



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 ($^{\circ}\text{C}$), 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

DoE Fractional Factorial
? ×

Available Designs

Custom

Fraction Relationship

a b c d abcd

Number of Center Points per Block

0

Number of Replicates

1

Number of Blocks

1

☐ Random Standard order

Excluded Columns

>>

>

<

<<

Included Columns

Col2 -- X1
Col3 -- X2
Col4 -- X3
Col5 -- X4
Col6 -- X5

Execute

Cancel

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)*

	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

Fractional factorial

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 -- X4

Col10 -- X5

Execute

Cancel

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

Select Column(s) ? X

Excluded Columns

Included Columns

>>

>

<

<<

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						

Multiple Spreadsheet Joiner

Join Configuration Steps

Step 1: Factors X Responses (Concatenat

Add

Delete

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 normalizer

Excluded Columns

Col7 -- Y1
Col8 -- Y2

Included Columns

Col2 -- X1
Col3 -- X2
Col4 -- X3
Col5 -- X4
Col6 -- X5

Min

-1.0

Max

1.0

Execute

Cancel

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*

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:** Col7 – Y1
- Excluded Columns:** Col8 – Y2
- Factors:** (Empty)
- Covariates:** Col2 – X1, Col3 – X2, Col4 – X3, Col5 – X4
- Formula Type:** Custom (selected), Main Effects, Full Factorial
- Specify Custom Model Formula:** X1 + X2 + X3 + X4 + X5 + X1:X2 + X1:X3 + X1:X4 + X1:X5 + X2:X3 + X2:X4 + X2:X5 + X3:X4 + X3:X5 + X4:X5
- Buttons:** Execute, Cancel

Custom Formula Creation Dialog:

- Included Columns:** X1, X2, X3, X4, X5
- Included Terms:** X1, X2, X3, X4, X5, X1:X2, X1:X3, X1:X4, X1:X5, X2:X3, X2:X4, X2:X5, X3:X4, X3:X5, X4:X5
- Buttons:** Add interactions, up to order (2), Add terms, up to order (2), Add Custom Interaction, Delete Selected, Reset Selections, Move up, Move down
- Formula:** X1 + X2 + X3 + X4 + X5 + X1:X2 + X1:X3 + X1:X4 + X1:X5 + X2:X3 + X2:X4 + X2:X5 + X3:X4 + X3:X5 + X4:X5
- Buttons:** Save, Cancel

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, Y₂. 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

	Value
Goodness of Fit	
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	38.010090	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	-2.187500000 000001	X1	-5.863365348 801607E-16
2		X2	-6.3125	X2	1.624999999 999987
3		X3	-19.8125	X3	1.5
4		X4	-3.187500000 000005	X4	0.999999999 999996
5		X5	-14.43749999 9999993	X5	0.624999999 999999
6		X1*X5	3.312499999 99999	X1*X5	-0.125000000 0000036
7		X1*X4	-2.687499999 999996	X1*X4	-1.249999999 9999993
8		X2*X5	1.187499999 999951	X2*X5	0.249999999 99999
9		X1*X3	4.187500000 000036	X1*X3	-0.249999999 99999814
10		X2*X4	-2.812499999 9999973	X2*X4	-0.874999999 9999991
11		X3*X5	7.187499999 99995	X3*X5	2.624999999 99999
12		X1*X2	-0.062499999 9999976	X1*X2	-2.125000000 000001
13		X2*X3	5.312500000 000036	X2*X3	-2.375000000 0000004
14		X3*X4	-0.062499999 99999695	X3*X4	2.250000000 000018
15		X4*X5	-4.3125	X4*X5	1.374999999 999996

Bar Chart
?
×

Chart Title

Coefficient plot - Y1

Horizontal Axis Title

Terms

Vertical Axis Title

Coef. Values

Category

Col2 -- Y1 - cat

Value

Col3 -- Y1 - coeff

☐ Include Subcategory

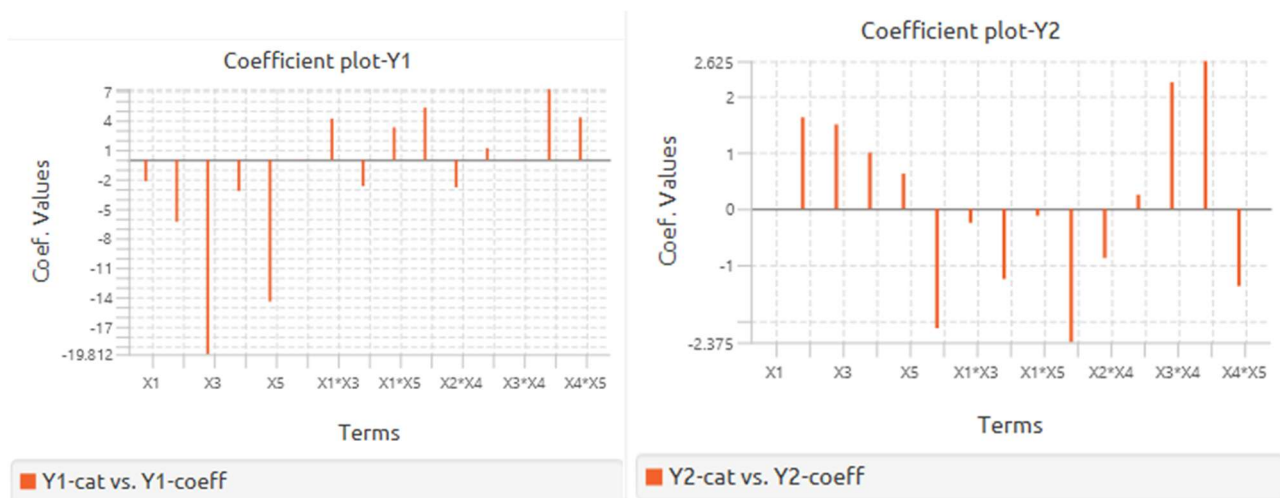
Subcategory

☐ Stacked Bar Chart

Execute

Cancel

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>.