Self-assembled liquid crystalline nanoparticles as an ophthalmic drug delivery system. Part I: influence of process parameters on their preparation studied by experimental design

This research paper aims to develop and optimize self-assembled liquid crystalline nanoparticles as a drug delivery system for keratoconus treatment. Design of experiments (DoE) methodology is implemented to estimate the main effects and interaction effects of process parameters on the examined responses.

The factors (independent variables) examined are: X_1 = homogenization heating temperature (°C), X_2 = duration of emulsification (min), X_3 = homogenization heating (without/with), X_4 = number of cycles and X_5 = pressure (bar). All the factors are continuous except X_3, which is categorical. The responses (dependent variables) examined are: Y_1 = particle size (nm) and Y_2 = encapsulation efficiency (%). The applied DoE method is fractional factorial design with a resolution of V.

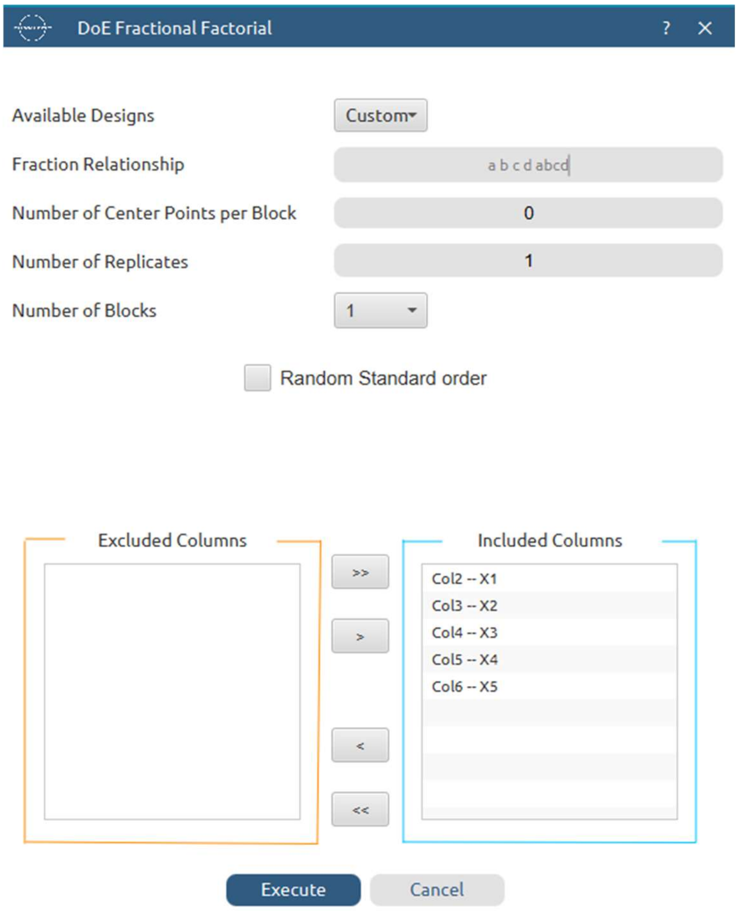
Isalos version used: 2.1.2

Scientific article: https://doi.org/10.3109/03639045.2013.850707

Step 1: Fractional 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 "Fractional Factorial". Afterwards, apply the fractional factorial method: *DOE → Screening → Fractional Factorial*

	Col1	Col2 (I)	Col3 (I)	Col4 (I)	Col5 (I)	Col6 (I)
User Header	User Row ID	X1	X2	X3	X4	X5
1		60	3	-1	5	350
2		80	5	1	8	700

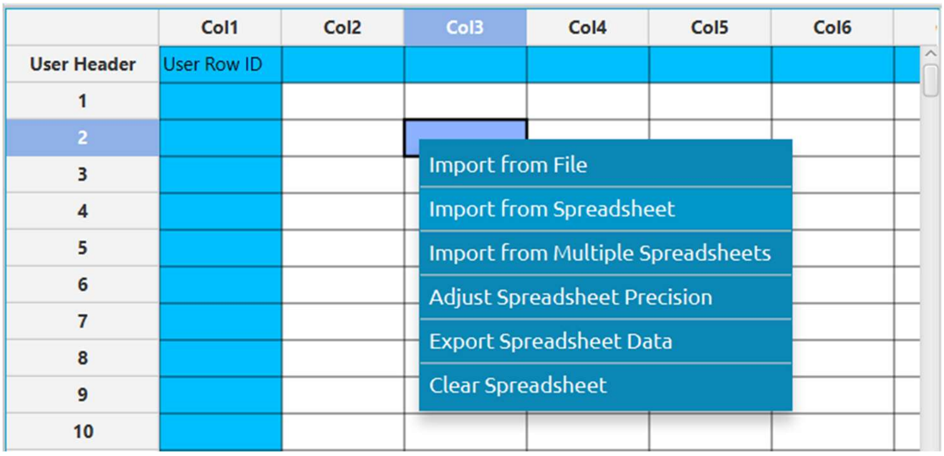


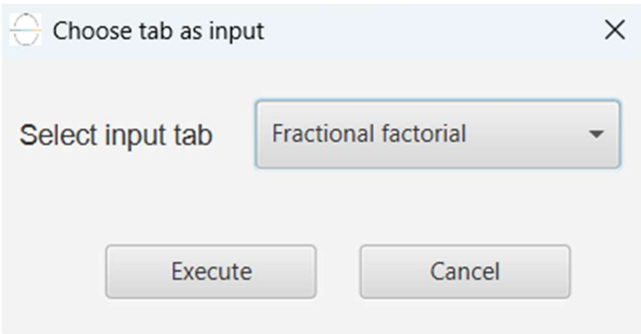
Results (right spreadsheet):

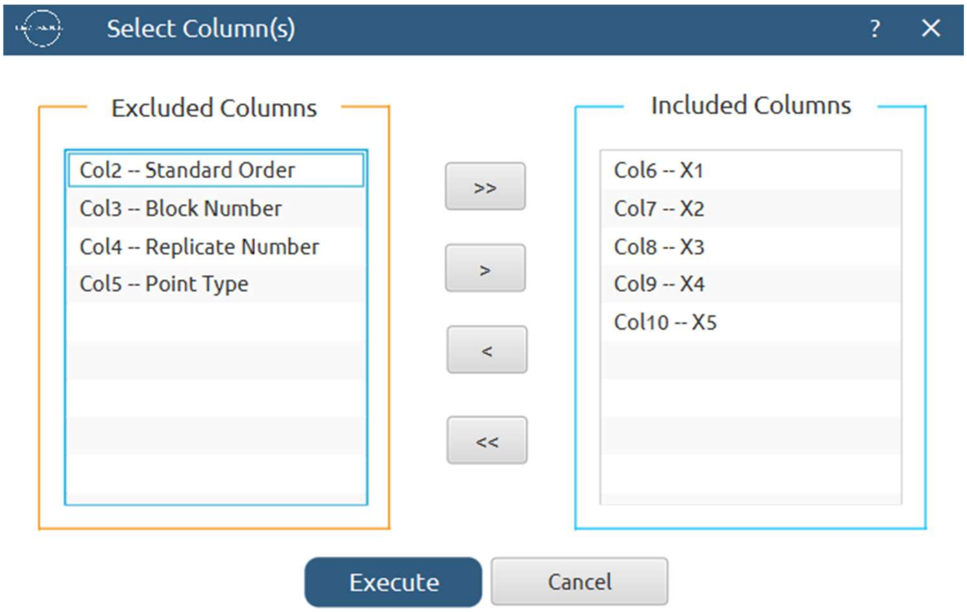
	Col2 (I)	Col3 (S)	Col4 (S)	Col5 (S)	Col6 (D)	Col7 (D)	Col8 (D)	Col9 (D)	Col10 (D)
User Header	Standard Order	Block Number	Replicate Number	Point Type	X1	X2	X3	X4	X5
1	1	Block: 1	Replicate: 1	Design Point	60.0	3.0	-1.0	5.0	700.0
2	2	Block: 1	Replicate: 1	Design Point	80.0	3.0	-1.0	5.0	350.0
3	3	Block: 1	Replicate: 1	Design Point	60.0	5.0	-1.0	5.0	350.0
4	4	Block: 1	Replicate: 1	Design Point	80.0	5.0	-1.0	5.0	700.0
5	5	Block: 1	Replicate: 1	Design Point	60.0	3.0	1.0	5.0	350.0
6	6	Block: 1	Replicate: 1	Design Point	80.0	3.0	1.0	5.0	700.0
7	7	Block: 1	Replicate: 1	Design Point	60.0	5.0	1.0	5.0	700.0
8	8	Block: 1	Replicate: 1	Design Point	80.0	5.0	1.0	5.0	350.0
9	9	Block: 1	Replicate: 1	Design Point	60.0	3.0	-1.0	8.0	350.0
10	10	Block: 1	Replicate: 1	Design Point	80.0	3.0	-1.0	8.0	700.0
11	11	Block: 1	Replicate: 1	Design Point	60.0	5.0	-1.0	8.0	700.0
12	12	Block: 1	Replicate: 1	Design Point	80.0	5.0	-1.0	8.0	350.0
13	13	Block: 1	Replicate: 1	Design Point	60.0	3.0	1.0	8.0	700.0
14	14	Block: 1	Replicate: 1	Design Point	80.0	3.0	1.0	8.0	350.0
15	15	Block: 1	Replicate: 1	Design Point	60.0	5.0	1.0	8.0	350.0
16	16	Block: 1	Replicate: 1	Design Point	80.0	5.0	1.0	8.0	700.0

Step 2: Factor isolation

Create a new tab named "Factors" and import the results from the "Fractional Factorial" 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)*







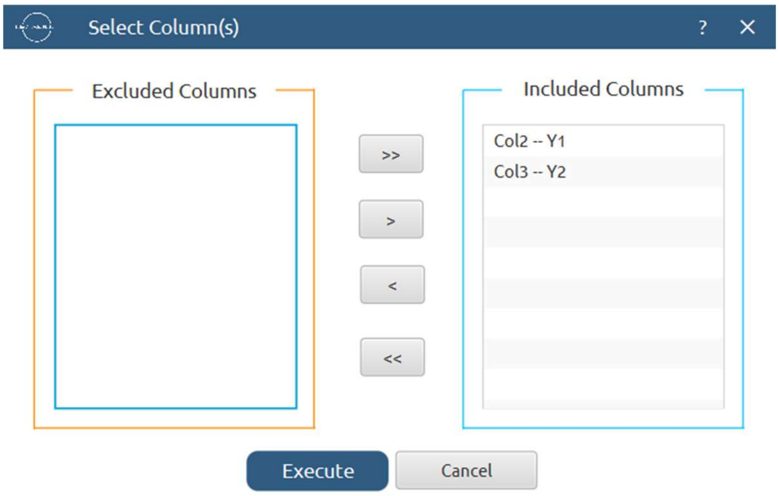
Results:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)
User Header	User Row ID	X1	X2	X3	X4	X5
1		60.0	3.0	-1.0	5.0	700.0
2		80.0	3.0	-1.0	5.0	350.0
3		60.0	5.0	-1.0	5.0	350.0
4		80.0	5.0	-1.0	5.0	700.0
5		60.0	3.0	1.0	5.0	350.0
6		80.0	3.0	1.0	5.0	700.0
7		60.0	5.0	1.0	5.0	700.0
8		80.0	5.0	1.0	5.0	350.0
9		60.0	3.0	-1.0	8.0	350.0
10		80.0	3.0	-1.0	8.0	700.0
11		60.0	5.0	-1.0	8.0	700.0
12		80.0	5.0	-1.0	8.0	350.0
13		60.0	3.0	1.0	8.0	700.0
14		80.0	3.0	1.0	8.0	350.0
15		60.0	5.0	1.0	8.0	350.0
16		80.0	5.0	1.0	8.0	700.0

Step 3: Definition of response variables

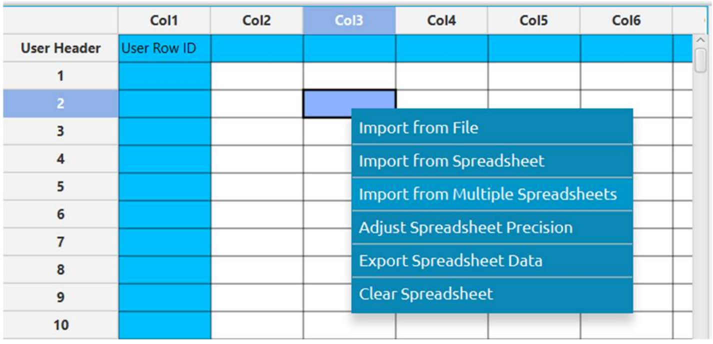
Create a new tab named "Responses" and define the responses in the column headers. Fill each column with the values of the corresponding responses that were observed and make sure the values follow the order of the experiments as given by the fractional factorial design. Then, select all columns to be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

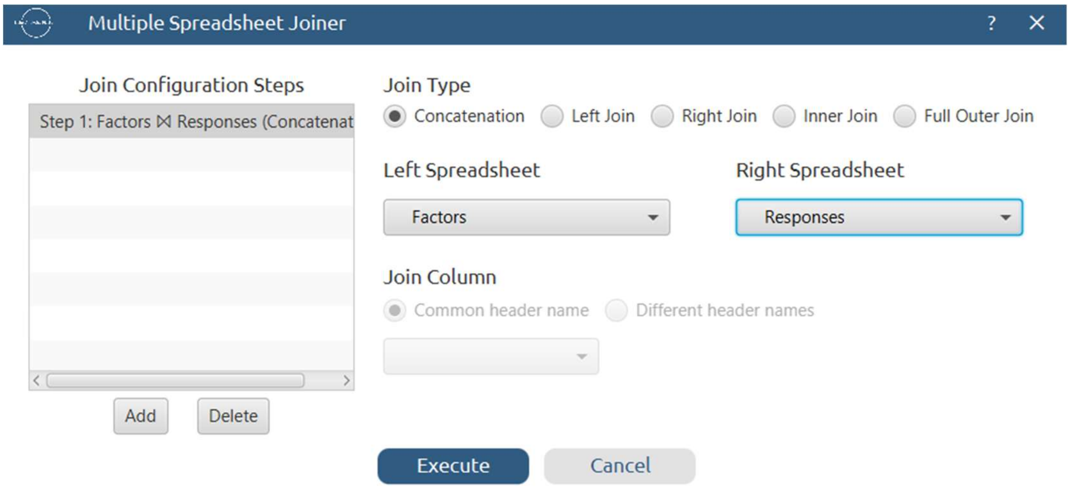
	Col1	Col2 (I)	Col3 (I)
User Header	User Row ID	Y1	Y2
1		176	29
2		223	38
3		217	44
4		160	42
5		164	29
6		148	44
7		138	43
8		168	31
9		233	35
10		178	30
11		163	39
12		177	39
13		145	46
14		152	44
15		150	43
16		145	40

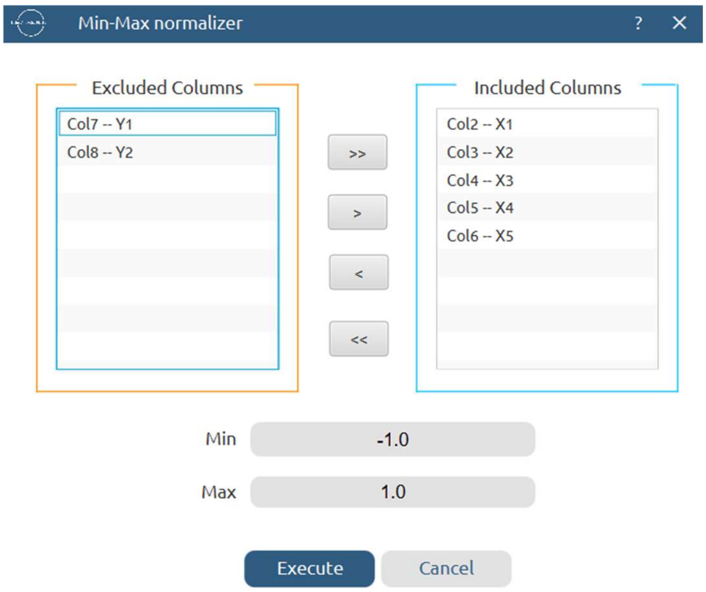


Step 4: Normalization

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]: *<u>Data Transformation → Normalizers → Min-Max</u>*







Results:

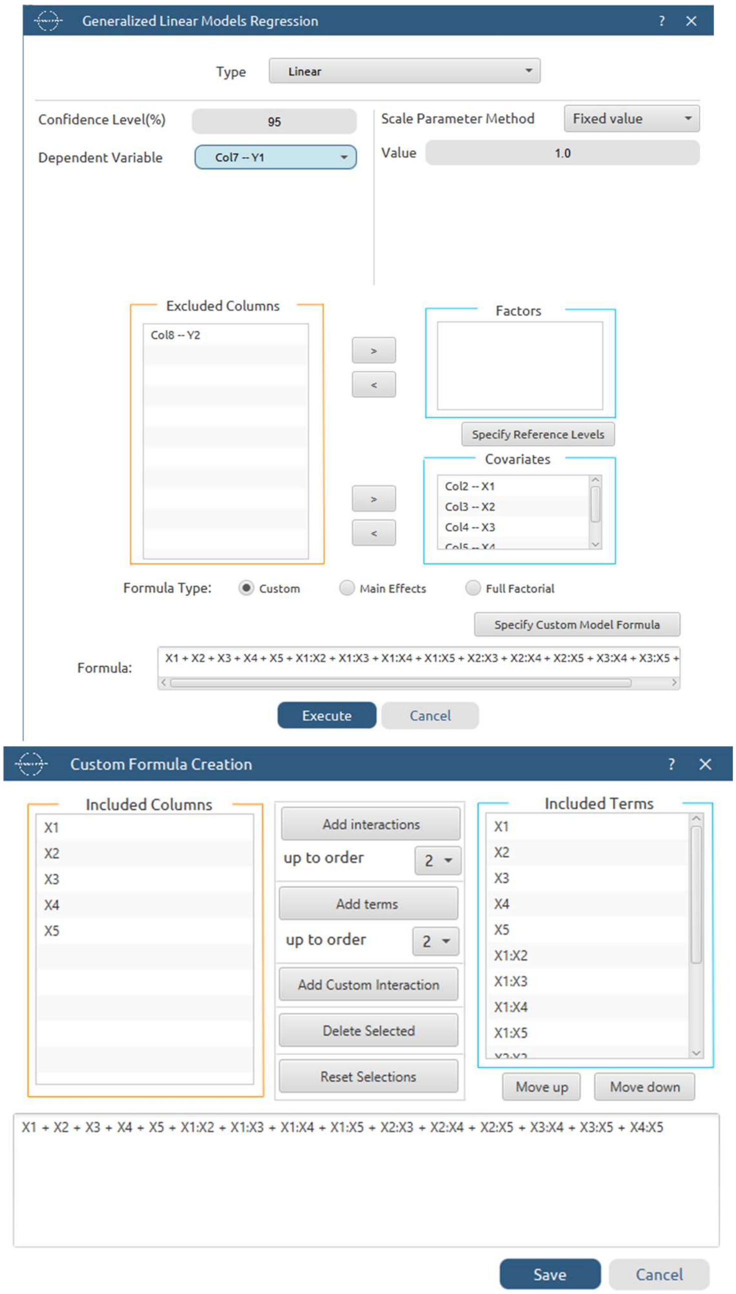
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)	Col8 (D)
User Header	User Row ID	X1	X2	X3	X4	X5	Y1	Y2
1		-1.0	-1.0	-1.0	-1.0	1.0	176.0	29.0
2		1.0	-1.0	-1.0	-1.0	-1.0	223.0	38.0
3		-1.0	1.0	-1.0	-1.0	-1.0	217.0	44.0
4		1.0	1.0	-1.0	-1.0	1.0	160.0	42.0
5		-1.0	-1.0	1.0	-1.0	-1.0	164.0	29.0
6		1.0	-1.0	1.0	-1.0	1.0	148.0	44.0
7		-1.0	1.0	1.0	-1.0	1.0	138.0	43.0
8		1.0	1.0	1.0	-1.0	-1.0	168.0	31.0
9		-1.0	-1.0	-1.0	1.0	-1.0	233.0	35.0
10		1.0	-1.0	-1.0	1.0	1.0	178.0	30.0
11		-1.0	1.0	-1.0	1.0	1.0	163.0	39.0
12		1.0	1.0	-1.0	1.0	-1.0	177.0	39.0
13		-1.0	-1.0	1.0	1.0	1.0	145.0	46.0
14		1.0	-1.0	1.0	1.0	-1.0	152.0	44.0
15		-1.0	1.0	1.0	1.0	-1.0	150.0	43.0
16		1.0	1.0	1.0	1.0	1.0	145.0	40.0

Step 5: Regression

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

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{15}X_1X_5 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{25}X_2X_5 + b_{34}X_3X_4 + b_{35}X_3X_5 + b_{45}X_4X_5$$

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*



Results:

Y1	Prediction
176	176.0
223	223.0
217	217.0
160	160.0
164	164.0
148	148.0
138	138.0
168	168.0
233	233.0
178	178.0
163	163.0
177	177.0
145	145.0
152	152.0
150	150.0
145	145.0

Goodness of Fit	
	Value
Deviance	0.0
Scaled Deviance	0.0
Pearson Chi-Square	0.0
Scaled Pearson Chi-Square	0.0
Log Likelihood	-14.7030165
Akaike's Information Criterion (AIC)	61.4060331
Finite Sample Corrected AIC (AICC)	-482.5939669
Bayesian Information Criterion (BIC)	73.7674526
Consistent AIC (CAIC)	89.7674526

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	171.0625	0.25	170.5725090	171.5524910	468198.0625	1	0.0
X1	-2.1875000	0.25	-2.6774910	-1.6975090	76.5625000	1	0.0
X2	-6.3125	0.25	-6.8024910	-5.8225090	637.5625	1	0.0
X3	-19.8125	0.25	-20.3024910	-19.3225090	6280.5625	1	0.0
X4	-3.1875000	0.25	-3.6774910	-2.6975090	162.5625000	1	0.0
X5	-14.4375000	0.25	-14.9274910	-13.9475090	3335.0625000	1	0.0
X1*X2	-0.0625000	0.25	-0.5524910	0.4274910	0.0625000	1	0.8025873
X1*X3	4.1875000	0.25	3.6975090	4.6774910	280.5625000	1	0.0
X1*X4	-2.6875000	0.25	-3.1774910	-2.1975090	115.5625000	1	0.0
X1*X5	3.3125	0.25	2.8225090	3.8024910	175.5625	1	0.0
X2*X3	5.3125000	0.25	4.8225090	5.8024910	451.5625000	1	0.0
X2*X4	-2.8125000	0.25	-3.3024910	-2.3225090	126.5625000	1	0.0
X2*X5	1.1875000	0.25	0.6975090	1.6774910	22.5625000	1	0.0000020
X3*X4	-0.0625000	0.25	-0.5524910	0.4274910	0.0625000	1	0.8025873
X3*X5	7.1875000	0.25	6.6975090	7.6774910	826.5625000	1	0.0
X4*X5	4.3125	0.25	3.8225090	4.8024910	297.5625	1	0.0

Repeat this step for the second response variable, Y_2. Results:

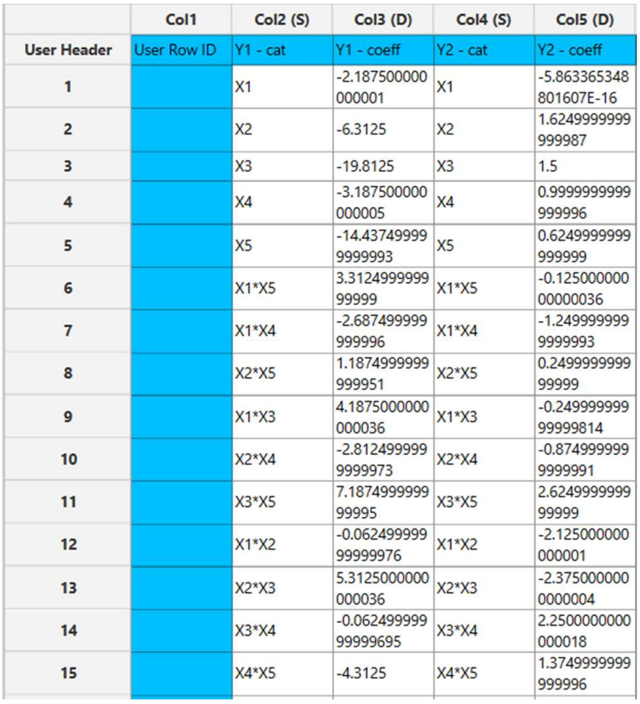
Y2	Prediction
29	29.0
38	38.0
44	44.0
42	42.0
29	29.0
44	44.0
43	43.0
31	31.0
35	35.0
30	30.0
39	39.0
39	39.0
46	46.0
44	44.0
43	43.0
40	40.0

Goodness of Fit	
	Value
Deviance	0.0
Scaled Deviance	0.0
Pearson Chi-Square	0.0
Scaled Pearson Chi-Square	0.0
Log Likelihood	-14.7030165
Akaike's Information Criterion (AIC)	61.4060331
Finite Sample Corrected AIC (AICC)	-482.5939669
Bayesian Information Criterion (BIC)	73.7674526
Consistent AIC (CAIC)	89.7674526

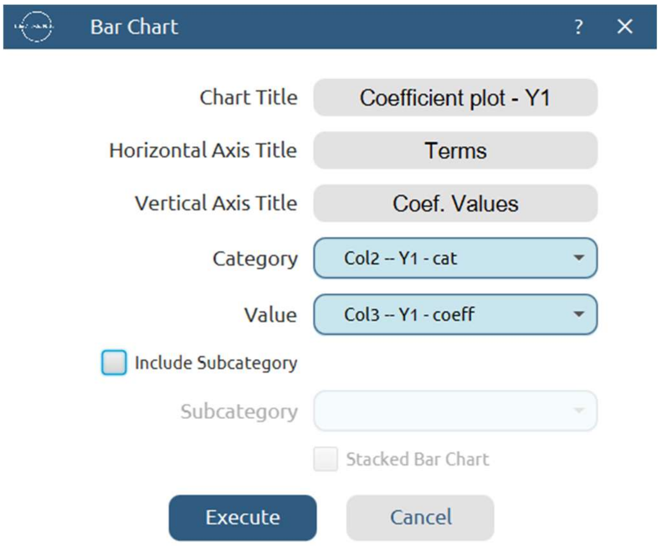
Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	38.5	0.25	38.0100090	38.9899910	23716.0	1	0.0
X1	0E-7	0.25	-0.4899910	0.4899910	0E-7	1	1.0000000
X2	1.6250000	0.25	1.1350090	2.1149910	42.2500000	1	0E-7
X3	1.5000000	0.25	1.0100090	1.9899910	36.0000000	1	0E-7
X4	1.0000000	0.25	0.5100090	1.4899910	16.0000000	1	0.0000633
X5	0.6250000	0.25	0.1350090	1.1149910	6.2500000	1	0.0124193
X1*X2	-2.1250000	0.25	-2.6149910	-1.6350090	72.2500000	1	0.0
X1*X3	-0.2500000	0.25	-0.7399910	0.2399910	1.0000000	1	0.3173105
X1*X4	-1.2500000	0.25	-1.7399910	-0.7600090	25.0000000	1	6E-7
X1*X5	-0.1250000	0.25	-0.6149910	0.3649910	0.2500000	1	0.6170751
X2*X3	-2.3750000	0.25	-2.8649910	-1.8850090	90.2500000	1	0.0
X2*X4	-0.8750000	0.25	-1.3649910	-0.3850090	12.2500000	1	0.0004653
X2*X5	0.2500000	0.25	-0.2399910	0.7399910	1.0000000	1	0.3173105
X3*X4	2.2500000	0.25	1.7600090	2.7399910	81.0000000	1	0.0
X3*X5	2.6250000	0.25	2.1350090	3.1149910	110.2500000	1	0.0
X4*X5	-1.3750000	0.25	-1.8649910	-0.8850090	30.2500000	1	0E-7

Step 6: Coefficient plots

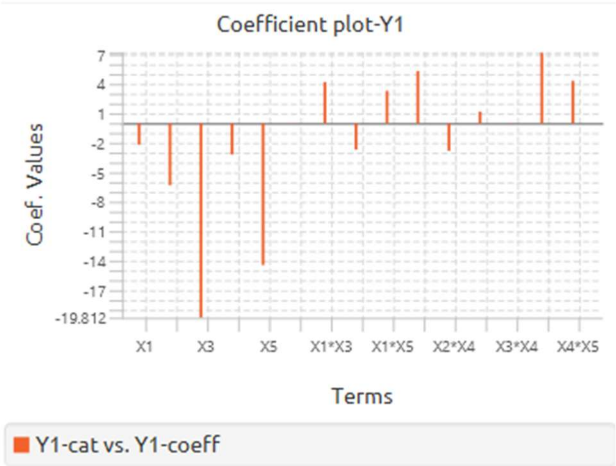
Create a new tab named "Coefficient plots". Copy and paste the regression coefficients from the spreadsheets "Regression – Y1" and "Regression – Y2" on the left spreadsheet. Afterwards, produce one bar chart for each response's coefficients: *Business Intelligence → Create New Dashboard → Add Plot → Comparison Charts → Add Bar Chart*

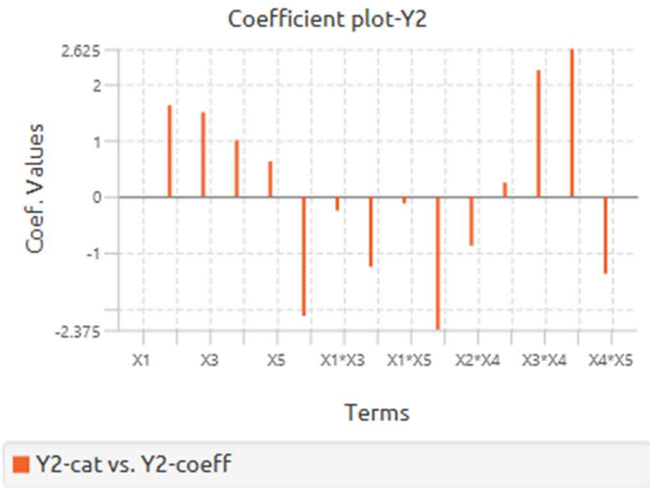


	Col1	Col2 (S)	Col3 (D)	Col4 (S)	Col5 (D)
User Header	User Row ID	Y1 - cat	Y1 - coeff	Y2 - cat	Y2 - coeff
1		X1	-2.187500000000001	X1	-5.863365348801607E-16
2		X2	-6.3125	X2	1.6249999999999987
3		X3	-19.8125	X3	1.5
4		X4	-3.187500000000005	X4	0.9999999999999996
5		X5	-14.437499999999993	X5	0.6249999999999999
6		X1*X5	3.312499999999999	X1*X5	-0.12500000000000036
7		X1*X4	-2.687499999999996	X1*X4	-1.2499999999999993
8		X2*X5	1.1874999999999951	X2*X5	0.249999999999999
9		X1*X3	4.1875000000000036	X1*X3	-0.24999999999999814
10		X2*X4	-2.8124999999999973	X2*X4	-0.8749999999999991
11		X3*X5	7.187499999999995	X3*X5	2.624999999999999
12		X1*X2	-0.06249999999999976	X1*X2	-2.125000000000001
13		X2*X3	5.3125000000000036	X2*X3	-2.3750000000000004
14		X3*X4	-0.06249999999999695	X3*X4	2.2500000000000018
15		X4*X5	-4.3125	X4*X5	1.3749999999999996



Results Y_1, Y_2:





Final Isalos Workflow

The final workflow is presented below:

References

(1) Achouri, D.; Hornebecq, V.; Piccerelle, P.; Andrieu, V.; Sergent, M. Self-Assembled Liquid Crystalline Nanoparticles as an Ophthalmic Drug Delivery System. Part I: Influence of Process Parameters on Their Preparation Studied by Experimental Design. *Drug Development and Industrial Pharmacy* **2015**, *41* (1), 109–115. https://doi.org/10.3109/03639045.2013.850707.