



## 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: 2.0.6*

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

>>

>

<

<<

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)

Excluded Columns

Included Columns

>>

>

<

<<

Execute

Cancel

### Step 3: Data isolation

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)

**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						

Import from File  
Import from Spreadsheet  
Import from Multiple Spreadsheets  
Adjust Spreadsheet Precision  
Export Spreadsheet Data  
Clear Spreadsheet

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

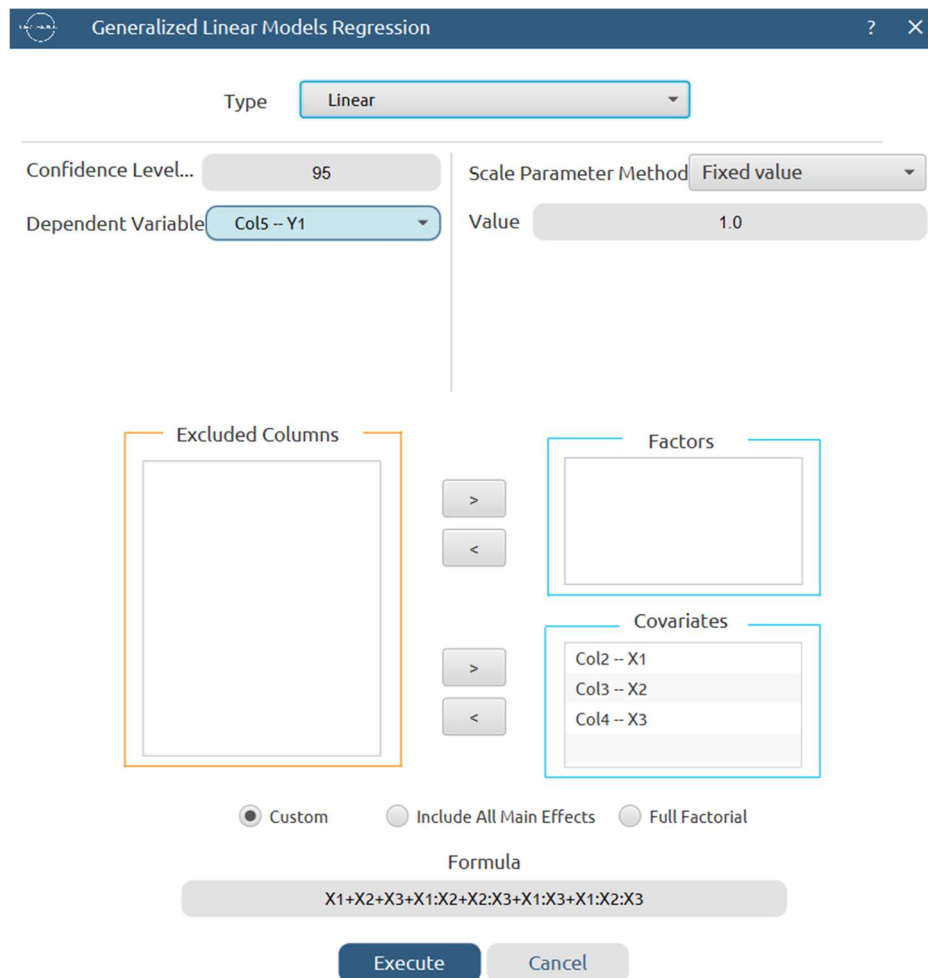
Results:

	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 quadratic effects for  $Y_1$ :  $Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$

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 dialog box is titled "Generalized Linear Models Regression". It contains the following fields and options:

- Type:** A dropdown menu set to "Linear".
- Confidence Level...:** A text input field containing "95".
- Scale Parameter Method:** A dropdown menu set to "Fixed value".
- Value:** A text input field containing "1.0".
- Dependent Variable:** A dropdown menu set to "Col5 -- Y1".
- Excluded Columns:** An empty rectangular box with an orange border.
- Factors:** An empty rectangular box with a blue border.
- Covariates:** A list box containing three items: "Col2 -- X1", "Col3 -- X2", and "Col4 -- X3".
- Buttons:** Between the Excluded Columns and Factors boxes are two buttons: ">" and "<". Between the Factors and Covariates boxes are two buttons: ">" and "<".
- Radio Buttons:** At the bottom, there are three radio buttons: "Custom" (selected), "Include All Main Effects", and "Full Factorial".
- Formula:** A text input field containing the formula "X1+X2+X3+X1:X2+X2:X3+X1:X3+X1:X2:X3".
- Buttons:** At the bottom, there are two buttons: "Execute" and "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	91.5937500	21.4366800	49.5786292	133.6088708	18.2564625	1	0.0000193
X1	-1.6743750	0.5199159	-2.6933914	-0.6553586	10.3714465	1	0.0012798
X2	-0.1186250	0.0325960	-0.1825120	-0.0547380	13.2441324	1	0.0002734
X3	-1.5622917	0.4519249	-2.4480482	-0.6765351	11.9506548	1	0.0005463
X1*X3	0.0277708	0.0109608	0.0062881	0.0492536	6.4193967	1	0.0112881
X1*X2	0.0022625	0.0007906	0.0007130	0.0038120	8.1902500	1	0.0042116
X2*X3	0.0021875	0.0006872	0.0008406	0.0035344	10.1332721	1	0.0014562
X1*X2*X3	-0.0000379	0.0000167	-0.0000706	-0.0000053	5.1756250	1	0.0229059

## Step 6: Regression Metrics

Create a tab named “Metrics – Y1” and import the results from the spreadsheet “Regression – Y1”. Then, produce the regression metrics for the  $Y_1$  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 7: 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<sub>1</sub>: *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)

Excluded Columns

>

<

Factors

>

<

Covariates

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

☒ Custom

☐ Include All Main Effects

☐ Full Factorial

Formula

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

Execute

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			

## 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>.