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RESEARCH ARTICLE

Formulation and Optimization of Floating Microspheres of Cefixime Trihydrate by Factorial Design

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ABSTRACT

Cefixime trihydrate is an orally active third generation cephalosporin having a wide range of activity. But its bioavailability is limited to about 40- 50% after oral administration. The development of floating microspheres is a possible alternative to overcome this problem. The floating microspheres of cefixime were prepared with this objective using the biocompatible natural polymers like alginate and chitosan by ionotropic gelation method. A 3^2 full factorial experiment was designed to study the effect of independent variables such as alginate and chitosan concentration. The response parameters investigated are buoyancy and cumulative drug release percentage and was statistically analyzed by applying ANOVA. Contour plots and three-dimensional surface response plots were drawn to evaluate the interaction of the independent variables on the chosen dependent variables. Two optimal formulations were developed by setting the constraints on the independent variables to maximize the buoyancy and drug release percentage. The values of the observed responses are compared with those predicted by the mathematical models along with the % prediction errors. The low value of error proved the ability of response surface methodology to predict the behavior of the drug-loaded floating microspheres. Surface morphology of the microspheres was studied by SEM analysis. Kinetic studies reveal that the optimized formulations release the drug in the zero order manner with non-Fickian diffusion mechanism based on the regression values of zero order, Higuchi, and Korsmeyer-Peppas model.

KEYWORDS

Cefixime, Alginate, Chitosan, Floating Microspheres, Optimization, Buoyancy, SEM, Kinetic studies.

INTRODUCTION

The incomplete release of drug from the dosage form and shorter residence time of the dosage forms in the upper gastrointestinal tract, a prominent site for absorption of many drugs, will lead to lower bioavailability.[1] Retention of dosage forms in the stomach prolongs overall gastrointestinal transit time and improves the oral bioavailability of the drugs that are having site-specific absorption from the stomach or upper part of small intestine. Both single and multiple unit systems have been developed. The single – unit systems are more popular but have a disadvantage owing to their "all-or-nothing" emptying process, leading to high variability of the gastrointestinal transit time.[2,3] In contrast, multiple-unit particulate dosage forms (e.g., microspheres) have the advantages that they can pass uniformly through the gastrointestinal tract to avoid the vagaries of gastric emptying and provide an adjustable release, thereby reducing the inter-subject variability in absorption and risk of local irritation. Floating microspheres with lower density than that of the

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gastrointestinal fluids remain buoyant in the stomach for a prolonged period of time and are useful for enhancing the bioavailability.

Statistical modeling and experimental design are two most essential tools in the field of formulation development.[4] While developing a sustained release floating microsphere dosage form; an important issue is to design an optimized formulation with an ideal release profile in a specific time period and a minimum number of trials. The factorial design enables all the factors to be varied simultaneously, allowing quantification of the effects caused by independent variables and interaction between them. In this study, a 3^2 full factorial experimental design was used to optimize the formulation of the floating microspheres. Preoptimization studies were under taken to decide the excipients and their levels in the experimental design.[5, 6]

Cefixime is an orally active 3rd generation cephalosporin antibiotic active against Enterobacteriaceae, *Haemophilus influenzae*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Moraxella*, *E.coli*, *Protease*, *Neisseria gonorrhea* and is resistant to many β lactamases. The absolute bioavailability of it is below 50-60%, which suggests an absorption mechanism through the mucosa with limited capacity. The biological half-life of cefixime 3±0.4 hours and dosing of cefixime is 200mg twice daily for 7-10 days.[7] In the present study floating microspheres of cefixime were prepared by ionotropic gelation method using two biocompatible and natural biodegradable polymers, alginate and chitosan. The aim of the work was to evaluate the polymer concentrations on floating properties and release characteristics of the formulation.

The drug release profile was optimized with the aid of design of experiments (DoE). The microspheres of cefixime using the polymers were designed according to 3^2 full factorial design, taking the concentration of alginate and chitosan as the independent variables. These two factors were varied at each of the 3 levels. Total nine batches of formulations were prepared. The dependent variables selected were buoyancy and cumulative drug release percentage. Floating matrix tablets of cefixime were developed, and optimization was done by Patel et al[8] to study the effect of formulation variables on dug release.

MATERIAL & METHODS

Cefixime was gifted from Sance Pharmaceuticals Pala, Kerala, India. Sodium alginate was purchased from Loba Chemie Pvt. Ltd. Mumbai. Chitosan was gifted by India Sea Foods, Kochi, Kerala. Calcium carbonate was purchased from S.D fine laboratories. All other chemicals used were analytical grade.

Experimental Design

A 3^2 full factorial design was utilized in the present study for the development of floating microspheres. In this design, two factors were evaluated each at three levels and experimental trials were performed at all nine possible combinations. The concentration of polymers such as alginate (3%) and chitosan 1.5% were selected as independent variables. Buoyancy, cumulative drug release percentage were selected as dependent variables. The actual and coded values of the independent variables are given in table 1. The full factorial experimental design lay out is given in table 2. The design was done using the Design Expert Software (Version 7.1.4, Stat- Ease Inc., Minneapolis, MN).[5.6]

Table 1: Actual and coded values of the independent variables

Code	Concentration of alginate (X_1)	Concentration of chitosan (X_2)
-1	2	1
0	3	1.5
+1	4	2

Table 2: Full factorial experimental design layout

Batch (Runs)	Factor1 (Concentration of alginate) X_1	Factor2 (Concentration of chitosan) X_2
A_1	-1	0
A_2	0	0
A_3	0	1
A_4	1	0
A_5	1	1
A_6	1	-1
A_7	-1	1
A_8	0	-1
A_9	-1	-1

Preparation of Optimized Floating Microspheres

Alginate was dissolved in 100ml distilled water 100 mg of the drug cefixime, and $CaCO_3$ was added to the solution at the ratio of 0.75:1 (gas forming agent: alginate, w/w). The solution was stirred thoroughly. The gelation medium was prepared by dissolving 0.5% of calcium chloride) in 2 % glacial acetic acid. To 100 ml of the gelation medium, chitosan was added. Homogenous alginate solution was extruded using a 21G syringe needle into the gelation medium. The dropping rate was 30 drops/minute, and the falling distance was 5cm. The solution containing the suspended microcarriers was stirred with a magnetic stir bar for 10 min to improve the mechanical strength of the microspheres and was allowed to complete the reaction to produce gas. The microspheres were collected, washed twice with distilled water and subsequently air dried.[14,15]

Determination of Drug Entrapment Efficiency

The drug content in the microspheres was determined by pulverizing the drug-loaded microspheres (equivalent to 100mg of the drug) followed by immersing them in 1000 ml simulated gastric fluid (pH 1.2 buffers) with agitation at room temperature for 24 hours. From this, 1ml of the solution was transferred to 10 ml volumetric flask and diluted with pH 1.2 buffer to the volume. Filtered the solution through Whatman No.1 filter paper, the drug concentration was determined spectrophotometrically at wave length 284nm using a UV spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan). The filtered solution from the empty microspheres was taken as blank. All samples were analyzed in triplicate.[9]

$$\text{Encapsulation efficiency (EE \%)} = W_A/W_T X100$$

EE: Encapsulation efficiency; W_A: Actual drug content; W_T: Theoretical drug content

***In vitro* Evaluation of Floating Ability (buoyancy) of Microspheres**

The floating properties of the beads were evaluated in a dissolution vessel filled with1000 ml simulated gastric fluid (pH 1.2) containing 0.02% of Tween 80. Paddle rotation speed was at 100 rpm, the temperature was maintained at 37 ± 0.5^0C. For each sample of microspheres, 50 individual microspheres were placed in the dissolution vessel. Both, the number of microspheres N_F (observed visually) and the floating duration F_T (which is the time during which the micro spheres remain buoyant on test solution) were then determined at fixed time intervals during a 24 hour period. Experiments were performed in triplicate, and the percentage of floating micro spheres were calculated according to the equation.[9]

$$F\% = [N_F/ N_T] \text{ x}100$$

Where, NF = Number of floating Microspheres

N_T = Total number of the microspheres

Equilibrium Swelling Studies

The prepared microspheres of 100 mg were placed in 900 ml of 0.1N HCl and allowed to swell for the required period of time at 37±0.5^{0}C using the USP dissolution apparatus with the dissolution basket assembly at 50 rpm. The microparticles were periodically removed, blotted with filter paper and their changes in weight were measured during swelling until equilibrium was attained. Finally, the weight of the swollen microparticles was recorded after a time period of 4 hours, and the swelling index was then calculated using the formula.[11]

Swelling Index = (We-Wo) / Wo

Where Wo is the initial weight of the dry microparticles, and We are the weight of the swollen microparticles at equilibrium swelling in the media. Each experiment was repeated three times, and the average value of ±S.D. was taken as the swelling index value.

Micromeritic Studies

The optimized floating microspheres were characterized by their micromeritic properties as follows: [12]

Bulk Density

The prepared microspheres of known weight were introduced into a graduated measuring cylinder of 10 ml capacity. The volume of the sample was taken, and bulk density can be calculated using the formula given below.

Bulk Density = Weight of the particle / Bulk volume of the particle

Tapped Density

Weighed quantities of microspheres were introduced into 10 ml measuring cylinder. After that, the initial volume was noted, and the cylinder was allowed to fall under on to a hard surface from the height of 2.5 cm at 2-second intervals. Tapping was continued until no further change in volume was noted.

Tapped Density = Mass of the microsphere / Volume of the microsphere after tapping

True Density

The true densities of floating microspheres were determined by immersing the microparticles in a 0.02% Tween 80 solution for 3 days in a metal mesh basket. The particles that were sunken after this process was used for density measurement. The determination was carried out by the liquid displacement method using n-hexane as a non-solvent. A specific gravity bottle of 25ml capacity was used. Initially, the specific gravity bottle was weighed, and it was noted as W_1. Then one fourth was filled with microspheres and weighed as W_2, the remaining volume was filled with n-hexane and again weighed as W_3. Now the contents are emptied and filled with n-hexane alone, and weight was noted as W4. From this, the weight of n-hexane displaced was calculated, and the true density was determined.[13]

Carr's Compressibility Index

The simplest way for measurement of the free flow of powder is compressibility, an indication of the ease with which a material can be induced to flow.

Compressibility index = [(Tapped density-Bulk density) / Tapped density] x 100

Hausner Ratio

This parameter was calculated from the values of tapped density and bulk density by using the equation:

Hausner ratio= [Tapped density/ Bulk density] x100

Values less than 1.25 indicate good flow (equivalent to 20% Carr's index), where as greater than 1.25 indicate poor flow (33% Carr's index)

Angle of Repose

It was determined by using a funnel whose tip was fixed at a constant height (H) of 2cm from the horizontal surface. The microspheres were allowed to pass freely through the funnel until the tip of the pile touches the tip of the funnel. The radius of the base of the pile was measured

as R (cm). The angle of repose was determined with the formula.[14]

$$\text{Angle of repose} = \text{Tan}^{-1}(\text{height}/\text{radius})$$

Particle Size Analysis

Particle sizes of different batches of optimized formulations were determined by optical microscopy with the help of ocular and stage micrometer. The sizes of around 100 particles were measured, and their average particle size was determined. The mean particle sizes of all formulations were determined by using the Edmondson's equation.

$$D \text{ mean} = \sum nd / n$$

Where, n = no. of microspheres observed,

d = mean size range.

***In vitro* Drug Release Studies**

The dissolution studies of microspheres equivalent to 100mg of cefixime were performed using USP dissolution type apparatus II (paddle type). The drug release study was carried out using 900ml of pH 1.2 buffer, maintained at 37± 0.5^0C. The speed of stirrer was maintained at 100 rpm. An aliquot of 5 ml of the solution was withdrawn at predetermined time intervals, and perfect sink condition was established during the dissolution study period by replacing an equivalent volume of fresh dissolution medium. The sample solution was filtered through Whatman No.1 filter paper and analyzed for the concentration of cefixime using a UV spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan) at a wavelength of 284 nm. The amount of cefixime released was calculated from the calibration curve of the same dissolution medium. All experiments were performed in triplicate.[9]

Regression Analysis

The targeted response parameters were statistically analyzed by applying one-way ANOVA using Design Expert Software (Version 7.1.4, Stat-Ease Inc., Minneapolis, MN). Statistical second –order model including interaction and polynomial terms were generated for all the response variables using Multiple Linear Regression Analysis (MLRA). The individual parameters were evaluated using F test. The general form of the model is represented as

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_1 X_2^2 + \beta_7 X_1^2 X_2 + \beta_8 X_1^2 X_2^2$$

Where β_0, is the intercept which is the arithmetic average of all quantities of outcomes for 9 runs, $\beta_1$to β_8 are the coefficients computed from the observed experimental values of Y, and X_1 and X_2 are the coded levels of the independent variables. X_1 X_2 is the interaction between the main effects. X_1^2 and X_2^2 are the quadratic terms of the independent variables that were used to simulate the curvature of the designed sample space. The quadratic models generated by regression analysis were used to construct the 3- dimensional graphs in which the response parameter Y was represented by a curvature surface as a function of X. The effect of the independent variables on each response parameter was visualized from the contour plots.[15,16]

Optimum Floating Microspheres

Numerical optimization using the desirability approach was employed to locate the optimal settings of the formulation variables to obtain the desired response. The optimal formulations (S_1 &S_2) were developed by setting constraints on the dependent and independent variables.[17,18] The new formulations were evaluated for the responses and the experimental values obtained were compared with those predicted by mathematical models.

Evaluation of the Optimum Floating Microspheres

Prepared microspheres were evaluated for drug entrapment efficiency, buoyancy %, cumulative drug release % and *in vitro* drug release studies. Also, micromeritic studies like bulk density, tapped density, true density, Carr's index, Hausner ratio and particle size determination were done.

Scanning Electron Microscopy

Scanning electron microscopy was used to study the morphology, surface topography and cross-section of the floating microspheres.The

floating microspheres from the optimized batch were mounted on the Scanning electron microscopy (SEM) sample stab, using a double-sided sticking tape and coated with gold (200 A^o) under reduced pressure (0.001 torr) for 5min using ion sputtering device (Jeol JFC-1100E, Tokyo, Japan). The gold-coated samples were observed under the scanning electron microscopy (SEM-Jeol JSM-840A, Tokyo, Tapan) and photomicrographs of suitable magnification were obtained.[19]

Drug Release Kinetics of the Optimized Formulations

The in vitro drug release data of the optimized formulations was evaluated to check the goodness of fit to the zero-order release kinetics, first-order kinetics, Hixson- Crowell cube root model, Higuchi's square root of time equation and Korsmeyer-Peppas power law equation for quantifying the phenomena controlling the release from swellable matrix in which the contribution of the relaxation or erosion mechanism and of the diffusive mechanism can be quantified. The goodness of fit was evaluated using the R^2 (correlation coefficient) values.[20,21]

RESULTS AND DISCUSSION

From the pre-optimisation studies , 3% of alginate and 1.5% of chitosan were optimized, and these two factors were taken as independent variables, and a 3^2 full factorial design was selected for the optimization. Accordingly, 9 formulations were prepared by varying the polymer concentration and subjected to evaluation. The results are shown in table 3.

In vitro Floating Studies

Buoyancy tests were performed at pH 1.2 with 0.02% w/v Tween 80 in order to simulate the surface tension of human gastric juice (35-50 mN/m^2).[22] The results are shown in table 4. These results showed that the formulations A_4 and A_5 had buoyancy greater than 83%, and all the other formulations had floating ability between 70% and 82%, but buoyancy for formulation A_1 was only 68.13%. All the formulations showed floating lag time (FLT) less than 1 minute which indicated that the original density of the microspheres prior to matrix swelling in the simulated gastric fluid

Table 3: Response parameters of the optimized formulations

Formulation code	Response 1 Buoyancy % (Y_1)	Response 2 Cumulative drug release% (Y_2)	Response 3 Entrapment efficiency% (Y_3)	Response 4 Correlation coefficient (R^2) (Y_4)
A_1	85.42	50.56	86.94	0.93
A_2	76.13	80.33	84.44	0.982
A_3	73.64	82.32	79.42	0.949
A_4	62.76	92.63	77.94	0.913
A_5	83.47	63.73	86.32	0.986
A_6	70.68	77.67	82.34	0.891
A_7	82.53	73.73	78.29	0.879
A_8	78.24	58.73	83.24	0.958
A_9	68.13	84.32	80.87	0.892

Table 4: *In vitro* floating and swelling characteristics of the optimized formulations

Formulation code	Floating lag time (seconds)	Total floating time (hours)	Buoyancy (%)	Swelling index
A_1	23±6	>18	68.13±1.34	70.08±2.28
A_2	28±4	>18	75.63±3.32	76.32±3.13
A_3	34±6	>18	78.24±2.06	80.21±0.98
A_4	26±3	>18	83.47±3.03	86.54±3.01
A_5	24±3	>18	85.42±1.98	88.04±1.67
A_6	30±5	>18	82.53±0.75	84.68±2.74
A_7	29±3	>18	70.68±2.58	74.42±2.45
A_8	27±3	>18	73.64±3.26	76.04±1.42
A_9	28±3	>18	62.76±2.57	72.43±0.78

was less than 1. The total floating time (TFT) of all the microspheres were found to be more than 18 hours. These results showed that microspheres remained floated in a continuous manner and percentage buoyancy was found to be increased with increasing amount of polymer concentration.[21]

Swelling Index

In order to study the behavior of the chitosan-alginate floating microspheres in the gastric fluid, the swelling index study of all the formulations were performed. All the formulations showed swelling and floating property without any sign of disintegration. The results of the study showed the swelling index between 70.08± 2.28 % to 88.04±1.67 % (table 4)

Micromeritic Studies

Micromeritic studies of the formulations were done, and the results are shown in table 5. The bulk density values of the floating microspheres were found to be in the range of 0.512±0.43 to 0.574±0.64g/cm^3. The tapped density ranged from 0.614±0.13 to 0.652±0.98g/cm^3,while their true densities ranged between 0.404± 0.03 to 0.728±0.04g/cm.^{3} The density values were less than 1 which indicated that the formulations exhibited excellent floating nature.[13] The percentage compressibility index (Carr's index) ranged between 9.46±0.95% and 19.07±1.23%, and the angle of repose ranged between 24.32±3.09 to 29.02±5.05 degree. So the formulations were free-flowing. The particle size of the formulations was found to be in the range of 548.98 ±4.23 µm to 592.20±0.03 µm. Hausner ratio of all the formulations was in the range of 1.13±0.22 to 1.24±0.67 (less than 1.25) which indicated good flow, further supporting the floating nature of the microspheres.

In *vitro* Drug Release Studies

In vitro dissolution studies of cefixime from floating microspheres were performed in 0.1N HCl (pH 1.2) using USP dissolution type apparatus II (paddle type). It was found that the

Table 5: Micromeritic properties of the optimized floating microspheres

Formulation code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	True density (g/cm^3)	Carr's Index (%)	Hausner ratio	Angle of repose (degree)	Particle size (µm)
A_1	0.525±0.04	0.651±0.03	0.721±0.04	19.07±1.23	1.24±0.67	26.34±3.98	578.23±2.98
A_2	0.528±0.12	0.643±0.74	0.404±0.03	18.50±0.08	1.21±0.98	24.52±4.12	563.19±3.83
A_3	0.530±0.54	0.648±0.05	0.605±0.13	18.20±1.02	1.22±0.04	29.02±5.05	592.20±0.03
A_4	0.543±0.84	0.614±0.13	0.728±0.04	18.41±2.43	1.13±0.22	26.49±2.98	568.43±4.34
A_5	0.512±0.43	0.621±0.21	0.621±0.04	17.55±0.88	1.21±0.96	26.76±3.43	574.76±4.94
A_6	0.562±0.12	0.652±0.98	0.563±0.18	13.8±1.04	1.16±1.43	28.12±2.98	585.23±5.06
A_7	0.563±1.34	0.641±1.09	0.716±0.43	12.16±0.48	1.13±0.90	25.68±4.12	548.98±4.23
A_8	0.525±0.07	0.620±1.30	0.632±0.04	15.32±1.93	1.18±1.10	24.32±3.09	585.02±3.09
A_9	0.574±0.64	0.634±0.54	0.701±1.21	9.46±0.95	1.23±0.05	27.43±3.34	564.12±3.05

Each value is the mean of three readings ± Standard Deviation

formulations showed 58.73± 2.79 to 92.63± 2.74 % of drug release for 24 hours. From the results, it was also clear that the drug release was significantly sustained (Figure 1). As the concentration of the polymer increased the cumulative release of drug decreased. These results were supported by the results obtained from pre-optimization studies.

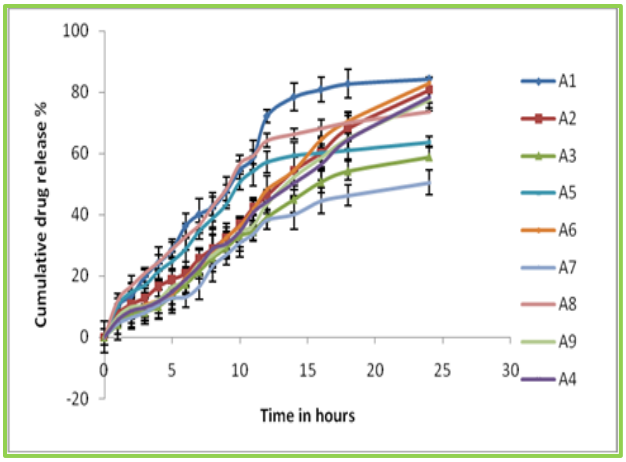


Figure 1: Cumulative drug release profiles of the optimized formulations in pH 1.2 buffer

Experimental Design and Statistical Analysis

The coefficients of the polynomial equations were generated using multiple linear regression analysis for % buoyancy and cumulative % drug release. All the data of the summary output of regression analysis for effect of X_1 and X_2 on Y_1 and Y_2 are enlisted in table 6 and 7.

Final equation in terms of coded factors

$Y_1 = +75.76 + 8.31X_1 + 2.57\ X_2 - 1.26X_1X_2 - 0.21X_1^2 - 0.073\ X_2^2$ ---- (1)

$Y_2 = + 78.77 - 11.10\ X_1 - 10.29X_2 - 2.05X_1X_2 - 1.60\ X_1^2 - 5.10\ X_2^2$----- (2)

Final equation in terms of actual factors

Buoyancy = + 29.2456 + 13.35635Alginate + 13.55270 Chitosan - 2.51500 Alginate. Chitosan - 0.21259 $Alginate^2$ - 0.29034 $Chitosan^2$.

Drug release = + 64.1836 +4.64922Alginate + 52.92511 Chitosan -4.10500 Alginate. Chitosan - 1.59862 $Alginate^2$ -2039448 $Chitosan^2$

Where Y_1 is the % buoyancy, Y_2 is the cumulative drug release%, X_1 - alginate concentration and X_2- chitosan concentration.

All the polynomial equations were found to be statistically significant ($p<0.01$), as determined using ANOVA as per the provision of Design expert software. The model F-value of 258.54 (table 6) in equation (1) implies that the model is significant. Values of "Prob > F" and less than 0.0500 indicate model terms are significant. In this case, X_1, X_2, and X_1X_2 are significant model terms. The "lack of fit F-value" of 3.54 implies that the lack of fit is not significant relative to the pure error. Nonsignificant lack of fit is good, and the model is fit.

The "Pred- R squared" of 0.9607 is reasonable agreement with the "Adj. R- Squared" of 0.9908. "Adeq precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The value was found to be 53.662 which indicated an adequate signal. So this model can be used to navigate design space.

Equation (1) suggests that the factors $X_1$and X_2 have a positive effect on the buoyancy of the dosage forms. As the concentration of the polymer increased the buoyancy also increased. The response surface plot, Figure 2 is used to visualize the impact of changing variables and was found that the buoyancy increased with increase in the concentration of the polymers. This is also supported by the contour plot (Figure 3).

Table 6: Summary of the results of regression analysis for responses and analysis of variance for buoyancy

Paramet-ers	**df**	**S.S**	**M.S**	**F**	**P**	**R^2**	**S.D**	**C.V %**	**Lack of fit F-value**	**Adeq Precisi-on**
Model	5	460.27	92.05	258.54	<0.0001	Adj. 0.9908 Pred. 0.9607	0.60	0.79	3.54	53.662
Residual	7	2.49	0.36	-	-	-	-	-	-	-
Cor total	12	462.77	-	-	-	-	-	-	-	-

Table 7: Summary of the results of regression analysis for responses and analysis of variance for cumulative drug release

Paramet-ers	**df**	**S.S**	**M.S**	**F**	**P**	**R^2**	**S.D**	**C.V %**	**Lack of fit F-value**	**Adeq. Precisi on**
Model	5	1503.31	300.66	24.83	0.0003	Adj. 0.9085 Pred. 0.8801	3.48	4.60	4.76	18.093
Residual	7	84.76	12.11	-	-	-	-	-	-	-
Cor total	12	1588.07	-	-	-	-	-	-	-	-

df is a degree of freedom, S.S is the sum of squares, M.S is mean sum of squares, F is Fischer's ratio, R^2 is correlation coefficient, S.D is standard deviation, C.V is coefficient of variance

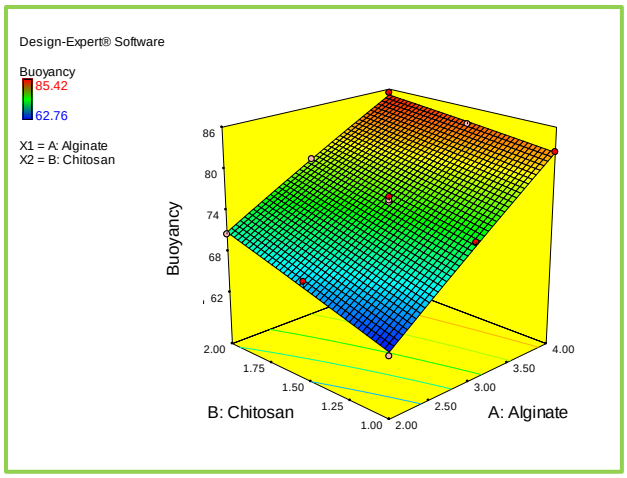


Figure 2: Response surface plot is showing the effect of polymer concentrations on buoyancy

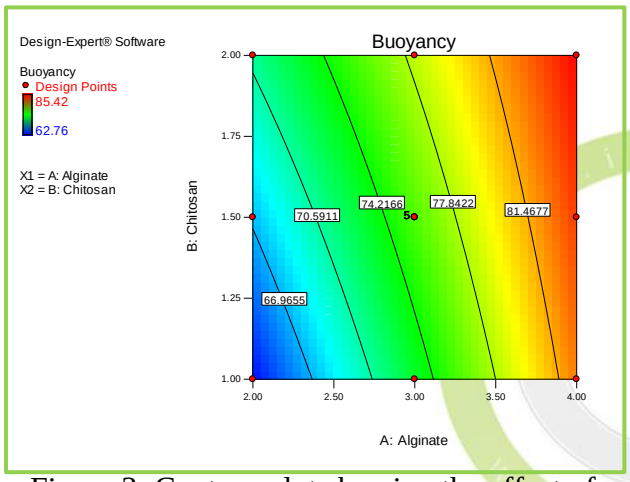


Figure 3: Contour plot showing the effect of polymer concentrations on buoyancy

The following model diagnostic plots can be plotted to investigate the goodness of fit of the proposed model.[23]

Predicted Vs. Actual

A graph is plotted between the actual and predicted response values. It helped in detecting a value or group of values that are not easily predicted by the model.Ideally, such plots passing through the origin should be highly linear, i.e.; with r^2 value close to unity.These plots were simple to construct and comprehend.They revealed the most pragmatic information of prognosis ie; whether the experimentally observed values of responses were analogs with those predicted using optimization methodology as shown in the Figure 4.

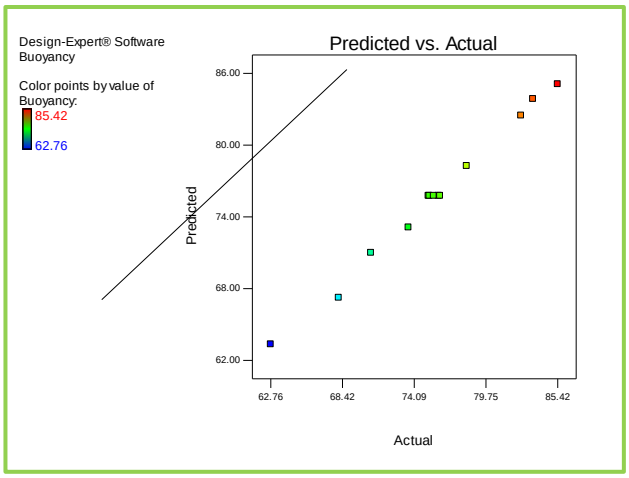


Figure 4: The model diagnostic plot of Actual Vs. Predicted showing the goodness of fit of the proposed model

Normal Probability Plot

The plot indicates whether the residual follow a normal probability distribution,in which case the points will follow a straight line when plotted on a probit scale.Shown in the Figure 5.

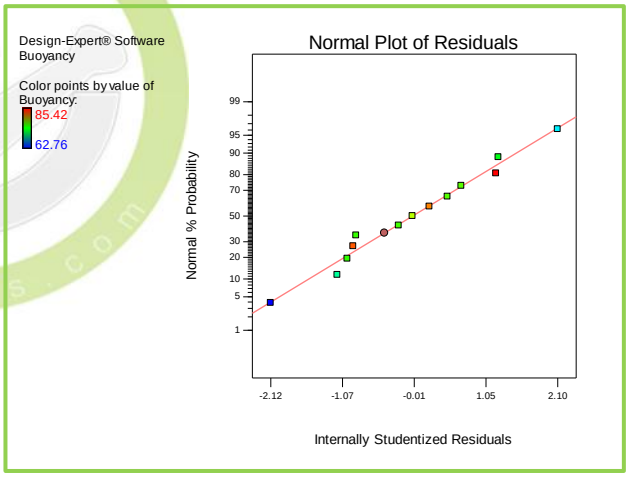


Figure 5: The model diagnostic plot of Normal probability plot showing the goodness of fit of the proposed model

Both of the plots are found to be linear and supporting the models.

The model F-value of 24.83 (table 7) in eqn.(2) implies the model is significant. Values of "Prob>F" and less than 0.0500 indicated that model terms are significant. In this case, X_1, X_2, X_2^2 are significant model terms. The" lack of fit F- value" of 4.76 implies the lack of fit is not significant. Nonsignificant lack of fit is good, and the model is fit.

The " Pred R –squared " of 0.8801 is in reasonable agreement with the " Adj R-squared" of 9085. "Adeq precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 18.093 indicated an adequate signal. So this model can be used to navigate design space.

Equation (2) suggests that the factors $X_1$and X_2 have negative effect on % drug release. As the concentration of the polymer increased % drug release from the floating microspheres gets decreased significantly. The response surface plot Figure 6 and contour plot Figure 7 were used to visualize the impact of changing variables.

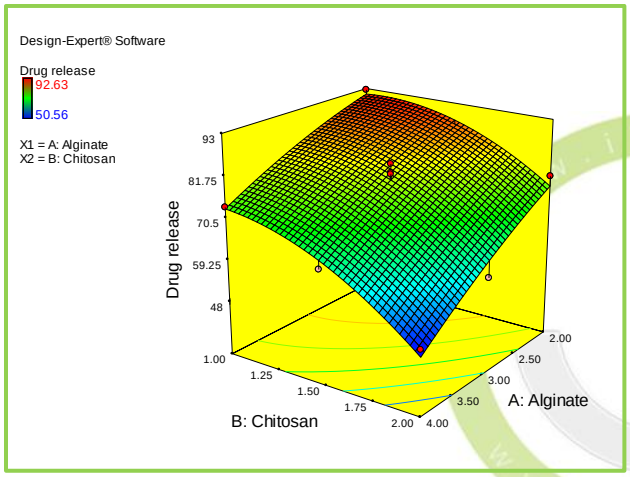


Figure 6: Response surface plot is showing the effect of polymer concentrations on cumulative drug release

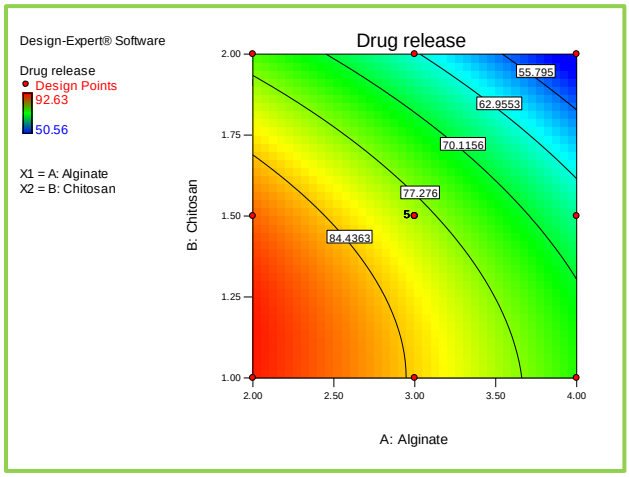
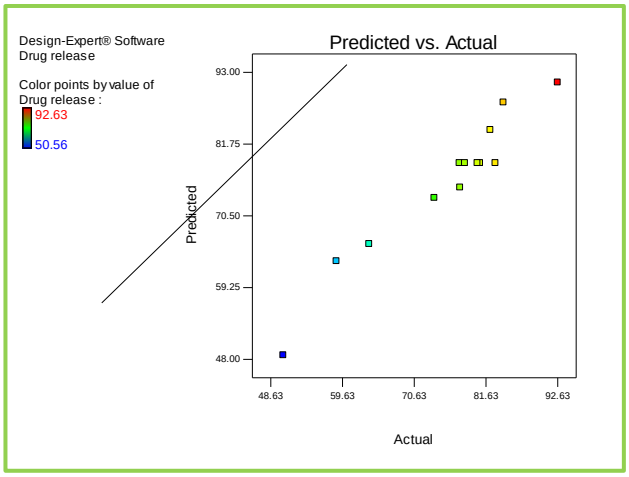


Figure 7: Contour plot showing the effect of polymer concentrations on cumulative drug release

The goodness of fit of the proposed model can be predicted by the Actual Vs. predicted response plot (Figure 8) and the normal probability plot (Figure 9). Both of them are found to be linear, supporting the models.



Figire 8: The model diagnostic plot of Actual Vs. Predicted showing the goodness of fit of the proposed model

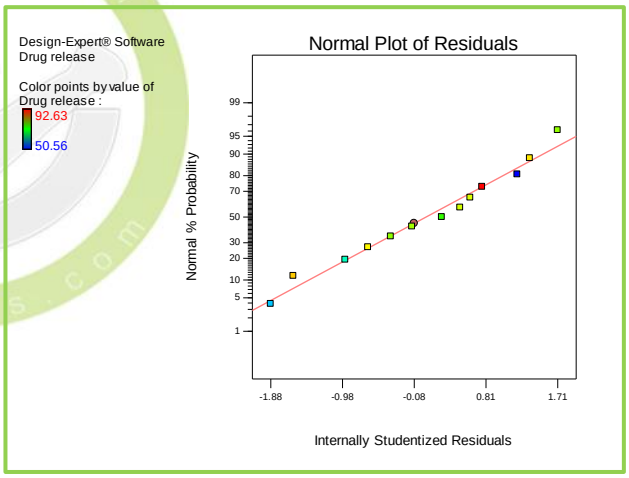


Figure 9: The model diagnostic plot of Normal probability plot showing the goodness of fit of the proposed model.

Validation of the Optimum Floating Microsphere Formulations

A numerical optimization technique using the desirability approach was employed to develop new formulations with the desired responses. Constraints like maximizing the buoyancy % (X_1) and drug release % (X_2) were set as goals to locate the optimum settings of the independent variables in the new formulation. The optimized floating formulations (S_1) was

developed using 2.92 % alginate and 1.48% chitosan and (S_2) using 3.205% alginates and 1.00% Chitosan. The optimized formulations were evaluated to find buoyancy and drug release. Table 8 enlists the value of the observed responses and those predicted by mathematical models along with the percentage prediction errors. The low value of error proved the ability of response surface methodology to predict the behavior of the drug-loaded floating formulations. Thus the low magnitude of error and significant values of R^2 in the current study indicated a high prognostic ability to float microsphere formulations of cefixime.

In vitro Drug Release Study of the Optimized Formulations

Dissolution study of the optimized formulation had been done Fig.10 shows the in vitro drug release profile.The formulations S_1 & S_2 showed 78.98 & 83.05 % drug release for 24 hours. A Prolonged release in a controlled manner was shown by the optimized formulations.

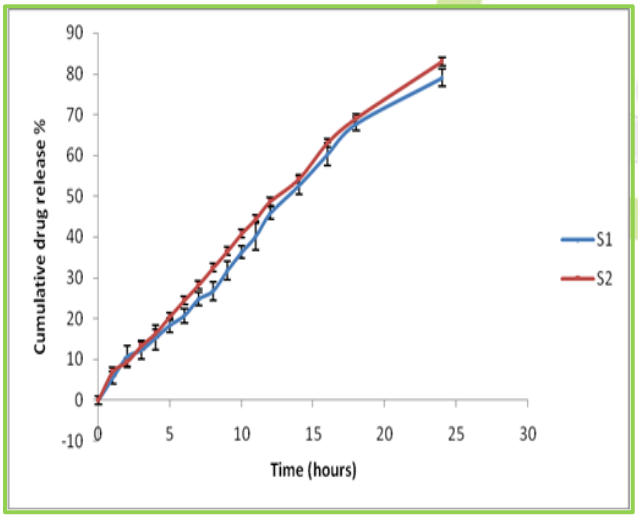


Figure 10: Cumulative drug release plots of the optimized formulations S_1& S_2 in pH 1.2 buffer

Micromeritic Studies

Micromeritic studies were conducted. The tapped density of the formulations S_1& S_2 were 0.365± 0.04, and 0.372 ± 0.02 and true density values were 0.404±0.03 and 0.420±0.02 respectively. Obviously, the density values of the floating microcapsules (<1.000 g/cm^3) were less than that of the gastric fluid (~ 1.004 g/cm^3) thereby implying that these microspheres will have the propensity to exhibit an excellent buoyancy effect in vivo. The percentage compressibility index (Carr's index) was 14.12 ±0.04 and 15.05 ±0.03. Hausner ratio values less than 1.25 indicated good flow (equivalent to 20% Carr's index) and the results are shown in table 9.

Particle Size

The particle sizes of the microspheres were determined using an optical microscope fitted with an ocular micrometer and stage micrometer. The mean particle sizes were calculated as 563.28 ±1.07 and 544.29 ±4.03 of S_1 and S_2. The particle size analysis plays an important role in determining the release characteristics and floating property.

Scanning Electron Microscopy

Surface topography, particle size, morphology and internal cross-sectional structures were investigated with a scanning electron microscope. The 3-dimensional information about the microspheres was found in the micrograph. Scanning electron micrograph of the formulation S_1 is shown in Figure 11. Figure 12 shows the cross-sectional view of the microsphere.

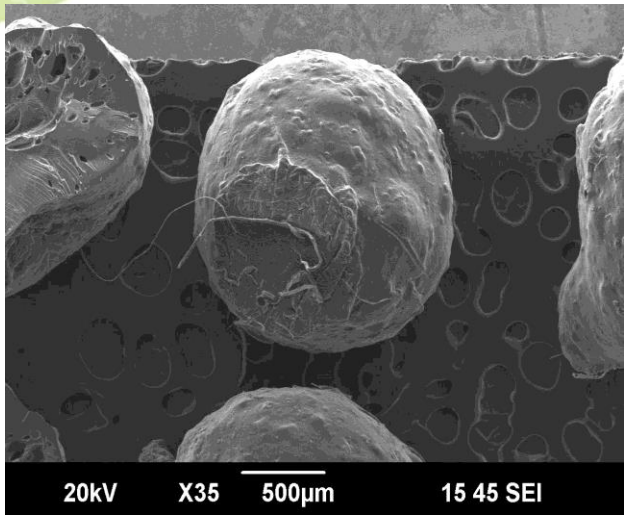


Figure 11: Scanning electron photomicrograph of optimized floating microsphere (S_1) showing the surface morphology , spherical structure, and porous nature

Table 8: Comparison of the experimentally obtained responses of the optimized formulation with that of the predicted responses

Batch code	Concentration of alginate (%)	Concentration of chitosan (%)	Response parameters	Observed value	Predicted value	%error
S_1	2.92	1.48	Buoyancy %	75.32	74.58	-0.981
			Cumulative drug release %	78.98	79.91	1.016
S_2	3.20	1.00	Buoyancy %	75.948	74.80	-1.0483
			Cumulative drug release %	80.79	79.85	1.0326

Table 9: Evaluated parameters of the optimized formulations S_1 & S_2

Batch code	Buoyancy (%)	Cumulative drug release (%)	Entrapment efficiency (%)	R^2 Value	Bulk density	Tapped density	True density	Carr's index	Hausner ratio	Particle size (µm)
S_1	75.32±3.05	78.98±2.10	83.76±3.05	0.990	0.314±0.05	0.365± 0.04	0.404±0.03	14.12 ±0.04	1.162 ± 0.032	563.28± 3.12
S_2	75.69±2.89	83.05±1.82	80.79±2.16	0.955	0.316±0.06	0.372 ± 0.02	0.420±0.02	15.05 ±0.03.	1.172 ± 0.01	544.29± 4.03

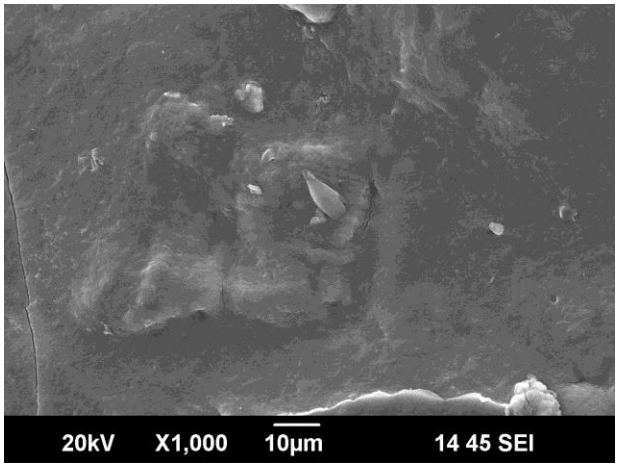


Figure 12: SEM - cross-sectional image of the floating microsphere S_1

Kinetics of Drug Release Studies

Zero-order Kinetics

The dissolution data of the optimized formulations S_1 and S_2 were plotted in accordance with zero order equation and the plot was made as the cumulative % of drug released vs. time,shown in Figure 13(a) and 13(b). The correlation coefficient R^2 was found to be 0.989 and 0.990 from the graph.

First order Kinetics

The dissolution data of the optimized formulations S_1 and S_2 were plotted in accordance with first order equation. Log cumulative of % drug remaining vs. time is

plotted and the plots are shown in Figure 14(a) and 14(b). The correlation coefficient R^2 is found to be 0.967 and 0.911 from the graph.

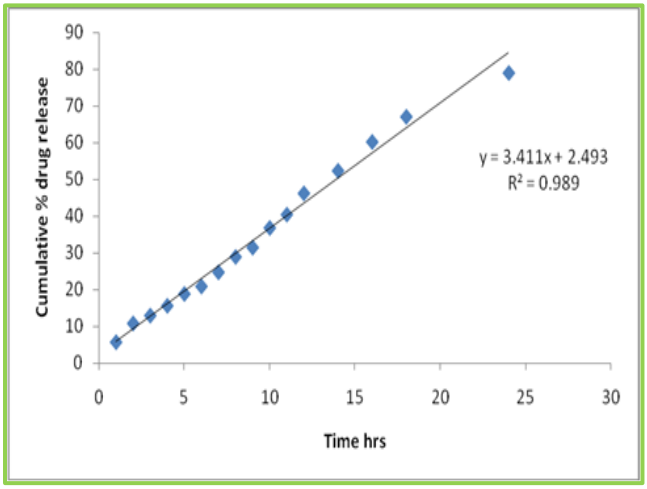


Figure 13 (a): Zero-order release model of S_1

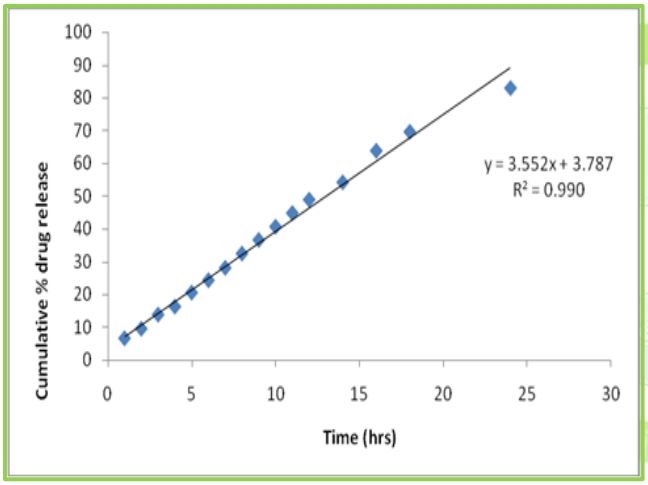


Figure 13 (b): Zero-order release model of S_2

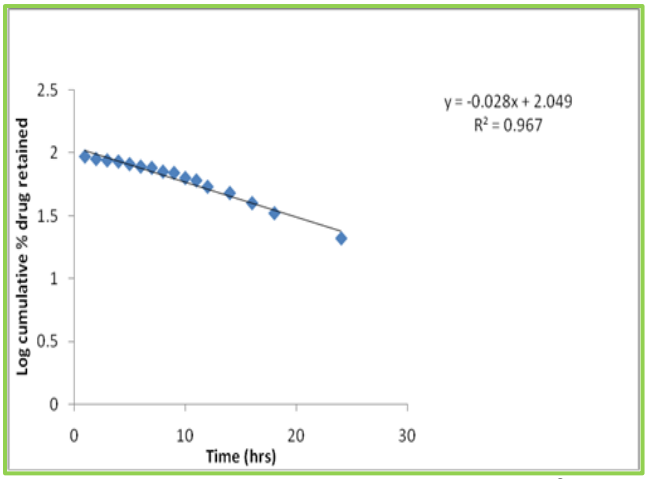


Figure 14 (a): First order release model of S_1

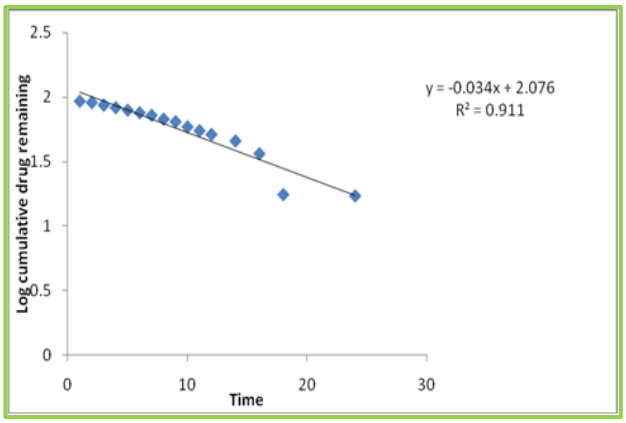


Figure 14 (b): First order release model of S_2

Higuchi Model

Higuchi model is useful to identify the mechanism of drug release. The dissolution data of the optimized formulations is plotted as cumulative % of drug released vs. square root of time. The plots for S_1 and S_2 are Figure 15(a) and 15(b) respectively. The correlation coefficient R^2 is found to be 0.957 and 0.973 from the graph.

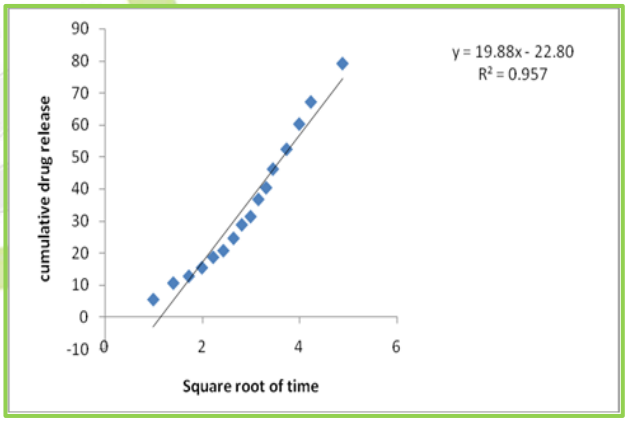


Figure 15(a): Higuchi release model of S_1

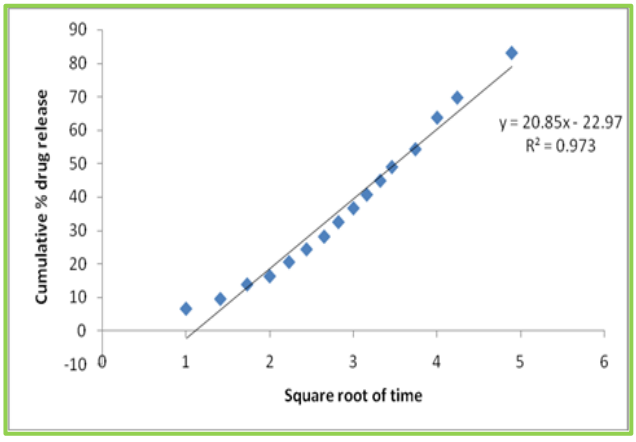


Figure 15 (b): Higuchi release model of S_2

Korsmeyer Peppas Model

The plot was used to decide the drug release which follows a Fickian or non Fickian release pattern. The dissolution data of the optimized formulations are plotted in accordance with Peppas model as log cumulative % drug released vs. log time and the plots are shown in Figure 16(a) and 16(b). The exponent 'n' is observed as 0.848 and 0.868 for the formulations S_1 and S_2 respectively.

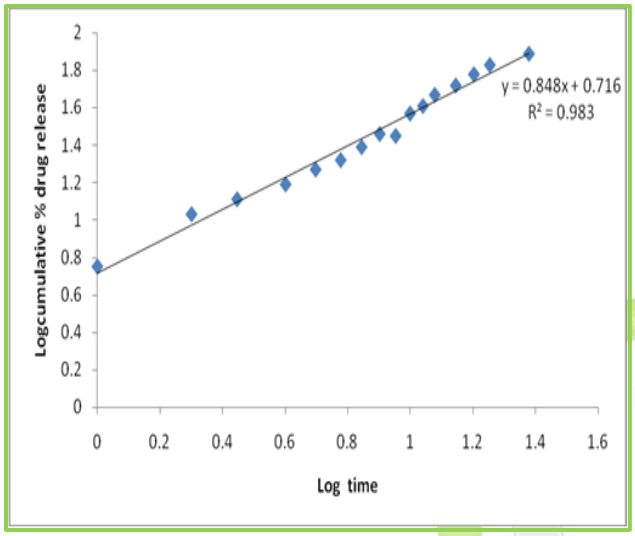


Figure 16(a): Korsmeyer- Peppas model for mechanism of drug release of S_1

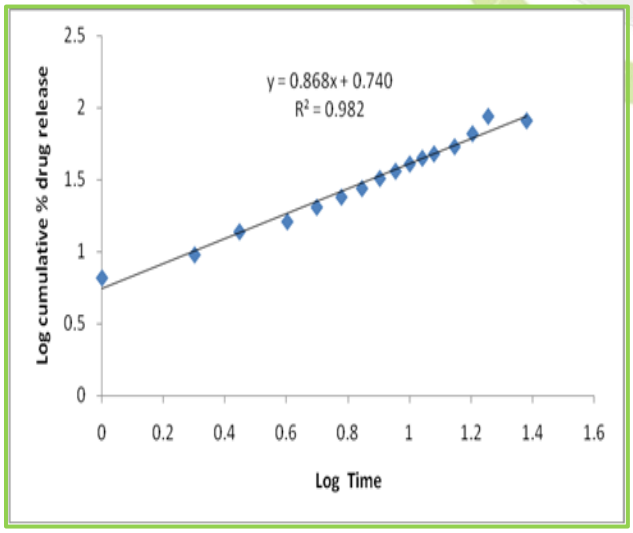


Figure 16(b): Korsmeyer- Peppas model for the mechanism of drug release of S_2

Hixson- Crowell Cube Root Model

The Hixson- Crowell cube root law described the release from systems where there was a change in surface area and diameter of particles or tablets with time. The plots were made according to the cube root of drug % remaining in matrix vs. time. The plots are shown in the Figure 17(a) and 17(b) for the formulations $S_1$and S_2. The correlation coefficient R^2 is found to be 0.985 and 0.984 from the graph.

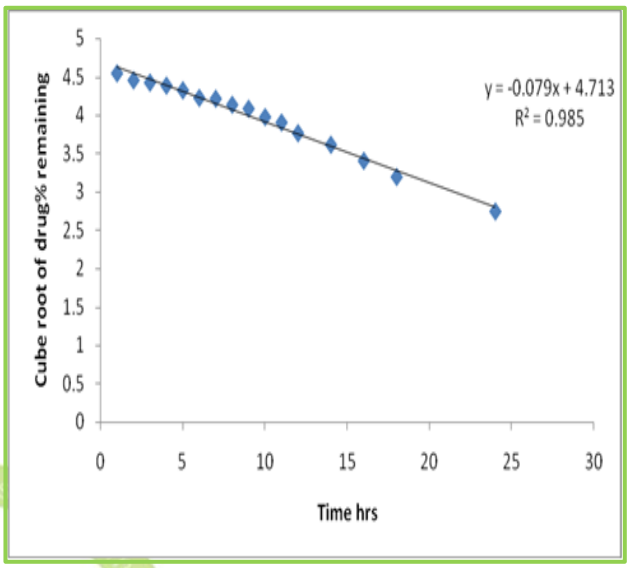


Figure 17(a): Hixson – Crowell cube root plot of the sustained release mechanism of S_1

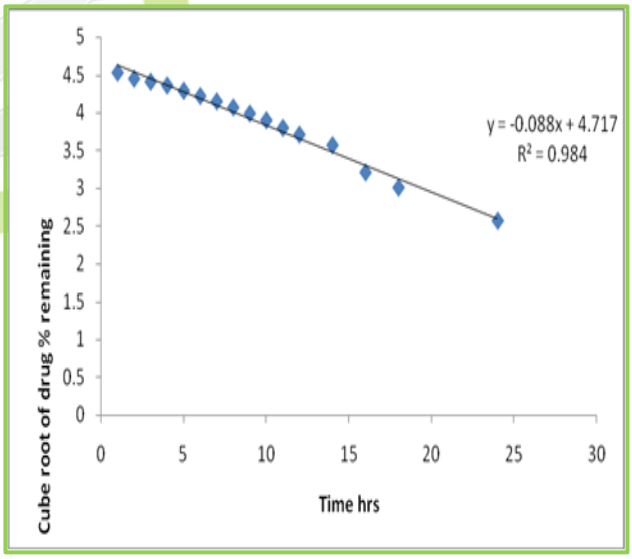


Figure 17(b): Hixson – Crowell cube root plot of the sustained release mechanism of S_2

Comparing the values of correlation coefficient (R^2) of different kinetic models (Table 10) reveal that the optimized formulations release the drug in the zero order manner with non-Fickian diffusion mechanism based on the regression values of zero order, Higuchi, and Korsmeyer-Peppas model.

Table 10: Comparison of different dissolution kinetics models of the optimized formulations

Formulation code	Kinetic models				
	Zero-order release (R^2)	First order release (R^2)	Higuchi (R^2)	Hixson-Crowell(R^2)	Korsmeyer-Peppas (n)
S_1	0.989	0.967	0.957	0.985	0.848
S_2	0.990	0.911	0.973	0.984	0.868

CONCLUSION

Alginate- chitosan floating microspheres of cefixime trihydrate were prepared successfully, and the process parameters were optimized using 3^2 full factorial design. Buoyancy and cumulative drug release % of all the nine batches were evaluated and compared. From the regression analysis, it is observed that increase in the concentration of polymers increased the buoyancy of the formulation and decreased the cumulative drug release percentage. The numerical optimization technique using the desirability approach was employed to develop optimized formulations with maximum buoyancy and cumulative drug release %. These formulations were evaluated, and the results were compared with those predicted by the mathematical models. The low magnitude of error and significant value of R^2 indicated the high prognostic ability of floating microspheres of cefixime. Morphology and internal cross-sectional structures were investigated using SEM. Kinetics of drug release were conducted to determine the mechanism of drug release. Further, in vivo studies are required to evaluate the therapeutic potential of the delivery system.

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