Research Article

FORMULATION AND EVALUATION OF FLOATING MICROSPHERES OF CEPHALEXIN

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ABSTRACT

The purpose of this research was to prepare a floating drug delivery system of Cephalexin. In the present study, preparation of Cephalexin floating microspheres, *in-vitro* evaluation of Floating Drug Delivery System (FDDS), prediction of the drug release, and optimization of stirring speed and polymers concentration to match target release profile was investigated. Floating microspheres were prepared by emulsion solvent evaporation technique using EthylCellulose (EC) as the rate controlling polymer. Particle size analysis, drug encapsulation efficiency, surface topography, buoyancy percentage and release studies were performed. Results showed that the polymer concentration and stirring speed affected the size, incorporation efficiency and drug release of microspheres (> 12 h) and its floating time (> 12 hr). The best results were obtained at the ratio of drug: EC (1:6). The mean particle size of prepared floating microspheres increased but the drug release rate from the microspheres decreased as the polymer concentration increased. The developed floating microspheres of Cephalexin may be used in clinic for prolonged drug release in stomach for at least 12 hrs, thereby improving the bioavailability, prevents degradation in stomach and patient compliance.

Keywords: Floating drug delivery system (FDDS), Cephalexin, Microspheres, Gastro retentive, *in-vitro* release.

INTRODUCTION

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then maintain the desired drug concentration. The most convenient and commonly employed route of drug delivery has historically been by oral ingestion. Drugs that are easily absorbed from the GIT and having a short half-life are eliminated quickly from the blood circulation. To avoid these problems oral controlled drug delivery systems have been developed as they releases the drug slowly into the GIT and maintain a constant drug concentration in the serum for longer period of time. However, incomplete release of the drug and a shorter residence time of dosage forms in the upper gastrointestinal tract, a prominent site for absorption of many drugs, will lead to lower bioavailability. Efforts to improve oral drug bioavailability have grown in parallel with the pharmaceutical industry. As the number and chemical diversity of drugs has increased, new strategies are required to develop orally active therapeutics. Thus, gastro retentive dosage forms, which prolong the residence time of the drugs in the stomach and improve their bioavailability, have been developed. One of the most feasible approaches for achieving a prolonged and predictable drug delivery profile in the GI tract is to control the gastric residence time i.e. Gastro Retentive Dosage Forms (GRDFs). These are primarily controlled release drug delivery systems, which gets retained in the stomach for longer periods of time, thus helping in absorption of drug for the intended duration of time. Gastric retentive drug delivery devices can be useful for the spatial and temporal delivery of many drugs[1]. Thus, control of placement of a DDS in a specific region of the GI tract offers numerous advantages, especially for drug exhibiting an 'absorption window' in the GI tract. The intimate contact of the DDS with the absorbing membrane and also the potential to maximize drug absorption may influence the rate of drug absorption. These considerations have led to the development of oral controlled release (CR) dosage forms in the form of floating microspheres of Cephalexin possessing gastric retention capabilities.

MATERIALS AND METHODS

Materials

Cephalexin was obtained as a gift sample from Innova Cap Tab (Chandigadh). Ethyl cellulose (EC) and PVA (0.5%) were obtained from Qualikems Fine Chemicals Pvt. Ltd (Gujarat). Dichloromethane (DCM) and Acetone were obtained from Aatur Instra Chemicals Pvt. Ltd. (Vadodara). All other chemicals / reagents used were of analytical grade, available commercially and used as such without further processing. A UV spectrophotometer (Shimadzu, UV-1700, Pharmaspec.) was used for drug analysis.

Methods

Preparation of Microspheres

Microspheres were prepared by emulsion solvent evaporation (w/o/w) technique[2]. Cephalexin was dissolved in aqueous media. Ethyl cellulose (EC) was dissolved in a mixture of Acetone and dichloromethane (2:1) at room temperature. The mixture of drug was poured to the mixture of organic solvent containing polymer by continuous stirring. This was poured into 200 ml water containing 0.5% PVA and subsequently stirred at ranging agitation speed (550rpm to 950rpm) for 2 to 3 hrs to allow the volatile solvent to evaporate. The

microsphere formed were filtered, washed with water and dried in vacuum.

Application of Full Factorial Design[3]

For the present work, factorial design was applied to develop an optimized formulation.

3^2 Full Factorial Designs

In the present investigation, the ratio of Stirring speed (X_1) and the polymer concentration (X_2) were selected as independent variables as shown in table 1a.

Table 1a: Independent variables

X_1:	POLYMER CONCENTRATION
X_2:	STIRRING SPEED

The time required for 80 % of drug release ($T_{80\%}$), the % drug encapsulation efficiency and particle size (µm) were selected as dependent variables as shown in table 1b.

Table 1b: Dependent variables

Y_1:	Particle size (micron)
Y_2:	% Drug encapsulation efficiency
Y_3:	$T_{80\%}$ (min)

The other optimized variables are listed below in table 1c:

Table 1c: Optimized variables

Volume of aqueous phase	10 ml
Volume of organic phase	20 ml
Concentration of PVA	0.5% v/v

Transformation of actual values

In this design, three factors were evaluated each at 3 levels in such a way that **low** level was **(-1), medium** level **(0)** and **high** (+**1).** Experimental trials were performed using all possible nine combinations as per the design layout shown in Table 1d.

Table 1d: *A full 3^2 factorial design layout*

Batch	$X_{1(\%)}$	$X_{2(rpm)}$
S_1	8.33	550
S_2	10.42	550
S_3	12.50	550
S_4	8.33	750
S_5	10.42	750
S_6	12.50	750
S_7	8.33	950
S_8	10.42	950
S_9	12.50	950

The results obtained from the experiment were statistically analyzed for response variables by using **Design expert 8.0.5.2 version.** The design was evaluated by a factorial linear interactive first order model:

$$Y = b_0 + b_1X_1 + b_2X_2 + + b_{12}X_1X_2$$

Where Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs, and b_i is the estimated coefficient for the factor X_i. The main effect (X_1 and X_2 represents the average result of changing one factor at a time from its low medium to high value. The interaction terms X_1, X_2 shows how the response changes when two factors are changed simultaneously.

In-vitro evaluation of floating microspheres of Cephalexin

Determination of percent yield[4]

Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was then calculated.

Particle size analysis[5]

Particle size of prepared microspheres was measured using an optical microscope, and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer.

Determination of drug encapsulation efficiency[6]

The floating microspheres equivalent to 10 mg of Cephalexin were accurately weighed and crushed. The powdered of microspheres were dissolved in *dichloromethane* (5 ml) in volumetric flask (100ml) and made the volume with 0.1 N HCl. This solution was then filtered through Whatman filter paper No. 44. After suitable dilution the absorbance was measured at 257 nm using UV spectrophotometer using 0.1N HCL as a blank and corresponding drug concentrations in the sample were calculated from calibration plot and the *percentage* drug encapsulated was calculated by following formula:

$$\% \text{ Drug content} = \frac{\text{Calculated amount of cephalexin} \times 100}{\text{Total weight of the hollow microspheres}}$$

$$\% \text{ Theoretical content} = \frac{\text{Total weight of cephalexin} \times 100}{\text{Total weight of cephalexin and Ethyl cellulose}}$$

$$\% \text{ Drug encapsulation} = \frac{\% \text{ Drug content} \times 100}{\%\text{Theoretical content}}$$

In-vitro dissolution studies in 0.1N HC1[7]

A USP basket apparatus has been used to study drug release from the prepared floating microspheres. The microspheres equivalent to 100 mg Cephalexin were filled in "0" size transparent hard gelatin capsules. In the present study, drug release was studied using a modified USP XXVII dissolution apparatus type I (basket mesh # 120) at 100 rpm in 0.1 mol/l hydrochloric acid (pH 1.2) as the dissolution fluid (900 ml) maintained at 37±0.5°C. The withdrawn samples (5ml) were analyzed spectrophotometrically as stated above. The volume was replenished with the same amount of fresh dissolution fluid each time to maintain the sink condition. The dissolution data of different batches are shown in (table 5) and respective release profiles are also depicted in (figures 7, 8 and 9).

Floating behaviour (buoyancy)[8]

50 mg of the microspheres were placed in 100 ml of simulated gastric fluid (pH 1.2) containing 0.02% w/v

Tween 20. The mixture was stirred at 100 rpm on a magnetic stirrer. After 4 h, the layer of buoyant microspheres was pipetted and separated by filtration; particles in the sinking particulate layer were also separated by filtration. Particles of both types were dried in desiccators. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

SEM study[9]

The surface topography and internal textures of the microspheres was observed by scanning electron microscopy.

Mechanism of release[10]

The mechanism of release was determined by fitting the release data to the various kinetic equations such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas and finding the R^2 values of the release profile corresponding to each model.

RESULTS AND DISCUSSION

Percent yield

Here on the basis of % yield batches are selected for formulation, Out of eight batches (E1-E8), two batches E1 & E2 showed a yield of more than 70%. Percentage yield is found to be higher with formulation batch E1. Percentage yield increases with increase in the amount of polymer concentration as shown in table 2.

Particle size analysis

Results showed that particle size of prepared microspheres was in the range of 239±1.56 µm to 491 ± 0.95 µm. It was concluded that with increase in polymer concentration, particle size of prepared microspheres increases as shown in table 3 and figure 1 and 2.

Table 2: % yield of Microspheres during formulation

Batch No	Polymer:drug ratio	Product characteristic	Aggregation	% yield
E1	6:1	Spherical	*	78
E2	5:1	Spherical	*	70
E3	4:1	Spherical	*	63
E4	3:1	spherical	*	59
E5	2:1	Spherical	*	55
E6	1:1	Spherical	**	53
E7	1:1.5	Spherical	**	49
E8	1:2	Irregular	***	-----

*= low aggregation; **= High aggregation

Table 3: Effect of Polymer concentration on Particle size

Batch code	Y_1 (Particle size)
S1	475±1.32
S2	481±1.53
S3	491±0.95
S4	375±0.99
S5	385±1.17
S6	390±1.11
S7	239±1.56
S8	250±0.99
S9	275+1.56

Mean ± S.D., n=3

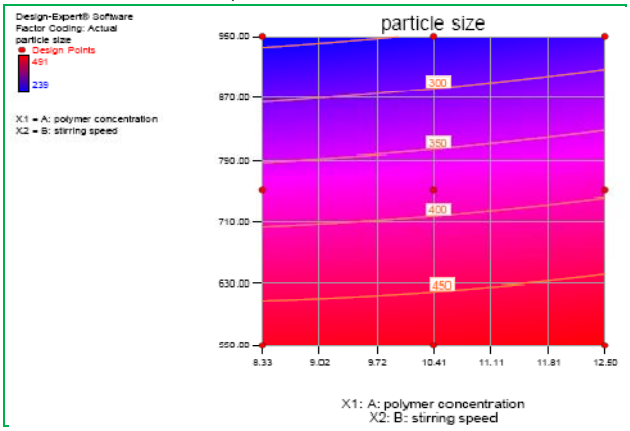

Figure 1: Counter plot of effect of X_1 and X_2 on (Y_1) particle size

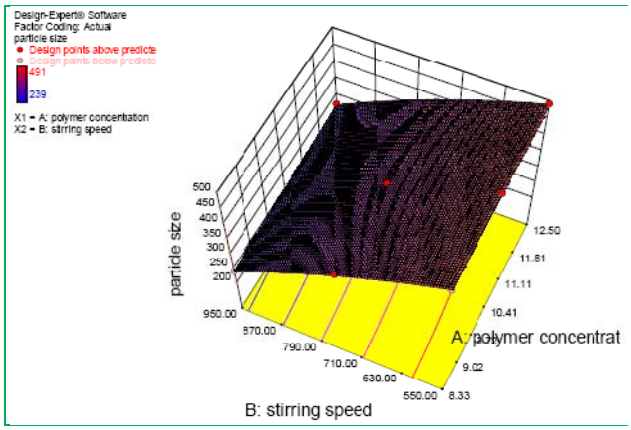

Figure 2: Response surface model of effect of X_1 and X_2 on (Y_1) particle size

Encapsulation efficiency

All batches show percent encapsulation more than 59% and it is found that encapsulation of drug increases with an increase in the amount of the polymer. Formulation S3 shows maximum entrapment whereas formulation S7 shows minimum entrapment of the Cephalexin in the polymer as shown in table 4 and in figure 3 and 4.

Table 4: Results of dependent variables (% encapsulation efficiency)

Batch code	Y_2 (% encapsulation efficiency)
S_1	67.95±1.20
S_2	74.28±0.80
S_3	81.93±0.90
S_4	64.74±1.17
S_5	72.23±0.75
S_6	77.45±1.11
S_7	59.52±1.56
S_8	70.47±0.30
S_9	74.16±0.80

Mean ± S.D, n=3

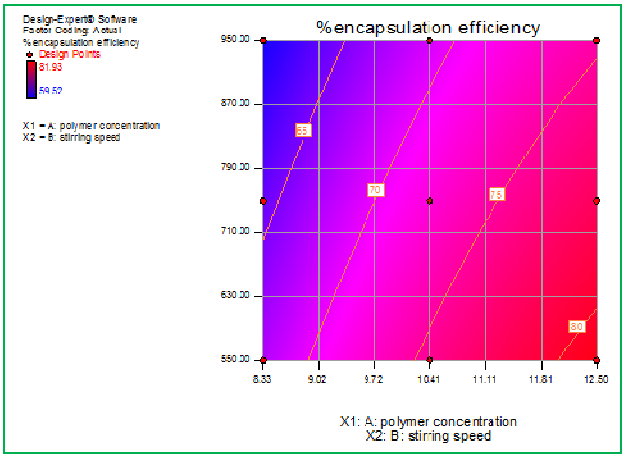

Figure 3: Counter plot of effect of X1 and X2 on (Y_2) encapsulation efficiency

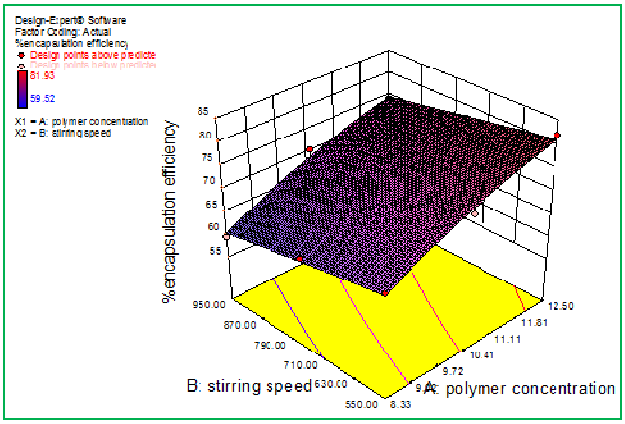

Figure 4: Response surface model of effect of X_1 and X_2 on (Y_2) Encapsulation efficiency

In Vitro Drug Release Study

In vitro dissolution studies of Cephalexin from floating Microspheres were performed in 0.1 N HCL (pH 1.2) for 12 hrs using USP basket type dissolution test apparatus. It was found that formulation S1, S2 and S3 showed 70.5% to 78.93 of release at 8hr and as drug release was not sustained considerably, as the EC concentration was increased there was further retardation in drug release. For formulation S4, S5 and S60, the drug release was 70.42% to 72.2 % within 8 hr (Table 5). Formulation S7, S8 and S9 showed 52.43% to 54.28 % of release at 8hr. Moreover, from the results it is also clear that no burst effect was seen and drug release was significantly sustained. It was observed that as the concentration of EC increased the % cumulative release of cephalexin decreased. The increase in EC concentration leads to the formation of high density polymer matrix.

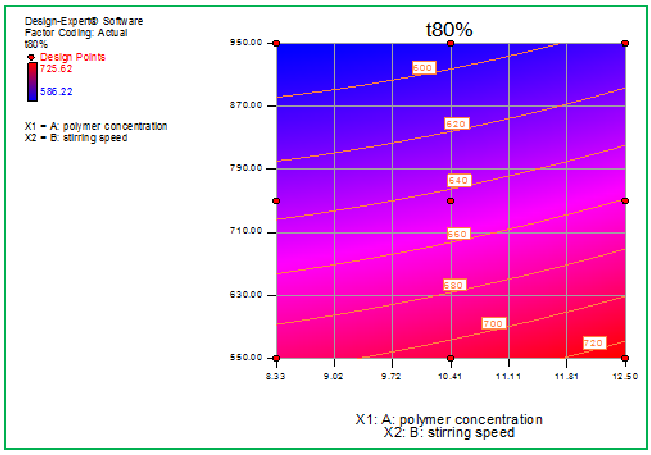

Figure 5: Counter plot of effect of X_1 and X_2 on t80% (Y_3)

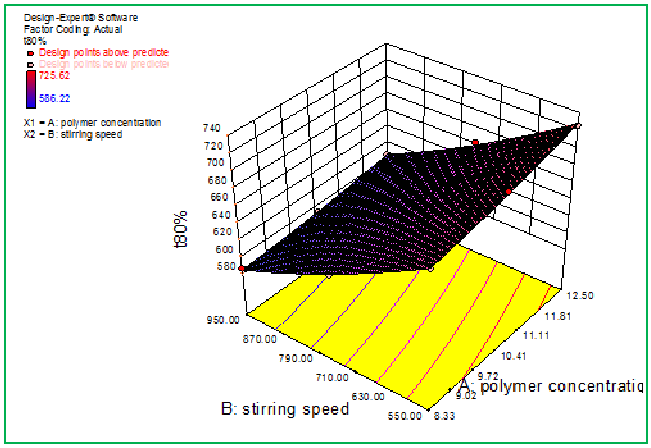

Figure 6: Response surface model of effect of X_1 and X_2 on $t_{80\%}$

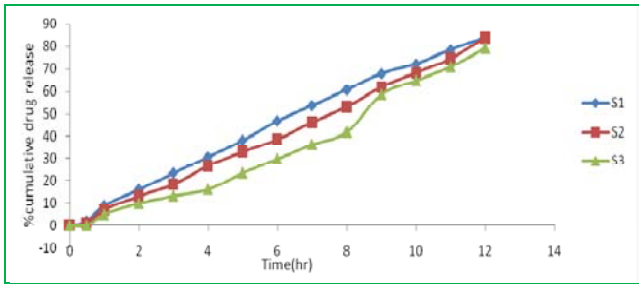

Figure 7: Graph of % cumulative drug release vs Time (hr) for batch S1 to S3

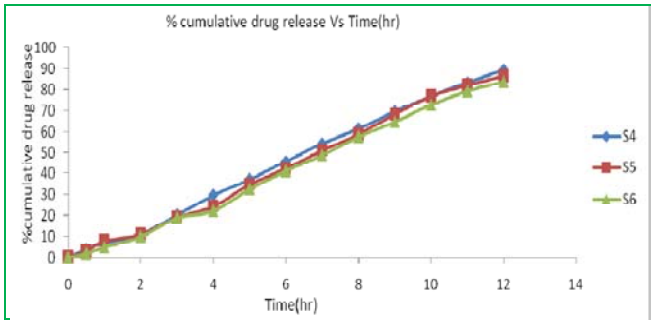

Figure 8: Graph of % cumulative drug release vs Time (hr) for batch S4 to S6

Table 5: % Cumulative Drug Release of batch S1 to S9

TIME (hr)	Cumulative % Drug Release of Batch S1 to S9								
	S1	S2	S3	S4	S5	S6	S7	S8	S9
0	0	0	0	0	0	0	0	0	0
0.5	1.97	0.987	0.074	3.94	2.96	1.97	6.9	4.93	2.96
1	8.92	6.92	4.93	6.99	7.93	4.93	10.94	8.92	7.93
2	16.02	12.99	9.86	11.09	11.09	10.01	18.08	14.07	13.42
3	23.27	18.21	13.2	20.2	18.98	18.89	27.34	24.26	23.18
4	30.67	26.49	16.13	29.51	24.32	22.26	36.8	33.65	31.57
5	38.22	32.96	23.38	37.04	34.7	32.6	44.48	42.25	39.14
6	46.91	38.58	29.79	45.71	42.34	41.18	53.3	52.01	47.85
7	53.79	46.28	36.33	54.54	51.11	48.94	60.31	58.02	54.76
8	60.81	53.15	42.02	61.57	59.07	57.85	67.71	64.13	63.77
9	67.95	61.93	58.75	69.73	68.42	64.95	76.7	74.3	71.96
10	72.26	68.27	64.86	77.04	77.04	73.16	81.18	81.74	79.32
11	78.61	74.57	71.08	83.5	82.15	79.55	89.8	88.28	86.8
12	84.1	84.1	79.38	89.5	86.74	84.1	98.17	96.91	93.22

Table 6: % Buoyancy with respect to time

Time (hr)	% Buoyancy of Cephalexin loaded Microspheres								
	S1 (%)	S2 (%)	S3 (%)	S4 (%)	S5 (%)	S6 (%)	S7 (%)	S8 (%)	S9 (%)
1	100	100	99	100	99	98	100	98	97
2	100	100	99	99	98	96	99	97	97
3	100	99	98	99	96	96	98	96	96.5
4	98	98	98.5	96	95	95	97	95	94
5	98	97.5	96	95	95	94	94.5	93	93
6	97.5	97	95	93	93	92	92	91	90.5
7	97	96	93	93	92	90	90	89	88
8	97	96	93	92	91	89	89	88.5	87.5
9	96	95.5	91.5	91	88	88.5	87	87	86
10	94.5	94	90	90.5	87	87	86.5	84.5	84
11	93	92	89	90	86.5	85.5	83	83	81
12	92	91	88.5	88	85	84	81.5	80	79

Table 7: Micromeritics properties

Batches	Angle of repose (°)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's index	Hausner's ratio
S_1	19.29±0.22	0.350±0.013	0.394±0.006	11.16±0.231	1.12±0.34
S_2	21.00±0.34	0.375±0.009	0.434±0.009	13.59±0.942	1.15±0.32
S_3	22.19±0.29	0.400±0.110	0.450±0.003	11.11±0.620	1.12±0.23
S_4	18.67±0.18	0.412±0.050	0.471±0.005	12.61±0.742	1.14±0.37
S_5	22.58±0.65	0.437±0.060	0.507±0.010	13.80±0.426	1.16±0.28
S_6	24.95±0.22	0.462±0.007	0.521±0.007	11.32±0.378	1.12±0.33
S_7	19.29±0.65	0.487±0.060	0.557±0.015	12.59±0.672	1.14±0.34
S_8	25.17±0.54	0.525±0.090	0.583±0.009	10.17±0.722	1.11±0.07
S_9	27.11±0.27	0.562±0.030	0.633±0.016	11.21±0.465	1.12±0.14

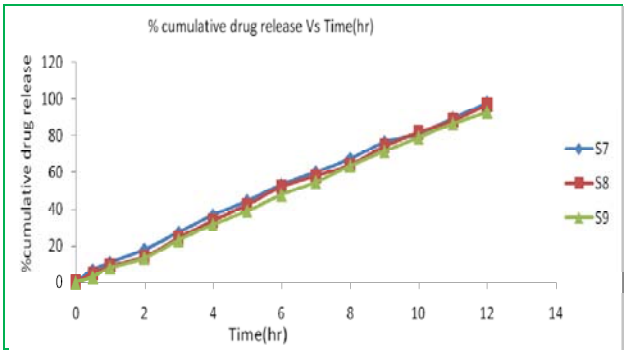

Figure 9: Graph of % cumulative drug release vs Time (hr) for batch S7 to S9

Optimized Formulation

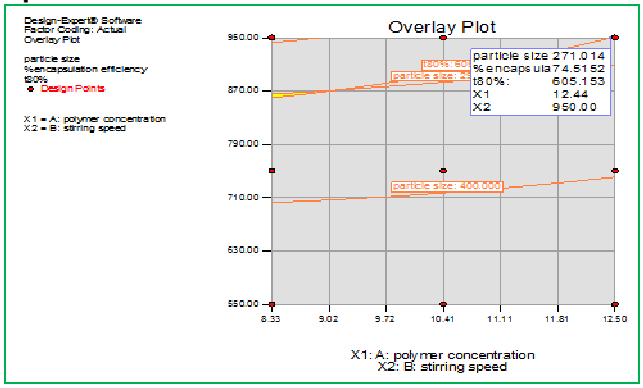

Figure 10: Overlay plot of all the responses

Here, overlay of all three responses were taken and optimized region was identified. Optimized region showed the optimum concentration of X_1 and X_2 with desired drug release ($T_{80\%}$), good % encapsulation efficiency and smaller particle size. Predicted values from the above overlay plot for responses Y_1, Y_2 and Y_3 are 605.15 mins, 74.51% and 271 µm respectively.

Floating ability (Percent buoyancy)

The formulated batches of floating microspheres of Cephalexin showed average buoyancy more than 90%. Amongst the batches of prepared microspheres, batch S1 showed highest buoyancy as shown in table 6.

SEM (scanning electron microscopy) study

Results showed that ethyl cellulose microspheres of Cephalexin were predominantly spherical in shape with smooth surface. The porous nature and characteristics internal structure of the microspheres, enclosed with the rigid shell constructed with drug and polymer was clearly evident. The porous nature and cavity formed in the microspheres would dictate the floating behaviour of microspheres of Cephalexin, as shown in figure 11.

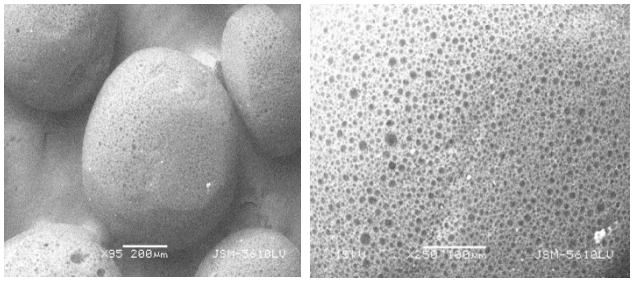

Figure 11: % SEM study of floating microspheres of Cephalexin

Mechanism of release

The results were used for the selection of the most appropriate model. The goodness of fit test proposed by Bemba and co-workers was used to determine the kinetics of drug dissolution profile. The release profile of the optimized batch, which showed, correlation co-efficient 0.998 of zero order model fitting to optimized (check point batch). Higuchi model also showed good correlation of 0.92. The values of slope and intercept for Zero order models are 0.332 and 8.24 respectively. Thus it may be concluded that the drug release from floating microspheres of cephalexin is best explained by zero order. In k-peppas release exponent (n) is higher than 1.0 so drug mechanism is super case 2 transport.

Kinetic Modelling of the drug release was carried out on the drug release was carried out on the optimized batch formulation.

Table 8: Results of Model Fitting of optimized Batch

	Intercept	Slope	R^2
Zero order plot	8.24	0.322	0.773
First order plot	0.0125	0.753	0.998
Higuchi	30.48	19.21	0.920
Korsmeyer peppas	1.17	0.749	0.828

Optimized Batch

Criteria for the optimized batch

The time required for 80% drug release: 8-10hr.

The % drug encapsulation efficiency: maximum and the particle size (µm): 250-400 µm.

Table 9: Evaluation of check point batch K_{10}

Parameter	Optimized formulation
% Floating of microspheres	98% up to 12hrs
Bulk density	0.355±0.003
Tapped density	0.421±0.002
Compressibility index	15.67±0.685
Hausner's ratio	1.18±0.32
% Encapsulation efficiency	73.21%
Particle size (µm)	270-275 µm

CONCLUSION

The present study reports the developments of drug loaded floating microspheres by emulsion solvent evaporation method. The concentration of EC and stirring speed affect the size and yield of microspheres. Concentration of EC has significant effect on the floating ability as well drug release. EC having good encapsulation efficiency and drug release retarding ability. It is non toxic in nature. Therefore, various concentration of EC was selected and optimization carried out for floating ability, Encapsulation efficiency and release study. 3^2 full factorial designs carried out for optimization of the parameter and it shows the effect of variables. The microspheres exhibited good encapsulation efficiency, excellent floating and micromeritics properties. Encapsulation efficiency of microspheres is around 70%. Thus, such floating microspheres of cephalexin prove to be formulating that can be used for prolonged gastric residence of the drug, better bioavailability and prevention of degradation.

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