Formulation and Optimization of Carbamazepine Microspheres by 2 Factor 2 Level Central Composite Design

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Abstract

The present investigation was designed to prepare controlled release microspheres of carbamazepine using two polymers of different solubility and permeability characteristics, Ethocel standard 45 premium and Eudragit RL 100. The drug release profile was optimized with the aid of design of experiments (DoE). Microspheres of combined polymers were designed according to 2^2 factorial central composite design (CCD), taking drug loading and polymeric ratio as the independent variables. Total thirteen batches were prepared. The dependent variables were percentage of drug released in 3 hours and 6 hours and mean dissolution time (MDT). The regression parameters of the developed model and graphical interpretation for each response with statistical significance were calculated by using Minitab 17. The relationship between the experimental variables and responses were evaluated by generating response surface plots. Increased amount of Eudragit RL 100 had impact on surface morphology of prepared microspheres. It produced larger holes on the surface due to its higher permeability characteristics. Polynomial mathematical models generated for various response variables using multiple linear regression analysis, were found to be statistically significant ($p < 0.05$). One optimum formulation (O1) was selected based on USP specification and the second optimum formulation (O2) was selected for the maximization of MDT (hours). Batch O1 showed 22.85 % and 48.78 % drug release after 3 and 6 hours, respectively which were found to be in close agreement with those predicted by the mathematical model. Another optimum formulation, batch O2 showed MDT as 160.61 hours.

Key words: DoE, optimization, Eudragit RL100, carbamazepine, microsphere

Introduction

Statistical modeling and experimental design are two most essential tools in the field of formulation development (Montgomery and Douglas, 2012; Singh *et al.*, 2011). While developing a sustained release microsphere dosage form, an important issue is to design an optimized formulation with an ideal release profile in a specific time period and minimum number of trials. For this purpose, in this current study formulations of microspheres are developed with response surface methodology (RSM) utilizing a polynomial equation (Mandal *et al.*, 2007; Singh *et al.*, 2006). Carbamazepine, a BCS Class II drug was chosen as a model drug because it shows poor water solubility and erratic oral absorption property. Peak plasma concentration of the conventional carbamazepine tablets varies from 4 to 8 hours but may be delayed up to 24 hours (Katzung *et al.*, 2015; Goodman *et al.*, 2006). Carbamazepine is also characterized by shorter biological half-life (~ 12 hours) during chronic dosing, due to auto-induction of hepatic metabolism and it may fall approximately to 8 hours in patients receiving other enzyme-inducing drugs (Katzhendler *et al.*, 1998; Filipović-Grčić *et al.*, 2003). The rate and extent of absorption and thus bioavailability of carbamazepine can be increased by enhancing dissolution profile of the drug. Various attempts were taken by several investigators to enhance rate of dissolution of

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carbamazepine (Filipović-Grčić *et al*., 2003; Gavini *et al*., 2006; Moneghini *et al*., 2001; Javadzadeh *et al*., 2007; Passerini *et al*., 2002). Among these it was reported that spray drying technique enhanced the rate of dissolution of carbamazepine because of reduction in crystallinity of carbamazepine (Filipović-Grčić *et al*., 2003). Another issue is an immediate release formulation of carbamazepine shows various central nervous system side effects (Olling *et al*., 1999).

Thus the present investigation was designed to prepare controlled release microspheres of carbamazepine using two polymers of different solubility and permeability characteristics, Ethocel standard 45 premium and Eudragit RL 100 (Haznedar and Dortunç, 2004; Kusum Devi *et al*., 2003). Emulsion solvent evaporation was the choice of technique. The drug release profile was optimized with the aid of design of experiments (DoE). The microspheres of combined polymers were designed according to 2^2 factorial central composite design (CCD), taking drug loading and polymeric ratio as the independent variables. Total thirteen batches were prepared. The dependent variables selected were percentage of drug released in 3 hours, percentage of drug released in 6 hours and MDT. Because in USP for extended release formulations of carbamazepine specifications were made for percentage of drug release after 3 hours and 6 hours. The percentage of amount dissolved should be between 10 % to 35 % and 35 % to 65 % after 3 hours and 6 hours, respectively (USP 29, 2007).

Materials and Methods

Carbamazepine was obtained as a generous gift from Beximco Pharmaceuticals Ltd. Ethocel standard 45 premium (Colorcon Asia Pvt. Ltd, USA), Eudragit RL 100 (Evonik Industries, Germany), liquid paraffin (Merck, Germany), Span 80 (Merck, Germany), acetone (Merck, Germany), *n*-hexane (Merck, Germany) were obtained from the mentioned sources. Sodium lauryl sulfate was purchased from local market.

Preparation of carbamazepine microspheres: The method of preparation of carbamazepine microspheres was based on emulsion solvent evaporation method using two different polymers: Ethocel standard 45 premium and Eudragit RL 100 (Rajkumar and Bhise, 2010; Behera *et al*., 2008). Weighed quantities of both polymers and carbamazepine were dissolved in acetone at room temperature. This solution of internal phase was then slowly poured drop by drop to the liquid paraffin which was previously emulsified with 1 % span 80 at same temperature. The whole system was stirred continuously at 1000 rpm for 3 hours. After that the microspheres were separated by filtration. Thereafter, the excess paraffin oil retained in the surface of microspheres was eliminated by repeated washing (4 to 5 times) with n-hexane and finally dried and preserved in a desiccator. The dried microspheres were stored in a glass vial after proper identification.

Experimental design: The microspheres of combined polymers are designed according to 2^2 factorial central composite design (CCD) (Bezerra *et al*., 2008). Drug loading (%) and the polymeric ratio of Ethocel standard 45 premium & Eudragit RL 100 were the two independent variables. The selected variables with the actual and coded levels as per the design are represented in Table 1. The higher, lower and the intermediate levels of each variable are coded as +1, −1 and 0 respectively. Final formulation of all batches with their respective amounts is represented in Table 2.

Table 1. Formulation protocol for the microspheres designed by 2^2 factorial CCD.

					Coded level		
Variables	Code	Units	$-\alpha$	-1	0	+1	$+\alpha$
Drug loading	X1	%	0.2379	0.30	0.45	0.60	0.6621
Polymer ratio	X2	-	0.1895	0.50	1.25	2.00	2.3105

Table 2. Composition of different microspheres formulations.

Formulation code	Coded variables		Independent variables		Carbamazepine (gm)	Ethocel Std. 45 P (gm)	Eudragit RL 100 (gm)
	X1	X2	Drug loading (%)	Ethocel Std 45 P: Eudragit RL 100			
B1	1	1	60	2:1	0.6	0.266	0.133
B2	1	-1	60	1:2	0.6	0.133	0.266
B3	-1	1	30	2:1	0.3	0.466	0.233
B4	-1	-1	30	1:2	0.3	0.233	0.466
B5	-1.414	0	23.79	1.25:1	0.2379	0.423	0.339
B6	1.414	0	66.21	1.25:1	0.6621	0.188	0.150
B7	0	-1.414	45	0.1895:1	0.45	0.088	0.462
B8	0	1.414	45	2.3105:1	0.45	0.384	0.166
B9	0	0	45	1.25:1	0.45	0.306	0.244
B10	0	0	45	1.25:1	0.45	0.306	0.244
B11	0	0	45	1.25:1	0.45	0.306	0.244
B12	0	0	45	1.25:1	0.45	0.306	0.244
B13	0	0	45	1.25:1	0.45	0.306	0.244

Variable X1 represents drug loading (%) and variable X2 represents ratio of the polymers, Ethocel standard 45 premium and Eudragit RL 100.

Morphology study by scanning electron microscope (SEM): Microspheres were observed by Scanning Electron Microscopy (SEM) to see the morphological changes that occurred due to the formulation variation. The microsphere from the selected batches were mounted on the SEM sample stab (aluminium stabs) which were coated with a double sided sticking tape, sealed and finally coated with gold (200 A°) under reduced pressure (0.001 torr) for 15 minutes using ion sputtering device. The gold coated samples were scanned using scanning electron microscope (s-3400N, Hitachi) under different magnification.

In vitro dissolution study of carbamazepine microspheres: The dissolution studies of the microspheres were carried out in a basket type (type II) USP dissolution apparatus. Weighed amount (50 mg) of microspheres were taken in 900 mL of dissolution medium (distilled water containing 1 % sodium lauryl sulfate) and were stirred at 100 rpm at $37 \pm 0.5°C$ (USP 29, 2007). A 10 mL dissolution sample was withdrawn from the dissolution medium at pre-determined intervals of 30 minute, 1st hour, 2nd hour, 3rd hour, 4th hour, 5th hour, 6th hour, 7th hour & 8th hour. Collected samples were analyzed spectrophotometrically through an UV spectrophotometer at 284 nm after suitable dilution to determine the amount of the drug released from the microspheres. The percentage of drug release was plotted *versus* time. The dissolution study was repeated three times for each batch.

ANOVA and response surface analysis: Analysis of variance and response surface analysis was conducted for cumulative percent of drug released after 3 hours & 6 hours and MDT using Minitab 17.

Results and Discussion

Observation of microspheres by scanning electron microscope (SEM): SEM images of the batches prepared with drug load variation and polymer ratio variation were taken and were analyzed morphologically.

Here, batch B1 is the microsphere of carbamazepine using Ethocel standard 45 premium and Eudragit RL 100 at the ratio of 2:1 having 60 % drug loading. As shown in SEM photographs Figure 1, the microspheres were spherical, smoother and less porous in nature. Batch B2 is the microsphere of carbamazepine using Ethocel standard 45 premium and Eudragit RL 100 at the ratio of 1:2 having 60 % drug loading. SEM image revealed that microspheres were spherical, smoother and had large holes on the surface.

Eudragit RL 100 has quaternary ammonium group in the structure, which increases its solubility characteristics (Sonje and Chandra, 2013). Comparing

SEM images of B1 and B2 it can be concluded that as the amount of Eudragit RL 100 was more in batch B2, it produced larger holes on the surface due to its higher permeability characteristics. These larger holes may further ensure higher drug release profile in the body

(USP 29, 2007). It is confirmed in the cumulative percent drug release data obtained from dissolution studies as illustrated in "Results and Discussion" section.
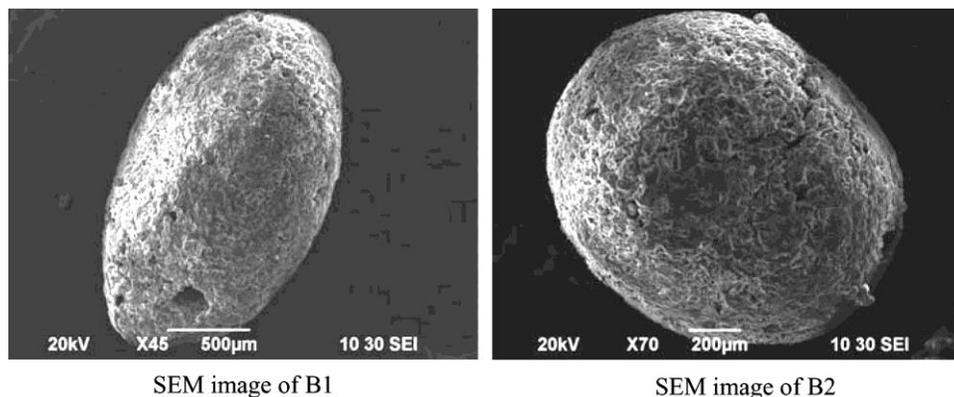

Figure 1. Effect of Ethocel standard 45 premium and Eudragit RL 100 on the surface morphology on batch B1, B2 and B3.

In vitro dissolution and kinetic studies of carbamazepine microspheres: Figure 2 represents the release of carbamazepine from microspheres in different drug: polymer ratio. The rate of drug release from the microspheres depended on the amount of polymer and the type of polymer used (Mukhopadhyay *et al*., 2014). As concentration of Ethocel standard 45 premium increased the release rate was decreased which may be due to increase in thickness of polymer matrix layer (Mane *et al*., 2013). The decrease in release rate with increasing content of the Ethocel can also be explained by the fact that the amount of uncoated drug decreases with the increase of Ethocel amount and drug diffusion becomes more retarded (Filipović-Grčić *et al*., 2003; Mukhopadhyay *et al*., 2014). As compare to Ethocel, microspheres having more amount of Eudragit RL 100 showed a little higher drug release rate, which is attributed to the difference in the permeability characteristics (USP 29, 2007).

The results of *in vitro* dissolution studies have shown that batches B2 and B6 had higher drug release profile. Comparing the formulation of batches B2 and B6 it was observed that as the drug loading increases, percent release of drugs also increases accordingly (Filipović-Grčić *et al*., 2003). Batch B2 showed 72.82 % cumulative drug release at the end of 8 hours and

batch B6 showed 70.43 % cumulative drug release at the end of 8 hours. Although batch B2 had 60 % drug loading compared to batch B6 which had 66.21 % drug loading but B2 showed little bit higher drug release profile than B6. Because it had relatively higher amount of Eudragit RL 100 in the composition.

Statistical analysis and model development: The regression parameters of the developed model and graphical interpretation for each response with statistical significance were calculated by using Minitab 17. The relationship between the experimental variables and responses were evaluated by generating response surface plots (Myers *et al*., 2016).

The quadratic model was selected by the software for both the responses. Multiple regression analysis was used to correlate the responses of percentage drug release and MDT with the two variables studied using a second order polynomial equation. The quadratic regression models for cumulative percentage of drug release after 3 hours (Y1), Cumulative percentage of drug release after 6 hours (Y2) and MDT can be represented by following equations of (1), (2) and (3):

Y1 (3 hours) = $48.47 + 4.88\,A - 7.31\,B - 9.33\,A^2 - 5.24\,B^2 + 1.19\,AB$ ……………… (1)

Y2 (6 hours) = $58.33 + 7.49\,A - 9.36\,B - 8.46\,A^2 - 4.83\,B^2 + 1.06\,AB$ ……………..... (2)

MDT (hours) = $7.35 - 18.55 A + 19.17 B + 14.36 A^2 + 9.38 B^2 - 19.28 AB$ ………….. (3)

Here, in Eq. (1), (2) and (3), A and B represents the coded values for X1 and X2.

Statistical significance of the model

Effect of formulation variables on percent release at the end of 3 hours (Y1): The adequacy and significance of the model was justified by analysis of variance (ANOVA). Analysis of the acquired data shows that the F value of the model is 17.75, which is significant. The F-value of the model has P-value 0.001 < 0.05 (significance level) which indicates that the overall model has significant capacity to explain variation in response variable Y1 (3 hours). At 5 % significance level, a model is considered significant if the P value (significance probability value) is less than 0.05.
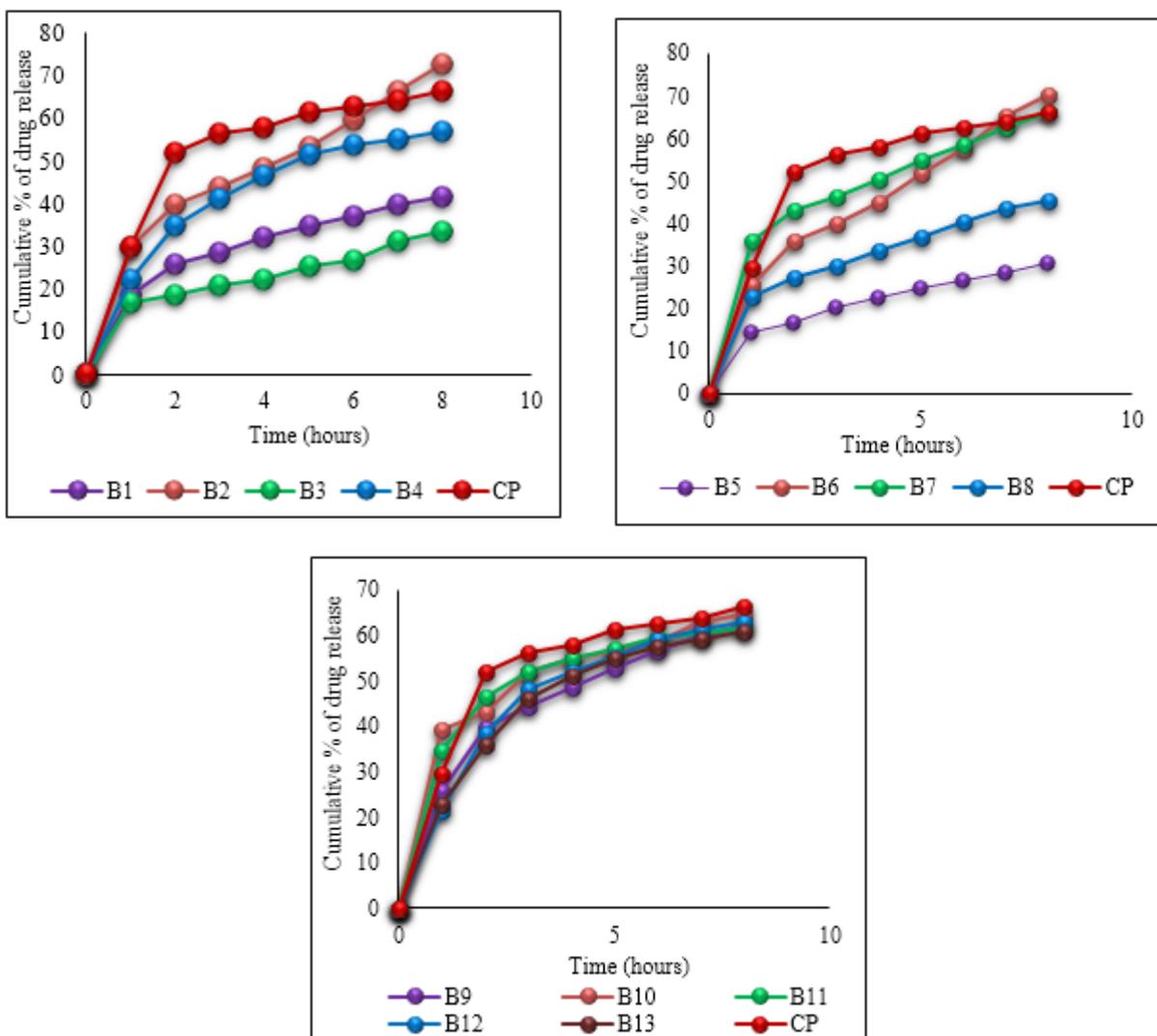

Figure 2. Cumulative percent drug release vs time curves.

However, both linear (effects of A and B) and quadratic effects (effects of A^2 and B^2) are also significant. But interaction effect (effect of AB) is not significant. Insignificant lack of fit also reflects the model adequacy. After all, the $R^2 = 92.69$ % implies that the model can explain 92.69 % variation in Y1.

The response surface plot revealed that the drug loading and polymer ratio had a significant opposing

impact on the drug release without creating any interaction effect. The surface plot (Figure 3) shows that highest yield (Y1) obtained when X1 level (drug loading) is high and X2 level (polymer ratio) is low. Drug release at the end of 3 hours increased from 20.52 % to 32.66 % and from 37.52 % to 44.90 % at high and low levels of polymer ratio (Ethocel standard 45 premium: Eudragit RL 100) respectively as the drug loading increased. That indicates when the amount of Ethocel standard 45 premium was comparatively high it showed greater release retarding property due to its hydrophobic nature (Jain *et al*., 2010; Shivakumar *et al*., 2008).

Effect of formulation variables on percent release at the end of 6 hours (Y2): The analysis of variance of the regression model explains that the model is highly significant, as is evident from the F-value (13.85). The P value of < 0.05 was considered statistically significant. However, both linear (effects of A and B) and quadratic effects (effects of A^2 and B^2) are also significant. Lack of fit was not significant for the model. After all, the R^2 = 90.82 % implies that the model can explain 90.89 % variation in Y2.

Figure 3. Response surface plot showing the effect of formulation variables on percent release at the end of 3 hours.
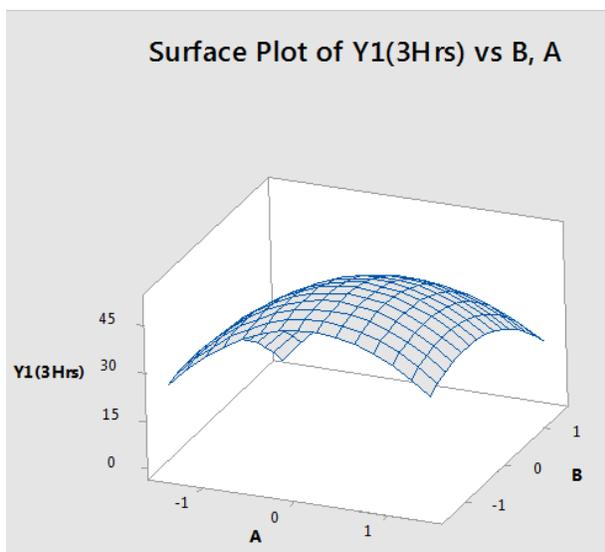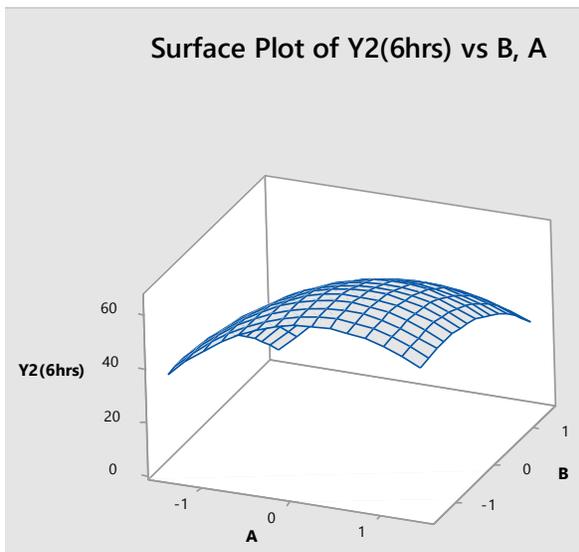

Figure 4. Response surface plot showing the effect of formulation variables on percent release at the end of 6 hours.

The surface plot (Figure 4) shows that highest yield (Y2) obtained when X1 level (drug loading) is high and X2 level (polymer ratio) is low. The 3D plot shows that the drug release at the end of 6 hours increased from 27.13 % to 44.23 % and from 47.97 % to 60.83 % at lower and higher levels of polymer ratio (Ethocel standard 45 premium: Eudragit RL 100) respectively as the drug loading levels increased. Eudragit RL 100 has greater permeability characteristic because of having greater quaternary ammonium group content in the polymer. This specific property can explain the

enhancement in percent drug release with comparative increase in Eudragit RL 100 amount.

Effect of formulation variables on MDT (hours): ANOVA data shows that the F-Value (10.12) of the model has P-value 0.004 < 0.05 (significance level) which indicates that the overall model has significant capacity to explain variation in response variable MDT (hours). Linear (effects of A and B) effects are significant but quadratic effects (effects of B^2) are not significant. Interaction effect (effect of AB) is significant. After all, the R^2 = 87.85 % implies that the model can explain 87.85 % variation in MDT.
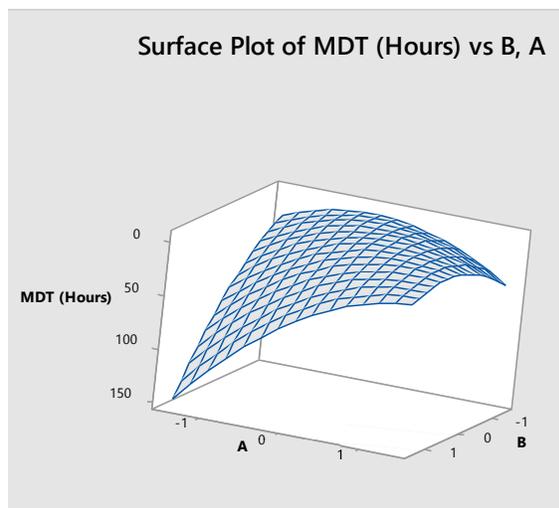
Figure 5. Response surface plot showing the effect of formulation variables on MDT.

The surface plot (Figure 5) shows that highest yield (MDT) obtained when X1 level (drug loading) is low and X2 level (polymer ratio) is high. The 3D plot shows that the MDT increased from 12.65 hours to 88.09 hours at lower and higher levels of polymer ratio (Ethocel standard 45 premium: Eudragit RL 100) respectively as the drug loading levels decreased.

Response optimization

Response optimization results for Y1 and Y2:

Parameters

Response	Goal	Lower	Target	Upper
Y2 (6 hrs)	Target	35 %	**50.0 %**	65 %
Y1 (3 hrs)	Target	10 %	**22.5 %**	35 %

Response optimization results for MDT (hours):

The optimization of the responses was conducted with the help of Minitab 17 software, which was used to construct the response surface plot for the fitted polynomial equations and to find the optimum area, at which the desired responses could be achieved. One optimum formulation (O1) was selected based on the following criteria: Y1 (percentage drug released at the end of 3 hours of dissolution) should be 22.5 % and Y2 (percentage drug released at the end of 6 hours of dissolution) should be 50 %. And the second optimum formulation (O2) was selected for the maximization of MDT (hours). The first formulation showed Y1 as 22.85 % and Y2 as 48.78 % (Table 3). Another optimum formulation (O2) showed MDT (hours) as 160.61 hours (Table 4).

For the optimum formulation, the results of the Y1 (percentage drug released at the end of 3 hours of dissolution) and Y2 (percentage drug released at the end of 6 hours of dissolution) were found to be in close agreement with those predicted by the mathematical model. Table 4 lists the compositions of the checkpoints, their predicted and experimental values of all the response variables, and the percentage error. Figure 6 shows linear correlation plots between the observed and predicted response variables. When observed responses were compared with that of the predicted responses, we found that the prediction error varied between 1.55 % and 2.45 %. As the prediction error for the response parameters was low, it establishes the high forecasting ability of response surface methodology. The linear correlation plots (Figure 6) drawn between the predicted and observed responses showed high values of R^2 (0.9995).

Table 3. Composition of the checkpoint formulations, the predicted and experimental values of response variables, and percentage prediction error.

Composition		Response variable	Experimented value	Predicted value	Percentage error
Drug loading (%)	Ethocel Std. 45 Premium: Eudragit RL 100	Y1 (3Hrs)	22.848	22.5	-1.55
66.21	1.87: 1	Y2 (6Hrs)	48.776	50	2.45

Y1 and Y2 represent the percentage drug released at the end of 3 and 6 hours of dissolution.
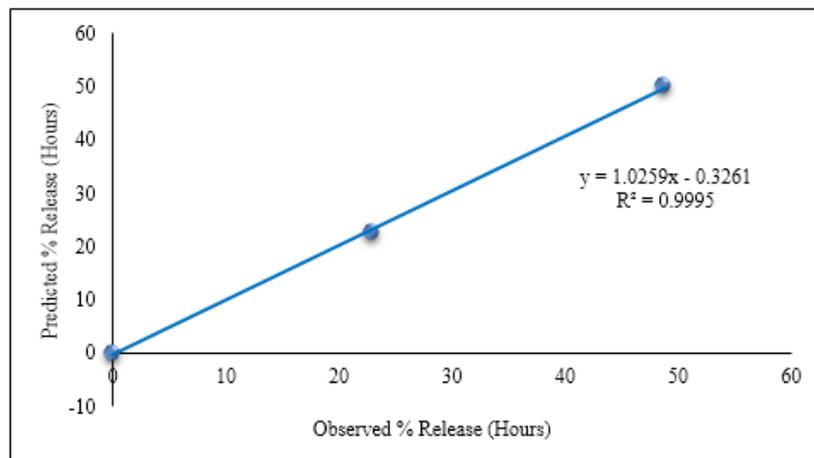
Figure 6. Predicted % release vs. observed % release.

Table 4. Composition of the checkpoint formulations and the experimental values of response variable.

Composition		Response variable	Experimental value
Drug loading (%)	Ethocel Std. 45 Premium: Eudragit RL 100		
23.79	2.31: 1	MDT (Hours)	160.61

High degree of prognosis obtained using RSM corroborates that a 2-factor CCD is quite efficient in optimizing drug delivery systems. Both the formulation variables studied exerted a significant influence on the drug release, which was confirmed by ANOVA data. An optimized formulation with desirable release properties was developed employing numerical optimization technique. Optimum formulation showed a maximum error of 2.45 % which is acceptable. Thus, it can be concluded that the response surface analysis has a reasonable power of prediction within the experimental design range.

Conclusion

Controlled release microspheres of carbamazepine were prepared and optimized successfully using response surface methodology (RSM). The results obtained indicate that response surface methodology can be employed successfully to quantify the effect of several formulation and processing variables thereby minimizing the number of experimental trials and cutting down the formulation development cost. Good correlation between the predicted responses and observed responses of the optimized formulation

indicated high degree of prognosis. Results of drug release profile showed that microspheres with greater proportion of Eudragit RL 100 had rapid carbamazepine release profile. So it can be concluded that to obtain better release profile Eudragit RL 100 could be a better option.

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