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.

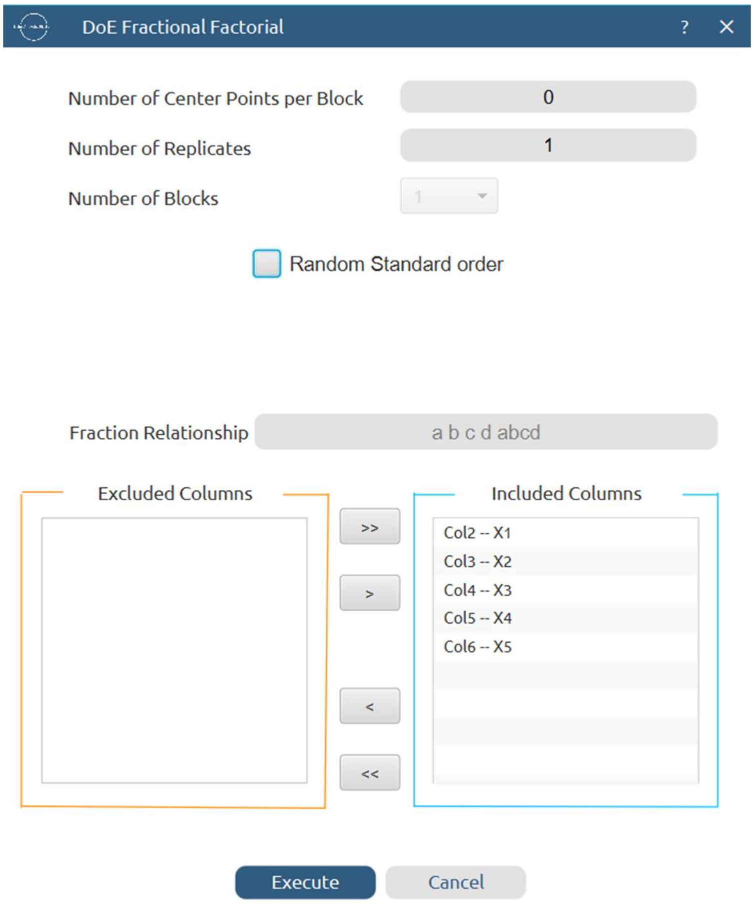
Isalos version used: 2.0.6

Scientific article: https://www.tandfonline.com/doi/abs/10.3109/03639045.2014.884113

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

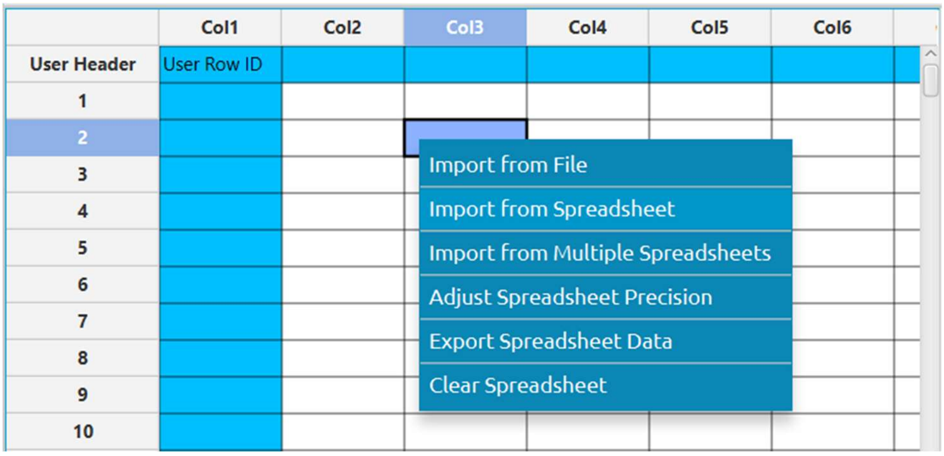


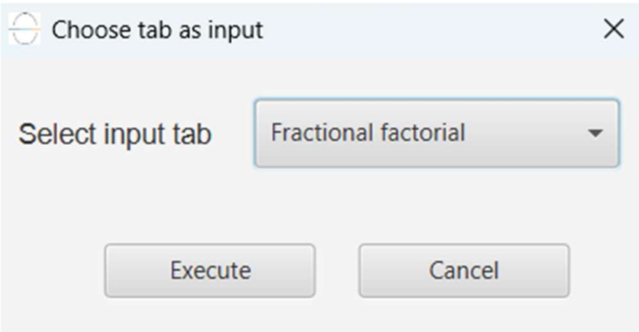
Results (right spreadsheet):

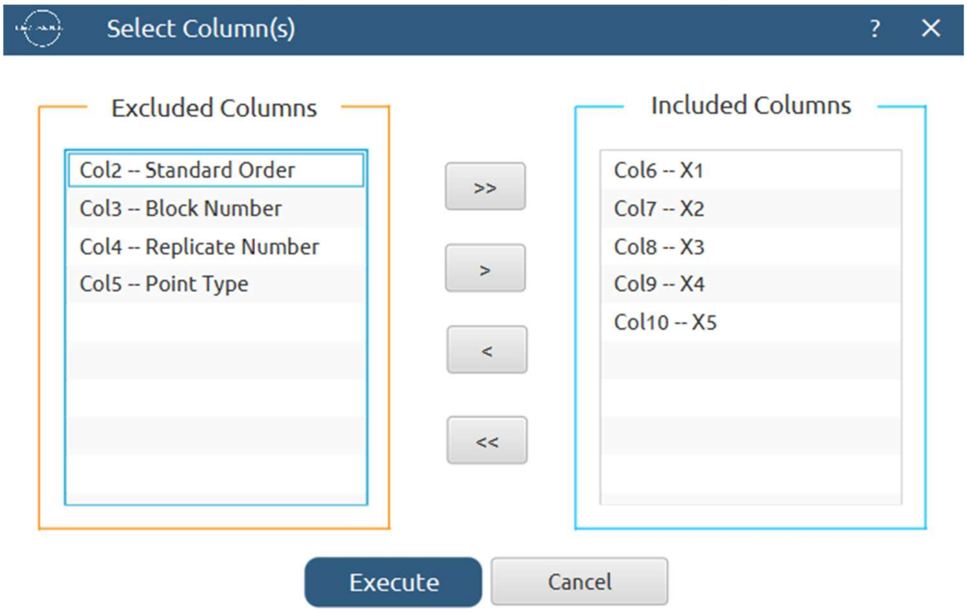
	Col2 (I)	Col3 (S)	Col4 (S)	Col5 (S)	Col6 (D)	Col7 (D)	Col8 (D)	Col9 (D)	Col10 (D)
User Header	Standard Order	Block Number	Replicate Number	Point Type	X1	X2	X3	X4	X5
1	1	Block: 1	Replicate: 1	Design Point	60.0	3.0	-1.0	5.0	700.0
2	2	Block: 1	Replicate: 1	Design Point	80.0	3.0	-1.0	5.0	350.0
3	3	Block: 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: <u>*Data Transformation → Data Manipulation → Select Column(s)*</u>







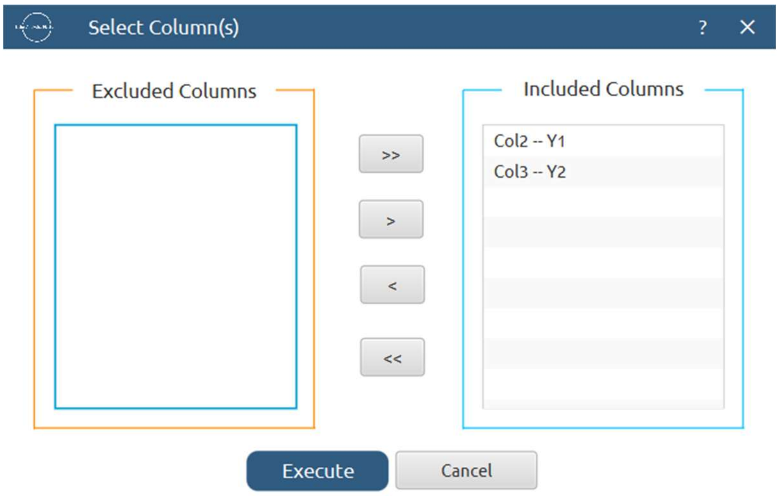
Results:

	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

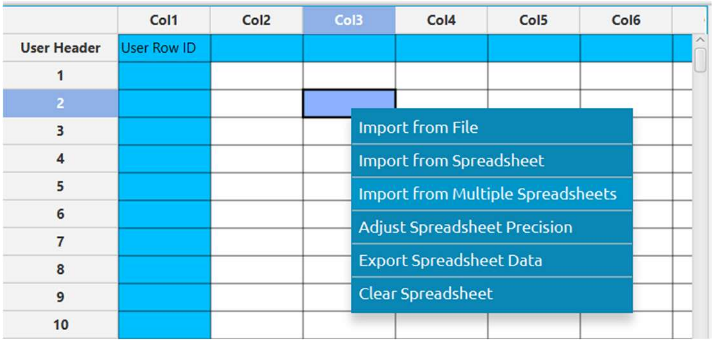
Create a new tab named “Responses” and define the responses in the column headers. Fill each column with the values of the corresponding responses that were observed and make sure the values follow the order of the experiments as given by the fractional factorial design. Then, select all columns to be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

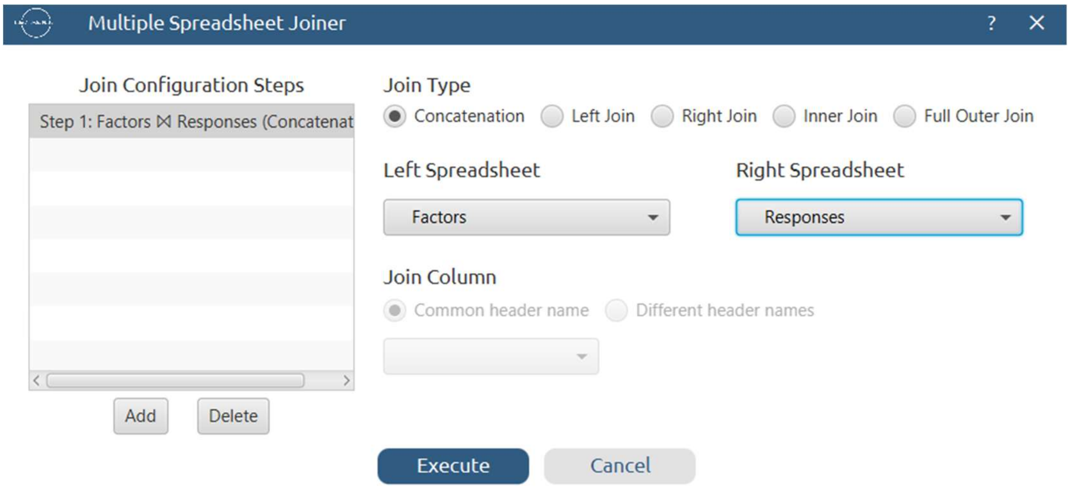
	Col1	Col2 (I)	Col3 (I)
User Header	User Row ID	Y1	Y2
1		176	29
2		223	38
3		217	44
4		160	42
5		164	29
6		148	44
7		138	43
8		168	31
9		233	35
10		178	30
11		163	39
12		177	39
13		145	46
14		152	44
15		150	43
16		145	40

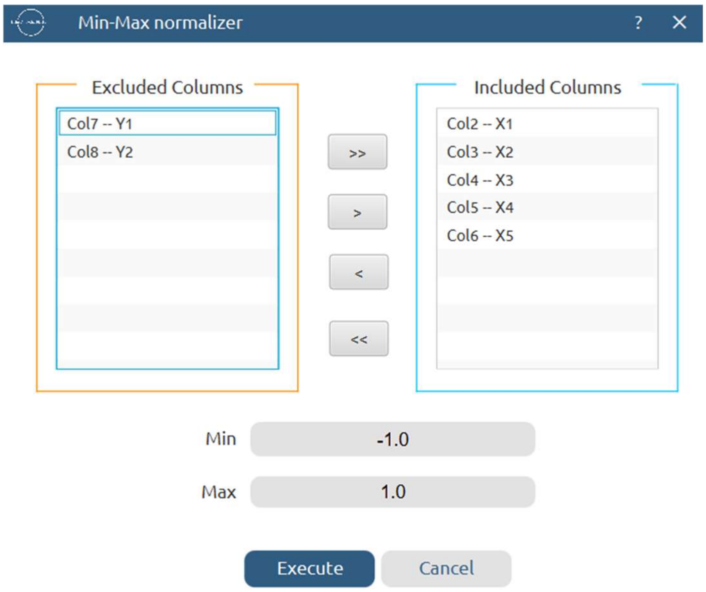


Step 4: Normalization

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:

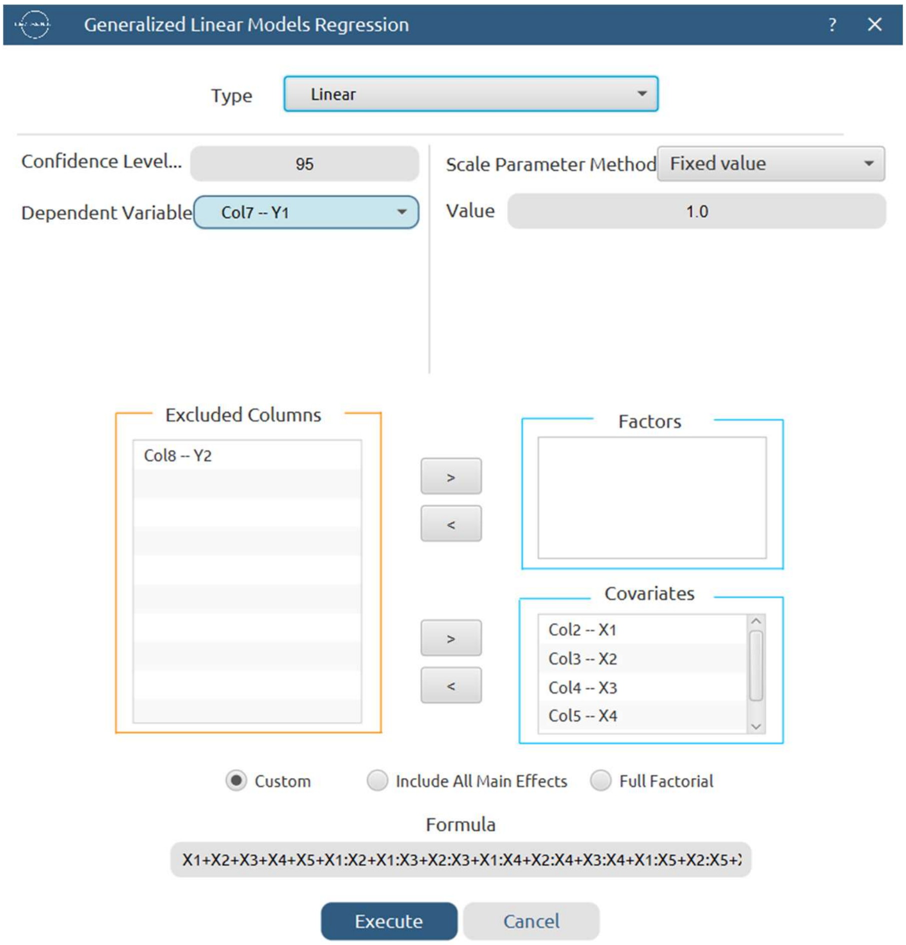
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)	Col8 (D)
User Header	User Row ID	X1	X2	X3	X4	X5	Y1	Y2
1		-1.0	-1.0	-1.0	-1.0	1.0	176.0	29.0
2		1.0	-1.0	-1.0	-1.0	-1.0	223.0	38.0
3		-1.0	1.0	-1.0	-1.0	-1.0	217.0	44.0
4		1.0	1.0	-1.0	-1.0	1.0	160.0	42.0
5		-1.0	-1.0	1.0	-1.0	-1.0	164.0	29.0
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
176.0	184.625
223.0	214.375
217.0	208.375
160.0	168.625
164.0	155.375
148.0	156.625
138.0	146.625
168.0	159.375
233.0	241.625
178.0	169.375
163.0	154.375
177.0	185.625
145.0	136.375
152.0	160.625
150.0	158.625
145.0	136.375

Goodness of Fit	
	Value
Deviance	1190.25
Scaled Deviance	1190.25
Pearson Chi-Square	1190.25
Scaled Pearson Chi-Square	1190.25
Log Likelihood	-609.8280165
Akaike's Information Criterion (AIC)	1251.6560331
Finite Sample Corrected AIC (AICC)	707.6560331
Bayesian Information Criterion (BIC)	1264.0174526
Consistent AIC (CAIC)	1280.0174526

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*X5	3.3125000	0.25	2.8225090	3.8024910	175.5625000	1	0.0
X1*X4	-2.6875000	0.25	-3.1774910	-2.1975090	115.5625000	1	0.0
X2*X5	1.1875000	0.25	0.6975090	1.6774910	22.5625000	1	0.0000020
X1*X3	4.1875000	0.25	3.6975090	4.6774910	280.5625000	1	0.0
X2*X4	-2.8125000	0.25	-3.3024910	-2.3225090	126.5625000	1	0.0
X3*X5	7.1875000	0.25	6.6975090	7.6774910	826.5625000	1	0.0
X1*X2	-0.0625000	0.25	-0.5524910	0.4274910	0.0625000	1	0.8025873
X2*X3	5.3125000	0.25	4.8225090	5.8024910	451.5625000	1	0.0
X3*X4	-0.0625000	0.25	-0.5524910	0.4274910	0.0625000	1	0.8025873
X4*X5	-4.3125	0.25	-4.8024910	-3.8225090	297.5625	1	0.0

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

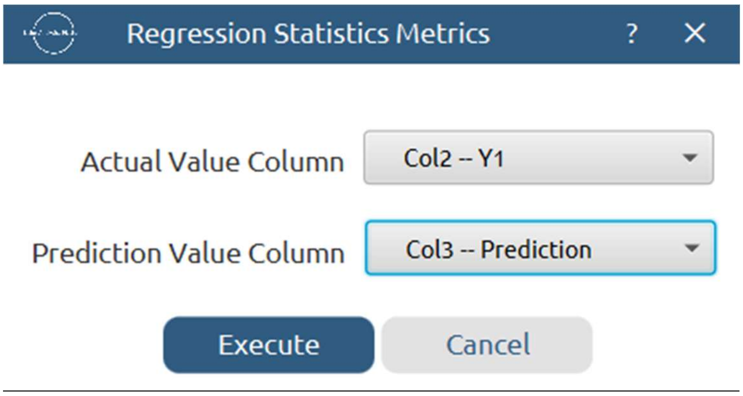
Y2	Prediction
29.0	26.25
38.0	40.75
44.0	46.75
42.0	39.25
29.0	31.75
44.0	41.25
43.0	40.25
31.0	33.75
35.0	32.25
30.0	32.75
39.0	41.75
39.0	36.25
46.0	48.75
44.0	41.25
43.0	40.25
40.0	42.75

Goodness of Fit	
	Value
Deviance	121.0
Scaled Deviance	121.0
Pearson Chi-Square	121.0
Scaled Pearson Chi-Square	121.0
Log Likelihood	-75.2030165
Akaike's Information Criterion (AIC)	182.4060331
Finite Sample Corrected AIC (AICC)	-361.5939669
Bayesian Information Criterion (BIC)	194.7674526
Consistent AIC (CAIC)	210.7674526

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	1.5	0.25	1.0100090	1.9899910	36.0	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*X5	-0.1250000	0.25	-0.6149910	0.3649910	0.2500000	1	0.6170751
X1*X4	-1.2500000	0.25	-1.7399910	-0.7600090	25.0000000	1	6E-7
X2*X5	0.2500000	0.25	-0.2399910	0.7399910	1.0000000	1	0.3173105
X1*X3	-0.2500000	0.25	-0.7399910	0.2399910	1.0000000	1	0.3173105
X2*X4	-0.8750000	0.25	-1.3649910	-0.3850090	12.2500000	1	0.0004653
X3*X5	2.6250000	0.25	2.1350090	3.1149910	110.2500000	1	0.0
X1*X2	-2.1250000	0.25	-2.6149910	-1.6350090	72.2500000	1	0.0
X2*X3	-2.3750000	0.25	-2.8649910	-1.8850090	90.2500000	1	0.0
X3*X4	2.2500000	0.25	1.7600090	2.7399910	81.0000000	1	0.0
X4*X5	1.3750000	0.25	0.8850090	1.8649910	30.2500000	1	0E-7

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*



Results:

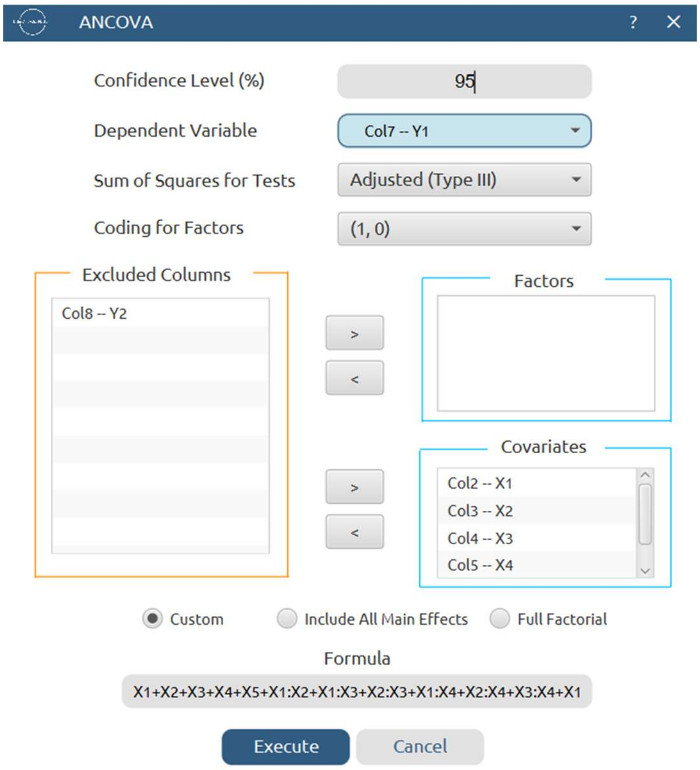
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		74.390625	8.625	8.625	0.9090967

Repeat this step for the second response variable, Y_2. 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		7.5625	2.75	2.75	0.7824145

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_1: *Statistics → Analysis of (Co)Variance → ANCOVA*



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	76.5625000	76.5625000	0.2572989	0.7011529
2		X2	1	637.5625000	637.5625000	2.1426171	0.3815517
3		X3	1	6280.5625000	6280.5625000	21.1067003	0.1364420
4		X4	1	162.5625000	162.5625000	0.5463138	0.5947863
5		X5	1	3335.0625000	3335.0625000	11.2079395	0.1847884
6		X1*X2	1	0.0625	0.0625	0.0002100	0.9907743
7		X1*X3	1	280.5625	280.5625	0.9428691	0.5093614
8		X2*X3	1	451.5625000	451.5625000	1.5175383	0.4340941
9		X1*X4	1	115.5625000	115.5625000	0.3883638	0.6452146
10		X2*X4	1	126.5625000	126.5625000	0.4253308	0.6320962
11		X3*X4	1	0.0625000	0.0625000	0.0002100	0.9907743
12		X1*X5	1	175.5625000	175.5625000	0.5900021	0.5830173
13		X2*X5	1	22.5625000	22.5625000	0.0758244	0.8289383
14		X3*X5	1	826.5625000	826.5625000	2.7777778	0.3440417
15		X4*X5	0	0.0	NaN	NaN	NaN
16		Error	1	297.5625000	297.5625000		
17		Total	15	12788.9375			

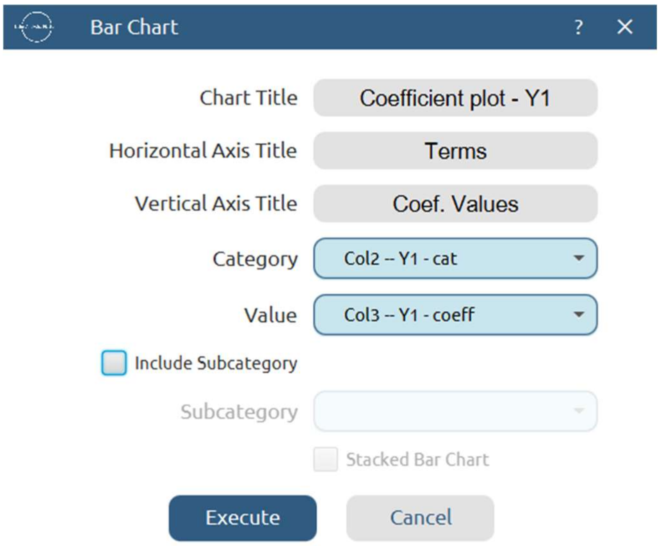
Repeat this step for the second response variable, Y_2. Results:

	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	0E-7	0E-7	0E-7	1.0000000
2		X2	1	42.2500000	42.2500000	1.3966942	0.4470706
3		X3	1	36.0000000	36.0000000	1.1900826	0.4723383
4		X4	1	16.0000000	16.0000000	0.5289256	0.5996959
5		X5	1	6.2500000	6.2500000	0.2066116	0.7284005
6		X1*X2	1	72.25	72.25	2.3884298	0.3656138
7		X1*X3	1	1.0000000	1.0000000	0.0330579	0.8855017
8		X2*X3	1	90.25	90.25	2.9834711	0.3340954
9		X1*X4	1	25.0000000	25.0000000	0.8264463	0.5302923
10		X2*X4	1	12.2500000	12.2500000	0.4049587	0.6392090
11		X3*X4	1	81.0000000	81.0000000	2.6776860	0.3492174
12		X1*X5	1	0.2500000	0.2500000	0.0082645	0.9422841
13		X2*X5	1	1.0000000	1.0000000	0.0330579	0.8855017
14		X3*X5	1	110.2500000	110.2500000	3.6446281	0.3071775
15		X4*X5	0	0.0	NaN	NaN	NaN
16		Error	1	30.2500000	30.2500000		
17		Total	15	524.0			

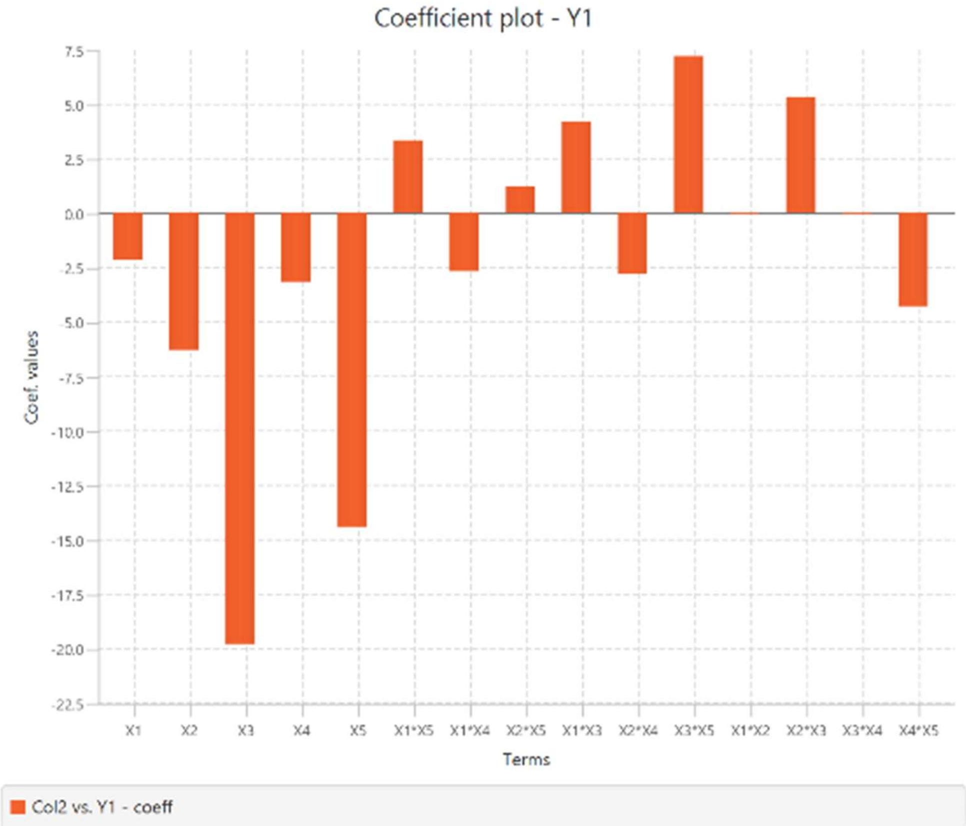
Step 8: Coefficient plots

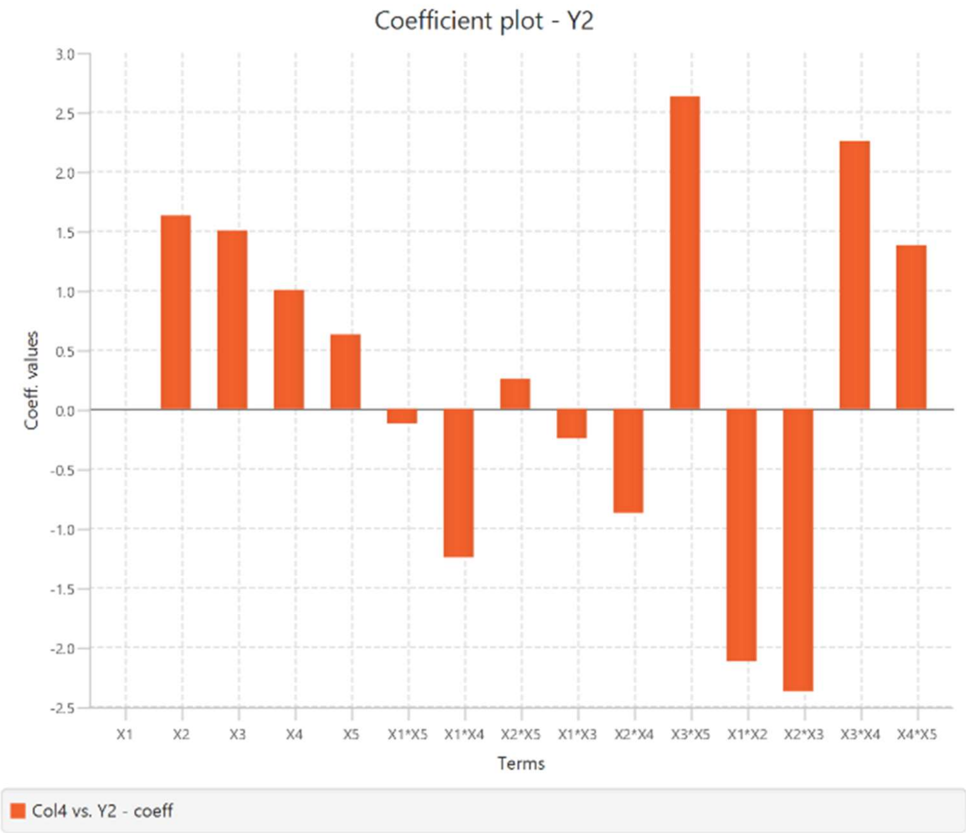
Create a new tab named "Coefficient plots". Copy and paste the regression coefficients from the spreadsheets "Regression – Y1" and "Regression – Y2" on the left spreadsheet. Afterwards, produce one bar chart for each response's coefficients: *Business Intelligence → Create New Dashboard → Add Plot → 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	4.187500000000036	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	5.312500000000036	X2*X3	-2.3750000000000004
14		X3*X4	-0.06249999999999695	X3*X4	2.2500000000000018
15		X4*X5	-4.3125	X4*X5	1.3749999999999996



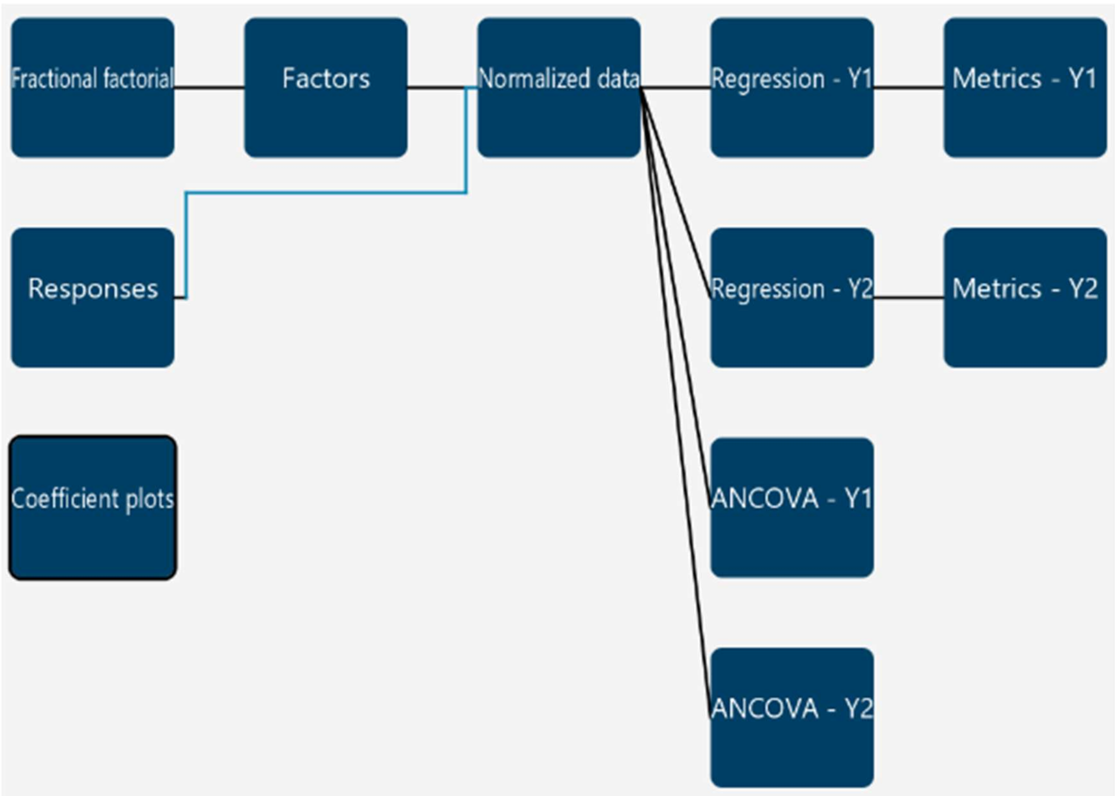
Results Y_1, Y_2:





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.