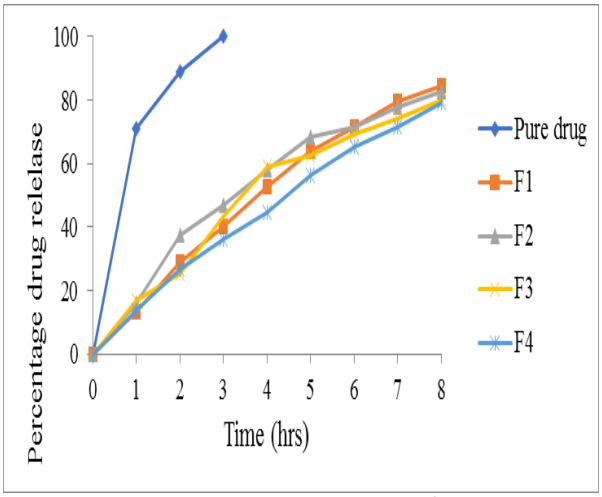

Fig. 2: *In vitro* percent drug release from pure drug (levofloxacin) and factorial batches F1, F2, F3 and F4 in 0.1N HCl.

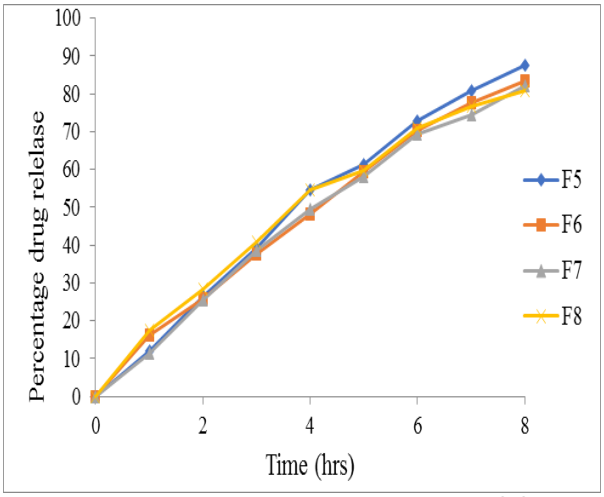

Fig. 3: *In vitro* percent drug release of factorial batches in 0.1N HCl for F5, F6, F7 and F8.

Table 10: *In vitro* drug release profile of factorial batches in 0.1N HCl.

Time (hrs)	Percent drug release (AM*± S.D) (%)			
	F5	F6	F7	F8
1	12.0±0.29	16.3±1.34	**11.4±0.85**	17.3±0.71
2	26.3±1.26	25.6±1.07	**25.3±1.30**	28.4±1.26
3	39.0±0.99	37.5±0.43	**38.6±0.49**	40.6±1.29
4	54.4±0.95	48.3±0.73	**49.5±1.32**	54.5±0.47
5	61.2±1.10	59.2±1.18	**58.0±0.61**	59.6±0.92
6	72.5±0.64	70.4±1.11	**69.3±0.98**	70.8±1.21
7	80.8±1.00	77.7±0.66	**74.2±0.50**	76.9±0.72
8	87.4±0.80	83.4±1.23	**82.1±0.98**	80.8±0.78

**Each value is average of three determinations*

Drug release kinetics

The drug release kinetics of the optimized batch F7 was determined in 0.1N hydrochloric acid solution and the results are shown in the table 11. The release kinetic data indicated that optimized batch F7 showed zero order release kinetics (high R^2 value for zero order) suggesting sustained release of drug. In Korsmeyer-Peppas model the n-value was 1.5352 which corresponds to non-fickian mechanism. Formulation followed higuchi model for drug release suggesting drug release is diffusion control.

Table 11: Release kinetics data of optimized batch F7.

Formulation			Kinetic models (R^2)				
			Zero order	First order	Higuchi	Hixon crowell	Peppas
F7	**0.9853**	0.9845	**0.9501**	0.7525		0.7495	

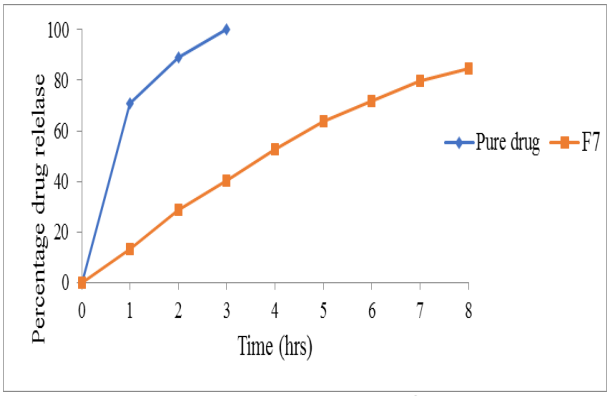

Fig. 4: *In vitro* percent drug release from optimized batch F7 and pure drug (levofloxacin).

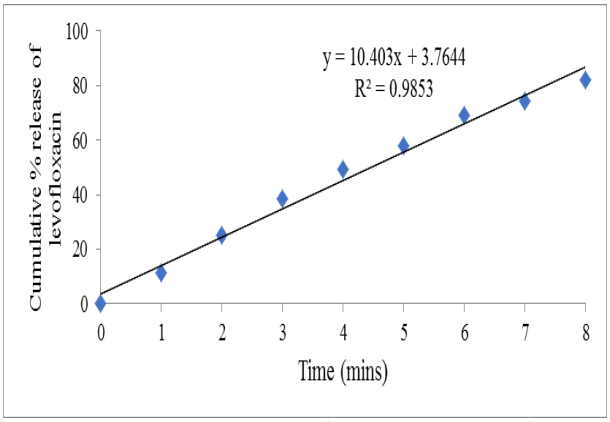

Fig. 5: Zero order kinetics of *in vitro* release of levofloxacin from the optimized batch F7.

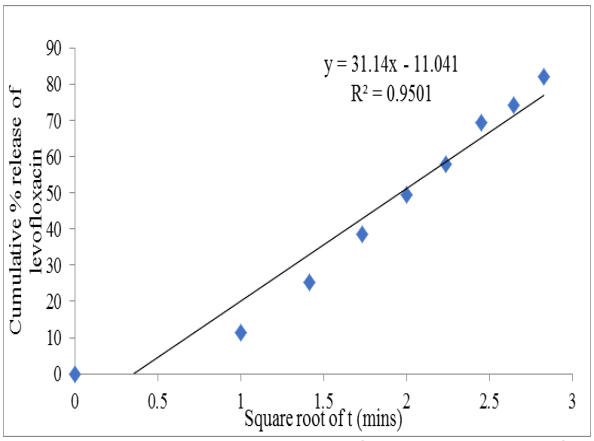

Fig. 6: Higuchi model kinetics of *in vitro* release of levofloxacin from the optimized batch F7.

Statistical Analysis by Design Expert Software

The 2^3 full factorial design was selected to study the effect of independent variables Carbopol 934P concentration (X_1), HPMC K4M concentration (X_2) and Stirring speed (X_3) on dependent variables mucoadhesion and drug release. A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

The responses of the formulations prepared by 2^3 factorial design batches are shown in tables 8, 9 and 10. The data clearly indicates that the mucoadhesion and percent drug release values are strongly dependent on the selected independent variables. The fitted regression equations relating the responses, mucoadhesion and % drug release are shown in the equations, respectively. The equation conveyed the basis to study the effects of variables. The regression coefficient values are the estimates of the model fitting. The polynomial equations can also be used to draw conclusions considering the magnitude of co-efficient and the mathematical sign it carries; i.e. positive or negative.

Final equation in terms of coded factors for mucoadhesion

Mucoadhesion = +82.38 –1.18*A + 2.82*B + 1.95*C

Final equations in terms of actual factors for mucoadhesion

Mucoadhesion = +66.72500 – 0.047000*Carbopol 934P + 0.113000*HPMC K4M + 0.007300*Stirring speed.

Final equation in terms of coded factors for drug release

Drug release = +82.46 – 0.9625*A – 2.06*B + 0.9625*C

Final equations in terms of actual factors for drug release

Drug release = +94.70000 – 0.038500*Carbopol 934P – 0.082500*HPMC K4M + 0.003850*Stirring speed.

The negative coefficient of variables indicates increase in variable level decreases the particular response and decrease in variable increases the response. On the other hand, the positive coefficient of variables indicates increase in variable level increases the response and decrease in level decreases the response.

The model obtained from the regression analysis used to build a 3-D graphs, in which the responses were represented by curvature surface as a function of independent variables. The relationship between the response and independent variables can be directly visualized from the response surface plots. The response surface plots were generated using Design Expert software to observe the effects of independent variables on the response studied such as mucoadhesion and drug release. Graphical presentation of the data helped to show the relationship between the response and the independent variables. The information given by graph was similar to that of mathematical equations obtained from statistical analysis.

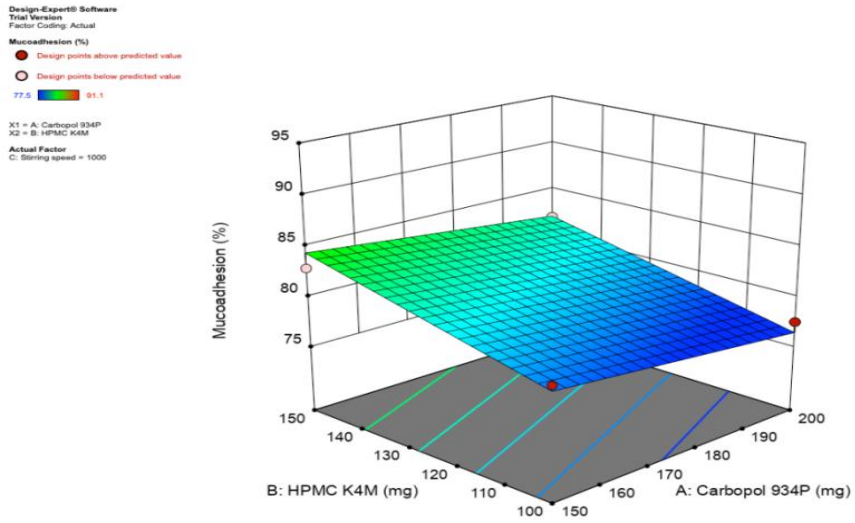

Fig. 7: Response surface plot for the study of the effect of polymers on mucoadhesion (at stirring speed 1000 rpm)

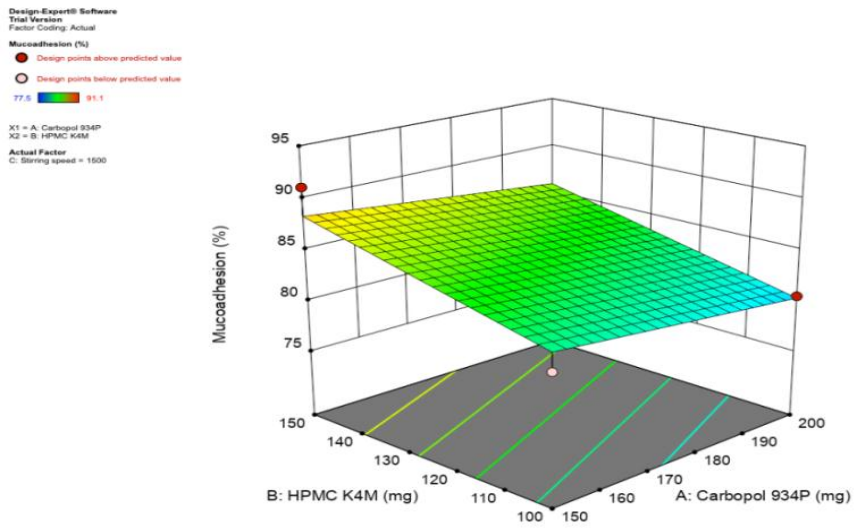

Fig. 8: Response surface plot for the study of the effect of polymers on mucoadhesion (at stirring speed 1500 rpm).

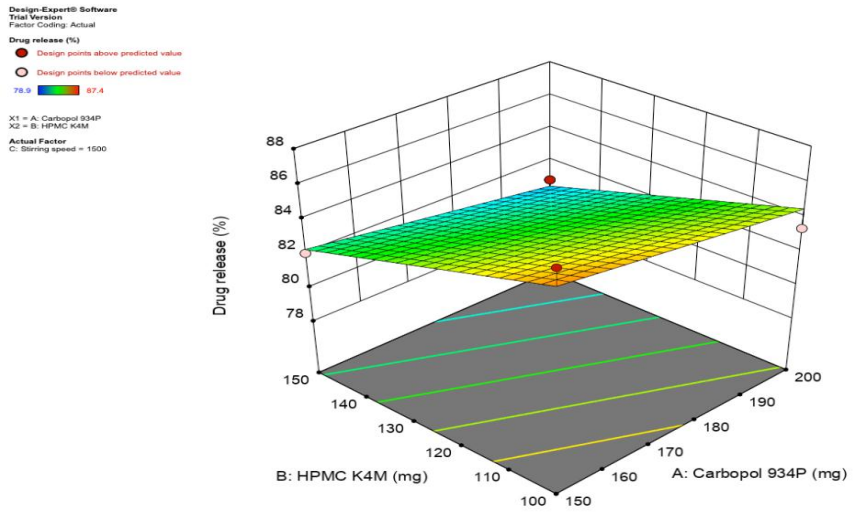

Fig. 9: Response surface plot for the study of the effect of polymers on drug release (at stirring speed 1000 rpm).

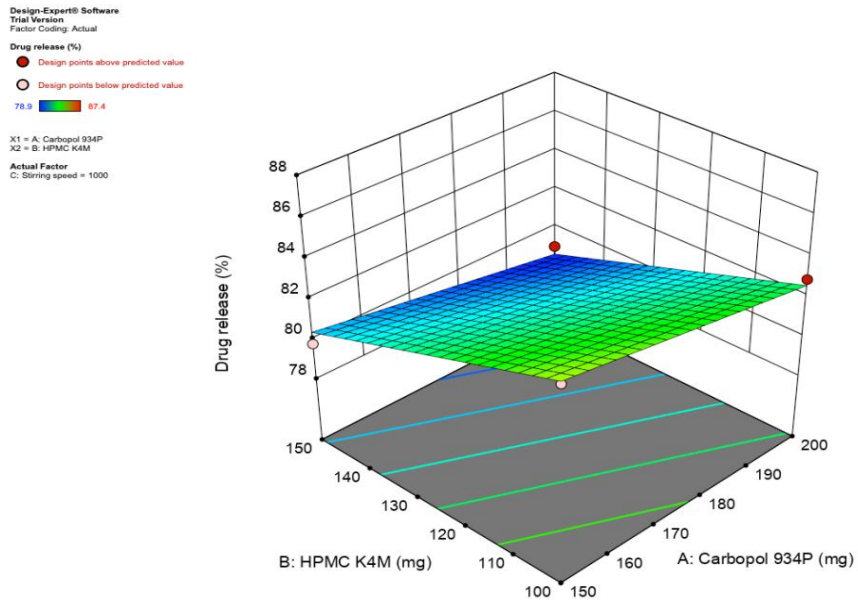

Fig. 10: Response surface plot for the study of the effect of polymers on drug release (at stirring speed 1500 rpm).

***ANOVA* Study**

Table 12: Analysis of variance for mucoadhesion of factorial batches.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	105.31	3	35.10	8.76	0.0312	**Significant**
A-Carbopol 934P	11.05	1	11.05	2.76	0.1722	
B-HPMC K4M	63.84	1	63.84	15.94	0.0162	
C-Stirring speed	30.42	1	30.42	7.59	0.0411	
Residual	16.02	4	4.01			
Cor Total	121.33	7				
R^2	0.8679					
Adjusted R^2	0.5695					
Predicted R^2	0.4717					
Adeq precision	8.4080					

Table 13: Analysis of variance for drug release of factorial batches.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	48.85	3	16.28	21.53	0.0062	**Significant**
A-Carbopol 934P	7.41	1	7.41	9.80	0.0352	
B-HPMC K4M	34.03	1	34.03	45.00	0.0026	
C-Stirring speed	7.41	1	7.41	9.80	0.00352	
Residual	3.03	4	0.7563			
Cor Total	51.88	7				
R^2	0.9417					
Adjusted R^2	0.8980					
Predicted R^2	0.7668					
Adeq precision	12.9692					

Evaluation and interpretation of research findings are most important and the p-value serves a valuable purpose in these findings. Tables 12 and 13 show ANOVA for the dependent variables mucoadhesion and drug release, respectively. The coefficients of X_1, X_2 and X_3 were found to be significant at $p < 0.05$, hence confirmed the significant effect of three variables on the selected responses. ANOVA and Multiple regression analysis were done using Stat-Ease Design-Expert 11.1.1 software.

Stability studies

Based on all the evaluation parameters, F7 was optimized batch. F7 showed optimum yield, entrapment,

drug content, extended release for 8 hours and maximum mucoadhesion of 91.1%. Thus, F7 was carried forward as optimized batch for further stability studies. The stability studies were carried out for the optimized batch F7 to assess the effect of temperature and humidity on the formulation.

Table 14: Stability data for the optimized batch F7.

Formulation	Drug content (mg/100mg)	Mucoadhesion (%)	Drug release after 8 hrs (%)
F7	**Accelerated temperature (40±2°C, 75±5% RH)**		
0 Month	35.9	91.1	82.1
3 Months	35.3	90.8	81.9

It was clearly observed from the results as shown in table 14 that there was negligible change in drug content, percentage mucoadhesion and percentage drug release after 8 hours, which therefore indicated that the optimized batch F7 was stable at accelerated storage condition for a period of 3 months.

CONCLUSION

Mucoadhesive microspheres of levofloxacin were made using different polymers to eradicate the *H. pylori* infection which can be achieved by the increase in concentration of the drug at the site of action. The preliminary batches were prepared to optimize the concentration of span 80. The batch with 1% span concentration resulted in formation of nearly spherical discrete particles, which was then selected for the formulation of factorial batches. Factorial batches were formulated based on 2^3 factorial design, where the variables include concentration of Carbopol 934P, concentration of HPMC K4M and stirring speed. The effect of independent parameters which include concentration of Carbopol 934P, concentration of HPMC K4M and stirring speed on dependent parameters was studied. Scanning Electron Microscopy for optimized batch (F7) suggested that the formulated microspheres were nearly spherical with rough surface. The formulated factorial batches showed mucoadhesion in the range of 77.5 to 91.1%. The optimized batch F7 showed maximum mucoadhesion of 91.1%. At higher levels of HPMC K4M and stirring speed, the resulted microspheres showed more mucoadhesion. Whereas, mucoadhesion decreased with low levels of HPMC K4M and stirring speed.

Microspheres obtained with higher polymer concentration resulted in sustained drug release, which means higher the concentration of HPMC and carbopol, lesser the drug release from the formulations. On the other hand, the microspheres obtained at higher stirring speed possessed low particle size, suggesting higher exposed surface area as compared to microspheres obtained at low stirring speed. This indicated that higher the speed, faster will be the drug release and lower the speed, slower will be the drug release. The design expert software suggested the significant ANOVA for mucoadhesion and drug release from factorial batches. For the response mucoadhesion, the selected parameters concentration of polymers and stirring speed shown positive effect, whereas for drug release the parameters concentration of polymers showed negative effect and stirring speed showed positive effect. Thus, it can be concluded that the selected independent parameters have significant effect on dependent variables.

Finally, it can be concluded that the stable gastroretentive mucoadhesive microspheres of levofloxacin can be successfully developed by using 2^3 factorial design in order to obtain sustain drug release, better treatment for *H. pylori* infection, reduce dosing frequency of conventional dosage form and improve patient compliance.

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