Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2014, 5(5):42-55

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Statistical optimization and characterization of prepared Fluconazole topical liposomal gel for improved skin permeation

Subhabrota Majumdar*[1], Rabindra Debnath[1], Arka Bhattacharjee[1], Ananya Banerjee[1] and Chandra Sekhar Patro[2]

[1]*Department of pharmaceutics, Calcutta Institute of Pharmaceutical Technology & Allied Health Sciences., Uluberia, Howrah, West Bengal, India*
[2]*Department of pharmaceutics, Raghu College of Pharmacy., Visakhapatanam, Andrapradesh, India*

ABSTRACT

The aim of the study is to prepare topical liposome of fluconazole by standard lipid film hydration method followed by incorporation into freshly prepared hydrogel for effective topical permeability. Different ratio of cholesterol and phospholipid was considered as a lipid carrier and 2^3 factorial design was used to evaluate the influence of different conditions on entrapment efficiency and drug release from liposomal gel. Prepared fluconazole liposomal gel was characterized by photo microscopic study, entrapment efficiency, drug content, stability studies, surface topography and in-vitro release study. DSC and FTIR analysis were performed to characterize the state of drug and lipid modification shape and surface morphology were determined by SEM which revealed white spherical shape of the formulation. The consequence of simultaneously varying two preparation factors that is Ratio of Phospholipid: Cholesterol and Hydration volume and their interaction on response were modeled by response surface methodology (RSM). Three-dimensional response surface plots and mathematical polynomial equations were used to correlate the index and control. In vitro dissolution study shows that R^2 value of formulation F9 was found to be 0.998 which is significant amongst all with a zero order drug release profile. The final optimized formulation having the composition of PC was 84.22 mg with cholesterol 20 mg and hydration volume 13.83 ml incorporated in 2% carbopol gel.

Key words: Fluconazole, Lipid film hydration, Hydration volume, Factorial design, Cholesterol.

INTRODUCTION

To achieve optimal and significant therapeutic efficacy, the drug molecules could be transported by a carter to the site of action to execute their assignment. However, the carrier should be biodegradable, non-toxic with proper size and shape to carry a wide range of medicinal agents. Liposomes are macroscopic, spherical, self-closed vesicles consists of one or more phospholipids. Both water soluble and lipophilic drug molecules may be encapsulated in the vesicle, either in the entrapped aqueous media, in the edge of phospholipid bilayer, or in the bilayer; the demand locality of the drug will be depended on the composition and physicochemical properties of its composed lipids. Due to these characteristics, liposomes were considered as a proficient drug transporter.

Subsequent administration into the body, the liposomes with reception to the target tissues are taking into cells passing through different types of endocytosis [1] [2]. Through passive diffusion process it is impossible to take

proteins, peptides and nucleic acids into the cells, as a result intracellular uptake of liposome is the prime aspect for liposome-mediated drug delivery system for these drugs to ensure satisfactory bioavailability. It was reported by many researchers that liposomes was taken up through clathrin endocytosis into the cells [3]. Furthermore, by modify the physico-chemical characteristics along with surface properties of the vesicle may influence the intracellular uptake system [4] [5] [6].

In the present study, Fluconazole was taken as a model drug which is a synthetic triazole anti fungal agent, which selectively inhibits the fungal cytochrome P-450 enzyme and C-14 alpha sterol demethylase. It has very negligible action on human sterol synthesis. The drug is absorbed completely after oral administration, thus the pharmacokinetic properties are alike following oral and intravenous administration. Subsequent to oral administration the peak plasma concentrations are achieved within one to two hours and elimination half life is about 30hrs. Following single oral doses daily the steady-state concentrations are reached within 5- 10 days [7] [8].

In the present study, response surface methodology (RSM) is a collection of mathematical and statistical techniques for empirical model building, used to develop and optimizing the liposome formulation by factorial design method and its therapeutic effect [9].

MATERIALS AND METHODS

Materials

Fluconazole is obtained as the gift sample from the Wallace Rivela (division of Wallace Pharmaceutical Pvt. Ltd.). Phosphatidylcholines (PC), Cholesterol (CHOL) and Dl-alpha tocopherol acetate were procured from HI- MEDIA Laboratories, Mumbai, India. All other chemicals and solvents used are of analytical grade.

Liposome preparation

In this investigation, the liposomes were formulated by adapting 'standard lipid film hydration method'. Different weight ratios of phospholipids: cholesterol, were weighed and dissolved in chloroform: methanol mixture (2: 1 v/v) in 250 ml rounds bottom flask, as provided in Table 1. A lipid film was formed on the inner side of round bottom flask by evaporating organic solvent under vacuum in rotary evaporator at 45-50 °C. Subsequently, the flask was kept overnight under vacuum to ensure the complete removal of residual solvent. The dry lipid film was hydrated with 20 ml phosphate buffer solution (pH 7.4) containing fluconazole at a temperature of 60±2 °C. Afterwards, the dispersion was left undisturbed at room temperature for 2-3 h to allow complete swelling of the lipid film and hence to obtain the vesicular dispersion [10].

Formulation design

The novel optimization technique, design of experiment (DOE) modeling was applied by means of factorial design to develop and optimize fluconazole liposome formulation and to distinguish the significant factors' effects influencing the investigated responses in the proposed liposome formulation. The weight ratio of PC : CHOL(X1) and hydration volume (X2) as independent variables each at three levels was studied for the interest of responses such as entrapment efficiency (Y1) and drug released at eight hours (Y2) as dependent variables.

Table 1. Formulation chart of nine batches

Formulation code.	Variable X1 Ratio of PC : CHOL (mg)	Variable X2 Hydration volume (ml)
F1	5:1 (+1)	10 (-1)
F2	4:1 (0)	20 (+1)
F3	3:1 (-1)	15 (0)
F4	3:1 (-1)	10 (-1)
F5	3:1 (-1)	20 (+1)
F6	4:1 (0)	10 (-1)
F7	5:1 (+1)	15 (0)
F8	5:1 (+1)	20 (+1)
F9	4:1 (0)	15 (0)

Statistical Analysis

Statistical validity of the polynomials was established on the basis of ANOVA provision in the Design expert Software. Three-dimensional (3D) response surface plots and two dimensional (2-D) contour plots were constructed based on the model polynomial functions using Design Expert software. These plots are very useful to see interaction effects on the factors on the responses. Eight optimum checkpoints were selected by intensive grid

search, performed over the entire experimental domain, to validate the chosen experimental design and polynomial equations. The formulations corresponding to these checkpoints were prepared and evaluated for various response properties. Subsequently, the resultant experimental data of response properties were quantitatively compared with that of their predicted values. Also, linear regression plots between observed and predicted values of the response properties were drawn using MS-Excel, forcing the line through origin.

Due to aforementioned factors, a 2^3 full factorial design by way of the two independent factors at three levels was used to provide rational proceed to organize the response model and optimal variable combinations. Table 1 describes an account of 9 experimental runs, with their independent factor's combination and coded level version using throughout the study. During the proposed study, the responses produce polynomial models along with their interactions and quadratic terminologies using multiple regression analysis techniques. The general form of the second-order polynomial model is represented as the following equation:

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

in which Y was the determination value of index, β_0 was the intercept and β_1- β_7 were regression coefficients, whilst $X_1 - X_2$ are investigated factors. The correlation in between each factor and response was fitted by using data processing software Design-Expert trial version 8.0.4 (Stat-Ease Inc., USA), subsequent regression coefficients and constants were calculated, as well, precision of regression formula obtained was evaluated by fitness and correlation coefficient. The sensibleness of statistical polynomial models was predictable by ANOVA. Three-dimensional response surfaces (3D) that show signs of the interaction between every factor, and their significant influence on the index were plotted according to fitted equation.

Validation and optimization of the proposed model

To authenticate this experimental model, eight check point solutions were selected and were screened for the selected responses. The ensuing observed responses were quantitatively compared with the corresponding predicted values to get the optimized formula. Afterward, linear regression plots were drawn between the obtained observed response properties and the consequent predicted values.

Photo microscopic study of liposomes

The liposomal suspension was subjected to size analysis under a microscope (10×400 magnification) fitted with a calibrated ocular micrometer. The shape of pre-pared liposomes was also studied [11].

Entrapment efficiency

To determine the amount of fluconazole, liposomes were centrifuged at 20000 rpm for 1 hour at the controlled temperature of 4^0C to obtain the supernatant containing unentrapped fluconazole. The total amount of drug was agreed on by UV spectrophotometrically at 261.5 nm against phosphate buffer saline (pH 7.4), and the amount of fluconazole entrapped in liposome was determined by using the following equation:

$$Entrapment\ Efficiency(\%) = \frac{C_f - C_d}{C_f} \times 100\%$$

Where C_d is concentration detected of the total amount of fluconazole, and C_f is the concentration of free fluconazole. The entrapment efficiency was obtained by repeating the experiment in triplicate and the values were expressed as mean standard deviation.

Drug content and content uniformity

100gm of gel sample was taking to determine drug content using UV spectrophotometer at 261.5 nm. Similarly, the content uniformity was resolute by analyzing drug concentration in gel taken from 3 to 4 different points from the container. However, for the liposomal gel, it was shaken with sufficient quantity of methanol to extract the drug and then analyzed by using UV spectrophotometer at 261.5nm.

Stability studies

The ability of vesicles to retain the drug (i.e., drug retentive behavior) was assessed by storing the liposomal suspensions at two different temperature conditions, i.e., 4-8°C (Refrigerator; RF) and 25±2 °C(Room temperature;

RT) for a period of 60 days. Samples were withdrawn sporadically to analyze the entrapment efficiency of liposomal suspension [12] [13].

In vitro release studies

In vitro, release of fluconazole was performed using modified Franz diffusion cell. For this study, rat was sacrificed by exposing to excess chloroform. To the abdominal skin, depilatory was applied and kept for 10 minutes to remove the hair from it. Now the skin was washed with water and excised from rat with a scalpel to confiscate fatty layer by keeping the skin in warm water at 60^{o} C. After 2 minutes, the fatty layer was peeled off smoothly, following washing with water and kept for saturation in the phosphate buffer saline pH 7.4 for about 30 minutes before it was used for permeation studies. Fresh skin was used every time. Skin permeation studies with liposomal formulations were carried out using abdominal rat skin, employing modified Franz-diffusion cells.

The liposomal gel was positioned in the donor compartment, while the receptor compartment was filled with phosphate buffer, pH 7.4 of 90 ml. The diffusion cells were maintained at 37±0.5^{o}C with stirring at 500rpm right through the experiment. At predetermined time intervals, 4ml of aliquots were withdrawn from receiver compartment through side tube and analyzed by UV-Visible Spectrophotometer at 261.5nm. Data obtained from *in vitro* release studies were fitted to various kinetic equations like Zero order, First order, Higuchi matrix and Krosmeyer-peppas for formulation F1to F9 to find out the mechanism of drug release from the liposomal gel [14].

Surface topography

The samples for the scanning electron microscopy analysis were prepared by sprinkling the dried liposomal formulation on one side of an adhesive stub to resolve details of the lipid bilayer. The liposomes were then mounted with gold in ion sputtering unit and finally the liposomes were mounted into the scanning electron microscope (FEI Quanta-200 MK2, Netherlands) [15].

Differential scanning calorimetry

This study was performed to explore the transition temperature of the liposomes incorporated with cholesterol at different ratios was detected by Perkin Elmer-Jeda DSC instrument equipped with an intra-cooler [16].

FT-IR spectral analysis

It is an excellent tool used for material quantitative analysis to study the drug-excipient interactions, with an intention of predicting rapidly and reasonably the long-term stability of the mixtures. Both morphological and thermal properties are sensitive to interactions, which leave mostly unmodified the IR spectra's. The FT-IR surface analysis was performed by Perkin Elmer FTIR, Spectrurux RX, UK [17].

RESULTS AND DISCUSSION

Calibration curve of fluconazole

The absorbance of fluconazole at different concentration was précised using UV-visible spectrophotometer at λmax 261.5nm. The calibration curve of fluconazole in distinctive concentration was plotted with regression value of 0.998 shown in Figure. 1. The curve obeys Beers –Lambert's law in a concentration of 50µg/ml, 100 µg/ml,200 µg/ml, 300 µg/ml, 400 µg/ml and the relation between the drug concentration and absorbance was linear and within the concentration range of fluconazole. Consequently, the entrapment efficiency, drug loading & in-vitro drug releases of different types of fluconazole liposomes were effortlessly calculated [18].

Surface topography (Scanning Electron Microscopy)

Scanning electron microscopy of the developed liposome of formulation F4 is shown in Figure 1 and Figure 2 respectively.

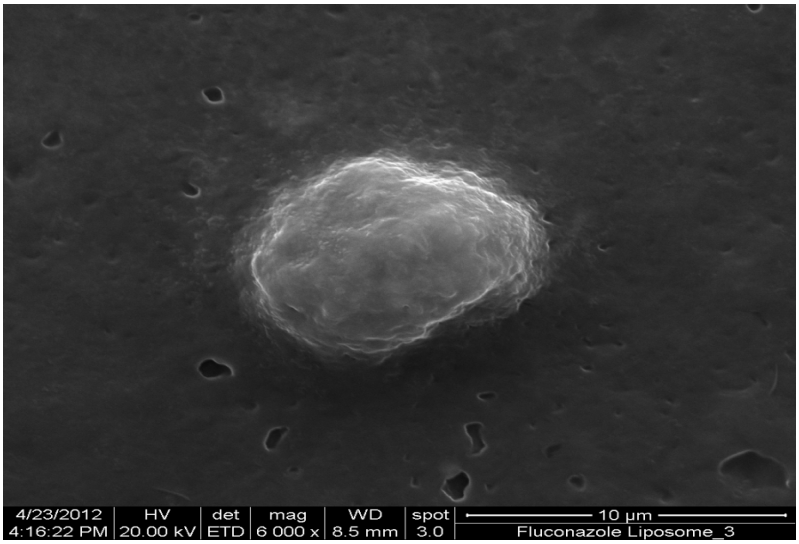

Figure 1. SEM of fluconazole liposome at 6000x magnification

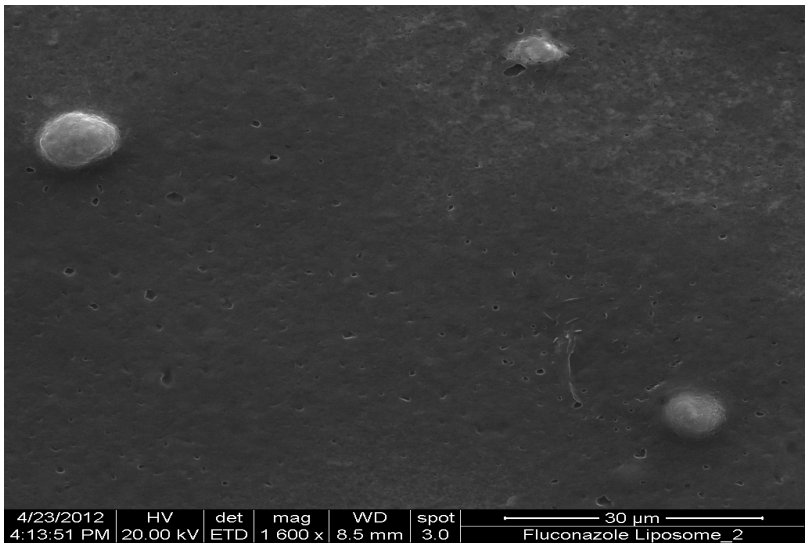

Figure 2. SEM of fluconazole liposome at 1600x magnification

FT-IR Spectral Analysis

The FT-IR study was performed to examine about the interactions between the drug and polymers used for preparation of liposomes. The spectra obtained for formulation of fluconazole with polymers are shown in Figure 3 and Figure 4. It is confirmed that there was no significant interaction between drug and polymer.

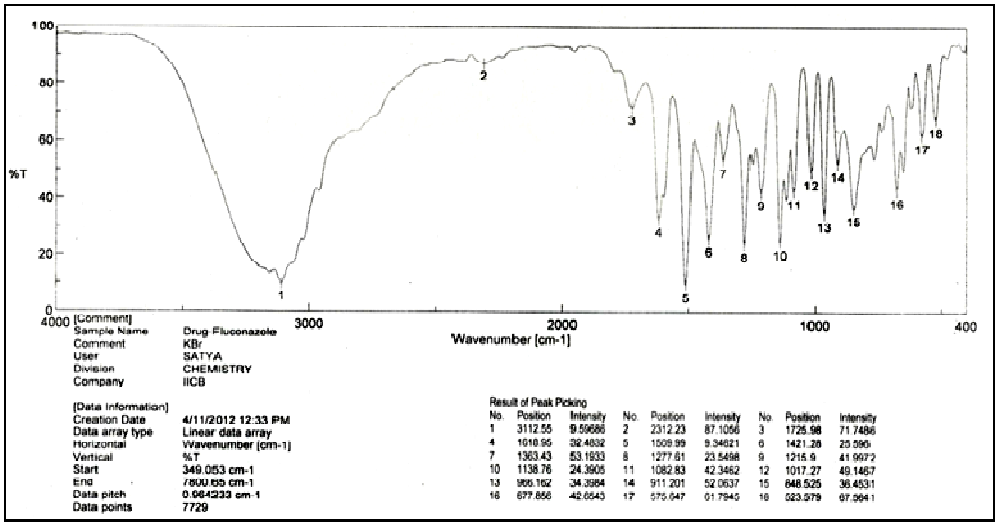

Figure 3. FT-IR spectra of Drug (Fluconazole)

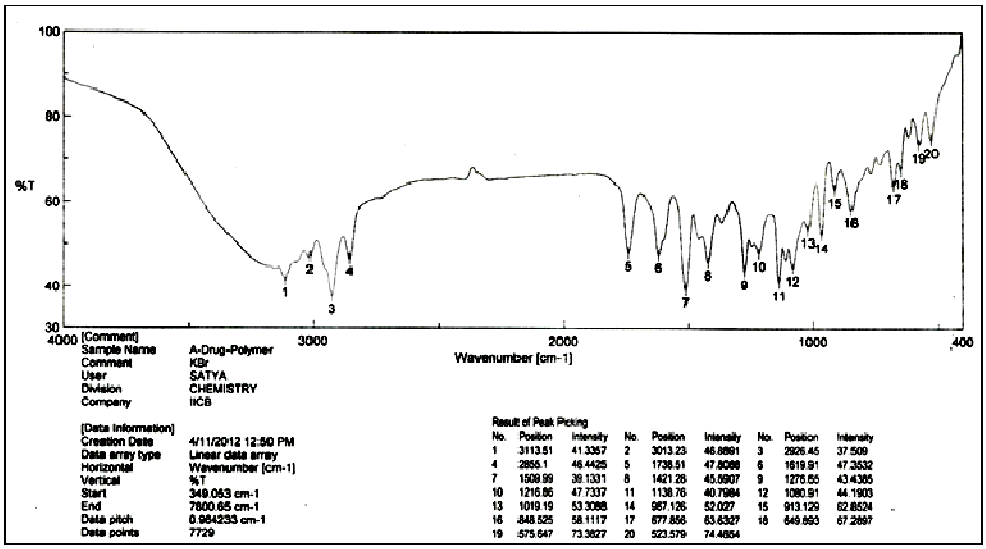

Figure 4. FT-IR Spectra of Drug+ polymer (fluconazole + PC)

Differential Scanning Calorimetry (DSC)

The membrane of liposome can subsist in either crystalline form or liquid state. At the transition temperature, the degree to which this transformation of liposome has resulted can be barred by DSC, which determines changes in heat capacity shown by thermal transition. For the DSC thermogram of fluconazole, sample consisted of a single sharp endotherm maximum at 145.60^0 C. The endotherm is assigned to the melting of the compound and characterized by an enthalpy of fusion = 252.5770 J/g. The quality of the thermogram indicates the purity of fluconazole. It is confirmed that there was no significant interaction between drug and polymer as shown in Figure 5 and Figure 6.

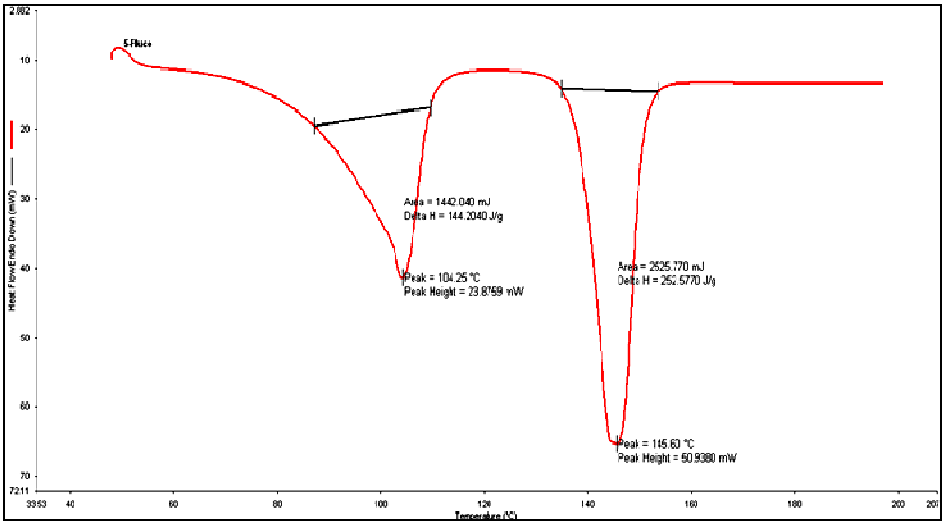

Figure 5. DSC thermogram of pure Drug (fluconazole)

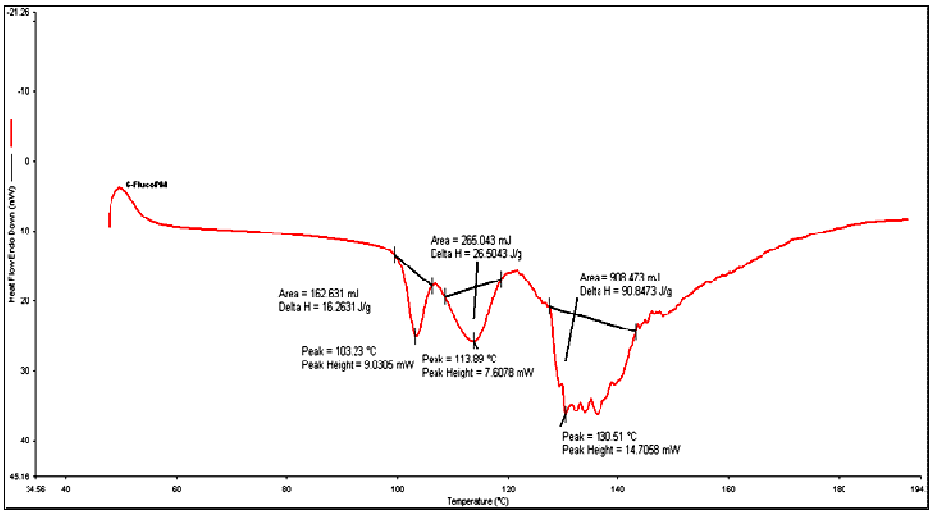

Figure 6. DSC thermogram of physical mixture (Fluconazole + PC)

In vitro drug released study

The in vitro drug release study of the desired formulations was carried in phosphate buffer pH 7.4 for 8 hrs using a diffusion cell apparatus.

From the obtained results of in vitro release studies for all the formulation were plotted in four different models of data treatments as follows:

Cumulative % drug release Vs. Time, Remaining log cumulative % drug release Vs. Time, Cumulative % drug release Vs. Square root of time & Log cumulative % drug release Vs. Log time. The release kinetics of the all formulations in zero order are shown in Figure. 7

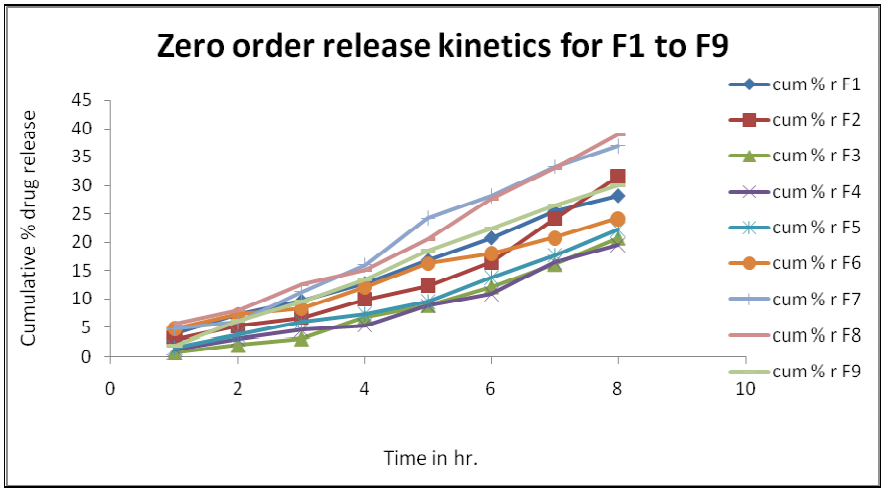

Figure 7. Zero order release fitted in formulation F1 to F9

Comparison of Various Release Kinetics Models of different formulations

It was observed that from Table 2, the n value lied between >0.5 but <1 in case of F1, F6 and F8, indicates anomalous Non-Fickian diffusion drug release and rest of the formulation shows the n value >1,indicates a Non-Fickian diffusion (supercase II) drug release.

Table 2. Comparison of the various release kinetics models of different formulation

Formulation code	Zero order model		First order model		Higuchi matrix model		Korsmeyer-peppas model		
	r^2	k_0	r^2	k_1	r^2	k_h	r^2	k_{kp}	n
F1	0.993	3.529	0.986	0.018	0.957	13.5	0.991	0.953	0.953
F2	0.922	3.865	0.895	0.020	0.843	14.4	0.950	1.089	1.089
F3	0.964	2.845	0.953	0.013	0.899	10.71	0.992	1.633	1.633
F4	0.945	2.578	0.933	0.012	0.876	9.678	0.978	1.288	1.288
F5	0.962	2.847	0.95	0.014	0.901	10.74	0.988	1.219	1.219
F6	0.988	2.817	0.986	0.014	0.956	10.8	0.969	0.801	0.801
F7	0.987	5.063	0.985	0.027	0.957	19.43	0.973	1.142	1.142
F8	0.979	4.862	0.962	0.027	0.925	18.42	0.966	0.952	0.952
F9	0.998	4.052	0.996	0.021	0.977	15.62	0.992	1.291	1.291

Drug content and content uniformity

It was observed that, there was no significant difference observed in the % drug at various locations, indicating that the method used to disperse the liposomal dispersion in the gel base is satisfactory.

Stability studies

Liposomal preparations were analyzed for stability for 60 months at 4-8 °C and room temperature. Responses obtained for different formulations for liposomal dispersion during stability period are exposed in Table 3. Liposomes were found to be rationally stable in terms of aggregation, fusion and/or vesicle disruption tendencies, over the studied storage period. From the above results it can be accomplished that at room temperature and freeze temperature there was slightly but insignificantly decrease in % entrapment efficiency.

Table 3. Effect of Entrapment efficiency during storage

No. of days	Entrapment Efficiency (%)			
	4-8^{0}C		Room temp	
	F1	F7	F1	F7
0	63.56	73.05	63.56	73.05
30	62.24	72.67	61.02	70.44
60	59.89	70.23	57.89	68.75

Experimental design and data acquiring

The influence of both ratio of PC: CHOL & hydration volume used to formulate fluconazole liposome was studied. Liposomes were obtained by lipid film hydration method. Response surface methodology with the aid of Central Composite Design was exploited to estimate the influence of both ratio of PC: CHOL & hydration volume as dependent variables and their interactions on the investigated responses. This experiment was aimed to identify considerable factor effect influencing the formulation performance and to set up to their excellent levels for the desirability of responses shown in Table 4.

Table 4. Response variables obtained from trial formulations of fluconazole liposomes.

Formulation code.	Ratio of PC : CHOL (mg) X_1	Hydration volume (ml) X_2	Entrapment Efficiency Y_1	%Drug released at 8hours Y_2
F1	5:1 (+1)	10 (-1)	63.46±1.2712	28.185±1.404
F2	4:1 (0)	20 (+1)	66.18±1.3216	31.45±1.575
F3	3:1 (-1)	15 (0)	56.66±1.1352	20.569±1.033
F4	3:1 (-1)	10 (-1)	55.56±1.1152	19.587±0.974
F5	3:1 (-1)	20 (+1)	58.82±1.1784	22.31±1.1105
F6	4:1 (0)	10 (-1)	60.73±1.2086	24.378±1.208
F7	5:1 (+1)	15 (0)	70.25±1.409	36.886±1.849
F8	5:1 (+1)	20 (+1)	73.25±1.461	38.878±1.94
F9	4:1 (0)	15 (0)	64.75±1.297	29.750±1.497

(n=3)

Statistical analysis and mathematical modeling of experimental data

To evaluate the quantitative effects of the combined ratio of factors and their levels on the preferred responses, the experimental values of the flux were analyzed by Design Expert software and mathematical models obtained for each response [19]. The mathematical relationship generated using multiple linear regression analysis for the studied response variables (entrapment efficiency and drug release at 8 hr.) that were relating different response and independent variables are expressed as following polynomial equations.

$$Y_1 (E.E.)=64.49+5.94A+3.05B+1.58AB-0.70A^2-1.05B^2 \quad (1)$$

$$Y_2 (\%DRat8hr.)=28.61+6.62A+3.40B+1.97AB-0.84A^2-1.62B^2 \quad (2)$$

The above equations expose the quantifiable effect of the dependent variables; Ratio of PC & CHOL and volume of hydration, on the responses such as E.E (Y1) and % DR at 8 hours (Y2) as dependent variables. The polynomial equation includes the coefficients intercept, first order of individual factor's influence, interaction and higher-order term [20]. In the above equations, the positive signs indicate synergistic effect, and the negative sign signifies the antagonistic affect. The positive regression coefficient of both factors in equations (1) and (2) propose that increase in E.E and % DR at 8 hours with an increase in concentration of the independent variables. It is also observed that influence of A > B on the both responses. In the equations (1) & (2) coefficients of factors with higher order term (A^2 & B^2) represent quadratic correlation. The negative regression coefficient of the quadratic term of A^2 & B^2, in equations (1) and (2) signifies that the respective responses decrease. There is a positive influence on the both responses by the interaction of the two factors [21]. For estimation of the significance of the model, the analysis of variance (ANOVA) was executed the linearity, interaction & quadratic term on responses. Using 5% significance level, a model is considered significant if the p-value (significance probability value) is less than 0.05 [22].

Table 5. Analysis of variance table of entrapment efficiency [Partial sum of squares - Type III]

Source	Sum of square	Df	Mean square	F- value	p-value prob>F	
Model	280.50	5	56.10	57.63	0.0035	significant
A-PC:CHOL	211.46	1	211.46	217.22	0.0007	
B- hydration volume	55.82	1	55.82	57.33	0.0048	
AB	10.02	1	10.02	10.29	0.0490	
A2	0.99	1	0.99	1.02	0.3877	
B2	2.22	1	2.22	2.28	0.2282	
Residual	2.92	3	0.97			
Cor- total	283.43	8				

Response 1 (entrapment efficiency)

ANOVA for response surface quadratic model

The Model F-value of 57.63 implies the model is significant shown in Table 5. There is only a 0.35% chance that a "Model F-Value" this large could occur due to noise. Values of "prob>F" less than 0.0500 indicate model terms are significant. In these case A,B,AB are significant model terms. Values greater than `1.000 indicate that models terms are not significant.

Response 2 (Drug release at 8 hr.)

ANOVA for response surface quadratic model

The Model F-value of 58.88 implies the model is significant. There is only a 0.34% chance that a "Model F-Value" this large could occur due to noise. Values of "prob>F" less than 0.0500 indicate model terms are significant. In these case A,B,AB are significant model terms. Values greater than `1.000 indicaste that models terms are not significant as revealed in Table 6.

Table 6. Analysis of variance table of drug released at 8 hr. [Partial sum of squares – Type III]

Source	Sum of square	Df	Mean square	F- value	p-value prob>F	
Model	354.15	5	70.83	58.88	0.0034	significant
A-PC:CHOL	262.55	1	262.55	218.26	0.0007	
B- hydration volume	69.36	1	69.36	57.66	0.0047	
AB	15.56	1	15.56	12.94	0.0368	
A2	1.41	1	1.41	1.17	0.3589	
B2	5.27	1	5.27	4.38	0.1274	
Residual	3.61	3	1.20			
Cor- total	357.76	8				

Formation of 3D response surface plots

To envisage the effect of independent factors on response, three-dimensional (3D) plots (Figure. 8 and Figure.9) for E.E and % DR at 8 hours were shaped based on the polynomial model respectively. All of the observed response surfaces formed hillsides with large curvatures confirms that they were typically influenced by the interaction effect of concentrations of both dependent factors.

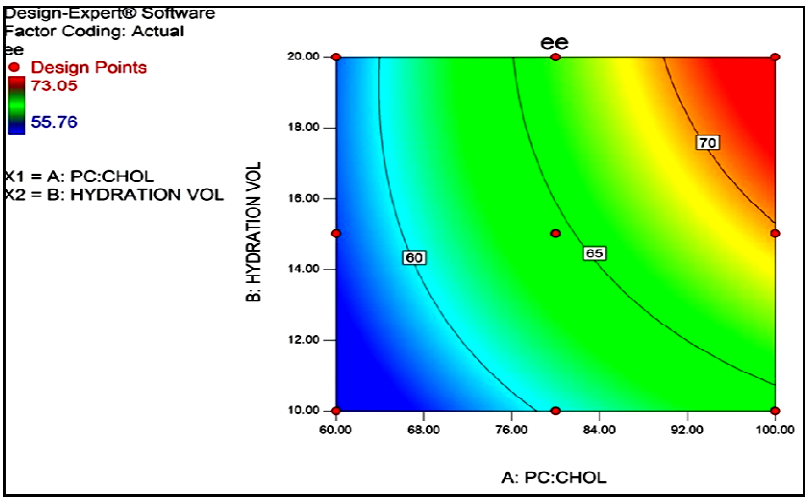

Figure 8. Contour plot showing the effect of ratio of PC:CHOL and hydration volume on Entrapment Efficiency from liposomal gel formulation

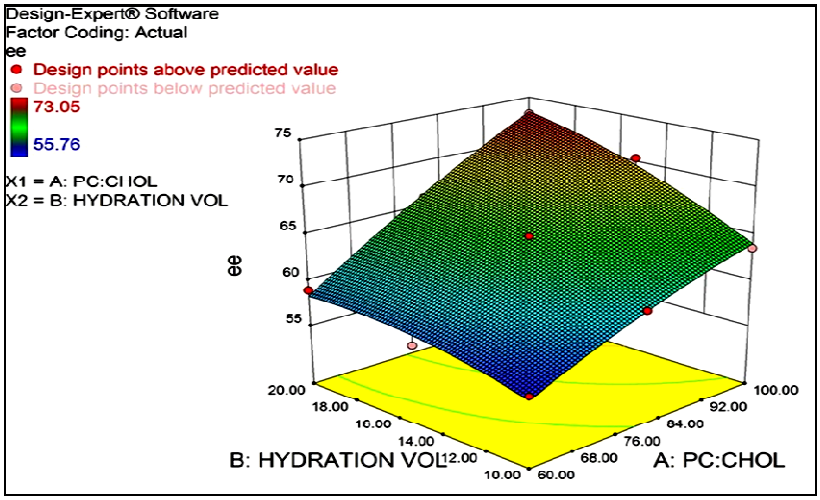

Figure 9. Response surface plot showing the effect of ratio of PC:CHOL and hydration volume on Entrapment Efficiency from liposomal gel formulation

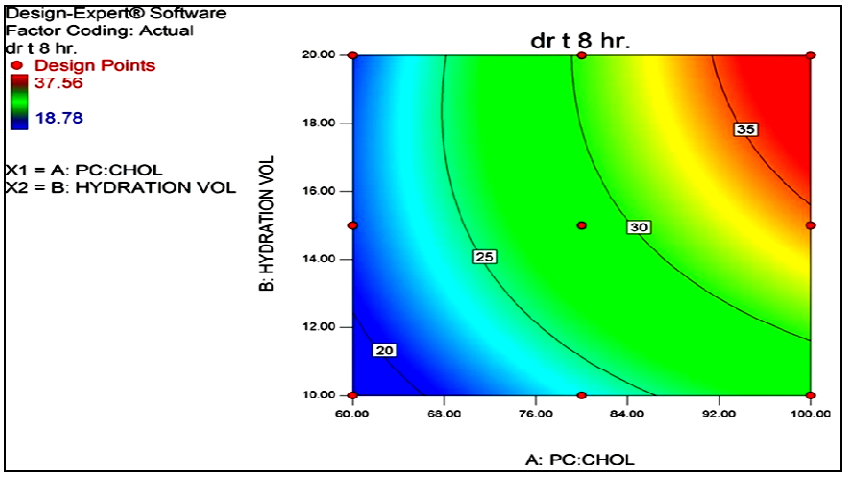

Figure 10. Contour plot showing the effect of ratio of PC:CHOL and hydration volume on % Drug release at 8 hr. from liposomal gel formulation

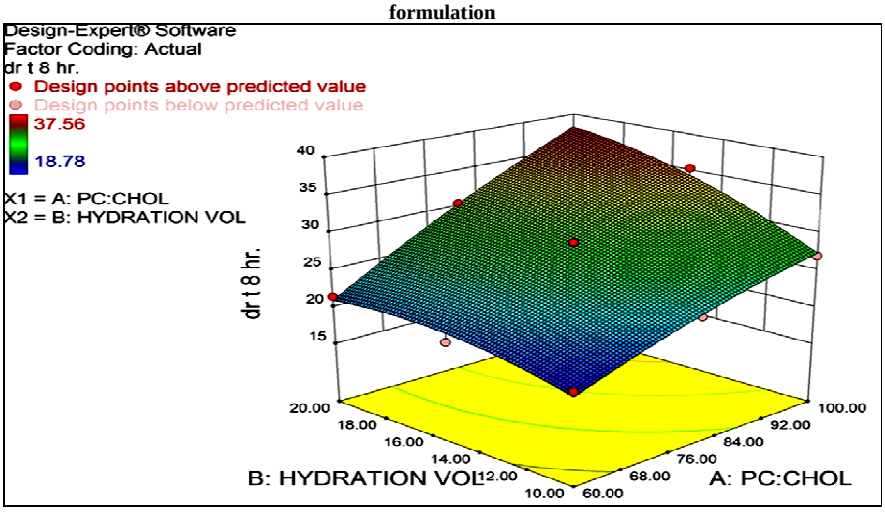

Figure 11. Response surface plot showing the effect of ratio of PC:CHOL and hydration volume on %Drug release at 8 hr. from liposomal gel formulation

Table 7. Comparison of the optimized formulation, the predicted and the experimental

Serial No.	Ratio of PC: CHOL (%)	Hydration volume (%)	Response variables	Observed response	Predicted response	Percentage error	Avg.
F-1	100:20(5:1)	15	(Y_1)	66.2883	69.7222	-3.4339	0.07
			(Y_2)	37.965	34.3822	3.5828	
F-2	60:20(3:1)	15	(Y_1)	56.033	57.8489	-1.8159	0.41
			. (Y_2)	23.789	21.1522	2.6368	
F-3	80:20(4:1)	10	(Y_1)	63.023	60.3856	2.6374	0.99
			(Y_2)	22.934	23.5822	-0.6482	
F-4	80:20(4:1)	20	(Y_1)	66.6732	66.4856	0.1876	0.22
			(Y_2)	30.6508	30.3822	0.2686	
F-5	60:20(3:1)	20	(Y_1)	56.873	58.2631	-1.3901	0.09
			(Y_2)	22.543	20.9564	1.5866	
F-6	100:20(5:1)	10	(Y_1)	66.234	64.0364	2.1976	1.77
			(Y_2)	28.745	27.3864	1.3586	
F-7	80:20(4:1)	15	(Y_1)	62.776	64.4889	-1.7129	0.55
			(Y_2)	31.432	28.6056	2.8264	
F-8	84.22:20 (4.211:1)	13.83	(Y_1)	63.956	64.8615	-0.9055	0.04
			(Y_2)	29.974	28.983	0.991	

values of response variables

Table 8. Final optimized formulation (F-8) of fluconazole liposomal gel

Part of liposomal gel	Ingredients	Amount
Liposome	PC	84.22mg
	CHOL	20mg
	Hydration volume	13.83ml
Gel	Carbopol	2%

Validation of RSM result

The optimization of the independent variables or the factors is another important step which was carried out by taking optimal release profile of the factors in range. The system had generated 39 solutions in the steepest ascent analysis [23] out of which 8 formulation were chosen as described in mathematical modeling part. For all of the 8 check point formulations, the result of the table assay was found to be within limits. Table 7 lists the composition, their predicted and experimental value of all response variables and percentage error. Figure 12 and Figure13 shows linear correlation plots between the observed and predicted response variables, and the residual plots showing the scatter of the residuals versus observed values.

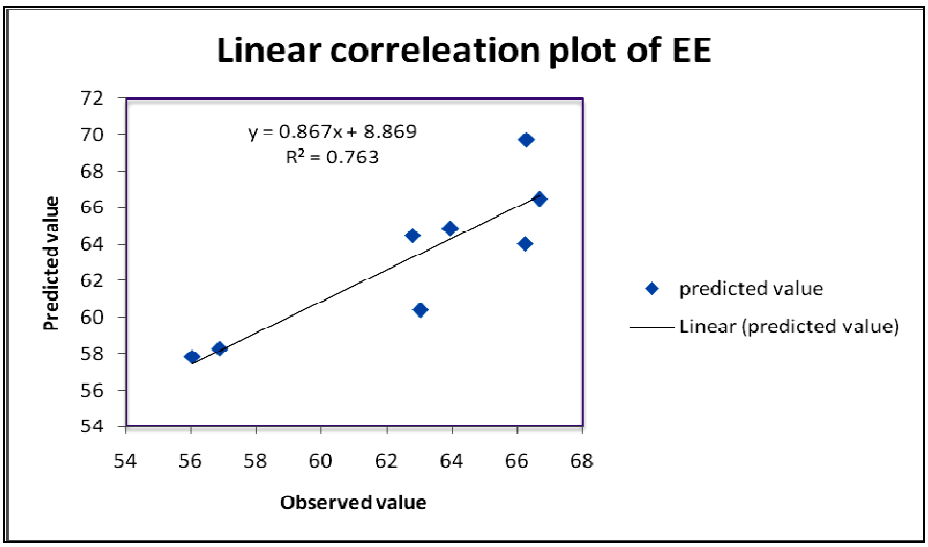

Figure 12. Linear correlation plot between observed and predicted values for % entrapment efficiency of check point formulation

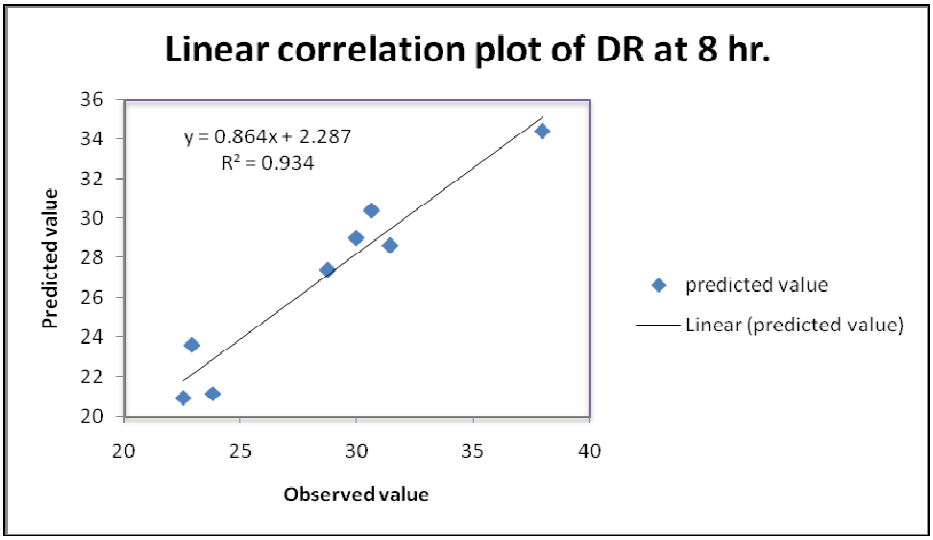

Figure 13. Linear correlation plot between observed and predicted values for % drug release at 8 hr. of check point formulation

By comparison between the observed responses with that of the anticipated responses, the prediction error varied between -3.4339 and 3.5828. The linear correlation plots drawn between the predicted and observed responses demonstrated high values of r^2 (ranging between 0.763 and 0.934), indicating excellent goodness of fit. The optimum formulation was selected by trading off various response variables and adopting the following maximizing criteria: EE = 63.956 and DR at 8 hr. = 29.974.

Upon comprehensive evaluation of grid searches, the formulation of F-8 as shown in Table 9 was chosen as the best optimized liposomal gel formulation of fluconazole with PC, CHOL as the error was low for the response of the dependable variables.

Table 9. Optimization of fluconazole liposomes formulation by surface response method

Response property	Range	Lower limit	Upper limit
Entrapment efficiency	In range	55.76	73.05
Drug release at 8 hr.	In range	18.78	37.56

CONCLUSION

The present study was done scientifically to achieve the above objective. The formulation of liposomal gel containing 5% fluconazole was prepared by dried film hydration method. From the different evaluation parameters and in vitro release study it is concluded that 29.950±1.497% drug released was observed in formulation F9 with drug entrapment efficiency 64.85±1.297 %. The in vitro diffusion study shows that r^2 value of formulation F9 was found 0.998. This is nearer to 0.999 and best among all. Diffusion study concluded that drug release profile follows Zero-order release. The optimum conditions were selected based on the drug release requirement and entrapment efficiency, by using RSM with the aid of CCD. The optimization sounded 39 solutions out of which 8 were selected through extensive grid search. After preparation and evaluation of these 8 batches F-8 came out with the coveted drug release pattern through the comparison of predicted and observed responses. The final optimized formulation is having the composition of PC 84.22mg with 20mg. The liposome was formulated with hydration volume 13.83ml and gel was prepared with 2% carbopol. Moreover, their mutual influences on studied parameters can be exploited and commercialized.

Acknowledgments
The authors gratefully acknowledge Prof. Rabindar Debnath, Executive Chairman of Calcutta Institute of Pharmaceutical Technology & Allied Health Sciences for his cooperation to avail all the laboratory facilities to carry out the research work.

REFERENCES

[1] Kauwe LK, Xie J, Hamm-Alvarez S, *Gene Ther.*, **2005**; 12,1734-51.
[2] Ziello JE, Huang Y, Jovin IS. *Mol.Med.*, **2010**, 16, 222-9.
[3] Rejman J, Oberle V, Zuhorn IS, Hoekstra D,.*Biochem.J*, **2004**, 377,159- 69.
[5] Wong AW, Scales SJ, Reilly DE, *J .Biol. Chem.*, **2007**, 282, 22953-63.
[6] Kheirolomoom A, Ferrara KW, *Biomaterials.*, **2007**, 28, 4311-20.
[7] Caracciolo G, Callipo L, De Sanctis SC, Cavaliere C, Pozzi D, Laganà, *Biochim.Biophys. Acta*, **2010**, 1798, 536-43.
[8] Aller AI, Martin,Mazuelos E, Lozano F, Gomez-Mateos J, Steele-Moore L, Holloway WJ, Gutierrez MJ, Recio FJ, A. Espinel-Ingroff, *Antimicrob. Agents. Chemother.*, **2000**, 44, 1544-8.
[9] Pfaller MA, Messer SA, Boyken L, Hollis RJ, Rice C, Tendolkar S, Diekema DJ., *Diagn Microbiol. Infect. Dis.*, **2004**, 48, 201-5.
[10] Montgomery, Douglas C. Design and analysis of experiments: response surface method and designs, New Jersey, John Wiley and Sons, Inc, **2005**.
[11] Mitkari BV, Korde SA, Mahadik KR, Kokare CR., *Indian.J.Pharm. Educ. Res*, **2010**, 44, 324-33.
[12] Prabhu P, Kumar N, Dhondge G, Koland M, Harish NM, Charyulu RN , Vijaynarayana K, *Int. J. Res. Pharm. Sci*, **2010**,1,502-8.
[13] Yadav AV, Murthy MS, Shete AS, and Sfurti Sakhare, *Indian.J.Pharm.Educ.Res*, **2011**, 45,402-413.
[14] Garg M, Dutta J, Jain N K. *AAPS. Pharm.Sci.Tech*, **2007**, 2, 156-89.
[15] Jithan AV, Swathi M, *Int.J.Pharm.Sci.Nanotech*, **2010**, 3,986-93.
[16] Fowler K, Lawrence A. Bottomley, Schreier H, *J. Controlled.Release*,**1992**, 22,283–91.
[17] Tseng LP, Liang HJ, Chung TW , Huang YU, Liu DZ., *Journal of medical and biological Engineering*, **2007**, 27,29-34.
[18] Ricci M, Sassi P,Nastruzzi C, Róssi C, *AAPS.Pharm.Sci.Tech*,**2000**, 1, 9-19.
[19] Panwar P,Pandey B,Lakhera PC, Singh KP. *Int.J. Nanomedicine*, **2010**,5,101-108.
[20] Chang JS, Huang YB, Hou SS, Wang RJ, Wu PC, Tsai YH, *Int.J.Pharm*, **2007**, 338, 48–54.
[21] Ahuja M, Yadav M, Kumar S. *Carbohyd. Polym*, **2010**, 80,161– 167.
[22] Pabari RM, Ramtoola Z, *Int. J. Pharm*, **2012**, 430, 18-25.
[23] Furlanetto S, Cirri M, Maestrelli F, Corti G, Mura P, *Eur. J. Pharma. Biopharm*, **2006**, 62, 77–84.
[24] Russell VL, *J. Stat. Software*, **2009**,32,1-17.