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Sustained Release Floating Microspheres Of Acyclovir: Formulation, Optimization, Characterization And *In Vitro* Evaluation

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Abstract

The aim of the present work was to prepare floating microspheres of acyclovir to prolong residence time in stomach and to sustain the release of acyclovir. Acyclovir loaded floating microspheres were prepared by double emulsion solvent evaporation method. The 3^2 full factorial design was applied to optimize the formulation. The resultant microspheres were evaluated for average particle size, percentage encapsulation efficiency, *in vitro* drug release and model fitting kinetics. Scanning electron microscopy, Fourier transform infrared (FT-IR) spectroscopy and differential scanning calorimetry were used to investigate the physical state of the drug in the microspheres. The particle size of microspheres was in the range of 275-340 µm. Percentage encapsulation efficiency was between 59%-77% w/w. Microspheres remained buoyant for more than about 12 h. The results of FT-IR spectroscopy and differential scanning calorimetry indicated the stable character of acyclovir in microspheres and also revealed absence of drug-polymer interaction. The *in vitro* drug release study showed that acyclovir release from the microspheres was slow and sustained for more than about 10 h. Drug release followed Korsemeyer-peppas model. The results of factorial batches revealed that the concentration of ethyl cellulose and stirring speed significantly affected drug encapsulation efficiency and particle size of the microspheres. Thus we can conclude that floating microspheres can successfully be developed to sustain the drug release.

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3^2 Full factorial design, floating microspheres, w/o/w emulsion solvent evaporation method, Acyclovir.

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INTRODUCTION

The oral drug administration is by far the most preferable route for administration of drugs. However, the short biological half-life and preferential absorption via defined segment of intestine limits the therapeutic potential of many drugs[1], [2]. Such a pharmacokinetic limitation result in to frequent dosing to maintain the therapeutically effective blood concentration. This results in pill burden and consequently, patient complaints. The phenomenon of absorption via a limited part of the GIT has been termed the narrow absorption window; once this dosage form passes the absorption window, the drug will be neither bioavailable nor effective. In extreme cases drugs that are insufficiently absorbed due to narrow absorption window cannot be delivered entirely and are either given by the parenteral route or by the development of such dosage form, which is otherwise safe.

Conventional oral dosage forms such as tablets, capsules provide specific drug concentration in systemic circulation without offering any control over drug delivery and also cause great fluctuations in plasma drug levels leading to peak plasma concentration at the absorption site and produce systemic toxicity and major draw-back is non-site specificity[3]. The drugs with site specific absorption, require drug release at specific site or such that maximum amount of drug reaches to the specific site. Pharmaceutical field is now focusing such drugs which require specificity. For oral solid-delivery systems, drug absorption is unsatisfactory and highly variable between the individuals despite excellent in vitro release patterns. There are several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the gastrointestinal tract (GIT) due to variable gastric emptying and motility. Furthermore, the relatively brief gastric emptying time (GET) in humans which normally averages 2-3 h through the major absorption zone, i.e., stomach and upper part of the intestine can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose. Therefore, control of placement of a drug delivery system in a specific region of the GI tract offers advantages for a variety of important drugs characterized by a narrow absorption window in the GIT or drugs with a stability problem[4], [5].

A rational approach to enhance bioavailability and improve pharmacokinetic and pharmacodynamic profile is to retain the drug reserve above its absorption region in GIT, i.e. in the stomach and to release the drug in controlled manner so as to achieve a zero order release kinetics (i.e. oral infusion) for prolonged period of time.

Several attempts have been made to extend the gastrointestinal transit time of the dosage form. Sophisticated gastro retentive floating drug delivery systems are used for increasing gastric residence time. They remain in the gastric region for several hours and hence prolong the gastric residence time of the drug. It has several advantages over immediate release dosage form including the minimization of fluctuations in drug concentration in plasma and at the site of action over prolonged periods of time, resulting in optimized therapeutic efficiencies and reduce the side effects, reduction of total dose administered and reduction of administration frequency leading to improved patient compliance.

Acyclovir, BCS class III/ IV drug [6], is widely used in the treatment of Herpes simplex virus infection as well as vericella zoster infection. It's short biological half life (2.5-3.3 h) and low absorption (15-30% of administered dose) only from upper part of GIT are the two major reasons for the need of development of novel drug delivery system. Hence, it was aimed to develop gastro retentive system of acyclovir which results in to complete absorption and higher bioavailability.

The present work consists of preparation and evaluation of floating microspheres of acyclovir using

ethyl cellulose in different proportions. The drug is slowly released from microspheres and complete and slow drug release in the stomach is expected to increase bioavailability of the drug as well as its complete utilization which may result in to lower side effects.

MATERIALS AND METHODS

Acyclovir was obtained as gift sample from Bakul Pharma Pvt. Ltd (Ankleshwar, India). Ethyl cellulose was procured from S. D. Fine Chem Labs (Mumbai, India). Acetone and dichloromethane (DCM) were purchased from fine chemicals Ltd (Mumbai, India). All the chemicals used in the study were of analytical grade.

Preparation of floating microspheres

Acyclovir loaded microspheres were prepared by w/o/w emulsion solvent evaporation method[7]. In this method, acyclovir was dissolved in 10 ml 0.1N NaOH solution. The drug solution was then added to the solvent blend consisting of acetone: dichloromethane containing of ethyl cellulose while being stirred and then this solution was ultrasonicated for 15 min [8]. The water/oil emulsion was poured into aqueous phase containing 0.2 %v/v Tween 80 as stabilizer and gently allowed to stirr using a mechanical stirrer at high speed to obtain w/o/w emulsion. The emulsion was then brought to 40°C and stirred continuously for 3 h to evaporate the organic solvent blend. Microspheres were centrifuged at 1200 rpm for 20 minutes, washed twice with water, collected on a whatmann filter paper and dried under vacuum.

Design of experiments

A 3^2 full factorial was applied to design the experiments [9]. Polymer to drug ratio and stirring speed were used as independent variables, whereas % encapsulation efficiency, time required for 80% drug release, and particle size were kept as dependent variables. Formulations F1 to F9 were prepared using three different levels of polymer to drug ratio and stirring speed. Microspheres thus obtained were filtered, washed with water, and dried overnight at room temperature. The responses of the dependent variables were evaluated. The polynomial equations were generated for each responses using Design Expert Software (7.1.4). The check point batch was prepared to validate the polynomial equation. A 3^2 full factorial design layout is shown in Table 1.

Evaluation of formulations subjected to optimization

Percentage drug encapsulation efficiency

The floating microspheres equivalent to 10 mg of acyclovir were accurately weighed and crushed. The powdered microspheres were dissolved in dichloromethane (5 ml) in volumetric flask and made the volume with 0.1 N HCl. This solution was then filtered through whatmann filter paper No. 44. After suitable dilution, the absorbance was measured at 254 nm using UV spectrophotometer and the percentage drug entrapped was calculated (equation 1). The drug entrapment study was conducted in triplicate.

$$\% \text{ drug encapsulation efficiency} = \frac{\text{Actual drugcontent}}{\text{Theoretical drug content}} \quad (1)$$

Size and Surface morphology of the microspheres

The size distribution in terms of average diameter (d_{avg}) of the microspheres was determined by an optical microscopic method. A compound microscope fitted with a calibrated ocular diameter and stage micrometer slide was used to count at least 100 particles (Olympus, NWF 10_x; Educational Scientific stores, India). The samples for the SEM analysis were prepared by sprinkling the microspheres on one side of a double adhesive stub. The stub was then coated with gold (Fine coat, ion sputter, JFC-1100). The microspheres were then observed with the scanning electron microscope at sophisticated instrumentation center for applied research and testing (SICART),

Vallabh Vidyanagar, India using model (ESEM TMP+EDAX, Philips, Netherland) at 30 kv.

Micromeritic properties and % yield

The microspheres were characterized for true density, tapped density, Carr's index (I_c), Hausner's ratio (H_R) and angle of repose using the following equations[10]. The tapping method was used to determine the tapped density and Carr's index as follows.

D_o = True density = W/V_o (2)

D_t = tapped density = W/V_t (3)

Hausner's ratio (H_R) = Tapped density/True density (4)

Carr's index = (Tapped density – True density)/Tapped density *100 (5)

V_o and V_t are the true volume and tapped volume respectively.

***In vitro* evaluation of floating ability**

The floating microspheres (300 mg) were spread over the surface of the dissolution medium 0.1N HCl, pH (1.2) containing 0.02%v/v of Tween 80 that was agitated by a paddle rotated at 100 rpm. After agitation for a predetermined time interval, the microspheres that floated over the surface of the medium and those settled at the bottom of the flask were recovered separately. After drying, each fraction of the microspheres was weighed and their buoyancy was calculated by the following equation:

Buoyancy (%) = $Q_f / Q_f + Q_s$ (6)

Q_f = Quantity of floating microspheres, Q_s = Quantity of settled microspheres

***In vitro* drug release study**

The *in-vitro* release of acyclovir from the ethyl cellulose microspheres was measured in 0.1N HCl at 37°C±0.5°C using USP paddle dissolution apparatus type II (LAB INDIA DISSO 2000). An accurately weighed amount of prepared microspheres equivalent to 100 mg of pure Acyclovir, were stirred in 900 ml dissolution medium at 100 rpm. Samples were withdrawn at predetermined time interval and replaced immediately with same volume of fresh medium. Aliquots following suitable dilution were analyzed spectrophotometrically at 254 nm. The drug release experiments were conducted in triplicate following the above procedure.

Drug- Excipients compatibility study

Fourier transform infrared spectroscopy

Infrared spectra of acyclovir, ethyl cellulose blank microspheres and acyclovir loaded microspheres were taken by using KBr pellet technique and were recorded on a Fourier transform Infrared spectrophotometer (FTIR-8400S, Shimadzu, japan).

Differential Scanning Calorimetry

Calorimetric analysis was performed at S. K. Patel Pharmacy College, Kherva, India using model DSC-&, Perkin Elmer, equipped with a measuring cell DSC 20. About 2 mg sample was placed in pierced aluminium pans and heated at a scanning rate of 10°C per minute from 50 to 250 °C. The instrument was calibrated with an indium standard.

Data analysis

A statistical model incorporating interactive terms was used to evaluate the responses:

$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2$ (7)

Where β_o, the intercept is the arithmetic average of all quantities outcomes of nine runs, β_1, β_2 and β_{12} 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. The terms X_1X_2 (*i* = 1, 2) is the interaction terms.

Stability studies

The stability studies were carried out at an optimized formulation, i.e., formulation F_9. The formulation was stored at 40° ± 2°C/75% ± 5% RH for 3 months[12], [13]. After intervals of 7, 15, 30, 60, and 90 days, samples were withdrawn and retested for drug release. Paired t-test was used to compare the dissolution profiles.

RESULTS AND DISCUSSION

Percentage drug encapsulation efficiency

The percentage encapsulation efficiency of microspheres varied from 59%-77%w/w (Table 2). Results demonstrated that an increase in polymer to drug ratio, increased the encapsulation efficiency of the drug. The drug encapsulation efficiency was good because the drug was sparingly soluble in the aqueous medium. Stirring speed had a negative effect on entrapment efficiency.

Size and surface morphology of microspheres by Scanning electron microscopy

The SEM micrographs revealed that the resulting microspheres were spherical in nature with rough surfaces containing cracks and holes over its surface. The micrographs showed almost spherical but the morphology appeared to be rough (Figure 1). The reason behind this morphology change can be attributed to the faster evaporation of dichloromethane forming a pore like structure. The mean particle size of the microspheres significantly increased with increase in polymer concentration and was in the range of 275-340 µm (Table 2).

Micromeritic properties and % yield

The compressibility index ranged from 13.70% to 16.75% (Table 2). All formulations showed excellent flowability as expressed in terms of angle of repose in the range of 17°-26° (Table 2). The percentage yield of prepared microspheres was in the range of 76%-95% (Table 2).

***In vitro* evaluation of floating ability**

Floating ability of batch F_9 microspheres was found to be more than about 12 h due to insolubility of the ethyl cellulose polymer in 0.1N HCl (pH 1.2). The results also showed a tendency that the larger the particle size, the longer the floating time. It should be noted, however, that the situation in vivo can be quite different and the residence time may vary widely depending on the phase of gastric motility.

***In vitro* drug release study**

The *in vitro* drug release profiles of all the formulations have been shown in figure 2. The release of acyclovir mainly depends upon the polymer concentration. The release rate of the drug from the microspheres was found to decrease drastically with increase in polymer concentration. Acyclovir release from all the formulations was found to be slow and sustained over 12 h. Bye the end of 12 h, Formulations F1, F2, F3, F4, F5, F6, F7, F8 and F9 were found to release 98.69%, 97.47%, 98.86%, 99.28%, 99.17%, 96.46%, 95.65%, 98.27% and 99.97% respectively (Figure 2).

Data Analysis

The floating microspheres of acyclovir were prepared by varying polymer to drug ratio (X_1) and stirring speed (X_2) as independent variables. The time required for 80% drug release ($T_{80\%}$ Y_1), percentage encapsulation efficiency (Y_2) and particle size (Y_3) were taken as dependent variables. On the basis of the data obtained from the formulations subjected to optimization, a general statistical model can be depicted with respect to the above data. The model developed can be characterized by using the polynomial equation representing the respective response data. This can be given as follows: In order to make prediction of Y_1, Y_2 and Y_3 mathematical models were evolved omitting the insignificant terms. Equations 1, 2 and 3 represents the refined

models for responses Y_1, Y_2 , and Y_3 with values of R^2 and Fischer's ratio (F).

$Y_1 = 519.11 + 29.66X_1 - 1.66X_2$ (R^2= 0.996, F=13.71, P<0.05) (8)

$Y_2 = 64.92 + 6.01X_1 + 0.92X_2$ (R^2 = 0.946 F= 24.79, P<0.05) (9)

$Y_3 = 287.89 + 20.50X_1 + 1.0X_2 + 10.50X_1X_2$ (R^2 = 0.986 F= 27.75, P<0.05) (10)

From the above equations, contour plots of the respective responses were generated, which were then used to predict the responses of dependent variables at the intermediate levels of independent variables. In the case of Y_1 ($T_{80\%}$), coefficients (β_1= 29.6) and (β_2= 1.66) were found to be significant. As the polymer to drug ratio (X_1) was increased, the $T_{80\%}$ was increases (Figure: 3(a))**.** In case of response Y_2, coefficients (β_1= 6.01) and (β_2= 0.92) were found to be significant (Figure: 3(b)). As the polymer to drug ratio (X_1) was increased, the percentage encapsulation efficiency was increased**.** In case of response Y_3, coefficients (β_1= 20.50) and (β_2= 1.0) were found to be significant. As the polymer to drug ratio was increased, the particle size was decreased (Figure: 3(c)).

Validation of optimum floating microspheres formulation

Table 5 lists the composition of the checkpoints, shows good correlation plots between the observed and the predicted values of drug entrapment efficiency, $T_{80\%}$, and particle size which confirms the practicability of the model.

Model fitting kinetic

The in vitro release data of batch F_{10} were analyzed for establishing kinetics of drug release. Model fitting was done using an in-house program FORTRAN. Zero order, First order, Higuchi[11], Hixson-Crowell, Korsemeyer-peppas and Weibull models were tested. The best fit was shown by Korsemeyer- Peppas model with least sum of square of residuals (SSR) and Fischer's ratio (Table 3).

Drug- Excipients compatibility study

Pure acyclovir showed prominent peaks at 3458 cm^{-1}, 1731 cm^{-1} and 3029 cm^{-1} because of hydroxyl, carboxy and aromatic amine respectively. These peaks were retained in acyclovir loaded microspheres indicating the stability of acyclovir during processing of microspheres (Figure 4). This was further supported by DSC**.** Acyclovir showed a sharp endothermic peak that corresponds to melting in the range of 250-300 ^oC, as shown in Figure 4(a). Acyclovir in the ethyl cellulose microspheres Figure 4(b) also showed a similar characteristic peak with decreased intensity showing its stability during the encapsulation.

Stability studies

Stability studies indicated that there was no significant difference observed between the release pattern of microspheres at 40^oC and 75%RH and at room temperature for three months[12],[13] (Figure 5).

CONCLUSIONS

The results of a 3^2 full factorial design revealed that the polymer to drug ratio (X_1) and stirring speed (X_2) significantly affected the dependent variables such as drug encapsulation efficiency, $T_{80\%}$ and particle size of the microspheres. The best fiited model was Korsemeyer-peppas. The microspheres of the check point batch (F_{10}) exhibited 70.76% drug encapsulation efficiency, mean particle size of 310 µm and 570 min $T_{80\%}$ (Table 3) which were nearer to predicted values obtained from overlay contour plot of all the responses, (Figure 3(d)). An appropriate balance between the levels of the polymer to drug ratio and stirring speed was imperative to acquire maximum drug encapsulation efficiency, sustained release of the drug and adequate particle size. Hence, it could be established that among the prepared formulations, F_9 was the optimum formulation. In

vitro data obtained for the floating microspheres of acyclovir showed excellent floating ability, good buoyancy and prolonged drug release.

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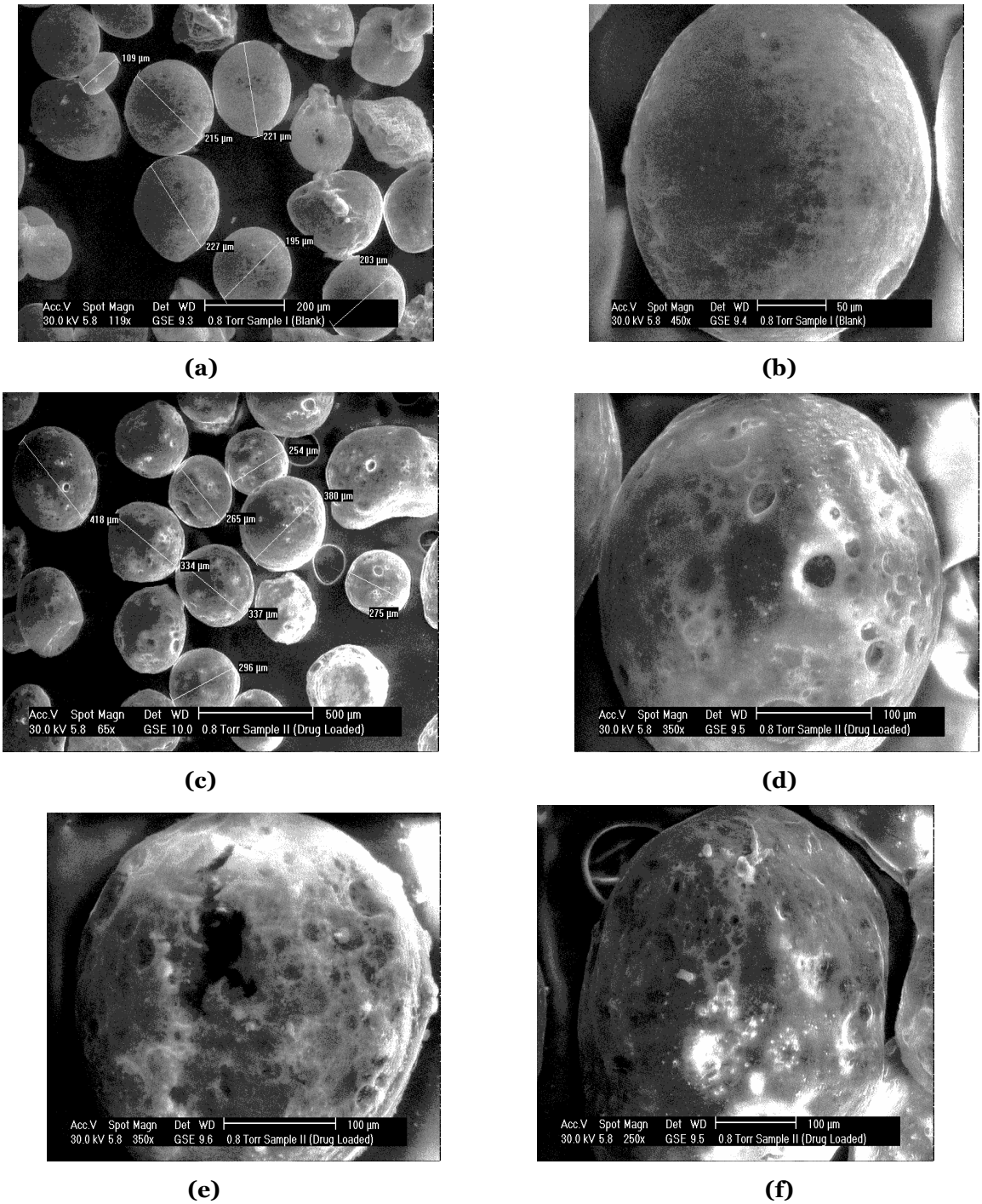
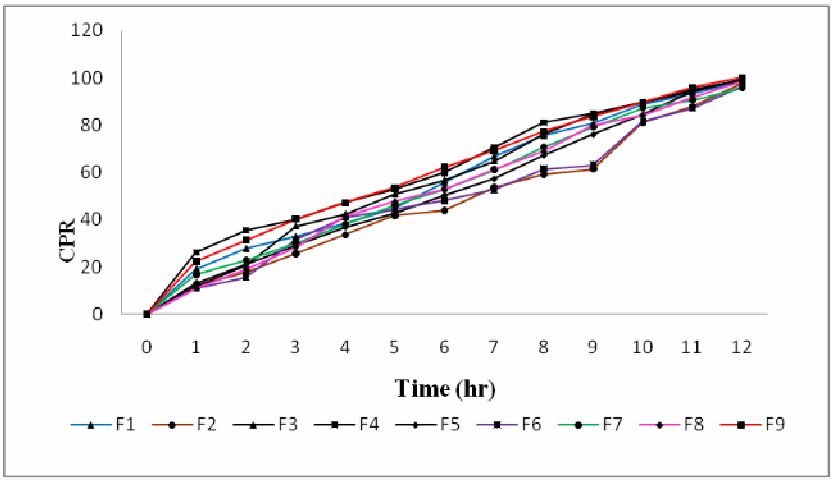


Figure 1: (a) and (b) Blank microspheres, (c) and (d) Acyclovir loaded microspheres, (e) and (f) Microspheres collected after dissolution



CPR = Cumulative percentage release

Figure 2: Comparative drug release profile of batch F_1 to F_9

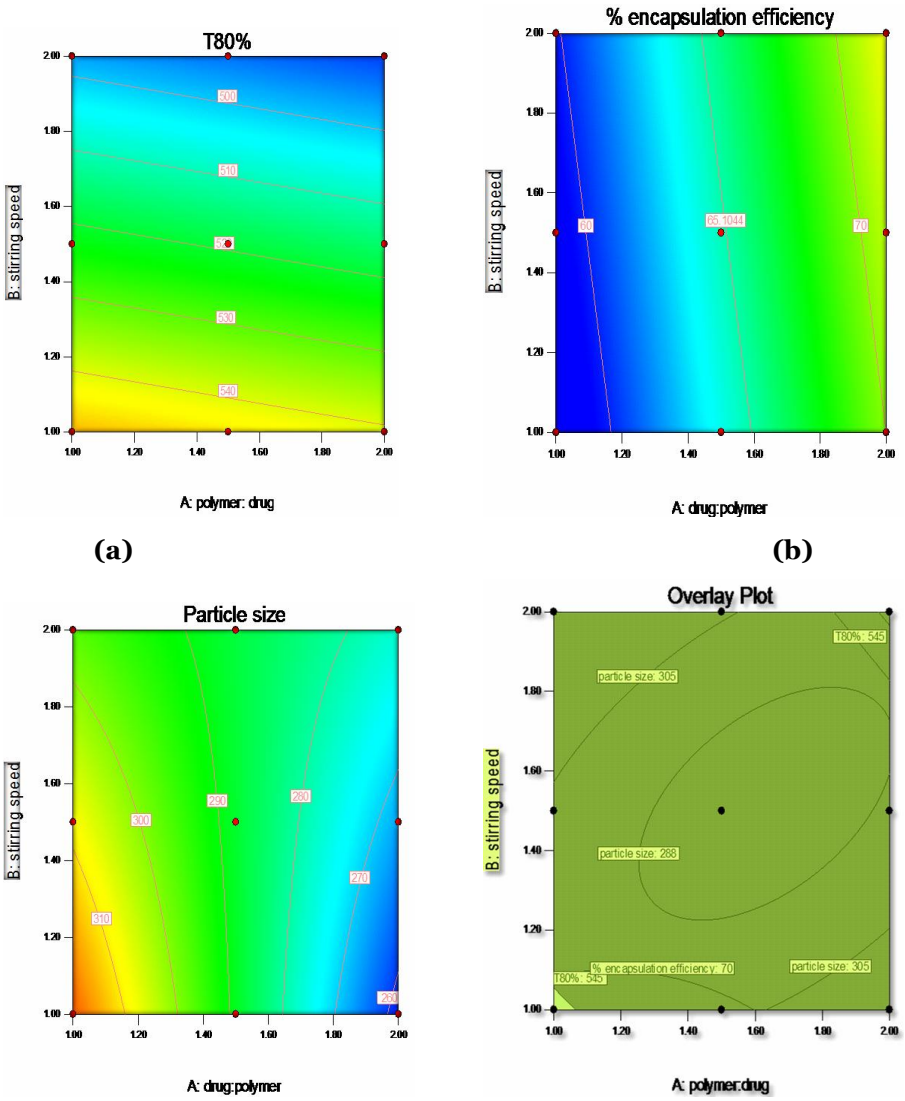


Figure 3: (a) Contour plot of the effects of X_1 and X_2 on Y_1 (b) Contour plot of the effects of X_1 and X_2 on Y_2 (c) Contour plot of the effects of X_1 and X_2 on Y_3 (d) Overlay plot of all the responses

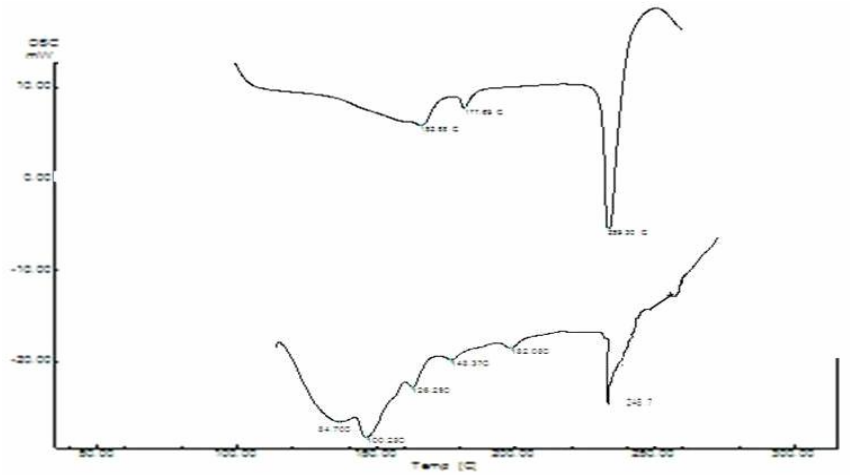


Figure 4: (a) DSC thermogram of acyclovir pure drug (b) DSC thermogram of acyclovir loaded microspheres

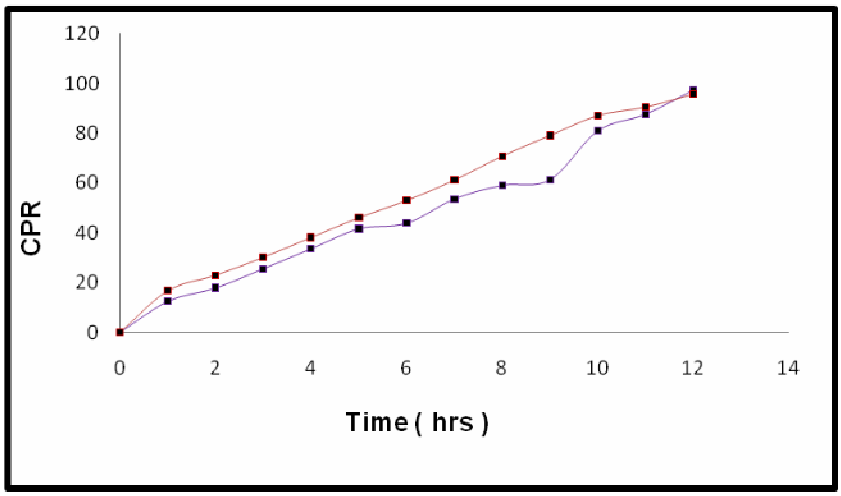


Figure 5: Drug release profile of batch F_{10} at room temperature and accelerated conditions.

Table 1: 3^2 full factorial design layout

Independent variables	F1	F2	F3	F4	F5	F6	F7	F8	F9
Polymer to drug ratio (X1)	1:1	1:1	1.5:1	1.5:1	1.5:1	1.5:1	2:1	2:1	2:1
Stirring Speed (X_2)	600	900	1200	600	900	1200	600	900	1200

Stirring speed (X_2)= rpm

Table 2: Results of optimized formulations

Batch Code	Yield(%)	% encapsulation efficiency (%w/w)	Mean particle size (µm)	Angle of repose (°)	Tapped density (g/cm³)	Carr's index
F1	76.49	64.10± 1.7	340±2.2	17.91±0.42	0.428±0.002	15.65±0.696
F2	83.68	59.46± 1.3	327±2.7	19.66±0.20	0.464±0.008	17.02±0.432
F3	89.47	62.00± 1.5	332± 3.1	19.81±0.54	0.474±0.010	17.93±1.465
F4	86.86	68.55± 2.5	274±1.9	19.25±0.48	0.493±0.009	14.40±0.537
F5	92.98	61.82± 2.1	310±2.5	17.98±0.61	0.518±0.013	13.70±0.426
F6	94.77	60.00± 2.3	295± 1.7	22.64±0.52	0.522±0.009	13.79±1.231
F7	84.15	60.68± 1.8	276± 1.6	24.16±0.63	0.570±0.015	17.01±0.742
F8	93.50	71.40± 2.9	282±1.4	26.22±0.43	0.631±0.016	15.68±0.882
F9	95.23	76.23± 3.1	320±2.9	25.00±0.47	0.695±0.021	16.75±0.765

Table 3: Comparison between the experimental and predicted value for the most probable optimal formulation F_{10}

Dependent variables	Optimized formulation F_{10}	
	Experimental	Predicted
$T_{80\%}$ (min)	570	548
% encapsulation efficiency	70.76	72.51
Particle size(µm)	310	302

Table 4: Results of kinetics drug release profile of Batch F_{10}

Paremeters	Zero order	First order	Higuchi model	Hixon- crowell	Korsemeyer- peppa's	Weibull
F*	3.40	2444.534	75.22	91.57	**1.45**	49.21
SSR+	34.00	24445.34	7.52	915.72	**13.09**	442.97
R^2	0.995	0.585	0.990	0.885	**0.997**	0.839

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