

# *Annexure- IV*

## Annexure IV: Minitab files

### 1.0 Model fitting of Entrapment efficiency of DOX

#### 1.1 Initial model fitting with 2-way and 3-way interactions: Results and discussion

##### Results- Full Factorial Design

Factors: 3 Base Design: 3, 8  
Runs: 8 Replicates: 1  
Blocks: 1 Center pts (total): 0

All terms are free from aliasing.

#### Factorial Regression: EE DOX (%) versus Ammonium Sulphate, PC:Chol ratio, pH of drug loading

##### Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	26.641	3.806	*	*
Linear	3	11.531	3.844	*	*
Ammonium Sulphate	1	5.314	5.314	*	*
PC:Chol ratio	1	2.599	2.599	*	*
pH of drug loading	1	3.618	3.618	*	*
2-Way Interactions	3	8.449	2.816	*	*
Ammonium Sulphate*PC:Chol ratio	1	1.862	1.862	*	*
Ammonium Sulphate*pH of drug loading	1	4.743	4.743	*	*
PC:Chol ratio*pH of drug loading	1	1.843	1.843	*	*
3-Way Interactions	1	6.661	6.661	*	*
Ammonium Sulphate*PC:Chol ratio*pH of drug loading	1	6.661	6.661	*	*
Error	0	*	*		
Total	7	26.641			

### Model Summary

S	R-sq	R-sq (adj)	R-sq (pred)
*	100.00%	*	*

### Regression Equation in coded Units

EE DOX (%) = 96.27 + 0.8150 Ammonium Sulphate - 0.570 PC:Chol ratio - 0.6725 pH of drug loading  
- 0.4825 Ammonium Sulphate\*PC:Chol ratio  
- 0.7700 Ammonium Sulphate\*pH of drug loading  
- 0.4800 PC:Chol ratio\*pH of drug loading  
- 0.9125 Ammonium Sulphate\*PC:Chol ratio\*pH of drug loading

### Alias Structure

Factor Name

A	Ammonium Sulphate
B	PC:Chol ratio
C	pH of drug loading

Aliases

I  
A  
B  
C  
AB  
AC  
BC  
ABC

**Discussion-** The results of the model fitting with all the possible interactions (including quadratic ones) results in improper model fitting including the absence of p values for the modelling. Based on prior experience with the formulation design and the evaluation of the formulation design with the contour plots, the quadratic terms used in the modelling were removed and the model was re-evaluated using minitab. The coefficient of simultaneous variation of the three variables was removed due to the lack of fit of the model during the screening study.

## 1.2 Model fitting with 2-way interactions: Results and discussion

### Factorial Regression: EE DOX (%) versus Ammonium Sulphate, PC:Chol ratio, pH of drug loading

#### Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	19.980	3.330	0.50	0.045
Linear	3	11.531	3.844	0.58	0.042
Ammonium Sulphate	1	5.314	5.314	0.80	0.028
PC:Chol ratio	1	2.599	2.599	0.39	0.032
pH of drug loading	1	3.618	3.618	0.54	0.029
2-Way Interactions	3	8.449	2.816	0.42	0.043
Ammonium Sulphate*PC:Chol ratio	1	1.862	1.862	0.28	0.036
Ammonium Sulphate*pH of drug loading	1	4.743	4.743	0.71	0.029
PC:Chol ratio*pH of drug loading	1	1.843	1.843	0.28	0.037
Error	1	6.661	6.661		
Total	7	26.641			

#### Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.58094	96.26%	90.18%	78.26%

#### Regression Equation in coded Units

$$\begin{aligned}
 \text{EE DOX (\%)} = & 96.2725 + 0.815 \text{ Ammonium Sulphate} - 0.57 \text{ PC:Chol ratio} - 0.6725 \text{ pH of drug loading} \\
 & - 0.4825 \text{ Ammonium Sulphate*PC:Chol ratio} \\
 & - 0.77 \text{ Ammonium Sulphate*pH of drug loading} \\
 & - 0.48 \text{ PC:Chol ratio*pH of drug loading}
 \end{aligned}$$

#### Alias Structure

##### Factor Name

A	Ammonium Sulphate
B	PC:Chol ratio
C	pH of drug loading

#### Aliases

I  
A  
B  
C  
AB  
AC  
BC

**Discussion-** The results of the model fitting showed the presence of linear correlation between the variables with significant effect of the variation in the values of the variables on that of the tested responses. The level of significance of the model was given by regression coefficient (R<sup>2</sup>) and p values while the F values represented the level of fit of the model. The coefficient of simultaneous variation of the three variables was removed due to the lack of fit of the model during the screening study. The results indicate that the concentration of ammonium sulphate, phosphatidylcholine to cholesterol molar ratio and the pH of drug loading had significant effect ( $p < 0.05$ ) on the tested responses. These results were further supplemented with the contour plots which indicated the linear regression between the tested variables.

## 2.0 Model fitting of Entrapment efficiency of VCR

### 2.1 Initial model fitting with 2-way and 3-way interactions: Results and discussion

#### Factorial Regression: EE VCR (%) versus Ammonium Sulphate, PC:Chol ratio, pH of drug loading

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value
Model	7	103.030	14.7186	*
Linear	3	91.995	30.6649	*
Ammonium Sulphate	1	74.970	74.9700	*
PC:Chol ratio	1	1.540	1.5400	*
pH of drug loading	1	15.485	15.4846	*
2-Way Interactions	3	10.158	3.3860	*
Ammonium Sulphate*PC:Chol ratio	1	4.606	4.6056	*
Ammonium Sulphate*pH of drug loading	1	2.588	2.5878	*
PC:Chol ratio*pH of drug loading	1	2.965	2.9646	*
3-Way Interactions	1	0.878	0.8778	*
Ammonium Sulphate*PC:Chol ratio*pH of drug loading	1	0.878	0.8778	*
Error	0	*	*	
Total	7	103.030		

Source	P-Value
Model	*
Linear	*
Ammonium Sulphate	*
PC:Chol ratio	*
pH of drug loading	*
2-Way Interactions	*
Ammonium Sulphate*PC:Chol ratio	*
Ammonium Sulphate*pH of drug loading	*
PC:Chol ratio*pH of drug loading	*
3-Way Interactions	*
Ammonium Sulphate*PC:Chol ratio*pH of drug loading	*
Error	
Total	

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
*	100.00%	*	*

Regression Equation in coded Units

EE VCR (%) = 198.6 - 0.4650 Ammonium Sulphate - 116.4 PC:Chol ratio  
- 15.26 pH of drug loading + 0.5118 Ammonium Sulphate\*PC:Chol ratio  
+ 0.07000 Ammonium Sulphate\*pH of drug loading  
+ 14.91 PC:Chol ratio\*pH of drug loading  
- 0.07162 Ammonium Sulphate\*PC:Chol ratio\*pH of drug loading

Alias Structure

Factor Name

A	Ammonium Sulphate
B	PC:Chol ratio
C	pH of drug loading

Aliases

I  
A  
B  
C  
AB  
AC  
BC  
ABC

**Discussion-** The results of the model fitting with all the possible interactions (including quadratic ones) results in improper model fitting including the absence of p values for the modelling. Based on prior experience with the formulation design and the evaluation of the formulation design with the contour plots, the quadratic terms used in the modelling were removed and the model was re-evaluated using minitab. The coefficient of simultaneous variation of the three variables was removed due to the lack of fit of the model during the screening study.

## 2.2 Model fitting with 2-way interactions: Results and discussion

### Factorial Regression: EE VCR (%) versus Ammonium Sulphate, PC:Chol ratio, pH of drug loading

#### Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	102.153	17.0254	19.40	0.018
Linear	3	91.995	30.6649	34.93	0.014
Ammonium Sulphate	1	74.970	74.9700	85.41	0.009
PC:Chol ratio	1	1.540	1.5400	1.75	0.041
pH of drug loading	1	15.485	15.4846	17.64	0.015
2-Way Interactions	3	10.158	3.3860	3.86	0.035
Ammonium Sulphate*PC:Chol ratio	1	4.606	4.6056	5.25	0.026
Ammonium Sulphate*pH of drug loading	1	2.588	2.5878	2.95	0.034
PC:Chol ratio*pH of drug loading	1	2.965	2.9646	3.38	0.032
Error	1	0.878	0.8778		
Total	7	103.030			

#### Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.936916	93.09%	89.04%	75.47%

#### Regression Equation in coded Units

$$\begin{aligned} \text{EE VCR (\%)} = & 90.459 + 3.061 \text{ Ammonium Sulphate} - 0.439 \text{ PC:Chol ratio} - 1.391 \text{ pH of drug loading} \\ & + 0.759 \text{ Ammonium Sulphate*PC:Chol ratio} \\ & - 0.569 \text{ Ammonium Sulphate*pH of drug loading} \\ & - 0.609 \text{ PC:Chol ratio*pH of drug loading} \end{aligned}$$

#### Alias Structure

##### Factor Name

A	Ammonium Sulphate
B	PC:Chol ratio
C	pH of drug loading

Aliases

I  
A  
B  
C  
AB  
AC  
BC

**Discussion-** The results of the model fitting showed the presence of linear correlation between the variables with significant effect of the variation in the values of the variables on that of the tested responses. The level of significance of the model was given by regression coefficient ( $R^2$ ) and p values while the F values represented the level of fit of the model. The coefficient of simultaneous variation of the three variables was removed due to the lack of fit of the model during the screening study. The results indicate that the concentration of ammonium sulphate, phosphatidylcholine to cholesterol molar ratio and the pH of drug loading had significant effect ( $p < 0.05$ ) on the tested responses. These results were further supplemented with the contour plots which indicated the linear regression between the tested variables.

### 3.0 Model fitting of Particle size

#### 3.1 Initial model fitting with 2-way and 3-way interactions: Results and discussion

#### Factorial Regression: Particle size versus Ammonium Sul, PC:Chol ratio, pH of drug I

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value
Model	7	307.462	43.923	*
Linear	3	293.486	97.829	*
Ammonium Sulphate	1	34.861	34.861	*
PC:Chol ratio	1	232.848	232.848	*
pH of drug loading	1	25.776	25.776	*
2-Way Interactions	3	11.490	3.830	*
Ammonium Sulphate*PC:Chol ratio	1	2.205	2.205	*
Ammonium Sulphate*pH of drug loading	1	3.125	3.125	*
PC:Chol ratio*pH of drug loading	1	6.160	6.160	*
3-Way Interactions	1	2.486	2.486	*
Ammonium Sulphate*PC:Chol ratio*pH of drug loading	1	2.486	2.486	*
Error	0	*	*	
Total	7	307.462		

Source	P-Value
Model	*
Linear	*
Ammonium Sulphate	*
PC:Chol ratio	*
pH of drug loading	*
2-Way Interactions	*
Ammonium Sulphate*PC:Chol ratio	*
Ammonium Sulphate*pH of drug loading	*
PC:Chol ratio*pH of drug loading	*
3-Way Interactions	*
Ammonium Sulphate*PC:Chol ratio*pH of drug loading	*
Error	
Total	

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
*	100.00%	*	*

Regression Equation in coded Units

Particle size (nm) = -4.918 + 0.6714 Ammonium Sulphate + 113.9 PC:Chol ratio  
+ 30.64 pH of drug loading - 0.6665 Ammonium Sulphate\*PC:Chol ratio  
- 0.1311 Ammonium Sulphate\*pH of drug loading  
- 26.68 PC:Chol ratio\*pH of drug loading  
+ 0.1205 Ammonium Sulphate\*PC:Chol ratio\*pH of drug loading

Alias Structure

Factor Name

A	Ammonium Sulphate
B	PC:Chol ratio
C	pH of drug loading

Aliases

I  
A  
B  
C  
AB  
AC  
BC  
ABC

**Discussion-** The results of the model fitting with all the possible interactions (including quadratic ones) results in improper model fitting including the absence of p values for the modelling. Based on prior experience with the formulation design and the evaluation of the formulation design with the contour plots, the quadratic terms used in the modelling were removed and the model was re-evaluated using minitab. The coefficient of simultaneous variation of the three variables was removed due to the lack of fit of the model during the screening study.

### 3.2 Model fitting with 2-way interactions: Results and discussion

#### Factorial Regression: Particle siz versus Ammonium Sul, PC:Chol rati, pH of drug I

##### Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	304.976	50.829	20.44	0.017
Linear	3	293.486	97.829	39.34	0.012
Ammonium Sulphate	1	34.861	34.861	14.02	0.017
PC:Chol ratio	1	232.848	232.848	93.65	0.028
pH of drug loading	1	25.776	25.776	10.37	0.019
2-Way Interactions	3	11.490	3.830	1.54	0.023
Ammonium Sulphate*PC:Chol ratio	1	2.205	2.205	0.89	0.032
Ammonium Sulphate*pH of drug loading	1	3.125	3.125	1.26	0.026
PC:Chol ratio*pH of drug loading	1	6.160	6.160	2.48	0.036
Error	1	2.486	2.486		
Total	7	307.462			

##### Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.57685	96.26%	92.34%	74.24%

##### Regression Equation in coded Units

Particle size (nm) = 106.552 - 2.088 Ammonium Sulphate - 5.395 PC:Chol ratio  
 + 1.795 pH of drug loading + 0.525 Ammonium Sulphate\*PC:Chol ratio  
 + 0.625 Ammonium Sulphate\*pH of drug loading  
 + 0.877 PC:Chol ratio\*pH of drug loading

##### Alias Structure

##### Factor Name

A	Ammonium Sulphate
B	PC:Chol ratio
C	pH of drug loading

Aliases

I  
A  
B  
C  
AB  
AC  
BC

**Discussion-** The results of the model fitting showed the presence of linear correlation between the variables with significant effect of the variation in the values of the variables on that of the tested responses. The level of significance of the model was given by regression coefficient (R<sup>2</sup>) and p values while the F values represented the level of fit of the model. The coefficient of simultaneous variation of the three variables was removed due to the lack of fit of the model during the screening study. The results indicate that the concentration of ammonium sulphate, phosphatidylcholine to cholesterol molar ratio and the pH of drug loading had significant effect ( $p < 0.05$ ) on the tested responses. These results were further supplemented with the contour plots which indicated the linear regression between the tested variables.

## 4.0 Model fitting of Zeta Potential

### 4.1 Initial model fitting with 2-way and 3-way interactions: Results and discussion

#### Factorial Regression: Zeta Potenti versus Ammonium Sul, PC:Chol rati, pH of drug I

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value
Model	7	53.5734	7.6533	*
Linear	3	53.4978	17.8326	*
Ammonium Sulphate	1	48.0200	48.0200	*
PC:Chol ratio	1	1.4450	1.4450	*
pH of drug loading	1	4.0328	4.0328	*
2-Way Interactions	3	0.0724	0.0241	*
Ammonium Sulphate*PC:Chol ratio	1	0.0450	0.0450	*
Ammonium Sulphate*pH of drug loading	1	0.0032	0.0032	*
PC:Chol ratio*pH of drug loading	1	0.0242	0.0242	*
3-Way Interactions	1	0.0032	0.0032	*
Ammonium Sulphate*PC:Chol ratio*pH of drug loading	1	0.0032	0.0032	*
Error	0	*	*	
Total	7	53.5734		

Source	P-Value
Model	*
Linear	*
Ammonium Sulphate	*
PC:Chol ratio	*
pH of drug loading	*
2-Way Interactions	*
Ammonium Sulphate*PC:Chol ratio	*
Ammonium Sulphate*pH of drug loading	*
PC:Chol ratio*pH of drug loading	*
3-Way Interactions	*
Ammonium Sulphate*PC:Chol ratio*pH of drug loading	*
Error	
Total	

Model Summary



## 4.2 Model fitting with 2-way interactions: Results and discussion

### Factorial Regression: Zeta Potenti versus Ammonium Sul, PC:Chol rati, pH of drug I

#### Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	53.5702	8.9284	2790.11	0.014
Linear	3	53.4978	17.8326	5572.69	0.010
Ammonium Sulphate	1	48.0200	48.0200	15006.25	0.005
PC:Chol ratio	1	1.4450	1.4450	451.56	0.030
pH of drug loading	1	4.0328	4.0328	1260.25	0.018
2-Way Interactions	3	0.0724	0.0241	7.54	0.026
Ammonium Sulphate*PC:Chol ratio	1	0.0450	0.0450	14.06	0.017
Ammonium Sulphate*pH of drug loading	1	0.0032	0.0032	1.00	0.045
PC:Chol ratio*pH of drug loading	1	0.0242	0.0242	7.56	0.022
Error	1	0.0032	0.0032		
Total	7	53.5734			

#### Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0565685	99.62%	95.96%	93.62%

#### Regression Equation in coded Units

$$\begin{aligned} \text{Zeta Potential (mV)} = & -5.57 - 2.45 \text{ Ammonium Sulphate} - 0.43 \text{ PC:Chol ratio} \\ & + 0.71 \text{ pH of drug loading} - 0.0750 \text{ Ammonium Sulphate*PC:Chol ratio} \\ & - 0.0200 \text{ Ammonium Sulphate*pH of drug loading} \\ & - 0.055 \text{ PC:Chol ratio*pH of drug loading} \end{aligned}$$

#### Alias Structure

Factor Name

A	Ammonium Sulphate
B	PC:Chol ratio
C	pH of drug loading

Aliases

I  
A  
B  
C  
AB  
AC  
BC

**Discussion-** The results of the model fitting showed the presence of linear correlation between the variables with significant effect of the variation in the values of the variables on that of the tested responses. The level of significance of the model was given by regression coefficient (R<sup>2</sup>) and p values while the F values represented the level of fit of the model. The coefficient of simultaneous variation of the three variables was removed due to the lack of fit of the model during the screening study. The results indicate that the concentration of ammonium sulphate, phosphatidylcholine to cholesterol molar ratio and the pH of drug loading had significant effect ( $p < 0.05$ ) on the tested responses. These results were further supplemented with the contour plots which indicated the linear regression between the tested variables.