



Development and evaluation of metronidazole loaded microsphere based gel for superficial surgical wound infections

The purpose of this research paper is to optimize, characterize and develop a microsphere drug delivery system for topical delivery. Design of experiments (DoE) methodology is implemented for the statistical optimization process.

The factors (independent variables) examined are: X_1 = amount of dichloromethane (mL), X_2 = amount of xanthan gum (% w/v) and X_3 = amount of sodium chloride (% w/v). All the factors are continuous. The responses (dependent variables) examined are: Y_1 = drug entrapment efficiency (%), Y_2 = particle size (μm) and Y_3 = cumulative permeability drug release at 8 h (%). The applied DoE method is Box Behnken design.

Isalos version used: 2.1.2

Scientific article: <https://www.sciencedirect.com/science/article/abs/pii/S1773224715300162>

Step 1: Box Behnken Design

In the first tab named "Action" define the factors in the column headers and fill each column with the low and high levels of the corresponding factors. This tab can be renamed "Box Behnken". Afterwards, apply the Box Behnken method: *DOE* → *Response Surface* → *Box Behnken*

	Col1	Col2 (I)	Col3 (D)	Col4 (D)
User Header	User Row ID	X1	X2	X3
1		10	0.1	0.05
2		50	1	0.1

DoE Box Behnken
?
X

Number of Center Points per Block
5

Number of Replicates
1

Number of Blocks
1

☐ Random Standard order

Excluded Columns

Included Columns

Col2 -- X1
Col3 -- X2
Col4 -- X3

Execute
Cancel

Results (right spreadsheet):

	Col1	Col2 (I)	Col3 (S)	Col4 (S)	Col5 (S)	Col6 (D)	Col7 (D)	Col8 (D)
User Header	User Row ID	Standard Order	Block Number	Replicate Number	Point Type	X1	X2	X3
1		1	Block: 1	Replicate: 1	Design Point	10.0	0.1	0.0750000
2		2	Block: 1	Replicate: 1	Design Point	50.0	0.1	0.0750000
3		3	Block: 1	Replicate: 1	Design Point	10.0	1.0	0.0750000
4		4	Block: 1	Replicate: 1	Design Point	50.0	1.0	0.0750000
5		5	Block: 1	Replicate: 1	Design Point	10.0	0.55	0.05
6		6	Block: 1	Replicate: 1	Design Point	50.0	0.55	0.05
7		7	Block: 1	Replicate: 1	Design Point	10.0	0.55	0.1
8		8	Block: 1	Replicate: 1	Design Point	50.0	0.55	0.1
9		9	Block: 1	Replicate: 1	Design Point	30.0	0.1	0.05
10		10	Block: 1	Replicate: 1	Design Point	30.0	1.0	0.05
11		11	Block: 1	Replicate: 1	Design Point	30.0	0.1	0.1
12		12	Block: 1	Replicate: 1	Design Point	30.0	1.0	0.1
13		13	Block: 1	----	Center Point	30.0	0.55	0.0750000
14		14	Block: 1	----	Center Point	30.0	0.55	0.0750000
15		15	Block: 1	----	Center Point	30.0	0.55	0.0750000
16		16	Block: 1	----	Center Point	30.0	0.55	0.0750000
17		17	Block: 1	----	Center Point	30.0	0.55	0.0750000

Step 2: Factor selection

Create a new tab named “Factors” and import the results from the “Box Behnken” spreadsheet by right clicking on the left spreadsheet. Then, select only the factor columns to be transferred to the right spreadsheet: *Data Transformation* → *Data Manipulation* → *Select Column(s)*

	Col1	Col2	Col3	Col4	Col5	Col6
User Header	User Row ID					
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

 Choose tab as input 

Select input tab

Box Behnken 

Execute

Cancel

 Select Column(s)  

Excluded Columns

Col2 -- Standard Order

Col3 -- Block Number

Col4 -- Replicate Number

Col5 -- Point Type

>>

>

<

<<

Included Columns

Col6 -- X1

Col7 -- X2

Col8 -- X3

Execute

Cancel

Results:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)
User Header	User Row ID	X1	X2	X3
1		10.0	0.1	0.0750000
2		50.0	0.1	0.0750000
3		10.0	1.0	0.0750000
4		50.0	1.0	0.0750000
5		10.0	0.55	0.05
6		50.0	0.55	0.05
7		10.0	0.55	0.1
8		50.0	0.55	0.1
9		30.0	0.1	0.05
10		30.0	1.0	0.05
11		30.0	0.1	0.1
12		30.0	1.0	0.1
13		30.0	0.55	0.0750000
14		30.0	0.55	0.0750000
15		30.0	0.55	0.0750000
16		30.0	0.55	0.0750000
17		30.0	0.55	0.0750000

Step 3: Definition of response variables

Create a new tab named “Responses” and define the responses in the column headers. Fill each column with the values of the corresponding responses that were observed and make sure the values follow the order of the experiments as given by the Box Behnken design. Then, select all columns to be transferred to the right spreadsheet: *Data Transformation* → *Data Manipulation* → *Select Column(s)*

	Col1	Col2 (D)	Col3 (D)	Col4 (D)
User Header	User Row ID	Y1	Y2	Y3
1		59.6	0.0000267	6.7268120
2		17.71	0.0000605	4.7822589
3		46.85	0.0000332	4.4855323
4		19.59	0.0000461	3.3630343
5		61.62	0.0035391	4.2107007
6		14.36	0.0000350	4.0074930
7		53.81	0.0001144	4.2107007
8		19.09	0.0000583	4.0681691
9		67.54	0.0002838	7.4020267
10		23.99	0.0000834	4.6043458
11		19.02	0.0001990	5.2028838
12		19.98	0.0000700	4.4888751
13		25.93	0.0005007	4.7518417
14		25.93	0.0000950	4.7455242
15		25.93	0.0000702	4.7822589
16		25.93	0.0005007	4.7518417
17		25.93	0.0000950	4.7455242


Select Column(s)
? ×

Excluded Columns

Included Columns

Col2 -- Y1
Col3 -- Y2
Col4 -- Y3

>>

>

<

<<

Execute

Cancel

Step 4: Normalization

Create a new tab named “Normalized data” and import the results from the “Factors” and “Responses” spreadsheets. Afterwards, normalize the factor columns to take values in the range [-1, 1]: Data Transformation → Normalizers → Min-Max

	Col1	Col2	Col3	Col4	Col5	Col6
User Header	User Row ID					
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Import from File

Import from Spreadsheet

Import from Multiple Spreadsheets

Adjust Spreadsheet Precision

Export Spreadsheet Data

Clear Spreadsheet

Multiple Spreadsheet Joiner

Join Configuration Steps

Step 1: Factors X Responses (Concatenate)

Join Type

☒ Concatenation ☐ Left Join ☐ Right Join ☐ Inner Join ☐ Full Outer Join

Left Spreadsheet: Factors

Right Spreadsheet: Responses

Join Column

☒ Common header name ☐ Different header names

Execute Cancel

Min-Max

?

X

Excluded Columns

Col5 - Y1

Col6 - Y2

Col7 - Y3

>>

>

<

<<

Included Columns

Col2 - X1

Col3 - X2

Col4 - X3

Min -1.0

Max 1.0

Execute

Cancel

Results:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)
User Header	User Row ID	X1	X2	X3	Y1	Y2	Y3
1		-1.0	-1.0	0E-7	59.6	0.0000267	6.7268120
2		1.0	-1.0	0E-7	17.71	0.0000605	4.7822589
3		-1.0	1.0	0E-7	46.85	0.0000332	4.4855323
4		1.0	1.0	0E-7	19.59	0.0000461	3.3630343
5		-1.0	0E-7	-1.0	61.62	0.0035391	4.2107007
6		1.0	0E-7	-1.0	14.36	0.0000350	4.0074930
7		-1.0	0E-7	1.0	53.81	0.0001144	4.2107007
8		1.0	0E-7	1.0	19.09	0.0000583	4.0681691
9		0.0	-1.0	-1.0	67.54	0.0002838	7.4020267
10		0.0	1.0	-1.0	23.99	0.0000834	4.6043458
11		0.0	-1.0	1.0	19.02	0.0001990	5.2028838
12		0.0	1.0	1.0	19.98	0.0000700	4.4888751
13		0.0	0E-7	0E-7	25.93	0.0005007	4.7518417
14		0.0	0E-7	0E-7	25.93	0.0000950	4.7455242
15		0.0	0E-7	0E-7	25.93	0.0000702	4.7822589
16		0.0	0E-7	0E-7	25.93	0.0005007	4.7518417
17		0.0	0E-7	0E-7	25.93	0.0000950	4.7455242

Step 5: Regression

The goal here is to produce a regression equation that includes main effects, two-factor interactions and quadratic effects for Y_1 :

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

Create a new tab named “Regression – Y1” and import the results from the spreadsheet “Normalized data”. Afterwards, fit a generalized linear model to the data: *Analytics* → *Regression* → *Statistical fitting* → *Generalized Linear Models*

The image shows two screenshots of the software interface for fitting a generalized linear model.

Generalized Linear Models Regression Dialog:

- Type:** Linear
- Confidence Level(%):** 95
- Scale Parameter Method:** Fixed value
- Value:** 1.0
- Dependent Variable:** Col5 – Y1
- Excluded Columns:** Col6 – Y2, Col7 – Y3
- Factors:** (Empty)
- Covariates:** Col2 – X1, Col3 – X2, Col4 – X3
- Formula Type:** Custom (selected), Main Effects, Full Factorial
- Specify Custom Model Formula:** X1 + X2 + X3 + X1:X2 + X1:X3 + X2:X3 + X1:X1 + X2:X2 + X3:X3
- Buttons:** Execute, Cancel

Custom Formula Creation Dialog:

- Included Columns:** X1, X2, X3
- Add interactions:** up to order 2
- Add terms:** up to order 2
- Add Custom Interaction:** (Button)
- Delete Selected:** (Button)
- Reset Selections:** (Button)
- Included Terms:** X1, X2, X3, X1:X2, X1:X3, X2:X3, X1:X1, X2:X2, X3:X3
- Buttons:** Move up, Move down
- Formula:** X1 + X2 + X3 + X1:X2 + X1:X3 + X2:X3 + X1:X1 + X2:X2 + X3:X3
- Buttons:** Save, Cancel

Results:

Y1	Prediction
59.6	65.1687500
17.71	20.0712500
46.85	44.48875
19.59	14.0212500
61.62	66.1975
14.36	22.1450000
53.81	46.025
19.09	14.5125000
67.54	57.3937500
23.99	21.7737500
19.02	21.2362500
19.98	30.12625
25.93	25.9300000
25.93	25.9300000
25.93	25.9300000
25.93	25.9300000
25.93	25.9300000

Goodness of Fit	
	Value
Deviance	452.0087250
Scaled Deviance	452.0087250
Pearson Chi-Square	452.0087250
Scaled Pearson Chi-Square	452.0087250
Log Likelihood	-241.6263176
Akaike's Information Criterion (AIC)	503.2526351
Finite Sample Corrected AIC (AICC)	539.9193018
Bayesian Information Criterion (BIC)	511.5847686
Consistent AIC (CAIC)	521.5847686

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	25.9300000	0.4472136	25.0534775	26.8065225	3361.8245000	1	0.0
X1	-18.8912500	0.3535534	-19.5842019	-18.1982981	2855.0346125	1	0.0
X2	-6.6825	0.3535534	-7.3754519	-5.9895481	357.2464500	1	0.0
X3	-6.9512500	0.3535534	-7.6442019	-6.2582981	386.5590125	1	0.0
X1*X3	3.1350000	0.5	2.1550180	4.1149820	39.3129000	1	0E-7
X1*X2	3.6575000	0.5	2.6775180	4.6374820	53.5092250	1	0E-7
X2*X3	11.1275000	0.5	10.1475180	12.1074820	495.2850250	1	0.0
X1*X1	7.2975	0.4873397	6.3423317	8.2526683	224.2252895	1	0.0
X2*X2	2.7100000	0.4873397	1.7548317	3.6651683	30.9225263	1	0E-7
X3*X3	3.9925	0.4873397	3.0373317	4.9476683	67.1160263	1	0E-7

Repeat this step for the rest of the response variables. Results, Y₂:

Y2	Prediction
0.0000267	0.0005178
0.0000605	-0.0003501
0.0000332	0.0004439
0.0000461	-0.0004450
0.0035391	0.0026753
0.0000350	0.0000730
0.0001144	0.0000765
0.0000583	0.0009220
0.0002838	0.0006565
0.0000834	0.0005365
0.0001990	-0.0002541
0.0000700	-0.0003027
0.0005007	0.0002523
0.0000950	0.0002523
0.0000702	0.0002523
0.0005007	0.0002523
0.0000950	0.0002523

Goodness of Fit	
	Value
Deviance	0.0000032
Scaled Deviance	0.0000032
Pearson Chi-Square	0.0000032
Scaled Pearson Chi-Square	0.0000032
Log Likelihood	-15.6219567
Akaike's Information Criterion (AIC)	51.2439133
Finite Sample Corrected AIC (AICC)	87.9105800
Bayesian Information Criterion (BIC)	59.5760468
Consistent AIC (CAIC)	69.5760468

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	0.0002523	0.4472136	-0.8762702	0.8767749	3E-7	1	0.9995498
X1	-0.0004392	0.3535534	-0.6933911	0.6925127	0.0000015	1	0.9990089
X2	-0.0000422	0.3535534	-0.6929941	0.6929097	0E-7	1	0.9999048
X3	-0.0004374	0.3535534	-0.6933894	0.6925145	0.0000015	1	0.9990128
X1*X3	0.0008620	0.5	-0.9791200	0.9808440	0.0000030	1	0.9986245
X1*X2	-0.0000052	0.5	-0.9799872	0.9799767	0E-7	1	0.9999916
X2*X3	0.0000179	0.5	-0.9799641	0.9799999	0E-7	1	0.9999715
X1*X1	0.0002835	0.4873397	-0.9548848	0.9554518	3E-7	1	0.9995359
X2*X2	-0.0004942	0.4873397	-0.9556625	0.9546741	0.0000010	1	0.9991909
X3*X3	0.0004009	0.4873397	-0.9547674	0.9555692	7E-7	1	0.9993436

Results, Y₃:

Y3	Prediction
6.7268120	6.3680462
4.7822589	5.1038210
4.4855323	4.1639701
3.3630343	3.7218002
4.2107007	4.8477759
4.0074930	3.9642402
4.2107007	4.2539534
4.0681691	3.4310939
7.4020267	7.1237174
4.6043458	4.2888327
5.2028838	5.5183969
4.4888751	4.7671845
4.7518417	4.7553982
4.7455242	4.7553982
4.7822589	4.7553982
4.7518417	4.7553982
4.7455242	4.7553982

Goodness of Fit	
	Value
Deviance	1.6346525
Scaled Deviance	1.6346525
Pearson Chi-Square	1.6346525
Scaled Pearson Chi-Square	1.6346525
Log Likelihood	-16.4392813
Akaike's Information Criterion (AIC)	52.8785626
Finite Sample Corrected AIC (AICC)	89.5452293
Bayesian Information Criterion (BIC)	61.2106960
Consistent AIC (CAIC)	71.2106960

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	4.7553982	0.4472136	3.8788756	5.6319207	113.0690582	1	0.0
X1	-0.4265988	0.3535534	-1.1195507	0.2663531	1.4558922	1	0.2275848
X2	-0.8965242	0.3535534	-1.5894762	-0.2035723	6.4300457	1	0.0112206
X3	-0.2817422	0.3535534	-0.9746941	0.4112097	0.6350292	1	0.4255165
X1*X3	0.0151690	0.5	-0.9648130	0.9951510	0.0009204	1	0.9757974
X1*X2	0.2055138	0.5	-0.7744682	1.1854958	0.1689437	1	0.6810523
X2*X3	0.5209181	0.5	-0.4590639	1.5009001	1.0854226	1	0.2974877
X1*X1	-0.6081279	0.4873397	-1.5632962	0.3470404	1.5571349	1	0.2120852
X2*X2	0.6921391	0.4873397	-0.2630292	1.6473074	2.0170803	1	0.1555380
X3*X3	-0.0230044	0.4873397	-0.9781727	0.9321639	0.0022282	1	0.9623506

Step 6: Data selection for Regression Metrics

Create a tab named “Metrics Data – Y1” and import the results from the spreadsheet “Regression – Y1”. Next, assign names to all unnamed columns containing data, using names such as Z1–Z9. Then, select only the response column (Y1) and the “Prediction” column to be transferred to the right spreadsheet:

Data Transformation → Data Manipulation → Select Column(s)

	Col1	Col2 (D)	Col3 (D)	Col4	Col5 (S)	Col6 (S)	Col7 (S)	Col8 (S)	Col9 (S)	Col10 (S)	Col11 (S)	Col12 (S)
User Header	User Row ID	Y1	Prediction	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8	Z9
1		59.6	65.1687500		Goodness of Fit							
2		17.71	20.0712500			Value						
3		46.85	44.48875		Deviance	452.0087250						
4		19.59	14.0212500		Scaled Deviance	452.0087250						
5		61.62	66.1975		Pearson Chi-Square	452.0087250						
6		14.36	22.1450000		Scaled Pearson Chi-Square	452.0087250						
7		53.81	46.0250000		Log Likelihood	-241.6263176						
8		19.09	14.5125000		Akaike's Information Criterion (AIC)	503.2526351						
9		67.54	57.3937500		Finite Sample Corrected AIC (AICC)	539.9193018						
10		23.99	21.77375		Bayesian Information Criterion (BIC)	511.5847686						
11		19.02	21.23625		Consistent AIC (CAIC)	521.5847686						
12		19.98	30.1262500									
13		25.93	25.9300000									
14		25.93	25.9300000		Parameter Estimates							
15		25.93	25.9300000		Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
16		25.93	25.9300000		intercept	25.9300000	0.4472136	25.0534775	26.8065225	3361.8245000	1	0.0

Results:

Select Column(s)

☒ Select Columns Manually

☐ Select Matching Columns from Node

☐ Select Columns Manually and from Node

Select Node

Select Node

Excluded Columns

Col5 -- Z2
 Col6 -- Z3
 Col7 -- Z4
 Col8 -- Z5
 Col9 -- Z6
 Col10 -- Z7
 Col11 -- Z8
 Col12 -- Z9

>>
 >
 <
 <<

Included Columns

Col2 -- Y1
 Col3 -- Prediction

Execute

Cancel

	Col1	Col2 (D)	Col3 (D)
User Header	User Row ID	Y1	Prediction
1		59.6	65.1687500
2		17.71	20.0712500
3		46.85	44.48875
4		19.59	14.0212500
5		61.62	66.1975
6		14.36	22.1450000
7		53.81	46.0250000
8		19.09	14.5125000
9		67.54	57.3937500
10		23.99	21.77375
11		19.02	21.23625
12		19.98	30.1262500
13		25.93	25.9300000
14		25.93	25.9300000
15		25.93	25.9300000
16		25.93	25.9300000
17		25.93	25.9300000

Repeat this step for the rest of the response variables.

Step 7: Regression Metrics

Create a tab named “Metrics – Y1” and import the results from the spreadsheet “Metrics Data– Y1”. Then, produce the regression metrics for the Y₁ regression equation: *Statistics* → *Model Metrics* → *Regression Metrics*


Regression Statistics Metrics
?
×

Actual Value Column

Col2 – Y1

Prediction Value Column

Col3 – Prediction

Execute

Cancel

Results:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		26.5887485	5.1564279	3.8417647	0.9094393

Repeat this step for the rest of the response variables. Results, Y₂:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		2E-7	0.0004345	0.0003677	0.7140028

Results, Y₃:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		0.0961560	0.3100904	0.2330987	0.8896206

Step 8: Analysis of Covariance

Create a new tab named “ANCOVA – Y1” and import the results from the spreadsheet “Normalized data”. Afterwards perform analysis of covariance for Y₁: Statistics → Analysis of (Co)Variance → ANCOVA

ANCOVA

?

✕

Confidence Level (%)

95

Dependent Variable

Col5 -- Y1

Sum of Squares for Tests

Adjusted (Type III)

Coding for Factors

(-1, 0, +1)

Excluded Columns

Col6 -- Y2
Col7 -- Y3

Factors

Specify Reference Levels

Covariates

Col2 -- X1
Col3 -- X2
Col4 -- X3

Formula Type:

☒ Custom

☐ Main Effects

☐ Full Factorial

Specify Custom Model Formula

Formula:

X1 + X2 + X3 + X1:X2 + X1:X3 + X2:X3 + X1:X1 + X2:X2 + X3:X3

Execute

Cancel

Custom Formula Creation

Included Columns

- X1
- X2
- X3

Add interactions

up to order 2

Add terms

up to order 2

Add Custom Interaction

Delete Selected

Reset Selections

Included Terms

- X1
- X2
- X3
- X1:X2
- X1:X3
- X2:X3
- X1:X1
- X2:X2
- X3:X3

Move up Move down

$X1 + X2 + X3 + X1:X2 + X1:X3 + X2:X3 + X1:X1 + X2:X2 + X3:X3$

Save Cancel

Results:

	Col1	Col2 (S)	Col3 (I)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)
User Header	User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value
1		X1	1	2855.0346125	2855.0346125	44.2142843	0.0002907
2		X2	1	357.2464500	357.2464500	5.5324710	0.0509283
3		X3	1	386.5590125	386.5590125	5.9864178	0.0443202
4		X1*X2	1	53.5092250	53.5092250	0.8286667	0.3929242
5		X2*X3	1	495.2850250	495.2850250	7.6701952	0.0277136
6		X1*X3	1	39.3129000	39.3129000	0.6088163	0.4607988
7		X1*X1	1	224.2252895	224.2252895	3.4724485	0.1046798
8		X2*X2	1	30.9225263	30.9225263	0.4788794	0.5112243
9		X3*X3	1	67.1160263	67.1160263	1.0393874	0.3418995
10		Error	7	452.0087250	64.5726750		
11		Total	16	4991.2242471			

Repeat this step for the rest of the response variables. Results, Y₂:

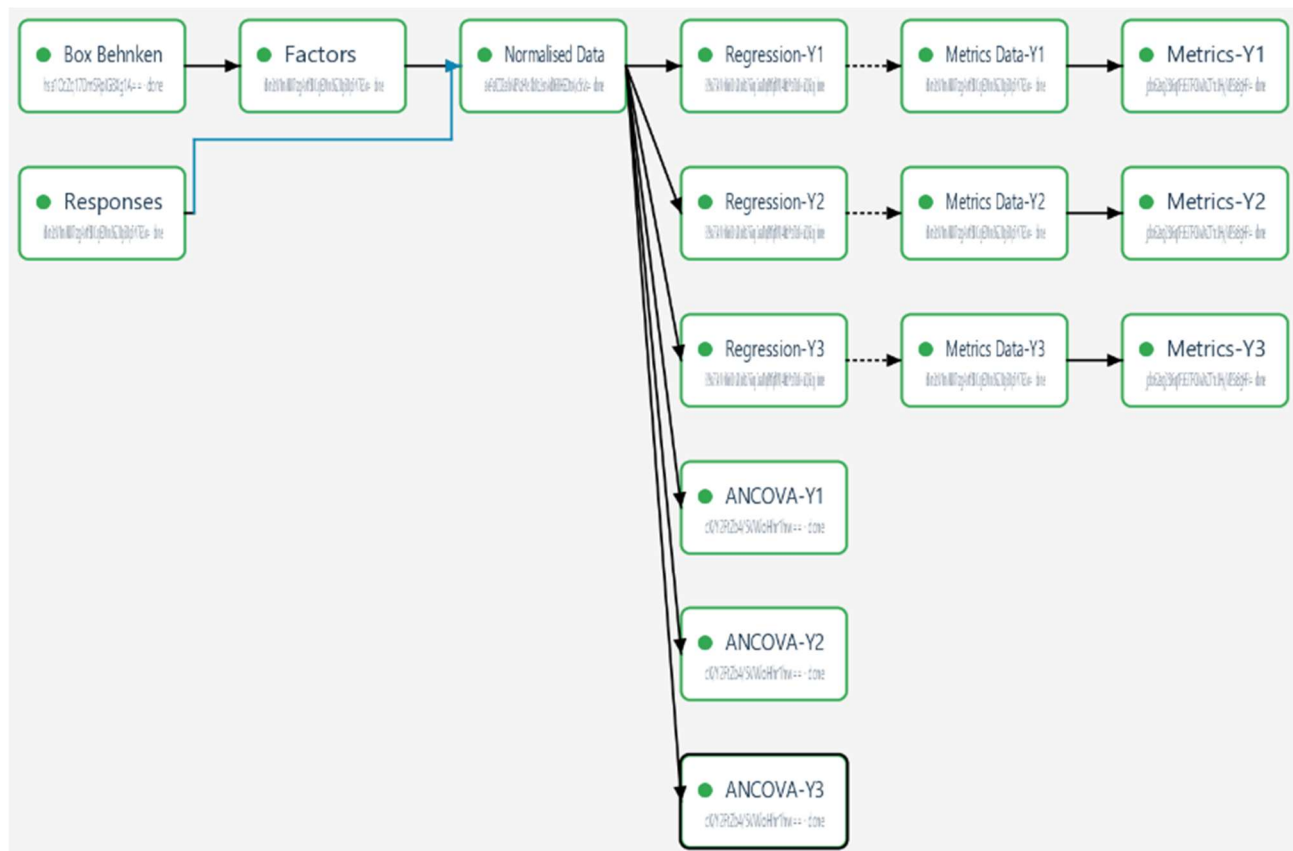
	Col1	Col2 (S)	Col3 (I)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)
User Header	User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value
1		X1	1	0.0000015	0.0000015	3.3661503	0.1091892
2		X2	1	0E-7	0E-7	0.0310394	0.8651411
3		X3	1	0.0000015	0.0000015	3.3394990	0.1103604
4		X1*X2	1	0E-7	0E-7	0.0002402	0.9880666
5		X2*X3	1	0E-7	0E-7	0.0027872	0.9593709
6		X1*X3	1	0.0000030	0.0000030	6.4830913	0.0383160
7		X1*X1	1	3E-7	3E-7	0.7381098	0.4187129
8		X2*X2	1	0.0000010	0.0000010	2.2429905	0.1778899
9		X3*X3	1	7E-7	7E-7	1.4761475	0.2637648
10		Error	7	0.0000032	5E-7		
11		Total	16	0.0000112			

Results, Y₃:

	Col1	Col2 (S)	Col3 (I)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)
User Header	User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value
1		X1	1	1.4558922	1.4558922	6.2345029	0.0411787
2		X2	1	6.4300457	6.4300457	27.5351006	0.0011896
3		X3	1	0.6350292	0.6350292	2.7193577	0.1431263
4		X1*X2	1	0.1689437	0.1689437	0.7234601	0.4231479
5		X2*X3	1	1.0854226	1.0854226	4.6480571	0.0680149
6		X1*X3	1	0.0009204	0.0009204	0.0039414	0.9516964
7		X1*X1	1	1.5571349	1.5571349	6.6680499	0.0363526
8		X2*X2	1	2.0170803	2.0170803	8.6376537	0.0217445
9		X3*X3	1	0.0022282	0.0022282	0.0095418	0.9249228
10		Error	7	1.6346525	0.2335218		
11		Total	16	14.8094032			

Final Isalos Workflow

The final workflow is presented below:



References

- (1) Mahesh Kumar, P.; Ghosh, A. Development and Evaluation of Metronidazole Loaded Microsponge Based Gel for Superficial Surgical Wound Infections. *Journal of Drug Delivery Science and Technology* **2015**, 30, 15–29. <https://doi.org/10.1016/j.jddst.2015.09.006>.