FORMULATION DEVELOPMENT

6.1 Introduction:

Formulation development was performed using Quality of design (QbD) approach. Quality by design in product development is systematic approach selected with defined purpose [1]. It encompasses the study of formulation and process development together. The objective is to develop a robust process that ensures the quality of the product throughout the shelf life [2-4]. The ICH Q8(R2) guideline describes QbD implementation in practice such as "multivariate experiments", "statistical process control methods" and a "risk-based control strategy" [1].

6.2 Selection of Polymers:

Polylactic-co-glycolic acid (PLGA) is one of the widely used polymers in the microparticle drug development programme [5]. PLGA is biodegradable, biocompatible and water insoluble polymer designated as safe by regulatory agencies such as USFDA [6,7].

Polycaprolactone (PCL) is a semi-crystalline, biodegradable synthetic polyester with a melting point of around 60 °C [8]. PCL degrades slowly and hence provide controlled drug release over a period of time [8].

To achieve drug release for up to one week using a combination of PLGA (polylactic-co-glycolic acid) and polycaprolactone (PCL), typically PLGA with a higher glycolic acid content that degrades faster is preferred [9].

PLGA with a 50:50 lactic to glycolic acid ratio is often suitable for relatively shorter-term drug release applications [9]. Combining this with PCL, which has a slower degradation rate, can help modulate the release kinetics to achieve a more controlled and sustained release over the desired period [10].

Hence, in the present study, PLGA [50:50] and PCL polymer were used for drug loading of Amisulpride and Granisetron using the application of QbD in process development.

6.3 Selection of method:

Amongst all the listed methods, emulsion-solvent evaporation method is most simple, widely used and easily scalable to commercial scale. Also, this method is suitable to incorporate both hydrophilic and hydrophobic drugs simultaneously. Hence, this method was selected for preparation of Janus microspheres.

For formulation optimization, identification of process parameters which can have highest impact on product quality is of paramount importance. The following steps can be implemented for the same;

1. Definition of quality target profile;
2. Identification of critical quality attributes
3. Identification of formulation variables and process parameters
4. Use of screening designs to screen the variables and identify the most impacting one
5. Use of response surface designs to optimize the formulation further
6. Establishment of design space

6.4 Defining the Critical Quality Attributes:

Quality target product profile and critical quality attributes for formulation are presented in table 6-1 and 6-2.

Table 6-1 Quality target Product profile (QTPP)

Drug name		Amisulpride	Granisetron
Dosage form		Injection	
Dosage design		Sustained release microspheres	
Route of administration		Intramuscular/Subcutaneous	
Dosage strength		30 mg	10 mg
Pharmacokinetics		Bioequivalence with Multiple dose Immediate release (IR) formulations	
Drug product quality attributes	Morphology	Spherical	
	Particle size (µm)	D90 50-150 (Target 100 ± 20)	
	Drug loading	Maximum	Maximum
	Entrapment efficiency	Maximum	Maximum
	Drug Release	1 hour- NMT 25%	1 hour- NMT 25%
		4 hour- 25-55%	4 hour- 25-55%
		24 hour- NLT 70%	24 hour- NLT 70%
Stability		Regulatory acceptable stability	

Table 6-2 Critical Quality Attributes (CQA)

CQA	Target Amisulpride	Target Granisetron	Justification
Pharmacokinetics	Bioequivalence with marketed formulations		Required to establish bioequivalence with marketed formulations
Morphology	Uniform		Required to achieve uniform drug release and minimize variability. This CQA will be evaluated after optimization on final formulations.
Particle size distribution (PSD)	D90 50-150 (µm) (Target 100 ± 20)		Required to achieve uniform drug release, ease of administration. Product as well as process variables will affect drug loading. This CQA will be considered for evaluation in overall the product development.
Drug loading (DL)	Maximum	Maximum	Drug loading will affect safety and efficacy. Product as well as process variables will affect drug loading. This CQA will be considered for evaluation in overall the product development.
Entrapment efficiency (EE)	Maximum	Maximum	Entrapment efficiency will affect safety and efficacy. Product as well as process will affect drug entrapment efficiency. This CQA will be considered for evaluation in overall the product development.
Drug Release (DR)	Meet the specs		Not meeting the drug release specifications will impact *in-vivo* performance. Product as well as process variables will affect drug release. This CQA will be evaluated after optimization on final formulations.
	1 hour- NMT 25%		Required to achieve desired Cmax and Tmax
	4 hour- 25-55%		Required to achieve desired AUC
	24 hour- NLT 70%		Required to ensure desired release from the formulation

6.5 Identification and Evaluation of Material attributes and Process Parameters

Identification of formulation and process variables is the first step before designing any experiment [1]. Risk assessment tool was used to identify the risk associated with each variable. To list down all variables, which can impact the product quality, an Ishikava diagram was used (Figure 6-1). The raw materials such as drug and excipients, the formulation composition, the process parameters and the container/equipment details were identified. For every main cause, corresponding sub-causes were identified (Figure 6-1).

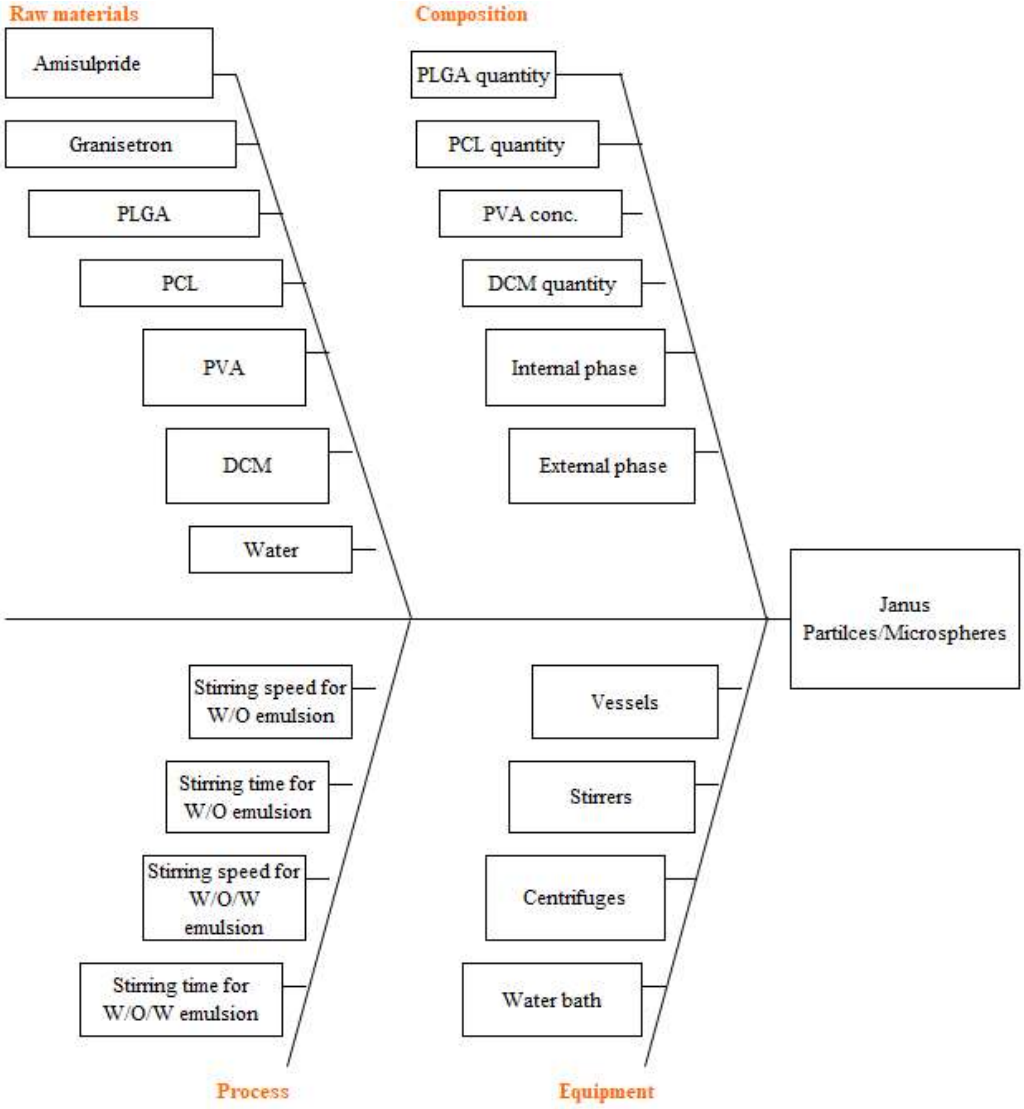

Figure 6-1 Ishikava diagram to assess the impact of different variables on microspheres

In the case of a complex manufacturing process with multiple variables, it is difficult to evaluate all factors/variables in an experimental design. The most critical variables were identified based on domain knowledge.

For the present investigation, the type and quality of API and excipients were fixed based on earlier study experience and domain knowledge [11]. For the double-emulsion-solvent-evaporation method, the selected organic solvent was dichloromethane which was volatile and water immiscible. Polyvinyl alcohol was selected as stabilizer and stabilizer concentration was assessed to study the impact on selected CQAs. Earlier studies showed that stirring time and speed can impact particle size and drug loading [12,13]. Therefore, the stirring speed and stirring time were selected as parameters for evaluation. Stirring the samples at environmental conditions was chosen due its simplicity and effectiveness to evaporate the organic solvent.

Mainardes et al. investigated the concentration of stabiliser, the polymer content to study impact on formulation CQAs [13].

Following variables were chosen for further investigations: **PLGA, PCL and PVA levels, stirring time and speed of preparation of the double emulsion**.

6.6 Risk assessment [1]

Risk assessment ranking system is shown in table 6-3

Table 6-3 Risk ranking system

Low	Risk is low, no further action is required.
Medium	Risk is medium, need to study further to lower the risk through experimentation.
High	Risk is high. Extensive studies are required to reduce the high risk to medium

For Active pharmaceutical ingredient (API), the risk was kept low as only one source API was used. For formulation variables, risk assessment was performed and presented in table 6-4 and risks were justified in table 6-5. For process variables, risk assessment was performed and presented in table 6-6 and risks were justified in table 6-7.

Table 6-4 Initial risk assessment of Formulation variables

Drug Product CQA ↓	**Formulation variables** →			
	API Level	**PLGA level**	**PCL level**	**PVA level**
PSD D90	Low	Medium	Medium	High
%DL	Low	High	High	Medium
%EE	Low	High	High	Medium
% DR	Low	High	High	Low

Table 6-5 Justification for Selected Formulation Variables

Formulation variables	**DP CQAs**	**Justification**
API Level	PSD D90	Risk is kept low as API level was finalized according to dose required to attain desired pharmacokinetics.
	%DL	
	%EE	
	% DR	
PLGA Level	PSD D90	Risk is medium. Polymer level can impact the particle size. Hence, it will be evaluated to study the impact of polymer level on particle size through experiments.
	%DL	Risk is high. Polymer level can impact the drug loading and encapsulation efficiency directly. Hence, it will be evaluated to study the impact of polymer level on these CQAs through experiments.
	%EE	
	% DR	Risk is high. Polymer level can impact the drug release directly. More the polymer concentration less is the drug release. Hence, it will be evaluated to study the impact of polymer level on drug release through experiments.

Formulation variables	DP CQAs	Justification
PCL level	PSD D90	Risk is medium. Polymer level can impact the particle size. Hence, it will be evaluated to study the impact of polymer level on particle size through experiments.
	%DL	Risk is high. Polymer level can impact the drug loading and encapsulation efficiency directly. Hence, it will be evaluated to study the impact of polymer level on these CQAs through experiments.
	%EE	
	% DR	Risk is high. Polymer level can impact the drug release directly. Hence, it will be evaluated to study the impact of polymer level on drug release through experiments.
PVA level	PSD D90	Stabilizer concentration is critical to achieve uniform particle size. Hence it will be evaluated through experiments to study the impact of stabilizer concentration on particle size.
	%DL	Stabilizer concentration is critical to achieve drug loading and encapsulation efficiency. Hence it will be evaluated through experiments.
	%EE	
	% DR	Stabilizer concentration will be finalized based on desired particle size and drug loading/entrapment efficiency. Hence its impact on drug release will be low considering fixed level.

Table 6-6 Initial risk assessment of Process variables

Drug Product CQA ↓	Process variables ⟶			
	Stirring speed W/O	Stirring Time W/O	Stirring speed W/O/W	Stirring Time W/O/W
PSD D90	Low	Low	High	High
%DL	Low	Low	Medium	Medium
%EE	Low	Low	Medium	Medium
% DR	Low	Low	Low	Low

Table 6-7 Justification for Selected Process Variables

Formulation variables	DP CQAs	Justification
Stirring speed W/O	PSD D90	The risk for Stirring speed for W/O emulsion was kept low based on initial feasibility trials and domain knowledge. The stirring speed in the initial feasibility trials was not found to be affecting drug loading and entrapment efficiency, hence it was decided not to evaluate this parameter further.
	%DL	
	%EE	
	% DR	
Stirring Time W/O	PSD D90	The risk for Stirring time for W/O emulsion was kept low based on initial feasibility trials and domain knowledge. The stirring time in the initial feasibility trials was not found to be affecting drug loading and entrapment efficiency, hence it was decided not to evaluate this parameter further.
	%DL	
	%EE	
	% DR	

Formulation variables	DP CQAs	Justification
Stirring speed W/O/W	PSD D90	Stirring speed in W/O/W stage is critical for achieving the desired particle size as it can affect the particle size. Hence it will be evaluated through experiments.
	%DL	Stirring speed in W/O/W stage can affect drug loading and entrapment efficiency. Hence it will be evaluated through experiments.
	%EE	
	% DR	The impact of stirring speed on drug release is not directly linked but rather it is governed through particle size and drug loading/entrapment efficiency. Hence the risk is considered low.
Stirring Time W/O/W	PSD D90	Stirring time in W/O/W stage is critical for achieving the desired particle size as it can affect the particle size. Hence it will be evaluated through experiments.
	%DL	Stirring time in W/O/W stage can affect drug loading and entrapment efficiency. Hence it will be evaluated through experiments.
	%EE	
	% DR	The impact of stirring time on drug release is not directly linked but rather it is governed through particle size and drug loading/entrapment efficiency. Hence the risk is considered low.

6.7 Materials:

Granisetron HCl and Amisulpride were obtained from Sun pharmaceutical Industries Ltd (India). PLGA and PCL polymers were obtained as gift samples from Sun pharma and Evonik respectively. All other chemicals and solvents used were of analytical grade and provided by Sun pharmaceutical Industries Ltd (India).

6.8 Equipment's:

Digital analytical balance (ATX224 Shimadzu, Japan)

Optical microscope (Leica Microsystems, India)

Magnetic stirrer (Remi sci. Equipment, India)

Overhead stirrer (Remi sci. Equipment, India)

Heating bath (PCI Analytics, India)

Analytical ultracentrifuge (Remi sci. Equipment, India)

6.9 Formulation development:

Feasibility trials were taken to check the feasibility of formulation and process. The process and formulation were screened and optimized on the basis of targeted particle size and maximum drug loading & entrapment efficiency for dual drug loaded formulations.

6.9.1 Preparation of microspheres for Amisulpride [14]

Amisulpride being insoluble in water, O/W emulsion method was found to be suitable for preparation of microspheres. Polymer (PLGA or PCL) and Amisulpride were dissolved in

dichloromethane. This solution was added dropwise in 0.5% - 1% PVA (stabilizer) solution to form emulsion under stirring. Stirring was continued for 30 mins and then added to excess water under continuous stirring. Stirring was continued for 8 hours. Emulsion was filtered to obtain microspheres. The obtained microspheres were washed three times with water and dried using vacuum drying (at 30°C for 24 hours).

6.9.2 Preparation of microspheres for Granisetron [14,15]

Granisetron being soluble in water, W/O/W emulsion method was found to be suitable for preparation of microspheres. Granisetron was dissolved in water. Polymer (PLGA or PCL) was dissolved in dichloromethane. Granisetron solution was added to polymer solution to form first W/O emulsion. This emulsion was added dropwise in 0.5% - 1% PVA (stabilizer) solution to form W/O/W emulsion under stirring. Stirring was continued for 30 mins and then added to excess water under continuous stirring. Stirring was continued for 8 hours. Emulsion was filtered to obtain microspheres. The obtained microspheres were washed three times with water and dried using vacuum drying (at 30°C for 24 hours).

6.9.3 Preparation of Janus microspheres for Amisulpride and Granisetron [11]

Novel drug loading strategy was used to incorporate hydrophobic (Amisulpride) and hydrophilic (Granisetron HCl) drugs in microsphere formulation using double emulsion-solvent evaporation technique. Two polymers (PLGA and PCL) were selected to enable formation of Janus microspheres. Granisetron was dissolved in water. PLGA, PCL and Amisulpride were dissolved in dichloromethane. Granisetron solution was added to PLGA-PCL-Amisulpride solution to form first W/O emulsion. This emulsion was added dropwise in 0.5% - 1% PVA (stabilizer) solution to form W/O/W emulsion under stirring. Stirring was continued for 30 mins and then added to excess water under continuous stirring. Stirring was continued for 8 hours. Emulsion was filtered to obtain microspheres. The obtained microspheres were washed three times with water and dried using vacuum drying (at 30°C for 24 hours).

6.9.4 Preparation of diluent:

Preparing the diluent for reconstituting microspheres for injection is a critical step to ensure the stability and efficacy of the final product. The diluent used for suspending the Janus microspheres consists of water for injection (WFI), suspending agent sodium carboxymethyl cellulose (CMC) and citrate and phosphate salts for pH adjustment to pH 7.4. The diluent was prepared by slowly dissolving sodium CMC in sterile WFI to avoid clumping, followed by addition of phosphate buffer pH 7.4. Considering the potential scale for commercial

production, the manufacturing process involves filtration through 0.2 micron PTFE filter for ensuring sterility. This step removes both particulates and bacteria before emulsification. Emulsification and solvent evaporation are carried out in closed systems to prevent contamination. Also, standard aseptic manufacturing processes were followed during manufacturing.

6.10 Design of experiments:

Minitab statistic software version 18 (Minitab Inc., State College, PA, USA) was used to design the experiments. The experiments for all DoE were conducted in randomised order with one replicate.

In present investigation, a definitive screening design used to screen the most impacting formulation and process variables. Screening design usually consists of less experiments but provide valuable information regarding the variables involved in the study [16].

The design used for screening in this study has 5 factors with 2 levels. The most significant factors were taken further based on level of significance (p value < 0.05). The response surface design was used to select optimum drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio. Even with a comparatively small number of runs, RSM can provide a good predictability for future outcomes [18]. Response surface designs are used to study the curvature effect that can occur in responses due to interaction of two variables [18, 19].

6.10.1 Screening design:

Five parameters were chosen for further investigation as presented in Table 6-8. The Minitab software was used to generate the DSD with five factors with two levels each (Table 6-9), resulting in 13 runs with one centre point. The experiments were carried out in randomised order and the respective responses for each trial were determined. The design was examined statistically for every response. The significance of all parameters on the response was compiled in Table 6-10. The main effect plots were used to study the effect of each variable on the respective response. The Pareto chart showed comparative scale of absolute effects and reference line on the plot indicates statistically significant effects. Further, contour plots were generated to understand the relationship between a fitted response and two continuous variables. Further using response optimizer, factor levels were adjusted to obtain best fit for all responses.

Table 6-8 Factors selected for Screening design

Factor	Factor Description	Low	High
A	Polymer PLGA level (mg)	100	250
B	Polymer PCL level (mg)	100	250
C	PVA Concentration (%)	0.1	1
D	Stirring speed in W/O/W (rpm)	400	800
E	Stirring time (ST) in W/O/W (hours)	4	8

The challenges were observed during formulation development are summarized below:

1. Drug leakage resulting in low drug loading:
    a) Initial trials with single polymer resulted in low drug loading. Hence, two polymers with different hydrophilicities were used such as PLGA (more hydrophilic) and Polycaprolactone (more hydrophobic). This resulted in improved drug loading and entrapment efficiency due to facilitated drug-polymer interaction in the two compartments.
    b) In screening DOE, low polymer levels resulted in lower drug loading and entrapment efficiency. Hence, Polymer levels were optimized to increase the drug loading efficiency.
    c) Drug: polymer ratio and polymer : polymer ratio was optimized to achieve maximum drug loading.
2. Low entrapment efficiency for water soluble drug granisetron:
    a) Use of double emulsion solvent evaporation method (W/O/W) to enhance entrapment efficiency of water soluble drug granisetron.
    b) PVA (as surfactant) level was optimized to control interfacial tensions to ensure uniform drug loading.
3. Uniform size and shape, which can also impact drug distribution:
    a) Stirring speed optimization: This ensured the achievement of desired particle size of around 100 microns.
    b) Solvent removal process optimization: This ensured the microspheres characteristics such as morphology and uniformity of distribution.
    c) Optimization of external aqueous phase

Table 6-9 Trial summary for screening design

Std Order	Run Order	Pt Type	Blocks	PLGA	PCL	PVA	SS	ST	D90 (µm)	% EE Ami	% EE Grani	% DL Ami	% DL Grani
6	1	2	1	100	100	0.55	400	8	108	32	35	10	4.1
8	2	2	1	100	250	0.1	600	4	95	60	40	14	4.5
10	3	2	1	100	250	1	400	6	106	50	48	16	6.5
3	4	2	1	250	175	1	400	4	108	50	50	13	5.5
2	5	2	1	175	100	0.1	400	4	103	39	50	11	5
4	6	2	1	100	175	0.1	800	8	95	45	32	12	4.2
11	7	1	1	250	250	0.1	400	8	110	62	60	15	6
1	8	2	1	175	250	1	800	8	92	55	52	15	6
13	9	0	1	175	175	0.55	600	6	80	62	65	17	6.5
12	10	1	1	100	100	1	800	4	85	30	32	8	4
5	11	2	1	250	250	0.55	800	4	85	60	61	16	7
9	12	2	1	250	100	0.1	800	6	95	40	60	12	6.5
7	13	2	1	250	100	1	600	8	86	52	65	14	6.9

PLGA: Polylactic-co-glycolic acid (mg), PCL: Polycaprolactone (mg), PVA: Polyvinyl alcohol (%), SS: Stirring speed (rpm), ST: Stirring time (hours), D90: Particle size, EE: Entrapment efficiency, DL: Drug loading

Minitab software Version 18 generated 13 screening DOE experiments for selected 5 factors in randomized order with one centre point and one block. Responses to individual trial were presented in respective columns.

Screening design experiments identified the most significant factors impacting the drug product CQAs. Table 6-10 shows the significance of the studied variables on the selected responses.

Table 6-10 Significance of parameters on responses and model summary for screening design

Outcome **Parameter**	**D90**	**% EE Ami**	**% EE Grani**	**% DL Ami**	**% DL Grani**
A: Polymer PLGA level (mg)	N	N	**Y (p =0.005)**	N	**Y (p =0.016)**
B: Polymer PCL level (mg)	N	**Y (p =0.004)**	N	**Y (p =0.011)**	N
C: Stirring speed in W/O/W (rpm)	**Y (p =0.016)**	N	N	N	N
D: Stirring time in W/O/W (hours)	N	N	N	N	N
E: PVA Concentration (%)	N	N	N	N	N
R^2 for model	61.6	75.9	70.9	68.2	64.6

Note: Y: Significant N: Not significant

6.10.2 Effect on particle Size in screening design:

Figures 6-2A, 6-2B and 6-2C shows the main effect plot, pareto chart and contour plot for particle size. Table 6-11 shows the screening design statistical summary for Particle size.

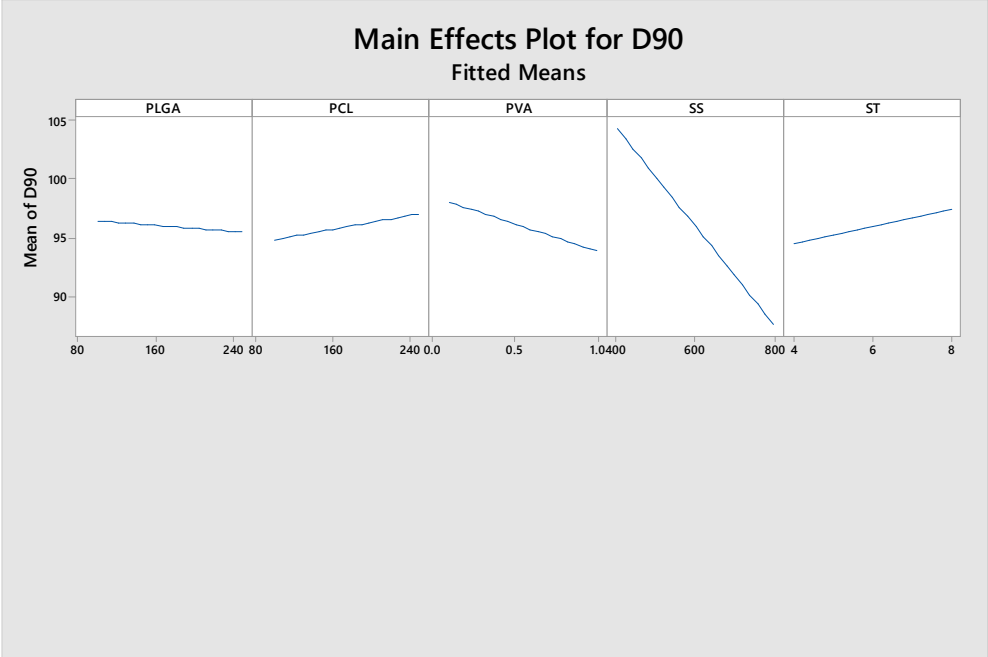

Figure 6-2A Main effect plot for Particle size (D90)

From fig 6-2A, it was observed that stirring speed was found to be most impacting factor for the particle size of formulation. More the stirring speed less was the particle size.

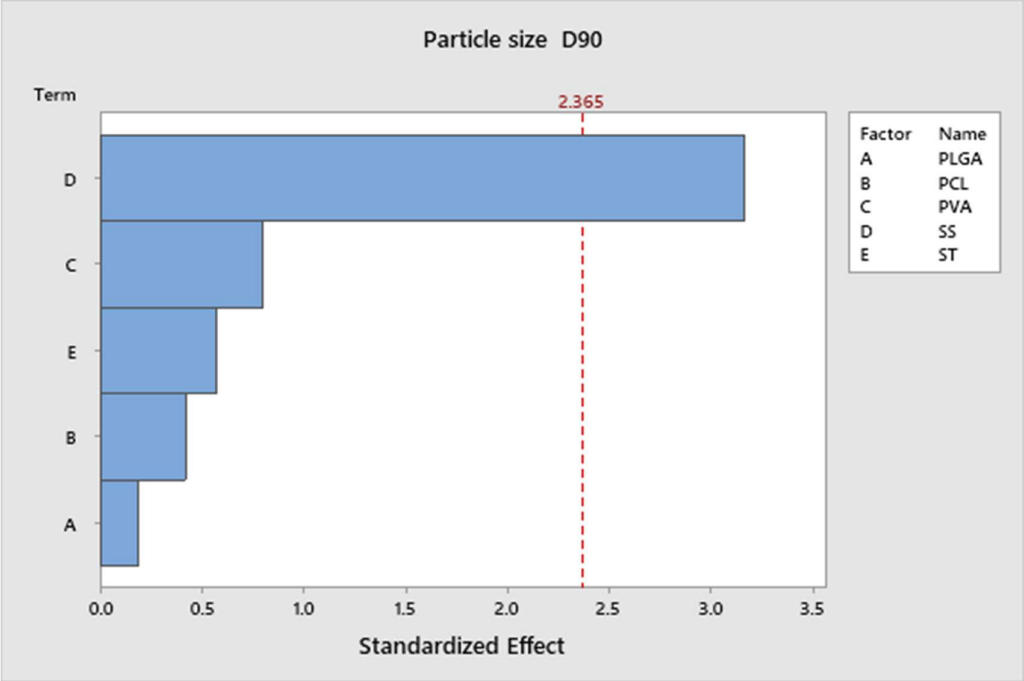

Figure 6-2B Pareto chart for Particle size (D90) in screening design

From fig 6-2B, it was observed that, stirring speed was found to be most impacting factor for the particle size of formulation as it crosses the reference line marked in dotted red.

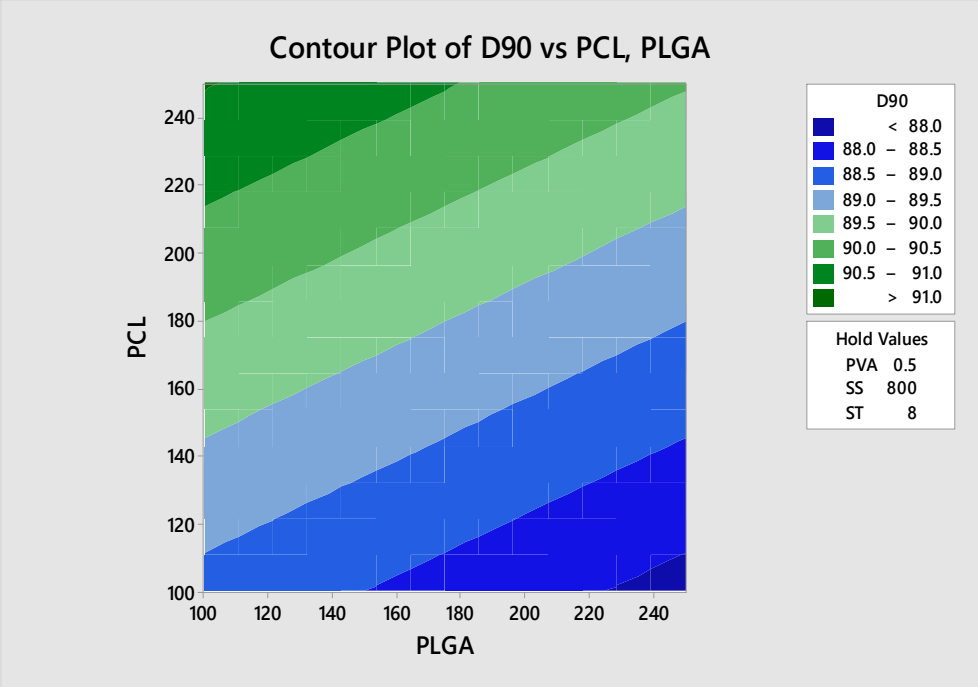

Figure 6-2C Contour plot for Particle size (D90)

From fig 6-2C, it was observed that, particle size was not significantly impacted by levels of both the polymers, which was also observed from table 6-10.

Screening design summary for Particle size:

The particle size varied between 80µm (run 13) and 110µm (run 11). The main effect plot shows stirring speed has inverse relationship with particle size (Fig 6-2A). The particle size was mainly affected by the most significant parameter: the stirring speed of the double emulsion as suggested by pareto chart (Fig 6-2B) and p value < 0.05 from Table 6-10. Unexpectedly, stirring time does not have a significant influence on the particle size as suggested by p-value > 0.05 from Table 6-10. None of the other parameters were seem to be significantly affecting particle size as suggested by p-value > 0.05 from Table 6-10. However, the target particle size has been achieved in all the trials. Hence, maximum stirring speed studied in these trials was finalized for confirmatory trials.

Contour plot shows more impact of PLGA over PCL levels in reducing the particle size (Fig 6-2C), however, as all the trials resulted in desired particle size, this effect was considered nonsignificant.

Table 6- 11 Screening design summary for Particle size (D90)

Screening design model: D90 versus PLGA, PCL, PVA, SS, ST					
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	770.1	154.02	2.25	0.16
Linear	5	770.1	154.02	2.25	0.16
PLGA	1	2.5	2.5	0.04	0.854
PCL	1	12.1	12.1	0.18	0.687
PVA	1	44.1	44.1	0.64	0.449
SS	**1**	**688.9**	**688.9**	**10.05**	**0.016**
ST	1	22.5	22.5	0.33	0.585
Error	7	479.9	68.557		
Total	12	1250			
Model Summary					
S	R-sq	R-sq(adj)	R-sq(pred)		
8.27992	61.61%	34.19%	9.80%		
Coded Coefficients					
Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	96	2.3	41.8	0	
PLGA	-0.5	2.62	-0.19	0.854	1
PCL	1.1	2.62	0.42	0.687	1
PVA	-2.1	2.62	-0.8	0.449	1
SS	-8.3	2.62	-3.17	0.016	1
ST	1.5	2.62	0.57	0.585	1
Regression Equation in Uncoded Units					
D90 = 117.6 - 0.0067 PLGA + 0.0147 PCL - 4.67 PVA - 0.0415 SS + 0.75 ST					
Alias Structure (up to order 2)					
Factor	Name				
A	PLGA				
B	PCL				
C	PVA				
D	SS				
E	ST				
Aliases					
I + 0.77 AA + 0.77 BB + 0.77 CC + 0.77 DD + 0.77 EE					
A					
B					
C					
D					
E					
Fits and Diagnostics for Unusual Observations					
Obs	D90	Fit	Resid	Std Resid	
9	80	96	-16	-2.01	R

As observed from table 6-11, Stirring speed was found to be most impacting factor for particle size based on p-value (<0.05). The R^2 value for model was found to be 61.6% which was fairly good.

6.10.3 Effect on entrapment efficiency in screening design:

Figures 6-3A, 6-3B and 6-3C shows the main effect plot, pareto chart and contour plot for entrapment efficiency of Amisulpride and Figures 6-3D, 6-3E and 6-3F shows the main effect plot, pareto chart and contour plot for entrapment efficiency of Granisetron.

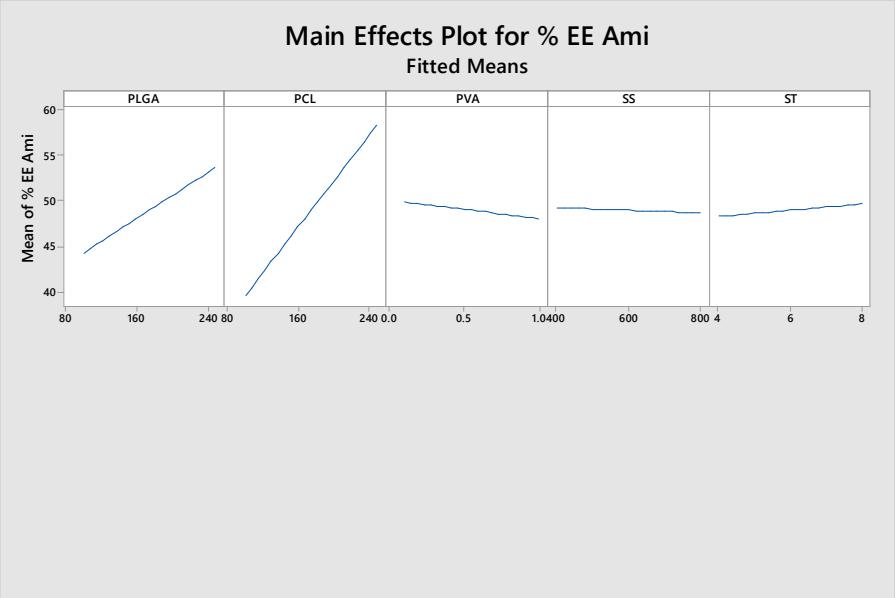

Figure 6-3A Main effect plot for Entrapment efficiency of Amisulpride

From fig 6-3A, it was observed that, concentration of both PCL and PLGA were found to be impacting % EE of amisulpride, PCL was the most impacting one among both. The concentrations were found directly proportional to % EE.

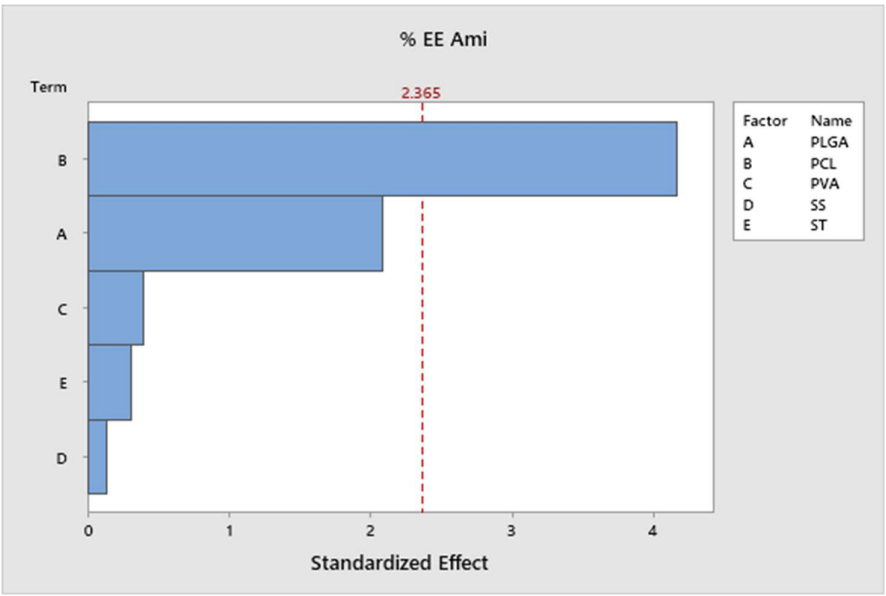

Figure 6-3B Pareto chart for Entrapment efficiency of Amisulpride in screening design

From fig 6-3B, it was observed that, PCL concentration was found to be most impacting factor for %EE of Amisulpride as it crosses the reference line marked in dotted red.

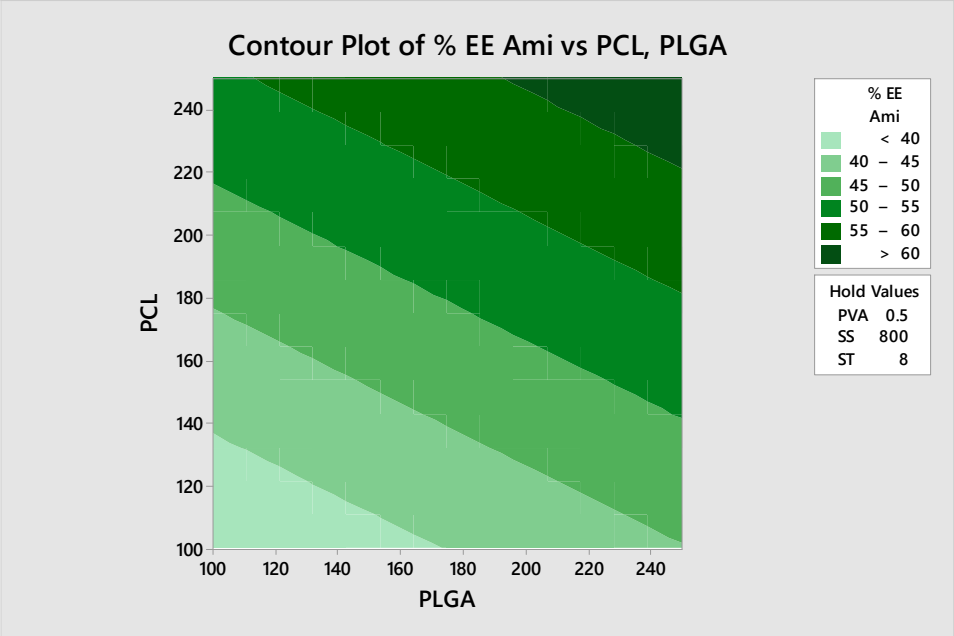

Figure 6-3C Contour plot for Entrapment efficiency of Amisulpride

From fig 6-3C, it was observed that, as the concentration of both polymers increased, the % EE of amisulpride was found to be increasing.

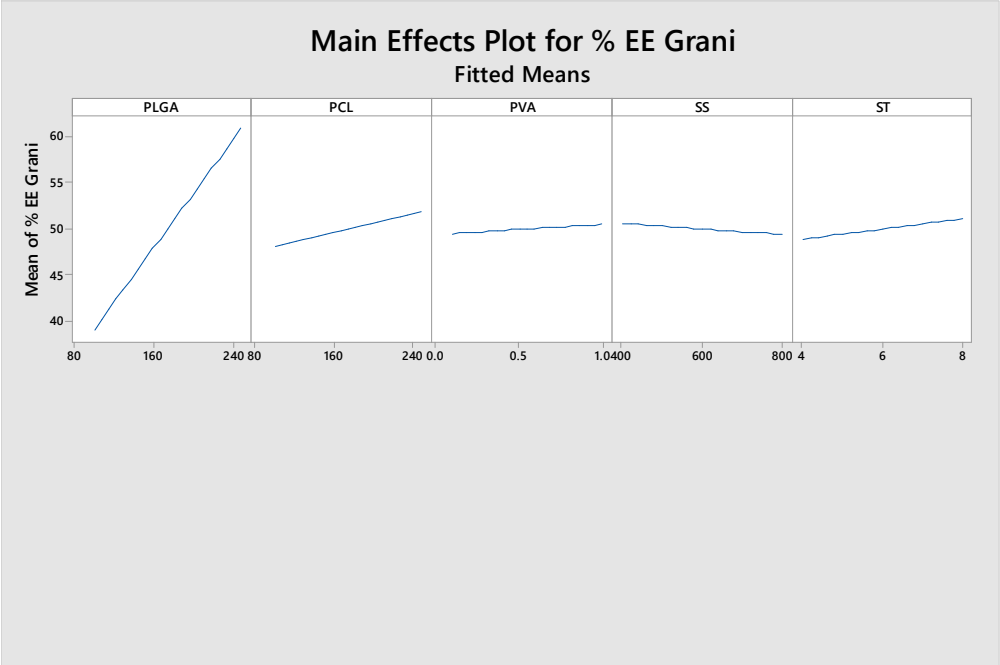

Figure 6-3D Main effect plot for Entrapment efficiency of Granisetron

From fig 6-3D, it was observed that, concentration of both PCL and PLGA were found to be impacting % EE of granisetron, PLGA was the most impacting one among both. The concentrations were found directly proportional to % EE.

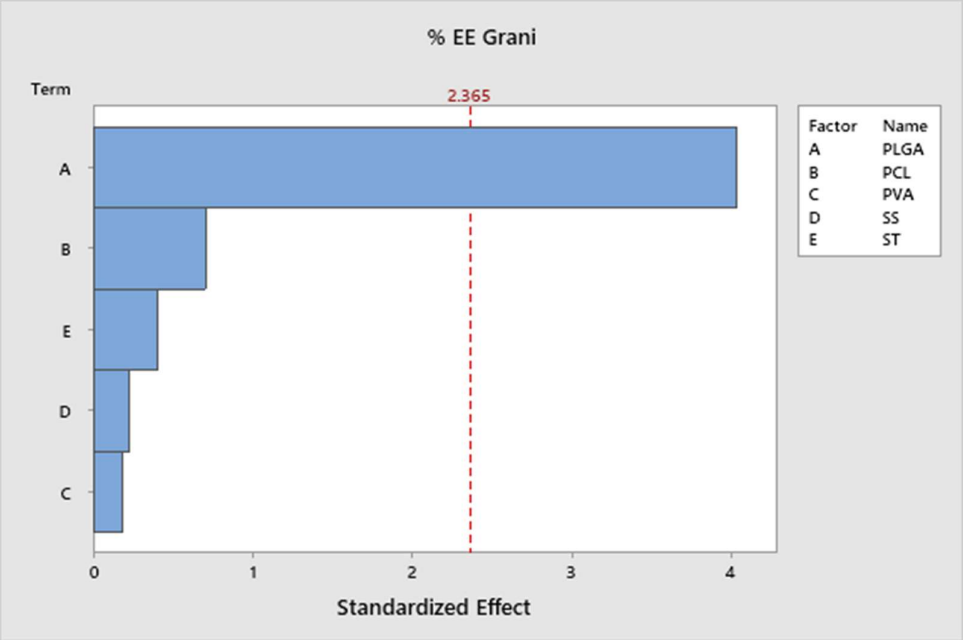

Figure 6-3E Pareto chart for Entrapment efficiency of Granisetron in screening design

From fig 6-3E, it was observed that, PLGA concentration was found to be most impacting factor for %EE of Granisetron as it crosses the reference line marked in dotted red.

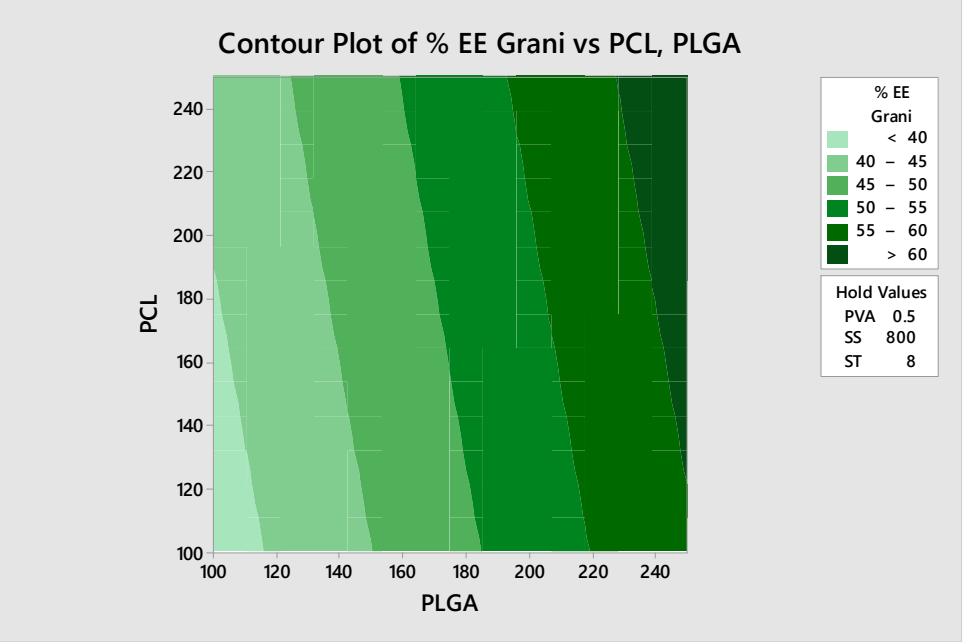

Figure 6-3F Contour plot for Entrapment efficiency of Granisetron

From fig 6-3F, it was observed that, as the concentration of both polymers increased, the % EE of granisetron was found to be increasing.

Screening design summary for Entrapment efficiency:

Within the DoE the % EE for amisulpride varied in a range from 32% to 62% and for granisetron from 32% to 65%. Main effects plot shows impact of both PLGA and PCL polymer levels on % EE of both the drugs in positive manner (Fig 6-3A and 6-3D). As per pareto chart % EE of amisulpride was significantly affected by PCL concentration and for % EE of granisetron was significantly affected by PLGA concentration (Fig 6-3B and Fig 6-3E). This was also supported by p values < 0.05 in Table 6-10. This observation was in-line with the literature [20] that hydrophobic drug amisulpride has more affinity towards relatively more hydrophobic polymer PCL and hydrophilic drug granisetron has more affinity towards hydrophilic polymer PLGA. Contour plots (Fig 6-3C and 6-3F) suggests 1:1 ratio of polymers (PLGA -250 mg: PCL-250 mg in this case) will result in maximum % entrapment efficiency. None of the other factors were found to be impacting the entrapment efficiency of either amisulpride or granisetron significantly as suggested by p-value > 0.05 from Table 6-10.

Table 6-12 and 6-13 shows the screening design summary for entrapment efficiency of amisulpride and granisetron respectively.

Table 6- 12 Screening design summary for Entrapment efficiency of Amisulpride

Screening design model: % EE Ami versus PLGA, PCL, PVA, SS, ST					
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	1118.4	223.68	4.4	0.039
Linear	5	1118.4	223.68	4.4	0.039
PLGA	1	220.9	220.9	4.35	0.075
PCL	**1**	**883.6**	**883.6**	**17.39**	**0.004**
PVA	1	8.1	8.1	0.16	0.702
SS	1	0.9	0.9	0.02	0.898
ST	1	4.9	4.9	0.1	0.765
Error	7	355.6	50.8		
Total	12	1474			
Model Summary					
S	R-sq	R-sq(adj)	R-sq(pred)		
7.12741	75.88%	58.64%	37.92%		
Coded Coefficients					
Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	49	1.98	24.79	0	
PLGA	4.7	2.25	2.09	0.075	1
PCL	9.4	2.25	4.17	0.004	1
PVA	-0.9	2.25	-0.4	0.702	1
SS	-0.3	2.25	-0.13	0.898	1
ST	0.7	2.25	0.31	0.765	1
Regression Equation in Uncoded Units					
% EE Ami = 16.0 + 0.0627 PLGA + 0.1253 PCL - 2.00 PVA - 0.0015 SS + 0.35 ST					
Alias Structure (up to order 2)					
Factor	Name				
A	PLGA				
B	PCL				
C	PVA				
D	SS				
E	ST				
Aliases					
I + 0.77 AA + 0.77 BB + 0.77 CC + 0.77 DD + 0.77 EE					
A					
B					
C					
D					
E					

From table 6-14, PCL concentration was found to be most impacting factor for % EE of amisulpride based on p-value (<0.05). The R^2 value for model was found to be 75.88% which was fairly good.

Table 6- 13 Screening design summary for Entrapment efficiency of Granisetron

Screening design model: % EE Grani versus PLGA, PCL, PVA, SS, ST					
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	1242.4	248.48	3.41	0.07
Linear	5	1242.4	248.48	3.41	0.07
PLGA	**1**	**1188.1**	**1188.1**	**16.32**	**0.005**
PCL	1	36.1	36.1	0.5	0.504
PVA	1	2.5	2.5	0.03	0.858
SS	1	3.6	3.6	0.05	0.83
ST	1	12.1	12.1	0.17	0.696
Error	7	509.6	72.8		
Total	12	1752			
Model Summary					
S	R-sq	R-sq(adj)	R-sq(pred)		
8.53229	70.91%	50.14%	22.03%		
Coded Coefficients					
Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	50	2.37	21.13	0	
PLGA	10.9	2.7	4.04	0.005	1
PCL	1.9	2.7	0.7	0.504	1
PVA	0.5	2.7	0.19	0.858	1
SS	-0.6	2.7	-0.22	0.83	1
ST	1.1	2.7	0.41	0.696	1
Regression Equation in Uncoded Units					
% EE Grani = 18.0 + 0.1453 PLGA + 0.0253 PCL + 1.11 PVA - 0.0030 SS + 0.55 ST					
Alias Structure (up to order 2)					
Factor	Name				
A	PLGA				
B	PCL				
C	PVA				
D	SS				
E	ST				
Aliases					
I + 0.77 AA + 0.77 BB + 0.77 CC + 0.77 DD + 0.77 EE					
A					
B					
C					
D					
E					

From table 6-13, PLGA concentration was found to be most impacting factor for % EE of granisetron based on p-value (<0.05). The R^2 value for model was found to be 70.91 % which was fairly good.

6.10.4 Effect on Drug Loading in screening design:

Figures 6-4A, 6-4B and 6-4C shows the main effect plot, pareto chart and contour plot for drug loading of Amisulpride and Figures 6-4D, 6-4E and 6-4F shows the main effect plot, pareto chart and contour plot for drug loading of Granisetron.

Figure 6-4A Main effect plot for Drug loading of Amisulpride

From fig 6-4A, it was observed that, concentration of both PCL and PLGA were found to be impacting % DL of amisulpride, PCL was the most impacting one among both. The concentrations were found directly proportional to % DL.

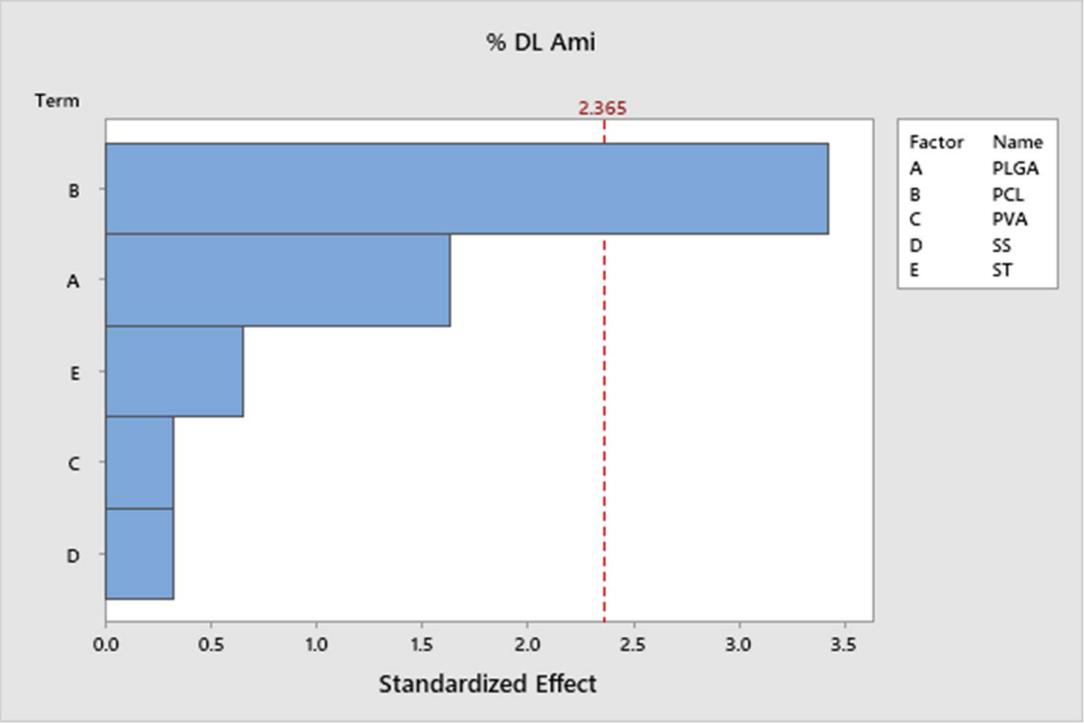

Figure 6-4B Pareto chart for Drug loading of Amisulpride in screening design

From fig 6-4B, it was observed that, PCL concentration was found to be most impacting factor for %DL of Amisulpride as it crosses the reference line marked in dotted red.

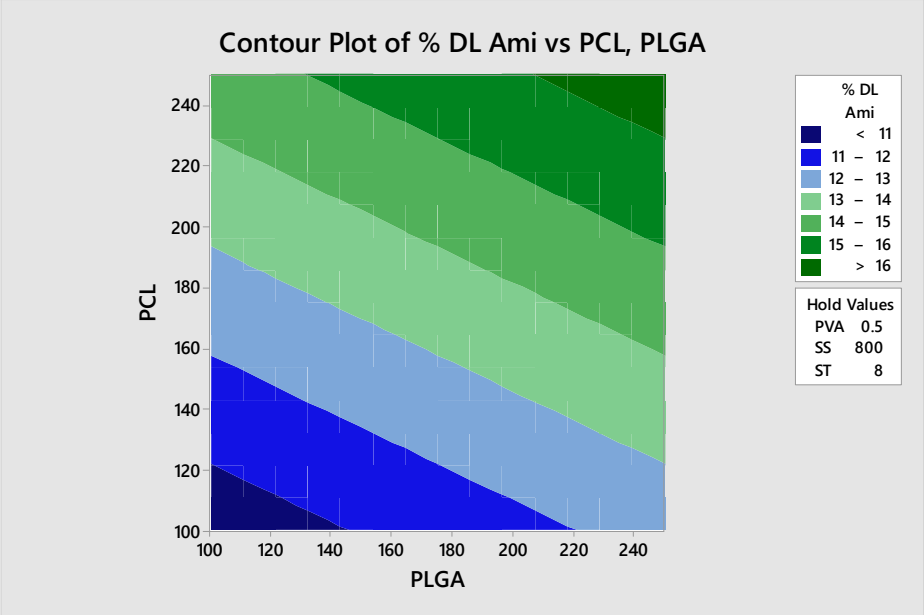

Figure 6-4C Contour plot for Drug loading of Amisulpride

From fig 6-4C, it was observed that, as the concentration of both polymers increased, the % DL of amisulpride was found to be increasing.

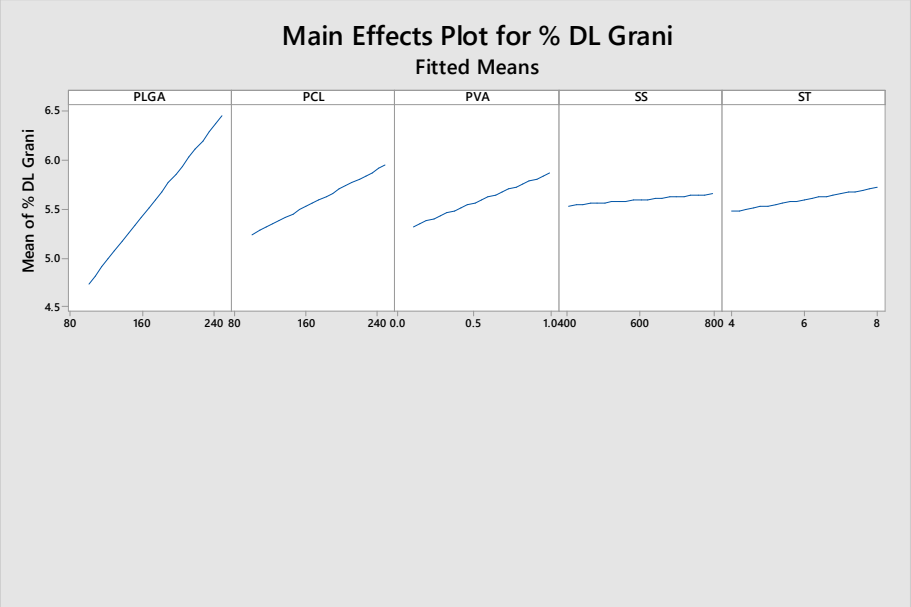

Figure 6-4D Main effect plot for Drug loading of Granisetron

From fig 6-4D, it was observed that, concentration of both PCL and PLGA were found to be impacting % DL of granisetron, PLGA was the most impacting one among both. The concentrations were found directly proportional to % DL.

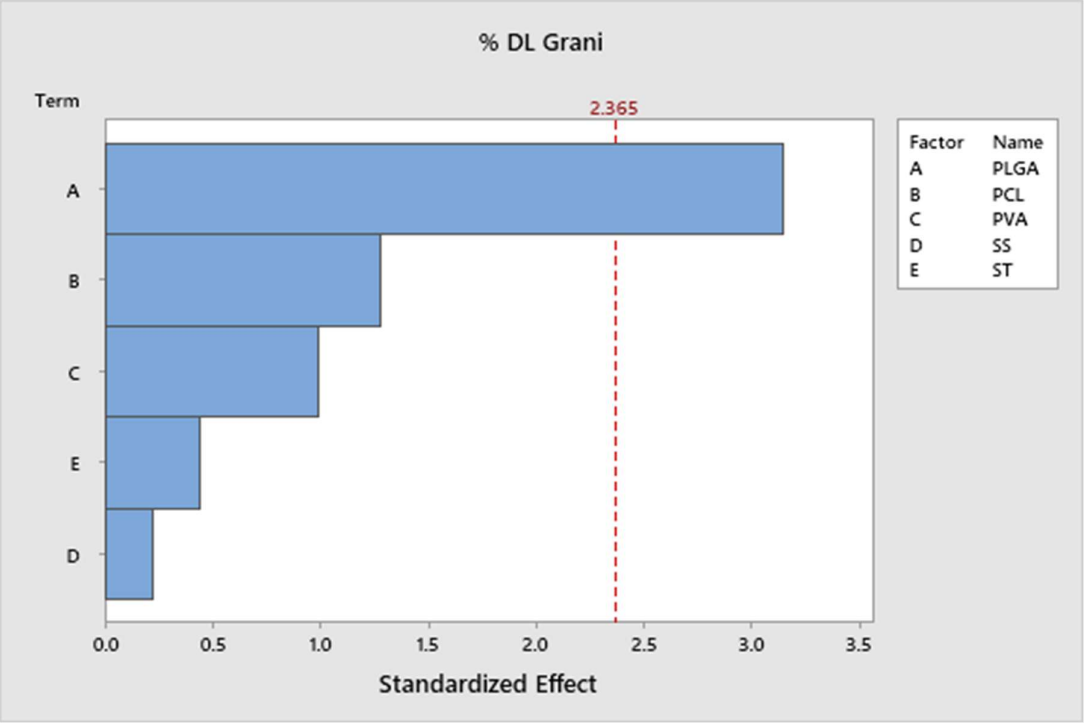

Figure 6-4E Pareto chart for Drug loading of Granisetron in screening design

From fig 6-4E, it was observed that, PLGA concentration was found to be most impacting factor for %DL of Granisetron as it crosses the reference line marked in dotted red.

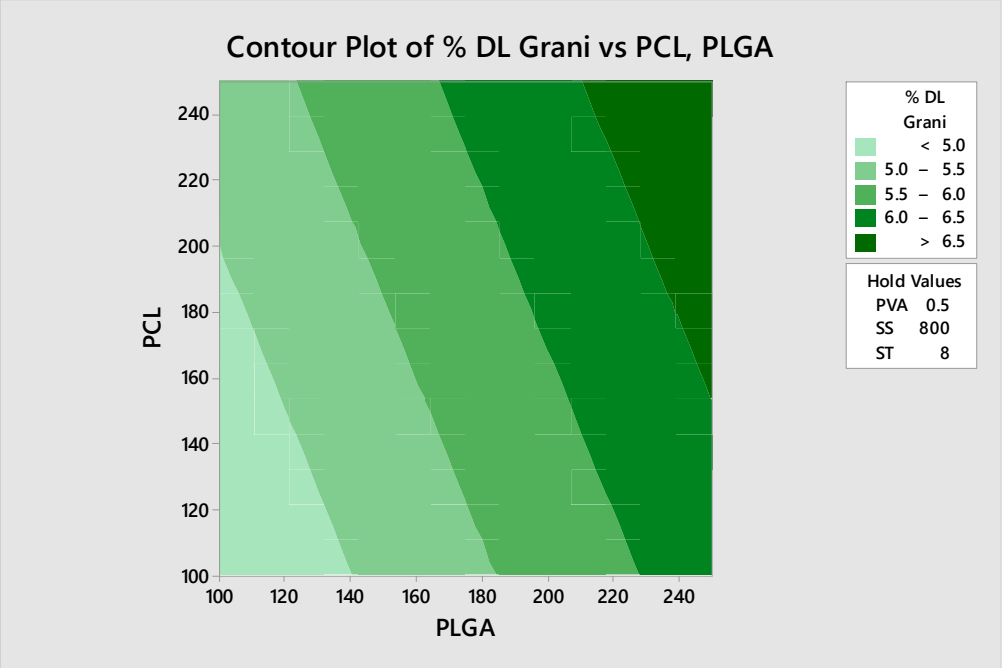

Figure 6-4F Contour plot for Drug loading of Granisetron

From fig 6-4F, it was observed that, as the concentration of both polymers increased, the % DL of granisetron was found to be increasing

Screening design summary for Drug loading:

Within the DoE the % DL for amisulpride varied in a range from 8% to 17% and for granisetron from 4% to 6.9%. Main effects plot shows impact of both PLGA and PCL polymer levels on % EE of both the drugs in positive manner (Fig 6-4A and 6-4D). Similar to % entrapment efficiency, for amisulpride % DL, significant effect was observed for PCL concentration and for granisetron % EE, significant effect was observed with PLGA concentration as per pareto chart (Fig 6-4B and Fig 6-4E) and p values < 0.05 from Table 6-16. Contour plots (Fig 6-4C and 6-4F) suggests 1:1 ratio of polymers (PLGA -250 mg: PCL-250 mg in this case) will result in maximum % drug loading.

Table 6-14 and 6-15 shows the screening design summary for drug loading of amisulpride and granisetron respectively.

Table 6- 14 Screening design summary for Drug loading of Amisulpride

Screening design model: % DL Ami versus PLGA, PCL, PVA, SS, ST					
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	56.5	11.3	3.01	0.092
Linear	5	56.5	11.3	3.01	0.092
PLGA	1	10	10	2.66	0.147
PCL	**1**	**44.1**	**44.1**	**11.75**	**0.011**
PVA	1	0.4	0.4	0.11	0.754
SS	1	0.4	0.4	0.11	0.754
ST	1	1.6	1.6	0.43	0.535
Error	7	26.2692	3.7527		
Total	12	82.7692			
Model Summary					
S	R-sq	R-sq(adj)	R-sq(pred)		
1.9372	68.26%	45.59%	9.62%		
Coded Coefficients					
Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	13.308	0.537	24.77	0	
PLGA	1	0.613	1.63	0.147	1
PCL	2.1	0.613	3.43	0.011	1
PVA	0.2	0.613	0.33	0.754	1
SS	-0.2	0.613	-0.33	0.754	1
ST	0.4	0.613	0.65	0.535	1
Regression Equation in Uncoded Units					
% DL Ami = 5.23 + 0.01333 PLGA + 0.02800 PCL + 0.44 PVA - 0.00100 SS + 0.200 ST					
Alias Structure (up to order 2)					
Factor	Name				
A	PLGA				
B	PCL				
C	PVA				
D	SS				
E	ST				
Aliases					
I + 0.77 AA + 0.77 BB + 0.77 CC + 0.77 DD + 0.77 EE					
A					
B					
C					
D					
E					

From table 6-14, PCL concentration was found to be most impacting factor for % DL of amisulpride based on p-value (<0.05). The R^2 value for model was found to be 68.26 % which was fairly good.

Table 6- 15 Screening design summary for Drug loading of Granisetron

Screening design model: % DL Grani versus PLGA, PCL, PVA, SS, ST					
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	9.53	1.906	2.56	0.127
Linear	5	9.53	1.906	2.56	0.127
PLGA	**1**	**7.396**	**7.396**	**9.92**	**0.016**
PCL	1	1.225	1.225	1.64	0.241
PVA	1	0.729	0.729	0.98	0.356
SS	1	0.036	0.036	0.05	0.832
ST	1	0.144	0.144	0.19	0.674
Error	7	5.2192	0.7456		
Total	12	14.7492			
Model Summary					
S	R-sq	R-sq(adj)	R-sq(pred)		
0.863484	64.61%	39.34%	0.00%		
Coded Coefficients					
Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5.592	0.239	23.35	0	
PLGA	0.86	0.273	3.15	0.016	1
PCL	0.35	0.273	1.28	0.241	1
PVA	0.27	0.273	0.99	0.356	1
SS	0.06	0.273	0.22	0.832	1
ST	0.12	0.273	0.44	0.674	1
Regression Equation in Uncoded Units					
% DL Grani = 1.90 + 0.01147 PLGA + 0.00467 PCL + 0.600 PVA + 0.00030 SS + 0.060 ST					
Alias Structure (up to order 2)					
Factor	Name				
A	PLGA				
B	PCL				
C	PVA				
D	SS				
E	ST				
Aliases					
I + 0.77 AA + 0.77 BB + 0.77 CC + 0.77 DD + 0.77 EE					
A					
B					
C					
D					
E					

From table 6-15, PLGA concentration was found to be most impacting factor for % DL of granisetron based on p-value (<0.05). The R^2 value for model was found to be 64.61 % which was fairly good.

Surprisingly, the stabilizer PVA concentration did not showed significant effect on any of the responses. This may be attributed to other factors masking the effect of PVA.

Minitab 18 was used to optimize the following parameters for a target size of 90-100 µm, maximised entrapment and drug loading: PLGA = 250 mg; PCL = 250 mg; SS = 800 rpm; ST = 8 hours and PVA = 0.5% as shown in Fig 6-5. The composite desirability was observed to be 0.90. These values resulted in desired optimum responses and hence increased confidence in the model.

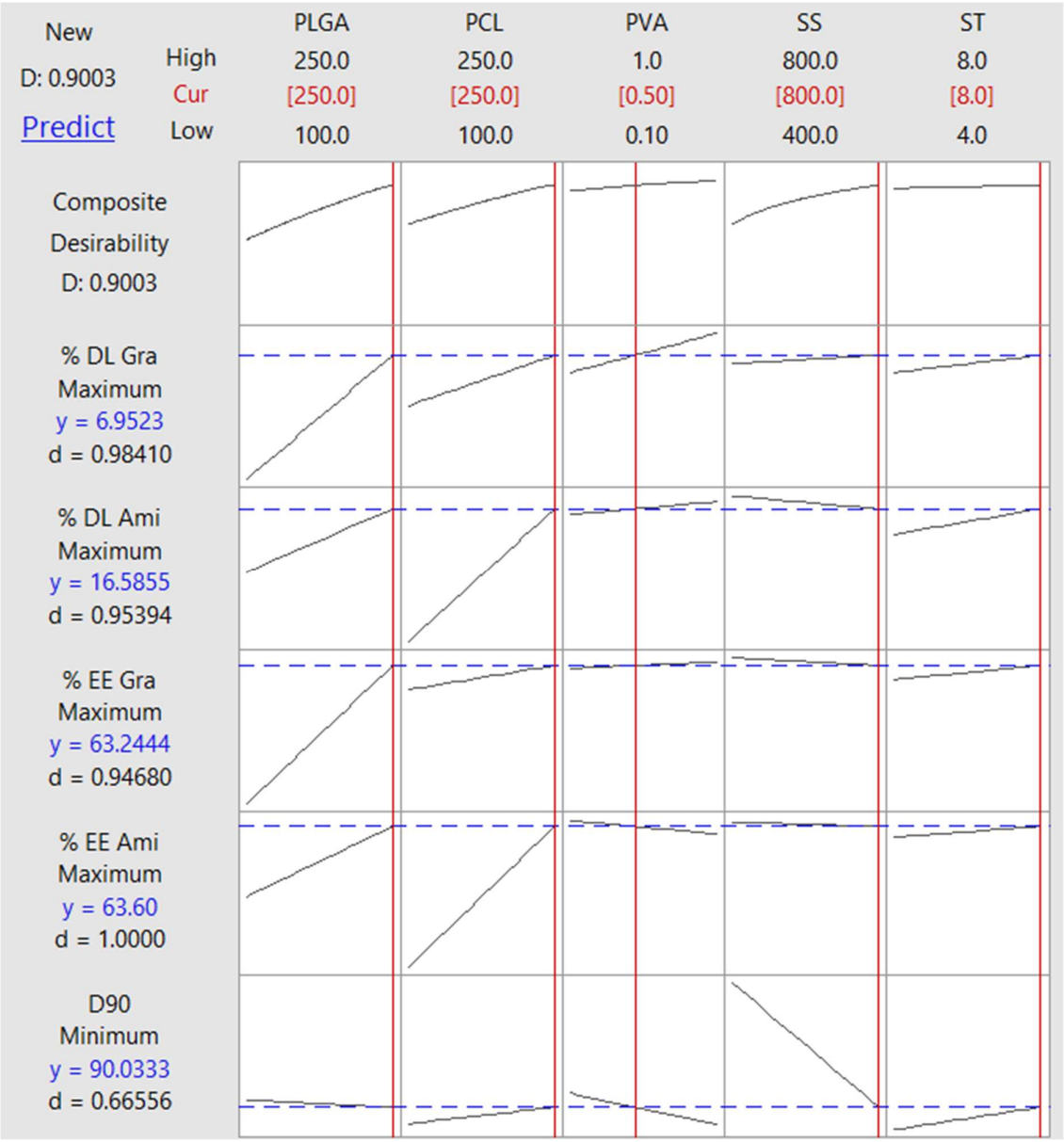

Figure 6-5 Response optimization plot

Response optimization plot in Figure 6-5 showed that, with the selected polymer levels of 250 mg each, PVA concentration of 0.5%, stirring speed of 800 and stirring time of 8 hours, desired formulation CQA's can be achieved with composite desirability of 0.9003.

6.10.5 Response surface design:

Confirmatory trials were taken for further optimization of formulation and study the effect of drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio. The trial summary is presented in table 6-16. The objective of this study was to check and optimize the drug release of the formulations, since drug release is governing factor for *in-vivo* response.

Table 6-16 Trial summary for confirmatory trials

Std Order	Run Order	Blocks	Pt Type	Drug: Polymer Ratio	(PLGA: PCL ratio)	D90 (µm)	% EE Ami	% EE Grani	% DL Ami	% DL Grani	% DR 4-hour Ami	% DR 4-hour Grani
1	1	1	1	0.2	0.25	85	65	58	16	6.5	48	37
2	2	1	1	0.2	0.67	92	60	55	15	6	42	36
3	3	1	1	0.2	1.00	89	58	56	15	5.7	46	34
4	4	1	1	0.2	1.50	88	55	58	14	6	48	29
5	5	1	1	0.2	4.00	87	55	63	13.9	6.4	52	27
6	6	1	1	0.3	0.25	93	62	55	15.5	5.8	41	35
7	7	1	1	0.3	0.67	85	60	55	14.5	5.9	42	35
8	8	1	1	0.3	1.00	83	61	58	16	6	45	33
9	9	1	1	0.3	1.50	82	55	62	15.6	6.1	48	30
10	10	1	1	0.3	4.00	88	55	65	15.2	6.6	53	28
11	11	1	1	0.4	0.25	95	63	55	15.9	5.8	48	42
12	12	1	1	0.4	0.67	92	62	56	15.5	5.6	43	35
13	13	1	1	0.4	1.00	91	59	59	14.4	5.9	44	32
14	14	1	1	0.4	1.50	93	52	60	14	6	48	30
15	15	1	1	0.4	4.00	94	58	62	14.5	6.2	51	32

Drug to polymer ratio means ratio of both drugs to total amount of polymers, where the two drugs were in their optimized ratio. Drug release at 4 hours was chosen as it was most significant CQA representing *in-vivo* area under the curve. Minitab software Version 18 was used to analyze the experiments using response surface platform. Responses to individual trial are presented in respective columns.

Confirmatory trial experiments showed the impact of drug: polymer ratio and polymer: polymer ratio (PLGA: PCL) on the drug product CQAs.

The table 6-17 shows significance of factors on selected responses. As can be seen from table 6-17, both drug: polymer ratio and the square interaction affected particle size as seen from p-value < 0.05. Polymer to polymer ratio has shown significant impact on all the responses except particle size as seen from p-value < 0.05. Some square terms of polymer: polymer ratio was found significant for % EE amisulpride and % DR granisetron as seen from p-value < 0.05.

Table 6-17 Significance of parameters on responses and model summary for confirmatory trials

Outcome Parameter	**D90**	**% EE Ami**	**% EE Grani**	**% DL Ami**	**% DL Grani**	**% DR Ami**	**% DR Grani**
Drug: Polymer ratio	**Y (p =0.048)**	N	N	N	N	N	N
Polymer: polymer ratio	N	**Y (p =0.00)**	**Y (p =0)**	**Y (p =0.038)**	**Y (p =0.029)**	**Y (p =0.003)**	**Y (p =0.00)**
Drug: Polymer Ratio*Drug: Polymer Ratio	**Y (p =0.033)**	N	N	N	N	N	N
Polymer to Polymer ratio*Polymer to Polymer ratio	N	**Y (p =0.001)**	N	N	N	N	**Y (p =0.002)**
Drug: Polymer Ratio*Polymer to Polymer ratio	N	N	N	N	N	N	N
R^2 for model	61.5	74.0	70.9	52.3	58.9	66.3	86.31

Note: Y: Significant N: Not significant

6.10.6 Effect on particle Size in confirmatory trials:

Figures 6-6A and 6-6B shows the pareto chart and surface plot for particle size. Table 6-18 shows the confirmatory trial statistical summary for Particle size.

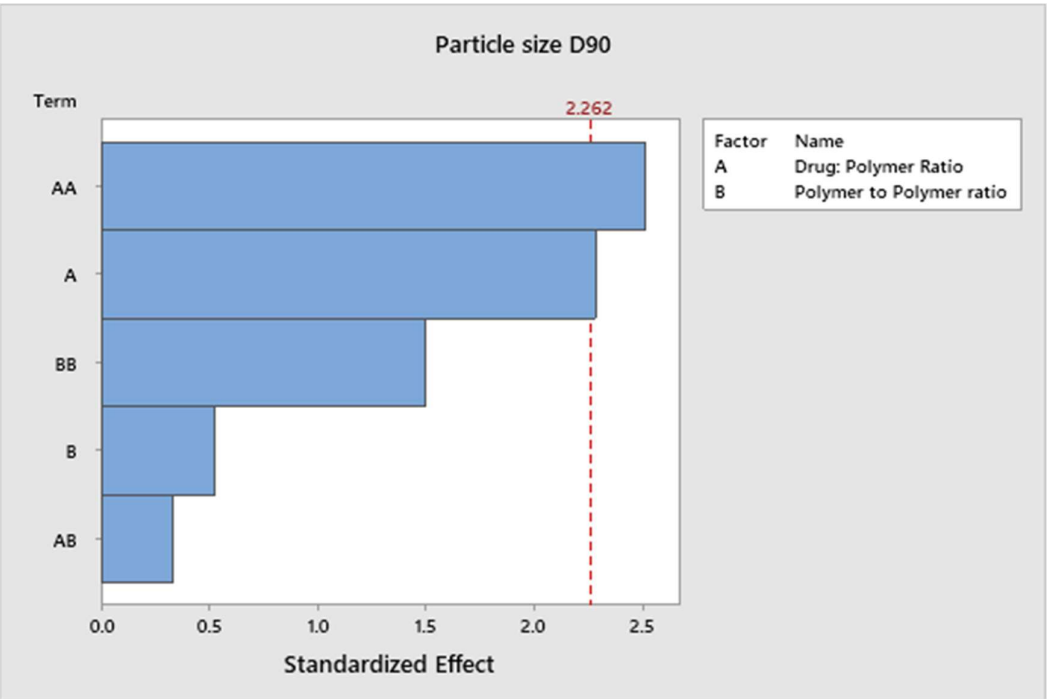

Figure 6-6A Pareto chart of Particle size D90 in confirmatory trials

From fig 6-6A, it was observed that, both drug: polymer ratio and its square term were found to be most impacting factor for particle size. However, as this CQA was found to be within acceptable limit in the studied ranges, this effect was not considered significant.

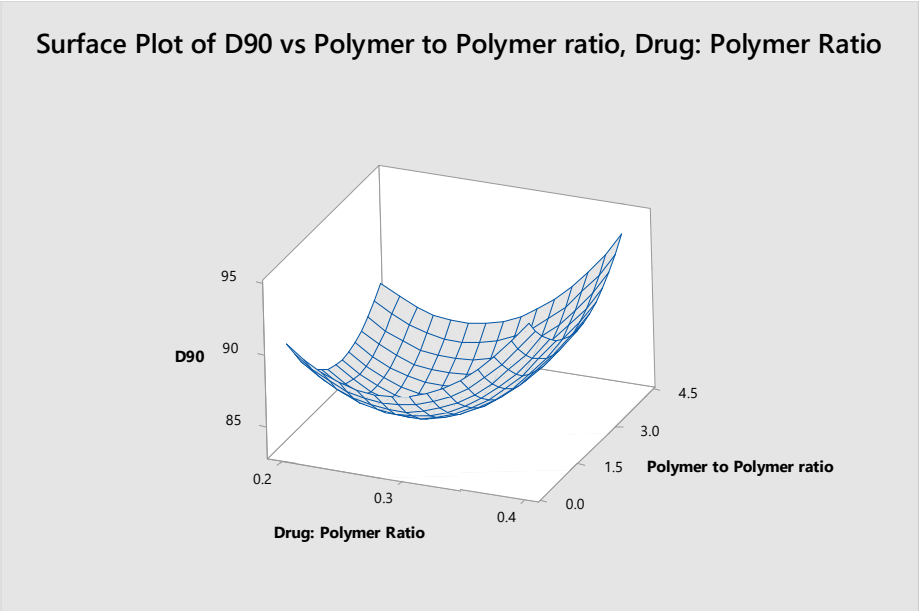

Figure 6-6B Surface plot for particle size D90

Fig 6-6B, surface plot of drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio against CQA particle size (D90) showed that the surface looks curved as the model contains statistically significant quadratic terms. The highest values of D90 are in the upper right corner of the plot, which corresponds with high values of both drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio. The lowest values of D90 are in the lower left corner of the plot, which corresponds with low values of both of both drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio.

Confirmatory trials summary for Particle size:

As seen from the screening design trials and also conformed from confirmatory trials, the desired particle size (D90 of 100 ± 20 microns) was achieved in all the trials. The selected parameters are consistently providing the desired particle size.

Table 6-18 Response surface for Particle size (D90)

Response Surface Regression: D90					
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	146.143	29.229	2.87	0.08
Linear	2	55.896	27.948	2.75	0.117
Drug: Polymer Ratio	**1**	**53.111**	**53.111**	**5.22**	**0.048**
Polymer to Polymer ratio	1	2.785	2.785	0.27	0.614
Square	2	87.264	43.632	4.29	0.049
Drug: Polymer Ratio*Drug: Polymer Ratio	**1**	**64.533**	**64.533**	**6.34**	**0.033**
Polymer to Polymer ratio*Polymer to Polymer ratio	1	22.731	22.731	2.23	0.169
2-Way Interaction	1	1.106	1.106	0.11	0.749
Drug: Polymer Ratio*Polymer to Polymer ratio	1	1.106	1.106	0.11	0.749
Error	9	91.59	10.177		
Total	14	237.733			
Model Summary					
S	R-sq	R-sq(adj)	R-sq(pred)		
3.19009	61.47%	40.07%	0.00%		
Coded Coefficients					
Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	83.74	2.19	38.3	0	
Drug: Polymer Ratio	2.56	1.12	2.28	0.048	1.24
Polymer to Polymer ratio	-0.63	1.21	-0.52	0.614	1.08
Drug: Polymer Ratio*Drug: Polymer Ratio	4.4	1.75	2.52	0.033	1
Polymer to Polymer ratio*Polymer to Polymer ratio	3.64	2.44	1.49	0.169	1.08
Drug: Polymer Ratio*Polymer to Polymer ratio	0.47	1.43	0.33	0.749	1.24
Regression Equation in Uncoded Units					
D90 = 122.7 - 244 Drug: Polymer Ratio - 5.49 Polymer to Polymer ratio + 440 Drug: Polymer Ratio* Drug: Polymer Ratio+ 1.035 Polymer to Polymer ratio*Polymer to Polymer ratio+ 2.51 Drug: Polymer Ratio *Polymer to Polymer ratio					
Fits and Diagnostics for Unusual Observations					
Obs	D90	Fit	Resid	Std Resid	
1	85	90.33	-5.33	-2.3	R
R Large residual					

From table 6-18, Drug: polymer ratio and its square term both were found to be impacting particle size based on p-value of <0.05. However, as the desired particle size was already achieved in the screening DOE, these factors are less significant at this stage of experimentation. The R^2 value of model is 61.47%, which is fairly good.

6.10.7 Effect on Entrapment efficiency in confirmatory trials:

Figures 6-7A and 6-7B shows the pareto chart and surface plot for % entrapment efficiency of amisulpride. Figure 6-7C and 6-7D shows the pareto chart and surface plot for % entrapment efficiency of granisetron. Table 6-19 and 6-20 shows the confirmatory trial statistical summary for % entrapment efficiency of amisulpride and granisetron respectively.

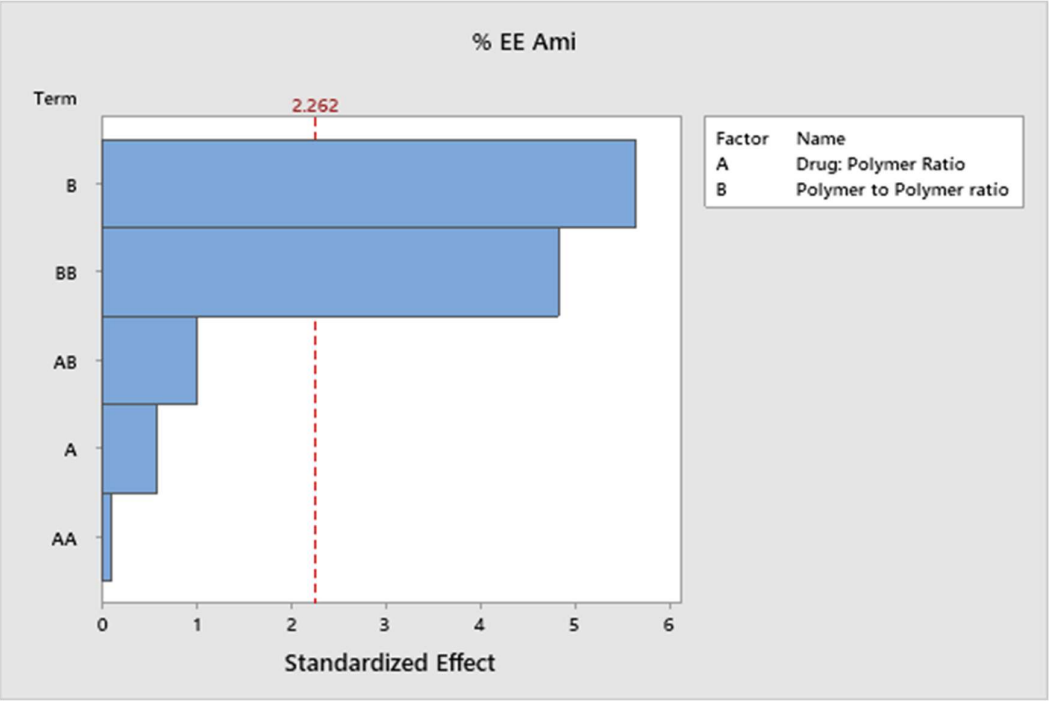

Figure 6-7A Pareto chart for EE of Amisulpride in confirmatory trials

From fig 6-7A, it was observed that, both polymer: polymer (PLGA: PCL) ratio and its square term were found to be most impacting factor for % EE of amisulpride.

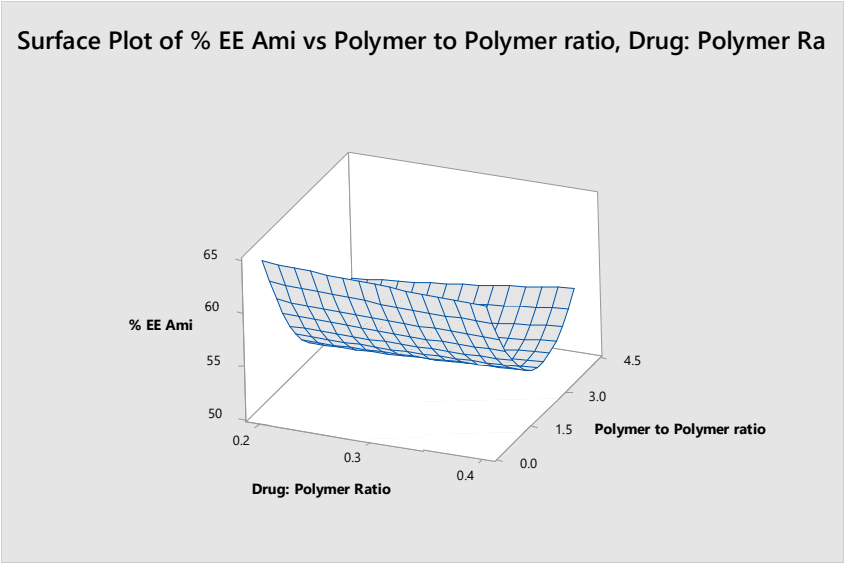

Figure 6-7B Surface plot for EE Amisulpride

Fig 6-7B, surface plot of drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio against CQA % EE of amisulpride showed that the surface looks curved as the model contains statistically significant quadratic terms. The highest values of % EE of amisulpride is in the upper right corner of the plot, which corresponds with high values of both drug: polymer ratio and polymer: polymer ratio. The lowest values of % EE of amisulpride is in the lower left corner of the plot, which corresponds with low values of both of both drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio.

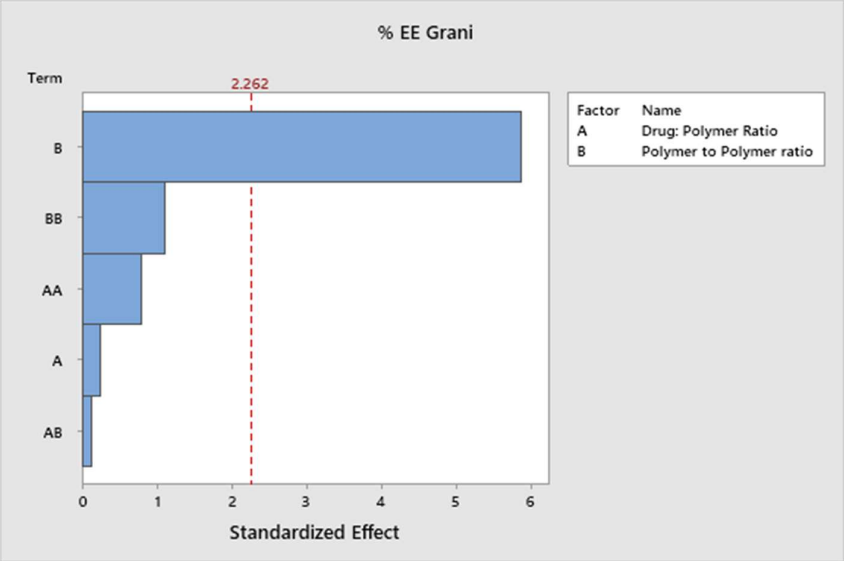

Figure 6-7C Pareto chart for EE of Granisetron in confirmatory trials

From fig 6-7C, it was observed that, polymer: polymer (PLGA: PCL) ratio was found to be most impacting factor for % EE of granisetron.

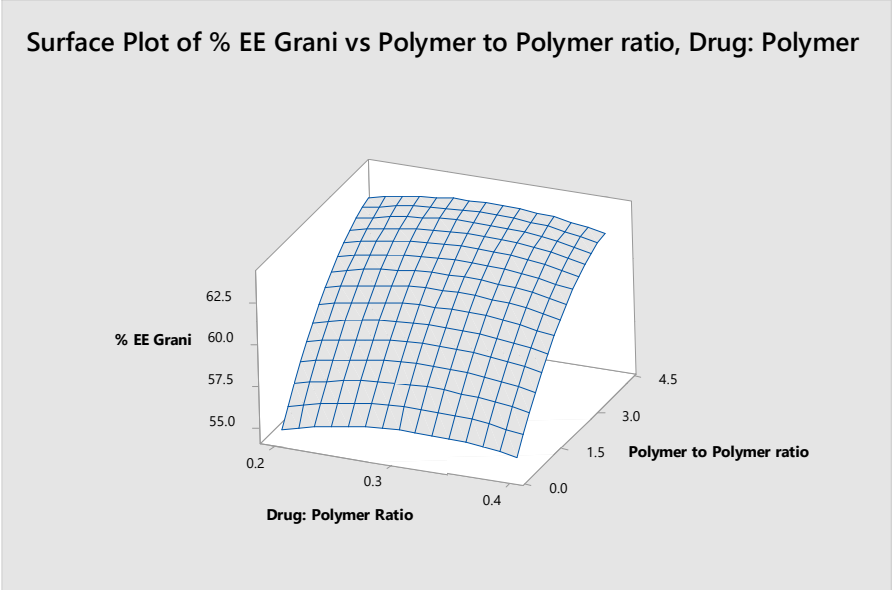

Figure 6-7D Surface plot for EE Granisetron

Fig 6-7D, surface plot of drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio against CQA % EE of granisetron showed that the surface is not curved because the model is not affected by interaction terms. The highest values of % EE of granisetron is in the upper right corner of the plot, which corresponds with high values of both drug: polymer ratio and polymer: polymer ratio. The lowest values of % EE of granisetron is in the lower left corner of the plot, which corresponds with low values of both of both drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio.

Confirmatory trials summary for % entrapment efficiency:

The % entrapment efficiency of both amisulpride and granisetron was affected by polymer: polymer (PLGA: PCL) ratio. The square term of polymer: polymer (PLGA: PCL) ratio also impacted % entrapment efficiency of amisulpride. Drug: polymer ratio did not seem to impact the CQA as p-value > 0.05 as seen from table 6-17.

Table 6-19 Response surface for Entrapment efficiency of Amisulpride

Response Surface Regression: % EE Ami					
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	157.668	31.534	8.96	0.003
Linear	2	113.349	56.674	16.11	0.001
Drug: Polymer Ratio	1	1.217	1.217	0.35	0.571
Polymer to Polymer ratio	**1**	**112.132**	**112.132**	**31.87**	**0**
Square	2	82.009	41.005	11.65	0.003
Drug: Polymer Ratio*Drug: Polymer Ratio	1	0.033	0.033	0.01	0.925
Polymer to Polymer ratio*Polymer to Polymer ratio	**1**	**81.976**	**81.976**	**23.3**	**0.001**
2-Way Interaction	1	3.519	3.519	1	0.343
Drug: Polymer Ratio*Polymer to Polymer ratio	1	3.519	3.519	1	0.343
Error	9	31.665	3.518		
Total	14	189.333			
Model Summary					
S	R-sq	R-sq(adj)	R-sq(pred)		
1.87572	83.28%	73.98%	51.99%		
Coded Coefficients					
Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	52.97	1.29	41.2	0	
Drug: Polymer Ratio	0.388	0.659	0.59	0.571	1.24
Polymer to Polymer ratio	-4.019	0.712	-5.65	0	1.08
Drug: Polymer Ratio*Drug: Polymer Ratio	0.1	1.03	0.1	0.925	1
Polymer to Polymer ratio*Polymer to Polymer ratio	6.91	1.43	4.83	0.001	1.08
Drug: Polymer Ratio*Polymer to Polymer ratio	0.841	0.84	1	0.343	1.24
Regression Equation in Uncoded Units					
% EE Ami = 69.00 - 11.6 Drug: Polymer Ratio - 11.84 Polymer to Polymer ratio + 10 Drug: Polymer Ratio*Drug: Polymer Ratio + 1.966 Polymer to Polymer ratio*Polymer to Polymer ratio + 4.48 Drug: Polymer Ratio*Polymer to Polymer ratio					
Fits and Diagnostics for Unusual Observations					
Obs	% EE Ami	Fit	Resid	Std Resid	
14	52	55.29	-3.29	-2.17	R
R Large residual					

From table 6-19, polymer: polymer (PLGA: PCL) ratio and its square term both were found to be impacting % EE of Amisulpride based on p-value of <0.05. The R^2 value of model is 83.28%, which shows model is sufficiently powered.

Table 6-20 Response surface for Entrapment efficiency of Granisetron

Response Surface Regression: % EE Grani					
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	121.107	24.221	7.12	0.006
Linear	2	117.992	58.996	17.34	0.001
Drug: Polymer Ratio	1	0.211	0.211	0.06	0.809
Polymer to Polymer ratio	**1**	**117.781**	**117.781**	**34.61**	**0**
Square	2	6.384	3.192	0.94	0.427
Drug: Polymer Ratio*Drug: Polymer Ratio	1	2.133	2.133	0.63	0.449
Polymer to Polymer ratio*Polymer to Polymer ratio	1	4.251	4.251	1.25	0.293
2-Way Interaction	1	0.063	0.063	0.02	0.895
Drug: Polymer Ratio*Polymer to Polymer ratio	1	0.063	0.063	0.02	0.895
Error	9	30.627	3.403		
Total	14	151.733			
Model Summary					
S	R-sq	R-sq(adj)	R-sq(pred)		
1.84471	79.82%	68.60%	17.46%		
Coded Coefficients					
Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	61.38	1.26	48.54	0	
Drug: Polymer Ratio	0.162	0.648	0.25	0.809	1.24
Polymer to Polymer ratio	4.119	0.7	5.88	0	1.08
Drug: Polymer Ratio*Drug: Polymer Ratio	-0.8	1.01	-0.79	0.449	1
Polymer to Polymer ratio*Polymer to Polymer ratio	-1.57	1.41	-1.12	0.293	1.08
Drug: Polymer Ratio*Polymer to Polymer ratio	-0.112	0.827	-0.14	0.895	1.24
Regression Equation in Uncoded Units					
% EE Grani=46.62 + 50.9 Drug: Polymer Ratio + 4.28 Polymer to Polymer ratio- 80 Drug: Polymer Ratio*Drug: Polymer Ratio- 0.448 Polymer to Polymer r atio*Polymer to Polymer ratio- 0.60 Drug: Polymer Ratio*Polymer to Polymer ratio					
Fits and Diagnostics for Unusual Observations					
Obs	% EE Grani	Fit	Resid	Std Resid	
1	58	54.61	3.39	2.53	R
R Large residual					

From table 6-20, polymer: polymer (PLGA: PCL) ratio was found to be impacting % EE of Granisetron based on p-value of <0.05. The R^2 value of model is 79.82%, which shows model is sufficiently powered.

6.10.8 Effect on drug loading in confirmatory trials:

Figures 6-8A and 6-8B shows the pareto chart and surface plot for % drug loading of amisulpride. Figure 6-8C and 6-8D shows the pareto chart and surface plot for % drug loading of granisetron. Table 6-21 and 6-22 shows the confirmatory trial statistical summary for % drug loading of amisulpride and granisetron respectively.

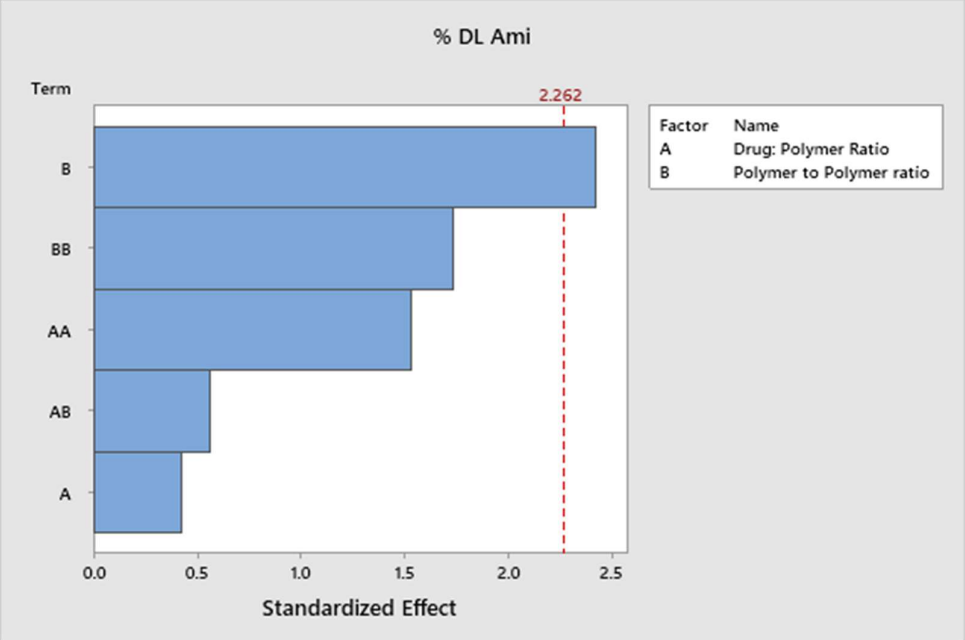

Figure 6-8A Pareto chart for Drug loading of Amisulpride in confirmatory trials

From Fig 6-8A, it was observed that, Polymer: polymer (PLGA: PCL) ratio was found to be most impacting factor for % DL of amisulpride.

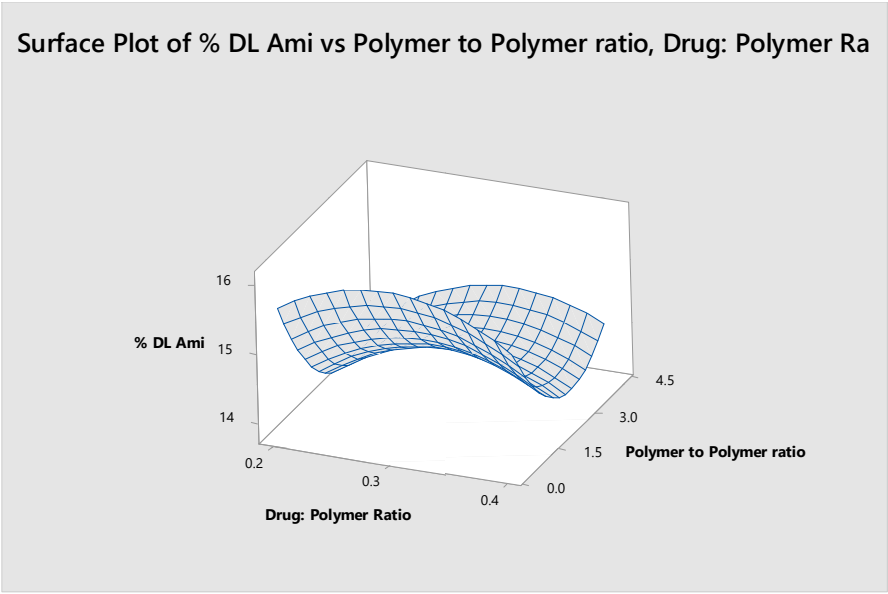

Figure 6-8B Surface plot for Drug loading of Amisulpride

Fig 6-8B showed surface plot of drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio against CQA %DL of amisulpride. The highest values of %DL of amisulpride is in the upper right corner of the plot, which corresponds with high values of both drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio. The lowest values of %DL of amisulpride is in the lower left corner of the plot, which corresponds with low values of both of both drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio.

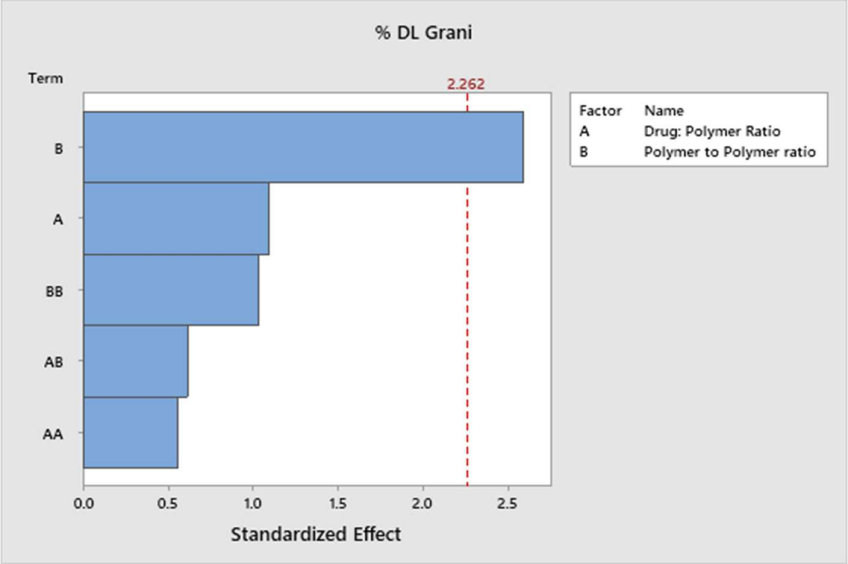

Figure 6-8C Pareto chart for Drug loading of Granisetron in confirmatory trials

From Fig 6-8C, it was observed that, polymer: polymer (PLGA: PCL) ratio was found to be most impacting factor for % DL of granisetron.

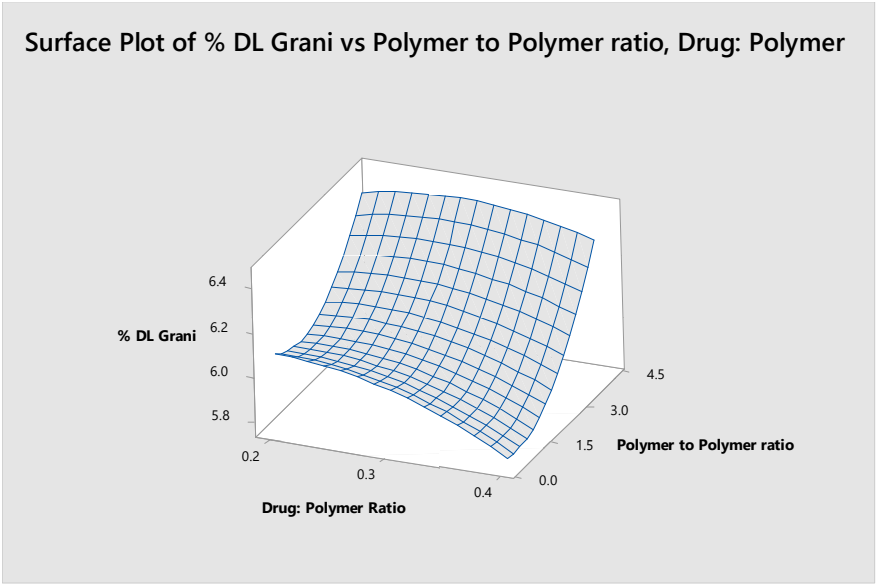

Figure 6-8D Surface plot for Drug loading of Granisetron

Fig 6-8D, surface plot of drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio against CQA % DL of granisetron showed that the surface is not curved because the model is not affected by interaction terms. The highest values of % DL of granisetron is in the upper right corner of the plot, which corresponds with high values of both drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio. The lowest values of % DL of granisetron is in the lower left corner of the plot, which corresponds with low values of both of both drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio.

Confirmatory trials summary for % drug loading:

The % drug loading of both amisulpride and granisetron was affected by polymer: polymer (PLGA: PCL) ratio. Drug: polymer ratio did not seem to impact the CQA as p-value > 0.05 as seen from table 6-17.

Table 6-21 Response surface for Drug loading of amisulpride

Response Surface Regression: % DL Ami					
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	4.07152	0.8143	1.98	0.177
Linear	2	2.49718	1.24859	3.03	0.099
Drug: Polymer Ratio	1	0.07386	0.07386	0.18	0.682
Polymer to Polymer ratio	**1**	**2.42331**	**2.42331**	**5.88**	**0.038**
Square	2	2.21544	1.10772	2.69	0.122
Drug: Polymer Ratio*Drug: Polymer Ratio	1	0.972	0.972	2.36	0.159
Polymer to Polymer ratio*Polymer to Polymer ratio	1	1.24344	1.24344	3.02	0.116
2-Way Interaction	1	0.13107	0.13107	0.32	0.587
Drug: Polymer Ratio*Polymer to Polymer ratio	1	0.13107	0.13107	0.32	0.587
Error	9	3.70848	0.41205		
Total	14	7.78			
Model Summary					
S	R-sq	R-sq(adj)	R-sq(pred)		
0.641914	52.33%	25.85%	0.00%		
Coded Coefficients					
Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	14.634	0.44	33.26	0	
Drug: Polymer Ratio	0.096	0.226	0.42	0.682	1.24
Polymer to Polymer ratio	-0.591	0.244	-2.43	0.038	1.08
Drug: Polymer Ratio*Drug: Polymer Ratio	-0.54	0.352	-1.54	0.159	1
Polymer to Polymer ratio*Polymer to Polymer ratio	0.851	0.49	1.74	0.116	1.08
Drug: Polymer Ratio*Polymer to Polymer ratio	0.162	0.288	0.56	0.587	1.24
Regression Equation in Uncoded Units					
% DL Ami= 11.80 + 31.5 Drug: Polymer Ratio - 1.604 Polymer to Polymer ratio- 54.0 Drug: Polymer Ratio*Drug: Polymer Ratio+ 0.242 Polymer to Polymer ratio*Polymer to Polymer ratio+ 0.87 Drug: Polymer Ratio*Polymer to Polymer ratio					

From table 6-21, Polymer: polymer (PLGA: PCL) ratio was found to be impacting % DL of Amisulpride based on p-value of <0.05. The R^2 value of model is 52.33%.

Table 6-22 Response surface for Drug loading of Granisetron

Response Surface Regression: % DL Grani					
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	0.67971	0.13594	2.58	0.102
Linear	2	0.41769	0.20884	3.97	0.058
Drug: Polymer Ratio	1	0.06324	0.06324	1.2	0.301
Polymer to Polymer ratio	**1**	**0.35445**	**0.35445**	**6.74**	**0.029**
Square	2	0.07246	0.03623	0.69	0.527
Drug: Polymer Ratio*Drug: Polymer Ratio	1	0.01633	0.01633	0.31	0.591
Polymer to Polymer ratio*Polymer to Polymer ratio	1	0.05612	0.05612	1.07	0.329
2-Way Interaction	1	0.01988	0.01988	0.38	0.554
Drug: Polymer Ratio*Polymer to Polymer ratio	1	0.01988	0.01988	0.38	0.554
Error	9	0.47363	0.05263		
Total	14	1.15333			
Model Summary					
S	R-sq	R-sq(adj)	R-sq(pred)		
0.229402	58.93%	36.12%	0.00%		
Coded Coefficients					
Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.046	0.157	38.45	0	
Drug: Polymer Ratio	-0.0884	0.0806	-1.1	0.301	1.24
Polymer to Polymer ratio	0.2259	0.0871	2.6	0.029	1.08
Drug: Polymer Ratio*Drug: Polymer Ratio	-0.07	0.126	-0.56	0.591	1
Polymer to Polymer ratio*Polymer to Polymer ratio	0.181	0.175	1.03	0.329	1.08
Drug: Polymer Ratio*Polymer to Polymer ratio	0.063	0.103	0.61	0.554	1.24
Regression Equation in Uncoded Units					
% DL Grani= 5.87 + 2.60 Drug: Polymer Ratio - 0.199 Polymer to Polymer ratio- 7.0 Drug: Polymer Ratio*Drug: Polymer Ratio+ 0.0514 Polymer to Polymer ratio*Polymer to Polymer ratio+ 0.337 Drug: Polymer Ratio*Polymer to Polymer ratio					
Fits and Diagnostics for Unusual Observations					
Obs	% DL Grani	Fit	Resid	Std Resid	
1	6.5	6.083	0.417	2.51	R
R Large residual					

From table 6-22, Polymer: polymer (PLGA: PCL) ratio was found to be impacting % DL of Granisetron based on p-value of <0.05. The R^2 value of model is 58.93%.

6.10.9 Effect on drug release in confirmatory trials:

Figures 6-9A and 6-9B shows the pareto chart and surface plot for % drug release of amisulpride. Figure 6-9C and 6-9D shows the pareto chart and surface plot for % drug release of granisetron. Table 6-23 and 6-24 shows the confirmatory trial statistical summary for % drug release of amisulpride and granisetron respectively.

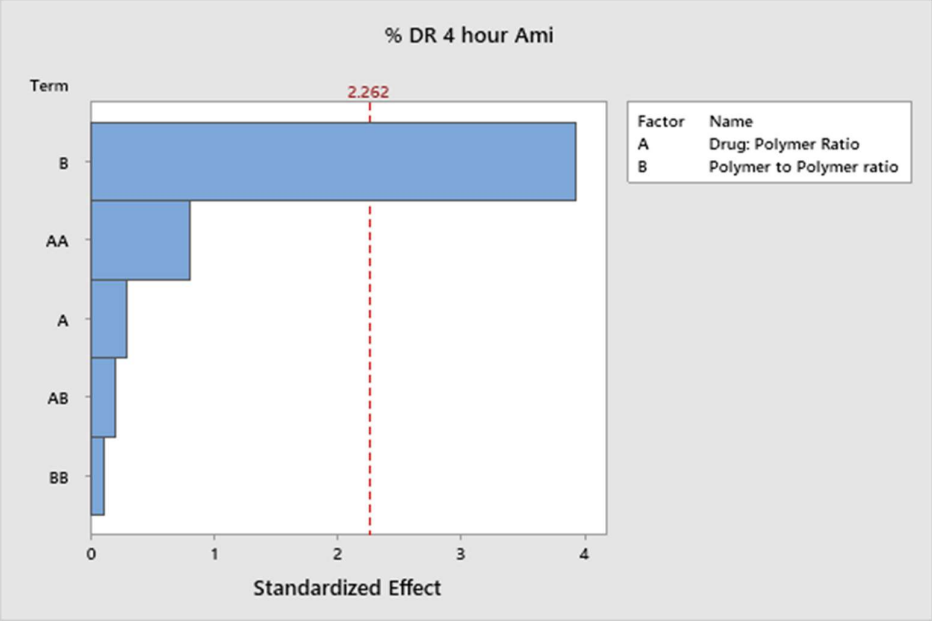

Figure 6-9A Pareto chart for DR at 4-hour for Amisulpride

From Fig 6-9A, it was observed that, polymer: polymer (PLGA: PCL) ratio was found to be most impacting factor for % DR of amisulpride.

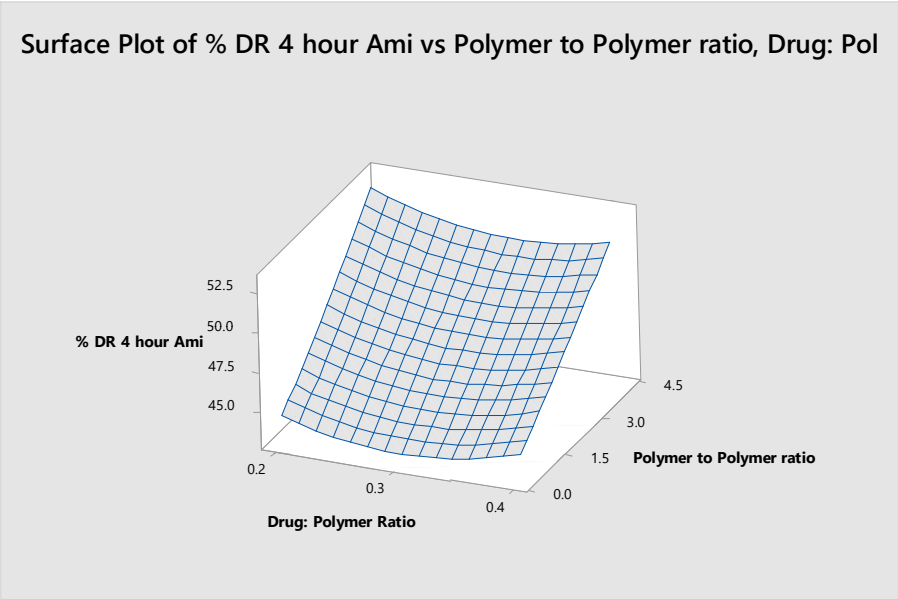

Figure 6-9B Surface plot for DR 4-hour for Amisulpride

From Fig 6-9B, surface plot of drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio against CQA % DR of amisulpride showed that the surface is not curved because the model is not affected by interaction terms. The highest values of % DR of amisulpride is in the upper right corner of the plot, which corresponds with high values of both drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio. The lowest values of % DR of amisulpride is in the lower left corner of the plot, which corresponds with low values of both of both drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio.

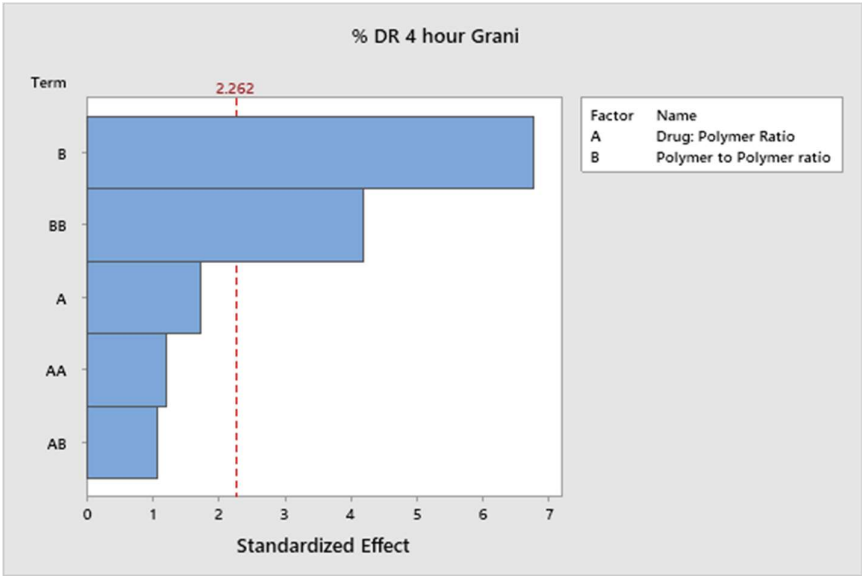

Figure 6-9C Pareto chart for DR at 4-hour for Granisetron

From Fig 6-9C, it was observed that, both polymer: polymer (PLGA: PCL) ratio and its square term were found to be most impacting factor for % DR of granisetron.

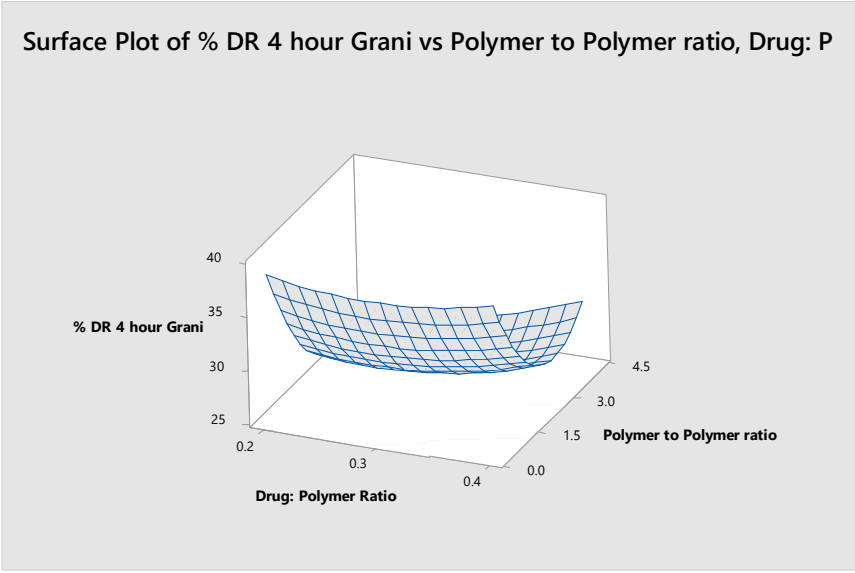

Figure 6-9D Surface plot for DR 4-hour for Granisetron

From Fig 6-9D, surface plot of drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio against CQA %DR of granisetron showed that the surface looks curved as the model contains statistically significant quadratic terms. The highest values of % DR of granisetron is in the upper right corner of the plot, which corresponds with high values of both drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio. The lowest values of % DR of granisetron is in the lower left corner of the plot, which corresponds with low values of both of both drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio.

Confirmatory trials summary for % drug release:

The % drug release of both amisulpride and granisetron was affected by polymer: polymer (PLGA: PCL) ratio. The square term of polymer: polymer (PLGA: PCL) ratio also impacted % drug release of granisetron. Drug: polymer ratio did not seem to impact the CQA as p-value > 0.05 as seen from table 6-17.

Table 6-23 Response surface for Drug release at 4-hour for Amisulpride

Response Surface Regression: % DR 4-hour Ami					
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	129.583	25.917	3.53	0.048
Linear	2	114.266	57.133	7.79	0.011
Drug: Polymer Ratio	1	0.666	0.666	0.09	0.77
Polymer to Polymer ratio	**1**	**113.6**	**113.6**	**15.49**	**0.003**
Square	2	4.881	2.441	0.33	0.725
Drug: Polymer Ratio*Drug: Polymer Ratio	1	4.8	4.8	0.65	0.439
Polymer to Polymer ratio*Polymer to Polymer ratio	1	0.081	0.081	0.01	0.919
2-Way Interaction	1	0.32	0.32	0.04	0.839
Drug: Polymer Ratio*Polymer to Polymer ratio	1	0.32	0.32	0.04	0.839
Error	9	66.017	7.335		
Total	14	195.6			
Model Summary					
S	R-sq	R-sq(adj)	R-sq(pred)		
2.70835	66.25%	47.50%	0.00%		
Coded Coefficients					
Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	47.05	1.86	25.34	0	
Drug: Polymer Ratio	-0.287	0.952	-0.3	0.77	1.24
Polymer to Polymer ratio	4.04	1.03	3.94	0.003	1.08
Drug: Polymer Ratio*Drug: Polymer Ratio	1.2	1.48	0.81	0.439	1
Polymer to Polymer ratio*Polymer to Polymer ratio	0.22	2.07	0.11	0.919	1.08
Drug: Polymer Ratio*Polymer to Polymer ratio	-0.25	1.21	-0.21	0.839	1.24
Regression Equation in Uncoded Units					
% DR 4-hour Ami=53.5 - 72.0 Drug: Polymer Ratio + 2.30 Polymer to Polymer ratio+ 120 Drug: Polymer Ratio*Drug: Polymer Ratio+ 0.062 Polymer to Polyme r ratio*Polymer to Polymer ratio- 1.35 Drug: Polymer Ratio*Polymer to Polymer ratio					

From table 6-23, polymer: polymer (PLGA: PCL) ratio was found to be impacting % DR of amisulpride based on p-value of <0.05. The R^2 value of model is 66.25%, which is fairly good.

Table 6-24 Response surface for Drug release at 4-hour for Granisetron

Response Surface Regression: % DR 4-hour Grani

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	186.42	37.284	11.34	0.001
Linear	2	160.873	80.437	24.47	0
Drug: Polymer Ratio	1	9.816	9.816	2.99	0.118
Polymer to Polymer ratio	**1**	**151.057**	**151.057**	**45.96**	**0**
Square	2	62.738	31.369	9.54	0.006
Drug: Polymer Ratio*Drug: Polymer Ratio	1	4.8	4.8	1.46	0.258
Polymer to Polymer ratio*Polymer to Polymer ratio	**1**	**57.938**	**57.938**	**17.63**	**0.002**
2-Way Interaction	1	3.855	3.855	1.17	0.307
Drug: Polymer Ratio*Polymer to Polymer ratio	1	3.855	3.855	1.17	0.307
Error	9	29.58	3.287		
Total	14	216			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)		
1.81291	86.31%	78.70%	54.00%		

Coded Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	27.03	1.24	21.75	0	
Drug: Polymer Ratio	1.101	0.637	1.73	0.118	1.24
Polymer to Polymer ratio	-4.664	0.688	-6.78	0	1.08
Drug: Polymer Ratio*Drug: Polymer Ratio	1.2	0.993	1.21	0.258	1
Polymer to Polymer ratio*Polymer to Polymer ratio	5.81	1.38	4.2	0.002	1.08
Drug: Polymer Ratio*Polymer to Polymer ratio	0.88	0.812	1.08	0.307	1.24

Regression Equation in Uncoded Unit

% DR 4-hour Grani=50.27 - 71.0 Drug: Polymer Ratio
- 10.92 Polymer to Polymer ratio+ 120.0 Drug: Polymer Ratio*Drug: Polymer Ratio+ 1.653 Polymer to Poly mer ratio*Polymer to Polymer ratio+ 4.69 Drug: Polymer Ratio*Polymer to Polymer ratio

Fits and Diagnostics for Unusual Observations

Obs	% DR 4 hour Grani	Fit	Resid	Std Resid	
11	42	38.93	3.07	2.34	R

R Large residual

From table 6-24, polymer: polymer (PLGA: PCL) ratio and its square term both were found to be impacting % DR of granisetron based on p-value of <0.05. The R^2 value of model is 86.31%, which shows model is sufficiently powered.

Finally, the model was used to set the optimal drug: polymer ratio and polymer: polymer (PLGA: PCL) ratio to check whether the desired responses can be achieved. The selected settings are shown in table 6-25 and the results in table 6-26.

Table 6-25 Selection of drug: polymer ratio and polymer: polymer ratio

Settings for final batches for desired responses	
Variable	Setting
Drug: Polymer Ratio	0.4
Polymer to Polymer ratio (PLGA: PCL)	1

Table 6-26 shows the results of fit for selected CQA along with standard error of fit and 95% confidence interval and 95% prediction interval. As can be seen from the results, all the desired CQAs are meeting the criteria set in CQA table 6-2.

Table 6-26 Prediction intervals for CQAs of final formulation

Sr. no.	Parameter	Prediction
1	**D90**	Fit: 92.1129; SE Fit: 1.59739; 95% CI: (88.4994, 95.7265); 95% PI: (84.0423, 100.184)
2	**% EE Ami**	Fit: 57.8553; SE Fit: 0.9392 36; 95% CI: (55.7306, 59.9800); 95% PI: (53.1099, 62.6007)
3	**% EE Grani**	Fit: 57.769; SE Fit: 0.923709; 95% CI: (55.6794, 59.8586); 95% PI: (53.1020, 62.4359)
4	**% DR 4-hour Ami**	Fit: 45.7672; SE Fit: 1.35616; 95% CI: (42.6993, 48.8350); 95% PI: (38.9153, 52.6191)
5	**% DR 4-hour Grani**	Fit: 33.6926; SE Fit: 0.907784; 95% CI: (31.6391, 35.7462); 95% PI: (29.1061, 38.2791)
6	**% DL Ami**	Fit: 14.7532; SE Fit: 0.321428; 95% CI: (14.0261, 15.4803); 95% PI: (13.1292, 16.3772)
7	**% DL Grani**	Fit: 5.77932; SE Fit: 0.114869; 95% CI: (5.51947, 6.03917); 95% PI: (5.19895, 6.35968)

6.11 Updated Risk assessment:

The initial identified risks form table 6-4 and 6-6 were updated to low as per the development results. The selected PLGA, PCL and PVA levels were able to provide desired CQAs within the proposed ranges. The selected process parameters like stirring speed and stirring time were optimized and fixed to ensure consistent results throughout the development cycle. The updated risk assessments for formulation variables and process parameters are given in table 6-27 and 6-28 respectively.

Table 6-27 Updated risk assessment of Formulation variables

Drug Product CQA ↓	Formulation variables ⟶			
	API Level	PLGA level	PCL level	PVA level
PSD D90	Low	Low	Low	Low
%DL	Low	Low	Low	Low
%EE	Low	Low	Low	Low
% DR	Low	Low	Low	Low

Table 6-28 Updated risk assessment of Process variables

Drug Product CQA ↓	Process variables ⟶			
	Stirring speed W/O	Stirring Time W/O	Stirring speed W/O/W	Stirring Time W/O/W
PSD D90	Low	Low	Low	Low
%DL	Low	Low	Low	Low
%EE	Low	Low	Low	Low
% DR	Low	Low	Low	Low

6.12 Design space:

In the context of Design of Experiments (DOE), the term "design space" refers to the multidimensional combination and interaction of input variables (such as material attributes) and process parameters that have been demonstrated to provide assurance of quality [21]. Essentially, it is the range within which these variables can be varied without affecting the quality of the final product. Design space is crucial for optimizing processes and ensuring robust performance. It allows researchers to understand the relationships between different factors and their impact on the outcome, enabling them to identify optimal conditions for their experiments [21]. The design space was created for the studied parameters.

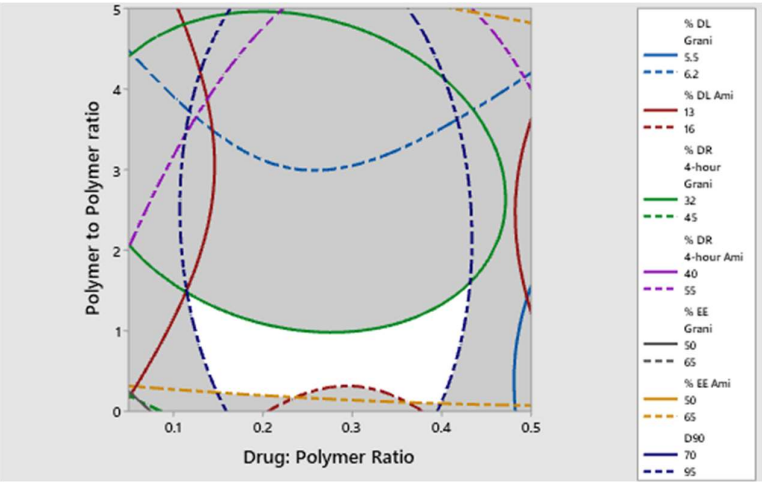

Figure 6-10 Design space for Formulation

White zone in Figure 6-10 shows design space (Polymer to polymer ratio :0.3 to 1.4; Drug to polymer ratio: 0.15 to 0.4). The design space showed that, within this region, the desired CQAs can be achieved successfully.

6.13 References:

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