



Development and characterization of miltefosine-loaded polymeric micelles for cancer treatment

This research paper aims to develop a formulation of miltefosine-loaded polymeric micelles of the copolymer Pluronic-F127 to decrease the hemolytic potential of miltefosine. Design of experiments (DoE) methodology is implemented in order to evaluate the effect of the independent variables on the responses.

The factors (independent variables) examined are: X_1 = hydration temperature ($^{\circ}\text{C}$), X_2 = stirring speed (rpm) and X_3 = stirring time (min). All the factors are continuous. The response (dependent variable) examined is: Y_1 = polydispersity index. The applied DoE method is 2^3 full factorial design.

Isalos version used: 2.1.2

Scientific article: <https://www.sciencedirect.com/science/article/pii/S0928493117320921>

Step 1: Full Factorial 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 “Full Factorial”. Afterwards, apply the full factorial method: *DOE* → *Factorial* → *Full Factorial*

	Col1	Col2 (I)	Col3 (I)	Col4 (I)
User Header	User Row ID	X1	X2	X3
1		30	550	30
2		50	750	60

DoE Full Factorial

?

×

Number of Center Points per Block

Number of Replicates

Number of Blocks

☐ Random Standard order

Excluded Columns

Included Columns

Col2 ~ X1
Col3 ~ X2
Col4 ~ X3

>>

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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	30.0	550.0	30.0
2		2	Block: 1	Replicate: 1	Design Point	50.0	550.0	30.0
3		3	Block: 1	Replicate: 1	Design Point	30.0	750.0	30.0
4		4	Block: 1	Replicate: 1	Design Point	50.0	750.0	30.0
5		5	Block: 1	Replicate: 1	Design Point	30.0	550.0	60.0
6		6	Block: 1	Replicate: 1	Design Point	50.0	550.0	60.0
7		7	Block: 1	Replicate: 1	Design Point	30.0	750.0	60.0
8		8	Block: 1	Replicate: 1	Design Point	50.0	750.0	60.0
9		9	Block: 1	Replicate: 2	Design Point	30.0	550.0	30.0
10		10	Block: 1	Replicate: 2	Design Point	50.0	550.0	30.0
11		11	Block: 1	Replicate: 2	Design Point	30.0	750.0	30.0
12		12	Block: 1	Replicate: 2	Design Point	50.0	750.0	30.0
13		13	Block: 1	Replicate: 2	Design Point	30.0	550.0	60.0
14		14	Block: 1	Replicate: 2	Design Point	50.0	550.0	60.0
15		15	Block: 1	Replicate: 2	Design Point	30.0	750.0	60.0
16		16	Block: 1	Replicate: 2	Design Point	50.0	750.0	60.0

Step 2: Definition of response variables

Create a new tab named “Responses” and define the response in the column headers. Fill a column with the values of the response variable that were observed and make sure the values follow the order of the experiments as given by the full factorial method. Then, select the column to be transferred to the right spreadsheet: *Data Transformation* → *Data Manipulation* → *Select Column(s)*

	Col1	Col2 (D)
User Header	User Row ID	Y1
1		8.8
2		4.4
3		5.2
4		5
5		4.5
6		4
7		7.2
8		6.6
9		9
10		4.5
11		4.9
12		5.2
13		4.2
14		4.1
15		6.4
16		6.3

Select Column(s) ? X

Excluded Columns

Included Columns

>>

>

<

<<

Execute

Cancel

Step 3: Data selection

Create a new tab named “Data” and import the results from the “Full Factorial” and “Responses” spreadsheets by right clicking on the left spreadsheet. Then, select only the factors and response 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						

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: Full factorial X Responses (Concatenation)

Add Delete

Join Type

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

Left Spreadsheet

Full factorial

Right Spreadsheet

Responses

Join Column

☒ Common header name
 ☐ Different header names

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
- Col9 -- Y1

>>

>

<

<<

Execute Cancel

Results:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	X1	X2	X3	Y1
1		30.0	550.0	30.0	8.8
2		50.0	550.0	30.0	4.4
3		30.0	750.0	30.0	5.2
4		50.0	750.0	30.0	5
5		30.0	550.0	60.0	4.5
6		50.0	550.0	60.0	4
7		30.0	750.0	60.0	7.2
8		50.0	750.0	60.0	6.6
9		30.0	550.0	30.0	9
10		50.0	550.0	30.0	4.5
11		30.0	750.0	30.0	4.9
12		50.0	750.0	30.0	5.2
13		30.0	550.0	60.0	4.2
14		50.0	550.0	60.0	4.1
15		30.0	750.0	60.0	6.4
16		50.0	750.0	60.0	6.3

Step 4: Normalization

Create a new tab named “Normalized data” and import the results from the “Data” spreadsheet. 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						

 Choose tab as input ×

Select input tab

Data ▾

Execute

Cancel

 Min-Max normalizer ? ×

Excluded Columns

Col5 -- Y1

>>

>

<

<<

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)
User Header	User Row ID	X1	X2	X3	Y1
1		-1.0	-1.0	-1.0	8.8
2		1.0	-1.0	-1.0	4.4
3		-1.0	1.0	-1.0	5.2
4		1.0	1.0	-1.0	5.0
5		-1.0	-1.0	1.0	4.5
6		1.0	-1.0	1.0	4.0
7		-1.0	1.0	1.0	7.2
8		1.0	1.0	1.0	6.6
9		-1.0	-1.0	-1.0	9.0
10		1.0	-1.0	-1.0	4.5
11		-1.0	1.0	-1.0	4.9
12		1.0	1.0	-1.0	5.2
13		-1.0	-1.0	1.0	4.2
14		1.0	-1.0	1.0	4.1
15		-1.0	1.0	1.0	6.4
16		1.0	1.0	1.0	6.3

Step 5: Regression

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

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3$$

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:** (Empty list)
- Factors:** (Empty list)
- Covariates:** Col2 -- X1, Col3 -- X2, Col4 -- X3
- Formula Type:** Custom (selected), Main Effects, Full Factorial
- Formula:** $X1 + X2 + X3 + X1:X2 + X1:X3 + X2:X3 + X1:X2:X3$
- Buttons:** Execute, Cancel

Custom Formula Creation Dialog:

- Included Columns:** X1, X2, X3
- Add interactions:** up to order 3
- 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:X2:X3 (selected)
- Buttons:** Move up, Move down
- Formula:** $X1 + X2 + X3 + X1:X2 + X1:X3 + X2:X3 + X1:X2:X3$
- Buttons:** Save, Cancel

Results:

Y1	Prediction
8.8	8.9000000
4.4	4.4500000
5.2	5.0500000
5	5.1000000
4.5	4.3500000
4	4.0500000
7.2	6.8000000
6.6	6.4500000
9	8.9000000
4.5	4.4500000
4.9	5.0500000
5.2	5.1000000
4.2	4.3500000
4.1	4.0500000
6.4	6.8000000
6.3	6.4500000

Goodness of Fit	
	Value
Deviance	0.5050000
Scaled Deviance	0.5050000
Pearson Chi-Square	0.5050000
Scaled Pearson Chi-Square	0.5050000
Log Likelihood	-14.9555165
Akaike's Information Criterion (AIC)	45.9110331
Finite Sample Corrected AIC (AICC)	66.4824616
Bayesian Information Criterion (BIC)	52.0917428
Consistent AIC (CAIC)	60.0917428

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	5.64375	0.25	5.1537590	6.1337410	509.6306250	1	0.0
X1	-0.6312500	0.25	-1.1212410	-0.1412590	6.3756250	1	0.0115698
X2	0.2062500	0.25	-0.2837410	0.6962410	0.6806250	1	0.4093716
X3	-0.2312500	0.25	-0.7212410	0.2587410	0.8556250	1	0.3549659
X1*X2	0.55625	0.25	0.0662590	1.0462410	4.9506250	1	0.0260812
X1*X3	0.46875	0.25	-0.0212410	0.9587410	3.515625	1	0.0607927
X2*X3	1.0062500	0.25	0.5162590	1.4962410	16.2006250	1	0.0000570
X1*X2*X3	-0.5687500	0.25	-1.0587410	-0.0787590	5.1756250	1	0.0229059

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		8.8	8.9		Goodness of Fit							
2		4.4	4.4500000			Value						
3		5.2	5.05		Deviance	0.5050000						
4		5	5.1000000		Scaled Deviance	0.5050000						
5		4.5	4.3500000		Pearson Chi-Square	0.5050000						
6		4	4.05		Scaled Pearson Chi-Square	0.5050000						
7		7.2	6.8		Log Likelihood	-14.9555165						
8		6.6	6.4500000		Akaike's Information Criterion (AIC)	45.9110331						
9		9	8.9		Finite Sample Corrected AIC (AICC)	66.4824616						
10		4.5	4.4500000		Bayesian Information Criterion (BIC)	52.0917428						
11		4.9	5.05		Consistent AIC (CAIC)	60.0917428						
12		5.2	5.1000000									
13		4.2	4.3500000									
14		4.1	4.05		Parameter Estimates							
15		6.4	6.8		Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
16		6.3	6.4500000		Intercept	5.64375	0.25	5.1537590	6.1337410	509.6306250	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		8.8	8.9
2		4.4	4.4500000
3		5.2	5.05
4		5	5.1000000
5		4.5	4.3500000
6		4	4.05
7		7.2	6.8
8		6.6	6.4500000
9		9	8.9
10		4.5	4.4500000
11		4.9	5.05
12		5.2	5.1000000
13		4.2	4.3500000
14		4.1	4.05
15		6.4	6.8
16		6.3	6.4500000

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 ? X

Actual Value Column

Col2 – Y1

Prediction Value Column

Col3 – Prediction

Execute

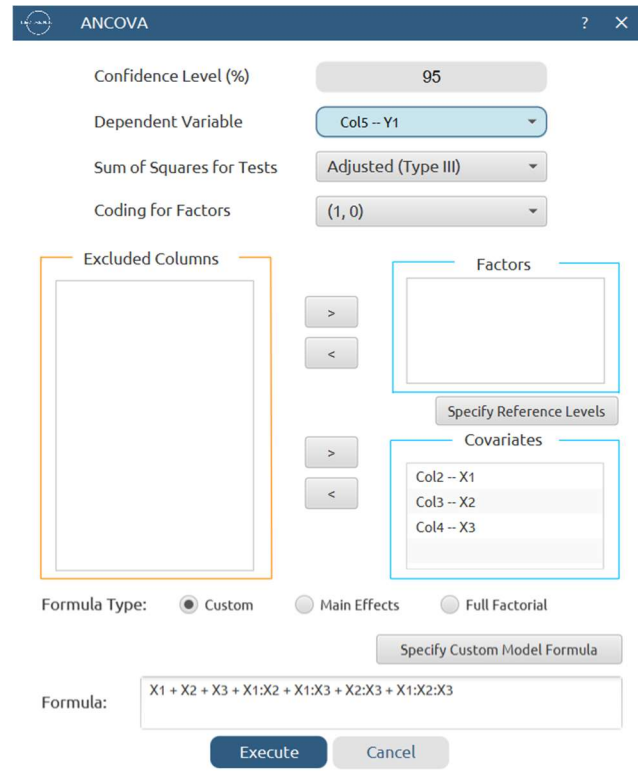
Cancel

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		0.0315625	0.1776584	0.1437500	0.9868006

Step 8: Analysis of Covariance

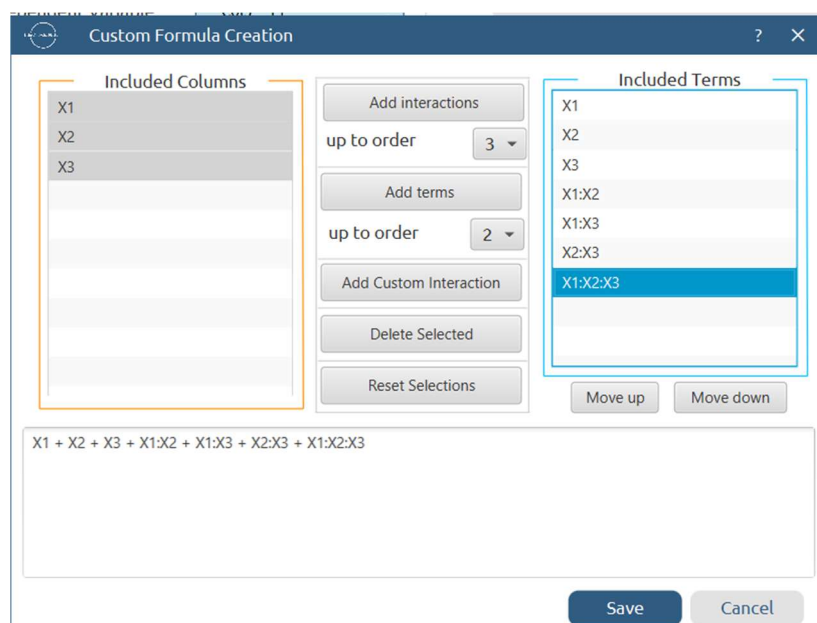
Create a new tab named “ANCOVA – Y1” and import the results from the spreadsheet “Normalized data”. Afterwards perform analysis of covariance for Y_1 : Statistics → Analysis of (Co)Variance → ANCOVA



The ANCOVA dialog box is shown with the following settings:

- Confidence Level (%): 95
- Dependent Variable: Col5 -- Y1
- Sum of Squares for Tests: Adjusted (Type III)
- Coding for Factors: (1, 0)
- Excluded Columns: (Empty list)
- Factors: (Empty list)
- Covariates: Col2 -- X1, Col3 -- X2, Col4 -- X3
- Formula Type: ☒ Custom, ☐ Main Effects, ☐ Full Factorial
- Formula: $X1 + X2 + X3 + X1:X2 + X1:X3 + X2:X3 + X1:X2:X3$

Buttons: Execute, Cancel



The Custom Formula Creation dialog box is shown with the following settings:

- Included Columns: X1, X2, X3
- Add interactions: up to order 3
- Add terms: up to order 2
- Add Custom Interaction: (Empty list)
- Delete Selected: (Empty list)
- Reset Selections: (Empty list)
- Included Terms: X1, X2, X3, X1:X2, X1:X3, X2:X3, X1:X2:X3
- Formula: $X1 + X2 + X3 + X1:X2 + X1:X3 + X2:X3 + X1:X2:X3$

Buttons: 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	6.3756250	6.3756250	101.0000000	0.0000082
2		X2	1	0.6806250	0.6806250	10.7821782	0.0111275
3		X3	1	0.8556250	0.8556250	13.5544554	0.0062045
4		X1*X2	1	4.9506250	4.9506250	78.4257426	0.0000209
5		X2*X3	1	16.200625	16.200625	256.6435644	2E-7
6		X1*X3	1	3.515625	3.515625	55.6930693	0.0000718
7		X1*X2*X3	1	5.1756250	5.1756250	81.9900990	0.0000177
8		Error	8	0.5050000	0.0631250		
9		Total	15	38.2593750			

Final Isalos Workflow



References

- (1) Valenzuela-Oses, J. K.; García, M. C.; Feitosa, V. A.; Pachioni-Vasconcelos, J. A.; Gomes-Filho, S. M.; Lourenço, F. R.; Cerize, N. N. P.; Bassères, D. S.; Rangel-Yagui, C. O. Development and Characterization of Miltefosine-Loaded Polymeric Micelles for Cancer Treatment. *Materials Science and Engineering: C* **2017**, 81, 327–333. <https://doi.org/10.1016/j.msec.2017.07.040>.