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FORMULATION AND DEVELOPMENT OF LULICONAZOLE MICROSPONGES FOR TOPICAL DELIVERY SYSTEM USING QbD APPROACH

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ABSTRACT

The present study deals with the design and optimization of Luliconazole microsponges loaded topical drug delivery system to facilitate the controlled release of active drug into the skin in order to reduce the systemic exposure and minimize local cutaneous reactions to active drugs by DoE method of QbD Approach. Luliconazole Microsponges were prepared by quasi emulsion solvent diffusion method using Eudragit-RS100 as a polymer, polyvinyl alcohol, Ethanol as Internal phase volume and Liquid paraffin as External phase volume. For the development of microsponges, Quality by Design approach was implemented. Based on risk assessment of critical quality attributes (CQAs), Optimization of Luliconazole loaded Microsponge was doneby Application of 2^4 Factorial Designs. Independent variable of formulation was Drug Concentration (X1), PVA Concentration(X2), Internal Phase Volume (X3) and Speed of Stirring (X4). The selected dependent variables were % Drug Content (Y1), Particle size (Y2), %CDR (Y3). The optimized batch of Luliconazole loaded Microsponge was evaluated by particle size, surface morphology and topography by SEM, Drug-Excipients compatibility study using FTIR Spectrum and further loaded into subsequent topical gel. For the optimized formulation, microsponges size was ranging from 8-50 µm and encapsulation efficiency was 80-90 %. The *in vitro* drug release from the microsponges was found to be extended up to 8 Hr. The scanning electron micrographs of microsponges revealed perfect spherical shape of microsponges as well as encapsulation of drug. FT-IR patterns of microsponges had shown compatibility with polymers. The values of micromeritics properties indicated good flow properties of these microsponges. The formulations Optimized Luliconazole Microsponges (LZLERMS2) loaded Gel was subjected to stability studies for 1 month and it was found that there was no significant change in their physical appearance and drug content after period of stability testing. Finally we may conclude that Optimized Luliconazole Microsponges (LZLERMS2) loaded Gel were best formulation of Eudragit RS 100 as a retarding polymer. It was demonstrated that the use of Quality by Design (QbD) principles, provide an effective means to achieve a greater understanding of process and formulation parameters for microsphere preparation. From the study it can be concluded that it is possible to design a topical polymeric microsponges formulation for anti-fungal drug Luliconazole, mainly for the treatment of Tinea Corporis and related where efficacy and patient compliance are of prime importance.

KEYWORDS: Microsponge, Quality by Design, Design of Experiment, Luliconazole, Microspongic gel.

INTRODUCTION

MS can be effectively absorbed into the TDS which may hold measurements structure on skinand has been utilized as oral conveyance utilizing bioerodible polymers especially for colonexact conveyance

which may improve tolerant lack of involvement because of its siteexplicitness and expanding dose interludes.[1] MS are characterized as permeable, inactive unitswhich is comprised of manufactured polymers and go about as a shield to the captured sedatefrom debasement which can be effortlessly ensnared as creams, salves, and powders. If there should arise an occurrence of Cosmetics and dermatological items, work just at out sided of skin.The dynamic part in traditional promoted measurement structure may surviving in a decentlyhigh focus and retained quickly on application upon skin. MDS may proposed to allow anadjusted pace of medication arrival of, along these lines presenting imminent decreasing in thesymptoms and keep up the restorative impact.[2] The polymers which have been utilized to getready MS are Ethyl cellulose, Eudragit RS 100, and so on which can shape 'confine' likestructure. Once in a while plasticizers might be utilized to balance out structure of MS.[3] Luliconazole is a novel azole antifungal medication having wide range antifungal movement. It iscompelling against dermatophytes, both in vitro and in vivo like Trichophyton spp., C. albicans,and A. fumigatus. It has a place with BCS class-II medicate for example low dissolvability andhigh penetrability, having Log P esteem 4.27 and atomic weight 354 g/mol which recommend itis appropriate for Topical DDS. It has exceptionally low fluid solvency constraining its dermalaccessibility and going about as boundary for topical conveyance. Additionally, dissolvability ofthe medication in lipid layer of layer corneum relates to rate restricting advance for pervasion. Soas to accomplish successful treatment for different contagious contaminations through topicalcourse, it is vital that it ought to be restricted in dermal and epidermal layers of the skin.Customary and advertised details have low skin pervasion and shorter maintenance of medicationon skin surface.

MS based topical medication conveyance framework to benefit from proportion of time which presents dynamic fixings at any rate portion, diminish symptoms by conveying drug at aroused site, adjust tranquilize discharge profiles and warehouse sedate inside the epidermis, however lessening transdermal infiltration into flowing framework. Consequently, they can be utilized as vehicles to convey different medications to the skin. Likewise MS based topical DDS has a high patient adequacy since they have the benefits of both miniaturized scale size ran molecule and gels.

MATERIALS AND METHODOLOGY

Method of Preparation of Luliconazole MS

The MS was prepared by Quasi-Emulsion Solvent Diffusion Method:

This is a two step process where the MS can be prepared by quasi emulsion solvent diffusion method using the different polymer amount.

To prepare the inner phase, the polymer was dissolved in a suitable solvent (Ethanol). Then, the drug can be added to the solution and dissolved. The inner phase was poured into outer phase containing PVA and Liquid paraffin as emulsifying agent.

Following 60 min of stirring, the mixture is filtered to separate the MS.

The MS are dried in an air-heated oven at 40 ° C and weighed & determine the % productionyield (% PY).[4]

FORMULATION AND DEVELOPMENT OF LULICONAZOLE MICROSPONGES FOR TOPICAL DELIVERY SYSTEM USING QbD APPROACH

STEP-I: Quality Target Product Profile (QTPP)

STEP-II: Risk Assessment of Critical Quality Attributes from Preliminary trial Batches to Develop QbD Approach

Risk assesement was done to select formulation and process variable which affect product quality for CQAs by process characterization that defines satisfactory changes in material and process parameters. Finally, this result in quality assurance by Process Design Space to understand and develop control strategy. The critical quality attributes are categorized into high, medium andlow risk parameters based on knowledge space. Usually high risk parameters are considered important for Design of Experiments as they are having more effect than others and need to be in accepting multivariate ranges.[5]

STEP-III: Formulation and Development of Luliconazole MS by Design of Experiment(DoE) Using QbD Approach

A design space had signify formulation and process understanding viz. attributes which are related to drug substance, materials, equipment, IP and finished product quality.[92] For this purpose, risk assessment was done based on the understanding process and formulation related parameters on MS quality. Preliminary studies and later Design of Experimentation (DoE) was carried out for high risk parameters. Based on effect of critical quality attributes of target product profile, we had propose design space for obtaining robust formulation. Characterization of MSwas done for various parameters viz. Particle size analysis, shape, micromeritics properties, encapsulation efficiency, percentage yield, *in vitro* drug releases shape and surface topography (SEM).

Characterization of Luliconazole MS [6, 7 ,8]

Percentage Yield

It was calculated by following formula.

% Yield = (Weight of MS obtained practically / Total weight of Drug + Polymertheoretically) X 100

Drug Content:

Weight accurate amount of 25 mg of MS and mix in 25 mL methanol with shaking filter this solution using whattman filter paper and withdraw 1 mL from this solution to volumetric flask with 10 ml dilution in volumetric flask. The quantitative determination of Luliconazole in MSwas carried out using a linear model UV absorbance detector at 296 nm against blank (methanol).

Entrapment Efficiency

It can be calculated by following formula.

$$\text{Drug Encapsulation efficiency} = \frac{\text{Actual Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

Mean Particle Size Analysis:

Particle size analysis of drug and MS was done using Optical Microscope and Malvern Instrument.

***In Vitro* Drug Release Study of MS**

The dissolution test was done in 900 mL Phosphate buffer (PH 7.4) at the 37±.5 °C, 150 RPM in USP-II Type dissolution apparatus. Aliquous was withdrawan every hour upto 8 hrs and replaced immediately with fresh solvent. The sample was estimated by absorbance of the solution at λmax 296 nm using UV- Visible spectrophotometer. And calculate % CDR.

Kinetics of Drug Release [8, 9]

The kinetic release study was performed to find drug release mechanism from dissolutionparameter by using different various kinetic model equations.

Zero Order Release KineticsQt = Q0 + K0t

Where,

Qt = amount of the drug dissolved in time t,

Q0 = initial amount of drug in the solution (most of the times, Q0 = 0) andK0 = zero order release constant expressed in units of concentration/time. **Plot:** Cumulative amount of drug remaining vs time.

First Order Kinetics

Log C = Log C0 - Kt / 2.303

Where,

C0 = initial concentration of drug,

K = first order rate constant, andt = time.

Plot: log cumulative percentage of drug remaining vs. time.

Higuchi Model [9]

Q = KH × t1/2

Where,

KH = Higuchi dissolution constant.

Plot: cumulative percentage drug release vs Square root of time.

Hixson-Crowell Model [10]

$$W_O^{1/3} - W_t^{1/3} = \kappa t$$

Where,

W_0 = initial amount of drug in the pharmaceutical dosage form, **Plot:** cube root of drug percentage remaining in matrix vs time. **Korsmeyer- Peppas Model [11]**

$$M_t / M_\infty = k t^n$$

Where,

Mt / M∞ = fraction of drug released at time t,k = release rate constant and

n = release exponent.

Plot: log cumulative percentage drug release vs log time.

STEP-IV: Validation Analysis (Predicted vs Observed Batches)

Validation Analysis will be done by Over Lay plot generated by **Stat-Ease** version 11.0.2.0 and batches were taken as suggested by software and responses (Obsereved batches) recorded which was compared with predicted Batches.

Preparation and Characterization of Luliconazole MS loaded Topical gel Method of Preparation

Luliconazole MS loaded Topical Gel: [7]

Weigh accurately Carbopol 934-P and liquefied in 100 mL of water for 2 hours soaking with 600 RPM agitation then penetration enhancer was added to the formulated gel which may prevent drying of gel. To this aqueous solution of Triethanolamine was added with slow agitation with continuous stirring. The Luliconazole Loaded MS was add in the gel.

Preliminary Trial batches of Topical Gel

Preliminary trials was undertaken to develop Luliconazole loaded MS gel. The various concentrations of Carbopol 934 was taken.

Characterization of Topical Gel: [12, 13, 14]

Physical evaluation:

It was done to evaluate organoleptic property, Occlusiveness and wash ability of gel.

Measurement of pH of gel:

The pH was check by a digital pH meter of formulated gel.

Viscosity study of gel:

50 gm of prepared gel was kept in 50 mL suitable beaker and spindle Groove was dipped at specific RPM in Brookfield Viscometer. This was done three times and recorded observation was considered as mean of viscosity.

Spreadability of gel:

An accurately weighed quantity of 1 g of gel was pushed among two slides and left as such for about 5 minutes. Diameters of speed circles was measure in cm and was taken as comparative values for Spreadability when no further spreading. The readings attained are mean of three determinations.

Homogeneity and grittiness

The consistency of prepared gel was determined by pressing between the thumb and the index finger. Minor quantity gel is wiped on skin of back of hand to check the homogeneity and grittiness.

Drug content:

1 gm of each gel formulation was dissolved in 20 mL of alcohol in volumetric flask with 30 min stirring. Finally, it was dilutde and filtered. Further dilution was made up to 10 mL alcohol and again 1 mL was withdrawn from above and diluted to 10 mL alcohol. The absorbance was measured at 296 nm in uv.

Antifungal activity:

Weighed 16.25 gm of sabouraud dextrose agar was transfered in a 500 ml of conical flask and 250 ml of purified water and some amount of heat is applied to dissolve it completely. Sterilized for 15 min at 121°C

at 15 lb pressure in autoclave for about 20 min. Then cooled it at room temperature and the fungal strain(*Candida albicans*) was dispersed in the medium and then the medium was poured it in to the three petridish and allowed it cool it for sometime at room temperature untill it forms solidifies at room temperature and then the three cups are bored in each petridish with the help of sterile steel bore of 6 mm and calculated concentration of the standard drug (Luliconazole), gel formulation(LZLERMS2), Marketed Luliconazole gel and placebo gel were placed in the bores and incubated the petri plates for 72 h at 37°C in incubators. Then the zone of inhibition was observed and calculated the radius of the zone of inhibition.[15]

Accelerated stability studies of Microspongic Gel

The drug or dosage form quality may affect under impact of by varying temperature, humidity and light with time which was found out by stability testing. It had been carried out at Room Temperature for the selected formulation for one month. Samples was withdrawn on 0th, 15th and 30th day and was analyzed for physical appearance and drug content.

Results and Discussion

STEP- I: QTPP of LZL MicrospongesQTPP Y_1: % Drug Content

QTPP Y_2: % Entrapment EfficiencyQTPP Y_3: Particle size

QTPP Y_4: %Cumulative Drug Release

STEP-II: Risk Assessment of Critical Quality Attributes from Preliminary trial Batches to Develop QbD Approach

The critical quality attributes are categorized in high, medium and low risk parameters based on knowledge space to check influence of formulation and process parameters. Usually high-risk parameters are considered important for Design of Experiments as they are having more effect than others and need to be in accepted multivariate ranges. The Critical parameters and critical quality attributes (CQAs) for selection of optimum formulation are shown in table 1.

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

Drug Product CQAs	Drug: Polymer Ratio	Volume Of Internal Phase	% Stabilizer Concentration	Agitation Speed
Drug Content	High	Low	Medium	Medium
Entrapment Efficiency	High	Low	High	Medium
Particle Size	High	Medium	High	High
Drug Release	High	Low	Medium	Medium

Selection of Formulation and Process Variables of Preliminary Trial Batches of Luliconazole MS

Table 2: Formulation Design of Trial batches for Luliconazole MS

Batch	Drug: Polymer Ratio	Type of Internal phase	Volume of Internal Phase (mL)	Volume of Liquid Paraffin (mL)	Surfactant Conc. (%)	Stirring Speed (R.P.M.)	Stirring Time (Mins)
PRELIMINARY SELECTION OF CONCENTRATION OF RETARDENT MATERIALS							
TLM1	**1:1**	Ethanol	10	50	0.75	1500	60
TLM2	**1:2**	Ethanol	10	50	0.75	1500	60
TLM3	**1:3**	Ethanol	10	50	0.75	1500	60
PRELIMINARY SELECTION OF DRUG:POLYMER RATIO							
TLM4	**7:1**	Ethanol	10	50	0.75	1500	60
TLM5	**9:1**	Ethanol	10	50	0.75	1500	60
TLM6	**11:1**	Ethanol	10	50	0.75	1500	60
TLM7	**13:1**	Ethanol	10	50	0.75	1500	60
TLM8	**15:1**	Ethanol	10	50	0.75	1500	60
PRELIMINARY SELECTION OF POLYMER							
TLM9	11:1	**Ethanol**	10	50	0.75	2000	60
TLM10	11:1	**Ethanol**	10	50	0.75	2000	60
PRELIMINARY SELECTION OF INTERNAL PHASE VOLUME							
TLM11	11:1	Ethanol	**5**	50	0.75	1500	60
TLM12	11:1	Ethanol	**10**	50	0.75	1500	60
TLM13	11:1	Ethanol	**15**	50	0.75	1500	60
PRELIMINARY SELECTION OF SURFACTANT CONCENTRATION							
TLM14	11:1	Ethanol	10	50	0.5	1500	60
TLM15	11:1	Ethanol	10	50	0.75	1500	60
TLM16	11:1	Ethanol	10	50	1	1500	60
PRELIMINARY SELECTION OF STIRRING SPEED							
TFM17	11:1	Ethanol	10	50	0.75	1500	60
TFM18	11:1	Ethanol	10	50	0.75	2000	60
TFM19	11:1	Ethanol	10	50	0.75	2500	60

A Microsponge Delivery System (MDS) is patented, highly cross-linked, porous, polymeric microspheres polymeric systems consisting of porous microspheres that can entrap wide range of actives and then release them onto the skin over a time and in response to trigger. It is a unique technology for the controlled release of topical agents. Luliconazole Microsponge was preparedby quasi emulsion diffusion method.

Selection of Concentration of Retardant Material (Polymer) in Internal Phase

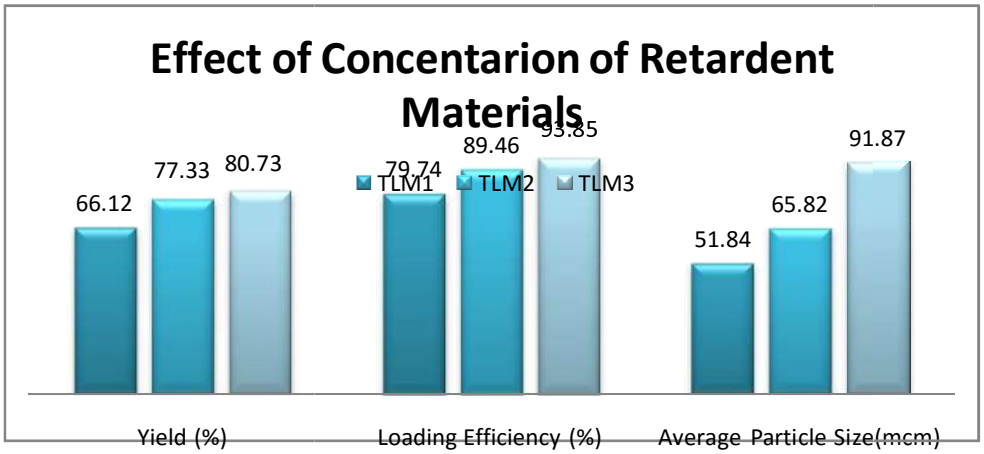


Figure 1: Effect of Concentration of Retardant Materials on Batches TLM1 – TLM3 Effect Concentration of Retardant Material in the Internal Phase:

The minimum concentration had found to be 1:1 of drug: polymer ration because at this concentration, the MS showed good physical characteristic like proper shape, size, porosity,

particle size distribution and did not collapse even after removal from the solvent and subsequentdrying. Hence, TLM1 has been selected as optimized batch.

Selection of Drug: Polymer Ratio

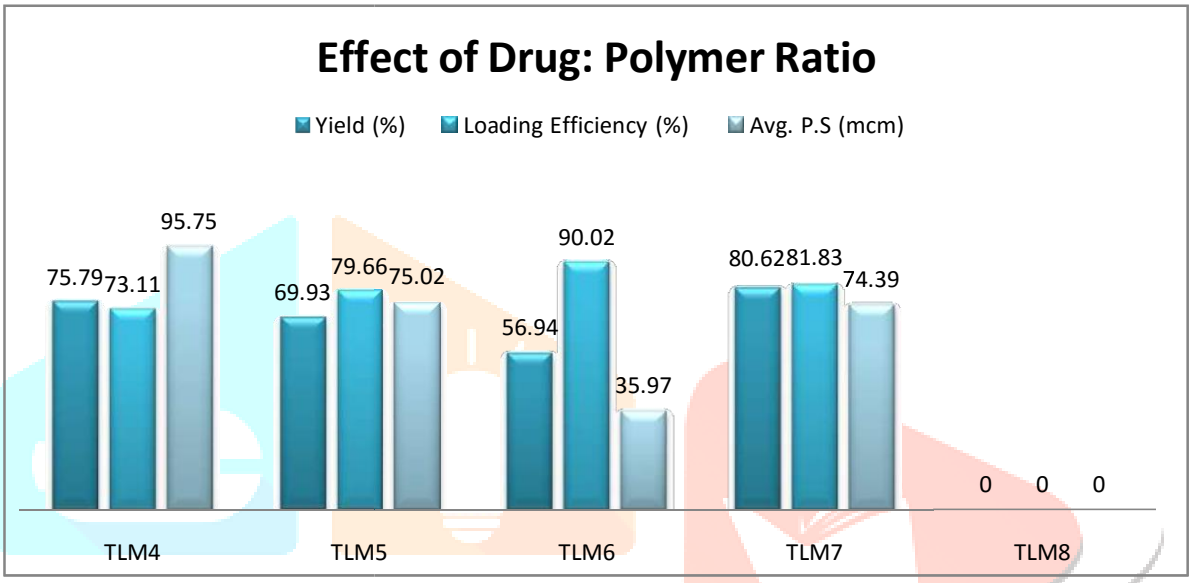


Figure 2: Effect of Drug: Polymer Ratio on Batches TLM4 - TLM8

Effect of Drug: Polymer Ratio:

The drug to polymer ratio in the internal phase had some effect on the particle size. The mean particle size decreases when the drug to polymer ratio was increased. The encapsulation efficiency and % yield gradually improved with an increase in Drug: Polymer ratio while mean particle size decreased, and the particle size distribution became narrower.

Selection of Retardant Materials

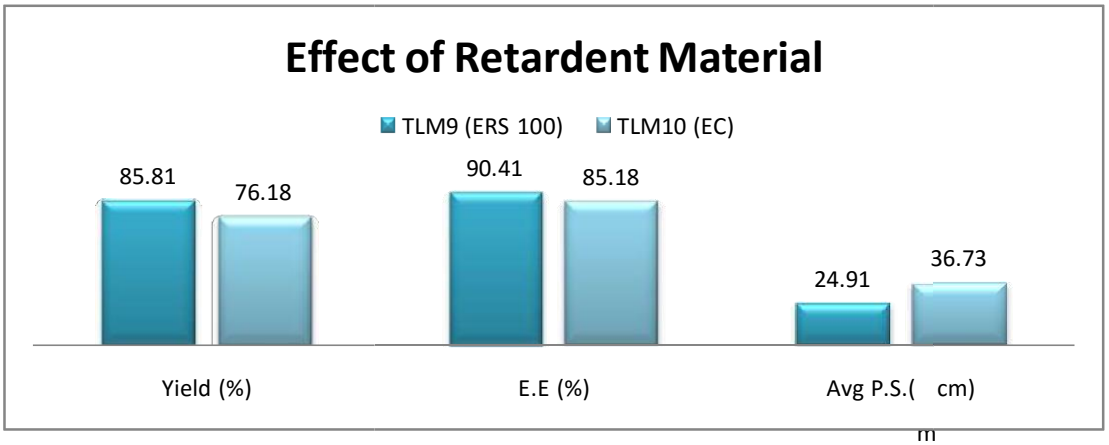


Figure 3: Effect of Retardant Material on Batches TLM9 – TLM10

Effect of Retardent Material:

It was examined on the formulation with 11: 1 drug: polymer ratio of Eudragit RS 100 atretardant material at stirring speed of 2000 RPM and at fixed duration of stirring. It was foundthat type of retardant material also played a crucial role in the formation of MS with reduced free drug content and particle size ranged from 24.91µm to 36.73µm with different type of retardant materials. Hence, TAM9 has been selected as optimized batch.

Selection of Internal Phase Volume

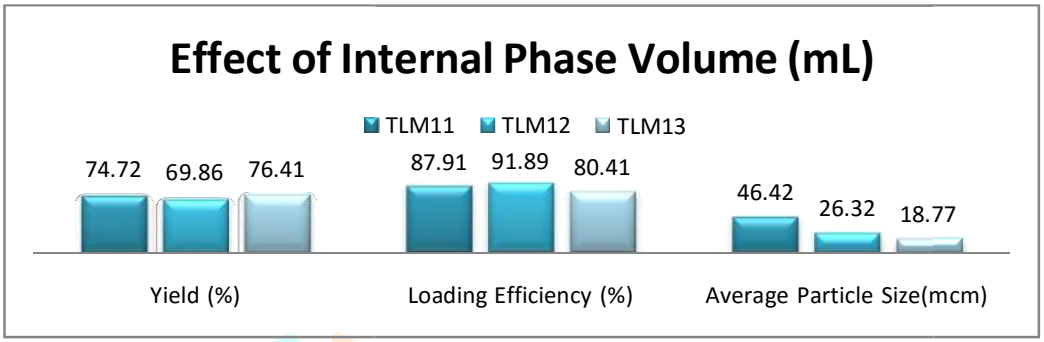


Figure 4: Effect of Internal Phase Volume TLM11 – TLM13

Effect of Internal Phase Volume.:

When the amount of ethanol was gradually increasing, % E.E. and drug content decreased. This was probably due to the lower concentration of the drug in the high volume of ethanol. It was observed that by reducing ethanol volume, the P.S. of prepared MS increases.

Selection of Surfactant Concentration

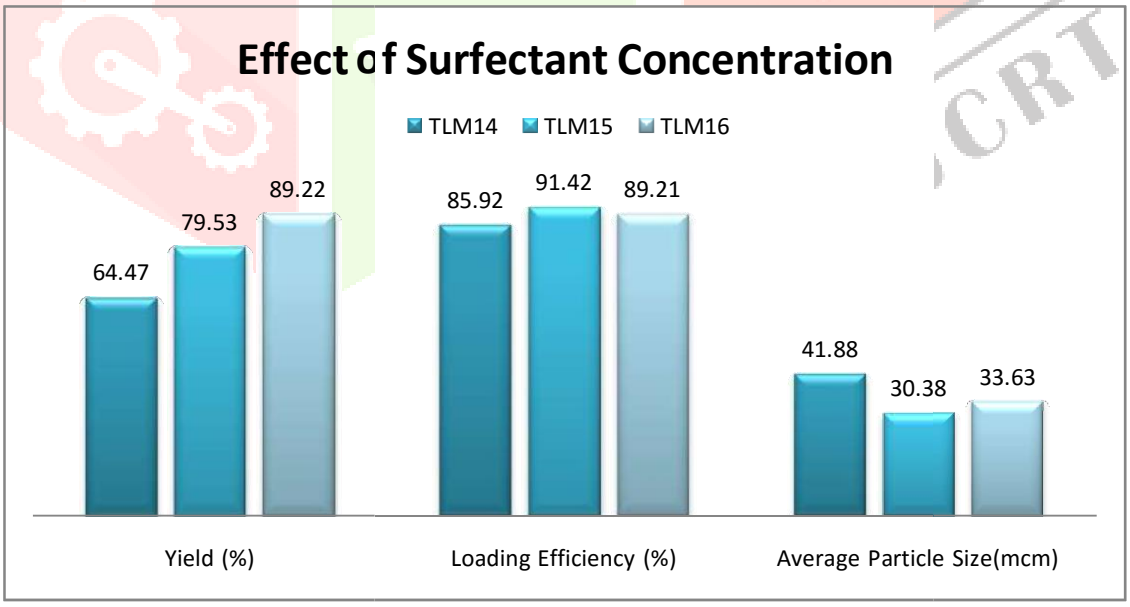


Figure 5: Effect of Surfactant Concentration on Batches TLM14 – TLM16 Effect of Surfactant Concentration:

MS did not form in the absence of surfactant. Though it was found that the particle size increased with the increase in the sur actant concentration attributed to an increase in the viscosity at increased emulsifier concentrations, high amounts of surfactant resulted in foaming.

Selection of Stirring Speed

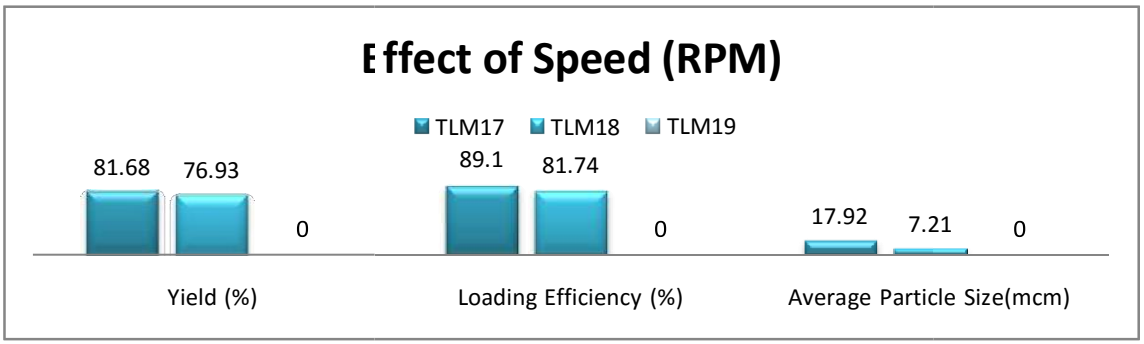


Figure 6: Effect of Stirring Speed on Batches TLM17 – TLM19

Effect of Stirring Speed:

It was observed that increasing the stirring speed from 1500, 2000 and 2500 RPM decreased the % Entrapment from 89.10% to 81.74% due to the turbulence produced within external phase.Hence, TLM17 and TLM18 have been selected as optimized batches.

STEP-III: Formulation and Development of Luliconazole – Eudragit-RS100 MS by usingQbD Approach

Various batches of Luliconazole MS by DoE Using QbD approach were prepared according to2^4factorial designs which are as follow:

Table 3: 2^4 Factorial Design

Independent Variables of Formulations		
Independent Variables	**Low(-)**	**High(+)**
Drug Concentration (X_1)	11:1	13:1
PVA (X_2)	0.75%	1%
Internal Phase volume (X_3)	10	15
Speed (RPM) (X_4)	1500	2000
Dependent Variables		
Y1 = % Drug Content		
Y2 = % Entrapment efficiency		
Y3 = Particle Size		
Y4 =% CDR		

Compositions of Factorial Batches in Coded Form

Various batches of Luliconazole MS with Eudragit RS 100 were prepared according to 2^4factorial designs which are as follow:

Table 4: Compositions of Factorial Batches in Coded Form

LZL- EC MS 2^4 =16 Batches				
Batch No	**Variable level in coded form**			
	Drug Con.-mg (X1)	**Int. Phase Vol.-mL(X2)**	**PVA Con.-mg (X3)**	**Speed-RPM(X4)**
LZLERSM1	-1	-1	+1	+1
LZLERSM2	-1	-1	-1	+1

LZLERSM3	-1	-1	-1	-1
LZLERSM4	+1	-1	+1	+1
LZLERSM5	-1	+1	-1	-1
LZLERSM6	-1	+1	+1	+1
LZLERSM7	+1	+1	-1	-1

LZLERSM8	+1	+1	+1	+1
LZLERSM9	-1	-1	+1	-1
LZLERSM10	+1	-1	-1	+1
LZLERSM11	+1	+1	+1	-1
LZLERSM12	-1	+1	+1	-1
LZLERSM13	-1	+1	-1	+1
LZLERSM14	+1	-1	-1	-1
LZLERSM15	+1	+1	-1	+1
LZLERSM16	+1	-1	+1	-1

Characterization of Batches LZLERSM1- LZLERSM16

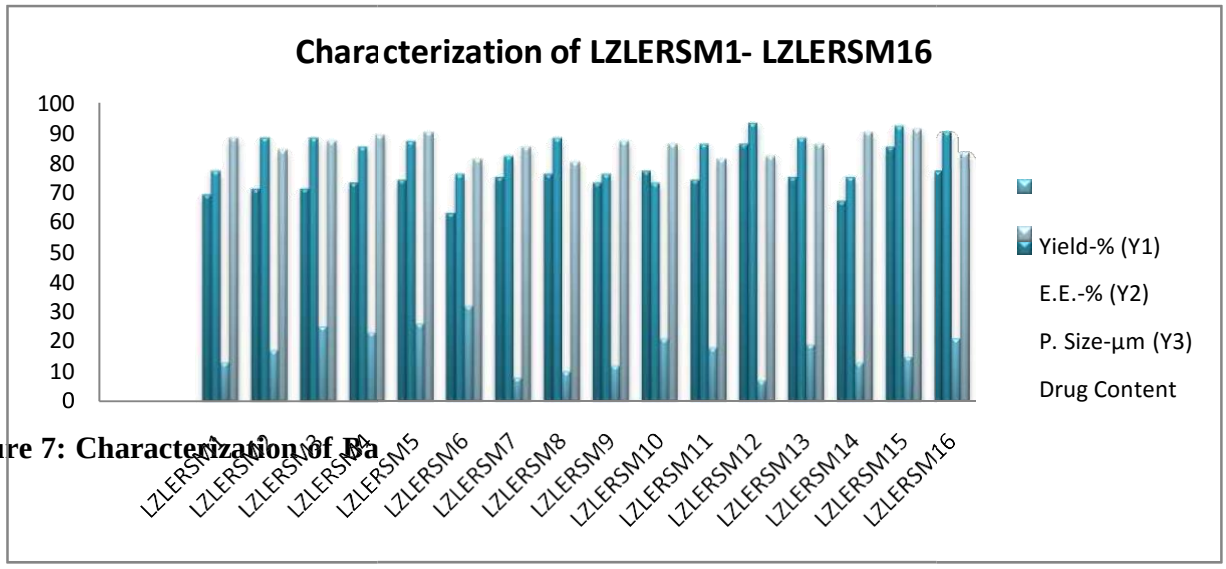


Figure 7: Characterization of Ba

% Cumulative Drug Release profile

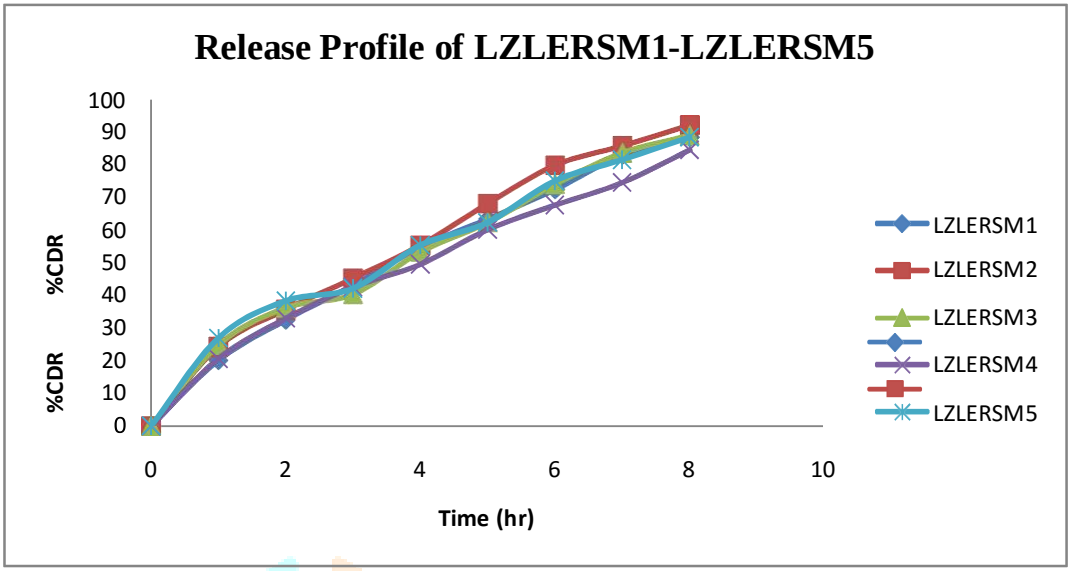


Figure 8:% Cumulative Drug Release profile of Batches LZLERSM1 - LZLERSM5

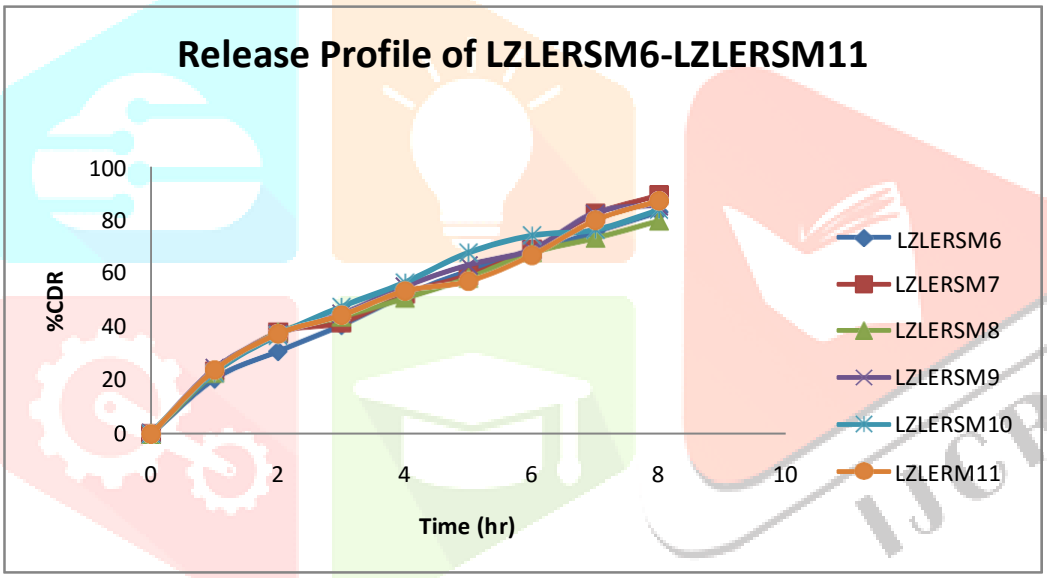


Figure 9:% Cumulative Drug Release profile of Batches LZLERSM6 – LZLERSM11

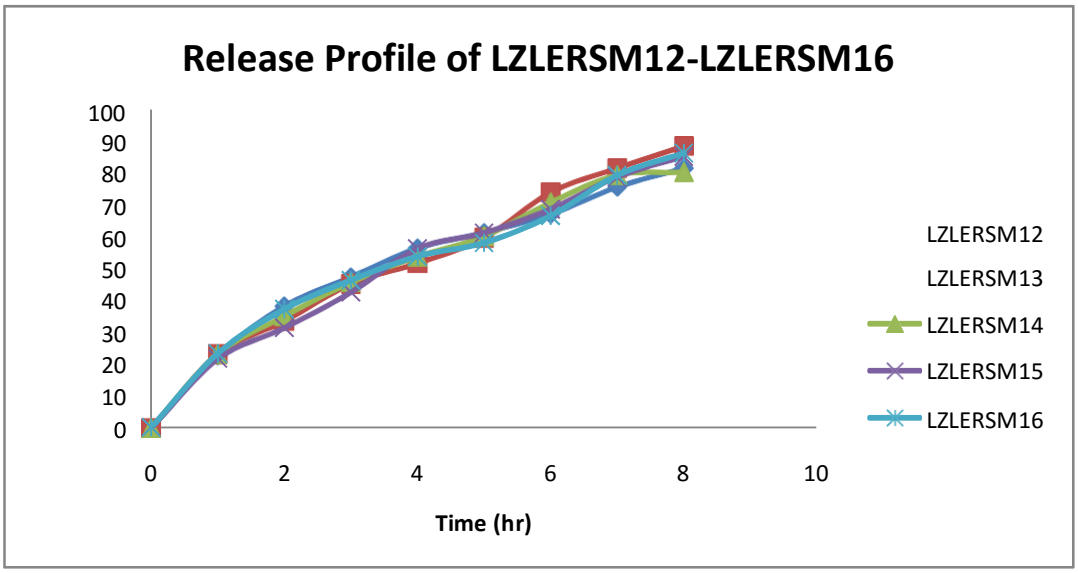


Figure 10:% Cumulative Drug Release profile of Batches LZLERSM12 – LZLERSM16 The % CDR

was found out between 80% to 94% to release almost fully from MS formulationwith intial comparatively high release in first 2 to 3 hrs.

Release Kinetic

The drug release mechanism was analyzed by fitting the release data into various equations like First order, Zero order, Higuchi, Korsmeyer – Peppas, Hixson – crowell with the aid of PCP V2.08 dissolution software. According to the results the best fit model for the Microsponge was Higuchi (matrix) model. By plotting the values for Higuchi model, near straight lines with parallel positive slopes were obtained indicating that, the best fit model for the formulations was Higuchi model.

Statistical Analysis

Two level factorial study was carried out using four different variable using Design ExpertSoftwere. In first factorial design, the amount of drug (ACF): polymer (EC) ratio (X1), amountof PVA Concentration (X2), Internal Phase Concentration (X3) and Speed (X4) was taken as independent variables while % Yield (Y1), % E. E (Y2). Particle sizes (Y3), % CDR (Y4) was selected as dependent variables for both factorial designs.

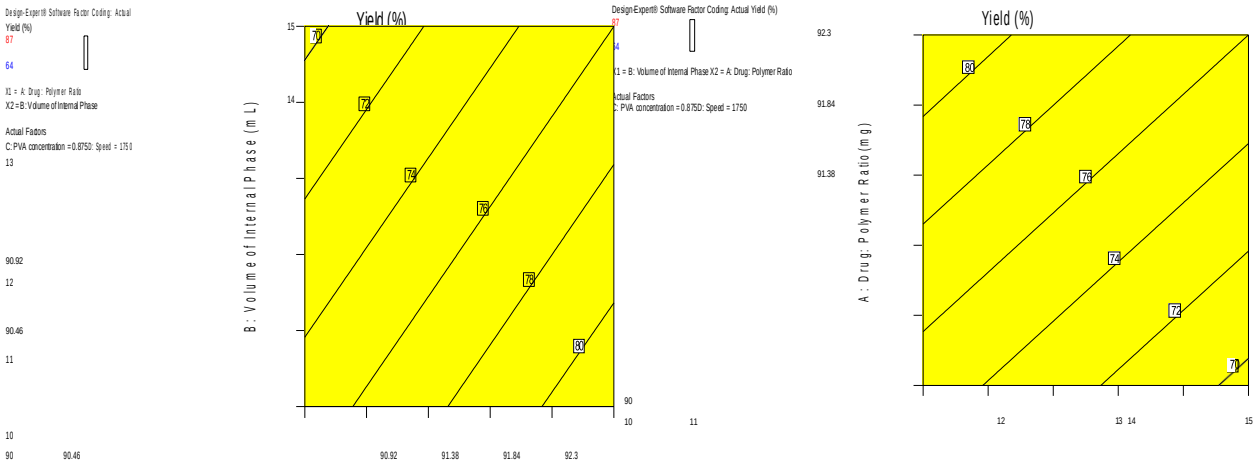
Effect on % Yield (Y1)- Surface Response Study

The positive value for the coefficient of X1 in the equation indicates decrease in the yield with Drug Concentration. The negative value of coefficient of X2 PVA concentration indicates decrease in response of Y1 i.e. % yield. The negative value of coefficient X3, time indicates decrease in yield.

Y1 (%Yield) =75.5 + 3.25 * X1 - 2.75 * X2 - 1.125 * X3 + 0.5 * X4

Table 5: ANOVA Table for Response Y1

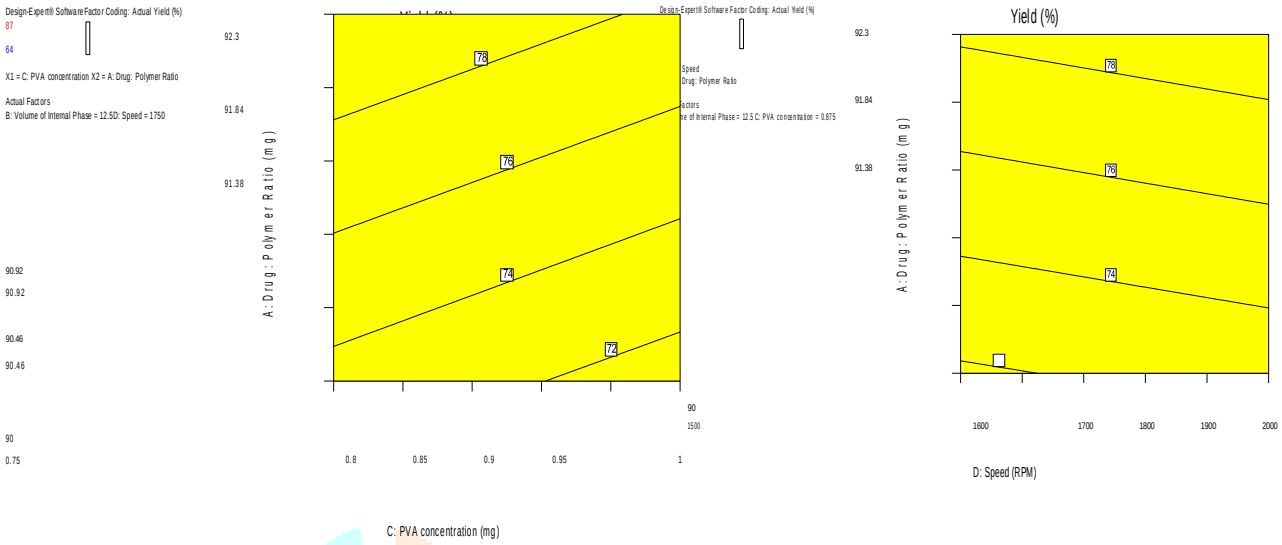
Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	314.25	4	78.56	4.65	0.0192	
A-Drug: Polymer	169.00	1	169.00	10.01	0.0090	
B-Volume of Internal Phase	121.00	1	121.00	7.17	0.0215	
C-PVA Concentration	20.25	1	20.25	1.20	0.0269	significant
D-Speed	4.00	1	4.00	0.24	0.0360	
Residual	185.75	11	16.89			
Cor Total	500.00	15				



B: Volume of Internal Phase (mL)

A: Drug: Polymer Ratio (mg)

(a) **(b)**

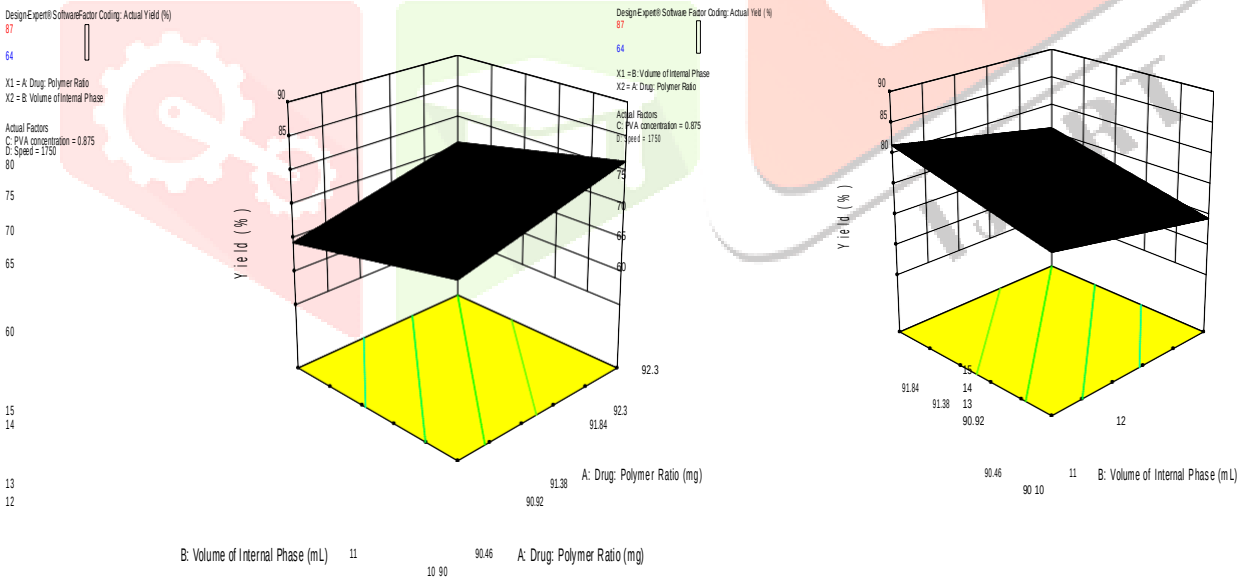


(d)

(c)

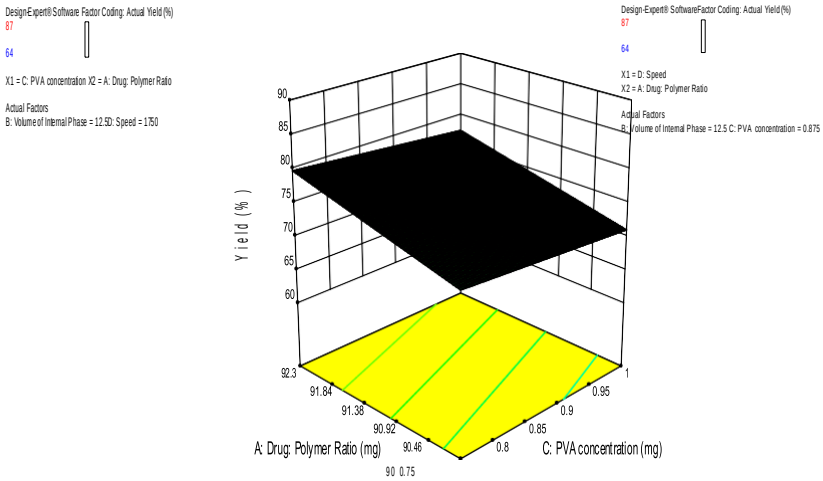
Figure 11: Response Surface Plot:

(a) Drug Polymer Ratio and Internal Phase Concentration, (b) Internal Phase Concentration and Drug Polymer Ratio, (c) PVA concentration and Drug Polymer Ratio and (d) Speed and Drug: Polymer Ratio on % Yield. (Y1)



(b)

(a)



(c) **(d)**

Figure 12: 3D Surface Plot: (a) Drug Polymer Ratio and Internal Phase Concentration, (b) Internal Phase Concentration and Drug Polymer Ratio, (c) PVA concentration and Drug Polymer Ratio and (d) Speed and Drug: Polymer Ratio on % Yield (Y1)

Effect on % Entrapment Efficiency (Y2) - Surface Response Study

The positive value for the coefficient of X1 in the equation indicates decrease in the yield with Drug Concentration. The negative value of coefficient of X2 PVA concentration indicates decrease in response of Y2 i.e. % E.E. The negative value of coefficient X3, time indicates decrease in yield.

Entrapment Efficiency (Y2) = 84.75 + 0.875 * X1 - 5 * X2 - 1.625 * X3 - 0.375 * X4 Table 6: ANOVA Table for Response Y2

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	456.75	4	114.19	6.82	0.0052	
A-Drug: Polymer	12.25	1	12.25	0.73	0.0107	
B-Volume of Internal Phase	400.00	1	400.00	23.88	0.0005	significant
C-PVA Concentration	42.25	1	42.25	2.52	0.0105	
D-Speed	2.25	1	2.25	0.13	0.0209	
Residual	184.25	11	16.75			
Cor Total	641.00	15				

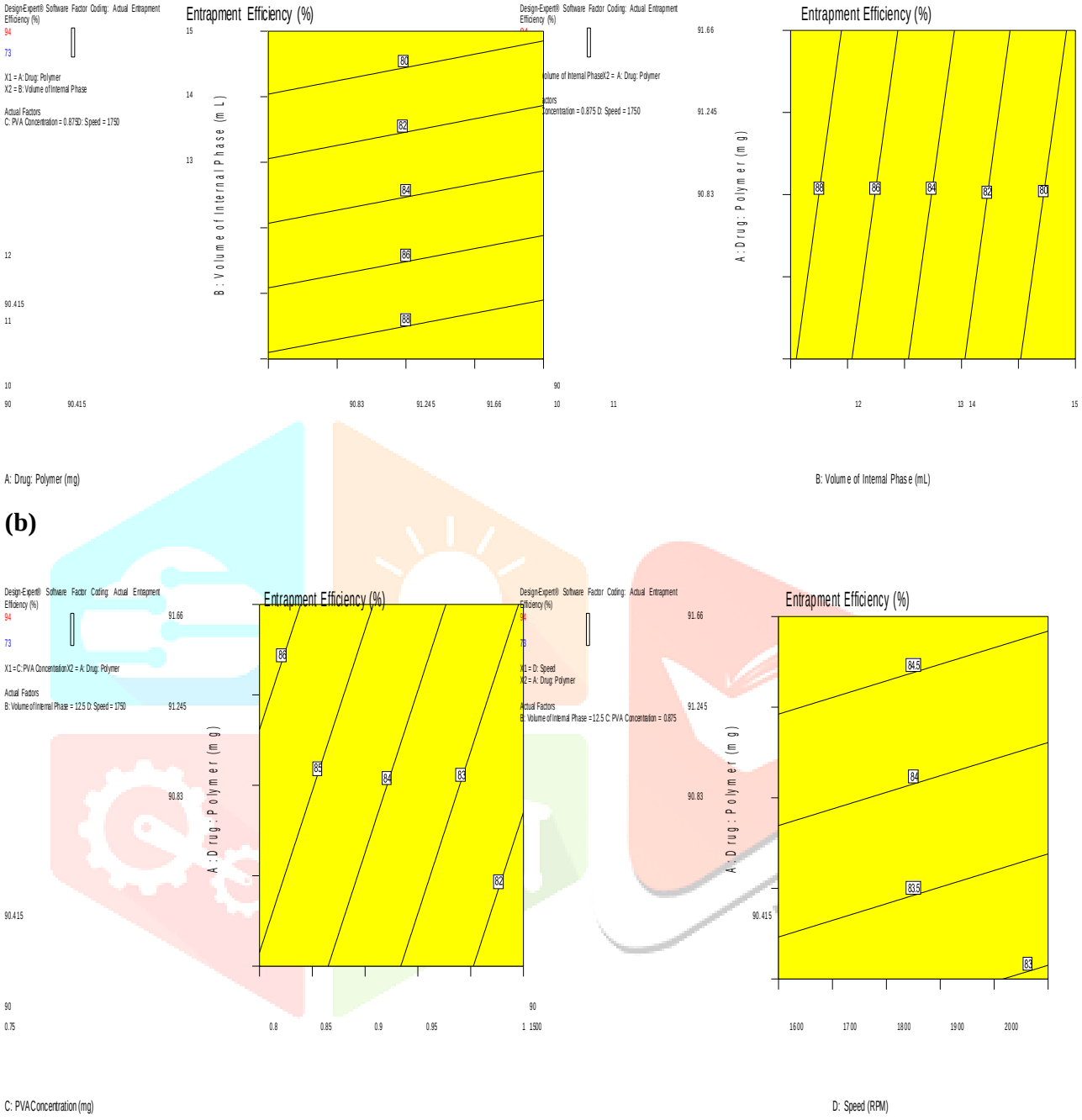


Figure 13: Response Surface Plot:

(a) Drug Polymer Ratio and Internal Phase Concentration, (b) Internal Phase Concentration and Drug Polymer Ratio, (c) PVA concentration and Drug Polymer Ratio and (d) Speed and Drug: Polymer Ratio on % E.E. (Y2)

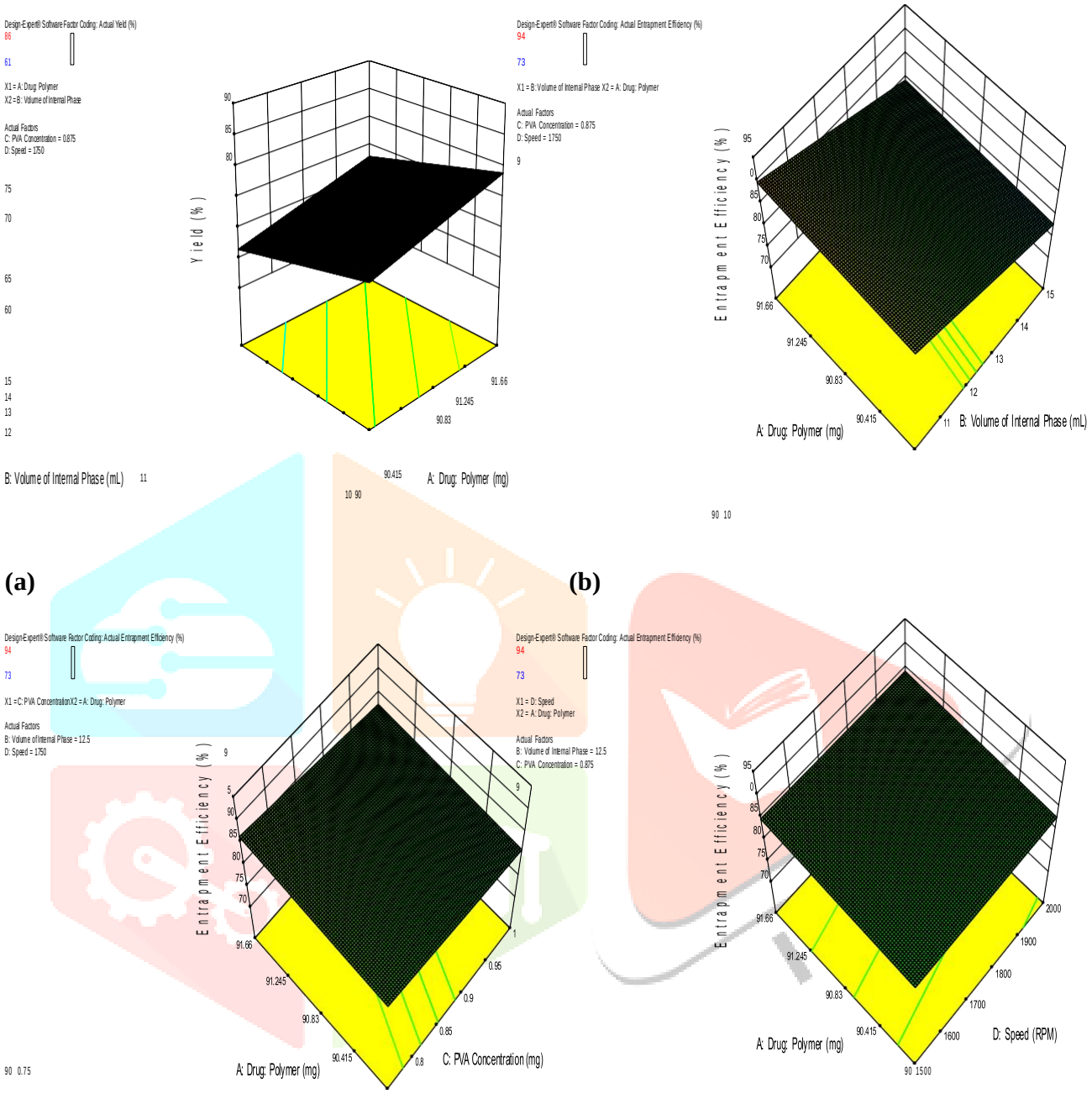


Figure 14: 3D Surface Plot: (a) Drug Polymer Ratio and Internal Phase Concentration, (b) Internal Phase Concentration and Drug Polymer Ratio, (c) PVA concentration and Drug Polymer Ratio and (d) Speed and Drug: Polymer Ratio on % E.E. (Y2)

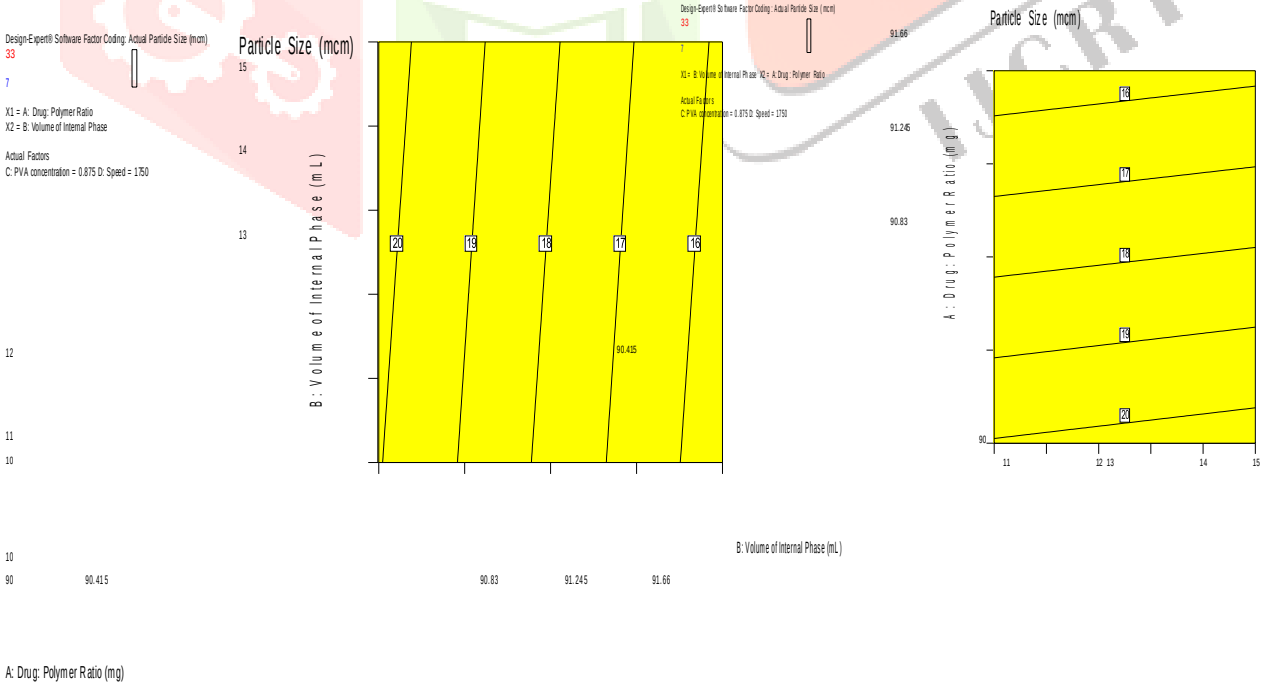
Effect on Particle Size (Y3)- Surface Response Study

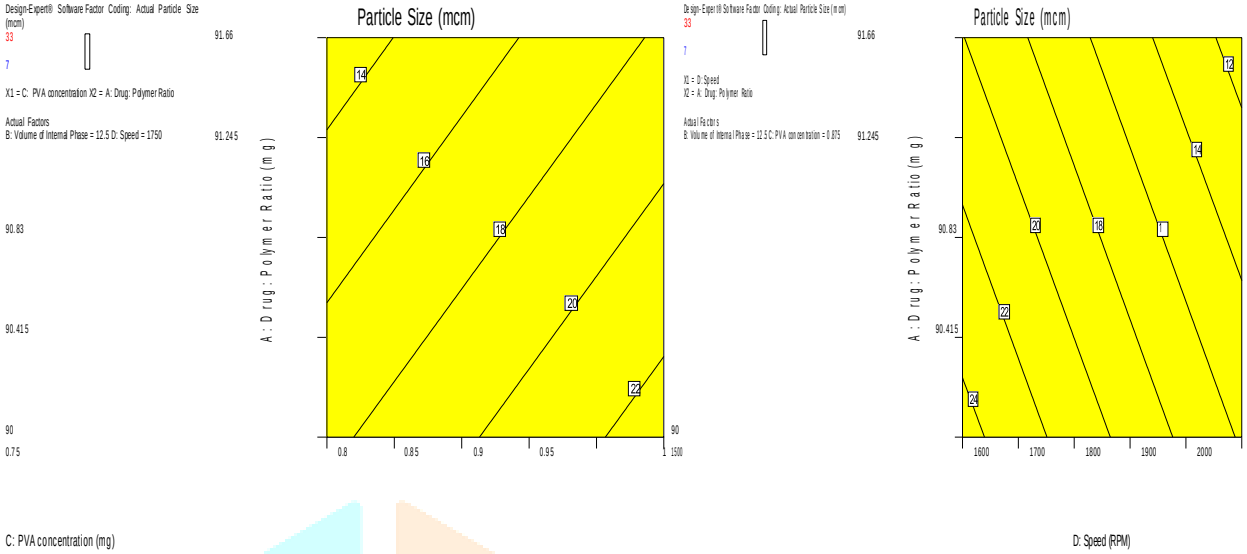
The positive value for the coefficient of X1 in the equation indicates decrease in the yield with Drug Concentration. The negative value of coefficient of X2 PVA concentration indicates decrease in response of Y3 i.e. P.S. The negative value of coefficient X3, time indicates decreasein yield.

P.S.(Y3) = 88.1875 + 1.8125 * X1 - 0.6875 * X2 - 1.4375 * X3 + 1.5625 * X4

Table 7: ANOVA Table for Response Y3

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	559.00	4	139.75	8.99	0.0018	Significant
A-Drug: Polymer	132.25	1	132.25	8.51	0.0140	
B-Volume of Internal Phase	12.25	1	12.25	0.79	0.0037	
C-PVA Concentration	72.25	1	72.25	4.65	0.0541	
D-Speed	342.25	1	342.25	22.02	0.0007	
Residual	171.00	11	15.55			
Cor Total	730.00	15				

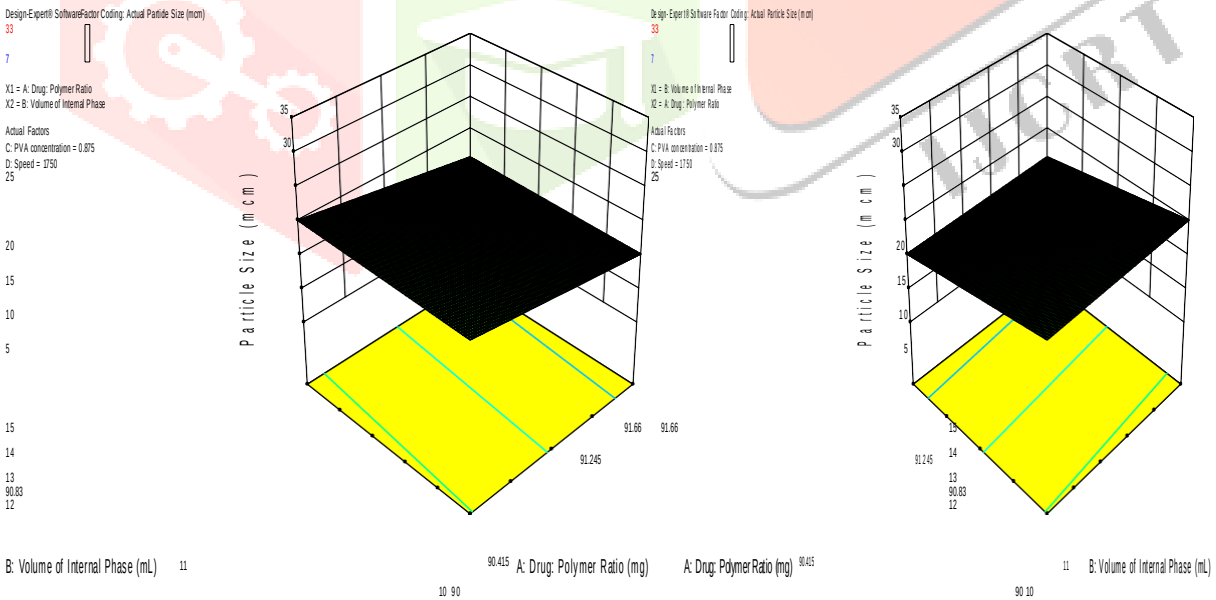




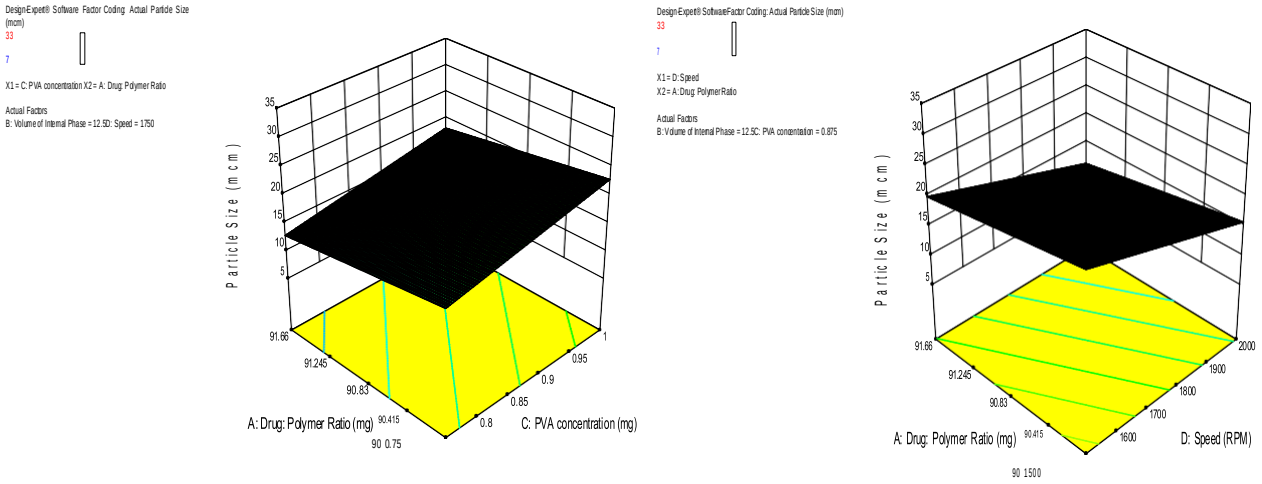
(d)

(c)

Figure 15: Response Surface Plot: (a) Drug Polymer Ratio and Internal Phase Concentration, (b) Internal Phase Concentration and Drug Polymer Ratio, (c) PVA concentration and Drug Polymer Ratio and (d) Speed and Drug: Polymer Ratio on % P.S. (Y3)



(a) **(b)**



(c) **(d)**

Figure 16: Response Surface Plot: (a) Drug Polymer Ratio and Internal Phase Concentration, (b) Internal Phase Concentration and Drug Polymer Ratio, (c) PVA concentration and Drug Polymer Ratio and (d) Speed and Drug: Polymer Ratio on % P.S. (Y3)

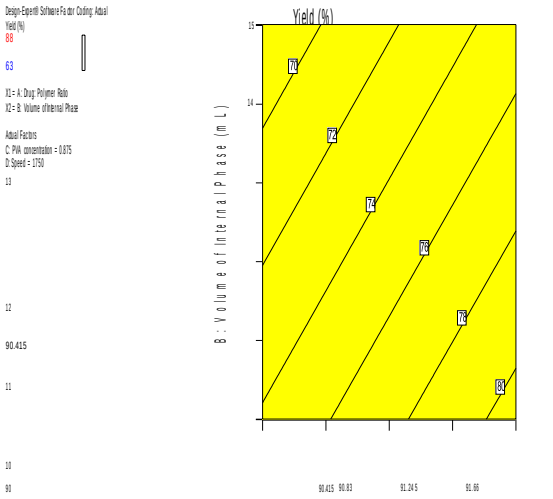
Effect on % CDR (Y4)- Surface Response Study

The positive value for the coefficient of X1 in the equation indicates decrease in the yield with Drug Concentration. The negative value of coefficient of X2 PVA concentration indicates decrease in response of Y4 i.e. % CDR. The negative value of coefficient X3, time indicates decrease in yield.

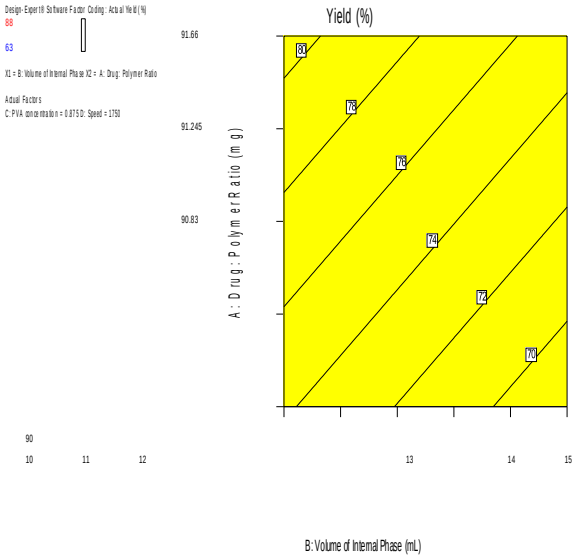
%CDR (Y4) = 87.0625 + 1.8125 * X1 - 0.9375 * X2 - 1.4375 * X3 + 1.5625 * X4

Table 8: ANOVA Table for Response Y4

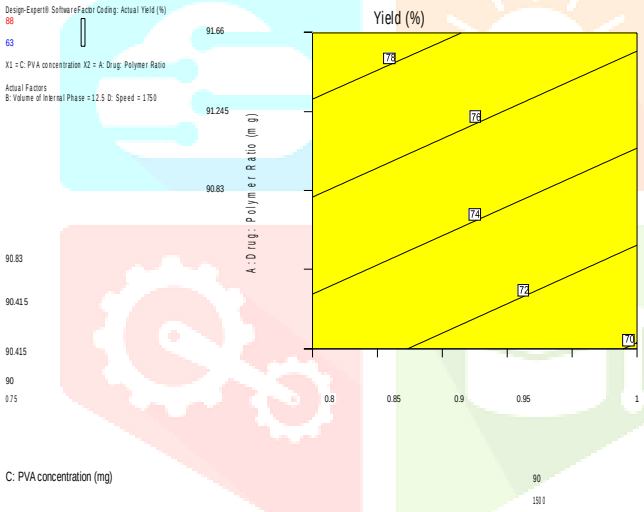
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	138.75	4	34.69	7.04	0.0046	Significant
A-Drug: Polymer	52.56	1	52.56	10.67	0.0075	
B-Volume of Internal Phase	14.06	1	14.06	2.85	0.0192	
C-PVA Concentration	33.06	1	33.06	6.71	0.0251	
D-Speed	39.06	1	39.06	7.93	0.0168	
Residual	54.19	11	4.93			
Cor Total	192.94	15				



(a)



(b)



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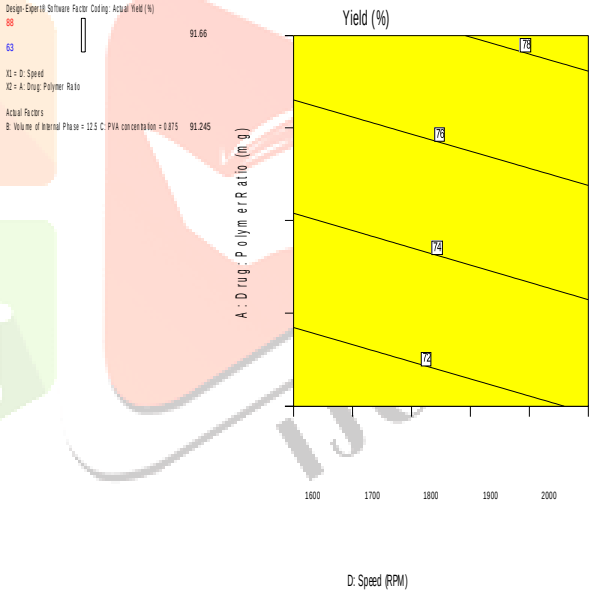
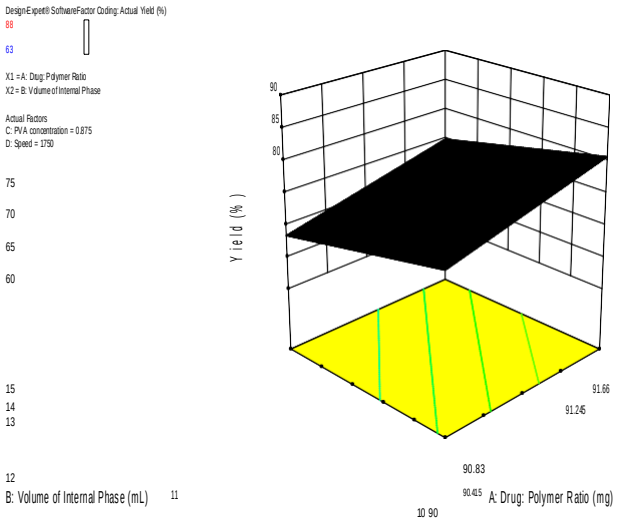
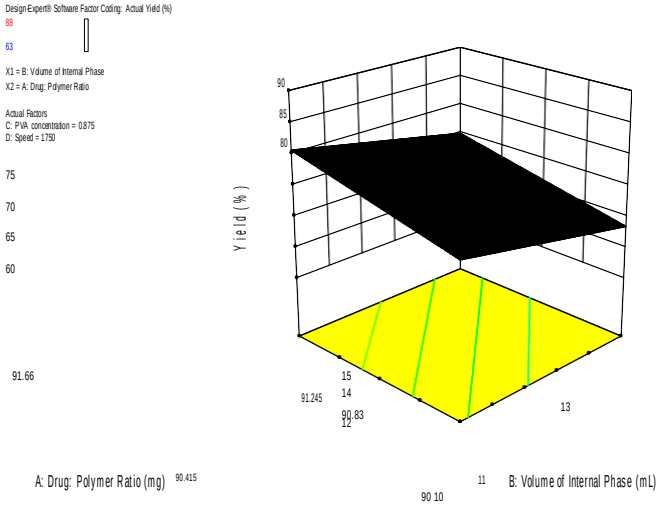


(d)

Figure 17: Response Surface Plot: (a) Drug Polymer Ratio and Internal Phase Concentration, (b) Internal Phase Concentration and Drug Polymer Ratio, (c) PVA concentration and Drug Polymer Ratio and (d) Speed and Drug: Polymer Ratio on % Yield (Y1)



(a)



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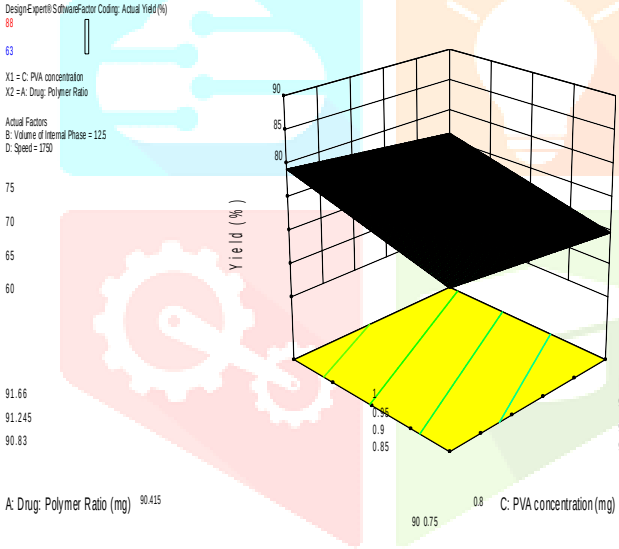

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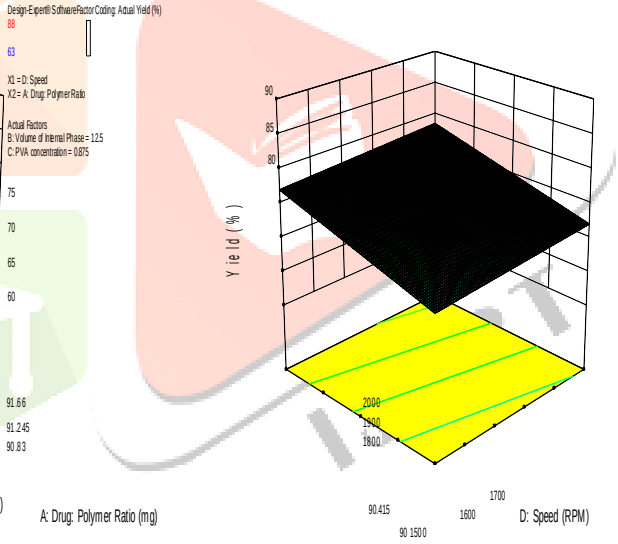


(d)

Figure 18: Response Surface Plot: (a) Drug Polymer Ratio and Internal Phase Concentration, (b) Internal Phase Concentration and Drug Polymer Ratio, (c) PVA concentration and Drug Polymer Ratio and (d) Speed and Drug: Polymer Ratio on % Yield (Y1)

Establishing Design Space and Control Strategy

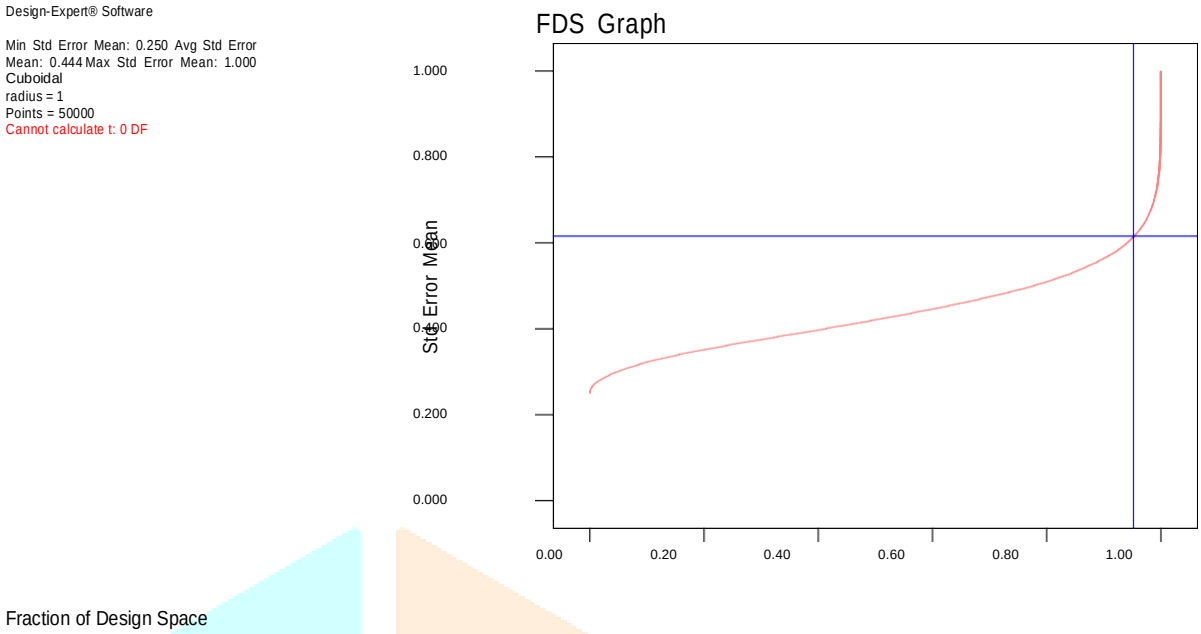


Figure 19: FDS Graph

FDS graph of good design will have a flatter and lower curve than a poor design as shown in figure 5.36. Flatter means the overall prediction error will be constant. Lower means the overall prediction error will be smaller. FDS should be at least 0.8 or 80% for exploration, and 100% for robustness testing.

Validation:

The intensive and integrated examine was done from the polynomial equations for response by Design Expert Software. Check Point Analysis indicates high prognosticability of using RSM optimization. The percentage error varied between 0.6 and 1.9. Thus indicating statistical equivalence between experimental data and the predicted ones and demonstrating the validity of the applied model.

During the independent variable characterization study, the impact of the parameters Drug Concentration (mg), internal Phase Volume (mL), PVA Concentration (mg) and Speed (RPM) were assessed. The criteria considered of response % yield-Y1, E.E. (Y2), Particle Size (μm) and

% CDR are between 79.65-83.17%, 86.82-90.43 %, 8.93-10.24 μm and 90.27-91.68 % respectively. This study lead to the knowledge space and ultimately design space from multidimensional combination of intensity, solvent volume and time leads to the acceptable operating ranges for isolating mucilage with respect to target product profile. Design space

shown in figure 20 and 21 also called as overlay plot which is shaded region with yellow colourindicates that region of successful operating ranges.

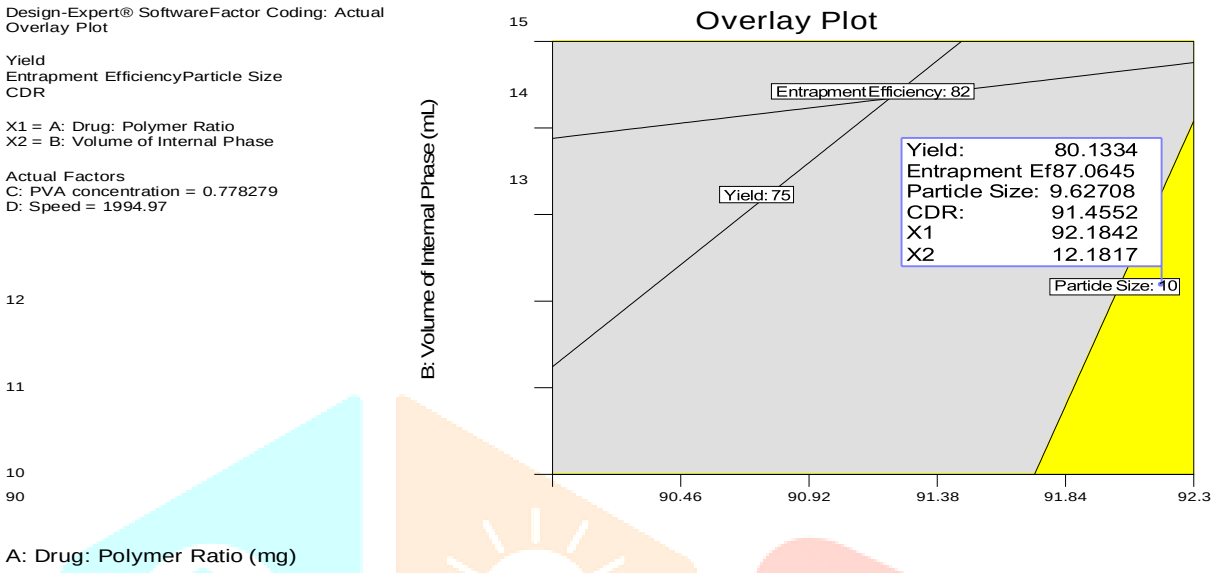


Figure 20: Overlay Plot

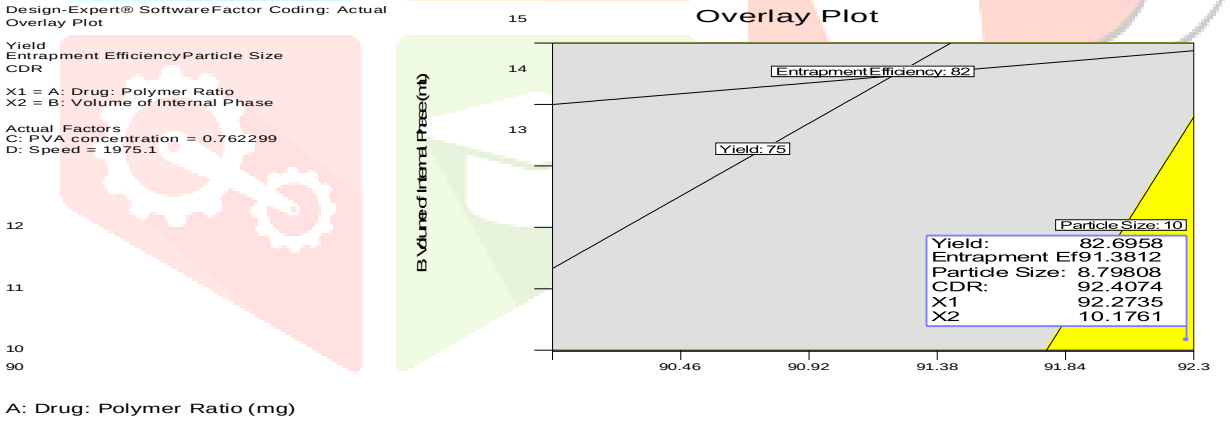


Figure 21: Overlay PlotSTEP-IV: Check point analysis of Validation Batches

LZLERMS1 & LZLERMS2 was designed and evaluated as check point formulation using pooled t-test at 95% confidence interval, degree of freedom 4 and $p < 0.05$. LZLERMS1 & LZLERMS2 shown no significance difference and hence establish validity of the generated model.

Table 9: Validation Batches: Predicted Response

BATCHNO	DRUG CON.-MG (X1)	INT. PHASE VOL.-ML (X2)	PVA CON.-MG (X3)	SPEED -RPM(X4)	YIEL D-% (Y1)	E.E.-% (Y2)	P.SIZE-MM (Y3)	CDR T8-%(Y4)
LZLER MS1	92.18	12.18	0.77	1995	80.13	87.06	9.62	91.45
LZLER MS2	92.27	10	0.762	1975	82.69	91.38	8.79	92.40

Table 10: Validation Batches: Actual Response

Batch No	Drug Con.- mg (X1)	Int. Phase Vol.-mL (X2)	PVA Con.-mg (X3)	Speed-RPM (X4)	Yield-% (Y1)	E.E.-% (Y2)	P. Size-µm (Y3)	CDR T8-%(Y4)
LZLER MS1	92.18	12.18	0.77	1995	79.65	86.82	10.24	90.27
LZLER MS2	92.27	10	0.762	1975	83.17	90.43	8.39	91.68

% Cumulative Drug Release profile:

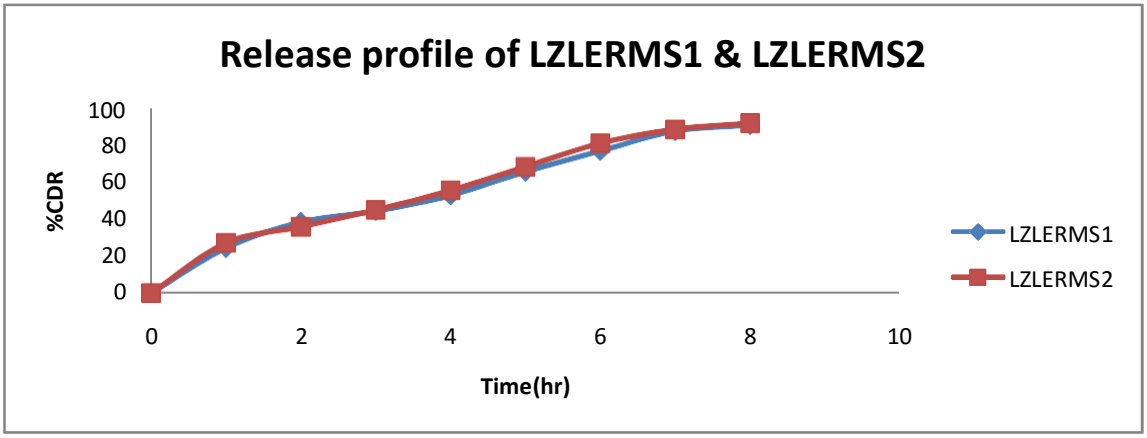


Figure 22:% Cumulative Drug Release profile of LZL Microsponge (LZLERMS1-LZLERMS2)

Selection of Optimized Formulation

LZLERMS2 was selected as validated optimized Batch and further consider for loading into gel which was having smallest particle size of 8.39 µm, % Yield of 83.17%, CDR of 91.61 % with desirability factor of 0.86.

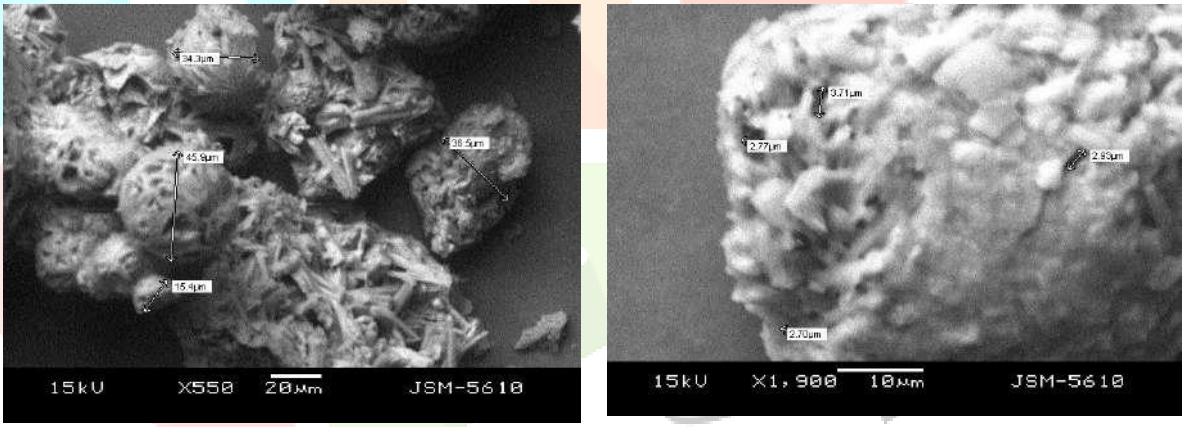
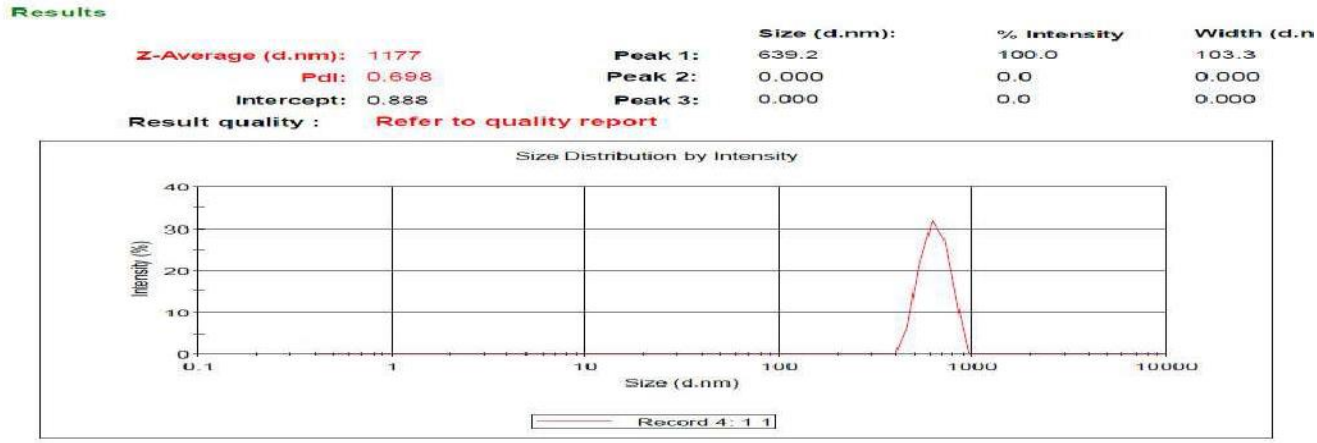


Figure 23: SEM image of Validated optimized Batch Microsponge (LZLERMS2)

From SEM studies it was found that samples had porous and spherical nature. comparison between blank and drug loaded MS showed that Microsponge containing drug was bulging. This showed that drug had been incorporated inside MS. MS of ERS 100 was highly porous.

Particle Size Analysis of LZLERMS2



Characterization

Table 11: Result of LZL Topical Gel

Parameter	**Pure Drug Gel**	**Marketed Luliconazole Gel**	**Optimized LZLG Microspongic (LZLERMS2) Gel**
Dose	300mg	1%	**300mg**
Strength	30 gm	30 gm	**30 gm**
Clarity	Transparent	Transparent	**Transparent**
Odour	odourless	odourless	**Odourless**
pH (Mean ± S.D.) (n = 3)	6.81±0.58	6.96±0.02	**6.92±0.003**
Spreadability (Mean ± S.D.) (n = 3)	10.6±0.79	11.28±1.03	**11.76±0.08**
Viscosity (Mean ± S.D.) (n = 3)	9479±123 cps	9896±43 cps	**9491±23cps**
% Drug content (Mean ± S.D.) (n = 3)	88.12±0.78	92.59±1.57	**90.87±0.81**
Anti-Fungal Activity (Zone of Inhibition-mm)	7.7	7.6	7.4

From these data we have found that Luliconazole Microspongic Topical Gel prepared from Eudragit RS 100 having greater drug content and spreadability mostly LZLERMS2 containing LZL-ER100 Microsponge.

Comparison Study

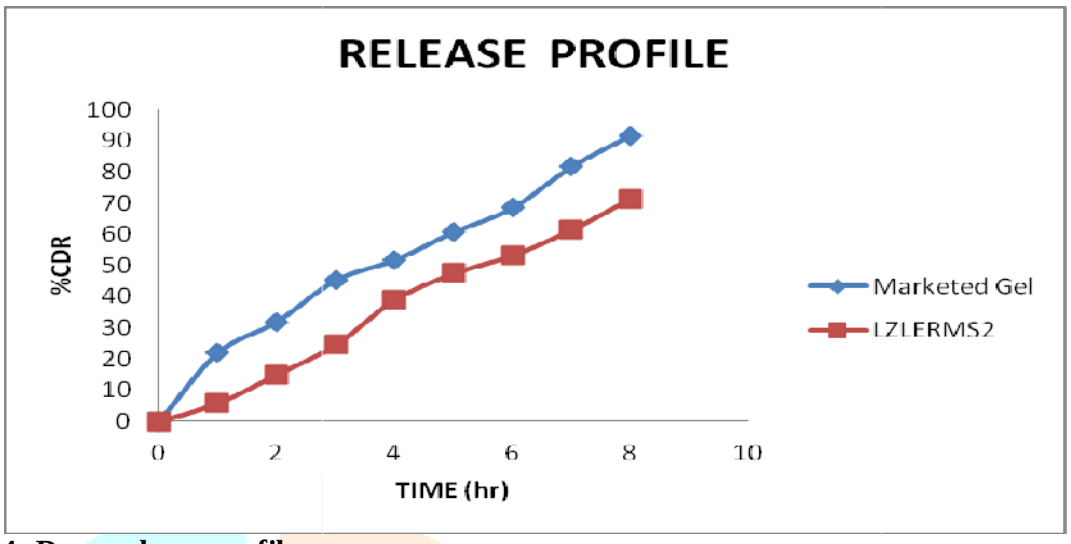


Figure 24: Drug release profile

Release Kinetic

Table 12: Release Kinetic of Luliconazole Conventional & Microspongic gel.

Model	Parameter	Pure Drug Gel	Marketed Luliconazole Gel	Optimized LZL Microspongic (LZLERMS2) Gel
Zero Order	R2	0.9509	0.9504	0.9564
	Slop	8.135	8.347	8.121
	Intercept	12.67	13.69	12.27
First Order	R2	0.9629	0.9972	0.9636
	Slop	0.152	-0.162	0.147
	Intercept	4.47	4.51	4.49
Higuchi Model	R2	0.9581	0.9974	0.9736
	Slop	26.64	26.76	26.93
	Intercept	1.79	1.85	1.79
Hixon Crowell	R2	0.8583	0.8469	0.8417
	Slop	0.876	0.82	0.86
	Intercept	2.9321	2.71	2.38
Corsmeyer Peppas	R2	0.4423	0.4309	0.4359
	Slop	0.52	0.59	0.53

The drug release mechanism was analyzed by fitting the release data into various equations like First order, Zero order, Higuchi, Korsmeyer– Peppas, Hixson –Crowell with the aid of PCP V2.08 dissolution software. Table 13 shows the data for *in-vitro* drug release kinetic study of LZL topical gel. The drug release mechanism was analyzed by fitting the release data intovarious equations like First order, Zero order, Higuchi, Korsmeyer– Peppas, Hixson –Crowellwith the aid of PCP V2.08 dissolution software. According to the results the best fit model for the Microspongic Gel was Higuchi (matrix) model. By plotting the values of Higuchi model, near straight lines with parallel positive slopes were obtained indicating that, the best fit model for the formulations was Higuchi model.

Stability Analysis

Stability studies were performed as per ICH guidelines. The results indicate that there was no evident change in the physical appearance and drug content of formulations after subjecting themto stability studies. Optimized Luliconazole MS (LZLERMS2) loaded Gel formulation was chosen for stability studies. At a fixed time interval drug content determination of these formulations shows that there were no significant changes in the values when compared to the initial formulations. Thus we may conclude that the drug does not undergo degradation on storage.

DISCUSSION

Concentration of Retardant Material in the Internal Phase

The polymer material required in the internal phase was found to be 1% w/v in case of Eudragit RS 100 and EC. At these concentrations, microsponge formation was initiated and below this concentration the MS were formed and showed good physical characteristic like proper shape,size, porosity, particle size distribution and did not collapse even after removal from the solvent and subsequent drying.

Effect of Drug: Polymer Ratio:

The drug to polymer ratio in the internal phase had some effect on the particle size. The mean particle size decreases when the drug to polymer ratio was increased. The encapsulation efficiency and % yield gradually improved with an increase in Drug: Polymer ratio while mean particle size decreased, and the particle size distribution became narrower due to greater viscosity and faster diffusion of the internal phase of the emulsion system.

Effect of Retardent Material:

It was found that type of retardant material also played a crucial role in the formation of MS which reduced free drug content and reduce particle size.

Effect of Internal Phase Volume.

When the amount of ethanol was gradually increasing, % E.E. and drug content decreased. This was probably due to the lower concentration of the drug in the high volume of ethanol. It was observed that by reducing Ethanol volume, the P.S. of prepared MS increases This might be due to the greater viscosity of ethanol, the globs of formed emulsion could not be further divided into reduced particles and greater droplets was formed and mean particle sizes increased.

Effect of Surfactant Concentration

MS did not form in the absence of surfactant. Though it was found that the particle size increased with the increase in the surfactant concentration attributed to an increase in the viscosity at increased emulsifier concentrations, high amounts of surfactant resulted in foaming. This resulted in formation of irregular particles and at increased concentration the encapsulation efficiency was also reduced. This may be due to less amount of polymer available for encapsulating the smaller droplets of LZL particles. When surfactant concentration decreases, % yield and drug content increased.

Effect of Stirring Speed:

It was concluded that increasing stirring speed, decreases the % Entrapment and fall of mean particle size. It was found that the stirring rate of the emulsion influences the particle size and its distribution greatly due to the turbulence created within the external phase. The study showedthat an increase in the stirring rate resulted. It was observed that lowering the stirring speed, leads to increase in mean particle due to the increased propensity of globs to aggregatation and coalescence while at higher stirring rates, forceful, even, improved mechanical shear might forced and resulted in a rapid dispersion of droplets having less chance of coalescing into bigger droplets.

SUMMARY AND CONCLUSION

Drug delivery via polymer systems had been proposed to be the prevailing type of controlled drug delivery devices both in present and future. For scientific as well as economic reasons, such delivery systems have potential advantage which includes enhanced therapeutic response, predictable rate of release and extent of absorption, topical retention and improved patient acceptance.

In the present work a topical polymeric MS formulation of a locally acting anti fungal agent, Luliconazole had been developed. The study includes formulation and development ofLuliconazole MS by DoE method of QbD approach. The idea behind developing a topical polymeric MS delivery system was to deliver Luliconazole in a sustained release pattern for an extended period which will lower application frequency and improve patient compliance. To begin with, the variables involved (viz. Selection of internal and external phase, selection of the type and concentration of emulsifier, selection of speed and time of stirring required forpreparation) in the preparation of the MS were identified CQAs to develop a QbD approach.

A topical polymeric MS formulation of Luliconazole was formulated using Eudragit RS100.

The internal phase suitable for the preparation of MS was found to be Ethanol and the externalphase was found to be Liquid Paraffin by solubility analysis of drug and polymer.

The concentration of the polymer required to produce MS with good physical and morphological characteristics was found to be 11.0% and 13% w/v of the internal phase for both the polymers.

The volume of internal and external phase required to prepare good MS was found to be 10mL ofinternal and 50mL of the external phase.

The minimum concentration of the emulsifier PVA required to produce MS was found to be0.75% w/v. The minimum speed and time of stirring was found to be 2000rpm for 60 Min.

The ratio of drug: polymer required to produce MS with good encapsulation efficiency was found to be from 7:1 to 13:1. Below this ratio, the MS formed had low capacity encapsulation of the drug and above this range there was no further increase in the encapsulation efficiency. Hence, it was concluded that 11: 1 to 13: 1 were optimum ratios of drug: polymer to produce good MS.

To begin with, the variables involved (viz. Selection of internal and external phase, selection of the type and concentration of emulsifier, selection of speed and time of stirring required for preparation) in the preparation of the MS were identified CQAs and their effect on the physical and morphological properties of the MS was to develop a QbD approach. In factorial design, the amount of drug (LZL): polymer (EC) ratio (X1), amount of PVA Concentration (X2), Internal Phase Concentration (X3) and Speed (X4) was taken as independent variables while % Yield (Y1), % E. E (Y2). Particle sizes (Y3), % CDR (Y4) was selected as dependent

variables for both factorial designs.

The MS after check point analysis which gave better physical, morphological and % encapsulation in the polymers were selected for incorporation into the gel formulations.

The release profile of the Luliconazole in the form of MS loaded Topical Gel was compared with that of the pure Luliconazole Topical Gel. From the results it can be concluded that the MS Topical Gel could sustain the drug release over a period of 8 hours when compared to the release after 6 hrs from the Marketed Luliconazole Gel. By model fitting of the data obtained from the drug release profile we can conclude that drug release mechanism was Higuchi (Matrix) Model.

The formulations Optimized Luliconazole MS (LZLERMS2) loaded Gel was subjected tostability studies for 1 months and significant changes observed.

Finally, we may conclude that Optimized Luliconazole MS (LZLERMS2) loaded Gel were bestformulated among the class of Eudragit RS 100 as a retarding polymer**.**

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