RESEARCH ARTICLE

Diclofenac Sodium-Loaded Eudragit® Microspheres: Optimization Using Statistical Experimental Design

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Abstract

Purpose In this study, polymeric microspheres containing diclofenac sodium were prepared by single emulsion (oil-in-water) solvent evaporation method and evaluated for their size, morphology, encapsulation efficiency, drug loading, and in vitro drug release.

Methods Two nonbiodegradable polymers, Eudragit® RS100 and RL100 were used in combination. Microspheres were prepared by varying the amount of polyvinyl alcohol as a surfactant (0.05, 0.125, and 2.0 %, w/v) to the external phase; varying the amount of polymer (1:1, 2:1, and 3:1, w/w) to the drug by employing 3^2 full factorial design using the Design Expert (Version 8.0.7.1). The drug polymer interactions were investigated by Fourier transform infrared spectroscopy (FTIR) and X-ray powder diffractometry (XRPD). Imaging of particles was performed by field emission scanning electron microscopy.

Results Graphical and mathematical analysis of the design showed a quadratic model was significant for the responses. Low magnitude of error and significant values of R^2 proves the high prognostic ability of the RSM. Encapsulation efficiency of microspheres (41.13 to 65.33 %) increases with an increase in surfactant concentration but decreases with an increase in polymer concentration. The microspheres were found to be discrete, spherical with smooth surface. The absence of drug polymer interactions was confirmed by FTIR spectroscopy. XRPD revealed the dispersion of drug within microspheres formulation. The Perfect pH-independent release profile was achieved from Eudragit® microspheres by anomalous transport mechanism.

Conclusions In conclusion, Eudragit® microspheres containing diclofenac sodium can be successfully prepared, and seem to be promising for sustained release application.

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Introduction

Musculoskeletal disorders are characterized by local inflammatory processes and are treated with nonsteroidal anti-inflammatory drugs (NSAIDs). Diclofenac sodium (DS; sodium(O-((2,6-dichlorophenyl)-amino)-phenyl)-acetate) is a NSAID that appears to be promising and most commonly used drug in the treatment of inflammatory processes because its potential anti-inflammatory and analgesic activity. DS is useful in the inflammatory disorders especially in rheumatoid arthritis, osteoarthritis, dental pain, and ankylosing spondylitis. A major disadvantage of DS therapy is the potential for upper gastrointestinal (GI) effects, increases a risk of ulcer bleeding resulting in an increase in rates of hospitalization or death from a GI complication [1–7]. With an increased use of NSAIDs comes an increased pressure to develop the new drug delivery strategies that will achieve the highest healing effect of DS with minimal GI events. For that purpose, sustained release formulation of DS is essential, and sustained release microspheres might be beneficial in overcoming the GI side effects of the conventional dosage forms thereby improving patient compliance.

Biodegradable and biocompatible microspheres are popularly investigated drug delivery system for therapeutic drugs. Administration of medication via such system is advantageous because microspheres can be tailored for desired release profiles. They are capable of providing the controlled release of the encapsulated drug for longer duration [8–11]. Solvent evaporation and organic phase separation techniques are widely used in pharmaceutical industries for the preparation of microspheres [8, 9, 12–14]. Eudragit RS 100 and RL 100 are water insoluble, pH independent polymers having 5 and 10 % functional quaternary ammonium groups and capable of limited swelling. Thus these polymers appear to be a good

polymeric carrier for the dispersion of drugs. These polymers are used for the sustained release product [15–18].

Response surface methodology (RSM) has been very popular in development and optimization of process variables for the new product as well as in the improvement of existing product without increasing the cost. RSM is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes in which a response of interest is influenced by several variables and the objective is to optimize this response [19–21]. Pharmaceutical scientist often faces the problem for optimization of formulation using a single factor optimization technique (one factor at a time). Multiple factor optimization is more efficient and generate a maximum point (an optimized condition) compared with the single factor optimization technique. Design of experiments approach is undertaken using RSM to generate the model that describes the optimum relationship between the process variable and responses for the development of the microspheres using 3^2 full factorial design. RSM is useful to understand about the process/formulation factors which affect the formulation development process. It also describes how the formulation varies when the values of process factors are changed [22–25]. Besides the process/formulation variables employed during the microspheres preparation very much determine the encapsulation efficiency and microspheres properties. In this study, the effect of formulation variables (the amount of polymers and surfactant concentration) was investigated to determine the microspheres properties.

The goal of this research was to develop, characterize, and optimize the DS loaded Eudragit microspheres to reduce the frequency of dosing, to overcome the side effect, and improve the patient compliance to treat musculoskeletal disorder. The screening design methodology was used to investigate the formulation variables affecting the microspheres properties. Microspheres were evaluated for encapsulation efficiency, practical drug loading, and in vitro drug release.

Materials and Methods

Materials

DS was provided by Natco Pharma Limited (Hyderabad, India) as a gift sample. Eudragit RL 100 and Eudragit RS 100 were kindly donated from Evonik Degussa India Pvt. Ltd. (Mumbai, India). Partially hydrolyzed polyvinyl alcohol (PVA; M_w, 140,000) was purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Dichloromethane was obtained from Merck Specialities Pvt. Ltd. (Mumbai, India). Methanol (HPLC grade) was purchased from RFCL Limited (New Delhi, India). All other solvents and reagents were of analytical grade and used as provided.

Methods

Factorial Design and Optimization

Before application of design, a number of preliminary experimentations were conducted to determine the formulation parameters and conditions at which the process resulted to microspheres [14, 26]. To optimize the formulation, a 3^2 full factorial design was employed using the Design-Expert® Software (Version-8.0.7.1, Stat-Ease Inc., Minneapolis, MN) which allows evaluation by nine experiments. The RSM was used to analyze the observed response [20, 21, 23–25, 27, 28]. The amount of partially hydrolyzed PVA as a surfactant (X_1, percent, w/v) [13, 14] and the amount of polymers (X_2, milligrams) with respect to drug were selected as independent variables. Such statistical models were used to evaluate the effect of independent variables on the dependent variables (responses) like encapsulation efficiency (Y_1, percent) and drug content (Y_2, percent). The actual and coded values of independent variables are shown in Table 1 along with their low (−1), medium (0) and high levels (+1). Statistical analysis was performed using the Design-Expert® Software. Linear, cross-product contribution (2FI), quadratic and cubic models were generated for the responses. The significance of the model was determined by the comparisons of statistical parameters, and the best model (suggested) was decided based on reasonable agreement between adjusted R^2 and predicted R^2 (within 0.2 of each other); higher values of adjusted R^2 and predicted R^2; model p value (should be less than 0.05) and small PRESS value of the model. PRESS is a measure of the fit of the model to data points in the design. The PRESS for the chosen model should be small relative to the other models under consideration [27–31]. Two-dimensional (2D) contour plots and three-dimensional (3D) response plots resulting from the equations were constructed using Design-Expert® software.

Table 1 Variables in a 3^2 full factorial design

Independent variables	Level used (actual; coded)		
	Low (−1)	Middle (0)	High (+1)
X_1=amount of PVA as a surfactant (%, w/v)	0.05	0.13	0.2
X_2=amount of Eudragit® polymers (mg) in combination	250	500	750

Preparation of Encapsulated Microspheres

DS loaded microspheres were prepared using oil-in-water (O/W) single emulsion solvent evaporation method. Briefly, 250 mg of DS was mixed with a mixture of dichloromethane (10 ml) and methanol (10 ml) containing a certain proportion (50–50 % of each polymer) of Eudragit RS 100 and RL 100 as per factorial design. The resulting oil phase was injected using a glass syringe with a 21.5 G needle into a 100-ml distilled water as an external phase containing varying concentrations of PVA as an emulsifier to produce an O/W single emulsion at a speed of 500 RPM using four bladed lab stirrers (Remi electrotechnik Limited, Thane, India). The pH of external aqueous phase was adjusted to pH 3.9 with acetic acid. After 60 min, *n*-hexane was added to the solution for hardening purpose of the microspheres. Stirring was continued for 4 h at the same speed for the complete evaporation of dichloromethane leaving solid microspheres. The microspheres were collected by filtration, rinsed with *n*-hexane, air dried for 48 h, and was used for further studies.

Microspheres Characterization

Evaluation of Encapsulation Efficiency (Percent) and Drug Contents (Percent) The amount of DS encapsulated in microspheres was determined by UV spectrophotometer (HITACHI U-2900, Tokyo, Japan). An accurately weighed 10 mg of microspheres was stirred with dichloromethane (5 ml) to dissolve the polymeric coat and extracted in phosphate buffer solution (pH 6.8). Stirring continued for 30 min to facilitate the evaporation of organic solvent. The dispersion was filtered, and the residue was washed with phosphate buffer solution. The drug content was determined in the filtrate after appropriate dilution with a phosphate buffer solution at 276 nm using a UV spectrophotometer [32, 33]. The encapsulation efficiency was expressed as the percentage of drug incorporated in the formulation relative to the total amount of drug used in the formulation. The percent encapsulation efficiency and percent drug loading of DS were calculated using the following equations:

$$\% \text{Encapsulation efficiency} = \frac{[\text{weight of drug determined}]}{[\text{weight of drug added}]} \times 100 \quad (1)$$

$$\% \text{Drug loading} = \frac{[\text{weight of drug determined}]}{[\text{weight of microspheres}]} \times 100 \quad (2)$$

Among all the formulation, microspheres having a higher encapsulation efficiency were chosen for further characterization study.

Field Emission Scanning Electron Microscopy The morphology of microspheres was investigated by field emission scanning electron microscopy (FESEM-S 4800, Hitachi, Japan) at a working distance of 8.6–8.7 mm and accelerating voltage of

1.0 kV. The particles were examined for shape, size, and surface characteristics.

Fourier Transform Infrared Spectroscopy Study The chemical structure of the DS, Eudragit RS 100, RL 100, and drug-loaded microspheres were analyzed using a Fourier transform infrared spectroscopy (FTIR) spectrophotometer (FTIR-8400; Shimadzu, Asia Pacific Pvt. Ltd. Singapore) by KBr pellet method. For that, sample (1 mg) was mixed with KBr (40 mg) and formed into a disc in a manual press. Spectra were recorded in the scan range of 4,000–400 cm^{-1}.

X-Ray Diffraction (XRD) To access the physical status characterization of drug i. e. amorphous or crystalline, X-ray diffraction (XRD) patterns of DS, Eudragit RS 100, RL 100, and drug-loaded microspheres were obtained in an X-ray diffractometer (Miniflex, Rikagu) with Cu Kα radiations (λ =1.5406 Å). The samples were analyzed over the angle range (2θ) 10°-70°.

In Vitro Drug Dissolution Studies Drug release from microspheres was performed in vitro using 0.1 N HCl for initial 2 h and phosphate buffer (pH 6.8) for 10 h in tablet dissolution tester (Electrolab, TDT-06T, India) USP XXVIII, type I (100 RPM, 37±0.5 °C). The dissolution medium of 0.1 N HCl and phosphate buffer (pH 6.8) was prepared according to Indian Pharmacopoeia 2010 [34]. Release media contain 0.05 % Tween 80 as a solubilizer. Aliquots of the dissolution medium were withdrawn at predetermined time intervals and replenished with fresh dissolution media to maintain the sink condition. The samples were filtered through a Whatman filter paper no. 41. The DS content of each sample after suitable dilution was assayed spectrophotometrically by UV spectroscopy (HITACHI U-2900, Tokyo, Japan) at 276 nm using a 1-cm cell. The experiments were done in triplicate. The release

Table 2 A 3^2 full factorial design with factor X_1=amount of PVA as a surfactant (percent, *w*/v) and X_2 = amount of polymers (milligrams) in combination and responses Y_1=encapsulation efficiency (percent) and Y_2=practical drug loading (percent)

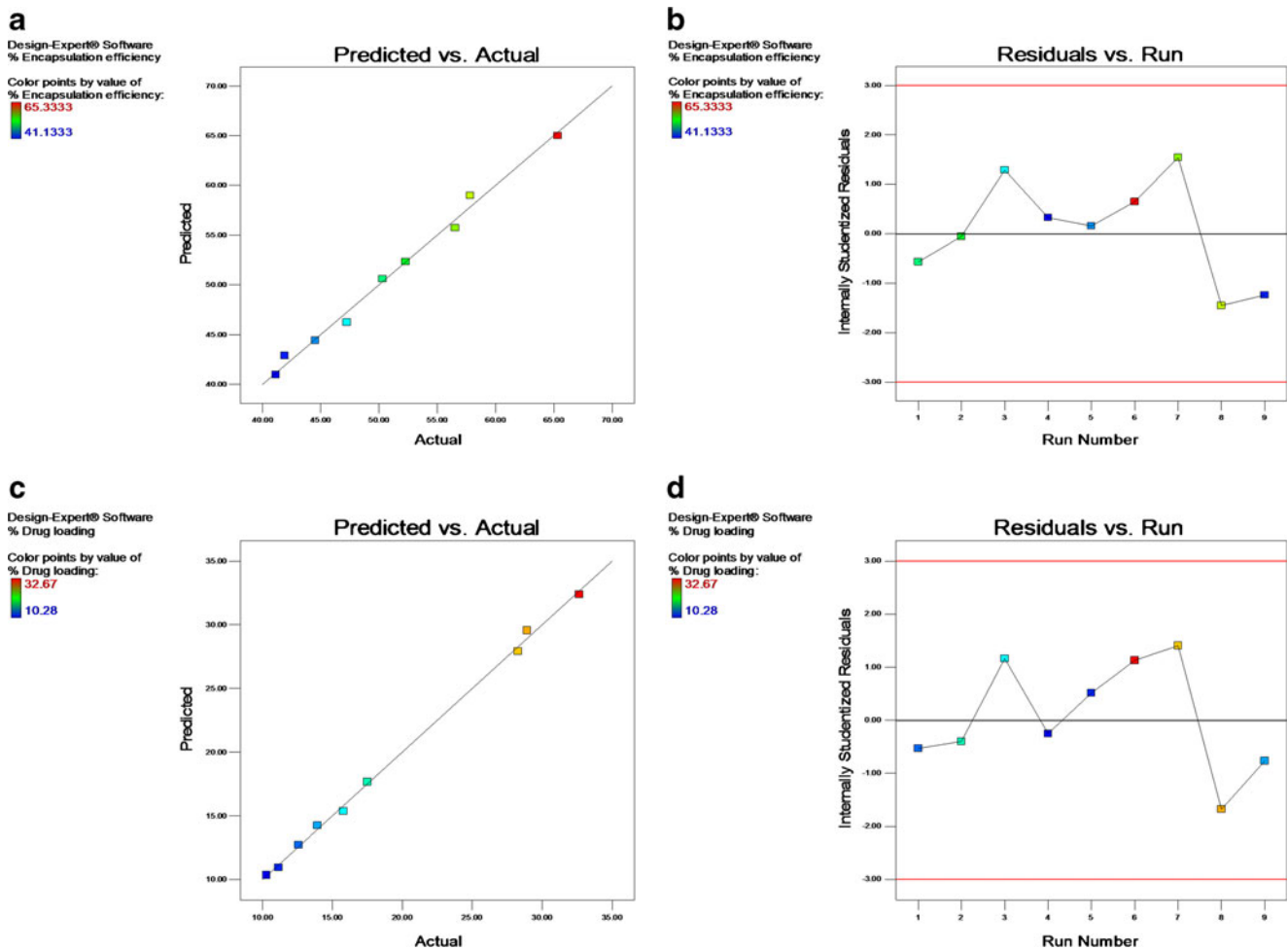| Experimental run	Independent variables		Dependent variables	
	X_1	X_2	Y_1	Y_2
1	0.05	250	56.54±5.5	28.27±2.7
2	0.13	250	57.83±8.1	28.92±4.0
3	0.2	250	65.33±12.4	32.67±6.2
4	0.05	500	41.89±6.2	13.95±2.0
5	0.13	500	47.26±3.0	15.80±1.0
6	0.2	500	52.29±5.8	17.51±1.8
7	0.05	750	41.13±10.2	10.28±2.5
8	0.13	750	44.54±9.4	11.13±2.3
9	0.2	750	50.31±5.9	12.58±1.4

Fig. 1 Linear correlation plots (**a**, **c**) between actual and predicted values and corresponding residual plots (**b**, **d**) for the responses

data were evaluated kinetically using a software program Kinet DS3.0 (revolution copy 2010) to study the possible mechanism of drug release from microspheres.

Release Kinetics To study the mechanism of drug release from the microspheres, zero order, first order, Higuchi equation, and Korsmeyer–Peppas equation were selected as a model dependent approach to characterize the dissolution profile [35–44]. These selected models are often used to describe the drug release from the polymeric system when the mechanism is not well known or when more than one type of release phenomenon is involved. The model which gave the

Table 3 Model summary statistics of responses Y_1=encapsulation efficiency and Y_2=practical drug loading

Response	Model	Standard deviation	R^2	Adjusted R^2	Predicted R^2	PRESS	Significance
Y_1	Linear	3.36	0.8696	0.8262	0.6986	156.88	–
	2FI	3.68	0.8697	0.7915	0.3204	353.74	–
	Quadratic	1.19	0.9917	0.9780	0.9214	40.87	Suggested
	Cubic	1.54	0.9954	0.9634	0.1679	433.16	–
Y_2	Linear	2.90	0.9140	0.8853	0.8099	111.79	–
	2FI	3.14	0.9159	0.8654	0.6230	221.77	–
	Quadratic	0.57	0.9983	0.9955	0.9833	9.80	Suggested
	Cubic	0.66	0.9992	0.9940	0.8637	80.19	–

Table 4 Coefficient estimate and p values of each factor for the measured responses Y_1=encapsulation efficiency and Y_2=practical drug loading

Factors	Y_1		Y_2	
	Coefficient estimate	p value	Coefficient estimate	p value
X_1	4.73	**0.0023**	1.71	**0.0052**
X_2	−7.29	**0.0007**	−9.31	**<0.0001**
X_1X_2	0.09	0.8827	−0.53	0.1628
X_1^2	1.37	0.2028	0.59	0.2375
X_2^2	5.46	**0.0075**	4.89	**0.0012**

highest coefficient of determination (R^2) was considered to be the most suitable kinetic model for describing the release of DS from the microspheres.

Optimization and Validation of Model A total of nine runs were generated by the Design-Expert software for the 3^2 full factorial design. Statistical validation of the equation was established using ANOVA. The models were evaluated using statistically significant terms and R^2 value. An intensive grid search was conducted to find out the composition of the optimized formulation having a higher value of responses. One optimum checkpoint formulation was selected to evaluate optimization capability of models generated using 3^2 full factorial design. Checkpoint formulation (run no. 10) was prepared using the optimal formulation variables setting and evaluated for the responses. The resultant experimental value was quantitatively compared with predicted value and the prediction error was calculated [20, 45, 46].

Results and Discussion

Statistical Analysis of Data

Using 3^2 factorial design, a total of nine runs was carried out for the preparation of microspheres and investigate the effects of two independent variables on the dependent variables (responses) using factorial design. The quadratic mathematical model (suggested) generated by 3^2 factorial design was used to evaluate the responses.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2 \quad (3)$$

Where, β_0 is the intercept; β_1 to β_5 are the estimated coefficient obtained from the observed experimental values of Y; X_1 and X_2 are the coded levels of the factor. The coefficient corresponding interaction (X_1X_2) and the quadratic effects (X_1^2 and X_2^2) were determined from the results of the experiments. Results of all the nine experiments carried out are summarized in Table 2. A study showed that the formulation parameters had an influence on the microspheres properties (encapsulation efficiency and drug content). The polynomial model describing the correlation between the formulation variables and the response can be represented by the following equation.

$$Y_1 = 46.23 + 4.73 X_1 - 7.29 X_2 + 0.097 X_1 X_2 + 1.37 X_1^2 + 5.46 X_2^2 \quad (4)$$

$$Y_2 = 15.36 + 1.71 X_1 - 9.31 X_2 - 0.53 X_1 X_2 + 0.59 X_1^2 + 4.89 X_2^2 \quad (5)$$

The equation represents the quantitative effects of factor (X_1 and X_2) upon the responses (Y_1 and Y_2). All the values of

Table 5 Experimental and predicted values of the responses

Response	Run	Experimental value	Predicted value	Residual	Prediction error[a] (%)
Y_1	1	56.54	55.73	0.81	1.43
	2	57.83	58.99	−1.16	−2.01
	3	65.33	64.99	0.34	0.52
	4	41.89	42.88	−0.99	−2.36
	5	47.26	46.23	1.03	2.18
	6	52.29	52.33	−0.042	−0.08
	7	41.13	40.96	0.17	0.41
	8	44.54	44.41	0.13	0.29
	9	50.31	50.61	−0.30	−0.60
Y_2	1	28.27	27.92	0.35	1.24
	2	28.92	29.56	−0.64	−2.21
	3	32.67	32.39	0.28	0.86
	4	13.95	14.24	−0.29	−2.08
	5	15.80	15.36	0.44	2.78
	6	17.51	17.66	−0.15	−0.86
	7	10.28	10.34	−0.063	−0.58
	8	11.13	10.93	0.20	1.80
	9	12.58	12.71	−0.13	−1.03

[a] Percent prediction error was calculated using the formula (Experimental value−predicted value)/experimental value×100

an estimated coefficient are reported for each response Y_1 and Y_2 in Fig. 1a, b. The sign of the coefficient shows how the factor influences the response. If the coefficient is positive, the response is increased (synergistic effect) as the factor moves from low level (−1) to high level (+1); the contrary is obtained (inverse relationship/antagonist effect) if the coefficient is negative [28, 47]. Linear, cross-product contribution (2FI), quadratic and cubic models were generated for the responses by the software. The quadratic model showed a best fit for the responses. Table 3 showed the model summary statistics of responses. Data in Table 4 showed the coefficient estimate and p values of each factor for the measured responses. Significant values indicated in bold faces. Significant factors affecting the response Y_1 were X_1 (amount of PVA as a surfactant, p value 0.0023), X_2 (ratios of polymer, p value 0.0007), and quadratic term X_2^2 (p value, 0.0075). Significant factors affecting the response Y_2 were X_1 (p value, 0.0052), X_2 (p value, <0.0001), and quadratic term X_2^2 (p value, 0.0012). To validate the model, all the points were selected and observed their experimental and predicted value for the responses. Table 5 showed

the prediction point of experimental and predicted values for the responses. Therefore, it can be concluded that the model is best suitable because of the difference between experimental and predicted value is very low. Figure 2 quantitatively compares the resultant experimental values with predicted values and corresponding residual of the responses.

Microspheres Characterization

Encapsulation Efficiency and Drug Content

Encapsulation efficiency and drug loading of different experimental runs of microspheres are reported in Table 2. DS exhibit poor aqueous solubility at lower values of pH. Hence, the pH of external aqueous phase was adjusted to 3.9 (pKa of DS=4.2) to prevent the leaching of drug [33]. The 3D response surface plots are useful in learning about the main and interaction effects of the independent variables, whereas 2D contour plots give a visual representation of values of the response [48]. To visualize the effect of independent variables
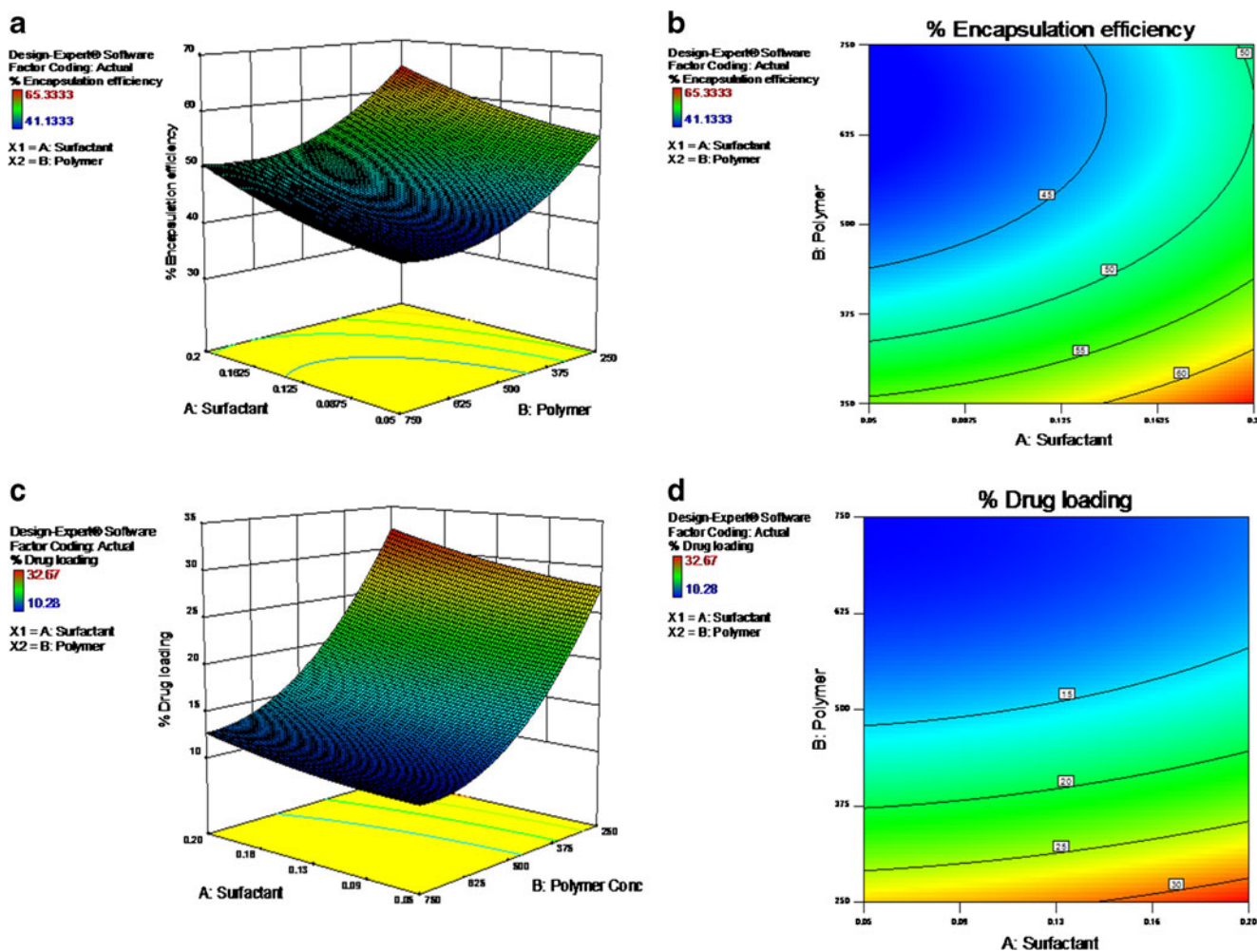

Fig. 2 Response surface plots (**a**, **c**) and contour plots (**b**, **d**) showing effect of independent variables on responses

on each response, 3D response surface plots and 2D contour plots (Fig. 2) were constructed. The percent encapsulation efficiency of the microspheres was ranged from 41.13 to 65.33 % of all the experimental runs. It was observed that the encapsulation efficiency was higher with a high amount of surfactant and the experimental run having equivalent drug-to-polymer concentration. Encapsulation efficiency of microspheres decreases with an increase in the amount of polymer in the organic phase. Surface response plots and contour plots (Fig. 2a, b) represent the increase in percent encapsulation efficiency (Y_1) with an increase in amount of PVA while percent encapsulation efficiency decreases with an increase in the amount of polymer. This may be due to the more time taken for the precipitation of polymer which was in higher amount. To minimize the potential risk in patients treated with sustained release diclofenac, the lowest effective dose should be used for the shortest possible duration. Increase in practical drug loading (Y_2) was observed in surface response plot and contour plots (Fig. 2c, d) with the increase in amount of PVA and vice versa with an increase in the amount of polymer.

Field Emission Scanning Electron Microscopy

Shape, size, and surface morphology of the DS loaded microspheres were examined by FESEM. Scanning electron microphotographs of microspheres prepared in this study are shown in Fig. 3. The particle size of the drug-loaded microspheres was found to be in the range of 10–200 μm. SEM images of the microspheres indicate that the microspheres are discrete, spherical, porous and with uniform surface.

Fourier Transform Infrared Spectroscopy Study

FTIR spectra of materials were obtained to analyze the prepared microspheres. Figure 4 shows typical spectra of pure DS (−(A)), Eudragit RS-100 (−(B)), RL-100 (−(C)), and encapsulated DS (−(D)) in the microspheres. In the spectrum of DS (−(A)), principal peaks were found at the range of 700–800 cm^{-1} attributed to substituted phenyl group, aromatic amines exhibit two strong bands at 1,180–1,360 cm^{-1} because of C–N stretching, signal at 1,575 cm^{-1} attributed to C=C stretching of aromatic ring, at 745 cm^{-1} because of C–Cl stretching, at 1,716 cm^{-1} attributed to C=O stretching of the carbonyl group, at 3,074 cm^{-1} attributed to C–H stretching of aromatic ring, at 3,385 cm^{-1} attributed to N–H stretching of amines. The FTIR spectra of Eudragit RS-100 and RL-100 displayed characteristic peaks at 2,949 and 2,947 cm^{-1} because of C–H aliphatic stretching and at 1,734 and 1,737 cm^{-1} because of C=O stretching [49, 50]. The FTIR spectra of optimized drug loaded microspheres displayed a characteristic peak at 3,321 cm^{-1} attributed to shifting of two N–H stretching peaks from 3,259 to 3,385 cm^{-1}. This could be due to the conversion of the DS to the acidic form of the drug in
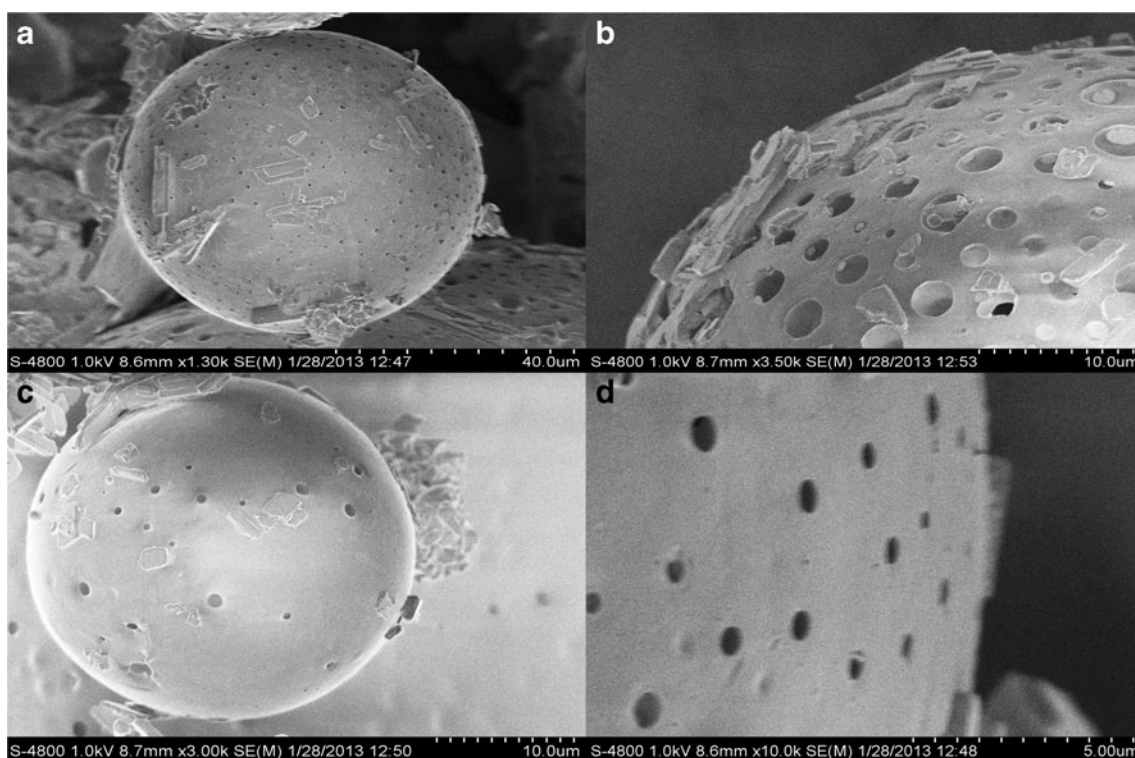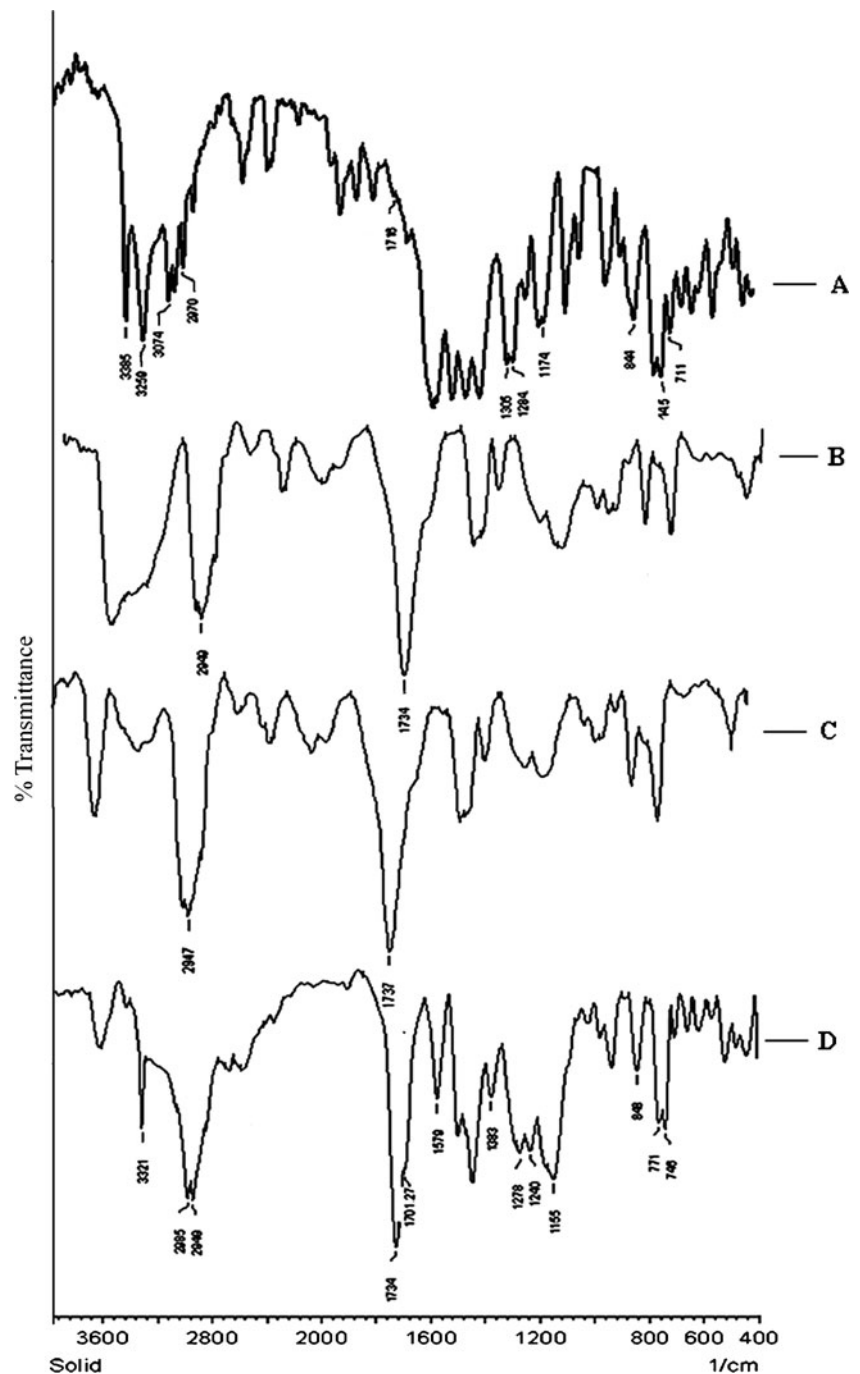

Fig. 3 SEM micrographs of microspheres (**a**, **c**) and microspheres surface (**b**, **d**)

Fig. 4 FTIR spectrum of DS (**a**), Eudragit® RS-100 (**b**), RL-100 (**c**), and microspheres (**d**)


contact with acidic external phase [18, 51]. Other values from drug and polymers remained very close in the FTIR spectra of drug-loaded optimized microspheres, indicating no existence of the different association form of the DS with polymers.

X-Ray Diffraction

The presence of numerous distinct peaks in the XRD patterns (Fig. 5) of DS (−(A)), at *2θ*; 11.26°, 15.22°, 19.91°, 23.48°, 24.15°, 27.04°, and 27.90° were present as crystalline material. There was no clear peak in the XRD patterns (−(B) and (C)) of Eudragit RS 100 and RL 100. XRD pattern of drug loaded optimized formulation (−(D)) was characterized by the absence (weak) of distinct diffraction signals at the respective scattering angles to that observed with DS and the X-ray profile is identical to that of pure polymers, i.e., typical of an amorphous material. Thus, XRD analysis suggested that

Fig. 5 XRD spectra of DS (**a**), Eudragit® RS-100 (**b**), RL-100 (**c**), and microspheres (**d**)
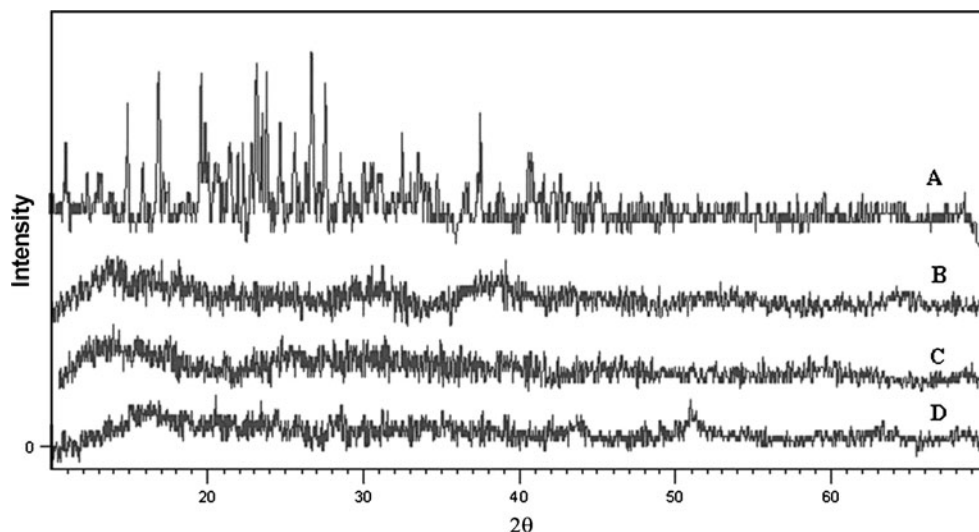
the degree of crystallinity of DS was reduced and most of the drug existed in amorphous state within the polymers.

In Vitro Drug Dissolution Studies

Eudragit RS100 and RL 100 are water insoluble polymers and able to control drug release from microparticles. Figure 6 shows the cumulative percent drug release as a function of the dissolution time from the experimental batch (run 2) and optimized DS loaded microspheres (run 10).

From the Fig. 6, it was found that the release rate of DS was extremely low at acidic pH (less than 10 % of the drug was released in 2 h). This was an expected result [52], as the solubility of the DS in the acidic medium is very less (<2.0 mg/L). However, when the pH was changed to 6.8, the drug was released more for the subsequent period of time.
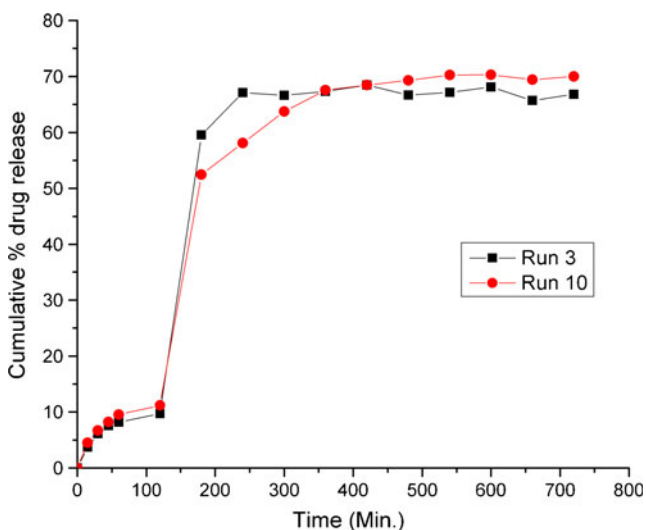

Fig. 6 Cumulative percent drug release from DS-loaded microspheres

After changing the media to phosphate buffer, sudden increase in drug release was observed in the range of 50–60 % at the 3rd hour of study, it might be due to the immediate release of surface associated drug. Furthermore, slow release phase (total 70 %) of encapsulated DS was observed in 12 h from Eudragit microspheres. The encapsulated microparticles showed a delayed drug dissolution rate, sustaining the drug for several hours. Four different kinetic models were employed to evaluate the possible changes in the release mechanism. The data were fitted into Korsmeyer–Peppas equation. The sample showed good linearity (R^2, 0.973 (run 3); R^2, 0.956 (run 10)) with 0.7 and 0.8 values of slope (n). This n value, however, appears to indicate that diffusion is the dominant mechanism of drug release with these formulations [53, 54].

Optimization and Validation of Response Surface Methodology

To access the reliability of the developed mathematical model, encapsulation efficiency of optimized formulation corresponding to the predicted values of the amount of Eudragit polymer and the amount of PVA was determined. The optimum formulation was selected based on the criteria of attaining the maximum encapsulation efficiency for the microspheres. The desirable range of the encapsulation efficiency was restricted to 41.13 % ≤ Y_1 ≤ 65.33 %. Upon extensive grid search, the formulation composition with the polymer level, 250 mg, and surfactant level of PVA, 0.2 % was found to fulfill the maximum requisite of an optimum formulation with high encapsulation efficiency. The desirability plot (2D) (Fig. 7a) indicating desirable regression ranges for optimal formulation variables and the overlay plot (Fig. 7b) indicating the region of optimal formulation variables. Optimized formulation of microspheres was prepared by solvent evaporation technique using optimal formulation variables setting to
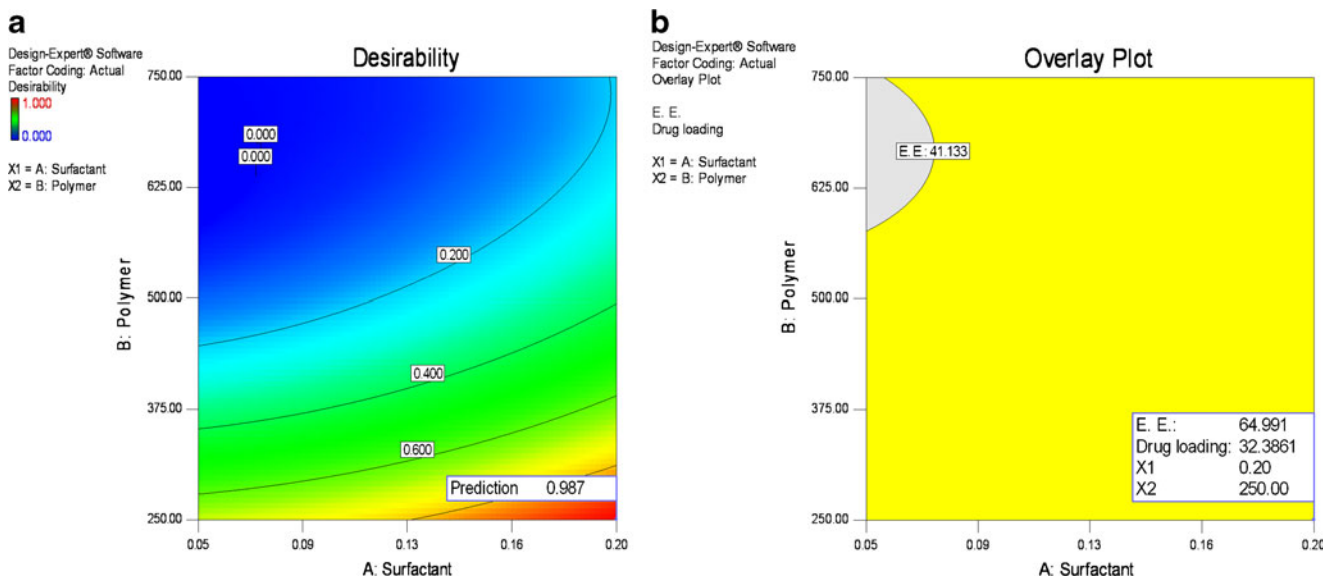
Fig. 7 Desirability (**a**) and overlay plots (**b**) for the responses

evaluate the optimization capability of the models generated according to the 3^2 full factorial design. Optimized formulation (run no. 10) was evaluated for responses. Table 6 lists the composition of the optimum checkpoint formulation, their predicted and observed values of the responses along with percentage error. Results show the low magnitudes of error in the present investigation prove the high prognostic ability of the RSM.

Conclusions

This work shows that it is possible to encapsulate DS in Eudragit (RS 100 and RL 100) polymer by employing the O/W solvent evaporation method using RSM. It is possible to obtain the desired information about the weight of each level of each factor on the responses with the help of statistical evaluation of the Design-Expert® Software. The mathematical model for the response developed using statistical analysis quantitatively describes the influence of the selected variables on the responses under study. Design and optimization through statistical experimental designs work well in solving

research and development problems. Concentration of PVA in the external aqueous phase and the amount of polymer in dichloromethane affected the percent encapsulation efficiency and percent practical drug loading. The percent encapsulation efficiency of the microspheres was ranged from 41.13 to 65.33 % of all the experimental runs. The microspheres were found to be discrete, spherical with smooth surface and in the range of 10–200 μm. Data of FTIR spectroscopy indicates the absence of drug polymer interactions. X-ray powder diffractometry revealed the dispersion of drug within microspheres formulation. The in vitro drug release from microspheres followed a sustained drug release pattern with diffusion mechanism. Microspheres prepared by such methods may represent a promising approach for efficient delivery of DS.

Table 6 Predicted and experimental values of responses according to optimized conditions

Run	Response	Factors X_1 (%)	X_2 (mg)	Experimental value	Predicted value	Prediction error[a] (%)
10	Y_1	0.2	250	64.39±1.3	64.99	−0.93
	Y_2			32.19±0.6	32.38	−0.59

[a] Percent prediction error was calculated using the formula (Experimental value−predicted value)/experimental value×100

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