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Original Article

DESIGNING AND DEVELOPMENT OF GASTRORETENTIVE MUCOADHESIVE MICROSPHERES OF CEFIXIME TRIHYDRATE USING SPRAY DRYER

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ABSTRACT

Objective: Cefixime is a weakly acidic drug primarily absorbed through the stomach and upper intestinal part and has incomplete absorption in lower GIT which leads to its poor bioavailability. The current research work is aimed to develop gastroretentive mucoadhesive microspheres of cefixime to enhance absorption in the stomach.

Methods: Cefixime trihydrate mucoadhesive microspheres formulation was developed by spray drying technique and optimized by DoE approach using Box-Behnken design. The independent variables selected in the formulation were HPMC K15M (X_1) as carrier polymer, Carbopol 971P (X_2) as mucoadhesive polymer and Cefixime trihydrate (X_3). The response variables studied were mean particle size (R_1), and percent cumulative drug release at different time points (R_2-R_8). The optimized batch was evaluated for mucoadhesion properties, DSC and SEM analysis.

Results: The *Ex-vivo* test of cefixime microspheres studied on goat intestinal mucosa showed strong mucoadhesion of 82% for an extended period of 6 h. The *in vitro* drug release studies of microspheres in 0.1 N HCl showed extended release up to 8 h. The DSC thermograph indicated the conversion of the drug from crystalline form to amorphous form following the formation of solid dispersion. SEM analysis reveals the microspheres were spherical and smooth.

Conclusion: It is concluded from the above studies that the current formulation has increased gastric residence time and prolonged release for better absorption of the drug, thus, the formulation will have better therapeutic and increased bioavailability.

Keywords: Cefixime trihydrate, Mucoadhesive microsphere, Gastroretention, Spray drying

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INTRODUCTION

Cefixime is a broad-spectrum antibiotic which prevents bacterial cell wall synthesis [1]. The drug has poor oral bioavailability (30-40 %) [2]. It also suffers from a short half-life of 3-4 h and is quickly eliminated from blood circulation [3]. Cefixime is very slightly soluble in water and acidic media. It is a weakly acidic drug and the hydrated form of the drug is unionized in the acidic environment of the stomach. The unionized form of the drug has a narrow absorption window which is mainly through the stomach [4].

Gastroretentive systems can localize the drug in the stomach and proximal small intestines for a few hours and hence the gastric residence time of the drug is increased. This increase in drug residence time in the stomach increases bioavailability, reduces wastage of the drug, and enhances the solubility of the drug, which is less soluble in the gastric environment. The mucoadhesive polymers are used to increase drug retention and improve the oral bioavailability of drugs due to close contact with the gastric mucus layer; this interaction of the bio-adhesive polymer with the mucus layer of a mucus membrane is known as mucoadhesion [5-10].

The objective of the current research work was to develop gastro-retentive mucoadhesive microspheres of cefixime trihydrate using a scalable spray drying process. It was planned to improve the *in vitro* release profile, decrease drug concentration fluctuation, improve mucoadhesion, and bioavailability in order to reduce the dose and dosing frequency required for patient treatment. The formulation of microspheres was optimized using the design of the experiment (DOE) [11-13]. The statistical methodology was incorporated to check the independent and response variable using the response surface methodology. The response variables, such as drug entrapment efficiency, particle size, and *in vitro* drug release, were evaluated for optimization of the formulation.

MATERIALS AND METHODS

Materials

Cefixime trihydrate was received from Schon Pharmaceuticals Ltd., Indore, India as a gift sample. Hydroxy propyl methyl cellulose (HPMC K15M) was received as a generous gift from Colorcon Asia Pvt. Ltd. Verna, India. Carbopol 971P, dichloromethane and methanol were purchased from SD fine chem, India.

Preparation of mucoadhesive microspheres of cefixime trihydrate

The mucoadhesive microspheres of cefixime trihydrate were prepared using the spray drying technique [14]. The Carbopol 971P and HPMC K15M were chosen as mucoadhesive polymers and release-controlling excipients, respectively. A weighed amount of Carbopol 971P and HPMC K15M was dissolved in 60 ml of methanol and 40 ml of dichloromethane, respectively by stirring separately on a magnetic stirrer for 1 h followed by mixing to form a clear solution. A weighed amount of cefixime trihydrate was dissolved in the above polymeric solution in a drug-polymer ratio of (1:4). The resultant solution was spray dried using a lab spray dryer (Spray mate, Jay Instruments and Systems Pvt. Ltd., Mumbai, India) to achieve drug-loaded microspheres. The solution was sprayed at a flow rate of 15 ml/min using a peristaltic pump at an inlet temperature of 120 °C and outlet temperature of 60 °C.

Experimental design

The response surface methodology was employed in optimizing the formulation variables [15]. The Box-Behnken design was selected to systemically investigate the effect of independent and dependent variables [16]. The 3 factors at the 3-level design were used in the experiments for preparing microspheres [17]. The independent variables selected in the design were: Cefixime concentration (X1), Carbopol 971P (X2) and HPMC K15M concentration (X3) at three different levels (-1, 0,+1) as mentioned in table 1. The prescreening of some process variables was determined from the studies performed earlier such as the solubility of drug-polymer ratio with solvent ratio (DCM: Methanol), the viscosity of feed, and the speed of the peristaltic pump. The three independent variables (factors) considered in the preparation of cefixime trihydrate microspheres were the quantity of carbopol 971P, HPMC K15M and cefixime trihydrate, while the particle size, cumulative % drug release till 8 h, were used as dependent variables (response variables)as shown in table 1.

Table 1: Variables and their levels in box-behnken design

Independent variables	Unit	Levels		
		Low	Middle	High
X1 = Carbopol 971P	mg	100	300	500
X2 = HPMC K15M	mg	500	750	1000
X3 = Cefixime trihydrate	mg	200	300	400
Response variables			Unit	
R1 = Particle size (μm)			Maximum	
R2 = Cumulative % drug release at 15 min			Minimum	
R3 = Cumulative % drug release at 30 min			Minimum	
R4 = Cumulative % drug release at 60 min			Minimum	
R5 = Cumulative % drug release at 120 min			Minimum	
R6 = Cumulative % drug release at 240 min			Minimum	
R7 = Cumulative % drug release at 360 min			Minimum	
R8 = Cumulative % drug release at 480 min			Minimum	

Table 2: Box-behnken experimental designed formulation composition of mucoadhesive microspheres

S. No.	Formulation code	Independent variable		
		X1: (Cefixime trihydrate)	X2: (Carbopol 971P) mg	X3: (HPMC K4M) mg
1.	CTMM-1	300	100	500
2.	CTMM-2	300	500	500
3.	CTMM-3	300	100	1000
4.	CTMM-4	300	500	1000
5.	CTMM-5	200	100	750
6.	CTMM-6	200	500	750
7.	CTMM-7	400	100	750
8.	CTMM-8	400	500	750
9.	CTMM-9	200	300	500
10.	CTMM-10	200	300	1000
11.	CTMM-11	400	300	500
12.	CTMM-12	400	300	1000
13.	CTMM-13	300	300	750
14.	CTMM-14	300	300	750
15.	CTMM-15	300	300	750

Characterization of cefixime microspheres

Determination of particle size of cefixime microspheres formulation

The particle size analysis of cefixime microspheres was performed by dispersing the microspheres in a small amount of water and analyzing them under an optical microscope (Leica microsystems) at a magnification of 100X. The particle size of 100 microspheres was observed and analyzed in each batch. The average particle size was determined using calibrated micrometer scale on an optical microscope.

Scanning electron microscopy of cefixime microspheres

The analyses of the surface morphology of optimized microspheres were done using scanning electron microscopy (Supra 55 Zeiss). The sample was placed on aluminium stubs and stick with carbon conductive double-faced adhesive tape (Oxon, Oxford Instruments, UK) and a very thin layer of gold coating was done using a sputtering unit before analysis at an acceleration voltage of 20 kV, at different magnification.

Determination of entrapment efficiency

The entrapment efficiency of microspheres was determined by dispersing the weighed quantity of drug-loaded microspheres in 5 ml of methanol, sonicating the dispersion for 2 min and the volume made up to 100 ml with 0.1 N HCl. The solution was appropriately diluted at 0.1 N HCl in the range of 0 to 100 µg/ml and analyzed on a UV-visible spectrophotometer (model No-1700, Shimadzu) at 283 nm. The entrapment efficiency of microspheres was determined by using the following formula [18].

$$\% \text{ Entrapment Efficiency} = \frac{\text{Amount of cefixime trihydrate present in microsphere}}{\text{Initial amount of cefixime trihydrate taken}} \text{ X } 100$$

Differential scanning calorimetric analysis

To check the physical parameters of cefixime in the microspheres, differential scanning calorimetry (Perkin Elmer 6000) was performed. The study enables us to determine whether the drug is completely entrapped and uniformly distributed throughout the microparticle forming solid dispersion. During the current work approximately weighed amount of 3 mg samples (Drug-cefixime trihydrate, Polymers-HPMC K15M and Carbopol 971P0, Microsphere formulation) were placed in an aluminium pan and crimped for DSC analysis. The samples were heated from 50 °C to 150 °C at a scanning rate of 20 °C/min under nitrogen flow (20 ml/min) [19].

***In vitro* drug release study**

An *in vitro* drug release study of optimized microspheres by direct addition of microsphere was performed by adding the microsphere to drug release media on a magnetic stirrer. The required amounts of drug-loaded microsphere were added to a beaker containing 200 ml of 0.1N HCl as dissolution media and kept at a magnetic stirrer at 100 rpm. At a predetermined time point, 5 ml aliquot of drug sample were withdrawn, centrifuged for 5 min al 11000 rcf (Eppendorf cooling centrifuge) filtered by 0.45-micron membrane filter and analyzed by UV-visible spectrophotometer at 283 nm. The samples withdrawn were replaced by the same amount of fresh medium. The experiments were carried out in triplicate and average values were recorded [20, 21].

Ex-vivo mucoadhesion study

The mucoadhesive property of the optimized microspheres batch was evaluated on a strip of stomach mucous membranes (3 cm long and 1 cm wide) isolated from a goat procured from a slaughterhouse at a local market. Stomach mucous membranes were removed and cleaned using a normal saline solution. The membrane was attached to a glass slide and an accurately weighed amount of microspheres (50 mg) were spread uniformly on the surface of the intestinal mucosa [22-24]. The microspheres present on mucosa were placed in a humidity chamber (90% relative humidity at room temperature) for 20 min and allowed to hydrate. The glass slide holding the hydrated mucosal surface of the membrane was fixed at

an angle of 45° and slowly drained with 0.1 N HCl using a syringe pump (Top Company, model 5300) at a flow rate of 20 ml/min. Washings were collected, centrifuged (Eppendorf Company, Minispin) at 7000 RPM for 15 min and dried [25, 26].

$$(\%)\ \text{Mucoadhesion} = \frac{Wa - WL}{Wa} \times 100$$

Where,

Wa = weight of microspheres applied

WL = weight of microspheres leached out

RESULTS AND DISCUSSION

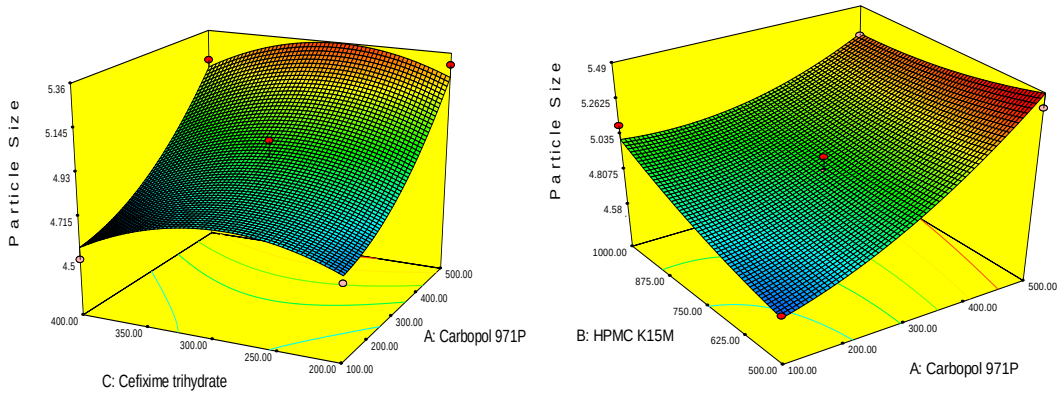
Optimization of cefixime microspheres by response surface methodology

The results obtained from the optimization formulations were statistically analyzed for response variables by using Design Expert 7.1.6 (trial version) software (Stat-Ease Inc., Minneapolis, USA). A total of 15 experiments were proposed by software according to Box Behnken design as shown in table 2. Models were selected on the basis of sequential comparison and lack of fit tests. The significance of the models was further confirmed using statistical analysis by ANOVA. The design was evaluated using statistical analysis by the sum of square and R-squared and p-value. On the above-mentioned tool, it was inferred that *in vitro* release followed the quadratic and mean model and drug content followed the 2FI model. The statistical summary of response variables and the polynomial equation followed by them is shown in table 4. The 3-D response surface plots were constructed as shown in fig. 1 to 5. The effect of independent variables on the response (particle size: R1) is schematically presented in fig. 1(A, B) as a 3-D surface plot. The plot showed that with increasing the concentration of cefixime and HPMC K15M there was no significant impact on the size of the particle, while with increasing the concentration of carbopol 971P the particle size was increased. The effect of independent variables on the response (cumulative % drug release: R2 to R8) was represented in fig. 2 to 5 as a three-dimensional response surface plot. Plots showed that increasing the concentration of carbopol 971P and HPMC K15M retarded the % drug release from the microspheres.

Table 3: Responses of the various optimization batches

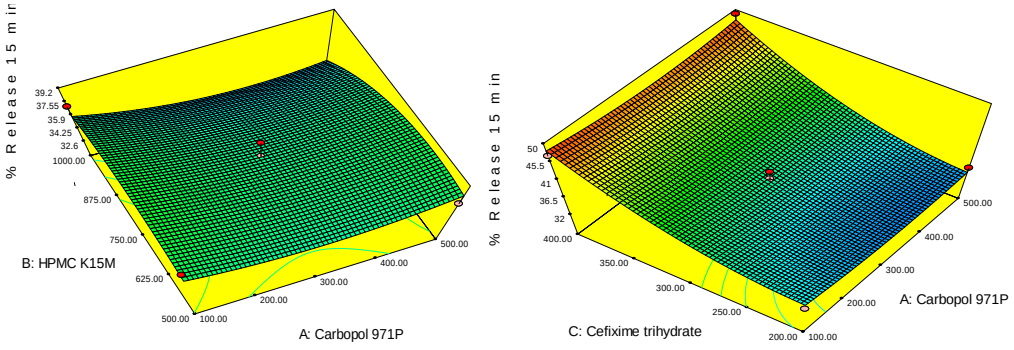
S. No.	Formulation code	Response variable							
		R1: Particle size (µm)	R2: Cumulative % drug release at 15 min	R3: Cumulative % drug release at 30 min	R4: Cumulative % drug release at 60 min	R5: Cumulative % drug release at 120 min	R6: Cumulative % drug release at 240 min	R7: Cumulative % drug release at 360 min	R8: Cumulative % drug release at 480 min
1.	CTMM-1	4.6±0.015	39.11±1.27	49.65±2.41	59.67±3.14	66.77±2.46	73.69±1.11	76.38±3.16	80.01±2.43
2.	CTMM-2	5.4±0.024	36.60±2.45	47.65±2.45	56.93±2.14	61.56±2.23	68.61±1.23	71.91±2.49	79.24±4.23
3.	CTMM-3	5.1±0.012	37.06±1.23	48.77±1.23	57.30±2.65	65.90±2.56	72.14±1.64	78.74±4.63	78.65±4.26
4.	CTMM-4	5.3±0.056	32.63±1.85	42.32±1.85	55.87±2.15	60.96±1.23	67.30±2.38	73.23±2.36	79.10±3.21
5.	CTMM-5	4.6±0.103	33.94±2.44	40.39±2.44	50.89±3.49	55.32±2.35	61.92±2.49	69.14±4.18	74.96±2.49
6.	CTMM-6	5.3±0.009	34.57±2.96	39.20±2.96	52.81±2.15	59.42±1.52	65.97±1.32	68.78±3.65	73.73±2.18
7.	CTMM-7	4.5±0.025	47.12±2.45	60.43±2.45	70.39±1.23	81.05±3.45	82.88±2.18	85.35±2.19	89.37±3.43
8.	CTMM-8	5.2±0.014	49.13±3.46	58.33±3.46	69.59±1.45	79.95±2.15	73.25±3.19	78.90±1.26	87.01±3.26
9.	CTMM-9	4.8±0.012	32.27±2.48	41.62±2.48	53.43±1.66	59.27±3.98	62.03±2.19	67.53±1.92	74.71±1.56
10.	CTMM-10	4.9±0.019	30.01±1.14	39.29±1.14	50.47±1.26	58.94±2.16	61.92±1.69	65.95±1.35	69.97±2.36
11.	CTMM-11	4.8±0.023	46.97±1.48	57.33±1.48	68.99±2.31	78.78±3.35	75.75±1.45	80.17±2.46	88.29±1.84
12.	CTMM-12	4.8±0.042	43.11±3.46	56.65±3.46	67.67±3.12	77.79±2.19	75.69±1.12	80.38±3.48	89.63±2.45
13.	CTMM-13	4.9±0.062	36.63±1.26	42.88±1.26	54.27±2.15	60.85±1.54	67.06±1.39	73.29±2.12	78.97±3.14
14.	CTMM-14	4.9±0.023	38.36±2.15	44.82±2.15	53.87±2.34	62.96±1.39	67.30±1.89	73.25±2.73	79.10±1.26
15.	CTMM-15	5.0±0.002	35.77±3.14	43.54±3.14	52.57±1.15	61.57±4.15	68.13±1.93	73.33±2.86	78.61±2.63

Particle size data are expressed as mean±SD, n=100, Cumulative drug release data are expressed as mean±SD, n=3



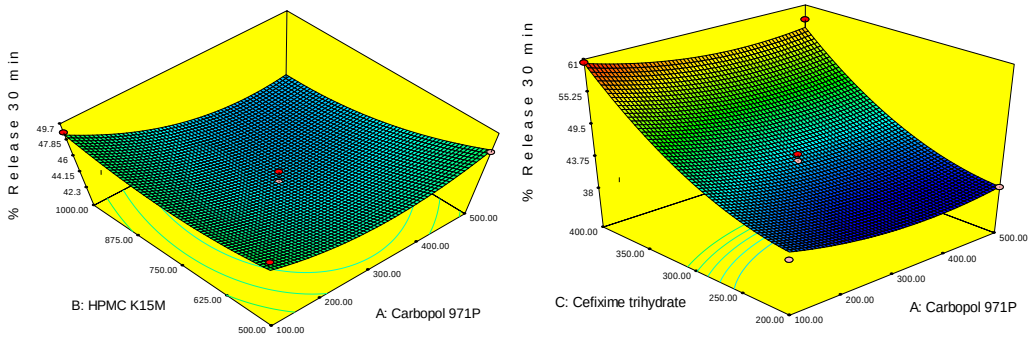
(A) **(B)**

Fig. 1: Three-dimensional response surface plot showing (A) the effect of cefixime trihydrate and carbopol 971 P concentration on particle size, and (B) the effect of HPMC K15M and carbopol 971P concentration on particle size



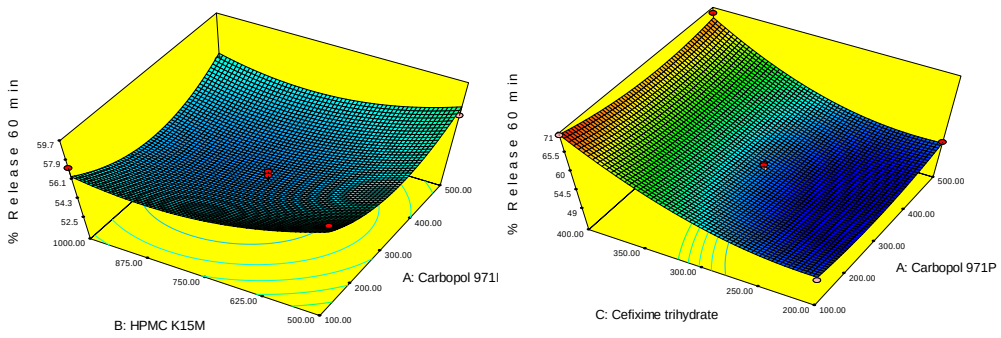
(A) **(B)**

Fig. 2: Three-dimensional response surface plot showing (A) the effect of HPMC K15M and concentration on % release in 15 min, (B) the effect of cefixime trihydrate and carbopol 971P concentration on % release in 15 min



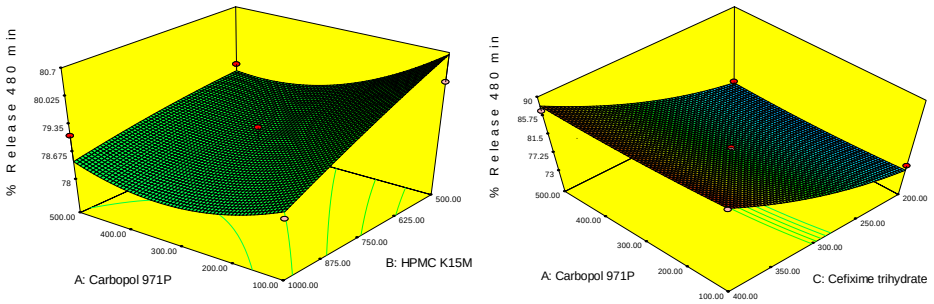
(A) **(B)**

Fig. 3: Three-dimensional response surface plot showing (A) the effect of Carbopol 971P and HPMCK15M concentration on % release in 30 min, and (B) the effect of cefixime trihydrate and Carbopol 971P concentration on % release in 30 min



(A) **(B)**

Fig. 4: Three-dimensional response surface plot showing (A) the effect of Carbopol 971P and HPMCK15M concentration on % release in 60 min, and (B) the effect of cefixime trihydrate and Carbopol 971P concentration on % release in 60 min



(A) **(B)**

Fig. 5: Three-dimensional response surface plot showing (A) The effect of carbopol 971P and HPMCK15M concentration on % release in 480 min, (B) The effect of cefixime trihydrate and carbopol 971P concentration on % release in 480 min

Table 4: Summary of ANOVA of measured responses

Model	Sequential p-value	Lack of fit p-value	Adjusted R^2 value	Predicted R^2 value	Remarks
Particle size (R1)					
Linear	0.0022	0.0982	0.6463	0.4112	
2FI	0.3614	0.0962	0.6669	0.0133	
Quadratic	0.0317	0.2441	0.8961	0.4934	Suggested
Cubic	0.2441		0.9558		Aliased
Cumulative % drug release at 15 min (R2)					
Linear	<0.0001	0.2014	0.8098	0.6977	Suggested
2FI	0.9700	0.1431	0.7459	0.3014	
Quadratic	0.0404	0.3279	0.9124	0.5993	Suggested
Cubic	0.3279		0.9490		Aliased
Cumulative % drug release at 30 min (R3)					
Linear	<0.0001	0.1008	0.8611	0.8245	Suggested
2FI	0.8929	0.0734	0.8223	0.7043	
Quadratic	0.0153	0.2488	0.9589	0.8002	Suggested
Cubic	0.2488		0.9822		Aliased
Cumulative % drug release at 60 min (R4)					
Linear	0.0001	0.0576	0.7881	0.7382	
2FI	0.9760	0.0397	0.7158	0.5332	
Quadratic	0.0015	0.3312	0.9741	0.8817	Suggested
Cubic	0.3312		0.9847		Aliased
Cumulative % drug release at 120 min (R5)					
Linear	0.0001	0.0580	0.7937	0.7058	
2FI	0.9521	0.0406	0.7276	0.3931	
Quadratic	0.0295	0.1068	0.9175	0.5581	Suggested
Cubic	0.1068		0.9850		Aliased
Cumulative % drug release at 240 min (R6)					
Linear	<0.0001	0.0363	0.8026	0.6951	
2FI	0.0481	0.0614	0.8936	0.7908	
Quadratic	0.0121	0.2291	0.9777	0.8898	Suggested
Cubic	0.2291		0.9911		Aliased
Cumulative % drug release at 360 min (R7)					
Linear	<0.0001	0.0005	0.9132	0.8630	
2FI	0.2867	0.0005	0.9236	0.8057	
Quadratic	0.0553	0.0011	0.9700	0.8285	Suggested
Cubic	0.0011		0.9999		Aliased
Cumulative % drug release at 360 min (R8)					
Linear	<0.0001	0.0194	0.9244	0.8816	Suggested
2FI	0.3266	0.0195	0.9309	0.8268	
Quadratic	0.0841	0.0333	0.9676	0.8185	Suggested
Cubic	0.0333		0.9982		Aliased
Regression equations of measured responses					
Particle Size (R_1): 4.93+0.30A+0.06B-0.037C-0.15AB+0.0001AC-0.025BC+0.12A^2+0.046B^2-0.15C^2					
Cumulative % drug release in 15 min (R_2): 36.92-0.54A-1.51B+6.93C-0.48AB+0.35AC-0.42BC+1.25A2-1.82B^2+3.02C^2					
Cumulative % drug release in 30 min (R_3):43.75-1.47A-1.15B+9.03C-1.11AB-0.23AC+0.41BC+2.11A^2+1.24B^2+3.73C^2					
Cumulative % drug release in 60 min (R_4):53.57-0.38A-0.96B+8.63C+0.33AB–0.68AC+0.41BC+2.33A^2+1.55B^2+5.03C^2					
Cumulative % drug release in 120 min (R_5):61.79-0.89A-0.35B+10.58C+0.067AB-1.30AC-0.16BC+1.12A^2+0.88B^2+6.02C^2					
Cumulative % drug release in 240 min (R_6):67.50-1.94A-0.38B+6.97C+0.060AB-3.42AC+0.012BC+2.55A^2+0.39B^2+0.96C^2					
Cumulative% drug release in 360 min (R_7):73.29-2.10A+0.29B+6.67C-0.26AB-1.52AC+0.45BC+1.91A^2-0.13 B^2+0.35C^2					
Cumulative % drug release in 480 min (R_8):78.96-0.49A+0.61B+7.62C+0.31AB-0.28AC+1.52BC+0.45A^2-0.16B^2+1.85C^2					

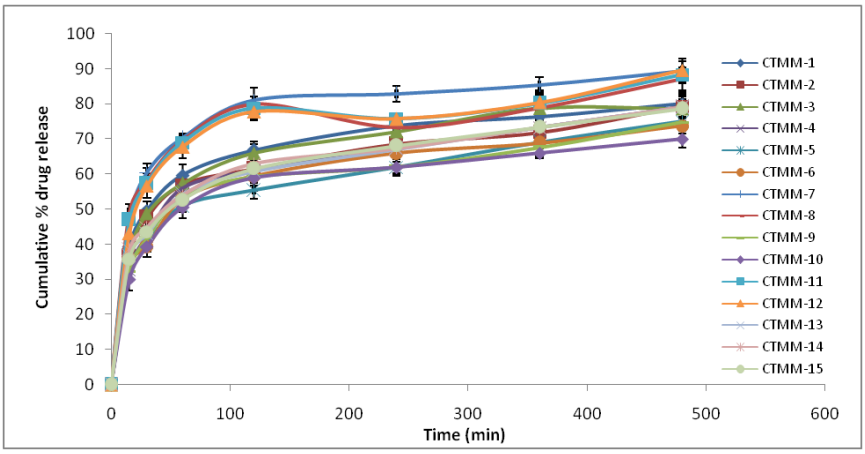


Fig. 6: Cumulative % drug release v/s time plot of cefixime trihydrate mucoadhesive microspheres optimization batches. All values shown in the graph are measured as mean±SD, n=3, Error bars indicate the standard deviation of replicates

Prediction of optimized cefixime trihydrate mucoadhesive microspheres formulation

Statistical analysis of the data was done by design expert software keeping the constraints and criteria on the desired characteristics of the final formulation of optimization batches i.e. desired particle size, and required sustained release drug release pattern as shown in table 1. the software predicted formulations with desirability close to 1. The desirability response surface plots and contour as shown in fig. 7 and 8, respectively, predict the formulation with maximum desirability and cumulative % drug release of the optimized batch. The formulation with maximum desirability of 0.902 was selected as the predicted optimum formulation. The relative percentage error between the response variables of the predicted batch and the prepared optimized batch was within the acceptance limit, and all relative percentage errors were below 8 % as shown in table 5.

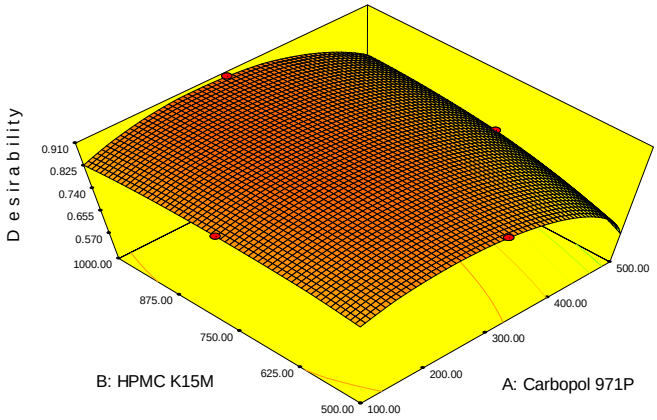


Fig. 7: Three dimension plot showing the microsphere formulation of maximum desirability

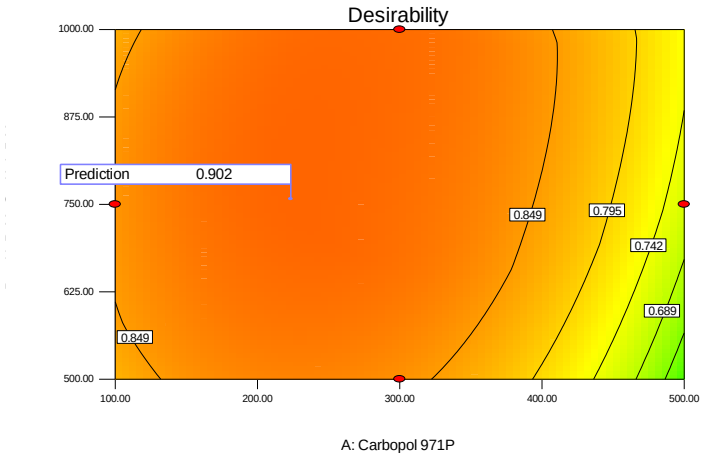


Fig. 8: Contour plot showing the microsphere formulation of maximum desirability

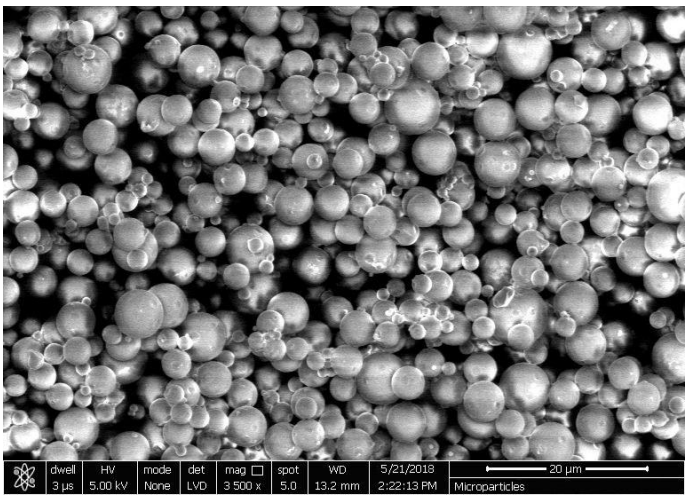


Fig. 9: Scanning electron microphotographs of optimized microspheres

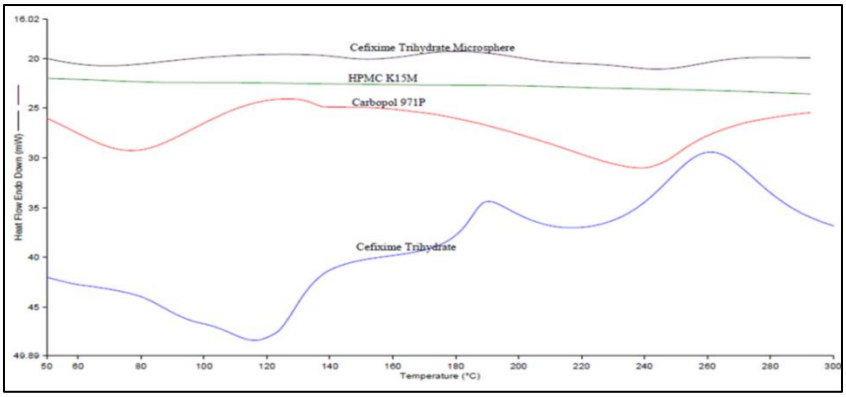


Fig. 10: Overlay graph of DSC analysis

Characterization of mucoadhesive microspheres of cefixime trihydrate

The preparation of microspheres by various techniques (solvent evaporation method, phase separation method) suffers the drawbacks of low entrapment efficiency of the drug in microparticles [27]. It was observed during the selection of various approaches of the preparation of microspheres that the entrapment efficiency does not change in the spray drying process. The highest entrapment efficiency of cefixime trihydate was found to be 92% for the microparticle prepared by spray drying process. The result shows that, the employment of the spray drying method is a very effective approach for achieving the highest entrapment efficiency in comparison to other techniques of microparticle preparation. The mean particle size of spray-dried microspheres obtained by optical microscopy was in the range of 4.5-5.3 µm. The scanning electron micrograph of cefixime trihydrate mucoadhesive microspheres is shown in fig. 9. Microspheres observed were of uniform size distribution with a smooth surface. The various approaches of cefixime trihydrate formulation [28, 29], such as tablet suffer the drawback of low surface area as compared to microparticle for gastro retention. The current formulation is a microparticle preparation and has an advantage of smaller particle size having a large surface area which relates to higher mucoadhesion. The release of the drug at the desired site of mucoadhesion will enhance the bioavailability of the drug.

The differential scanning colorimetric patterns of the microspheres are shown in fig. 10. which provides valuable information regarding the physicochemical properties of the formulation. The DSC thermograph shows the endothermic peak of cefixime trihydrate at 100 °C-117 °C showing evaporation of water molecules and exothermic peak at 190 °C and 250 °C showing crystalline stage transition and decomposition. The excipients, such as carbopol and HPMC did not show any endothermic peak, which reveals their amorphous characteristics. The absence of any specific peak at 100 °C–117 °C, 190 °C and 250 °C in the microsphere formulation confirmed the conversion of the physical form of cefixime trihydrate from the crystalline peak into an amorphous form. The results show that cefixime trihydrate is distributed uniformly in the polymeric matrix and forms a solid dispersion in the microsphere providing better control of the release characteristics of the drug.

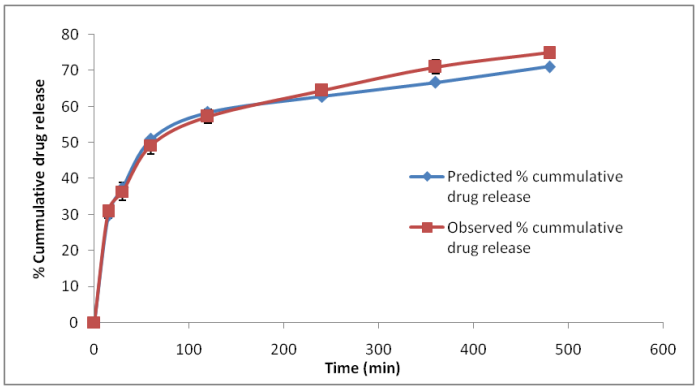


Fig. 11: Release profile of predicted and observed formulation of cefixime trihydrate mucoadhesive microspheres. Data are expressed as mean±SD, n = 6

Table 5: Predicted and optimized variables of cefixime trihydrate mucoadhesive microsphere formulation

Independent variable			Dependent variables			
Carbopol 971P (mg)	**HPMC K15M (mg)**	**Cefixime trihydrate (mg)**	**Responses**	**Predicted value**	**Observed value***	**Relative error (%)**
392	1000	200	Particle size (micron)	4.72	5.10±0.016	7.45
			Cumulative % drug release at 15 min	29.73	30.8±1.86	3.47
			Cumulative % drug release at 30 min	37.49	36.36±2.4	3.11
			Cumulative % drug release at 60 min	50.92	49.18±2.43	3.54
			Cumulative % drug release at 120 min	58.39	57.26±1.97	1.97
			Cumulative % drug release at 240 min	62.75	64.44±1.72	2.62
			Cumulative % drug release at 360 min	66.69	70.99±1.92	6.06
			Cumulative % drug release at 480 min	71.04	74.87±1.07	5.12

*Data are expressed as mean±SD, n=6

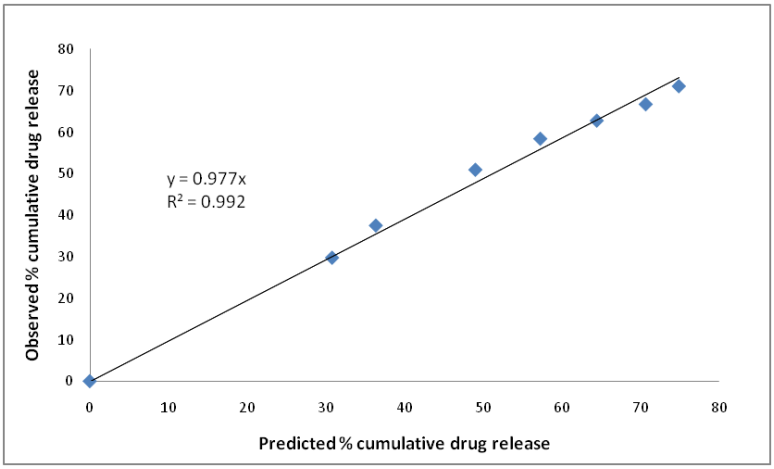


Fig. 12: Linear plots between observed and predicted values of % cumulative drug release

In vitro drug release study

The results for *in vitro* release studies of 15 optimization batches are reported in table 3 and graphically represented in fig. 6. The *in vitro* release data of 15 optimization batches were fed into the design expert software and desired constraints were provided to the software to predict the *in vitro* release data for optimized batch. The software-predicted and experimentally observed *in vitro* release data of the optimized formulation batch is shown in table 6 and fig. 11 and the regression plot is shown in fig. 12. The *in vitro* release data of the optimized batch shows that there was an initial burst release of about 30% of cefixime trihydrate was observed during the initial 15 min of the dissolution study of the formulations due to the drug adsorbed on the surface of microspheres further *in vitro* release followed prolonged drug release pattern up to 8 h which was beneficial to achieve the effective plasma concentration after administration of cefixime trihydrate mucoadhesive microsphere.

Evaluation of mucoadhesion of microspheres

Numerous research works have been reported on cefixime gastroretentive drug delivery systems which are mainly based on matrix tablet formulation. These tablet formulations either use a floating mechanism or mucoadhesion to achieve gastro retention [28, 29]. The matrix tablet formulations based on mucoadhesion suffers the drawbacks of lower surface area for mucoadhesion and can slip in the presence of food, whereas the demerit of the floating tablet is it requires sufficient gastric fluid to perform its action [30]. The *ex-vivo* mucoadhesive properties of the optimized batch of microspheres were found to be 82% after six hours of microsphere application. The percentage of mucoadhesion was notably increased with the incorporation of carbopol in the microspheres, which indicated that carbopol has a strong ability to interact with mucus. A better retention effect was observed with a higher amount of carbopol. The developed microspheres possess a high surface-to-volume ratio that demonstrates close contact with the mucous membrane and releases the medication for a better prolonged period showing the advantage of higher bioavailability.

CONCLUSION

Cefixime trihydrate mucoadhesive microspheres formulation was developed by spray drying technique and optimized by DoE approach using Box-Behnken design. The results obtained from the experiments were statistically analyzed for response variables. The Carbopol 971P and HPMC K15M aided in controlling the release of the drug from the polymeric matrix and also helped in mucoadhesion. The *in vitro* drug release study of the optimized batch provided a consistent drug release up to 8 h and the *ex-vivo* mucoadhesive studies showed mucoadhesion of 82% up to 6 h, which shows that the prepared formulation possesses both sustained release and mucoadhesion properties for the desired period. The developed formulation of mucoadhesive gastro-retentive microspheres cefixime trihydrate has a high surface-to-volume ratio which shows close contact with the mucous membrane and releases the medication for a longer period showing the advantage of prolonged activity than the conventional formulation.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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