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Statistical Evaluation and Optimization of Influence of Viscosity and Content of Polymer on Floating Microspheres of Clarithromycin

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Abstract: The purpose of the present study was to develop an optimized gastroretentive drug delivery system (GRDDS) of clarithromycin floating microspheres by the optimization technique. The clarithromycin microspheres were prepared by non aqueous solvent evaporation method using different grades of hydroxylpropyl methylcellulose (HPMC) such as HPMC 15M (15cps), HPMC K4M (4000cps), HPMC 100LV (100cps) and ethyl cellulose (EC). The prepared microspheres were characterized by polymer compatibility, percentage yield, buoyancy percentage, drug entrapment efficiency and in vitro drug release. An optimized formulation investigated for morphology and particle size analysis by scanning electron microscopy. A 3^2 factorial design was employed in formulating the GRDDS with different viscosity grades of HPMC (X_1) and polymer-to-polymer ratio Ethyl cellulose: HPMC (X_2) as independent variables. Four dependent variables were percentage of yield, drug entrapment efficiency, buoyancy percentage and percentage of cumulative drug release of microspheres after 12h (R_{12h}). The main effect and interaction terms were quantitatively evaluated using a mathematical model. Regression analysis and numerical optimization were performed to identify the best formulation. The predicted values agreed well with the experimental values, and the results demonstrate the feasibility of the model in the development of GRDDS.
Keywords: HPMC, factorial design, floating microspheres, clarithromycin.

Introduction

The development of oral controlled-release drug delivery systems has been hindered by the fluctuation in gastric emptying time, the variation in pH in different segments of the gastrointestinal (GI) tract and the difficulty of localizing an oral delivery system in a selected region of the GI tract[1]. A gastric floating drug delivery system (GFDDS) [1-8] can overcome at least some of these problems and is particularly useful for drugs that are primarily absorbed in the duodenum and upper jejunum segments. The GFDDS is able to prolong the retention time of a dosage form in the GI tract, thereby improving the oral bioavailability of the drug[2]. Some studies have been conducted to evaluate various pharmaceutical excipients that could be used to achieve the floating of dosage forms[1-10].

Eradication of H.pylori, therapeutic agents have to penetrate the gastric mucus layer to disrupt and resist the mechanism of colonization. When conventional tablet of Clarithromycin are produces large plasma peak of drug after 2h of administration, which rapidly declines to below minimum inhibitory concentration of most pathogenic microorganisms, before subsequent doses of 250/500mg are administered at 8/12h intervals, which has low bioavailability of drug to 50-60% due to its first pass metabolism. The gastroretentive drug delivery system will release the drug over an extended period in stomach and upper GIT, which will increase opportunity of drug absorption, bioavailability and useful in eradication H.pylori by local action.

Microspheres are useful as a drug carrier to improve the performance of therapeutic system. Floating drug delivery is able to prolong the gastric retention of microspheres, thereby improving oral bioavailability of clarithromycin. Various studies had been conducted to evaluate the suitability of various excipients to achieve floating dosage forms. In the present study, HPMC was selected as hydrophilic polymer with different viscosity grades to demonstrate the effect of viscosities on drug release.

Statistical optimization designs have been previously documented for the formulation of many pharmaceutical solid dosage forms. Additionally, it is a powerful, efficient and systematic tool that shortens the time

required for the development of pharmaceutical dosage forms. [12-14]

Materials and Methods

Materials

Clarithromycin was obtained as a gift sample from Cipla (Mumbai; India), HPMC K4M, HPMC 100LV were provided by Colorcon Asia Private Limited (Goa; India). Ethylcellulose were obtained from Signet chemicals and HPMC 15M was purchased from SD fine chemicals. All other chemicals used were of analytical grade.

Full Factorial Design

A 3^2 factorial design applied to establish the inter-relationship between the selected variables. The variables studied were viscosity grades of HPMC (X_1) and ethyl cellulose: HPMC (X_2) at three different levels. The coded and the actual values of the experimental design are given in table 1. The data analysis of values obtained from various batches percentage of yield, drug entrapment efficiency, buoyancy percentage and percentage of cumulative drug release of microspheres after 12h (R_{12h}) was subjected to multiple regression analysis using statistical software Design Expert V 7.1.6. The equation fitted was

$$Y= \beta_0+ \beta_1X_1+ \beta_2X_2 + \beta_{12}X_1X_2 + \beta_{11}X_1^2+ \beta_{22}X_2^2$$

where Y: measured response; X: levels of factors; β: coefficient computed from the responses of the formulations.

Preparation of Microspheres:

Microspheres containing clarithromycin drug as a core material were prepared by a non aqueous solvent evaporation method[15]. Drug, HPMC and EC were mixed in the mixture dichloromethane and ethanol solvent system at the ratio of 1:1. The slurry was slowly introduced into 30 ml of liquid paraffin containing 0.01% tween 80 which is being stirred at 1200 rpm using mechanical stirrer equipped with three bladed propellers at room temperature. The solution was stirred for 2h and the solvent was allowed to evaporate completely and filtered by using filter paper. The microspheres obtained were washed repeatedly with petroleum ether (40°-60°C) to make it free from oil. The collected microspheres were dried at room temperature and subsequently stored in desiccator. Same procedure was repeated for all batches with different viscosity grades of HPMC such as HPMC K4M, HPMC 100LV and HPMC 15M. Drug polymer ratios used in different formulations were given in the table:1.

Fourier transform infra-red analysis

FTIR measurements of drug, and optimized microspheres were obtained on PERKIN ELMER FTIR. Samples were prepared by mixing with KBr and placing in the sample holder. The spectra were scanned over the wave number rang e of 4000-400 cm^{-1} at the ambient temperature.

Morphology and Particle Size Analysis

The shape and surface morphology of various batches of microspheres prepared were determined by scanning electron microscopy (SEM-JEOL Model 8404, Japan at magnification 500). Particle size was determined using an optical microscope (Olympus, New Delhi, India) fitted with a stage and an ocular micrometer.

Buoyancy percentage

The microspheres weighed about 0.3 g were spread over the surface of USP XXIV dissolution apparatus (Type II) which was filled with 900 ml of 0.1 mol L^{-1} HCl containing 0.02% of tween 80. The medium was agitated with a paddle rotating at 100 rpm for 12h. The floating and the settled portions of microspheres were recovered separately. The microspheres were dried and weighed. Buoyancy percentage was calculated as the ratio of the mass of the microspheres that remained floating and the total mass of the microspheres.[16]

Drug Entrapment Efficiency (DEE)

Microspheres equivalent to 50 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N HCl repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1N HCl. Clarithromycin was analyzed by RP-HPLC as per reported method.[17] The amount of drug entrapped in the microspheres was calculated by the following formula:

DEE = Amount of drug actually present / Theoretical drug load expected

Yield of Microspheres

The prepared microspheres with a size range of 245-260μm were collected and weighed. The measured weight was divided by the total amount of all non-volatile components, which were used for the preparation of the microspheres.

% Yield = Actual weight of products / Total weight of excipients and drug

Dissolution Studies

The release rate of clarithromycin from microspheres was determined using USP dissolution test apparatus Type I (basket type). The dissolution test was performed using 900 ml of 0.1N HCl at 50 rpm. The temperature of the medium was maintained at 37 ± 0.5°C and the study was carried out for 12h. The withdrawn samples were replaced with fresh dissolution medium. The withdrawn samples were filtered through 0.45μm syringe filter and neutralized with NaOH solution (0.014 M) to adjust the pH of sample to approximately 5.0 in order to prevent the further degradation of drug before analyzed by RP-HPLC as described above. These experiments were conducted in triplicate.

Result and discussion

The IR spectra of clarithromycin showed the characteristic band of hydrogen bonds between –OH groups vibration at 3466 cm^{-1} characteristic band C=O vibration of lactone group at 1733 cm^{-1} and strong absorption band at 1691 cm^{-1} belonging to the carbonyl ketone, peak for N-CH_3 stretching of aromatic ring at 1424 cm^{-1}. The IR spectra of optimized microspheres showed all the above mentioned peaks of clarithromycin.

These results showed that there is no interaction between drug and excipients shown in fig.1.

The mean particle size of floating microspheres prepared by the non aqueous solvent evaporation process found between 245 to 260 µm. The mean particle size was slightly reduced when low viscosity grade of polymer used. This can be explained by the increased deposition rate of polymer due to the decrease in solubility of polymer with increasing viscosity effects on the system.

The scanning electron photomicrographs of floating microspheres of clarithromycin are shown in

fig.2. revails a smooth and uniform texture for all the microspheres which shows there were no drug crystals on the surface .

The experimental runs with independent variables and corresponding responses for the 9 formulations are presented in table 2. The dependent variables were the percentage of yield (Y_1), drug entrapment efficiency (Y_2), buoyancy percentage (Y_3) and percentage of cumulative drug release of microspheres after 12h (R_{12h}) (Y_4). Based on the 3^2 factorial design, the factor combinations resulted in different drug release rates. Various models, such as linear, 2FI, quadratic and cubic, were fitted to the data for three responses simultaneously using Design Expert software and adequacy and good fit of the model was tested using analysis of variance (ANOVA). The multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (adjusted R^2) and the predicted residual sum of square (PRESS) provided by Design-Expert software were used as factors for selection of adequate models. The lack of fit analysis (data not shown) shows that a quadratic model is appropriate for the description of all responses except for Y_2 appropriate model is reduced quadratic model.

From the results, the quadratic model was selected as a good fit for the model because its PRESS was the smallest. PRESS is a measure of the fit of the model to the points in design; the smaller PRESS the better the model fits to the data points. The quadratic model generated by the design is of the form:

$$Y= \beta_0+ \beta_1X_1+ \beta_2X_2 + \beta_{12}X_1X_2 + \beta_{11}X_1^2+ \beta_{22}X_2^2$$

where β_0 is an intercept and β_1-β_{22} are the coefficients of respective factors and their interaction terms. Mathematical relationships in the form of quadratic equations for all responses and their standardized main effects are shown in Tables 3 and 4, respectively. Positive or negative signs before a coefficient in quadratic models indicate a synergistic effect or an antagonistic effect for the factor. The data clearly indicate that the dependent variables are strongly dependent on the independent variables.

Floating Microspheres were prepared by non aqueous solvent evaporation method with different grades of HPMC (X_1) of 15,100 and 4000cps in various proportions (X_2). Three batches of each formulation, which contains of 250mg of drug and various ratios of polymer (different grades of HPMC) from 1:1 to 1:3 were taken. The parameters which were evaluated for microspheres are given in the table 2. Percentage yield for different batches were determined, it was found to be that percentage yield of F8 was 75.8%. Fig.3. shows the interaction effects of the viscosity grades (X_1) and polymer ratio (X_2) on the percentage yield. Equation for % yield (table 3) showed β_1 and β_2 positive but β_{11} and β_{22} negative this revails that up to certain level increase in X_1 and X_2 from -1 to 1 increase % yield after that point again decrease in % yield seen. In general the percentage yield of batches containing HPMC 100LV i.e. X_1 at 0 levels were satisfactory. The lowest yield was with F1 (50.5%).

The encapsulation efficiency was found to be 14.1% to 16.4% for batches containing X_1 at -1 level, 27.5% to 29.8% for batches containing X_1 at +1 level and 20.1% to 39.4% for batches containing X_1 at 0 level. Fig.4. shows the interaction effects of the viscosity grades (X_1) and polymer ratio (X_2) on the encapsulation efficiency. Equation for encapsulation efficiency (table 3) showed β_1 and β_2 positive but β_{11} negative this revails that up to certain level increase in X_1 and X_2 from -1 to 1 increase encapsulation efficiency after that point again decrease in encapsulation efficiency seen. Encapsulation efficiency observed maximum for batches containing X_1 at 0 level.

The Buoyancy percentage for all batches was almost above 50% which was studied for 12hours. Fig.5. shows the interaction effects of the viscosity grades (X_1) and polymer ratio (X_2) on the buoyancy percentage. The highest percentage for batches containing X_1 at 0 level. Average buoyancy in percentage for all batches was found to be in the range of 68.1% to 76.4%. In general the buoyancy percentage increased with increase in the amount of polymers and decreases with increase in viscosity level. The increase in the buoyancy percentage may be attributed to air which caused swelling because of increased amount of the polymers present.

Microspheres were subjected to in vitro release studies using USP dissolution apparatus Type I in 900ml of simulated gastric pH medium (0.1M HCl). Fig 6 shows the interaction effects of the viscosity grades (X_1) and polymer ratio (X_2) on the buoyancy percentage. With all the formulation there was an initial intermittent burst release. But the release seems to be somewhat sustained with increase in the amount of polymer and viscosity grades. As equation for R_{12h} shows (table 3) all β values negative except β_0.

After generating the model polynomial equations to relate the dependant and independent variables, the formulation was optimized for all three responses. The final optimal experimental parameters were calculated using the canonical analysis, which allows the compromise among various responses and searches for a combination of factor levels that jointly optimize a set of responses by satisfying the requirements for each response in the set. In this study, the optimization was performed with constraints for independent variable X_1 i.e. viscosity grade kept constant at 0 level for all four responses, with constraints percentage yield, entrapment efficiency, buoyancy percentage for maximizing and R_{12h} in range of 95 to 102 %.The optimal calculated parameters were:

Viscosity grades HPMC at 0 level i.e. HPMC 100LV, Ethyl cellulose:HPMC at 0.13 level i.e.1:2.3 ratio. From the results presented in Table 5, it can be concluded that optimized formulation of investigated independent variables ensured the response variables, which were very close to the predicted values.

Conclusion

The method for preparation of floating microspheres of clarithromycin with optimal yield, entrapment efficiency and buoyancy as well as optimal release properties was determined using experimental design methodology. After determination of significant parameters by using the 3^2 factorial design was applied. The model reliability and estimation of quantitative effects of different levels of investigated factors was performed using the Design expert 7.1.6 statistical software. The levels of these factors were predicted to obtain an optimal response with reference to set constraints. The observed responses were close to the predicted values for the optimized drug release method. From the above results, it can be concluded that optimization of the floating microsphere of clarithromycin was performed in a very short time period and with small number of experimental runs.

Table 1: Variables in 3^2 factorial Design

Independent Variables	Levels used		
	-1	0	1
X_1= Viscosity grades HPMC	HPMC15M	HPMC100LV	HPMCK4M
X_2= Ethyl cellulose:HPMC	1:1	1:2	1:3
Response Variables			
Y_1= percentage yield Y_2= drug entrapment efficiency Y3= buoyancy percentage Y_4= percentage of cumulative drug release of microspheres after 12h (R_{12h})			

Table 2: Experimental Runs and Observed Responses for 3^2 Factorial Design

BATCH CODE	X_1	X_2	% yield (Y_1)	% entrapment (Y_2)	% buoyancy (Y_3)	Rel_{12h} (Y_4)
F1	-1	-1	50.5	14.1	70.1	101.9
F2	0	-1	67.5	20.1	69.5	100.1
F3	1	-1	50.5	27.5	68.1	100.1
F4	-1	0	65.3	15.6	75.6	97.67
F5	0	0	69.3	35.3	70.3	101.8
F6	1	0	65.9	25.3	69.3	87.82
F7	-1	1	66.5	16.4	76.4	90.8
F8	0	1	75.8	39.4	75.8	65.09
F9	1	1	69.1	29.8	70.4	61.06

Table 3: Quadratic Equations for the Quantitative Effect of Independent Variables (X_1, X_2) on the Responses (Y_1, Y_2, Y_3 and Y_4)

Percentage yield (Y_1) $=+72.07+0.53X_1+7.15X_2+0.65X_1X_2-8.87X_1^2-2.83X_2^2$
Drug entrapment efficiency (Y_2) $=+32.70+6.08X_1+3.98X_2-11.25X_1^2$
Buoyancy percentage (Y_3) $= +71.21-2.38X_1+2.48X_2-1.00X_1X_2+0.19X_1^2+0.39X_2^2$
Rel_{12h} (Y_4)$=+96.94-6.90\ X_1-14.21X_2-6.98\ X_1X_2-0.19X_1^2-10.29X_2^2$

Table 4: Results of Analysis of Variance for Measured Response*

Parameters	df	SS	MS	F	Significance
For percentage yield (Y_1)					
Regression	5	538.52	107.70	6.31	0.0493
Residual	4	68.32	17.08		
Total	9	606.83			
Drug entrapment efficiency (Y_2)					
Regression	3	620.99	207.00	8.94	0.0124
Residual	6	138.95	23.16		
Total	9	759.94			
Buoyancy percentage (Y_3)					
Regression	5	75.58	15.12	5.51	0.0497
Residual	4	10.96	2.74		
Total	9	86.55			
Percentage of cumulative drug release of microspheres after 12h (R_{12h}) (Y_4)					
Regression	5	1947.76	389.55	9.79	0.0231
Residual	4	159.13	39.78		
Total	9	2106.89			

**df indicates degree of freedom; SS, sum of square; MS, mean sum of square; and F, Fischer's ratio.*

Table 5: Predicted and Observed Responses of the Optimized Formulation

Responses	Predicted	Observed	Residuals[a]
Y_1	72.9181	73.2	0.2819
Y_2	33.1993	32.9	-0.2993
Y_3	71.5245	71.0	-0.5245
Y_4	95	96.1	1.1

Residual[a] = observed value - predicted value

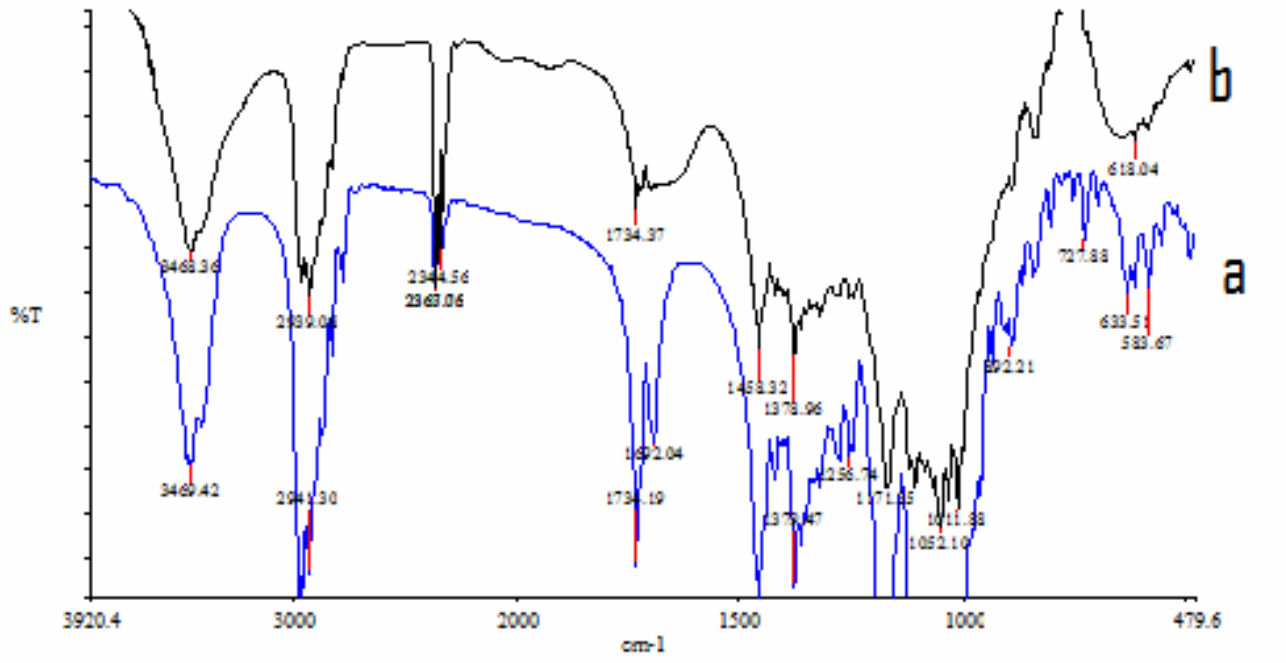

Fig. 1: Drug polymer interaction study by FTIR (a.drug b.optimized microspheres).

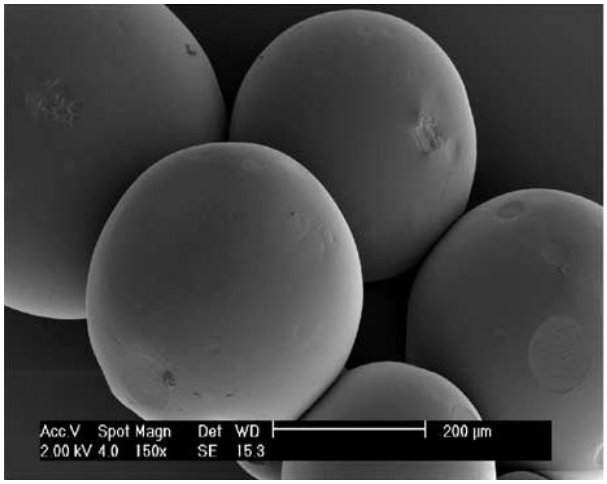

Fig. 2: Scanning electron micrograph of microspheres prepared by solvent evaporation method at a resolution of 2kv × 150.

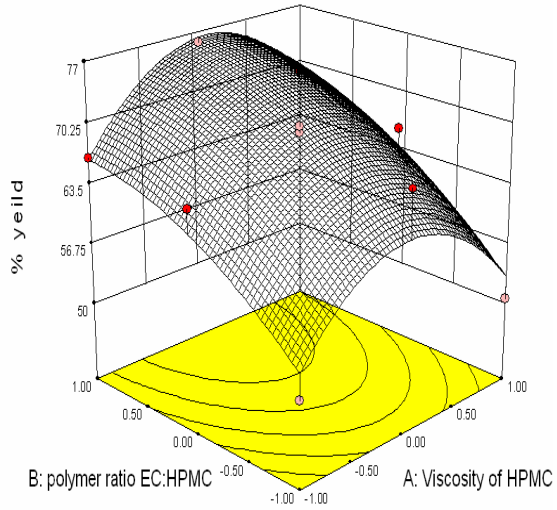

Fig. 3: Response surface plot of percentage yield.

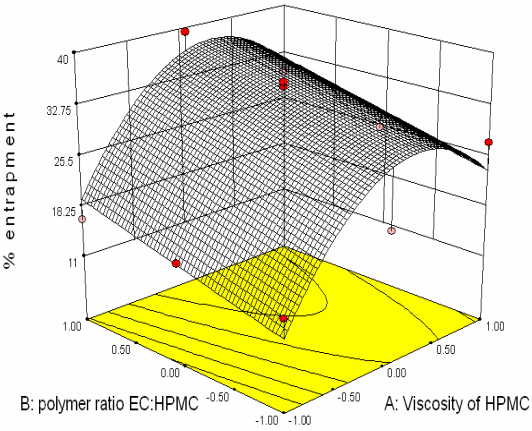

Fig. 4: Response surface plot of drug entrapment efficiency.

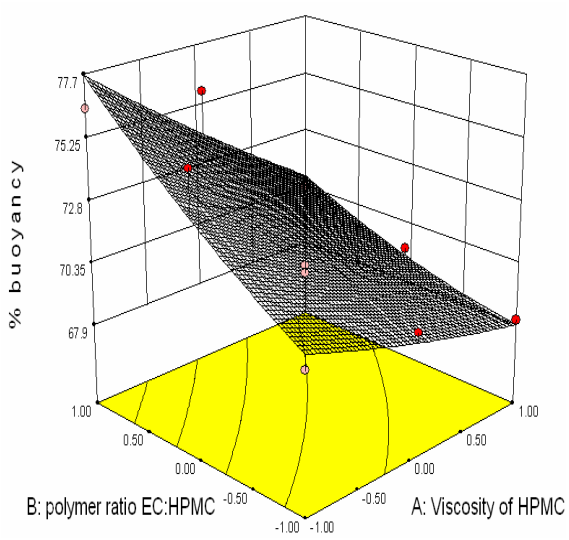

Fig. 5: Response surface plot of buoyancy percentage.

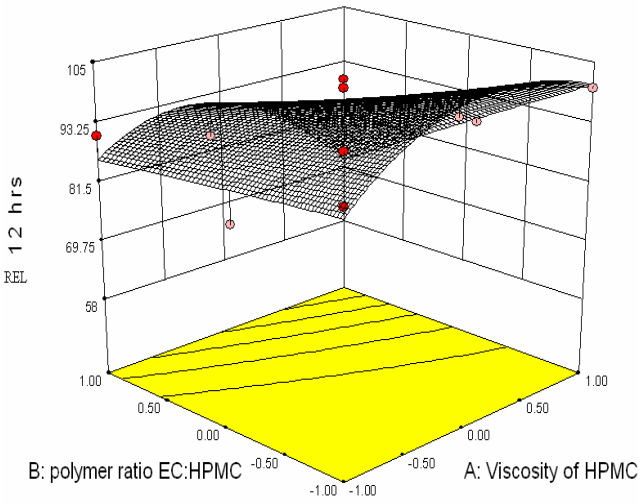

Fig. 6: Response surface plot of percentage of cumulative drug release of microspheres after 12h.

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