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Experimental design to predict process variables in the preparation of cellulose based sustained release microspheres system loaded with prednisolone-cyclodextrin complex

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Abstract−The purpose of this study was to evolve experimental design, to prepare the sustained release microspheres loaded with prednisolone-hydroxypropyl-β-cyclodextrin complex, and develop a successful mathematical model to predict various characteristics of microspheres. Response surface methodology (RSM) has been employed to develop model equations that correlate process variables such as ethyl cellulose (EC, mg), hydroxypropyl methyl cellulose (HPMC, mg), stirring speed (rpm) and surfactant (%) with the response variables such as entrapment efficiency (%), particle size (μm) and release rate (%) of the drug. The adequacy of model equations is confirmed by ANOVA result. Results as predicted by model equations are in good agreement with that of experimental results. *In vitro* drug release shows that drug (93%) is released from a check point formulation (CPF 2) over the period of 24 h with a sustained release fashion with Quasi-Fickian kinetics. Surface morphology of microspheres varies with the experimental conditions as evidenced by scanning electron microscopy.

Key words: Response Surface Methodology, Sustained Release Microspheres, Prednisolone-cyclodextrin Complex, Entrapment Efficiency, Average Particle Size, Drug Release

INTRODUCTION

Frequent administration of some drugs with higher doses often causes severe side effects, and many patients are bound to withdraw the recommended course of therapy [1]. The prolonged and controlled drug delivery systems are found more advantageous over the immediate-release-conventional dosage forms in terms of the reduction of dosing interval, side effects and improvement of patient compliance [2]. The use of multiunit-dosage systems as drug carriers has been studied extensively by numerous investigators for many years to evaluate its potential in delivering and targeting the bioactive molecules at the specified sites [3-5]. The preparation techniques such as coacervation phase separation [6], cross linking [7] and solvent evaporation [8] are widely used in the recent research of pharmaceuticals. The emulsion solvent evaporation method is used extensively to prepare drug-loaded-polymer based microspheres as it presents a very simple method [8-10]. Various biodegradable and compatible polymers such as PLGA, cellulose derivatives and methacrylate are used to entrap various classes of drugs [8,11-13]. Prednisolone (PRD), a glucocorticoid is highly potent anti-inflammatory and immunosuppressive drug. These are also used in substitution therapy for adrenal insufficiency. Even at a moderate-dose repeated administration of drug for prolonged period causes many side effects such as diabetes, hypertension, Cushing syndrome and osteoporosis [14]. Earlier investigators performed numerous works to evaluate the drug release characteristics of prednisolone loaded in microspheres [15-18], but no one has considered the mathematical model design of the process that involves various process-variables for prednisolone. Only a few investigators have reported the effect of process variables on the characteristics of microspheres that have been prepared by the emulsion solvent evaporation method [5,8-10]. The aforesaid design is initiated with judicial selection of controlling variables to achieve desired results. To achieve a desirable release rate of drug the key process-parameters for the preparation of sustained release microspheres are polymer/polymer-mixture/concentration, stirring speed, preparation method, volume of continuous phase and emulsifier concentration [19-21]. Therefore, systematic work is needed to analyze and optimize the process parameters to understand their effects on the response variables such as encapsulation efficiency (%), average particle size (μm) and drug release (%) from the microspheres. The response surface methodology (RSM) is an experimental design used for optimization, which helps develop model equations and carry out the analysis of experiments with the least number of experiments. Basically, this methodology is a collection of mathematical and statistical techniques that are useful for the modeling and analysis of problems [21]. Chemical technologists first ventured the application of RSM in experimental design, but with the advent of software technology it gained popularity in the pharmaceutical field also. The aim of the present work is to design the experiments for the preparation of sustained release microspheres loaded with prednisolone - hydroxypropyl-β-cyclodextrin complex and to develop mathematical model by RSM to predict various characteristics of microspheres.

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MATERIALS AND METHODS

1. Materials

The gifted samples are prednisolone (Medopharm, India), ethyl cellulose (Signet Chemical Corporation Pvt. Ltd, India), hydroxypropyl-β-cyclodextrin (HPβCD) (Roquette, Lestren), HPMC E15 (Colorcon Asia Pvt. Ltd, GAO, India). Span 80 (Loba Chemie, Mumbai, India) and Heavy liquid paraffin (Merck Specialties Pvt. Ltd, Mumbai, India) were purchased. All the reagents are of analytical grade.

2. Experimental Design

In the practical application of RSM, it is necessary to develop an approximate model of the true response surface. The approximate model is based on observed data from the processor system and is an empirical model. Usually, a second-order polynomial Eq. (1) is used in RSM,

$$\mathbf{y} = \beta_o + \sum_{j=1}^{k} \beta_j \mathbf{x}_j + \sum_{j=1}^{k} \beta_{jj} \mathbf{x}_j^2 + \sum_{i<j}^{k} \sum_{j=2}^{k} \beta_{ij} \mathbf{x}_i \mathbf{x}_j \quad (1)$$

where parameters β_{ij}=0, 1, …, k are called the regression coefficients, y is the response variable and x_i the independent variables.

In the present study, central composite design with four factor five level RSM modeling tool is employed to predict the effect of process variables on entrapment efficiency (R1), particle size (R2), and release of prednisolone from the prepared matrix microspheres at 1 h (R3) and % release at 8 h (R4).

The following independent process parameters have been identified during the trial experimental studies: (A) amount of ethyl cellulose in mg, (B) amount of HPMC - E15 in mg, (C) stirring speed in rpm and (D) surfactant Concentration in %v/v. The drug release rate is controlled by the variation of polymer composition and interfacial surface area of the microspheres. Ethyl cellulose at higher concentration retards the drug release from the polymeric matrix owing to its hydrophobic nature. Surfactant and high stirring speed result in the formation of smaller size of emulsion droplet. In the present study, higher entrapment efficiency, and % release of drug at desired rate are achieved by designing experiments with variables within their levels. Trial runs are conducted by varying one of the process parameters at a time, while other parameters are kept at constant value. The range of level is fixed according to extreme values (low and high) of the entrapment efficiency and drug release.

The selected process parameters and their limits, units and notations are given in Table 1. Design of experiment (DOE) software Design-Expert v7 is used to code the variables and to establish the design matrix. RSM is applied to the experimental data by using the same software to obtain the regression equations and to generate the statistical and response plots.

Table 1. Process control parameters and their limits

Parameters	Units	Notations	Limits				
			−2	−1	0	1	2
Ethyl cellulose	mg	A	100	200	300	400	500
HPMC	mg	B	0	50	100	150	200
Stirring speed	rpm	C	800	1000	1200	1400	1600
Surfactant	% v/v	D	0	1	2	3	4

3. Preparation of Prednisolone-cyclodextrin Complex

By using HPâCD (carrier), solid dispersion of prednisolone was prepared by the solvent evaporation method. Accurately weighed amount of prednisolone (3 gm) and carrier (15 gm) was dissolved in 300 ml mixture of ethanol and water at a ratio of 7 : 3 v/v. Then, the mixed solvent was evaporated under room temperature (25 °C) for 48 h. After complete evaporation of solvent, the obtained solid dispersion was pulverized by an agate mortar and pestle. The 120 μm sieve fraction was then used for further studies. Actual drug content was found as 50 mg in 320 mg of solid dispersion (i.e. 15.62%) after its assay in UV spectrophotometer.

4. Preparation of Microspheres

Drug-loaded microspheres were prepared by modified oil-in-oil (O/O) emulsion solvent evaporation method [22]. In brief, 320 mg of PRD - HPβCD was dissolved in 10 ml of solvent mixture (chloroform and ethanol (1 : 1 v/v)). To this solution specified amount of polymers (ethyl cellulose and HPMC-E15), as per the experimental design showed in Table 2, was added and stirred for 15 min in a magnetic stirrer and subsequently ultrasonicated (Takashi, Japan) for 5 min to make homogeneous dispersion. This dispersion was added dropwise to 125 ml of heavy liquid paraffin containing Span 80. Span 80, a surfactant acts as emulsifying agent. The resultant mixture was stirred at specified rpm at room temperature for 3 h. After the formation of primary emulsion, solvent present in the emulsion droplet diffuses into the continuous paraffin phase and gets evaporated [23]. Heavy paraffin was used to retard the fast diffusion of solvent, and this retardation of diffusion facilitates bridging between drug and polymer. Gradually, soft droplets turn to hard microsphere when solvent diffuses out of the droplets. To solidify the microspheres further, 25 ml of petroleum ether (non solvent) was added to it and the stirring was continued for next 2 h. The hardened microspheres were collected by filtration and washed with 100 ml of petroleum ether and air dried for 12 h. After wash with excess quantity of petroleum ether, microspheres turned from pale yellow color to white. Later, the used petroleum ether was collected and recovered by distillation process for reuse.

5. Determination of Yield, Drug Loading and Entrapment Efficiency

A 50 mg microsphere was pulverized and dissolved in 5 ml of methanol and diluted up to 50 ml with double distilled water in a volumetric flask and then necessary dilution was made. Absorbance of the sample was noted at 247 nm and content of drug in microspheres was determined. Encapsulation efficiency was determined in triplicate for all batches using Eq. (2). Values were expressed as a percentage:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{actual weight of prednisolone in sample}}{\text{theoretical weight of prednisolone}} \times 100 \quad (2)$$

6. Particle Size Determination by Microscopy

The average particle size of the microspheres was determined by using an optical microscopy method. Approximately 100 microspheres were taken on a glass slide and the particle size was measured using a calibrated optical microscope (KYOWA Getner microscope, TOKYO) under regular polarized light.

7. *In vitro* Release Studies

In vitro release studies were performed in USP basket apparatus

Table 2. Design matrix and measured responses

Run order	Formulation parameters				Responses			
	A (mg)	B (mg)	C (rpm)	D (%v/v)	% Entrapment efficiency	Average particle size (μm)	Release at 1 h (0.0%)	Release at 8 h (0.0%)
1	200	150	1000	1	72.36±4.21	312.48±30.484	48.42±2.48	86.81±2.42
2	300	100	1200	4	74.87±6.721	286.31±24.546	43.34±1.663	75.96±1.776
3	300	100	1200	2	77.65±3.762	425.73±19.869	40.19±3.088	75.03±2.51
4	400	50	1000	1	75.97±3.258	526.52±8.549	26.47±2.514	54.81±3.711
5	200	50	1400	3	64.74±3.535	180.23±17.675	55.28±1.713	91.9±2.32
6	100	100	1200	2	61.64±3.495	164.9±14.457	63.37±1.793	99.02±0.176
7	400	50	1400	3	71.74±4.632	273.59±10.557	32.37±1.445	60.27±2.102
8	300	100	1600	2	74.93±5.208	216.08±11.027	44.44±1.483	78.27±3.106
9	400	150	1000	1	78.93±4.007	523.75±21.398	32.78±1.035	60.9±2.343
10	200	50	1400	1	69.82±4.293	238.74±18.293	48.42±1.445	85.73±2.138
11	400	150	1400	3	81.37±5.074	278.46±19.738	35.8±1.425	70.79±3.024
12	300	100	800	2	78.07±2.861	440.43±9.432	34.56±2.291	72.32±2.144
13	400	50	1000	3	79.39±5.904	450.69±12.283	31±1.257	57.18±3.731
14	300	100	1200	2	76.32±5.105	430.23±25.13	38.68±1.483	73.18±1.368
15	200	150	1400	3	69.01±3.113	190.38±11.039	60.49±1.793	94.41±1.332
16	400	50	1400	1	74.19±5.306	440.64±23.159	29.08±2.411	59.92±2.673
17	200	50	1000	1	68.8±1.506	310.91±18.906	41.01±2.266	81.77±1.492
18	300	100	1200	2	75.84±3.979	428.08±17.898	38.4±1.663	74.29±1.989
19	500	100	1200	2	76.32±5.569	480.47±16.396	25.24±1.445	49.93±1.19
20	200	150	1400	1	75.58±3.522	255.22±12.302	54.45±1.257	91.43±3.305
21	200	50	1000	3	69.47±2.934	283.54±27.967	47.32±1.234	85.57±2.396
22	300	100	1200	2	76.8±4.204	420.65±23.952	39.36±3.548	76.84±0.931
23	300	100	1200	2	76.3±6.005	415.21±7.796	40.19±2.266	74.49±1.175
24	200	150	1000	3	73.39±2.67	290.65±13.205	52.53±1.445	90.59±2.639
25	400	150	1400	1	82.14±6.093	445.19±23.670	33.88±1.855	66.78±1.774
26	300	100	1200	0	75.82±2.554	435.51±12.370	34.84±1.713	72.3±2.621
27	400	150	1000	3	83.51±5.181	452.05±11.589	33.88±1.035	64.36±1.974
28	300	0	1200	2	72.86±4.354	375.12±5.292	34.29±2.266	66.62±3.05
29	300	200	1200	2	83.94±4.454	386.16±15.458	43.89±1.944	81.93±1.31
30	300	100	1200	2	77.09±4.354	425.67±26.196	39.91±2.291	72.86±2.914

(TDT 06P Electro lab, India). Microspheres containing drug equivalent to 30 mg were added to 500 ml of dissolution medium (pH 7.4, phosphate buffer) thermostated at 37±0.5 ºC and stirred at 50 rpm. At suitable time intervals, 5 ml samples were withdrawn from the dissolution vessels and immediately replaced with the same volume of the fresh dissolution medium. Samples were withdrawn from the dissolution medium at intervals of 1 h upto 12 h and at 24 h, and the samples were filtered by a Whatman filter paper (pore size 11 μm). Drug content in the filtrate was determined by UV spectrometric method (ANALAB UV - 180 Spectrophotometer) at λ max 247 nm [24]. No interference in the measurement of the drug due to presence of other ingredients was observed. The percent of drug re-

Table 3. Drug release kinetics for check point formulation (CPF)

S. No.	Zero order		First order		Korsmeyer peppas		Higuchi		Cube root	
	k_1	R^2	K_2	R^2	k_3	R^2	k_4	R^2	K_5	R^2
CPF 1	2.0994	0.6369	0.3366[a]	0.9202	0.2438	0.9495	14.169	0.8383	0.2364[a]	0.9899
CPF 2	2.4801	0.847	0.0978	0.9951	0.3191	0.993	15.237	0.8835	0.0923	0.9663
CPF 3	2.0947	0.7045	0.0635	0.835	0.3109	0.9648	13.831	0.8875	0.0668	0.7943
CPF 4	2.1032	0.8378	0.0567	0.9551	0.3102	0.9938	13.287	0.9662	0.0622	0.9221
CPF 5	1.7845	0.6476	0.1951	0.9338	0.1992	0.9571	12.007	0.8472	0.1916	0.9934
CPF 6	3.2015	0.5594	0.1812[a]	0.9981	0.283	0.9613	15.237	0.8835	0.1663[a]	0.9827

[a]Calculated with data upto t=12 hr

leased was plotted against time. Each experiment was repeated thrice.

8. Mathematical Modeling of Drug Release

In the design of new drug delivery system, it is essential to characterize release data (*in vitro*) by various kinetics equations and empirical/semi-empirical models. These are zero-order equations: $M=k_1*t$; First-order equation: $\ln(100-M)=\ln 100-k_2*t$; Hixon-Crowel's cube root law: $(100-M)^{1/3}=100^{1/3}-k_3*t$; Higuchi's-model: $M=k_4*t^{1/2}$; Korsmeyer-Peppas-model or power law equation: $(M_t/M)*100=k_5*t^n$ [25-27]. Here, M is the cumulative amount of drug (%) at time t; k_1-k_4 are the release rate constants. In the Korsmeyer-Peppas model, M_t and M_∞ are the amounts of drug released at time t and at equilibrium, respectively. In the present work, M_∞ represents total amount of drug incorporated in microspheres, k_5 is a constant related to structural characteristics of dosage form, and n is the diffusional exponent. Plots were made by fitting data into these equations and kinetic parameters (k_1-k_4, n), and r^2 values were obtained and listed in Table 3. Data (r^2, n) were analyzed to determine drug release mechanism.

9. Surface Morphology

The surface morphology of checkpoint formulation was examined using scanning electron microscopy, SEM (JEOL, JSM5200, Tokyo, Japan). Prior to examination, the samples were fixed on a brass stub and coated with a gold-palladium layer under argon atmosphere using a gold sputter module in a high vacuum evaporator. The pictures were then taken in the instrument set at an excitation voltage of 20 kV.

RESULTS AND DISCUSSION

1. Development of Mathematical Models

In the preliminary investigation, we observed the changes in different characteristics of microspheres such as entrapment efficiency, particle size, drug release and surface morphology are due to various kinds of experimental conditions. Thus we considered further to optimize the processing variables with the application of multivariate analysis. The microspheres were prepared using O/O emulsion solvent evaporation technique as per the experimental design shown in Table 2. The prepared microspheres were white, discrete and spherical. A biphasic release pattern was observed from the *in vitro* release profiles as depicted in Fig. 1. Response data (Table 2) were fitted in experimental design and analyzed by DOE software, design expert v7. The software-generated ANOVA and models were analyzed, and finally with the elimination of insignificant terms mathematical model equations were obtained.

2. Analysis of ANOVA for All Responses (R1, R2, R3, R4)

The fit summary for entrapment efficiency (R1) and particle size (R2) suggests a quadratic relationship where some of the additional terms are significant (i.e., α=0.05) and the models are not aliased. Quadratic models Eq. (3), (4) & Eq. (5), (6) obtained for R1 and R2 explained 98.57% and 99.51% of the behavior of entrapment efficiency and particle size, respectively. The regression equations best represent the description of responses, after the non significant parameters ($p>0.05$) are eliminated from the initial analysis and these are summarized in Table 4. The ANOVA result of R1 shows the main effects (A-ethyl cellulose; B-HPMC; C-stirring speed; D-surfactant), the quadratic effects of A^2, B^2 and D^2 along with the interaction effects of AB, AD, BC and CD as significant terms. Though factor D shows p value >0.05, it is included in the model since other

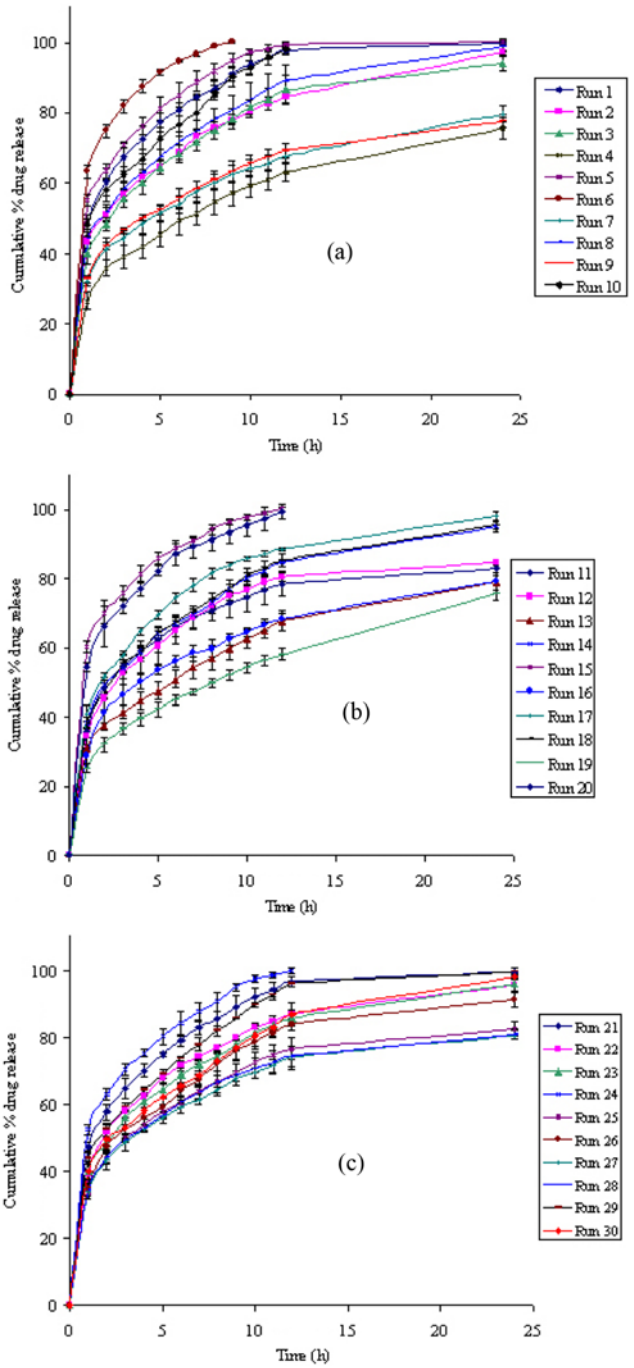

Fig. 1. Comparative *in vitro* drug release profiles of prednisolone microspheres as per experimental design formulation (Run order 1-10).

related factors (D^2, AD and CD) are found significant. Thus, factor D is added as hierarchical term. The ANOVA result of R2 shows the main effects (A, B and C) and quadratic effects (A^2, B^2, C^2 and D^2) along with interaction effects AC, AD and CD. This indicates that the particle size depends on ethyl cellulose, stirring speed and surfactant. The other adequacy measures R^2, adjusted R^2 and predicted R^2 are in reasonable agreement and these were close to 1 both for R1 and R2, which indicates adequacy of the models. The adequate model differentiation is observed from adequate precision

Table 4. Regression coefficients and their p-values (based on coded values) for the regression models for predicting optimized responses (Entrapment efficiency (%), Average Particle size (μm))

Entrapment efficiency (%)			Average particle size (μm)		
Factor	b-Coefficient	p-Value	Factor	b-Coefficient	p-Value
Intercept	76.54	<0.0001	Intercept	424.26	<0.0001
A	3.89	<0.0001	A	81.66	<0.0001
B	2.68	<0.0001	B[a]	2.72	0.168
C	−0.81	<0.0001	C	−54.03	<0.0001
D[a]	−0.29	0.0772	D	−39.67	<0.0001
AB	0.44	0.0322	AC	−11.38	0.000115
AD	0.92	0.00014	AD	−19.29	<0.0001
BC	0.81	0.00049	CD	−16.27	<0.0001
CD	−1.53	<0.0001	A^2	−26.61	<0.0001
A^2	−1.97	<0.0001	B^2	−12.12	<0.0001
B^2	0.38	0.0173	C^2	−25.21	<0.0001
D^2	−0.38	0.0171	D^2	−17.05	<0.0001
Other statistics			Other statistics		
R^2=0.9857; Adjusted R^2=0.9771			R^2=0.9951; Adjusted R^2=0.9921		
Predicted R^2=0.9550			Predicted R^2=0.9840		
Adequate precision=46.992			Adequate precision=64.89		

	Sum of squares	df	p-Value		Sum of squares	df	p-Value
Model	740.72	11		Model	316379.22	11	
Residual	10.67	18		Residual	1553.42	18	
Lack of fit*	8.57	13	0.325	Lack of fit*	1404.11	13	0.0821
Pure error	2.10	5		Pure error	149.31	5	
Corr total	751.39	29		Corr total	317932.65	29	
F-value of model=113.57				F-value of model=333.27			

D[a], B[a], Hierarchical term added after backward elimination regression=D, B
*Lack-of-fit is non significant (p>0.05)

value (signal/noise ratio), which was found satisfactory. The lack of fit is not significant and this is desired. Table 5 exhibits fit summary of responses R3 and R4, and a similar explanation is applicable here. The result indicates that the drug release depends on the factors which control both particle size and entrapment efficiency. The regression equations Eq. (7), (8) and Eq. (9), (10) represent best the description of responses (R3 and R4) after the non significant parameters (P>0.05) are eliminated from the results. The ANOVA result of R3 shows the main effects, some interaction effects (AB, AC, AD and BD) and one quadratic effect (A^2). It seems ethyl cellulose is the prominent parameter among others. ANOVA of R4 revealed that the controlling factors are only main factors. The model is significant. Other statistics for the both responses R3 and R4 are also satisfactory. The lack-of-fit F-value is not significant relative to pure error. These models for all responses can be used for prediction of responses within the same design space.

2-1. For Entrapment Efficiency

In terms of coded factors

$$R1=76.5+3.89A+2.68B-0.813C-0.295D+0.447AB+0.921AD+0.816BC-1.54\,CD-1.97A2+0.381B2-0.382D2 \quad (3)$$

In terms of actual factors

$$R1=46.8+0.130A-0.102B+0.00314C+7.69D+8.94E-005AB+0.00921AD+8.16E-005BC-0.00768CD-0.000197A^2+0.000153B^2-0.382D^2 \quad (4)$$

2-2. For Average Mean Particle Size

In terms of coded factors

$$R2=424.+81.7A+2.72B-54.0C-39.7D-11.4AC-19.3AD-16.3CD-26.6A^2-12.1B^2-25.2C^2-17.1D^2 \quad (5)$$

In terms of actual factors

$$R2=-1.20E+003+3.48A+1.02B+1.58C+184D-0.000569AC-0.193AD-0.0814CD-0.00266A^2-0.00485B^2-0.000630C^2-17.1D^2 \quad (6)$$

2-3. For Cumulative Drug Release at 1 h

In terms of coded factors

$$R3=39.7-9.54A+2.52B+2.34C+2.13D-0.402AB-1.40AC-0.780AD-0.489BD+1.34A^2 \quad (7)$$

In terms of actual factors

$$R3=22.8-0.0681A+0.0941B+0.0327C+5.45D-8.05E-005AB-6.99E-005AC-0.00780AD-0.00978BD+0.000134A^2 \quad (8)$$

2-4. For Cumulative Drug Release at 8 h

In terms of coded factors

Table 5. Regression coefficients and their p-values (based on coded values) for the regression models for predicting optimized responses (release at 1 h (%), release at 8 h (%))

Release at 1 h (%)		
Factor	b-Coefficient	p-Value
Intercept	39.72	<0.0001
A	−9.53	<0.0001
B	2.52	<0.0001
C	2.33	<0.0001
D	2.13	<0.0001
AB	−0.40	0.0441
AC	−1.39	<0.0001
AD	−0.78	0.00047
BD	−0.48	0.0168
A^2	1.33	<0.0001
Other statistics		
R^2=0.9958; Adjusted R^2=0.9939		
Predicted R^2=0.9894		
Adequate precision=88.201		

	Sum of squares	df	p-Value
Model	2675.11	9	
Residual	11.22	20	
Lack of fit*	8.21	15	0.599
Pure error	3.01	5	
Corr total	2686.34	29	
F-value of model=529.51			

Release at 8 h (%)		
Factor	b-Coefficient	p-Value
Intercept	74.87	<0.0001
A	−12.97	<0.0001
B	3.31	<0.0001
C	2.132	<0.0001
D	1.42	<0.0001
Other statistics		
R^2=0.9879; Adjusted R^2=0.9860		
Predicted R^2=0.9823		
Adequate precision=86.11		

	Sum of squares	df	p-Value
Model	4460.71	4	
Residual	54.45	25	
Lack of fit*	44.17	20	0.518
Pure error	10.28	5	
Corr total	4515.16	29	
F-value of model=511.97			

*Lack-of-fit is non significant (p>0.05)

$$R4=74.9-13.0A+3.32B+2.13C+1.43D \quad (9)$$

In terms of actual factors

$$R4=91.5-0.130A+0.0663B+0.0107C+1.43D \quad (10)$$

3. Validation of the Developed Models

To validate the developed response surface equations, derived from multiple regression analysis, six confirmative experiments were conducted from a random selection of different formulations within the ranges for which the equations were derived. The actual results are calculated as the average of three measured results for each response. The actual results, predicted values and calculated percentage error of confirmatory experiments (check point formulation (CPF)) are furnished in Table 6 and the drug release profile is depicted in Fig. 2. It is observed from the validation experiments that there is a negligible percentage error between the estimated and the experimental values. Therefore, the developed models are found satisfactory. Fig. 3 shows the relationship between the actual and predicted values of response variables. These figures also indicate that the developed models are adequate and predicted results are in good agreement with the measured data. The check point formulation (CPF2) with the composition of HPMC: ethyl cellulose (1 : 6 ratio), 1,200 rpm stirring speed and 2% surfactant yields better sustained effect (93.11% release) at end of 24 h (Fig. 2). It shows burst release of ~35%, it releases 2.5%/h at steady state of release, and it shows 3.95% error in 8h release with respect to the predicted value. This is found to be optimized composition.

4. Effects of Process Parameters on the Responses

Both EC and HPMC have shown a prominent effect on entrapment of the drug, when other variables are kept constant. Surface plot (Fig. 4(a)) shows 75.4% entrapment efficiency even at low mass fraction of either polymer. Maximum entrapment efficiency (79.7%) is found when both the polymers are at high level. This is because the viscosity of the polymer mixture increases with increasing amount of polymers in the dispersion phase (emulsion globule). Diffusion of drug from the emulsion globule toward continuous phase is retarded due to viscosity of polymer, hydrophilicity of drug and non aqueous nature of continuous phase. Some quantity of drug migrates towards inter surface along with volatile solvent (chloroform, ethanol) and it is lost finally in the continuous phase owing to shearing action. Higher quantity of polymer combination adequately entraps maximum amount of drug complex [28]. After removal of solvent from globule, polymers precipitate at the interface and prevent further diffusion of drug across the phase boundary [29]. Entrapment efficiency increases by controlling the factors that minimize drug loss. As all the factors have some effects on the responses, so we analyzed these effects by varying two independent variables while keeping others at a constant level. Fig. 4(b) depicts the effects of ethyl cellulose and surfactant concentration on entrapment efficiency. The entrapment efficiency decreases with the increase of surfactant concentration at any fixed level of ethyl cellulose, whereas it in-

Table 6. Validation test results

S. No.	Experimental composition				Response variable	Experimental value	Predicted value	Percentage error
	A (mg)	B (mg)	C (rpm)	D (%v/v)				
CPF* 1	200	100	1200	3	Entrapment efficiency (%)	72.57	69.04	−5.11
					Average particle size (μm)	335.19	319	−5.07
					Release in 1 h (%)	50.06	47.67	−5.01
					Release in 8 h (%)	89.95	86.47	−4.02
CPF 2	300	50	1200	2	Entrapment efficiency (%)	70.16	74.2	5.44
					Average particle size (μm)	382.5	409.18	6.52
					Release in 1 h (%)	35.25	37.18	5.19
					Release in 8 h (%)	68.75	71.58	3.95
CPF 3	300	100	1000	1	Entrapment efficiency (%)	72.49	75.68	4.21
					Average particle size (μm)	477.69	459.1	−4.04
					Release in 1 h (%)	33.19	35.23	5.79
					Release in 8 h (%)	70.03	71.34	1.83
CPF 4	400	100	1400	2	Entrapment efficiency (%)	72.72	77.6	6.28
					Average particle size (μm)	403.85	388.5	−3.95
					Release in 1 h (%)	31.13	32.44	4.03
					Release in 8 h (%)	60.69	64.03	5.21
CPF 5	200	150	1200	2	Entrapment efficiency (%)	70.53	73.25	3.71
					Average particle size (μm)	319.23	306.32	−4.21
					Release in 1 h (%)	56.37	53.5	−5.36
					Release in 8 h (%)	89.88	91.22	1.46
CPF 6	300	100	1400	3	Entrapment efficiency (%)	70.91	73.47	3.48
					Average particle size (μm)	286.36	271.7	−5.39
					Release in 1 h (%)	41.97	44.17	4.98
					Release in 8 h (%)	84.78	78.46	−8.05

*CPF is check point formulation

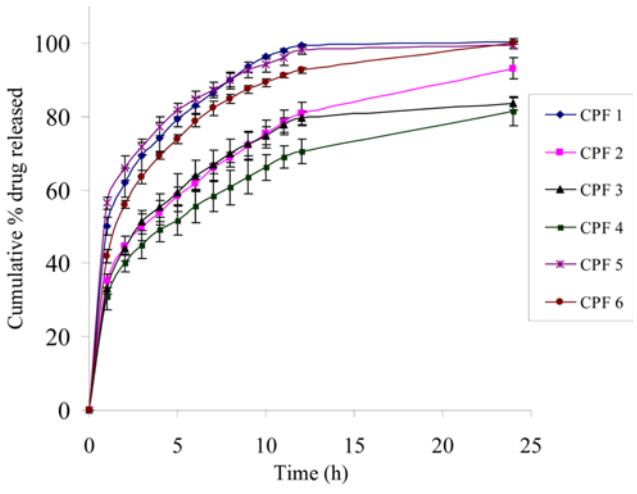

Fig. 2. Comparative *in vitro* release profile of check point formulations.

creases with increasing the level of ethyl cellulose at any concentration of surfactant. With increasing surfactant concentration, total interfacial area increases due to formation of large number of smaller globules. These smaller globules and some tiny globules are continuously sheared at the interface and these result in the loss of drug present at the interface into the continuous phase. Fig. 4(b) clearly shows that ethyl cellulose causes manifold change in entrapment efficiency (%) in comparison to that of surfactant.

The composite effect of polymer HPMC and stirring speed (SS) on entrapment efficiency is depicted in the surface plot of Fig. 4(c). This is based on fixed levels (centre) of ethyl cellulose and surfactant. Entrapment efficiency decreases with increasing levels of SS at any concentration of HPMC, and it increases with increasing concentration of HPMC at any constant level of SS. Change of entrapment efficiency with respect to these variables, HPMC and stirring speed is not so much greater as observed in Fig. 4(a) which shows the effects of polymers. The combination of polymers plays a major role on entrapment efficiency (%). This is due to fact that viscosity of dispersion phase increases with increasing polymer concentration and with high hydrophilicity of drug complex could retard the migration of the drug to the continuous phase (heavy liquid paraffin) and thus improve its entrapment [19,30]. Fig. 4(d) shows the effect of surfactant and stirring speed at fixed level (center) of ethyl cellulose and HPMC. At lower SS, entrapment of drug increases with increasing level of surfactant. With the increase of SS beyond the center point, entrapment efficiency gradually decreases with increasing levels of surfactant. Both the number of globules and the interfacial area increase with the increase of SS and surfactant concentration. Higher loss of drug and less entrapment are expected owing to high shearing action on finer globules.

A minor effect was observed in particle size with increasing level

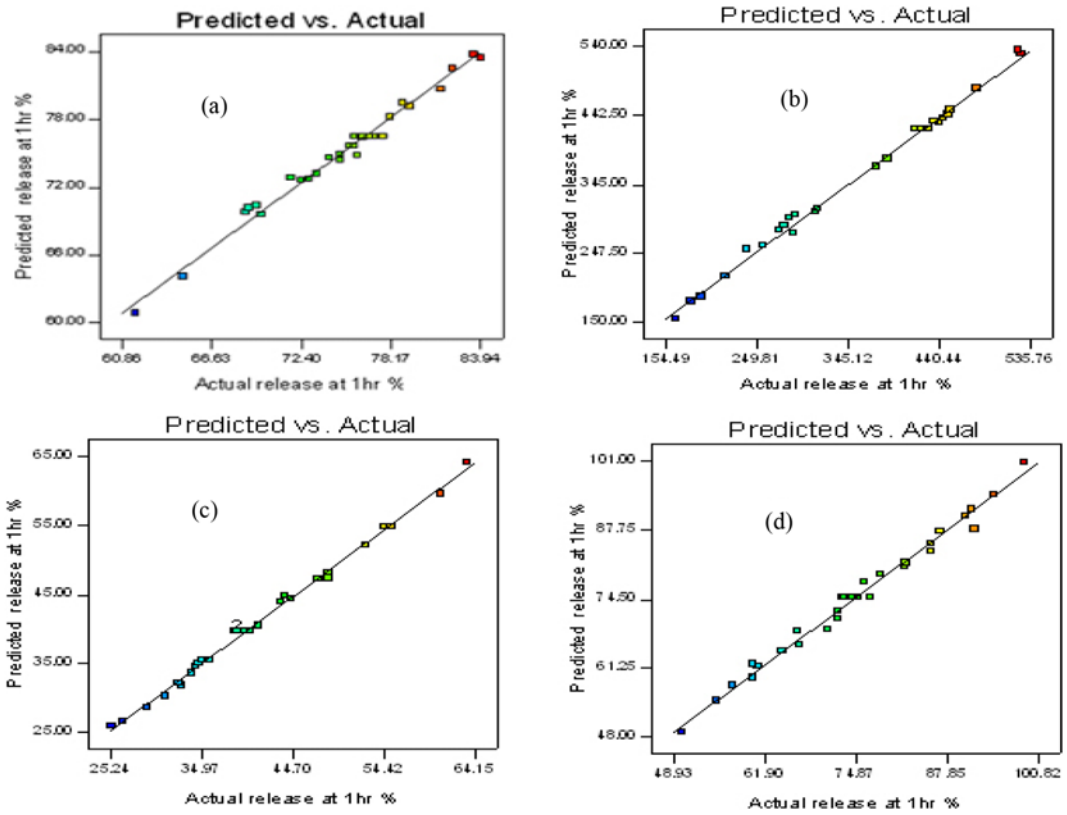

Fig. 3. Plot of actual vs. predicted response of (a) entrapment efficiency; (b) particle size; (c) release at 1 hr; (d) release at 8 hr.

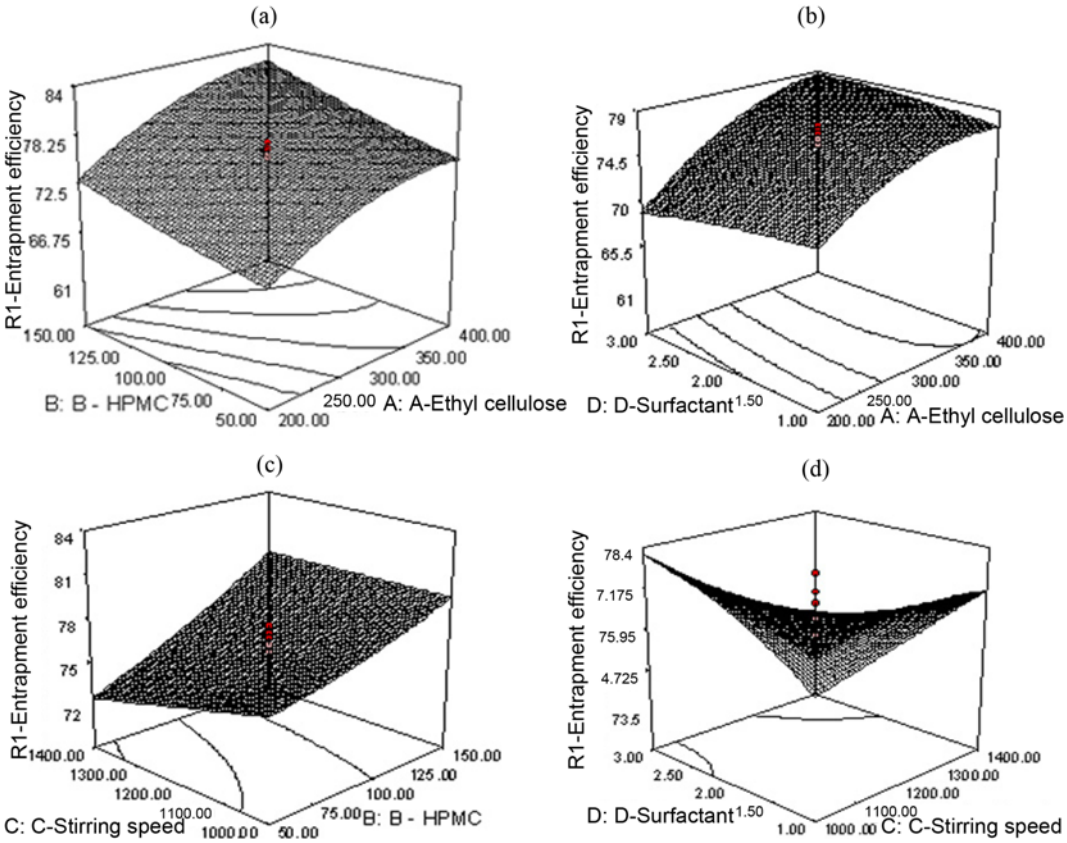

Fig. 4. Response surface plot showing effect of (a) ethyl cellulose (A) and HPMC (B), (b) Surfactant (D) and ethyl cellulose (A), (c) Stirring speed (C) and HPMC (B) and (d) Surfactant (D) and stirring speed (C) on entrapment efficiency.

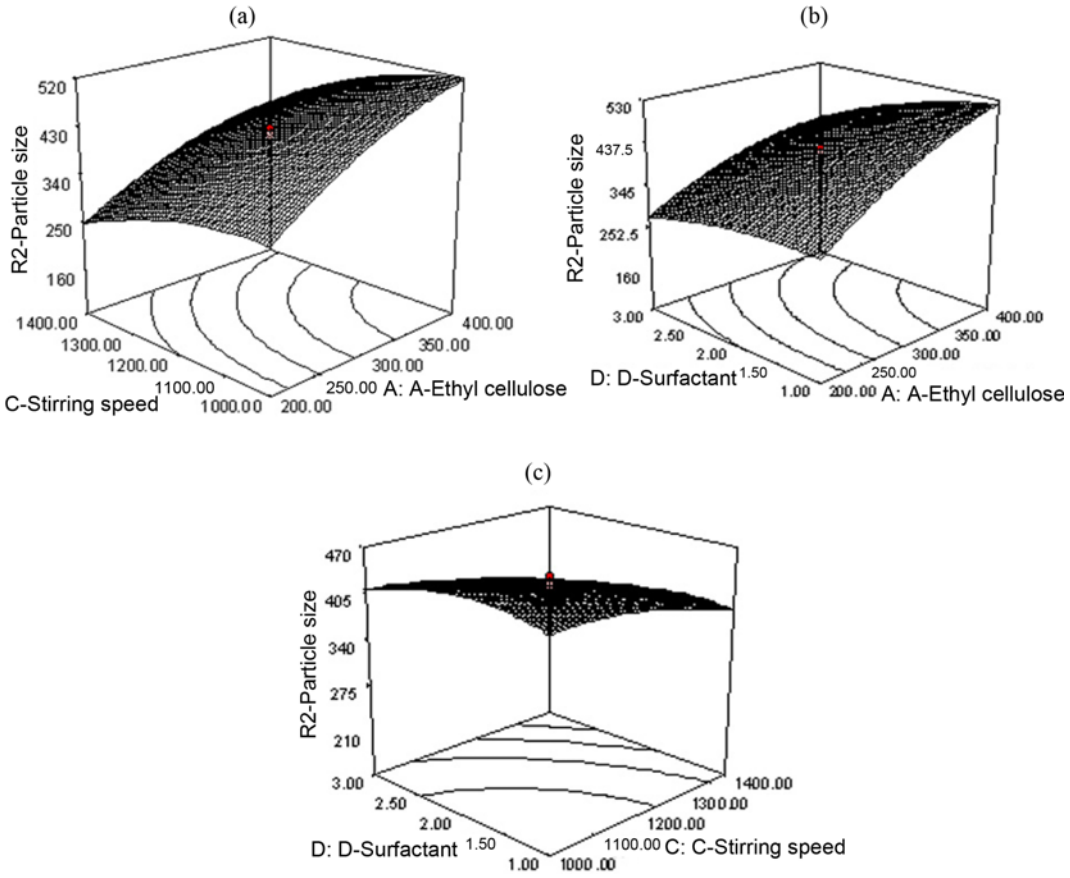

Fig. 5. Response surface plot showing effect of (a) stirring speed (C) and ethyl cellulose (A), (b) surfactant (D) and ethyl cellulose (A), and (c) surfactant (D) and stirring speed (C) on particle size.

of HPMC. Higher mass fraction of ethyl cellulose in the polymer combination and its physical properties such as porosity, bulk volume determine the particle size of microspheres besides the control of stirring speed and surfactant. The latter two variables, e.g., surfactant and stirring speed decrease particle size with their increasing levels. Previously, we observed that the microspheres with low particle size had showed lower entrapment efficiency. So, these two responses have common controlling variables. Both particle size and entrapment efficiency increase with increasing levels of EC, whereas these two responses decrease with increasing levels of surfactant concentration and stirring speed. Contour plots and response surface plots in Fig. 5(a) and 5(b) display the effects of a pair of controlling variables (ethyl cellulose-stirring speed and ethyl cellulose-surfactant concentration) on particle size while keeping constant level of HPMC, surfactant concentration and HPMC, SS, respectively.

At higher rate of agitation large numbers of finer globules are formed even at high level of ethyl cellulose; this fact supports the hypothesis that the effect of increasing stirring speed is dominating over the effect of ethyl cellulose [31]. In Fig. 5(b), the response plot shows that particle size decreases with the increase of surfactant concentration even at higher level of ethyl cellulose. It may be due to stabilization of emulsion droplets against coalescence [10,32]. Fig. 5(c) shows that both the variables (surfactant and stirring speed) are effective in lowering of particle size when polymer levels are fixed at center values.

The effects of controlling variables on entrapment of drug and particle size of microsphere have been explained with evidence. Next step is to explain the role of these variables on the release rate of entrapped drug from the microspheres in the dissolution medium. Owing to hydrophilicity of polymer (HPMC) and decreased particle size, factors B, C and D have similar increasing effect on R3 when their levels are increased. Whereas, ethyl cellulose exhibits negative effect; this happens as ethyl cellulose is hydrophobic in nature and its water permeability and gel forming ability are lesser in comparison to HPMC. So, it retards drug release and R3 declines steadily with increase of level of A. Effects of pair of factors such as A, B; A, C and A, D on R3 have been displayed in Fig. 6(a), 6(b), and 6(c), respectively. While keeping other factors fixed at centre level, these contour plots and response surface plots clearly exhibit retarding effect of factor (A) on R3 at any level of B, C, and D on R3. The factors that control particle size are found also to control release rate. Coupling ethyl cellulose with HPMC in proper ratio, burst release (initial) can be manipulated at desired level. Minimum burst release (25%) is recorded in Table 2, when levels of factors are A-500; B-100; C-1200; D-2. In the aforesaid condition, particle size is 480 μm. Here incomplete release (76.06%, at 24 h) of drug is observed. Smaller size globules produced at lower level of A and higher levels of C and D show higher burst release of drug at the initial hour. Though HPMC affects feebly on particle size, it enhances drug release as its water permeability and gel forming ability

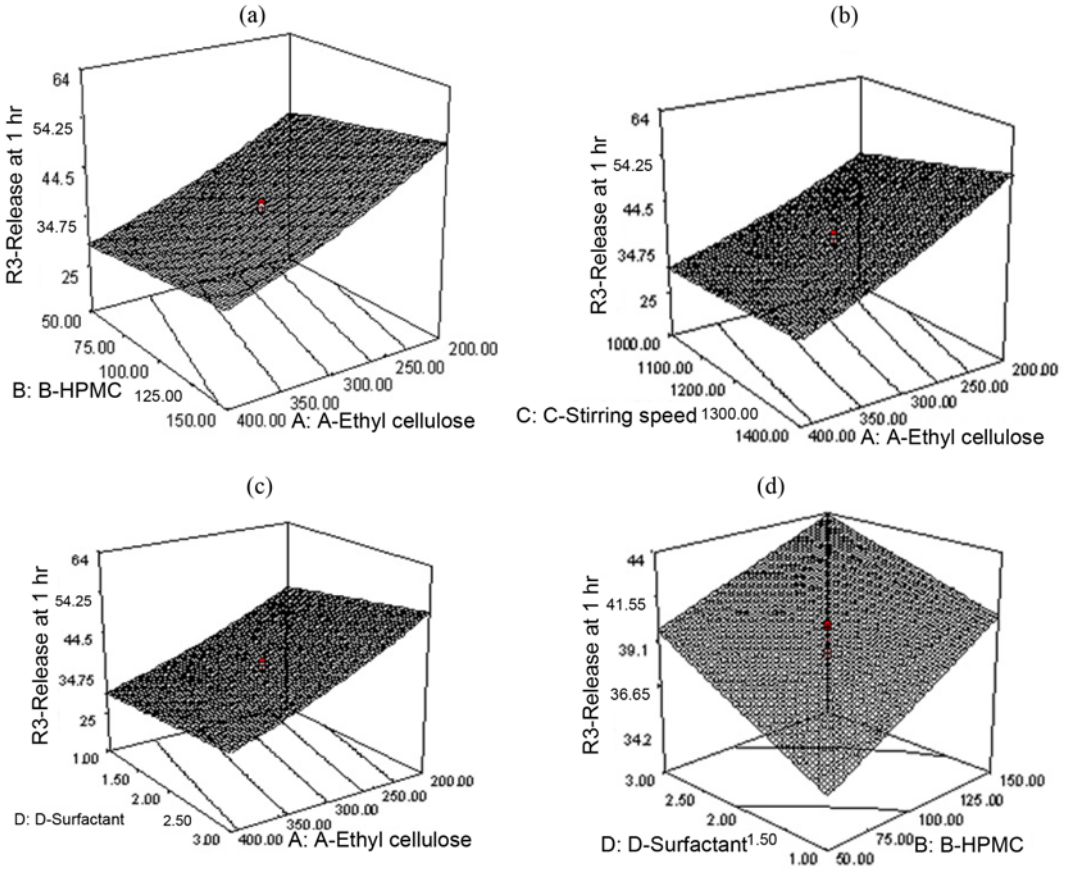

Fig. 6. Response surface plot showing effect of (a) ethyl cellulose (A) and HPMC (B), (b) stirring speed (C) and ethyl cellulose (A), (c) surfactant (D) and ethyl cellulose (A), and (d) surfactant (D) and HPMC (B) on release at 1 hr.

are formidable. This results in easy diffusion of soluble drugs into dissolution medium. And also, the rupture of polymeric membrane occurs by in taking the dissolution medium would cause the rapid release of drug as demonstrated [33]. In the present experiment its level is controlled to achieve the desired rate of drug release. Fig. 6(d) displays a response surface plot which shows the positive effect of HPMC and surfactant on initial release (R3). Initial burst release also depends on the volatility of solvent used to prepare disperse phase during preparation of microspheres. Chosen solvent should not have high solubilizing capacity on a selected drug. If solvent evaporates at faster rate, it will drive some of drug-complex from the inner core towards the interface. Uniform drug distribution may be affected and high amount of drug reaches the interface and this gives high burst effect when microspheres come into contact with the dissolution medium. Extent of burst release highly depends on main effects, interaction effects and quadratic effects. These multiple effects make the correlation nonlinear. Once burst effect is over, the thin layer around the particle becomes saturated with the drug and this saturated layer maintains a concentration gradient between this layer and bulk liquid in the subsequent release period, though the thickness of this layer depends on the stirring speed of the paddle in the dissolution vessel. At steady rate of release under a concentration gradient, it was observed that responses (R4) is linearly related to the main factors (A, B, C, and D). R4 increases with the increase of B, C and D and it decreases with the increase of A. Since, ethyl cellulose is less permeable to water and this feature makes it relatively viscous. Polymer matrix with increasing fraction of ethyl cellulose when coming in contact with water becomes relatively impermeable to dissolution medium, and this event causes slower diffusion of drug [34,35]. The incomplete liberation of drug from the microspheres is observed because the polymers are not in disintegrated/degraded state [19].

5. Drug Release Mechanism

The release mechanism of prednisolone from the cellulose microspheres is also evaluated on the basis of established equations such as zero order, first order, Higuchi equation, Korsmeyer Peppas and cube root model. The slope and the correlation coefficient (r^2) value are shown in Table 3. It shows that the drug release pattern of prednisolone from the microspheres fits both with the Korsmeyer-Peppas model and with the Higuchi equation and more satisfactorily than the other equations. Owing to high burst release, the (r^2) values are not satisfactory when fitted in zero-order and first-order model equations. The presence of pores on the surface of microspheres induces faster release of drug which gives low slope value obtained in cube root model. According to (Ritger and Peppas, 1987) [36], a value of the exponent n=0.5, 0.5<n<1, n=1.0 indicates Fickian diffusion, non-Fickian diffusion and zero-order transport, respectively. The values of diffusion exponent, n, for the microspheres are between 0.1992 and 0.3191 which suggest that drug release follows Quasi-Fickian kinetics.

6. Surface Morphology

Check point formulations (Table 6) were used to examine the

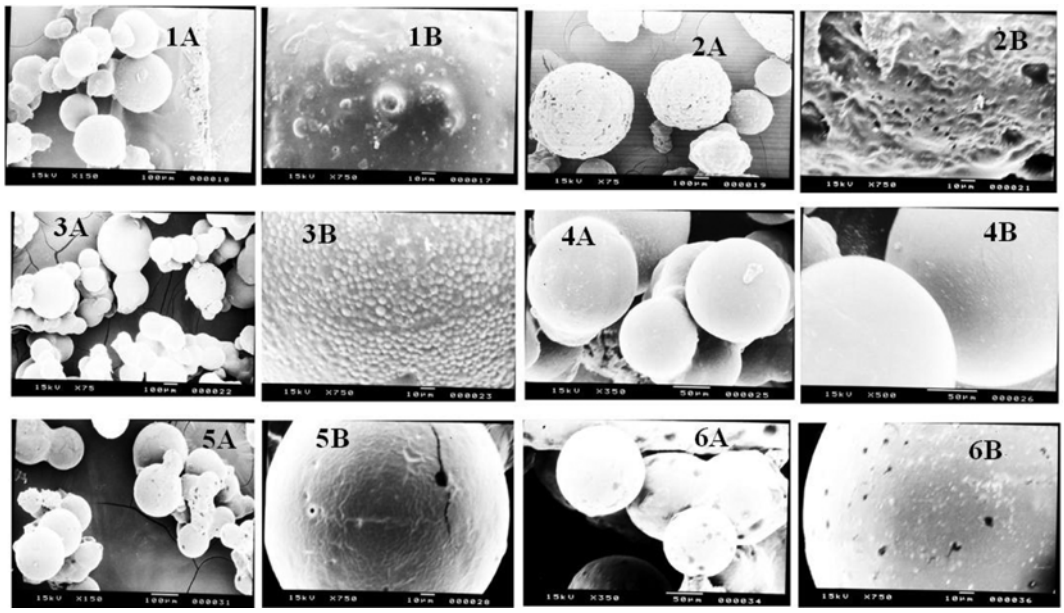

Fig. 7. SEM images of microspheres formulated as per the check point formulation composition.

effects of process parameters on surface morphology of microspheres by scanning electron microscopy. The microspheres (1-5) were found nearly spherical and the surface texture (roughness, porosity) was found differing as to processing parameters (Table 6, Fig. 7). Photographs 2a and 2b show the porous rough surface of microspheres that contain lesser amount of HPMC in comparison to others, though lower burst release is found in formulation 2 (Table 6). The surface texture is not a dominant factor here. Photographs 3a and 3b depict the mixture of spherical and irregular shaped spherical particles with rough surface. This was prepared at low stirring speed. It is suggested that solvent from emulsion droplets diffuses out at slow rate, so these droplets solidify slowly and microspheres become irregular in shape with rough surface owing to continuous shearing action. It is observed that microspheres prepared with higher quantity of HPMC (5b) and higher stirring speed (4a/4b, 6a/6b) are comparatively regular spheres with smooth surface led to faster solidification of dispersed phase favors to reduce porosity and crystallinity of drug in the microspheres [37,38]. Percent release mainly depends on particle size/interfacial area, porosity rather than on surface roughness. Since polymers form a network it is expected that pores are distributed uniformly throughout the volume of spherical particles.

CONCLUSIONS

The following conclusions can be drawn from this study based on the range of values of parameters considered.

- The response surface methodology and multiple response optimizations utilizing polynomial equation could be efficiently applied for the development of a drug delivery system to predict adequately the responses within the limit. The predicted results are in good agreement with the measured data as evidenced from the validation experiments.
- *In vitro* release study showed that the drug is released over the period of 24 h in a sustained release fashion and follows Fickian diffusion kinetics.
- Scanning electron microscopy reveals that the prepared microspheres are porous and their morphology changes with experimental conditions.
- The optimized combination for effective sustained release of prednisolone was found with the microspheres CPF2 [HPMC: ethyl cellulose (1 : 6 ratio), 2% surfactant and 1,200 rpm stirring speed].
- The present work clearly demonstrates how designing an experiment can be made economical to obtain maximum information in a short period of time and with fewer experiments.

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