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Tutorial for the Excel spreadsheets *DOE_R4, DOE_R8* and *DOE_R16*

You must have a basic understanding of Design of Experiment, DoE, to use these workbooks.

A single response or many responses?

You may have a single response (e.g. one result for one analyte) or many responses (could be areas for many analyst or it could be many responses for a single analyte, e.g. area, S/N-ratio, skewness etc).

With one response you should enter all input in the sheet named *"R4 xxxx", "R8 xxx" or "R16 xxxx"*as described below.

With many responses you may analyse them one-by-one in the same manner as for a single response, but you may also enter the responses in the sheet *Input* which simplifies the workflow.

- Instructions for entering data in sheet *Input* and for the format of the data are given in sheet *Input,* starting in cell AF4.

Workflow

There are 6 steps:

1) Set up the design
    a. Decide what design to use (depends on the number of factors, levels and possible runs)
    b. Enter the names and levels of the factors in the spreadsheets named **RX_Design Y-Z**
        - ***X**:* No of runs (4, 8 or 16); ***Y*** and ***Z***: No of factors (2 to 8)
        - **The number of factors and center points MUST be entered in Table 2**
    c. For the sample preparation course fill in all tables in this sheet, see the Teaching Video *DoE_Part_1* on Absalon or follow the instructions in the sheet itself
2) Run the experiments
    a. Experiments should be run in random order, and runs may be blocked
3) Enter the results in the spreadsheet
    - Enter a single result manually in the appropriate sheet – or
    - Enter multiple results in sheet *Input* and press *Start* in the appropriate sheet
4) Analyse the results and decide which **effects** are statistically significant
    a. Look at the statistics (if any)
    b. Look at the Normal Probability plot
5) Decide which effects are important for your application based on statistics and your own judgement. You may enter a manual limit for annotating important effects.
6) To save the results in sheet *Output*
    - For a single result press *Save*
    - For results from sheet *Input* press *Save and load next analyte*
7) For multiple responses repeat 3) – 6) for all data.
8) Save the workbook under a new name.

1a) You can choose between the following designs here:

Screening designs: Only main effects can be estimated, and they are confounded with 2-factor interactions. When there are many factors and few runs are possible.

Resolution IV designs: The effects of main factors are only confounded with 3-factor interactions. A compromise between screening and full factor designs.

Resolution V design: The effects of main factors are only confounded with 4-factor interactions. Two factor interactions are confounded with 3-factor interactions. A good design.

Full factor designs: No confounding. The best but requires many runs.

The workbooks *DOE_XXX* cover the following designs (all with 2 levels and with or without center points). The runs could be repeated up to 4 times and there could be up to 2 x 8 center points.

Workbook	Design	Runs	Factors	Resolution
R4	2^2	4 + center	2	Full
R4	2^{3-1}	4 + center	3	III - screening
R8	2^3	8 + center	3	Full
R8	2^{4-1}	8 + center	4	IV
R8	2^{7-4}	8 + center	5 to 7	III - screening
R16	2^4	16 + center	4	Full
R16	2^{5-1}	16 + center	5	V
R16	2^{8-4}	16 + center	6 to 8	IV

For this tutorial we will use workbook ***DOE_R8_1.0.xlsm*** for a synthesis, where we have 3 factors: temperature, time and volume of reagent. The experiments are relatively easy and fast, and we do expect interactions to be very important. In this case a 2^3 full factor design is to be referred, and we choose to add 4 runs at the center.

Ad 1b) Open the sheet ***R8 Designs 3-4*** and enter values for the design in cell C20:F25:

Table 3: Design factors				
Factor	**1**	**2**	**3**	**4**
Name	Temp	Time	Vol	
Unit	oC	min	ul	
Low	25	10	0	
Center 1	35	20	200	
Center 2				
High	45	30	400	

You must enter the number of factors and center points in Table 2 (enter 3 and 4 for this tutorial).
You can Copy-Paste or write the data by hand. The input in Table 3 is not used for calculations, only for documentation. You may use numbers or codes, no restrictions here.
The calculations are made with coded values (-1, 0, +1).

Ad 2) At this point you should go and do the experiments in random order and get some results – but for this tutorial use the numbers below.

Ad 3) Copy-Paste Special (Choose *text*)… or enter the following results in the input area cells F18:F25 in sheet **R8 3 factors FF** for the corner points:

Results 1
16.01
9.11
5.53
20.23
3.74
4.70
4.65
12.40

Now – press the button *Update sheet* and look on the results – the effects in the green table B37:F46:

Term	Effect	Coeff	P	Sign?
Mean	9.5	9.5	1.000	
1	4.1	2.1	1.000	
2	2.3	1.2	1.000	
3	-6.3	-3.2	1.000	
12	7.1	3.5	1.000	
13	0.2	0.1	1.000	
23	2.0	1.0	1.000	
123	-3.7	-1.9	1.000	
Center p				

There is no statistics, because we do not have any repeat measurements.

If you have replicates in your real experiment, enter them in the following columns G18:I25, but here we will add 4 results (replicates) for the center points :

Enter the center points in cell F26:G29:

10.3
14.2
12.33
14

After you entered all results you may have to press the button *Update sheet.*

Ad 4) Analyse the results and the statistics.

Statistics	
d.f.	3
s(pool)	1.8
s(effect)	1.3

First look in the green cells M30:N33. Here you can see that the standard deviation for *one* run, s(pool), which is calculated from the center points with 4-1 = 3 degrees of freedom. The standard deviation for an effect is also calculated, and is used to determine which effects are statistically significant.

The calculated effects and statistics can be seen in the green cells B37:F46.

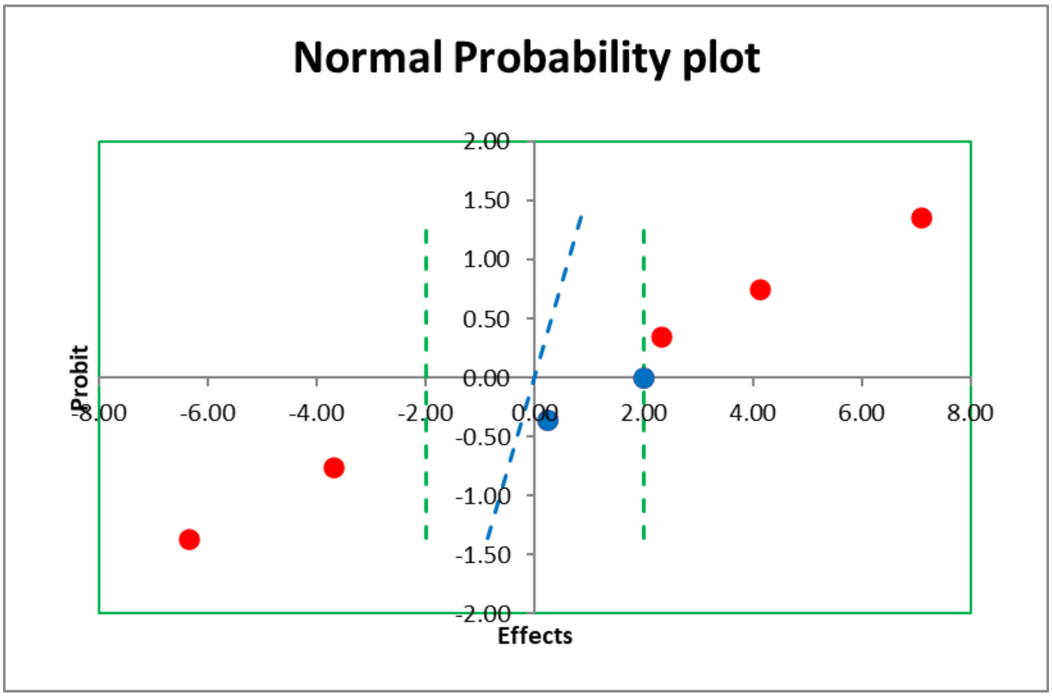
Term	Effect	Coeff	P	Sign?
Mean	9.5	9.5	0.001	***
1	4.1	2.1	0.048	*
2	2.3	1.2	0.169	
3	-6.3	-3.2	0.016	*
12	7.1	3.5	0.012	*
13	0.2	0.1	0.870	
23	2.0	1.0	0.218	
123	-3.7	-1.9	0.063	
Center p	3.2	3.2	0.065	
Manual	P<0.05	+	P<0.05	*
limit	P<0.01	++	P<0.01	**
<> blank:	P<0.001	+++	P<0.001	***
	If no s(effect) all P=1		If no s(effect) all P=1	

The first column is the name of the effect, next column is the value for each of the effects and the third column is the coefficients for the model, which is Effect/2 for main effects and interactions. Then follow the statistics. If the probability *P* for an effect is smaller than 0.05, we can assume that this effect is statistically significant, i.e. that its absolute magnitude is higher than could be caused by random uncertainty. All statistically significant effects are marked with asterisks. We can see that the main effect of factor 1 and 3 and the 12-interaction are significant.
If the center point were significant, this means that the response is *not linear*, i.e. we should extend the design to determine quadratic terms, i.e. effect of the *factors*^{2}, if we want an accurate determination of the effects and interactions. The design should be extended to a *Response Surface design* such as a *Central composite design* or a *Box-Behnken design* requiring at least 6 more runs. Quadratic terms may also be included by use of *D-Optimal designs*.

The green table in cell H39:J46 shows the effects sorted according to 1) magnitude from low to high, and 2) if they are significant (**red**) or if they can be considered to be only caused by noise (**blue**), see next page.

Effectes sorted from low to high		
Terms	Effect	Noise
3	-6.3	
123		-3.7
13		0.2
23		2.0
2		2.3
1	4.1	
12	7.1	
	CI(Noise)	6.7

The same statistical information is shown in the graph *Normal Probability plot,* where significant effects are **red** and noise effects are **blue**. The **green lines** are the limits for noise, i.e. the 95 % confidence interval for the noise according to the experimental standard deviation and number of degrees of freedom.



Now **the blue line** indicates where the noise effects should be, if they were 100 % caused by noise alone. We can see that the blue points do deviate somewhat from the blue line, which indicates that there is also some effect of the corresponding factor for these results – but this effect is not big enough to make the difference *statistically significant.*

So – from statistics alone, we should keep the main factors 1 & 3 and the interaction 12 and reject the rest of the terms as noise.

But – there are some restrictions here. If we want to take the 12-interaction into account, we need to control the factor 2 also, so the minimum model with the terms 1 & 3 & 12 must also include factor 2, so the simplest model contains the terms 1 & 2 & 3 & 12, thus we could skip the interactions 13 & 23 & 123. Let's look at the possible models in this case:

Ad 5) The effect of using different models can be tested by entering a -1, 0 or 1 (coded values) for settings of each factor in the blue boxes in cells B53:J64. Try to enter 1, 1 and -1 for factor **1, 2** and **3** in Model 1:

Model 1			
Term	**X**	**Coeff**	**Value**
Mean	1	9.5	9.5
1	1	2.1	2.1
2	1	1.2	1.2
3	-1	-3.2	3.2
12	1	3.5	3.5
13	-1	0.1	-0.1
23	-1	1.0	-1.0
123	-1	-1.9	1.9
Y			**20.2**
Center p			**12.7**

The response **Y** is now equal to the best run, because it is a full factor design, and we have used the best settings for all 3 factors. The value of the center point is the total response **Y** when all factors are 0 and we do include the experimental value for the center point. This value is NOT included in the model for **Y** in the table.

You can judge the effect of the interactions, by using the Model 2, where they can be set independent of the main factors. This is meaningful, when the interactions are small and we assume that they are only due to random uncertainty:

Model 2			
Term	**X**	**Coeff**	**Value**
Mean	1	9.55	9.55
1	1	2.06	2.06
2	1	1.16	1.16
3	-1	-3.17	3.17
12	0	3.55	0.00
13	0	0.11	0.00
23	0	1.00	0.00
123	0	-1.85	0.00
Y			**15.94**
Center p			**12.71**

You can see that the sum of interactions are important, and that the center point is not a good setting. Try to set 12 = 1 and see the effect of this interaction.

Decide which effects are important from your own judgement:

In cell D35 you can enter your own manual limit for an important effect. This is used to fine tune the model in two cases:

1) When there is no experimental determination of the uncertainty, i.e. if you did not do any

repetitions.

2) When there is an experimental determined uncertainty, but:
    - For your purpose, even statistically insignificant effects may be important
    - For your purpose, even statistically significant effects may be unimportant
    - You suspect that the experimentally determined uncertainty is wrong

If you enter a *Manual limit*, the significant effects will be marked with +, ++ and +++ instead of *, ** and *** for statistically significant effects.

In the example we had a mean response of 9.55 and the effect of factor 2 alone was 2.31. Now even if this is *not statistically significant* (***P*** = 0.169), an increase in yield of 24 % may be quite important, thus for your purpose, factor 2 is important, and you may want to include effects of this magnitude.

So enter the magnitude of an effect that you want to be included, in cell D35, try e.g. 2. Now all statistics is recalculated with you manually set limit, and a warning is given in cell K35 (**Set manually**)**,** but now you can see the model, which you have selected excluding the interaction **13** and **23**. Try also some other values. Finally set D35 to blank to return to experimental confidence intervals.

Term	Effect	Coeff	P	Sign?
Mean	9.5	9.5	0.000	+++
1	4.1	2.1	0.007	++
2	2.3	1.2	0.035	+
3	-6.3	-3.2	0.002	++
12	7.1	3.5	0.001	++
13	0.2	0.1	0.741	
23	2.0	1.0	0.050	
123	-3.7	-1.9	0.010	++
Center p	3.2	3.2	0.010	+
Manual	P<0.05	+	P<0.05	*
limit	P<0.01	++	P<0.01	**
<> blank:	P<0.001	+++	P<0.001	***
	If no s(effect) all P=1		If no s(effect) all P=1	

For comparison the spreadsheet shows two additional CI's in cell J35 and J47:

- CI(exp) = 4.07 is the CI calculated from the experimental determined uncertainty (the same value as before you entered the manual CI).
- CI(Noise) = 6.10. The effects that are considered to be noise can be used as an estimate of the standard deviation of an effect, and the corresponding CI is given here for your information (for advanced users).

Assume that we did not have any of the 4 center points. Start by deleting the 4 results in cell F26:F29 and press *Update sheet*. Now all statistics disappear (provided cell D35 is blank). In such cases, you look at the effects in cell C33:C41 and at the Normal Probability plot and decide how big an effect should be to be significant, and then enter this value in cell D35 as we did before. Try different values, e.g. 4-2-1-?

Output from the statistical software Minitab 14 for the 2^3 example for comparison:

Full Factorial Design

Factors:	3	Base Design:	3; 8
Runs:	12	Replicates:	1
Blocks:	1	Centre pts (total):	4

All terms are free from aliasing.

Factorial Fit: X versus A; B; C

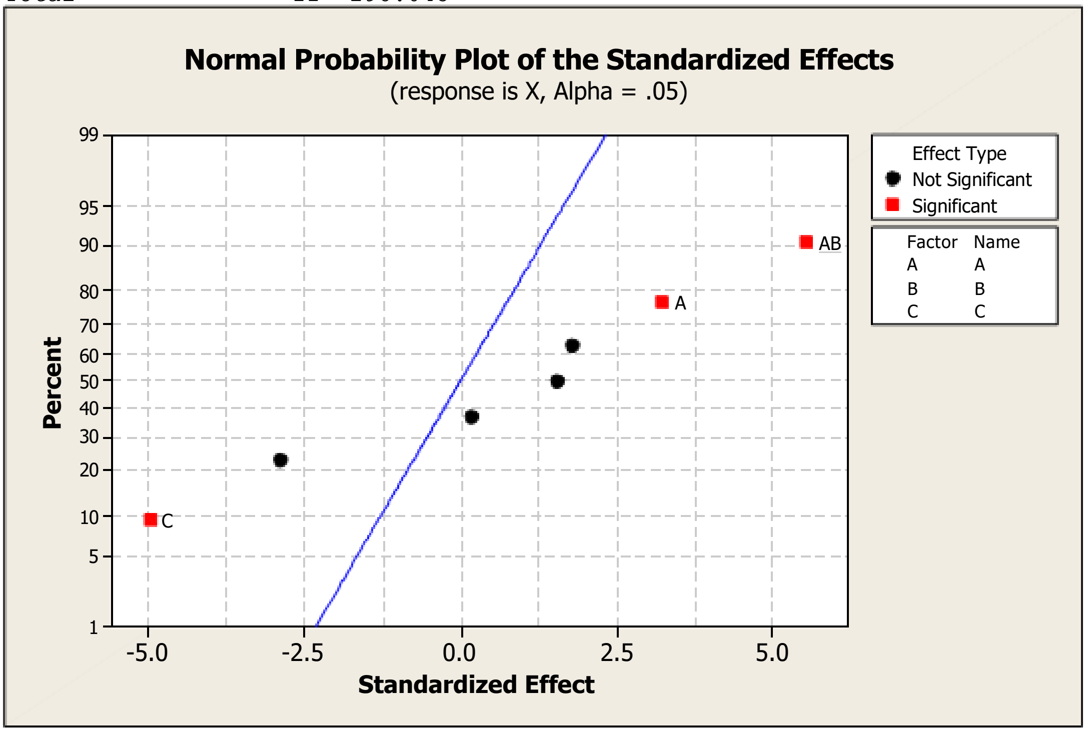
Estimated Effects and Coefficients for X (coded units)

Term	Effect	Coef	SE Coef	T	P
Constant		9.546	0.6402	14.91	0.001
A	4.127	2.064	0.6402	3.22	0.048
B	2.312	1.156	0.6402	1.81	0.169
C	-6.347	-3.174	0.6402	-4.96	0.016
A*B	7.098	3.549	0.6402	5.54	0.012
A*C	0.228	0.114	0.6402	0.18	0.870
B*C	1.993	0.996	0.6402	1.56	0.218
A*B*C	-3.703	-1.851	0.6402	-2.89	0.063
Ct Pt		3.161	1.1089	2.85	0.065

S = 1.81077   R-Sq = 96.70%   R-Sq(adj) = 87.90%

Analysis of Variance for X (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	3	125.349	125.349	41.783	12.74	0.033
2-Way Interactions	3	108.793	108.793	36.264	11.06	0.040
3-Way Interactions	1	27.417	27.417	27.417	8.36	0.063
Curvature	1	26.649	26.649	26.649	8.13	0.065
Residual Error	3	9.837	9.837	3.279		
Pure Error	3	9.837	9.837	3.279		
Total	11	298.045				



Please note, that P = 0.065 for the term *Ct Pt* (centre point) is the same as P for *Curvature* in the ANOVA