Formulation of extended release cefpodoxime proxetil chitosan-alginate beads using quality by design approach

The purpose of this research paper is to develop and characterize chitosan-alginate beads for the extended delivery of cefpodoxime proxetil (CFP), to understand the impact of formulation and process parameters on the critical quality attributes using quality-by-design approach. Design of experiments (DoE) methodology is implemented for the optimization of the formulation parameters.

The factors (independent variables) examined are: X_1 = amount of sodium alginate (w/v %), X_2 = amount of chitosan (w/v %) and X_3 = amount of calcium chloride (w/v %). All the factors are continuous. The responses (dependent variables) examined are: Y_1 = time for 80% of the drug to be released (h), Y_2 = particle size of the beads during manufacturing (μm) and Y_3 = maximum drug encapsulation (%). The applied DoE method is Box Behnken design.

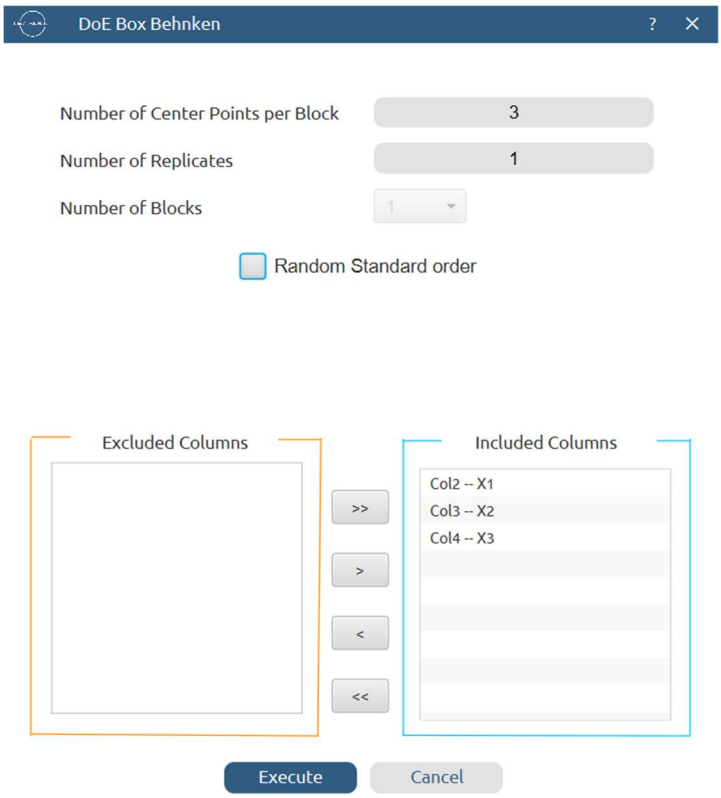
Isalos version used: 2.0.6

Scientific article: https://www.sciencedirect.com/science/article/pii/S0141813014003730

Step 1: Box Behnken 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 “Box Behnken”. Afterwards, apply the Box Behnken method: *DOE → Response Surface → Box Behnken*

	Col1	Col2 (I)	Col3 (D)	Col4 (I)
User Header	User Row ID	X1	X2	X3
1		4	0.5	5
2		6	2	10

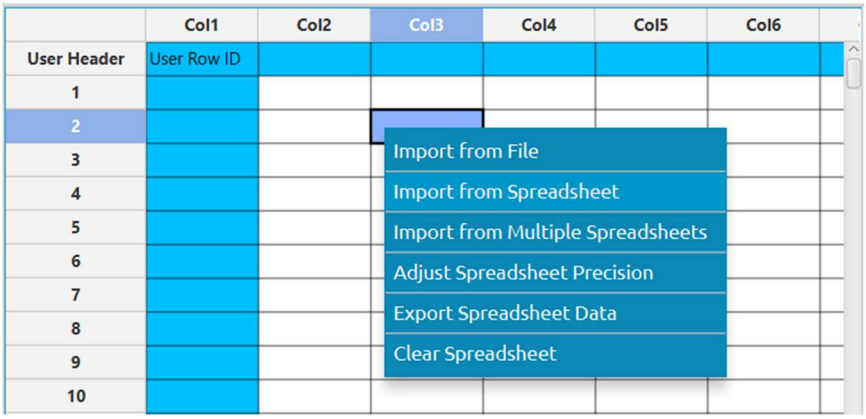


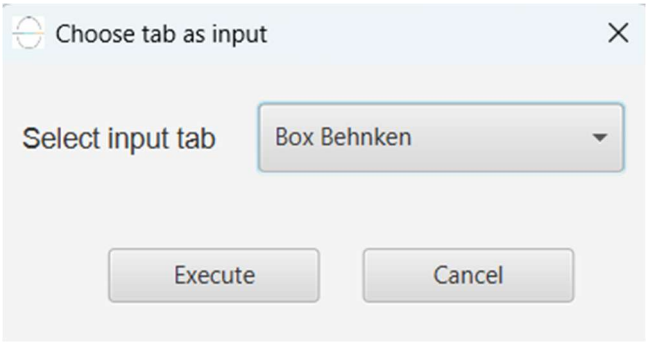
Results (right spreadsheet):

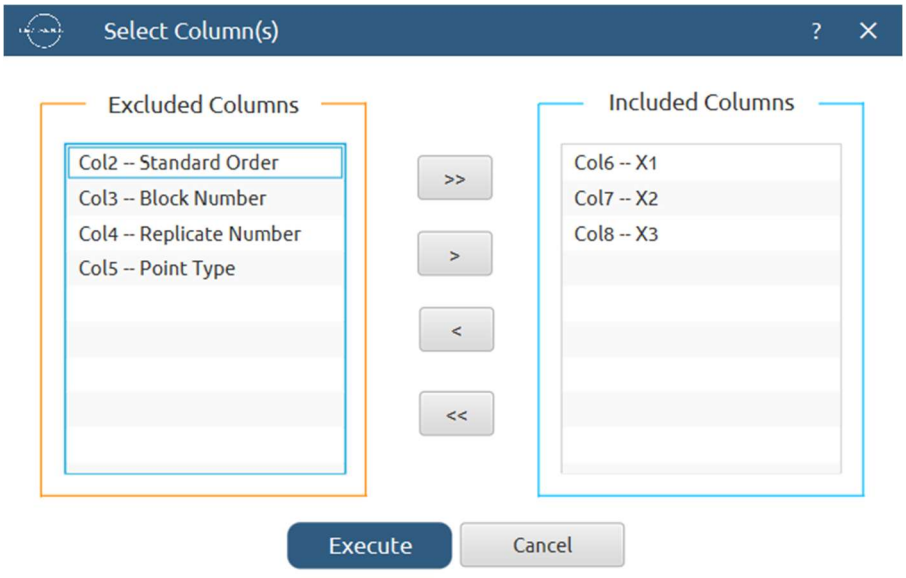
	Col1	Col2 (I)	Col3 (S)	Col4 (S)	Col5 (S)	Col6 (D)	Col7 (D)	Col8 (D)
User Header	User Row ID	Standard Order	Block Number	Replicate Number	Point Type	X1	X2	X3
1		1	Block: 1	Replicate: 1	Design Point	4.0	0.5	7.5
2		2	Block: 1	Replicate: 1	Design Point	6.0	0.5	7.5
3		3	Block: 1	Replicate: 1	Design Point	4.0	2.0	7.5
4		4	Block: 1	Replicate: 1	Design Point	6.0	2.0	7.5
5		5	Block: 1	Replicate: 1	Design Point	4.0	1.25	5.0
6		6	Block: 1	Replicate: 1	Design Point	6.0	1.25	5.0
7		7	Block: 1	Replicate: 1	Design Point	4.0	1.25	10.0
8		8	Block: 1	Replicate: 1	Design Point	6.0	1.25	10.0
9		9	Block: 1	Replicate: 1	Design Point	5.0	0.5	5.0
10		10	Block: 1	Replicate: 1	Design Point	5.0	2.0	5.0
11		11	Block: 1	Replicate: 1	Design Point	5.0	0.5	10.0
12		12	Block: 1	Replicate: 1	Design Point	5.0	2.0	10.0
13		13	Block: 1	----	Center Point	5.0	1.25	7.5
14		14	Block: 1	----	Center Point	5.0	1.25	7.5
15		15	Block: 1	----	Center Point	5.0	1.25	7.5

Step 2: Factor isolation

Create a new tab named “Factors” and import the results from the “Box Behnken” 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)*







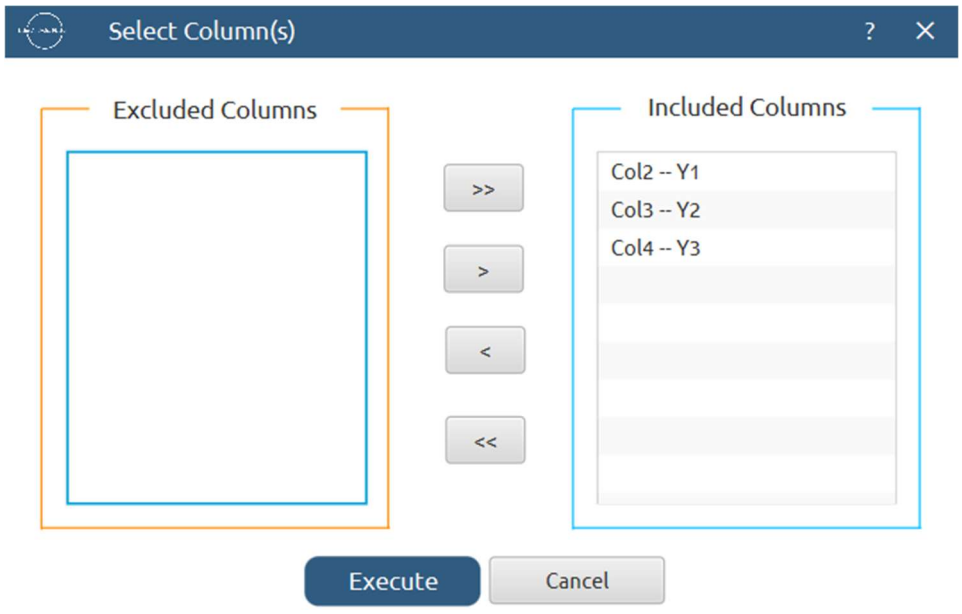
Results:

	Col1	Col2 (D)	Col3 (D)	Col4 (D)
User Header	User Row ID	X1	X2	X3
1		4.0	0.5	7.5
2		6.0	0.5	7.5
3		4.0	2.0	7.5
4		6.0	2.0	7.5
5		4.0	1.25	5.0
6		6.0	1.25	5.0
7		4.0	1.25	10.0
8		6.0	1.25	10.0
9		5.0	0.5	5.0
10		5.0	2.0	5.0
11		5.0	0.5	10.0
12		5.0	2.0	10.0
13		5.0	1.25	7.5
14		5.0	1.25	7.5
15		5.0	1.25	7.5

Step 3: Definition of response variables

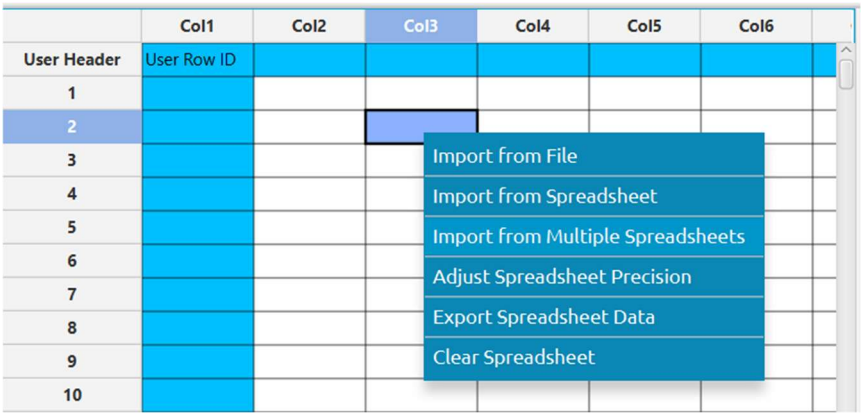
Create a new tab named “Responses” and define the responses in the column headers. Fill each column with the values of the corresponding responses that were observed and make sure the values follow the order of the experiments as given by the Box Behnken design. Then, select all columns to be transferred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

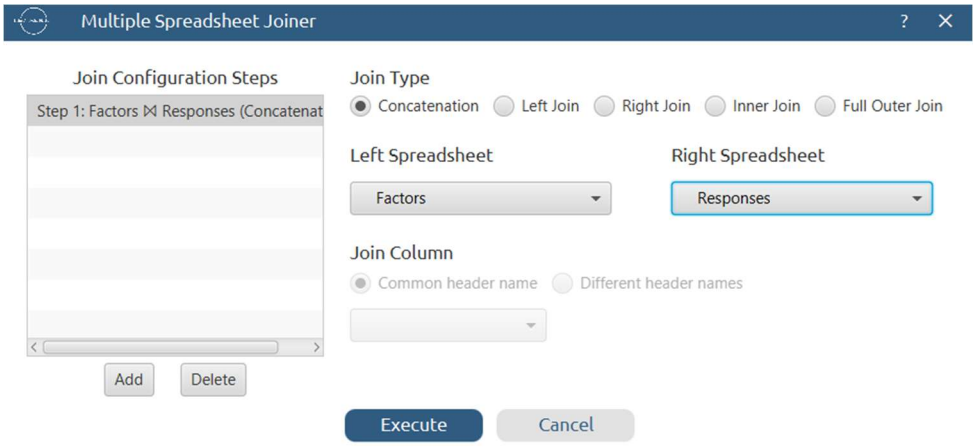
	Col1	Col2 (D)	Col3 (I)	Col4 (D)
User Header	User Row ID	Y1	Y2	Y3
1		12	1236	73.5
2		18	1372	80.9
3		14.5	1401	91.5
4		20.5	1526	96.4
5		14.3	1350	85.8
6		20	1487	88.7
7		16	1395	82.4
8		22.5	1522	83.9
9		15	1290	77.4
10		17.3	1465	93.7
11		16.4	1325	72.6
12		19.5	1505	90.2
13		18.5	1450	84.6
14		18	1435	82.8
15		18.3	1465	85.3

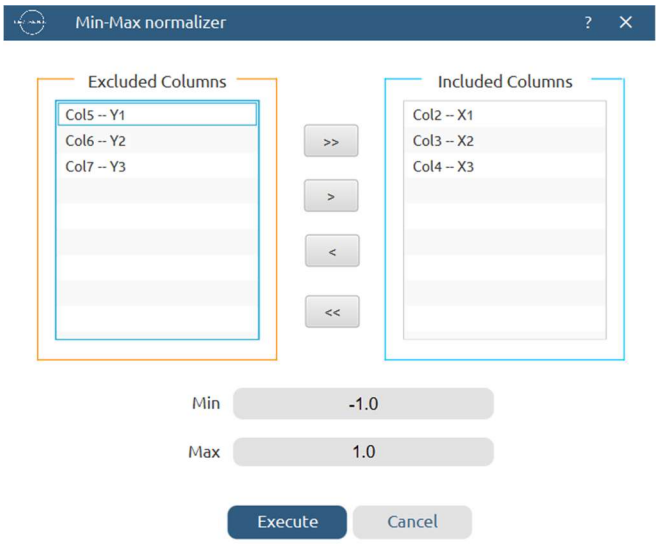


Step 4: Normalization

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*







Results:

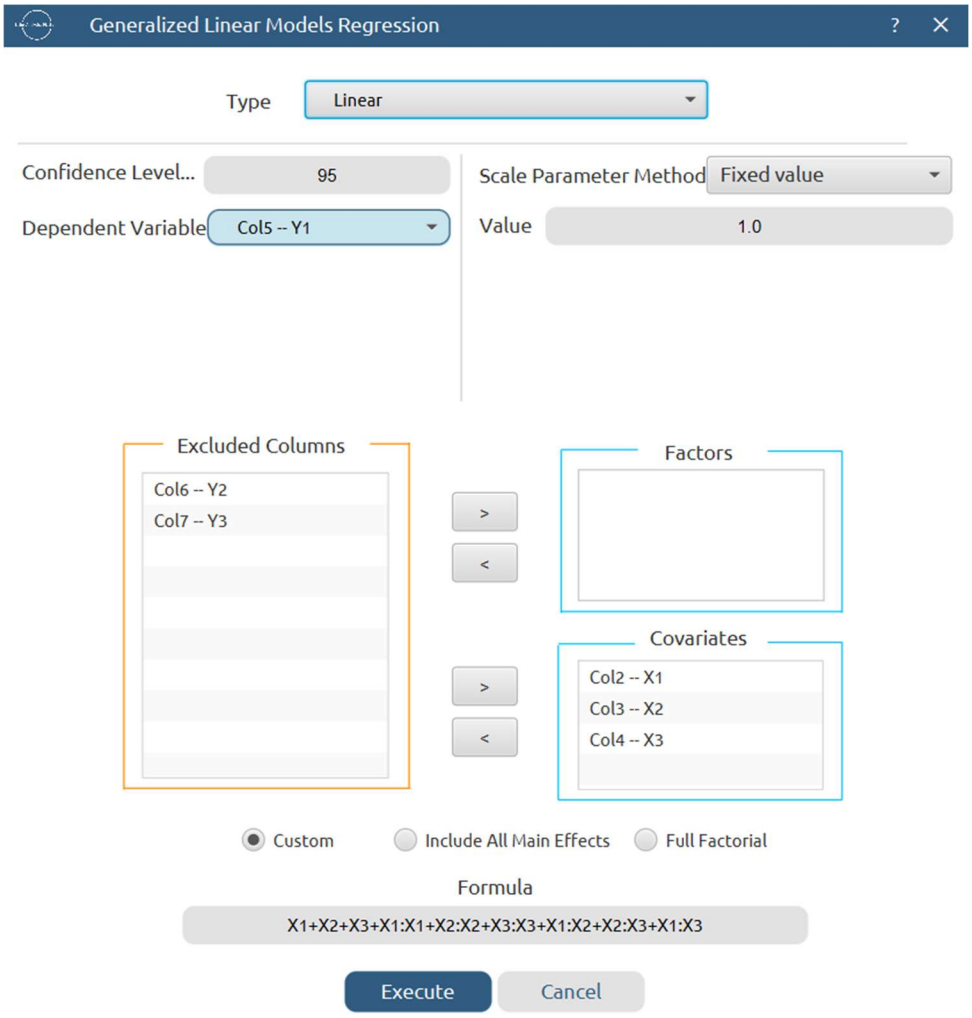
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)
User Header	User Row ID	X1	X2	X3	Y1	Y2	Y3
1		-1.0	-1.0	0.0	12.0	1236.0	73.5
2		1.0	-1.0	0.0	18.0	1372.0	80.9
3		-1.0	1.0	0.0	14.5	1401.0	91.5
4		1.0	1.0	0.0	20.5	1526.0	96.4
5		-1.0	0.0	-1.0	14.3	1350.0	85.8
6		1.0	0.0	-1.0	20.0	1487.0	88.7
7		-1.0	0.0	1.0	16.0	1395.0	82.4
8		1.0	0.0	1.0	22.5	1522.0	83.9
9		0.0	-1.0	-1.0	15.0	1290.0	77.4
10		0.0	1.0	-1.0	17.3	1465.0	93.7
11		0.0	-1.0	1.0	16.4	1325.0	72.6
12		0.0	1.0	1.0	19.5	1505.0	90.2
13		0.0	0.0	0.0	18.5	1450.0	84.6
14		0.0	0.0	0.0	18.0	1435.0	82.8
15		0.0	0.0	0.0	18.3	1465.0	85.3

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:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}{X_1}^2 + b_{22}{X_2}^2 + b_{33}{X_3}^2$$

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*



Results:

Y1	Prediction
12.0	11.9250000
18.0	17.9750000
14.5	14.5250000
20.5	20.5750000
14.3	14.4000000
20.0	20.05
16.0	15.9500000
22.5	22.4000000
15.0	14.9750000
17.3	17.1750000
16.4	16.5250000
19.5	19.5250000
18.5	18.2666667
18.0	18.2666667
18.3	18.2666667

Goodness of Fit	
	Value
Deviance	0.1966667
Scaled Deviance	0.1966667
Pearson Chi-Square	0.1966667
Scaled Pearson Chi-Square	0.1966667
Log Likelihood	-13.8824113
Akaike's Information Criterion (AIC)	47.7648227
Finite Sample Corrected AIC (AICC)	102.7648227
Bayesian Information Criterion (BIC)	54.8453247
Consistent AIC (CAIC)	64.8453247

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	18.2666667	0.5773503	16.6460250	19.8873084	1001.0133333	1	0.0
X1	3.0250000	0.3535534	2.0325637	4.0174363	73.2050000	1	0.0
X2	1.3000000	0.3535534	0.3075637	2.2924363	13.5200000	1	0.0002360
X3	0.9750000	0.3535534	-0.0174363	1.9674363	7.6050000	1	0.0058207
X1*X3	0.2000000	0.5	-1.2035169	1.6035169	0.1600000	1	0.6891565
X1*X2	0E-7	0.5	-1.4035169	1.4035169	0E-7	1	1.0000000
X2*X3	0.2000000	0.5	-1.2035169	1.6035169	0.1600000	1	0.6891565
X1*X1	-0.4333333	0.5204165	-1.8941600	1.0274934	0.6933333	1	0.4050329
X2*X2	-1.5833333	0.5204165	-3.0441600	-0.1225066	9.2564103	1	0.0023467
X3*X3	0.3666667	0.5204165	-1.0941600	1.8274934	0.4964103	1	0.4810817

Repeat this step for the rest of the response variables. Results, Y_2:

Y2	Prediction
1236.0	1231.1250000
1372.0	1367.8750000
1401.0	1405.1250000
1526.0	1530.8750000
1350.0	1351.0000000
1487.0	1487.2500000
1395.0	1394.7500000
1522.0	1521.0000000
1290.0	1293.8750000
1465.0	1459.8750000
1325.0	1330.1250000
1505.0	1501.1250000
1450.0	1450.0000000
1435.0	1450.0000000
1465.0	1450.0000000

Goodness of Fit	
	Value
Deviance	616.25
Scaled Deviance	616.25
Pearson Chi-Square	616.25
Scaled Pearson Chi-Square	616.25
Log Likelihood	-321.9090780
Akaike's Information Criterion (AIC)	663.8181560
Finite Sample Corrected AIC (AICC)	718.8181560
Bayesian Information Criterion (BIC)	670.8986580
Consistent AIC (CAIC)	680.8986580

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	1450.0000000	0.5773503	1448.3793583	1451.6206417	6307500.0000000	1	0.0
X1	65.6250000	0.3535534	64.6325637	66.6174363	34453.1250000	1	0.0
X2	84.2500000	0.3535534	83.2575637	85.2424363	56784.5000000	1	0.0
X3	19.3750000	0.3535534	18.3825637	20.3674363	3003.1250000	1	0.0
X1*X3	-2.5000000	0.5	-3.9035169	-1.0964831	25.0000000	1	6E-7
X1*X2	-2.7500000	0.5	-4.1535169	-1.3464831	30.2500000	1	0E-7
X2*X3	1.2500000	0.5	-0.1535169	2.6535169	6.2500000	1	0.0124193
X1*X1	-12.0000000	0.5204165	-13.4608267	-10.5391733	531.6923077	1	0.0
X2*X2	-54.2500000	0.5204165	-55.7108267	-52.7891733	10866.6923077	1	0.0
X3*X3	0.5000000	0.5204165	-0.9608267	1.9608267	0.9230769	1	0.3366684

Results, Y_3:

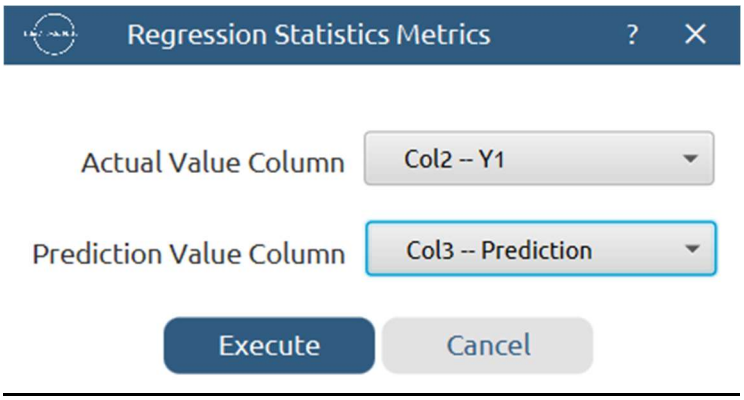
Y3	Prediction
73.5	74.4375
80.9	79.8625000
91.5	92.5375
96.4	95.4625
85.8	84.8250000
88.7	89.7000000
82.4	81.4
83.9	84.875
77.4	77.4375000
93.7	93.6375
72.6	72.6625000
90.2	90.1625000
84.6	84.2333333
82.8	84.2333333
85.3	84.2333333

Goodness of Fit	
	Value
Deviance	11.1491667
Scaled Deviance	11.1491667
Pearson Chi-Square	11.1491667
Scaled Pearson Chi-Square	11.1491667
Log Likelihood	-19.3586613
Akaike's Information Criterion (AIC)	58.7173227
Finite Sample Corrected AIC (AICC)	113.7173227
Bayesian Information Criterion (BIC)	65.7978247
Consistent AIC (CAIC)	75.7978247

Parameter Estimates							
Variable	Coefficient	Std. Error	Lower CI	Upper CI	Test Statistic	df	p-value
intercept	84.2333333	0.5773503	82.6126916	85.8539750	21285.7633333	1	0.0
X1	2.0875000	0.3535534	1.0950637	3.0799363	34.8612500	1	0E-7
X2	8.4250000	0.3535534	7.4325637	9.4174363	567.8450000	1	0.0
X3	-2.0625	0.3535534	-3.0549363	-1.0700637	34.03125	1	0E-7
X1*X3	-0.3500000	0.5	-1.7535169	1.0535169	0.4900000	1	0.4839273
X1*X2	-0.6250000	0.5	-2.0285169	0.7785169	1.5625000	1	0.2112995
X2*X3	0.3250000	0.5	-1.0785169	1.7285169	0.4225000	1	0.5156922
X1*X1	1.5333333	0.5204165	0.0725066	2.9941600	8.6810256	1	0.0032154
X2*X2	-0.1916667	0.5204165	-1.6524934	1.2691600	0.1356410	1	0.7126535
X3*X3	-0.5666667	0.5204165	-2.0274934	0.8941600	1.1856410	1	0.2762106

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		0.0131111	0.1145038	0.0888889	0.9981348

Repeat this step for the rest of the response variables. Results, Y_2:

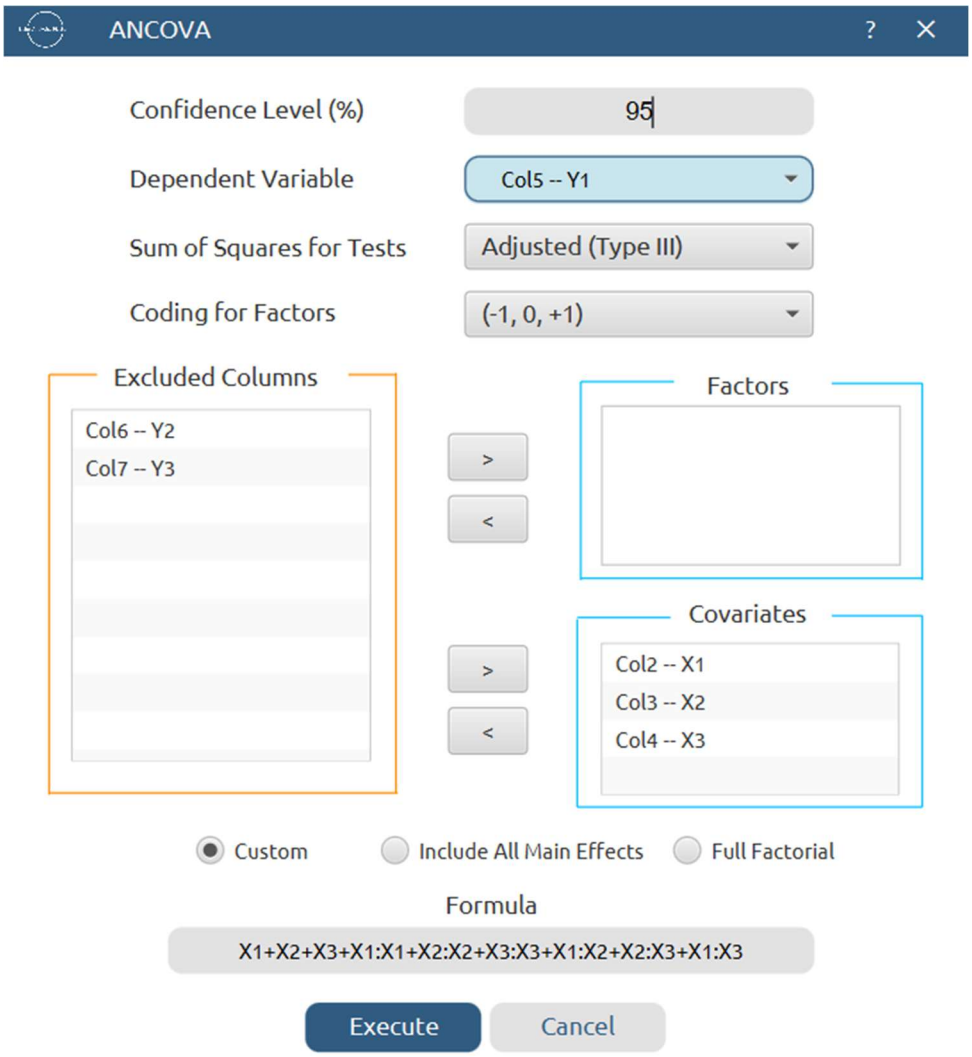
	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)
User Header	User Row ID	Mean Squared Error	Root Mean Squared Error	Mean Absolute Error	R Squared
1		41.0833333	6.4096282	4.5666667	0.9941926

Results, Y_3:

	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		0.7432778	0.8621356	0.7311111	0.9831338

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	73.2050000	73.2050000	1861.1440678	1E-7
2		X2	1	13.5200000	13.5200000	343.7288136	0.0000084
3		X3	1	7.6050000	7.6050000	193.3474576	0.0000346
4		X1*X1	1	0.6933333	0.6933333	17.6271186	0.0085021
5		X2*X2	1	9.2564103	9.2564103	235.3324641	0.0000214
6		X3*X3	1	0.4964103	0.4964103	12.6205997	0.0163432
7		X1*X2	1	0E-7	0E-7	0E-7	0.9999999
8		X2*X3	1	0.1600000	0.1600000	4.0677966	0.0997668
9		X1*X3	1	0.1600000	0.1600000	4.0677966	0.0997668
10		Error	5	0.1966667	0.0393333		
11		Total	14	105.4373333			

Repeat this step for the rest of the response variables. Results, Y_2:

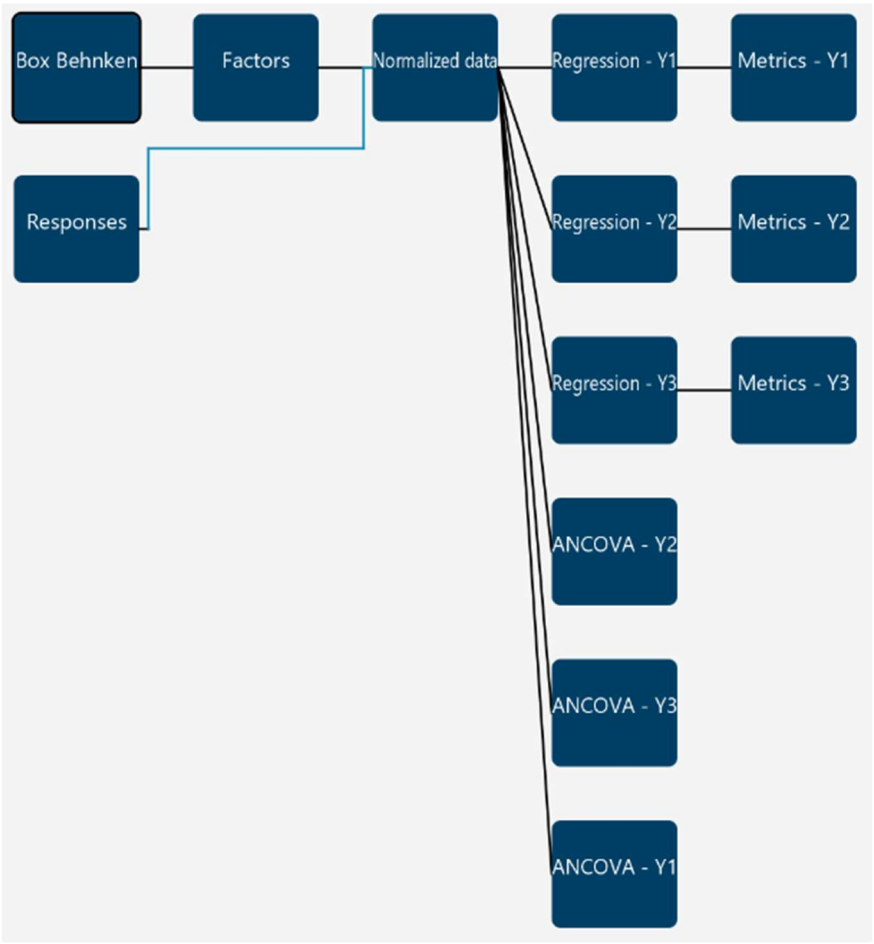
	Col1	Col2 (S)	Col3 (I)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)
User Header	User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value
1		X1	1	34453.1250000	34453.1250000	279.5385396	0.0000140
2		X2	1	56784.5000000	56784.5000000	460.7261663	0.0000041
3		X3	1	3003.125	3003.125	24.3661258	0.0043363
4		X1*X1	1	531.6923077	531.6923077	4.3139335	0.0924135
5		X2*X2	1	10866.6923077	10866.6923077	88.1678889	0.0002310
6		X3*X3	1	0.9230769	0.9230769	0.0074895	0.9343946
7		X1*X2	1	30.2500000	30.2500000	0.2454361	0.6413098
8		X2*X3	1	6.2500000	6.2500000	0.0507099	0.8307468
9		X1*X3	1	25.0000000	25.0000000	0.2028398	0.6712948
10		Error	5	616.2500000	123.2500000		
11		Total	14	106114.9333333			

Results, Y_3:

	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	34.8612500	34.8612500	15.6340160	0.0108076
2		X2	1	567.8450000	567.8450000	254.6580462	0.0000176
3		X3	1	34.0312500	34.0312500	15.2617909	0.0113333
4		X1*X1	1	8.6810256	8.6810256	3.8931276	0.1055047
5		X2*X2	1	0.1356410	0.1356410	0.0608301	0.8149947
6		X3*X3	1	1.1856410	1.1856410	0.5317173	0.4985955
7		X1*X2	1	1.5625000	1.5625000	0.7007250	0.4407027
8		X2*X3	1	0.4225000	0.4225000	0.1894760	0.6815018
9		X1*X3	1	0.4900000	0.4900000	0.2197474	0.6589595
10		Error	5	11.1491667	2.2298333		
11		Total	14	661.0373333			

Final Isalos Workflow

The final workflow is presented below:



References

(1) Mujtaba, A.; Ali, M.; Kohli, K. Formulation of Extended Release Cefpodoxime Proxetil Chitosan–Alginate Beads Using Quality by Design Approach. *International Journal of Biological Macromolecules* **2014**, *69*, 420–429. https://doi.org/10.1016/j.ijbiomac.2014.05.066.