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DESIGN AND STATISTICAL OPTIMIZATION OF ACYCLOVIR LOADED HOLLOW MICROSPHERES USING CELLULOSE ACETATE

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ABSTRACT
Acyclovir is an antiviral drug, having absorption window in the upper intestinal tract and an absolute bioavailability of 30%. Its short biological half-life and recommended adult oral dosage necessitates the development of a controlled release formulation. The present investigation conceptualizes a specific technology to ascertain the gastro retentivity of acyclovir by non-aqueous solvent evaporation method using cellulose acetate. A 3^2 full factorial design was employed to elucidate the effect of formulation factors of drug loaded hollow microspheres. The effect of two factors, polymer concentration (X_1) and stirring speed (X_2) as independent variables were studied on Y_1(% yield value), Y_2(% entrapment efficiency), Y_3(size of microsphere), Y_4(% buoyancy), Y_5(T_{50}, time to release 50 % of drug) and Y_6(T_{100}, time to release 100% drug) selected as dependent variables. The micromeritic properties like angle of repose, Hausner's ratio and Carr's index were within the limits suggesting good flow properties. The percent yield value, entrapment efficiency and buoyancy were about 79-97%, 49-70% and 10-92%, respectively. The size of microspheres (76-254 µm) was also found to be increasing with increase in polymer concentration and decreasing with rpm. *In vitro* dissolution test shows T_{50} at 2.0-2.9 h and T_{100} at 4-11 h for different formulations exhibiting zero order release kinetics following non-fickian diffusion release mechanism. The optimal formula contains X_1 (polymer) as 1095 mg and X_2 (stirring speed) as 1183 rpm with a desirability of 0.91. The prepared hollow microspheres of acyclovir could be used as twice a day capsule for enhancing the therapeutic activity.

KEYWORDS: Acyclovir, Bioavailability, Factorial Design, Microspheres.

INTRODUCTION
Acyclovir is an antiviral drug, belonging to the deoxiguanosine family. It is widely prescribed for the treatment of herpes simplex virus infections, as well as in the treatment of herpes zoster. Bioavailability of acyclovir is 10-20% when given orally owing to an important first pass metabolism. It has an elimination half-life of 2-3 h and has an absorption zone from the upper intestinal tract. The recommended adult oral dosage of acyclovir is 200 mg twice daily or 400 mg once daily. The effective treatment of genital herpes simplex requires administration of 1000 mg of acyclovir in 5 divided doses a day. An alternative dose of 800 mg leads to plasma fluctuations; thus a controlled release dosage form of acyclovir is desirable. The short biological half-life of drug (~2.5 hours) also favors development of a controlled release formulation.[1,2]

Acyclovir is absorbed only in stomach and the initial part of the small intestine and has 30% absolute bioavailability. In context of the above principles, a strong need was recognized for the development of a dosage form to deliver acyclovir in the stomach and to increase the efficiency of the drug, providing controlled action. Some of the most promising excipients that have been used commonly in these drug delivery systems include cellulose acetate, chitosan, eudragit, acrycoat, methocil, polyacrylates, polyvinyl acetate, carbopol, agar, polyethylene oxide, polycarbonates, acrylic resins and polyethylene oxide etc.[3,4] Previous research disclosed that floating matrix tablets and floating effervescent microspheres of acyclovir were prepared by using various bio adhesive polymers.[5-8] Therefore the present research endeavor directed towards development non-effervescent floating hollow microspheres of acyclovir to enhance the drug absorption, bioavailability, gastric residence time and therapeutic efficacy of the drug.[9,10]

A 3^2 full factorial design was developed to study the effect of formulation variables on the floating microspheres.[11] The prepared microspheres were evaluated for their micrometric properties like bulk density, tapped density, angle of repose, Carr's index and Hausner's ratio. All the prepared formulations were evaluated for particle size, percent yield, entrapment efficiency, buoyancy, surface morphology, *in vitro* dissolution studies and release kinetics.[12-15] The optimized formula with maximum desirability was cross validated with the predicted values at 5% level of significance using the statistical package Design-Expert®.

MATERIALS AND METHODS

Materials

Acyclovir was a gift sample from Dr. Reddy's Laboratories. Cellulose acetate, span 80 was purchased from S.D chemicals. Light liquid paraffin and petroleum ether were purchased from Finar chemicals and Qualigens fine chemicals, respectively. All other ingredients used in the present investigation are of analytical grade and generally regarded as safe.

Method of preparation of floating microspheres

Floating microspheres were prepared by non-aqueous solvent evaporation method according to the formula given in Table 1. In the present study, the ratio of drug with respect to polymer (cellulose acetate) was evaluated at three different ratios of 1:1, 1:2 and 1:3. The grounded polymer was added to 20 ml of organic solvent (acetone) kept under magnetic stirrer for about 15 mins, to form a uniform polymer solution. To this polymer solution, weighed amount of acyclovir (500 mg) was added and continued stirring for 15 mins to form a uniform dispersion of drug in polymer solution. This solution was slowly poured into the dispersion medium containing 100ml of light liquid paraffin and 0.25% v/v of SPAN 60 (oil soluble surfactant). The whole system was stirred at three different rpm (1000, 1500 and 2000 rpm) at room temperature (Table 2). Stirring was continued over a period of 1-2 h to ensure complete evaporation of solvent. As the solvent evaporates, it leaves an empty space or hollowness within the micro particles and simultaneously, this hollowness is filled with air and makes microspheres less dense and resulting in the formation of floating microspheres.[4] After completion of stirring, the formed floating microspheres are separated by filtration through Whatmann filter paper, and washed thrice with petroleum ether and air dried for 24 h. The dried microspheres were stored in airtight container for further evaluation.

Evaluation of Floating Microspheres

Drug–excipient interaction studies using FTIR

Drug-excipient interactions were studied by Fourier-transform infrared spectroscopy on a BRUKER IR system using KBr disc method. The spectra were recorded for pure drug (acyclovir), individual polymers and the optimized formula. Samples were triturated with KBr in a motor and pestle to ensure uniform mixing. This mixture was pressed into discs using hydraulic press at 7 tons pressure for 3 mins. The pellet was then placed in the light path and spectrum was recorded in the range of 500-4000 cm^{-1}.

Micromeritic properties

Bulk density

It is the ratio of mass of the blend to bulk volume. It was measured by pouring powder in measuring cylinder and measuring the volume occupied by powder.

$$Bulk\ density = \frac{mass\ of\ microspheres}{bulk\ volume}$$

Tapped density

It is defined as ratio of mass of blend to the tapped volume. Tapped volume was measured by using digital tap densitometer, the volume after 100 standard taps is noted.

$$Tapped\ density = \frac{mass\ of\ microspheres}{tapped\ volume}$$

Carr's (compressibility) index

Compressibility index (C.I.) or Carr's index value of micro particles was computed according to the below equation;

$$Carr's\ index = \frac{tapped\ density - bulk\ density}{tapped\ density} \times 100$$

The value given below 15% indicates a powder with usually give rise to good flow characteristics, whereas above 25% indicate poor flow ability.[16]

Hausner's ratio

Hausner's ratio of microspheres was determined by comparing tapped density to bulk density.

$$\text{Hausner's ratio} = \frac{\text{bulk density}}{\text{tapped density}}$$

Values less than 1.25 indicate good flow (= 20% Carr), whereas greater than 1.25 indicates poor flow (= 33% Carr).

Angle of repose

Angle of repose (q) of the microspheres was measured by using funnel method. The microspheres were poured through a funnel that can be raised vertically until a maximum cone of height was obtained. The radius of heap was measured and angle of repose was calculated.

$\tan \theta = h/r$

Where, θ = Angle of repose,

h = height of granules above the flat surface

r = radius of the circle formed by the granule heap

Particle size

The particle size of the microspheres was measured by microscopic method, with the help of calibrated ocular micrometer. The ocular micrometer was calibrated by

using a stage micrometer. On an average of 100 spheres were measured and mean diameter was calculated.

Yield of microspheres

The prepared microspheres were collected and weighed. The measured weight was divided by the total amount of polymer and drug to get the yield.

$$\text{Percent yield} = \frac{\text{weight of dried microspheres}}{\text{Total weight of polymer} + \text{drug}} \times 100$$

Entrapment efficiency (%EE)

The amount of drug entrapped was estimated by crushing the 50 mg microspheres and extracting with aliquots of 0.1N HCl repeatedly. The extract was transferred to a 100 ml volumetric flask and made up to final volume using 0.1N HCl. The solution was filtered and the absorbance was measured at 254 nm against 0.1N HCl. The amount of drug entrapped in the microspheres was calculated by the formula given below.[17]

$$\textit{Entrapment efficiency} = \frac{\text{Amount of drug actually present}}{\text{Theoritical drug load expected}} \times 100$$

Buoyancy percentage

Microspheres (0.2 g) were spread over the surface of a USP XXIV type II dissolution apparatus filled with 900 ml of 0.1N HCl containing 0.02% tween 80. The medium was agitated with a paddle rotating at 100 rpm for 12 h. The floated and the settled portions of microspheres were recovered separately. The microspheres were dried adequately before weighing. Buoyancy percentage was calculated using equation given below.

$$\text{Buoyancy (\%)} = \frac{\text{weight of floating microspheres after 12 hrs}}{\text{total weifht of microspheres after 12 hrs}} \times 100$$

In vitro dissolution studies

In vitro dissolution of acyclovir from floating microspheres was carried out using the USP type II dissolution test apparatus. A weighed amount of microspheres equivalent to 200 mg of acyclovir were dispersed over the 900 ml of 0.1 N HCl (pH 1.2) maintained at 37 ± 0.5°C and stirred at 50 rpm. At predetermined time intervals, 5 ml of sample was withdrawn and replaced with equal amount of fresh medium 0.1 N HCl (pH 1.2). The collected samples were filtered and suitably diluted with 0.1 N HCl. The amount of drug released in the dissolution medium was determined spectrophotometrically at 254 nm using Eilico SL-210 UV-Visible double beam spectrophotometer.

Drug release kinetics

The analysis of drug release kinetics and mechanism from a pharmaceutical dosage form is an important but complicated process. According to model dependent approach the order of drug release from matrix systems was described by using zero order or first order kinetics.[18-20] Appropriate models for the release kinetics and mechanism can be judged by their highest correlation coefficient values compared to other models. The dissolution data was fitted to the models to assess the dissolution behavior of the prepared floating microspheres. The mechanism of drug release from matrix systems was studied by using Higuchi diffusion model and Hixon-Crowell erosion model.[21,22] The release exponent 'n' value from the Korsemeyer-Peppas support the drug release mechanism from a dosage form for further judgment.[23,24]

Scanning electron microscopy

Scanning electron microscopy (SEM) studies was performed to study the surface morphology of the microspheres.[25] The specimens were mounted on a metal stub with double-sided adhesive tape and coated under vacuum with gold or palladium in an argon atmosphere. The electron micrograph of crystals was obtained by scanning it through a beam of 10-20 kv electrons.

Data analysis, optimization and cross-validation of model

Data analysis

Responses, Y_1 (% yield), Y_2 (% entrapment efficiency), Y_3 (particle size), Y_4 (% buoyancy), Y_5 (T_{50}), Y_6 (T_{100}) were used for statistical analysis and optimization as shown in Table 2. Responses obtained from the nine runs were simultaneously fitted to linear, interactive and quadratic models using the Design Expert software.[26-28] The software will select and suggest the highest order polynomial model as a suitable model based on coefficient of determination (R^2) and predicted residual sum of squares (PRESS) values where the additional terms are significant. Analysis of variance (ANOVA) was performed on the suggested model for the responses Y_1, Y_2, Y_3, Y_4, Y_5 and Y_6 to identify significant effect. Multiple regression analysis was performed on the dependent variables to know the significance of the regression coefficients on the model. The models generated were used to construct contour (2D) and response surface (3D) plots for Y_1, Y_2, Y_3, Y_4, Y_5, Y_6 responses of formulations to understand the main and the interaction effects of these two factors.

Optimization

A multi-criteria decision approach, numerical optimization technique (desirability) and graphical optimization technique (overlay plots) were employed to optimize the formulations with the desired responses (responses from theoretical profile values). Optimization was performed with constraints of Y_1 (% yield) = 96.8%, Y_2 (% EE) = 69.7%, Y_3 (particle size) = 215.1 nm, Y_4 (% buoyancy) = 64.1%, Y5 (T_{50}) = 2.9 hrs, Y6 (T_{100}) = 10.0 hrs, which were obtained from the theoretical profile. For finalizing the optimum formulation, targets were set for these constraints for getting respective desirability function response and overlay plots.

Cross-validation of model

The chosen experimental design was validated by preparing the optimized formulation as per the procedure described using predicted optimal independent values. Optimized formulation was evaluated for % yield, % EE, particle size, % buoyancy, *in vitro* dissolution test. The experimental values of the responses Y_1, Y_2, Y_3, Y_4, Y_5 and Y_6 were determined for the optimized formulation. The percentage relative error between predicted values and experimental values of each response was calculated using the below equation.

Relative error (%) = $\frac{P.V - E.V}{P.V} \times 100$

Where, P.V. is predicted value and E.V. is experimental value.

RESULTS

Micronization of Acyclovir

Preliminary studies showed that in absence of the stabilizer the particle size was larger than in the presence of a stabilizer. Span being oil soluble acts as a droplet stabilizer and prevents coalescence of the droplet by localizing at the interface between dispersed phase and dispersion medium. When the drug was recrystallized without any stabilizer the particle size was 250-500 µm. The results also showed that in absence of a stabilizer the tendency of particle to agglomerate is very high. Therefore, due to the presence of larger particles and aggregates no further studies were carried out on particles produced without a stabilizer. On the basis of the preliminary studies under the following conditions micro particles of acyclovir with the least aggregation can be produced. The condition was as follows: the amount of stabilizer, dispersion medium, drug concentration and time for complete evaporation at a stirring speed of 1000-2000 rpm were 0.25% (w/v), 20 ml acetone and 100 ml light liquid paraffin, 100 mg and 1-2 h, respectively. In the present study the effects of three different polymer concentrations (cellulose acetate) with respect to drug namely; 1:1, 2:1 and 3:1 on micrometric, morphology and dissolution behavior of micronized acyclovir were investigated.

Fourier Transform Infrared Spectroscopy

The interaction study was done by FT-IR spectroscopy. The spectrum of pure drug polymer and optimized formula were studied in the range of 500-4000 cm^{-1}. The spectrum of acyclovir shows a characteristic peak of primary amine $–NH_2$ at 3471 cm^{-1}, carbonyl group –CO- at 1716 cm^{-1} and CN at 1631 cm^{-1}. The characteristic peaks are observed as it is in the spectrum of optimized formula with additional polymer peaks (Figure 1). This indicates there were no interactions between drug and excipients in the formulation.

Micromeritic Properties of Microspheres:

The micrometric behaviors (angle of repose, Carr's Index and Hausner's ratio) of acyclovir were evaluated in all the formulated microspheres. Angle of repose and Hausner's ratio obtained was less, indicating the excellent flow property of all the prepared hollow microspheres. Carr's index which is an indication of powder flow behavior showed that original acyclovir particles have lower Carr's index values than treated samples. It is evident that the original acyclovir (untreated drug) has larger mean particle size than all other acyclovir samples obtained via solvent evaporation technique.

SEM Studies

As morphology of drug particles has an impact on micromeritic properties and dissolution behavior, so, the morphology of the micro particles was investigated using scanning electron microscope (SEM). SEM study is performed by coating the microspheres with gold and allowing a beam of electrons at 15.0 kv and viewed at x2.5k. The results of this study confirmed the spherical structure of microspheres. Figure 2 shows the digital photograph images of prepared hollow microspheres and their transverse section along with SEM images.

Particle Size

The particle size of the obtained microspheres was measured by using microscope with the help of calibrated ocular micrometer. A total of 100 particles were measured and the value was multiplied by correction factor. The average particle size value (µm) of 100 particles was taken and values are represented in the Table 3. The size of hollow microspheres was showing a direct relation with polymer concentration and inverse relation with stirring speed. The effect of rpm and polymer concentration on size of microspheres was illustrated by counter plots and 3D response surface plots given in Figure 3.

Percent Yield Value

The percent yield values of the obtained microsphere were calculated and the values are reported in Table 3. The effect of RPM and polymer concentration was studied by using 3^2 full factorial designs. There was an increase in solution viscosity as the concentration of polymer is increased, which often results in tailing of spheres and sometimes in coalescence of droplets in the given experimental conditions. The percent yield of spherical microspheres was more at lower rpm value only (Figure3).

Entrapment Efficiency

The entrapment efficiency (% EE) of the prepared microspheres was determined and values are represented in Table 3. The effect of rpm and polymer concentration on % EE was studied by 3^2 full factorial designs. As the polymer concentration increases the entrapment efficiency decreased. At lower rpm the rate of solvent evaporation and rigidization rate will be low, where there is a complete entry of drug to entrap. This effect of rpm and concentration of polymer on % EE is illustrated by contour and response surface graph given in Figure 3.

Buoyancy Percentage

The buoyancy percentage was calculated and the values are represented in the Table 3. The obtained values are subjected to 3^2 full factorial designs. The effect of rpm and polymer concentration on buoyancy percentage was observed. The buoyancy percentage showed an inverse relation with rpm, with decrease in rpm the buoyancy percentage was increased. The buoyancy percent shows a direct relation with polymer concentration, as the polymer concentration increases the size of microsphere also increases (Figure 3).

***In Vitro* Dissolution Studies**

In vitro dissolution studies were performed using USP dissolution test apparatus Type II (paddle type) and the dissolution profiles of acyclovir micro particles are shown in Figure 4. For better comparison of dissolution profiles, time taken for 50% (T_{50}) and 100% (T_{100}) drug dissolution data were fitted in 3^2 full factorial design. T_{50} and T_{100} showed a direct relation with polymer concentration, as the polymer concentration increased. T_{50} and T_{100} values increased indicating controlled drug release. This might be due to increased polymer coating. The same was illustrated by contour plot and 3D response surface in Figure 3.

Drug Release Kinetics

The drug release kinetics and mechanism is determined according to a model dependent approach.[29] The data was fitted into popular exponential equations namely zero order, first order, Higuchi, Hixon-Crowell and Peppas and release kinetics for all the prepared formulations. The obtained correlation coefficient 'r' values are higher for first order (0.9359-0.9972) than zero order (0.9106-0.9851). Among all formulations F1, F2 and F3 followed zero order release kinetics and all remaining formulations followed first order release kinetics. The drug release mechanism was further estimated by subjecting the data to Higuchi and Hixson-Crowell models. The drug release mechanism was found to be diffusion which is indicated by their higher correlation coefficient r-values, Higuchi (0.994). By subjecting the data to peppas model, 'n' value obtained is in the range of 0.45-0.66 which indicates non-fickian diffusion mechanism.

Data Analysis, Optimization and Cross-Validation of Model

In this 3^2 full factorial design of experiment, two factors polymer concentration (X_1) and stirring speed (X_2) are studied each at three levels low, medium and high. By using this data 6 responses Y_1 % yield value, Y_2 percent entrapment efficiency(% EE), Y_3 size of microsphere, Y_4 percent buoyancy, Y_5 time for 50% release of drug (T_{50}) and Y6 time for 100 % drug release (T_{100}) were selected for statistical optimization and fitted to linear, interactive and quadratic models. The comparative R^2, adjusted R^2, predicted R^2, PRESS, s.d., F-values and p-values were determined using the Design Expert. A suitable polynomial model for describing the data was selected based on correlation (R^2) and PRESS values. Response Y_1 and Y_2 follows quadratic model, response Y_3, Y_4 andY_6 follows interactive model and response Y_5 follows linear model respectively. Hence these models are selected for further optimization. These models showed higher R^2 and F-values and lower PRESS and p-values.

The results of the second order response surface model fitting in the form of ANOVA are given in Table 4 for all formulations. These parameters were used to construct the independent variables on the response. The F-value for the responses, Y_1, Y_2, Y_3, Y_4, Y_5 and Y_6 were found to be 19.57, 6.41, 124.65, 149.84, 44.62 and 72.75 respectively, which indicated that the models were significant. The values of probability >F (less than 0.05) for all the responses indicated the significance of the models. The application of response surface methodology yielded the following regression equations.

$Y_1=96.04+0.000X_1-2.00X_2+1.50X1X2-13.75X_1X_1+0.38 X_2X_2$

$Y_2=66042+5.50X_1-3.50X_2+2.75X_1X_2-13.75X_1X_1+2.25 X_2X_2$

$Y_3 = 178.65+12.17X_1-57.74X_2+21.11X1X_2$

$Y_4 = 48.33+21.17X_1-18.17X_2-3.75X_1X_2$

$Y_5 = 2.83+1.30X_1-1.06X_2$

$Y_6 = 8.95+0.95X_1-1.48X_2+0.58X_1X_2$

Where, X_1 and X_2 are the coded values of the test variables of the polymer concentration and stirring speed respectively.

The detailed summary of results of multiple regression analysis of dependent variables for all responses was shown in Table 4. The significant parameters in the equations can be selected using a stepwise forward and backward elimination for the calculation of regression analysis. However, in the present study full model having both significant and non-significant p-values were used for obtaining dependent variables. Coefficients with one factor indicate the effect of that particular factor, while the coefficients with more than one factor and those with second order terms represent the interaction between those factors and the quadratic nature of the phenomena, respectively. Positive sign of the term indicates positive (additive) effect, while negative sign indicates negative (antagonistic) effect of the factor on the response. Main effects of all the selected independent variables like polymer concentration (X_1) and stirring speed (X_2) are highly significant ($p<0.05$).

Additive and negative effects were observed for all responses respectively with polymer concentration (X_1) and stirring speed (X_2), indicating increased responses with increase in polymer concentration and decrease in rpm. The variance of inflation factor (VIF) measures the extent to which the variance of particular model coefficient was inflated by the lack of orthogonality in the design. The VIF values for all the models were found to be ≥ 1, indicating good estimation of coefficient.

The contour plots were built to evaluate the relationship between two independent factors X_1-polymer concentration and X_2-stirring speed and their effect on dependent factors i.e. responses Y_1, Y_2, Y_3,Y_4, Y_5 and Y_6. Similarly response surface plots were also generated to establish the effect on response factors. For all the responses the independent factor X_1shows an additive affect and factor X_2 shows negative effect/antagonistic effect. For all the formulations there is an increase in response value with increase in factor X_1 and decrease in factor X_2. These results suggest that polymer concentration shows a direct relation and stirring speed shows an inverse relation for achieving optimum response.

Table 1: Experimental range and coded values of the independent variables

Run No.	Variable level in coded form	
	X_1	X_2
1	-1	-1
2	0	-1
3	+1	-1
4	-1	0
5	0	0
6	+1	0
7	-1	+1
8	0	+1
9	+1	+1
10	0	0
11	0	0
12	0	0
Coded values	Actual values	
	X_1 (amount of polymer in mg)	X_2 (stirring speed in RPM)
-1	500	1000
0	1000	1500
+1	1500	2000

Table 2: Formulation of drug loaded non-effervescent hollow microspheres

Formula	Ratio	Acyclovir Drug in mg	Cellulose acetate Polymer in mg	RPM
1	1:1	500	500	1000
2	1:2	500	1000	1000
3	1:3	500	1500	1000
4	1:1	500	500	1500
5	1:2	500	1000	1500
6	1:3	500	1500	1500
7	1:1	500	500	2000
8	1:2	500	1000	2000
9	1:3	500	1500	2000
10	1:2	500	1000	1500
11	1:2	500	1000	1500
12	1:2	500	1000	1500

Table 3: Values of particle size, % yield value, % entrapment efficiency, % buoyancy

Formula	Particle size (µm)	% Yield value	% Entrapment efficiency	% Buoyancy
1	243.8	89.7	54.51	42
2	232.8	95.3	69.05	60
3	233.5	85.5	65.20	92
4	174.6	83.2	49.56	28
5	178.0	96.3	68.91	50
6	183.8	83.5	50.85	70
7	77.35	79.1	42.08	10

8	134.8	97.33	62.92	30
9	151.5	82.6	63.02	45
10	175.7	95.3	68.97	52
11	177.8	97.3	70.91	50
12	180.2	96.7	62.27	51

Table 4: Summary for model statistics for response Y_1, Y_2, Y_3, Y_4, Y_5 and Y_6

Response	Model	R^2	Adjusted R^2	Predicted R^2	PRESS	s.d	F-value	P-value	remark
Y_1	Linear	0.045	-0.167	-0.862	992.3	7.52	92.03	0.002	
	Interactive	0.062	-0.299	-2.507	1869.1	7.91	108.5	0.001	
	Quadratic	0.942	0.894	0.454	290.8	2.27	10.20	0.044	Suggested
Y_2	Linear	0.267	0.104	-0.308	1251.1	8.83	7.00	0.069	
	Interactive	0.298	0.035	-0.840	1759.8	9.16	8.02	0.059	
	Quadratic	0.842	0.711	-0.045	999.9	5.02	2.23	0.264	Suggested
Y_3	Linear	0.902	0.880	0.729	6271.1	15.87	111.3	0.001	
	Interactive	0.979	0.971	0.916	1951.0	7.79	28.09	0.010	Suggested
	Quadratic	0.980	0.964	0.806	4498.4	8.76	44.34	0.006	
Y_4	Linear	0.971	0.964	0.934	316.6	3.95	25.0	0.012	
	Interactive	0.983	0.976	0.950	240.7	3.24	17.8	0.020	
	Quadratic	0.991	0.983	0.919	388.8	2.70	14.9	0.026	Suggested
Y_5	Linear	0.908	0.888	0.818	0.85	0.22	28851.2	<0.0001	
	Interactive	0.913	0.881	0.715	1.33	0.23	3241.5	<0.0001	
	Quadratic	0.962	0.930	0.611	1.82	0.17	2388.9	<0.0001	Suggested
Y_6	Linear	0.901	0.879	0.758	50.1	0.48	4.98	0.108	
	Interactive	0.965	0.951	0.944	1.16	0.30	1.74	0.344	Suggested
	Quadratic	0.979	0.961	0.903	2.01	0.27	1.32	0.411	

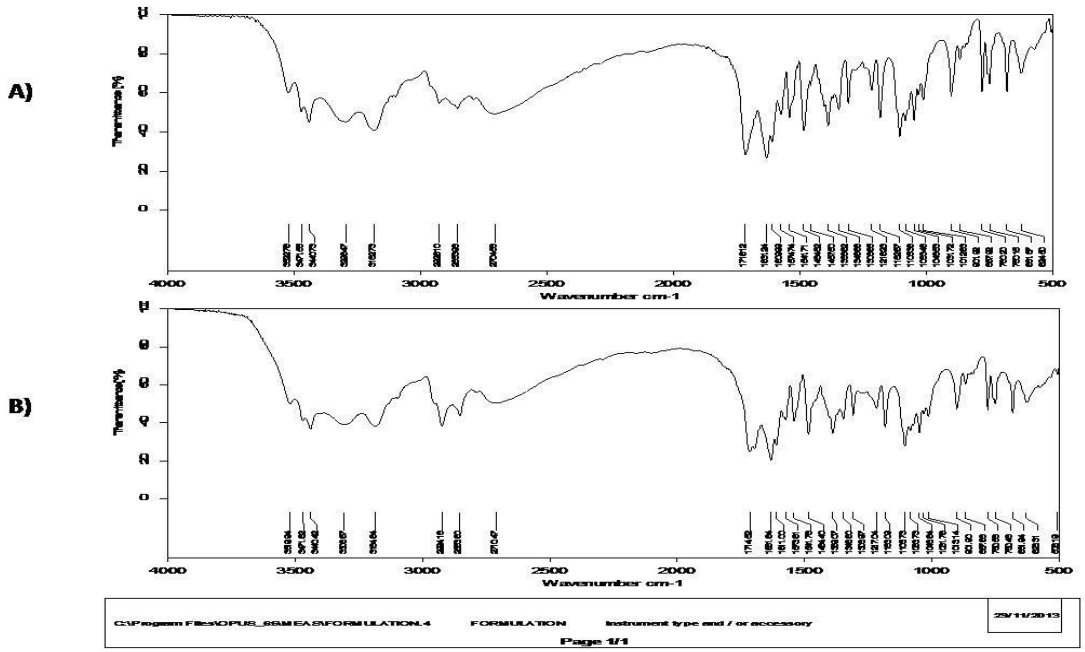

Figure 1: FTIR Spectra of A) pure drug (acyclovir) and B) optimized formulation

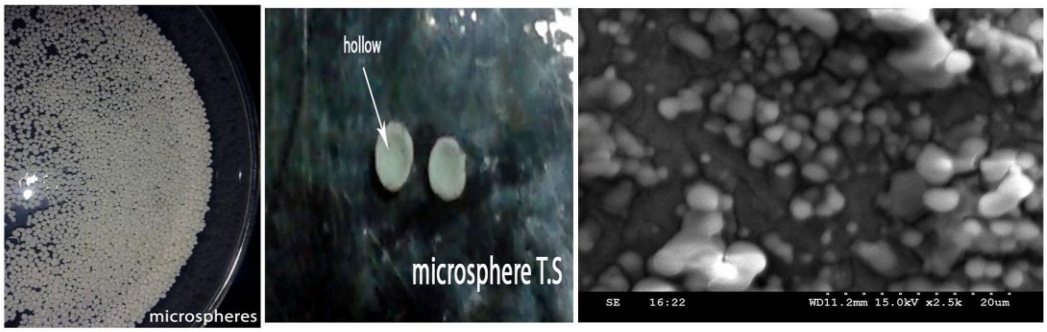

Figure 2: Digital images of A) Hollow microspheres and B) transverse section of microspheres and C) SEM photograph of microspheres

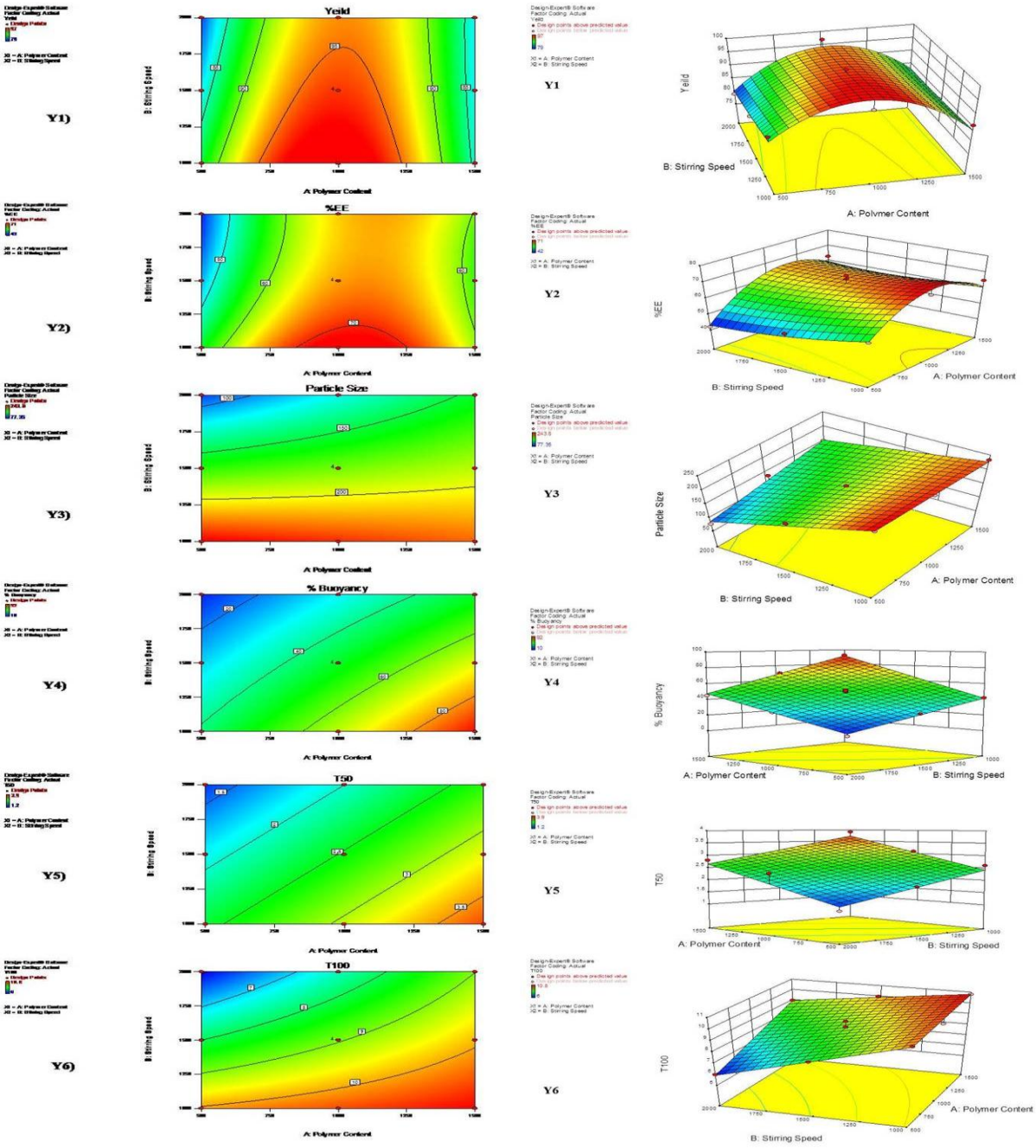

Figure 3: A) Contour plots and B) response surface plots showing effect of X_1-polymer concentration and X_2-stirring speed on responses Y_1, Y_2, Y_3, Y_4, Y_5 and Y_6

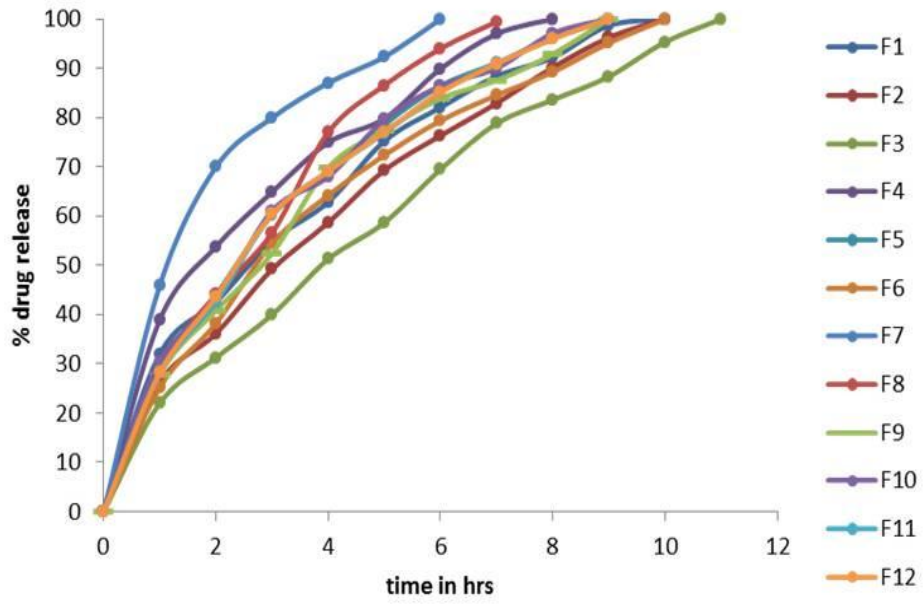

Figure 4: Dissolution profiles of acyclovir loaded hollow microspheres

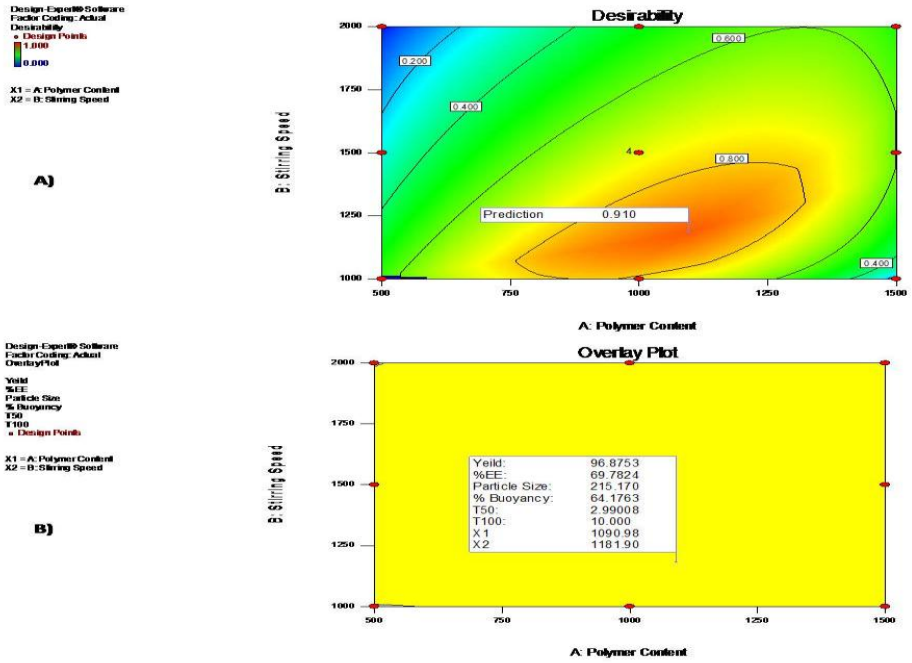

Figure 5: A) Desirability and B) Overlay plot of formulated microspheres

DISCUSSION

FT-IR spectra were used to further characterize the possibility of interactions between acyclovir and additives in the solid state. Figure 1 shows the FT-IR spectra of untreated drug, recrystallized in presence of PEGs and its physical binary mixtures. The spectra of all samples and all physical mixtures showed that they were identical with no incompatibility.

The prepared microspheres are evaluated for flow properties like Angle of repose, Carr's index, Hausner's ratio. Angle of repose was found to be lesser than 20 for all formulations, which indicates excellent flow property of all the prepared hollow microspheres. Carr's index was found to be lesser than 10 for all formulations, which indicates excellent flow property. Hausner's ratio was found to be in the range of 1.03 to 1.13 for all formulations, which indicate excellent flow property. The excellent flow property of microspheres is because of its spherical shape.

As the rpm decreases from 2000 to 1500 to 1000 there was an increase in size of microspheres, this was because of the droplets of the dispersed phase (polymer & drug in acetone) are broken down to a less extent as the stirring speed decreases, so there was an increase in size of

hollow microsphere. The case is reverse as RPM increases, the droplets of dispersed phase are subjected to more extent as the RPM increases so there was a decrease in size of microsphere. As the polymer concentration increases, there was an increase in the size of microspheres this was because of thicker coating of polymer as the concentration of polymer increases.

The polymer concentration have direct effect on % yield value to a certain extent i.e. upto1250 mg and then there was decrease in yield value due to increased concentration of polymer. As the rpm increases there are formation of irregular micro particles, resulting in decrease of % yield of spherical microspheres. The effect of rpm and polymer concentration on % yield value was illustrated in the counter-plot and 3D-response surface graph which are given in Figure 3.

As the polymer concentration increases the entrapment efficiency decreased because for same quantity of drug the drug loading will be decreased due to increased distribution for a given polymer concentration. As rpm increases the rate of solvent evaporation and rate of rigidization increases, thus the drug may escape the entrapment phase resulting in drug loss and poor entrapment.

As the polymer concentration increases the size of microsphere also increases resulting increased hollowness in the spheres which makes less dense and increased buoyancy. With decreased rpm, the rate of solvent evaporation will be slower and the size of the microspheres becomes more, which result in more hollowness within the sphere. During evaporation of solvent, this hollowness created will be filled with air, which makes the particle less dense and allowing more number of microspheres to float.

According to the results of *in vitro* dissolution studies, the dissolution rates of the micro particles are remarkably faster with increase in rpm ($P < 0.05$). The responses, T_{50} and T_{100} were fitted in 3^2 full factorial designs. T_{50} and T_{100} show an inverse relation with rpm. The agitation of the medium becomes more with increase in rpm, resulting the poor/thinner coating of the polymer and thus increasing the drug release.

As it is clear from Figure 2, the micro particles produced were very fine particles with spherical/granular shape. It can be concluded that the type of stabilizer had significant effect on drug particle morphology which is apparent from SEM results.

In the present work acyclovir, loaded hollow microspheres using cellulose acetate were prepared by non-aqueous solvent evaporation method and statistically optimized by 3^2 full factorial designs. In this design the effect of two factors X_1 (polymer concentration) and X_2 (stirring speed) are selected and responses Y_1(% yield value), Y_2(% entrapment efficiency), Y_3(size of microsphere), Y_4(% buoyancy), Y_5($T_{50,}$ time to release 50 % of drug) and Y_6($T_{100,}$ time to release 100% drug) were studied. All the effects of factors and responses of parameters are shown by contour and response surface plots. An optimized formula having X_1 and X_2 1098mg and 1183 with their expected values are given in the overlay plot (Figure 5). The optimized formula is cross validated using experimental values and percent relative error calculated within 5% which shows the correctness of the values.

CONCLUSION

Among all the approaches non-effervescent floating microspheres is one of the most appropriate method for increasing the bioavailability and therapeutic action of acyclovir. The hollow microspheres (non-effervescent floating microspheres) of acyclovir were prepared by non-aqueous solvent evaporation method using cellulose acetate. The prepared microspheres were evaluated for micromeritic properties, % yield value, % entrapment efficiency, particle size, % buoyancy and *in vitro* drug release kinetics. All the effects of factors and responses of parameters were statistically optimized by 3^2 full factorial designs. The micromeritic properties like angle of repose, Hausner's ratio and Carr's index were within the limits suggesting good flow properties. The SEM results exhibited that spherical morphology of the micro particles and no incompatibility of the samples as confirmed by FTIR spectra. The results of the present study showed that drug release is more controlled with increase in polymer concentration and decreases with increase in rpm. *In vitro* dissolution studies of optimized formula carried out in 0.1N HCl at 254 nm showed complete drug release in 10 hrs. Since sufficient controlled release formulations of acyclovir have not been reported for enhancing its bioavailability and therapeutic activity, there is a necessity for investigation of oral gastro retentive controlled release microspheres of acyclovir that can be used as twice a day capsule.

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