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Preparation and Evaluation of Etodolac Loaded Eudragit Rs 100 Microcapsules using Quality by Design Approach

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ABSTRACT

The objective of the current study was to design and optimize Etodolac loaded Eudragit RS 100 microcapsules using solvent evaporation method. Quality by design approach was implemented for development of Etodolac microcapsules using I-Optimal RSM design. Based on initial risk assessment and preliminary study, two independent variables, Drug: Eudragit RS 100 ratio and stirring speed were selected for Design of Experimentation (DoE) to see effect on critical quality attributes (CQAs); entrapment efficiency and particle size. The prepared Etodolac loaded Eudragit RS 100 microcapsules were characterized for particle size, entrapment efficiency, in-vitro dissolution study, DSC, XRD and SEM. Acceptance criteria for CQAs were considered as particle size in the range of 22-200 µm and entrapment efficiency in the range of 72.86-97.25 %, which generated the design space with combination of selected critical parameters leading to the acceptable operating ranges for formulating microcapsules with respect to desired Target Quality Product Profile (TQPP). The obtained Etodolac loaded Eudragit RS 100 microcapsules were spherical in shape, having better entrapment efficiency and sustained drug release profile. Validation of model was carried out by comparing experimental values with predicted value of optimized formulation. Optimized formulations within design space were capable of sustaining drug release for about 22 hours and were expected to reduce dosing frequency thus reducing side effects associated with therapy.

Keywords: Etodolac, Eudragit RS 100, Quality by design, entrapment efficiency, particle size.

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INTRODUCTION

Research, development and sales of drug-delivery systems are increasing at a rapid pace throughout the world. This worldwide trend will intensify in the next decade as cuts in public health expanses demand lower costs and higher efficacy. To meet this demand, many efficient drugs currently in use will be reformulated within delivery systems that can be value-added for optimal molecular activity[1].With advancement of pharmaceutical science; new concepts have evolved various modern dosage forms which give advancement in drug release pattern. In addition, problems related with conventional dosage forms are significant enough to make it less desirable than modified release drug therapy[2]. A sustained constant drug level at the therapeutic optimum is needed in the blood in a number of pathological conditions since they provide better control of plasma drug levels, less dosage frequency, less side effect, increased efficacy and constant delivery. Microencapsulation is one of the most frequently used approaches for both oral and parenteral use to achieve sustained release profile.

In the present study, an attempt has been made to formulate and evaluate sustained release microcapsules of Etodolac. Etodolac is a Non-Steroidal Anti-inflammatory Drug (NSAID) with potent analgesic and anti-arthritic properties. It has recommended oral dose of 200 to 400 mg given every 6 to 8 h to a maximum of 1.2 g daily[3,4]. Moreover, Etodolac causes the gastro intestinal disturbances such as peptic ulceration with bleeding if present in larger concentration in gastro intestinal tract. To overcome this problem sustained release microcapsules were prepared that can improve the patient's comfort and decrease the side effects associated with the therapy. In the present investigation, water insoluble polymer Eudragit RS 100 was used since it is widely used as wall material for sustain release microcapsules[5]. Solvent Evaporation technique was found to be suitable for preparation of biocompatible microcapsules though it is widely used to prepare microspheres.

Recently, Quality by Design (QbD) is becoming a widely used approach in the pharmaceutical industry. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use introduced the quality by design (QbD) concept to encourage new initiatives and provide guidance to pharmaceutical process developers. Implementation of QbD concept in product development provide quality medicines to patients, production improvements to Manufacturers with significantly reduced batch failures and drug regulatory bodies will have greater confidence in the robust quality of products they are being asked to approve[6]. Thus, for optimization of Etodolac microcapsules we demonstrated use of

QbD approach.

MATERIAL AND METHOD

Materials

Etodolac was provided by Emcure Ltd (Pune) as a gift sample. Eudragit RS 100 was kindly supplied Evonik Degussa India Pvt. Ltd. (Mumbai, India).Polyvinyl alcohol was purchased from Loba Chemie Pvt. Ltd, Mumbai. All other chemicals used were of analytical reagent grade.

Formulation of Microcapsules

Preparation of Etodolac loaded Eudragit RS 100 microcapsules

Etodolac loaded Eudragit RS 100 microcapsules were prepared using solvent evaporation method. Eudragit RS 100 and Etodolac were dissolved in organic solvent and further added to the aqueous solution containing stabilizer (polyvinyl alcohol) under continuous stirring at room temperature. To allow complete evaporation of organic solvent, the dispersion was allowed to stir for 3hrs. The dispersion containing microcapsules was centrifuged at 5000 rpm for 30 min. Supernatant was analyzed for free drug content and sediment containing microcapsules was washed three times with distilled water and then dried at room temperature.

Optimization of PS-PLGA nanoparticles

Initial Risk assessment

Prior to application of design, a number of preliminary trials were conducted to identify the potential formulation and process parameters significantly affecting ultimate product quality. Based on literature and preliminary knowledge initial risk assessment was performed to identify those formulation and process parameters whose variability may influence the potential CQAs. The relative risk that each of these parameters presented was categorized as high, medium, or low. Those attributes that could have a high impact on the drug product CQAs are considered important for Design of Experiment and are needed to be in accepted multivariate ranges.

Risk assessment of Critical Quality Attributes

Design of experimentation study using I optimal RSM design

Based on risk assessment, Drug : Eudragit RS 100 ratio (X1) and stirring speed (X2) were selected as independent variables (factors) and entrapment efficiency and particle size were considered as dependent variables (responses) which were varied at three levels (low, medium and high). The levels of critical variables were set based on literatures and preliminary studies to achieve desired product quality It is noteworthy to mention that from preliminary studies it was concluded that stabilizer (PVA) concentration equal to 0.2% wt could efficiently produce the

desired microcapsules. Hence concentration of stabilizer was not included as formulation parameter in design of experimentation (DoE) even though it had high impact on particle size. I Optimal RSM design was applied for optimization of Etodolac loaded Eudragit RS 100 microcapsules using 'Design Expert version 9' (State Ease). I Optimal RSM design generated 16 experimental runs, which were prepared and evaluated for selected responses. The experimental design and analysis, including effect estimates, response surface analysis and optimization, were conducted in software 'Design Expert version 9' (State Ease).

Table 1 Risk assessment to identify variables affecting drug product quality.

Drug Product CQAs	Initial risk assessment of the Process and Formulation variables					
	Coat: core ratio	Stabilizer Conc.	Stirring Speed	Type of Organic solvent	Organic: Aqueous Phase ratio	Rate of Addition of Organic phase
Entrapment Efficiency	High	Medium	Medium	Low	Low	Low
Drug Release	High	Low	Medium	Low	Medium	Low
Particle Size	High	High	High	Medium	Medium	Medium

Table 2. Levels of Independent variables

Sr.No	Independent variables	Levels		
		Low (-1)	Medium (0)	High (+1)
1.	Drug : Eudragit RS 100	0.25	0.2	0.167
2.	Stirring speed (rpm)	700rpm	900rpm	1100rpm

Table 3. Etodolac loaded Eudragit RS 100 microcapsules design of experimentation using I-Optimal RSM design

RUN	Independent Factors		Dependent Responses	
	X_1: Drug : Eudragit RS100 Ratio	X_2: Strring Speed	Y_1: Particle Size (µm)	Y_2: Encapsulation Efficiency (%)
1	0.167	700	200.2	94.02
2	0.167	900	163.1	91.55
3	0.2	900	28.71	96.05
4	0.25	1100	55.57	73.5
5	0.2	900	22.94	97.25
6	0.2	900	31.58	95.83
7	0.25	700	70.9	86.09
8	0.2	1100	43	75.49
9	0.167	700	198.12	93.47
10	0.25	700	70.69	85.19
11	0.2	900	34.71	94.5
12	0.167	900	162.81	89.32
13	0.25	900	66.32	83.1
14	0.2	700	51.2	91.04
15	0.25	1100	64.46	72.86
16	0.167	1100	89.04	78.42

Evaluation of microcapsules

Particle size analysis

All microcapsules were characterized for average particle size using laser diffraction particle size analyzer (Malvern Mastersizer 2000 SM, Malvern Instruments Corp.U.K.)

Drug Entrapment efficiency

The drug entrapment efficiency of prepared microcapsules was determined by centrifugation method. Prepared dispersion containing microcapsules was centrifuged at 5000rpm for 30 mins. Supernatant liquid containing free drug was collected and appropriately diluted with distilled water and absorbance was measured using UV spectrophotometer at 224.40 nm. Three replicates were prepared and average value was reported. The % drug entrapment efficiency was calculated by following formula.

% Drug Entrapment Efficiency = [Total drug (mg) - Free drug (mg)/Total weighed drug (mg)] ×100

Dissolution studies

In vitro drug dissolution study was performed by successively changing the dissolution media. For first two hours the dissolution was carried out in 900 ml 0.1 N HCl followed by replacing dissolution medium with 900 ml phosphate buffer pH 6.8 for next 20 hours. The in vitro drug dissolution was carried out in USP Apparatus I (basket) at stirring rate 100 rpm. The addition of 1% v/v Polysorbate 80 to the medium improved the dispersion and wettability of the microcapsules[7]. The samples of Etodolac microcapsules were added to dissolution medium and the samples were withdrawn at time intervals of 5, 15, 30 min, 1,2,4,8,12,14,18, 22 hours respectively. The volume of dissolution medium was adjusted to 900 ml by replacing it with fresh dissolution medium. The samples were immediately filtered through 0.45µm membrane filter, suitably diluted and analyzed spectrophotometrically at 223.6nm

Scanning electron microscopy (SEM)

The morphology of prepared microcapsules was evaluated through Scanning Electron Microscopy (SEM-Jeol Instruments, JSM-6360, Japan). Samples were mounted on a double-faced adhesive tape, sputtered with gold. Scanning electron photographs were taken at an accelerating voltage of 20 kV and obtained micrographs were examined at 1000 Xmagnification.

Differential Scanning Calorimetry (DSC)

The DSC thermograms of pure drug, Eudragit RS 100, PVA and optimized batch were recorded using a differential scanning calorimeter (DSC 823E, Mettler Toledo, Switzerland).

Approximately 2–5 mg of each sample was heated in a pierced aluminum pan from 35 to 435°C at a heating rate of 10 °C/min under a stream of nitrogen at a flow rate of 50 mL/min.

X-ray powder diffractometry (XRPD)

The powder XRD patterns of pure drug, Eudragit RS 100, PVA and optimized batch were recorded using Philips X-ray powder diffractometry ((PW 1729, Philips, The Netherlands*)* with Cu as anode material, operated at a voltage of 40 kV and a current of 30 mA. The samples were analyzed in the 2θ angle range of 5- 80^0.

Optimization of design space and validation of model

The optimization of design space was done by setting the criteria for the CQAs. Validation of the applied model was carried out by performing the 3 checkpoints formulations at different levels of variables within obtained design space and actual results were compared with predicted results. Linear regression line was drawn between actual and predicted values and percentage error was calculated.

RESULTS AND DISCUSSION

Etodolac loaded Eudragit RS 100 microcapsules were successfully prepared using solvent evaporation method with certain advantages, higher yield, better entrapment efficiency and simple method as compared to other microencapsulation methods. For optimization of microcapsule formulation, DoE was used in context to QbD since it ensures a predefined quality of the product. Based on prior knowledge and preliminary studies, the critical formulation variables (independent variables), the responses and design space were defined. *I*-Optimal RSM design was selected and '16' experimental runs were performed to see the effect of Etodolac: Eudragit RS 100 ratio (X1) and stirring speed (X2) on the critical quality attributes particle size of microcapsules (Y1) and entrapment efficiency (Y2) using 'Design expert' software (Version 9.0.1, State Ease. Inc. USA). Analysis of variance (ANOVA) was applied for testing the significance and validity of the postulated model, using a 1% significance level. ANOVA results shown in table 4 indicated that the assumed quadratic model was significant and valid for the examined responses. This means that the found relationship was able to describe the response variation in function of selected variables variations and thus leads to carefully describe the design space.

For selected statistical design, it is important that any experimental design has sufficient power to ensure that the conclusions drawn are meaningful. Power can be estimated by calculating the signal to noise ratio from fraction design space (FDS) graph (Figure 1). We evaluated *I* Optimal

RSM design for suitability to give fitted surface as precisely as possible by using FDS tool. 100% FDS indicates that design will provide a fitted response surface that is precise throughout the region of interest at 95% Tolerance Interval.

Table 4 ANOVA for CQAs of Etodolac loaded Eudragit RS 100 microcapsules

	Source	Sum of Squares	Df	Mean Square	F – Value	p-value Prob > F
Response(Y1)	Model	53300.86	5	10660.17	47.57	<0.0001
Particle size	X_1	18389.01	1	18389.01	82.07	<0.0001
	X_2	4110.90	1	4110.90	18.35	0.0016
	X_1X_2	2952.71	1	2952.71	13.18	0.0046
	X_1^2	22410.41	1	22410.41	100.01	<0.0001
	X_2^2	6.42	1	6.42	0.029	0.8690
	Residual	2240.72	10	224.07		
	Lack of Fit	2119.28	3	706.43	40.72	<0.0001
	Pure Error	121.44	7	17.35		
	Cor Total	55541.59	15			
Response(Y1)	Model	995.65	5	199.13	30.24	<0.0001
% EE	X_1	85.42	1	85.42	12.97	0.0048
	X_2	458.18	1	458.18	69.58	<0.0001
	X_1X_2	6.55	1	6.55	0.99	0.3421
	X_1^2	61.52	1	61.52	9.34	0.0121
	X_2^2	189.45	1	189.45	28.77	0.0003
	Residual	65.85	10	6.59		
	Lack of Fit	58.80	3	19.60	19.44	0.0009
	Pure Error	7.06	7	1.01		
	Cor Total	1061.50	15			

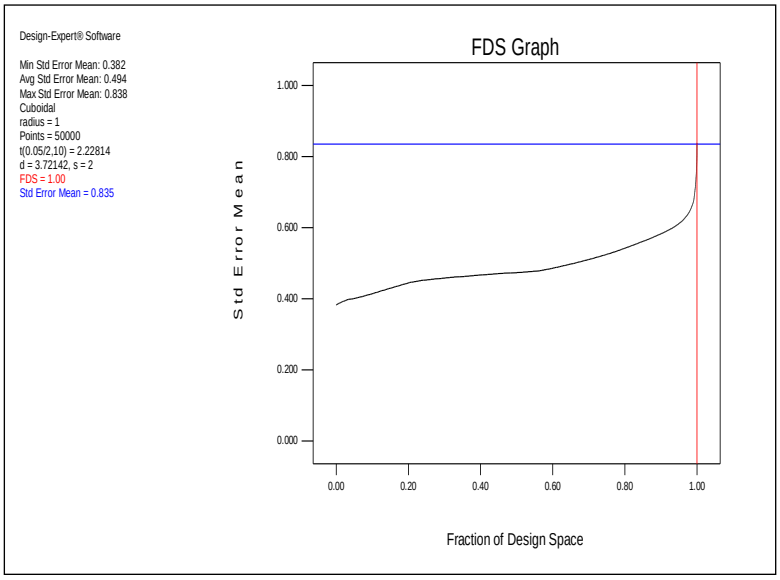


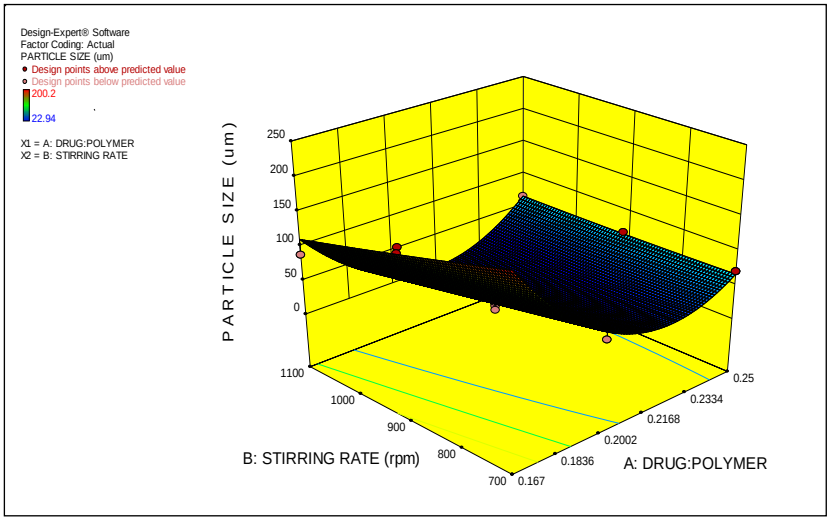
Figure 1. FDS plot from I Optimal RSM design of Etodolac loaded Eudragit RS 100 microcapsules

Table 5:Validation of design space from *I* Optimal RSM design.

Formulation Code	Composition (mg/tab)		Response	Predicted Value	Experimental Value	Standard Error
	X_1	X_2				
F 17	0.241	749	Y_1 - Entrapment efficiency	88.34	89.14	0.8
			Y_2 - Particle Size	46.83	45.55	1.28
F 18	0.210	707	Y_1 - Entrapment efficiency	92.32	91.03	1.29
			Y_2 - Particle Size	41	42.36	1.36
F 19	0.170	968	Y_1 - Entrapment efficiency	88.34	89.24	0.9
			Y_2 - Particle Size	121.99	119.19	2.8

Response Surface Response Surface analysis for CQAs

Particle size of Etodolac microcapsules was significantly affected by Etodolac/Eudragit RS 100 ratio and stirring speed which can be depicted from 'Prob > F' (< 0.05) value from ANOVA table 4. 3D surface plots were generated (Figure 2), in order to investigate more in depth the effects of Etodolac/Eudragit RS 100 ratio and stirring speed on the particle size. All batches showed higher particle size values, with decrease in Etodolac/Eudragit RS 100 ratio in Etodolac microcapsules and decrease in particle size with increase in stirring speed as response follow quadratic model. Entrapment efficiency was significantly affected by Etodolac/Eudragit RS 100 ratio and stirring speed (Prob > F is less than 0.05); entrapment was increased with decrease in Etodolac/Eudragit RS 100 ratio, this might be due to higher ratio of Etodolac/Eudragit RS 100 leads to more availability of polymer to encapsulate the drug resulting into good entrapment upto ratio 0.2 but above which entrapment gradually decreases due to very high polymer concentration and thus formation of placebo microcapsules. Stirring speed showed the same pattern as that of particle size, entrapment decreased with increasing stirring speed from low level to high level. This negative effect of stirring speed on EE was attributed due to attrition of microcapsules with other particles, propeller shaft and wall of container upto certain extent and due to reduction of particle size. This is one of the critical issues which need to focus prominently at scale up level and optimization of this will lead to robust and controlled formulation with respect to entrapment efficiency and drug release.



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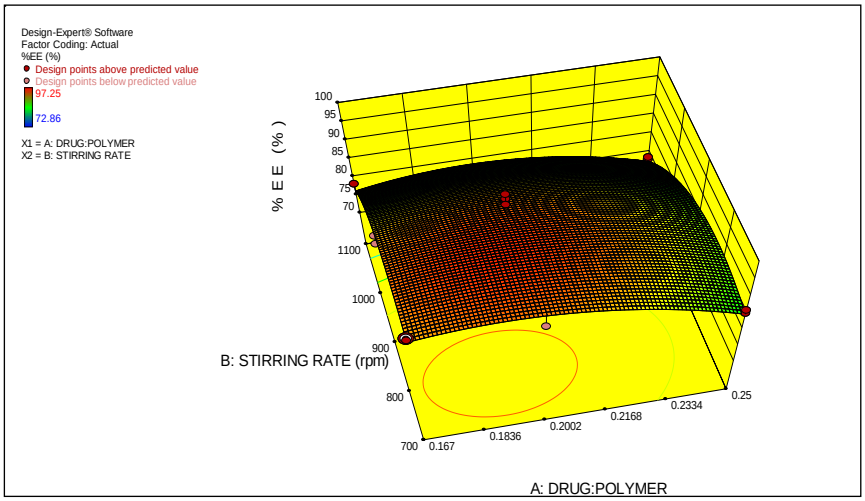


(b)

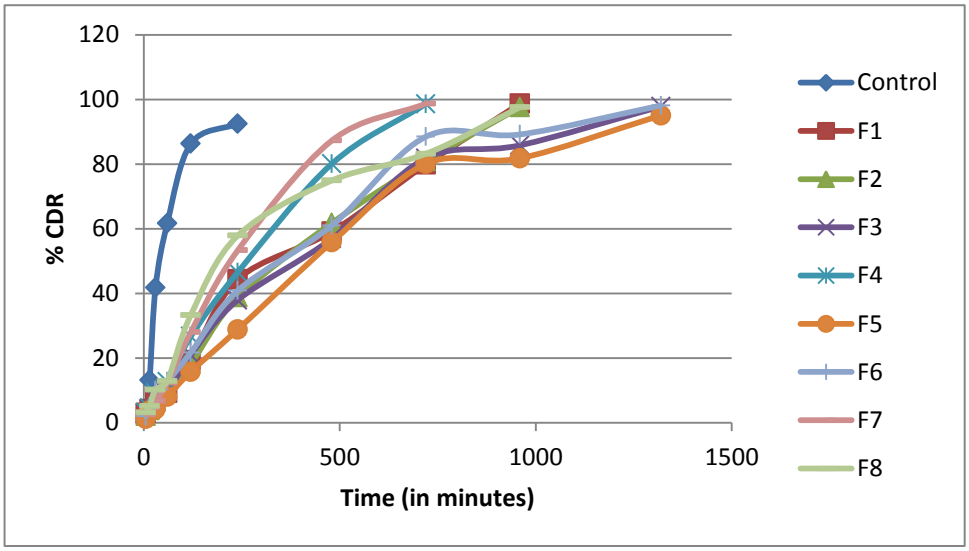
Figure 2. 3D Surface plot showing effect of Etodolac/Eudragit RS 100 ratio and stirring speed on a) particle size, b) entrapment efficiency of Etodolac loaded Eudragit RS 100 microcapsules.

In Vitro Drug Release

The release of etodolac from the microcapsules depends on the polymer concentration and the type of polymer used[5,8]. At high drug : Eudragit RS 100 ratio, low Eudragit RS 100 availability might have resulted into reduction in particle size of microcapsules leading to higher drug release due to increase in surface area. Also low Eudragit RS 100 availability might have resulted in increase in amount of drug present close to the surface further leading to higher drug release. Whereas decrease in release rate with increasing content of Eudragit RS 100 (low drug : Eudragit RS 100 ratio) can be explained by a decreased amount of drug present close to the surface and

also by the fact that the amount of uncoated drug decrease with increase in polymer concentration. When optimum ratio of drug : Eudragit RS 100 ratio was taken, it could fabricate microcapsules with desired particle size and entrapment with sustained *in-vitro* drug release. This might be due to formation of uniform coating of Eudragit RS 100 which resulted into increase in coat thickness surrounding the drug particles thereby increasing the distance traveled by the drug throughout coat.

The dissolution studies of Etodolac microcapsules were performed in pH 6.8 buffer by using USP-I basket method. The drug release from all formulations was found to be sustained as compared to pure drug. It was found that the formulation F5 showed better sustained release profile for about 22 hours.



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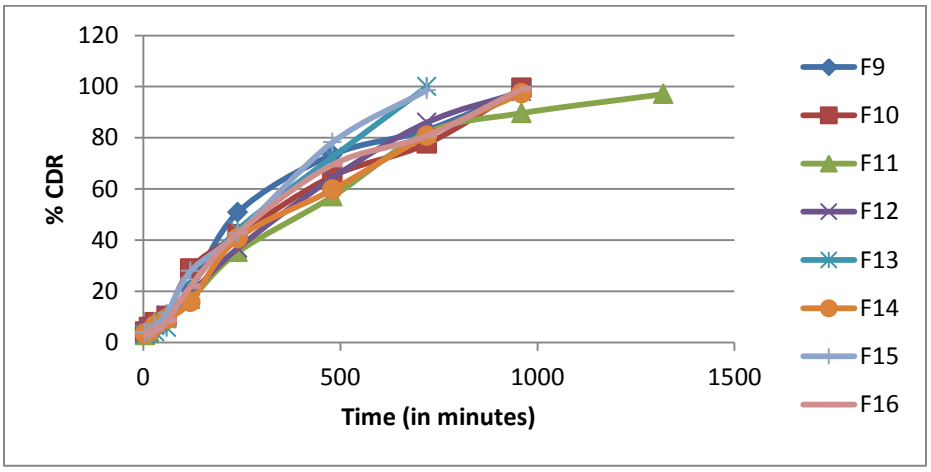

(b)

Figure 3.Percentage Cumulative Drug Release from Etodolac microcapsules in Buffer pH 6.8

Scanning Electron Microscopy

Figure 4 shows the scanning electron microscopic picture of optimized batch F5. The images showed that Etodolac loaded Eudragit RS 100 microcapsules were regularly spherical in shape. The surface of microcapsules showed large number of small pores. It might be due to mutual repulsion of cationic groups present in Eudragit RS 100[9]. The size results from SEM analysis were in agreement with those by laser size analyzer in the present study.

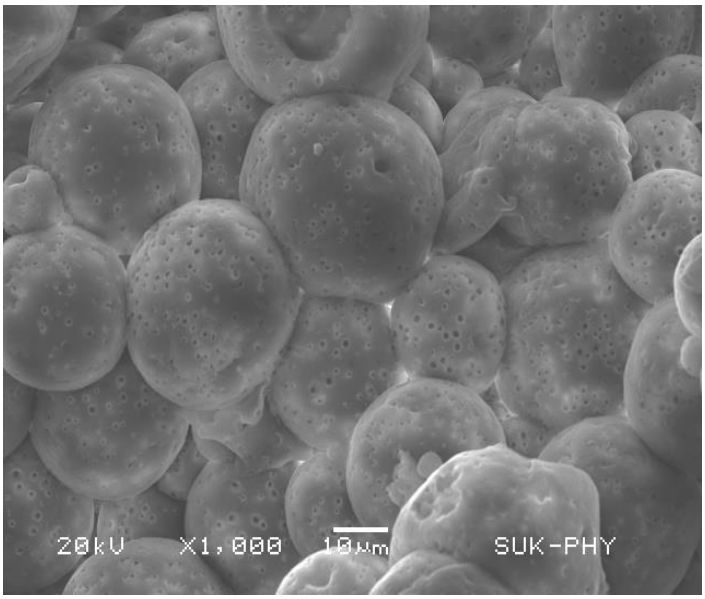

Figure 4. SEM Photomicrograph of Etodolac loaded Eudragit RS 100 microcapsules

Differential scanning calorimetry

DSC thermograms of Etodolac, Eudragit RS 100 and Etodolac loaded Eudragit RS 100 microcapsules are shown in fig 5. DSC thermogram of pure drug showed sharp endothermic peak at its melting point at 145.53^{0}C revealing the crystalline nature. Eudragit RS 100 thermogram exhibited a glass transition peak at 64.61^{0}C and was observed, as Eudragit RS 100 appears amorphous in nature. Physical mixture of Etodolac and Eudragit RS 100 showed peak at melting point temperature but with less intensity might be due to physical masking of peak as higher drug: polymer ratio (0.2), whereas Etodolac loaded Eudragit RS 100 microcapsules exhibit peak with change in intensity but no significant shifting of the position of peak. This indicates that the drug is only physically entrapped in the polymer matrix and there is no interaction between drug and polymers.

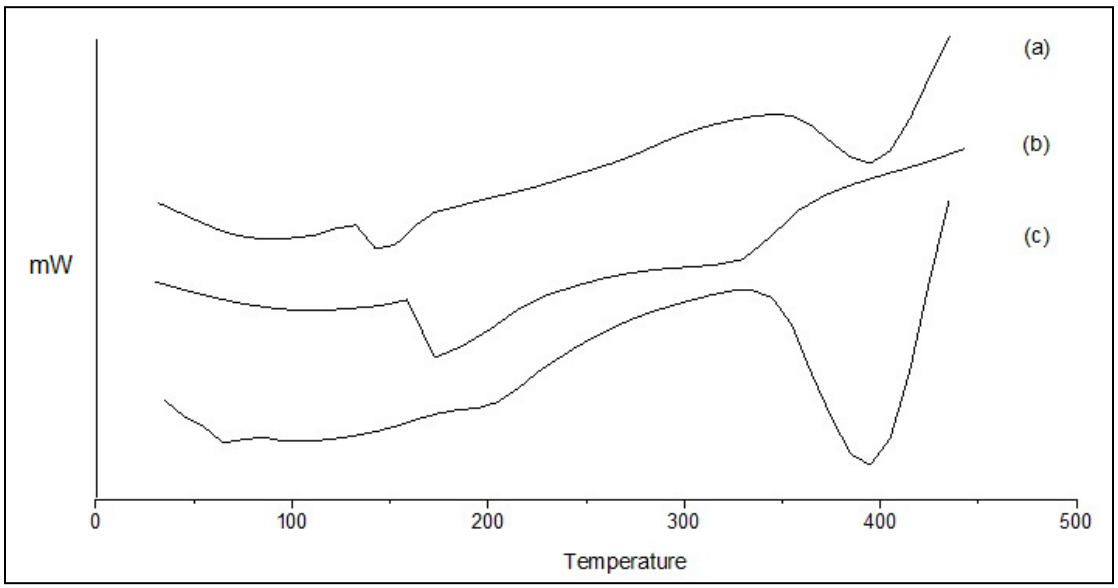

Figure 5. DSC thermogram for (a) Etodolac loaded Eudragit RS 100 microcapsules, (b) Etodolac and (c) Eudragit RS 100

X-ray diffraction pattern for Etodolac loaded Eudragit RS 100 microcapsules.

X-ray diffraction pattern for Etodolac, Eudragit RS 100, PVA physical mixture and Etodolac loaded Eudragit RS 100 microcapsules are shown in fig 6.27. XRD of Etodolac at 2θ showed crystalline nature with sharp peaks at 9.34^0, 14.49^0, 22^0 and 18.82^0. Eudragit RS 100 and PVA exhibit amorphous nature which shows absence of sharp peaks whereas microcapsules formulation shows peaks at almost same degree but with less intensity as compared to pure drug, which might be due to physical coating over drug or matrix formation with polymer or drug in embedded form inside the microcapsules. This indicates that there was no any interaction of drug and polymer in Etodolac loaded Eudragit RS 100 microcapsules.

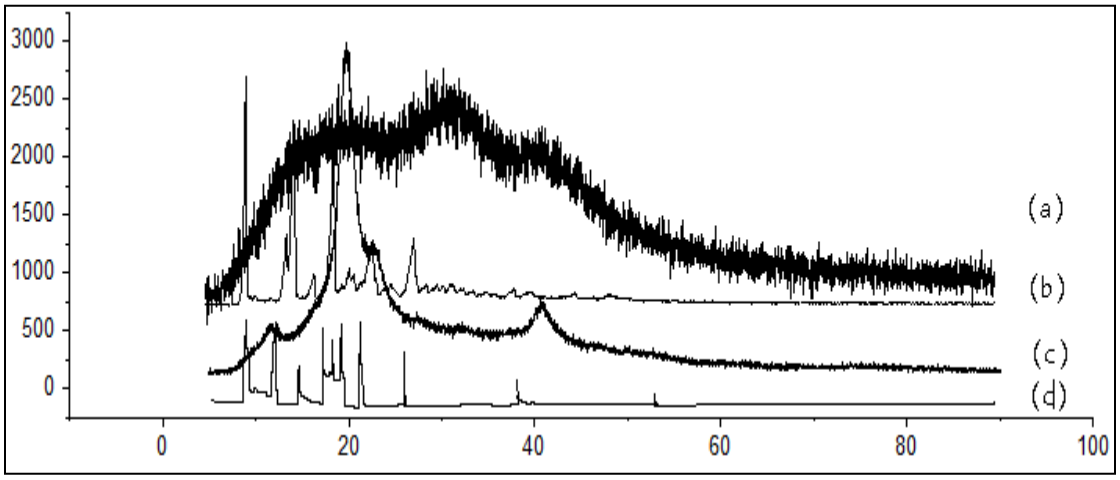

Figure 6. X-ray diffraction pattern for (a) Etodolac,(b) Eudragit RS 100, (c) PVA,(d) Etodolac loaded Eudragit RS 100 microcapsules.

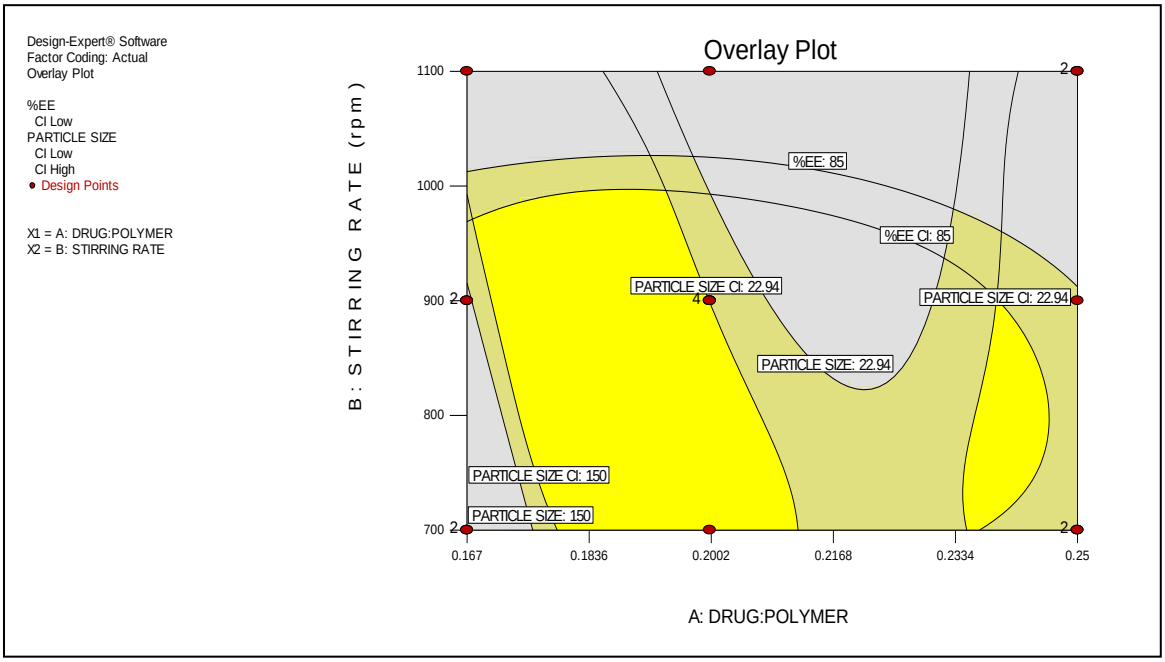

Figure 7 Functional design space for Etodolac loaded Eudragit RS 100 microcapsules framed with tolerance intervals

Optimization of design space and validation of model

The design space was generated from design of experimentation (DoE) and multivariate analysis (superimposing contour plots of particle size and entrapment efficiency) performed to ensure desired particle size (22-200 um) and entrapment efficiency (72.86-97.25 %) for Etodolac loaded Eudragit RS 100 microcapsules.

Design space shown in figure 7 characterizes acceptable ranges of Drug: Eudragit RS 100 ratio and stirring speed which provides assurance that CQAs will be within acceptable criteria, which helps to provide an "assurance of quality." Robustness can be assured for Etodolac loaded Eudragit RS 100 microcapsules formulation if operated in the range of design space.

Validation of the applied model was carried out by preparing the 3 formulations with combinations of independent variables within obtained design space and prepared microcapsules were evaluated for particle size and entrapment efficiency and obtained results were compared with predicted results.

CONCLUSION

Formulation and optimization of Etodolac loaded Eudragit RS 100 microcapsules was performed successfully by solvent evaporation method using Quality by Design approach. Optimized formulations showed better entrapment efficiency and sustain drug release for period of 22 hours. Based on initial risk assessment, Drug: Eudragit RS 100 ratio and stirring speed were

taken for DoE and *I*-Optimal design was selected to see the effect on CQAs; particle size, entrapment efficiency and drug release characteristics. Morphological studies of the optimized batch showed that the Etodolac microcapsules were spherical. The release kinetics from Etodolac microcapsules exhibited that both swelling and diffusion play an important role in the release. The application of RSM design demonstrates a useful tool for optimization of Etodolac microcapsule. The results of multiple regression analysis led to a statistical model that described adequately the influence of the selected variables at different levels on the chosen response and thereby, minimize the number of experimental trials and reduce the formulation development cost. Thus, it can be concluded that Etodolac microcapsules are capable of providing sustain drug delivery after oral administration.

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