Chapter 13
Two-Level Factorial Designs

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ABSTRACT

The term design of experiments in analytical chemistry is associated to the establishment of adequate experimental conditions when working in the laboratory or process conditions used in industry to improve the instrumental conditions and/or extract the highest information from the experimental data. This chapter presents practical problem-solving strategies used to obtain a product or chemical process with desirable characteristics in an efficient mode, focused on the use of full and fractional (2-level) designs. The information is presented as a tutorial and the main advantages and disadvantages are presented and discussed, emphasizing the effect of reduction of experimentation in the data quality.

INTRODUCTION

Design of experiments in analytical chemistry is a term associated to the establishment of adequate process conditions in order to: improve the instrumental conditions and/or extract the highest information from the experimental data (Otto, 2017). Before apply a design of experiments strategy it is appropriate to consider the following questions (Miller & Miller, 2010): a) the initial knowledge of the system, what variables or factors (and their levels) are important to consider in our study? b) the weight of each variable in the system, what variable has a higher contribution? and are there interaction between variables? c) optimize the response, what is the response associated to the highest quality of the product/process? and d) evaluate the system robustness, what is the effect of uncontrolled variables?

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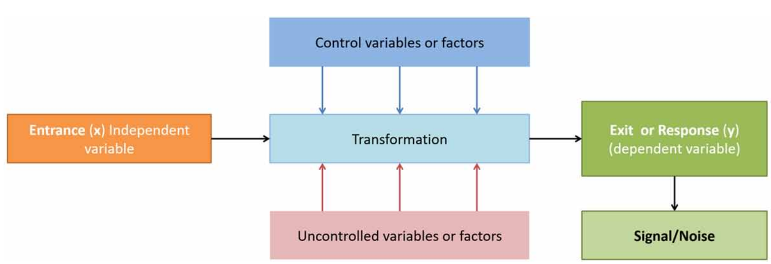
A system can be considered as a conjunct of elements interdependent forming and integrating whole (Deming & Morgan, 1993). The system has an entrance (generally the analyte) expressed as a quantity or quality, the factors involved in the response of the system, an output variable (signal) influenced by the processes and chemical reactions which took place during the transformation of entrance into response.

The main drawback in all cases is the existence of factors so-called uncontrolled which are included in all the experiments, these factors cannot be eliminated and affect the response by adding a variability or variance to the output variable. The variance related to the response has then two contributions: variance of the signal and a second one known as uncertainty or noise. In this sense, the design of experiments can be applied to estimate and quantify the contributions of the controlled factors and minimize the uncontrolled (Figure 1).

The proposed chapter is planned to be a tutorial for application and resolution of experimental problems based on the use of *full and fractional (2-level) designs* of experiments. Taking into account the proposed aim, we must consider the following steps commonly employed when it is applied design of experiments (Goupy, 1993):

1. Problem statement
    a. Definition of the response or output variable which must describe adequately the process.
    b. Identification of the factors (continue or discrete)
    c. Selection of the levels and experimental domain
    d. Identification of uncontrolled factors (noise)
    e. Definition of signal/noise ratio
    f. Evaluation of the system robustness
    g. Selection of the experimental methodology (experimental arrangement)
2. Experimentation
    a. Randomization
    b. Blocks
    c. Replicates
3. Results and analysis (interpretation)
    a. Analysis of variance (ANOVA)
4. Optimization
    a. Selection of the experimental conditions which generate the adequate signal/noise ratio
5. Conclusions
    a. Feed-back

Figure 1. Scheme of a system



FULL FACTORIAL DESIGN

The first practical case is the evaluation of the instrumental conditions employed for analysis of metallic ions in water samples by differential pulse polarography at hanging mercury electrode. The evaluation of the factors was performed employing a complete factorial design (Masetto et al. 2011). The proposed example would be useful to demonstrate the importance of identification, selection and randomization of the experiments. It is proposed the inclusion a tutorial to determine the analysis of variance terms and the interpretation of the results obtained. Additionally, the possible of minimize the experimentation would be discussed.

In this sense, the proposed problem is achieving the best limit of detection possible which is directly associated to the highest peak height (or area). Once defined the output variable, it is possible to propose the chemical transformation related to our goal, in this case it can be the instrumental technique employed (i.e. spectroscopy, chromatography, electrochemical). The technique employed must be studied in order to identify the possible control factors involved. If metal ions are determined in water samples by polarography followed by anodic stripping voltammetry the main factors are the electrolyte composition (pH, presence of complexing agents, ionic strength) and the instrumental conditions (drop size, deposition time, pulse height, etc.) (Zinoubi, et al. 2017). All these numeric factors are continue, which means that they their variation in a scale is continue despite they are limited (i.e. pulse height adequate interval is from -10 to -100 mV). The values evaluated are so-called levels and the set selected is the experimental domain.

The factors involved in the problem must be evaluated deeply, they must be identified and separate them in control and noise factors with the corresponding experimental domain. This information is necessary to determine the methodology adequate to establish the factors that have a significant effect, the levels which generate the highest signals and in consequence the conditions with a minimal contribution of un-controlled factors.

The experimental tool useful for this purpose is the complete factorial design. This is a randomized block experimental design with replicates. The complete factorial design studies all the possible combinations of the levels and factors, randomizing the order and replicating each combination in order to have a degree of freedom useful to determine the residual variance (associated to noise). The use of random experimentation minimize the inclusion of systematic error (which invalidate the results) and the blocks are required when it is required continue experimentation. The factorial design is antithesis of the classical design that evaluates one variable at time while the others remain constants.

In order to explain the construction of a experimental matrix, it is presented the evaluation of pH and ionic strength (I) on the peak height. The following experimental matrix is presented in Figure 2, where X_{ijk}= mean of each pair of experiments.

According to Figure 2, it is required to be performed 16 randomized experiments (in duplicate: 16x2=32). The experimental conditions of each pair (1,2) (19,20), etc are equal, and the differences observed would be a consequence of the random error (noise). On the other hand, it is possible to estimate the variance employing the mean value of each row (effect of pH), each column (effect of ionic strength) and the uncertainty (random error or residual). The last one is attributed to the interaction between the control factors and this contribution cannot be determined employing classical methodologies such as: one variable at time.

Figure 2. Factorial design for two factors
**subscripts make reference to the evaluated levels of each factor*

		Ionic strength (I)			
		I_1	I_2	I_3	I_4
pH	pH_1	1,2	3,4	5,6	7,8
	pH_2	x_{ijk}			
	pH_3	17, 18	19,20	21,22	23,24
	pH_4	25,26	27,28	29,30	31,32

Determination of the contribution of each variable and the interaction is shown in Figure 3,; r=rows number; n=columns number; p=number of replicates in each cell; N=prn=number of total experiments, T_{ij}= sum of each cell=X_{ij}; T_{if}=sum of row i, T_{cj}=sum of column j; $T=\Sigma T_{if}=\Sigma T_{cj}$ and $C=T^2(rnp)^{-1}$=correction term. The result most interesting is the residual (noise).

The main disadvantage of the complete design of experiments is the high amount of experiments required; this fact limits its applicability (Kobilinsky et al. 2017). However, considering the potential of this statistical tool it was proposed the use of two-level factorial designs (2^{k+1}), where k is the number of factors (Tounsadi et al 2019). This type of design of experiments limits the levels evaluated. The reduction of the experimental domain produces two problems: the first assume a linear response of the out-variable *vs.* each factor (which cannot be true) and the second is the loss of a degree of freedom, then an algorithm must be used.

A practical example is described, considering the example mentioned above it is desired to evaluate the effect of the mercury dropping time (t) and the pulse potential (U) in the peak height of the response obtained during the analysis of copper in water samples. Considering that minimize of experimentation is required, it was selected a two-level factorial design (2^{2+1}), which means the evaluation of two factors at two levels with two replicates to determine the effect of the following factors:

Figure 3. Equations employed for the analysis of variance of a full factorial design

Source of variation	Square Sum	Degrees of freedom
Between rows	$\sum \frac{T_{if}^2}{pn} - C$	**r - 1**
Between columns	$\sum \frac{T_{cj}^2}{pr} - C$	**n - 1**
Interaction	**By subtraction**	**By subtraction**
Residual	$\sum x_{ijk}^2 - \left(\sum \frac{T_{ij}^2}{p}\right)$	**r.n.(p - 1)**
Total	$\sum x_{ijk}^2 - C$	**p.r.n - 1**

t (s) = 0.4 (low level, -1) and 0.8 (high level, +1)

U (mV) -20 (low level, -1) and -100 (high level, +1)

The number of experiments is: $2^{2+1} = 2^3 = 8$ ($2^2 = 4$ experiments with 2 repetitions = 8 experiments). The experimental matrix obtained is shown in Figure 4.

A graphical representation of the experimental data is presented in Figure 5. The variable that changes in experiments Y_1 and Y_2 is U (from low to high level) and the variable t remains constant (low level), while in experiments Y_3 and Y_4 U changes and t is at high level. This is represented in blue line, and the effect can be determined as the mean sum of the differences:

$$Effect\,of\,U = \frac{(Y_2 - Y_1) + (Y_4 - Y_3)}{2} = \frac{(39.25 - 23.95) + (41.20 - 25.40)}{2} = 15.85$$

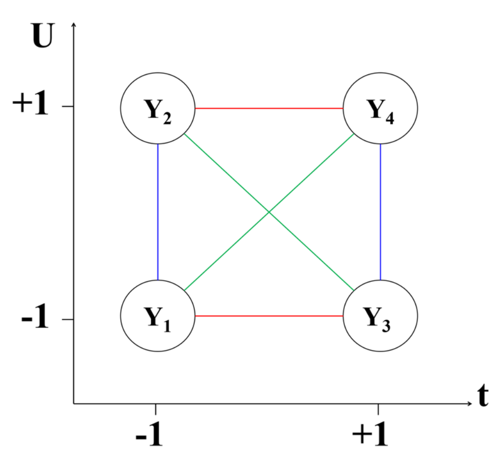
Considering the associative property of the sum, the effect can be estimated considering the difference between the sum of the response at high (Y_2 and Y_4) and low (Y_1 and Y_3) levels:

$$Effect\,of\,U = \frac{(Y_2 + Y_4) - (Y_1 + Y_3)}{2} = \frac{(39.25 + 41.80) - (23.95 + 25.40)}{2} = 15.85$$

Figure 4. Experimental matrix 22+1

Factors and levels		Denomination	Individual responses (μA)	Mean response (μA)
t	U			
-1	-1	Y_1	24.0 23.9	23.95
-1	+1	Y_2	39.5 39.0	39.25
+1	-1	Y_3	25.9 24.9	25.40
+1	+1	Y_4	40.9 42.7	41.80

Figure 5. Graphical representation of the 22 two level factorial design



The effect of t can be obtained following the red line in Figure 5, the low levels (-1) are Y_1 and Y_2 while the high level (+1) are Y_3 and Y_4.

$$Effect\ of\ t = \frac{\left(Y_3 + Y_4\right) - \left(Y_1 + Y_2\right)}{2} = \frac{\left(25.40 + 41.80\right) - \left(23.95 + 39.25\right)}{2} = 2.00$$

The interaction is obtained from Figure 5 applying the rules of signs for multiplication, generating a new column tU which represents the interaction between these factors. The Figure 6 shows the complete experimental design. The effect of tU is represented in green (Figure 5) and its effect was calculated following the explanation described above: high levels (Y_1, Y_4) low levels (Y_2, Y_3).

$$Effect\ of\ tU = \frac{\left(Y_1 + Y_4\right) - \left(Y_2 + Y_3\right)}{2} = \frac{\left(23.95 + 41.80\right) - \left(39.25 + 25.40\right)}{2} = 0.55$$

The effect values are semi-quantitative are useful to evaluate the behavior of each evaluated factor in the response. In order to demonstrate their significance, it is required to determine the variance for each variable. It must be remembered that a negative consequence of the two-level factorial designs is the loss of one degree of freedom. Yates' algorithm has been applied to determine the square sum (SS) using the expression (Massart et al. 1997):

$$Square\ sum\left(SS_t\right) = \frac{N(Effect\ of\ t)^2}{4} = \frac{8(2.00)^2}{4} = 8.00$$

where: N is total number of experiments. SS estimation has one degree of freedom and the value obtained is equal to the mean squares (MS) or variance. Critical factors are selected using *F* test, the $SS_{residual}$ was estimated:

$$SS_{residual} = \sum x_{ijk}^2 - \frac{\sum\left(pT_{if}^2\right)}{p}$$

$$\sum x_{ijk}^2 = \left[24.0^2 + 23.9^2 + 39.5^2 + 39.0^2 + 25.9^2 + 24.9^2 + 40.9^2 + 42.7.0^2\right] = 9015.38$$

Figure 6. Complete experimental design (factors+ interaction)

Factors and levels			Interaction	Denomination
t	U		tU	
-1	-1	⇨	+1	Y_1
-1	+1		-1	Y_2
+1	-1		-1	Y_3
+1	+1		+1	Y_4

$$\frac{\sum\left(pT_{if}^{2}\right)}{p}=\frac{(2*23.95)^{2}+(2*39.25)^{2}+(2*25.20)^{2}+(2*41.80)^{2}}{2}=9013.13$$

$$SS_{residual}=9015.38-9013.13=2.25$$

The $MS_{residual}$ was obtained as:

$$MS_{residual}=\frac{SS_{residual}}{Degrees\,of\,freedom}$$

$$MS_{residual}=\frac{2.25}{7-3}=0.5625$$

Once determined the variance of each factor, the experimental F (F_{exp}) are calculated as $MS_{variable}/MS_{residual}$. The results are contrasted to the critical F ($F_{1,4}$=7.71) and a variable is considered critical when $F_{exp}>F_{crit}$. The results obtained are collected in Figure 7, it can be concluded that in the proposed case both factors are critical, in addition the contribution of U is higher than t. It is important to observe that interaction tU is not critical and it can be estimated without additional experimentation.

The analysis of three factors involves the use of a 3 dimension graphic which is still suitable. In order to explain its applicability, it is presented the evaluation of a spectrophotometry methodology for analysis of Fe(III) based on liquid-liquid extraction of its complex with oxine (Chobot et al. 2018, Bahar, Zakerian 2012). The response variable (out-put or response) is the absorbance value and the control factors evaluated are: pH of the aqueous phase (Factor A), extraction time (Factor B) and volume of chloroform employed (Factor C). According to Figure 8 it is possible to observe the presence of different oxine species depending on the acidity of the media, the time is associated to the extraction yield and affects the analysis time while the solvent volume ratio (aqueous:organic) employed is related to the maximum concentration and in consequence affects the limit of detection (Hanson, 2013)..

Figure 9 shows the required experimental matrix, it contemplates to perform 2^{3+1}=16 experiments (eight combinations in duplicate). Each row shows the combination of the factors and the individual results, additionally it is included the mean values used for the analysis of the experimental data.

Figure 7. Analysis of variance of the analysis of a 22+1 factorial design

^{a} $F_{crit\,(1,4)}$=7.71

Factor	Effect	Sum of Squares (SS)	Degree of freedom	Mean Squares (MS)	F_{exp} [a]
t	2.00	8.000	1	8.0000	14.22
U	15.85	502.445	1	502.4450	893.24
tU	0.55	0.605	1	0.6050	1.08
Residual		2.25	4	0.5625	

Figure 8. Schematic representation and reactions involved on separation of Fe(III) by liquid-liquid extraction using oxine

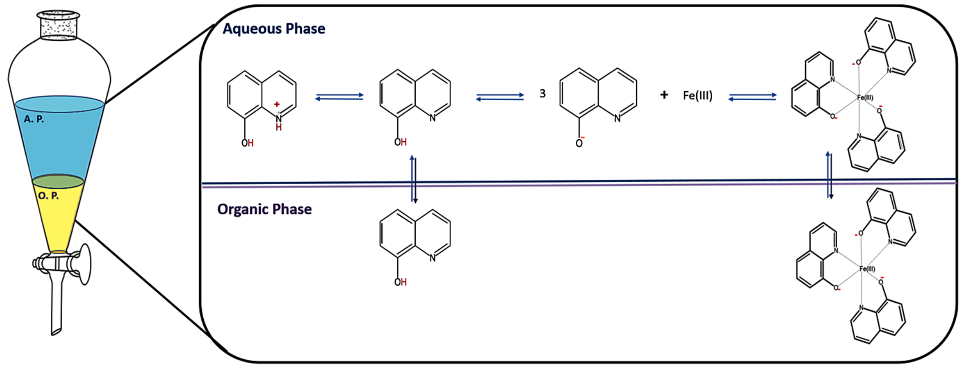
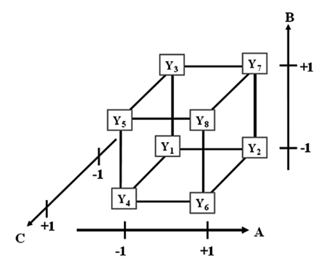


Figure 9. Experimental matrix 23+1 and 3D graphic used to evaluate 3 factors two level factorial designs

A	B	C	Denomination	Individual responses (A.U.)	Mean response (A.U.)
-1	-1	-1	Y_1	0.290 0.286	0.288
+1	-1	-1	Y_2	0.550 0.479	0.515
-1	+1	-1	Y_3	0.304 0.306	0.305
-1	-1	+1	Y_4	0.250 0.243	0.247
-1	+1	+1	Y_5	0.255 0.257	0.256
+1	-1	+1	Y_6	0.438 0.450	0.444
+1	+1	-1	Y_7	1.320 1.330	1.325
+1	+1	+1	Y_8	1.350 1.320	1.335



Considering the experimental data, the first step is the determination of the individual effects. Figure 10 includes the corresponding expression and the corresponding cubic part analysed. It must be remembered that it is used the Mean response to perform the determinations.

Once evaluated the individual contributions, it is determined the interactions. For three factors there are 2-way interactions (AB, AC and BC) and one 3-way interaction (ABC). The corresponding column was obtained applying the rules of signs for multiplication of the experimental matrix presented in Figure 9. The complete experimental matrix is composed of 7 columns (Figure 11) and it is useful to determine the effect of 2- and 3-way interactions, the graphical representation and the equations employed are presented in Figure 12.

Once determined the effect of all the factors included in the experimental design, it was determined the SS applying the Yates' algorithm, as example:

$$SS_A = \frac{16(Effect\, of\, A)^2}{4} = \frac{16(0.631)^2}{4} = 1.592$$

the effect of A=. The $SS_{residual}$ was determined employing the individual and mean data as mentioned above:

Figure 10. Determination of individual effects in a 23+1 experimental design

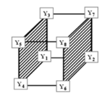
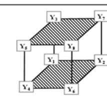
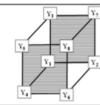
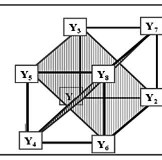
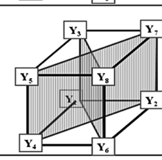
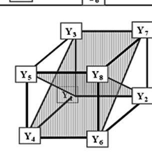
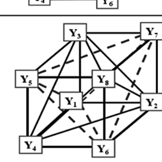
Factor	Effect	Representation
A	$\frac{(Y_2+Y_6+Y_7+Y_8)-(Y_1+Y_3+Y_4+Y_5)}{4}$	
B	$\frac{(Y_3+Y_5+Y_7+Y_8)-(Y_1+Y_2+Y_4+Y_6)}{4}$	
C	$\frac{(Y_4+Y_5+Y_6+Y_8)-(Y_1+Y_2+Y_3+Y_7)}{4}$	

Figure 11. Complete experimental matrix of a 23+1 design

Factor			2-way interaction			3-way interaction	Denomination
A	B	C	AB	AC	BC	ABC	
-1	-1	-1	+1	+1	+1	-1	Y_1
+1	-1	-1	-1	-1	+1	+1	Y_2
-1	+1	-1	-1	+1	-1	+1	Y_3
-1	-1	+1	+1	-1	-1	+1	Y_4
-1	+1	+1	-1	-1	+1	-1	Y_5
+1	-1	+1	-1	+1	-1	-1	Y_6
+1	+1	-1	+1	-1	-1	-1	Y_7
+1	+1	+1	+1	+1	+1	+1	Y_8

Figure 12. Determination of 2 and 3 way effects in a 23+1 experimental design

Factor	Effect	Representation
AB	$\frac{(Y_1+Y_4+Y_7+Y_8)-(Y_2+Y_3+Y_5+Y_6)}{4}$	
AC	$\frac{(Y_1+Y_3+Y_6+Y_8)-(Y_2+Y_4+Y_5+Y_7)}{4}$	
BC	$\frac{(Y_1+Y_2+Y_5+Y_8)-(Y_3+Y_4+Y_6+Y_7)}{4}$	
ABC	$\frac{(Y_2+Y_3+Y_4+Y_8)-(Y_1+Y_5+Y_6+Y_7)}{4}$	

$$SS_{residual} = \sum x_{ijk}^2 - \frac{\sum\left(pT_{if}^2\right)}{p} = 8.607056 - \frac{17.207854}{2} = 0.003129\,.$$

The results obtained are collected in Figure 13. All the selected factors have a significant statistical contribution to the methodology, however A (pH), B(extraction time) and the 2-way interaction AB have a greater contribution. The AC, BC and ABC interactions are not significant, since their F_{exp} is not greater than the critical F_{value}= 5.32.

Duplicate experiments cannot be obtained in all cases, in these circumstances the use of non-parametric test are an interesting option (Fraser, 1956). The ranking test (Figure 14) is the statistical tool for evaluation of critical parameters (Conover & Iman, 1981). If it is supposed that results of mean values (Figure 9) corresponds to a unique experiment, it can be determined the effect of each value into the response (second column in Figure 13). The effects must be then ordered ascending (low to high) and identified. One ranks the effects from the most negative to the most positive and then proceeds to visualize the distribution (Massart, *et al*. 1997). The probability of each point was determined and it is represented the effect of each variable *vs.* probability.

A single line must be obtained when the experimental data are compatible with a normal distribution. If there are factors that do not belong to this distribution (critical variables) they would be outside the line. The graphic representation is shown in Figure 15, the factors AC, BC and ABC are align, the variable C is not clear enough. However, the factors A, B and AB are clearly critical factors which contribution is higher than random errors. The results obtained are congruent to previous analysis but the information is not accurate because of the loss of information in the experimental data (Cornell & Gorman, 1984).

Factorial designs are an interesting alternative to select better experimental conditions (Montgomery. 2017). The mean graphic can also be obtained from the experimental data and it can be used to select and evaluate qualitatively the contribution of each factor. Using the experimental data in Figure 9, it must be determined the contribution of each variable at low and high levels. The evaluation of factor A (pH) is explained in Figure 16. The contribution of A at low and high levels is the mean value of the variable at -1 and +1 levels.

The same scheme was followed to evaluate the contribution of each variable and the values are connected by a single line. The Figure 17 shows the mean graphic obtained, contribution of each factor can be estimated by the distance between low and high levels for each factor, which means that A and

Figure 13. Analysis of variance of the analysis of a 23+1 factorial design
[a] $F_{crit\,(1,8)}$=5.32

Factor	Effect	Sum of Squares (SS)	Degree of freedom	Mean Squares (MS)	F_{exp} [a]
A	0.631	1.592	1	1.592	4068.72
B	0.432	0.746	1	0.746	1908.59
C	-0.038	0.006	1	0.006	14.57
AB	0.419	0.701	1	0.701	1793.31
AC	0.007	0.000	1	0.000	0.58
BC	0.018	0.001	1	0.001	3.41
ABC	0.022	0.002	1	0.002	4.95
Residual		0.003129	8	3.91×10^{-4}	

Figure 14. Ranking for non-replicate two level factorial design (23)

Factor	Effect
A	0.631
B	0.432
C	-0.038
AB	0.419
AC	0.007
BC	0.018
ABC	0.022

Factor	Effect	Ranking	Probability
C	-0.038	1	$100\frac{1-0.5}{7}=7.1$
AC	0.007	2	$100\frac{2-0.5}{7}=21.4$
BC	0.018	3	$100\frac{3-0.5}{7}=35.7$
ABC	0.022	4	$100\frac{4-0.5}{7}=50.0$
AB	0.419	5	$100\frac{5-0.5}{7}=64.3$
B	0.432	6	$100\frac{6-0.5}{7}=78.6$
A	0.631	7	$100\frac{7-0.5}{7}=92.9$

Figure 15. Ranking test for analysis of two level factorial design

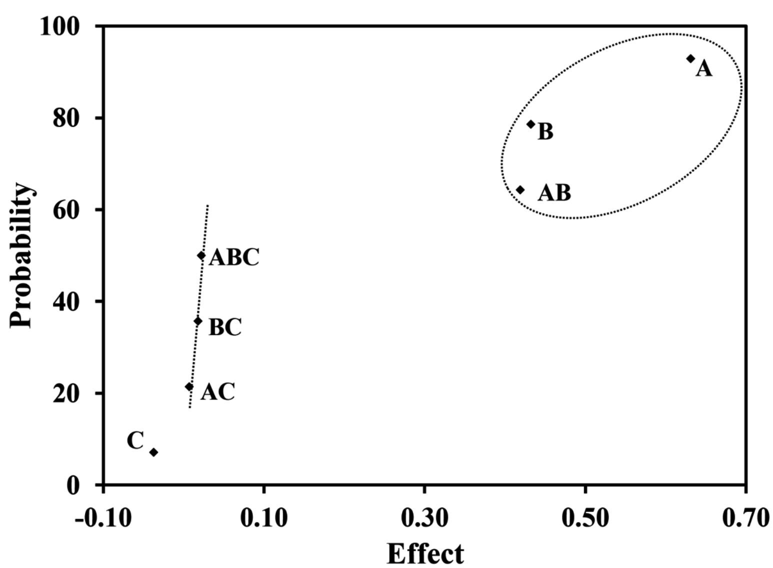


Figure 16. Determination of mean contribution at low (-) and high (+) levels for factor A

A	Denomination	Mean response (A.U.)	Mean low/high levels
-1	Y_1	0.288	$(A-)=\frac{Y_1+Y_3+Y_4+Y_5}{4}=\frac{0.288+0.305+0.247+0.256}{4}=0.274$
+1	Y_2	0.515	
-1	Y_3	0.305	
-1	Y_4	0.247	
-1	Y_5	0.256	$(A+)=\frac{Y_2+Y_6+Y_7+Y_8}{4}=\frac{0.515+0.444+1.325+1.335}{4}=0.90475$
+1	Y_6	0.444	
+1	Y_7	1.325	
+1	Y_8	1.335	

B contribute more to the response than C. The conditions selected to obtain the highest response are: A+, B+ and C-, which corresponds to experiment Y_7. From the experimental data it was inferred that experiment Y_8 has the highest response, nevertheless the contribution of C is not enough and there are no statistical differences between experiments Y_7 and Y_8.

The main problem is the analysis of more variables, the increment of the experiments increase exponentially. The analysis of 4 and 5 factors requires 32 and 64 experiments when considering replicates of the experiments, otherwise the number of experiments required would be 16 and 32, respectively. It is possible to minimize the amount of experiments by applying the fractional experimental designs.

FRACTIONAL DESIGN

The use of fractional factorial designs requires a deep knowledge of the studied system. In this section, it must be explained the advantages and disadvantages of the reduction of information. In the last example, it was concluded that factors A (pH) and B (extraction time) and their interaction AB were critical. In this case notation must be changed in order to minimize the mistake. The two level factorial design employed for 2 factors require performing 4 experiments in duplicate (8 in total) while the 3 factors involves 8 experiments in duplicate (16 experiments). The 2^{2+1} design generates 3 columns: two for factor and a two-way interaction. If the effect of interaction is not critical it can be neglected and we can evaluate an additional variable using this column. In the previous case, the AB interaction was critical and the column cannot be substituted (Robinson et al., 2004).

The interaction between pH (A) and organic solvent volume (C) was the value with less variance. These factors were selected to generate a 2^{2+1} matrix and the interaction was used to evaluate the effect

Figure 17. Means graphic for analysis of a two level factorial design

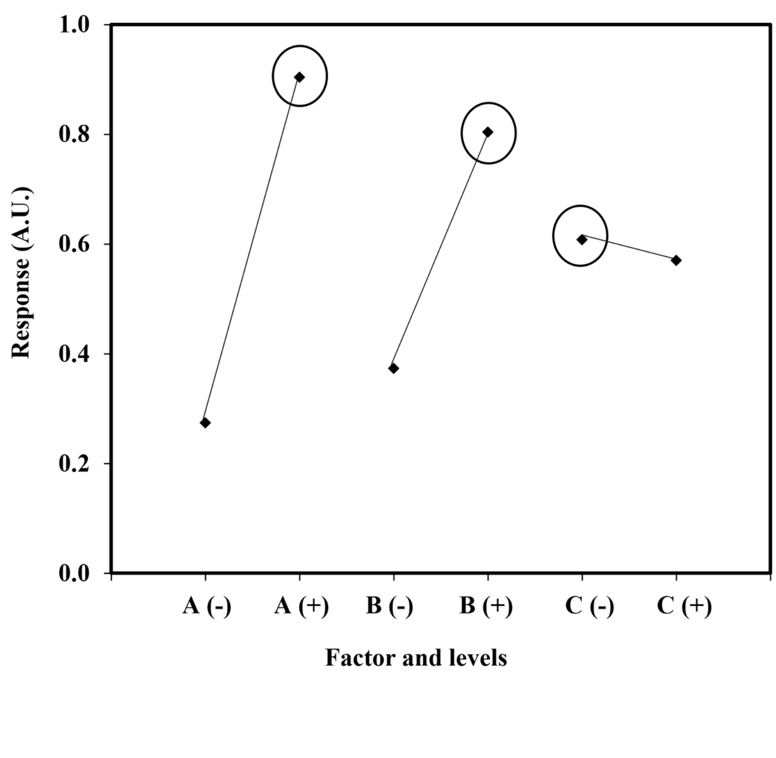


Figure 18. Generating of a Fractional factorial design

Factors		Interaction
A	C	AC
-1	-1	+1
+1	-1	-1
-1	+1	-1
+1	+1	-1

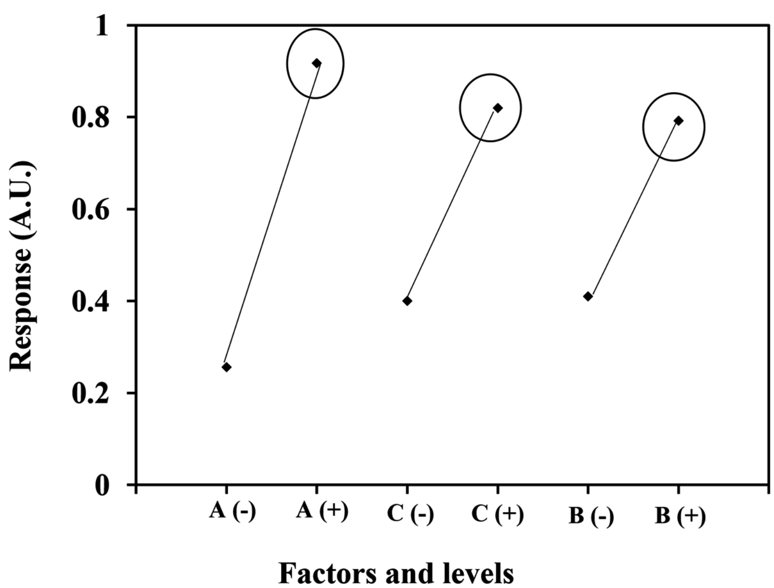
Factors		Factor added	Individual Responses	Mean Responses
A	C	B		
-1	-1	+1	0.304 0.306	0.305
+1	-1	-1	0.550 0.479	0.515
-1	+1	-1	0.250 0.243	0.247
+1	+1	+1	1.350 1.320	1.335

of extraction time. The initial 2^{2+1} design and the fractional one are presented in Figure 18, it must be focused that it would be evaluated 3 factors with 4 experiments in duplicate (8 total), promoting a reduction of the experiments needed.

The mean graphic is presented in Figure 19, it can be observed that the optimal conditions corresponds to the experiment Y_8, however the contribution is similar for the 3 factors which is a different conclusion that the obtained with the complete design (2^{3+1}). The difference is attributed to the inclusion of the variance of AC and the variance of the variable. However, the three factors have significant contribution and this result is congruent with the former design but using the half amount of the experiments.

Fractional factorial designs allow the user to improve the experimental conditions reducing the number of experiments needed, in this way the analysis is more economical and the responses are obtained quickly, this is desirable especially at industrial level.

Figure 19. Means graphic for analysis of a fractional factorial design



FUTURE RESEARCH DIRECTIONS

In perspective, evaluation and identification of the factors with significant effect must be a previous step before application of an optimization procedure. Therefore, one of the objectives of the 2-level designs is to find the factors that are significant. Once these factors have been identified, then optimization designs can be used (with at least 3 levels). It must be considered the knowledge of the system in order to minimize the experimentation required. Therefore, there are different designs of experiments options used to evaluate a process, however in chemistry it should be considered the economic, environmental and the time required for each test.

CONCLUSION

The use of factorial (2- level) design of experiments is an alternative to minimize experimentation and obtain the highest amount of information when experimental work is carried out. The use of two level factorial designs is a screening technique to evaluate a system; it can be applied not only for chemical purposes it can be extrapolated to industrial, pharmaceutical and food area. It is important to remember that any system can be evaluated without a previous knowledge of it. Before apply a design of experiments it must be considered the real experimental conditions than can be modified, with factorial designs the optimal conditions cannot be achieved due to practical consideration, since 2 level designs are just for monitoring, but it is possible to increase the response in order to find better experimental conditions. If an optimization is required, experimental designs with at least 3 levels can be used.

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REFERENCES

Bahar, S., & Zakerian, R. (2012). Speciation of Fe(II) and Fe(III) by using dispersive liquid-liquid microextraction and flame atomic absorption spectrometry. *Journal of the Brazilian Chemical Society*, *23*(5), 944–950. doi:10.1590/S0103-50532012000500021

Chobot, V., Hadacek, F., Bachmann, G., Weckwerth, W., & Kubicova, L. (2018). Antioxidant properties and the formation of iron coordination complexes of 8-hydroxyquinoline. *International Journal of Molecular Sciences*, *19*(12), 3917. doi:10.3390/ijms19123917 PMID:30544490

Conover, W. J., & Iman, R. L. (1981). Rank transformation as a bridge between parametric and non-parametric statistics. *The American Statistician*, *35*(3), 124–129.

Cornell, J. A., & Gorman, J. W. (1984). Fractional design plans for process variables in mixture experiments. *Journal of Quality Technology*, *16*(1), 20–38. doi:10.1080/00224065.1984.11978883

Deming, S. N., & Morgan, S. L. (1993). *Experimental design: A chemometric approach*. Amsterdam, The Netherlands: Elsevier.

Fraser, D. A. S. (1956). *Nonparametric methods in statistics*. Hoboken, NJ: John Wiley & Sons. doi:10.1037/13191-000

Goupy, J. L. (1993). *Methods for experimental design*. Amsterdam, The Netherlands: Elsevier.

Hanson, C. (2013). *Recent advances in liquid-liquid extraction*. Elsevier.

Kobilinsky, A., Monod, H., & Bailey, R. A. (2017). Automatic generation of generalised regular factorial designs. *Computational Statistics & Data Analysis*, *113*, 311–329. doi:10.1016/j.csda.2016.09.003

Masetto, A., Flores-Cotera, L. B., Díaz, C., Langley, E., & Sanchez, S. (2001). Application of a complete factorial design for the production of zeaxanthin by Flavobacterium sp. *Journal of Bioscience and Bioengineering*, *92*(1), 55–58. doi:10.1016/S1389-1723(01)80199-7 PMID:16233058

Massart, D. L., Vandegiste, B. G. M., Buydens, L. M. C., Lewi, S., de Jong, P. J., & Smeyers-Verbeke, J. (1997). Handbook of chemometrics and qualimetrics. Amsterdam, The Netherlands: Elsevier.

Miller, J. N., & Miller, J. C. (2010) *Statistics and chemometrics for analytical chemistry*, Pearson Education Limited, Exxes.

Montgomery, D. C. (2017). *Design and analysis of experiments*. John Wiley & Sons.

Otto, M. (2017). *CHEMOMETRICS: Statistics and computer application in analytical chemistry*. Weinheim, Germany: Wiley-VCH.

Robinson, T. J., Borror, C. M., & Myers, R. H. (2004). Robust parameter design: A review. *Quality and Reliability Engineering International*, *20*(1), 81–101. doi:10.1002/qre.602

Tounsadi, H., Khalidi, A., Farnane, M., Machrouhi, A., Elhalil, A., & Barka, N. (2019). Efficient removal of heavy metals by koh activated diplotaxis harra biomass: Experimental design optimization. *Environmental Engineering and Management Journal*, *18*(3).

Zinoubi, K., Majdoub, H., Barhoumi, H., Boufi, S., & Jaffrezic-Renault, N. (2017). Determination of trace heavy metal ions by anodic stripping voltammetry using nanofibrillated cellulose modified electrode. *Journal of Electroanalytical Chemistry*, *799*, 70–77. doi:10.1016/j.jelechem.2017.05.039