Journal of Materials Science and Chemical Engineering, 2023, 11, 91-103
https://www.scirp.org/journal/msce
ISSN Online: 2327-6053
ISSN Print: 2327-6045

Optimization by the Taguchi Method of a Robust Synthesis Protocol of Calcium Carbonate Phosphate

Mohamed Nohair[1*], Meryem Nini[1], Hassan Chaair[2], Omar Ait Layachi[1], ElMaati Khoumri[1], Mohssine ElMarrakchi[1], Abdelhake ElBrouzi[1]

[1]Physical Chemistry and Biotechnologies Laboratory of Biomolecules and Materials (LCP2BM), Mohammedia, Morocco
[2]Faculty of Science and Technology, Hassan II University of Casablanca, Mohammedia, Morocco
Email: *nohairmohamed@yahoo.fr

How to cite this paper: Nohair, M., Nini, M., Chaair, H., Layachi, O.A., Khoumri, E., ElMarrakchi, M. and ElBrouzi, A. (2023) Optimization by the Taguchi Method of a Robust Synthesis Protocol of Calcium Carbonate Phosphate. *Journal of Materials Science and Chemical Engineering*, **11**, 91-103.
https://doi.org/10.4236/msce.2023.117006

Received: April 13, 2023
Accepted: July 28, 2023
Published: July 31, 2023

Abstract

The experimental processes are difficult to model by physical laws, because a multitude of factors can intervene simultaneously and are responsible for their instabilities and their random variations. Two types of factors are to be considered; those that are easy to manipulate according to the objectives, and those that can vary randomly (uncontrollable factors). These could eventually divert the system from the desired target. It is, therefore, important to implement a system that is insensitive to fluctuations in factors that are difficult to control. The aim of this study is to optimize the synthesis of an apatitic calcium carbonate phosphate characterized with a Ca/P ratio equal to 1.61 by using the experimental design method based on the Taguchi method. In this process, five factors are considered and must be configured to achieve the previously defined objective. The temperature is a very important factor in the process, but difficult to control experimentally, so considered to be a problem factor (noise factor), forcing us to build a robust system that is insensitive to the last one. Therefore, a much simpler model to study the robustness of a synthetic solution with respect to temperature is developed. We have tried to parameterize all the factors considered in the process within a wide interval of temperature variation (60˚C - 90˚C). Temperature changes are no longer considered as a problem for apatitic calcium carbonate phosphate synthesis. In this finding, the proposed mathematical model is linear and efficient with very satisfactory statistical indicators. In addition, several simple solutions for the synthesis of carbonate phosphate are proposed with a Ca/P ratio equal to 1.61.

Keywords

Design of Experiments, Synthesis of Apatitic Calcium Carbonate Phosphate, Optimum Design, Robustness, Taguchi Approach

1. Introduction

The design of an experiment technique is a tool widely used in the optimization and modeling of nondeterministic processes, such as environmental management [1] [2] [3] [4], chemical, pharmaceutical, and industrial engineering [5] [6]. This study provides important insights into the optimization process conditions for the synthesis of apatitic calcium carbonate phosphate. The investigation is a continuation of a previous research [7], which proposes a unique combination for the synthesis of this product. This configuration seems difficult to achieve because it depends on the temperature. As it is known that the temperature is a difficult factor to stabilize, we propose in this work a synthetic configuration that is insensitive to the random variations of the temperature between 60˚C and 90˚C, by using the Taguchi method as a design of experimental techniques.

Literature review: It is well known that in the pure state, calcium phosphates are whitish solids that constitute the mineral part of bones and teeth. They are also found in blood plasma and cytoplasm. Furthermore, because they are immune to body toxicity, calcium phosphates are used as bioactive biomaterials in dental and bone surgery. Their physicochemical characteristics are responsible for their bioactivity, and these characteristics are mostly in the Ca/P atomic ratio. The stoichiometry of these solids is defined precisely by Ca/P ratio and may vary from 1.50 to 1.67. Therefore, good control of the stoichiometry of the final compound is the crucial parameter for optimizing the synthesis of calcium phosphates.

In general, hydroxyphosphate (HAp) $Ca_{10}(PO_4)_6(OH)_2$ is chemically the most similar calcium phosphate to bone crystals. However, it is very poorly soluble in biological medium and its degradation is very low [8] [9] [10] [11] [12]. In recent years, the search for compounds that replace human bone, offering an alternative to bone grafting, has been geared toward the study of the preparation of two-phase compounds with different hydroxyapatite and tricalcium phosphate compounds that may be partially absorbable *in vivo* [13] [14]. It was reported that *in vitro* cell culture research in a series of two-phase products containing 70% hydroxyapatite and 30% tricalcium phosphate with a Ca/P ratio of 1.61, showed no cytoxicity of these compounds [15]. Furthermore, cell growth occurred exponentially for each sample and each cell type.

Synthesis process: Generally, calcium phosphate is produced by a wet route using a double decomposition method, but the synthesis process proposed in this study is based on the hydrothermal method [16]. This synthesis consists of a mixture of $CaCO_3$ calcium salt and phosphate salt $(NH_4)_2HPO_4$ under water vapour flow over a given time and temperature. However, the synthesis process is governed by several parameters that must be optimized. For this purpose, synthesis conditions were established using the design of experiments method. A mathematical model was established between the Ca/P ratio of the precipitate and the factors that influence the precipitation. In this process, the factors retained are the pH (*A*), the initial Ca/P ratio (*B*), the calcium mass m_{Ca}(*C*), the reaction time (*D*), and the temperature *T* (*E*). The areas of their experimental variation are presented in **Table 1**.

For a full factorial design, 32 experiments are performed for the five retained factors. According to the principle of DOE, experiments are carried out at the center of the range of variation of all considered factors to verify the effectiveness of the proposed model. Furthermore, to reduce the number of experiments, a fractional factorial design of $2^{(5-1)} = 16$ experiments is carried out by confusing the levels of factor *E* with the interaction of other factors (*E* = *A*, *B*, *C*, *D*). All the main effects are confused with interactions of order IV and interactions of order II are confused with interactions of order III. Interactions of an order greater than or equal to III are negligible. Therefore, the resolution does not align with the problem of ambiguity.

$I = ABCDE$ $A = BCDE$ $B = ACDE$ $C = ABDE$ $D = ABCE$

$E = ABCD$ $AB = CDE$ $AC = BDE$ $AD = BCE$ $AE = BCD$

It should be noted that the interaction of order 5 is confused with the general mean, *i.e.* I. Moreover, for synthesis conditions optimization, a composite plan can be proposed by adding experiments on the axes for the five factors. Under these conditions, a simple polynomial model is proposed. This model is very simple because, after a statistical study of variance analysis, several actions (some main factors and interactions) are not significant [17].

$$Y = \frac{\mathrm{Ca}}{\mathrm{P}} = 1.5721 + 21 \cdot B - 10 \cdot E - 21 \cdot BE + 10 \cdot CD - 8 \cdot CE + 31 \cdot A^2 + 25 \cdot B^2 \quad (1)$$

The main objective is to establish the synthesis conditions for an apatitic calcium carbonate phosphate with a Ca/P ratio = 1.61. Through a multitude of combinations in the iso-response curves, the study carried out previously made it possible to find a configuration that meets this objective. The synthesis conditions are as follows:

pH equal to 7.25 ($A = 0.5$);

Initial Ca/P ratio equal to 1.565 ($B = -0.5$);

Table 1. The areas of experimental variation intervals of the different factors.

	Factors	Low-level	High-level
A	pH	5.5	7.5
B	Ca/P	1.53	1.67
C	m_{Ca} (g)	0.666	1
D	*t* (hours)	3	5
E	*T* (˚C)	60	90

Mass of calcium m_{Ca} equal to 1.033 g ($C = 1.2$);

Reaction time equal to 48 hours ($D = 1$);

Temperature equal to 60˚C ($E = -1$).

On the other hand, a statistical study with ANOVA analysis highlights the weakness of the pH effect with a statistically insignificant impact. However, the effect of temperature is by far the most important. Physicochemical characterization, such as X-ray diffraction (XRD) and infrared spectrum (IR), reveals that the synthesized solids present poorly crystallized calcium carbonate phosphate with an apatitic structure. Its chemical formula is presented below.

$Ca_{9,49}(PO_4)_{5,49}(HPO_4)_{0,39}(CO_3)_{0,13}(OH)_{1,49}$

Objectives of this study: The proposed response surface methodology (RSM) leads to a single solution that is realized and verified experimentally. Synthesis conditions may not be reproducible because some factors are unstable and may affect the synthesis process. It is very important to synthesize a product with a Ca/P ratio equal to 1.61. However, it is difficult to stabilize some retained synthesis conditions because they can vary and act on the process independently of the experimenter. This is the case for both the temperature and the pH in their areas of experimental variation.

In this study, a new approach with double optimization is proposed. Certainly, the Ca/P ratio = 1.61 is an important condition to optimize carbonated apatitic calcium phosphate synthesis, but it is not sufficient. The synthesis conditions must also minimize the influence of temperature, which is considered to be an interfering factor with the synthesis of apatitic calcium carbonate phosphate. As a result, the system is reconfigured taking into account only three factors, namely the mass of calcium, the Ca/P ratio in the initial mixture, and the reaction time. Furthermore, temperature and pH are discarded since they are involved in the construction of a product design to study the variability of the response (Ca/P ratio) as a function of random variability of both temperature and pH. An optimal configuration is possible when the following two objectives are achieved:

1) A product with a Ca/P ratio = 1.61 (this is the desired response);

2) Minimization of variability as a function of temperature and pH (as mentioned, the pH does not have a significant impact).

This double optimization has an original character and is achieved through a product design using the Taguchi methodology [18] [19]. It consists of classifying the various factors into two categories. The first includes factors that the experimenter can vary them easily, such as the mass of the calcium, the Ca/P ratio, and the reaction time. The second consists of the temperature, which randomly varies in an interval between 60˚C and 90˚C. The pH is also considered to be a disturbing factor, but its effect is weak. For the double optimization study, a product design has been used to obtain and develop a robust synthesis process. As a result of the optimization, the effect of noise generated by temperature will be reduced, and the number of main factors that impact the Ca/P ratio will be limited. In general, calcium carbonate phosphate will be developed by looking for optimal operating conditions that guarantee good accuracy (good accuracy and less dispersion) through a robustness study.

2. Modeling and Statistical Analysis

The product design consists of repeating the experiments of the main factorial design for the two limit values of the temperature. Three factors require eight experiments. Therefore, the main mathematical model is written in the following form (Equation (2)) keeping only the main factors and their interactions.

$$\tilde{Y} \sim M + B + C + D + BC + BD + CD \tag{2}$$

where M corresponds to the average of the responses; $B = m_{Ca}$; C = Ca/P ratio and D = duration of the reaction.

Both factors A (pH) and E (temperature) are obviously not included in the model, and the third-order interaction is neglected because it is often without any impact and its effect is very small. This is in accordance with the principle of the Taguchi methodology [18]. **Table 2** shows the experimental results of a complete design of eight experiments with the two responses of the Ca/P ratio at the two limit temperatures. Mean responses $\bar{Y}$ and variances σ^2 are calculated for each experiment (**Table 2**).

We observed a variation of the order of 0.01 on the Ca/P ratio when the temperature varies between 60˚C and 90˚C. This variation reflects the average; it can easily be amplified depending on the operating conditions, which justifies our approach for a robust synthesis process. It must be insensitive to random variations in temperature.

The resolution of the system for the 8 equations allows calculating the values of all the coefficients with their common variance.

$$a_i = \frac{\sum_8 Y_i}{8} \quad \text{et} \quad \operatorname{var}(a_i) = \frac{\sum_8 \operatorname{var}(Y_i)}{8^2} = \frac{8 \cdot \operatorname{var}(Y_i)}{8^2} = \frac{\operatorname{var}(Y_i)}{8} \tag{3}$$

Table 2. Experimental results with the values of the mean of the Ca/P ratio and its calculated variance.

N˚	B	C	D	Ca/P(T= 60)	Ca/P (T= 90)	$\bar{Y}$	$\sigma^2 \cdot 10^4$
1	−1	−1	−1	1.626	1.595	1.6105	4.805
2	−1	−1	1	1.563	1.607	1.585	9.68
3	−1	1	−1	1.612	1.616	1.614	0.08
4	−1	1	1	1.658	1.615	1.6365	9.245
5	1	−1	−1	1.661	1.622	1.6415	7.605
6	1	−1	1	1.636	1.664	1.65	3.92
7	1	1	−1	1.586	1.659	1.6225	26.645
8	1	1	1	1.739	1.615	1.677	76.88
		Average		**1.635**	**1.624**		

An analysis of variance by decomposing the total variation would make it possible to obtain the coefficient of determination and estimate the residual error. The validity of the model is verified by a Fisher significance test [20].

$$\sum\left(y_{i(obs)}-\bar{y}\right)^2=\sum\left(y_{i(calc)}-\bar{y}\right)^2+\sum\left(y_{i(obs)}-y_{i(calc)}\right)^2 \tag{4}$$

where $y_{i(calc)}$ is the value of the response after adjustment by the proposed model. **Table 3** below shows the analysis of variance model.

For a prespecified risk, Fisher's F-test allows the comparison of the ratio F_{obs} = CML/CMR that was calculated in the previous table with a critical value read from the Fisher-Snedecor' table with a couple of freedom degrees of (6; 1) [17]. For a good model, the residual variation must be very low compared to the one explained by the model. Moreover, the proportion of the variation explained by the model in the total variation corresponds to the coefficient of determination and noted R^2.

$$R^2=\frac{\sum\left(y_{i(calc)}-\bar{y}\right)^2}{\sum\left(y_{i(obs)}-\bar{y}\right)^2} \tag{5}$$

In addition, the standard error of the model is estimated by the following equation:

$$s=\sqrt{\frac{\sum\left(y_{i(obs)}-y_{i(calc)}\right)^2}{n-7}} \tag{6}$$

$$n=8$$

For both responses, all statistical analysis is performed jointly, including the Ca/P ratio and variance. Indeed, a double optimization would identify the configuration that would generate a product with a Ca/P ratio equal to 1.61 (which is the desired response) with the minimum dispersion. Therefore, two mathematical models are constructed corresponding to the Ca/P ratio and the variance, respectively.

Building a robust system means designing it to handle noise. Here, the noise is the temperature. Although double optimization consists of obtaining a desired response, by making the dispersion as small as possible, it is possible to find a

Table 3. Analysis of variance by decomposing the total variation.

Source	Sum of squares	Degree of freedom	Medium square	Ratio
Model variation	$SCL=\sum\left(y_{i(calc)}-\bar{y}\right)^2$	7-1	$CML=\frac{SCL}{6}$	CML/CMR
Residual variation	$SCR=\sum\left(y_{i(obs)}-y_{i(calc)}\right)^2$	1	$CMR=\frac{SCR}{1}$	
Total variation	$SCT=\sum\left(y_{i(obs)}-\bar{y}\right)^2$	8-1		

Note: 8 represents the number of measurements and therefore of responses, 7 is the number of unknowns in the model.

situation in which one of the factors acts effectively and positively on the Ca/P response. However, it acts negatively on the dispersion with an undesired response. In such a situation, it is in our interest to take advantage of the signal/noise ratio introduced by Taguchi [18]. This ratio is indicative of the loss of performance of the synthesis process when the Ca/P ratio deviates from its nominal value or the dispersion is degraded. Whereas this ratio will be optimal when the loss is very small. In the case where a nominal value of the response is sought, this ratio takes two forms depending on the interdependence between the standard deviation and the nominal value.

$$\vartheta_i = \frac{\text{Signal}}{\text{noise}} = -10\log\left[\sigma^2 + \left(\bar{Y} - 1.6\right)^2\right] \quad \text{case of independence} \tag{7}$$

$$\vartheta_i = \frac{\text{Signal}}{\text{noise}} = -10\log\left(\frac{\sigma^2}{\bar{Y}^2}\right) \quad \text{case of dependence} \tag{8}$$

Because the loss will be minimal when this ratio is very high, it would be useful to look for a configuration that maximizes this ratio. It is also possible to use an approach based on multi-criteria decision making as the Pareto front [21] technique to find the best compromise for this tow-objective optimization. This concept is widely used in engineering. In the present study, a double optimization is performed, and whenever there is ambiguity, the signal/noise ratio will be optimized or, alternatively, the Pareto front technique will be adopted.

3. Results and Interpretation

3.1 The Ca/P Ratio

The proposed linear model presumes the solution of a system of eight equations with as many unknowns. The focus is only on the values of the main effects and the second-order interactions. Solving this system leads to the estimated values of the effects of the three factors considered and their interactions (**Table 4** and **Figure 1**).

It should be noted that the t statistic represents the centred and reduced deviation from the null value of the factor under consideration. In order to study

Table 4. Statistical analysis of various factors.

Factor	Estimated value	Statistic t	Prob. > t
M	1.630	0.00025	0.000
B	0.018	0.00025	0.009
C	0.008	0.00025	0.020
D	0.008	0.00025	0.021
*B***C*	−0.006	0.00025	0.027
*B***D*	0.008	0.00025	0.019
*C***D*	0.012	0.00025	0.014

the statistical significance of each factor, the student's t-test is used. On the other hand, the effect of the factor B (the mass of calcium) is very important, while the effects of the other two factors are less important, but they act through their interaction. The main goal in this step is to obtain a nominal response with a ratio of 1.61. Therefore, there are many solutions. It is possible to visualize the multitude of solutions offered by our model by means of iso-response curves. At this stage, no constraints are imposed to discuss their feasibility.

The model produced leads to a coefficient of determination equal to 99% and a standard error evaluated about 0.0007. In addition, the analysis of variance shows that the variation explained by the model is very large (**Table 5**). This result is confirmed by the value of the constant, which is equal to 1.629. This value is almost equal to that observed experimentally for the following configuration ($Y_{obs} = 1.62$; $B = 0$, $C = 0$ and $D = 0$), confirming that the proposed linear model is valid and very significant.

In the following **Figures 2-4**, three iso-response curves are presented for a target ratio of 1.61 corresponding to the different combinations of two factors. The third factor is taken to obtain the maximum number of solutions for the goal of the process. Interestingly, a ratio of 1.6 is obtained when factor D is high and factors B and C are low. Furthermore, the iso-response curves illustrate the different combinations of synthesis of the considered phosphate. This means that synthesis is a challenge because the ranges of variation of the different factors are limited. For instance, when the reaction time (factor D) is at the high level, the ranges of variation of the other phosphate synthesis factors are very small. Clearly, low levels of calcium mass and Ca/P ratio offer more possibilities.

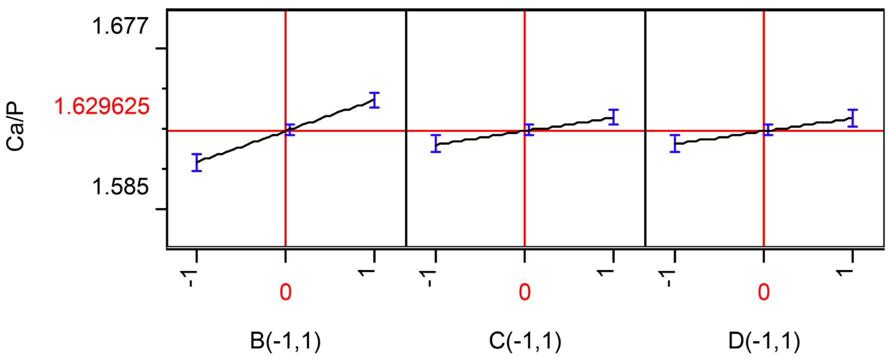

Figure 1. Profile of the effects of different factors.

Table 5. Results of the variance analysis.

Source	DDL	Sum of squares	Mean of squares	Ratio
Model	6	0.0055	0.0009	1833.1250
Error	1	0.0000	0.0000	
Total variation	7	0.0055		

Note: DDL: Degree of freedom.

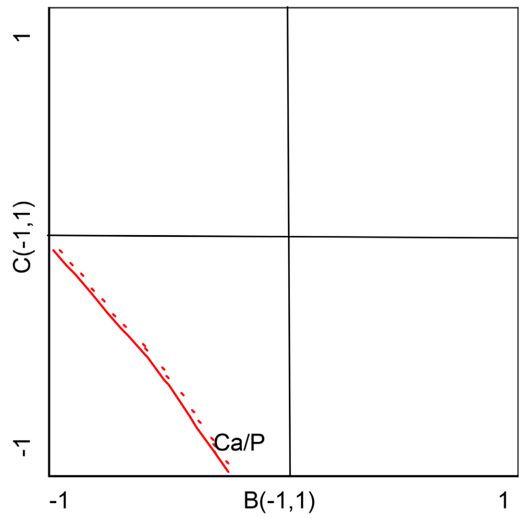

Figure 2. Iso-response curve (Ca/P = 1.61) for the factors (Ca/P; m_{Ca}); $D = 1$.

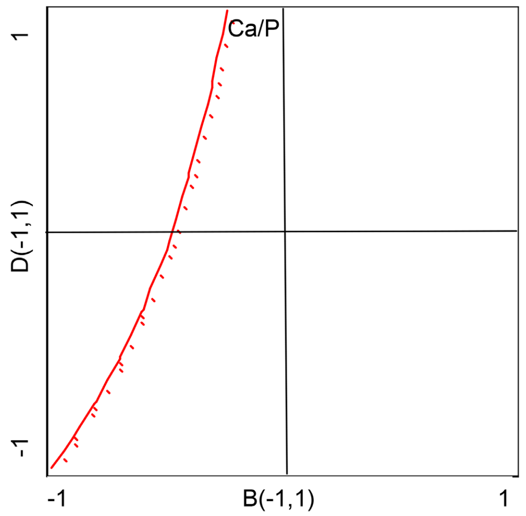

Figure 3. Iso-response curve (Ca/P = 1.61) for the factors (Ca/P; reaction time); $C = -1$.

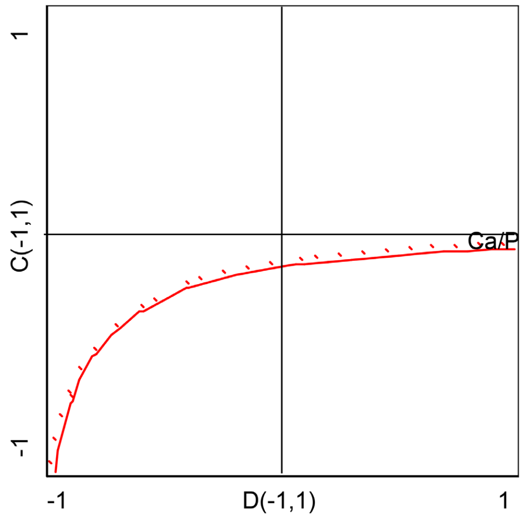

Figure 4. Iso-response curve (Ca/P = 1.61) for the factors (m_{Ca}; reaction time); $B = -1$.

Table 6. Estimation of the different parameters of the model.

Factor	Estimated value
M	17.35
$B(-1, 1)$	11.40
$C(-1, 1)$	10.85
$D(-1, 1)$	7.57
$B(-1, 1)*C(-1, 1)$	12.14
$B(-1, 1)*D(-1, 1)$	4.06
$C(-1, 1)*D(-1, 1)$	7.27
$B(-1, 1)*C(-1, 1)*D(-1, 1)$	6.20

3.2. Variance or Dispersion

In this section, the same procedure is used for the dispersion with the purpose of reducing it as much as possible. The proposed linear model assumes the resolution of a system of eight equations and eight unknowns. The resolution of this system led to estimated values of the effects of the three factors considered (Table 6).

All factors act similarly, and their effects are of the same order of magnitude. However, the effects of interactions are also important. In this paper, the different combinations are proposed to be explored in order to minimize the variance based on the proposed solutions for the different combinations and for a Ca/P ratio equal to 1.61.

***First case*: *when* $B = -1$**

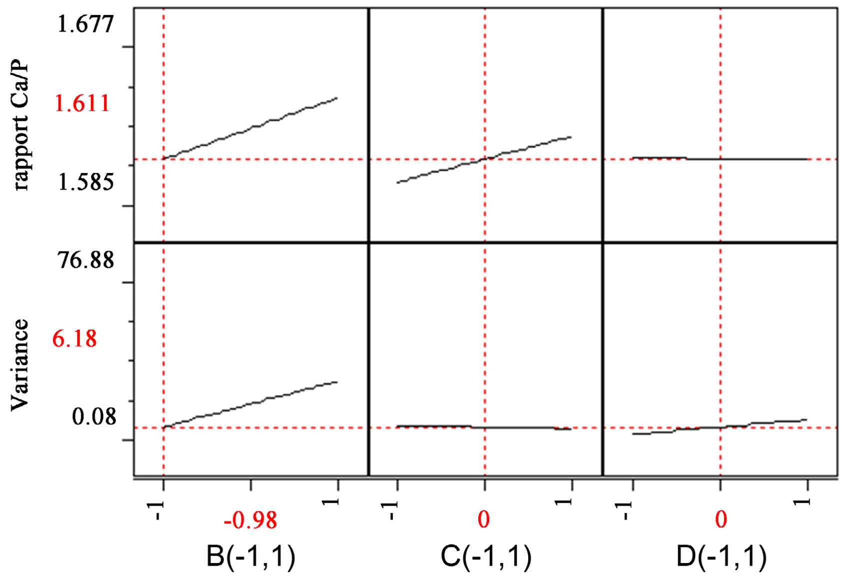

Figure 5. Profiles of the effects of the different factors on both the ratio Ca/P and the variance for $B = -1$.

***Second case*: *when* $C = -1$**

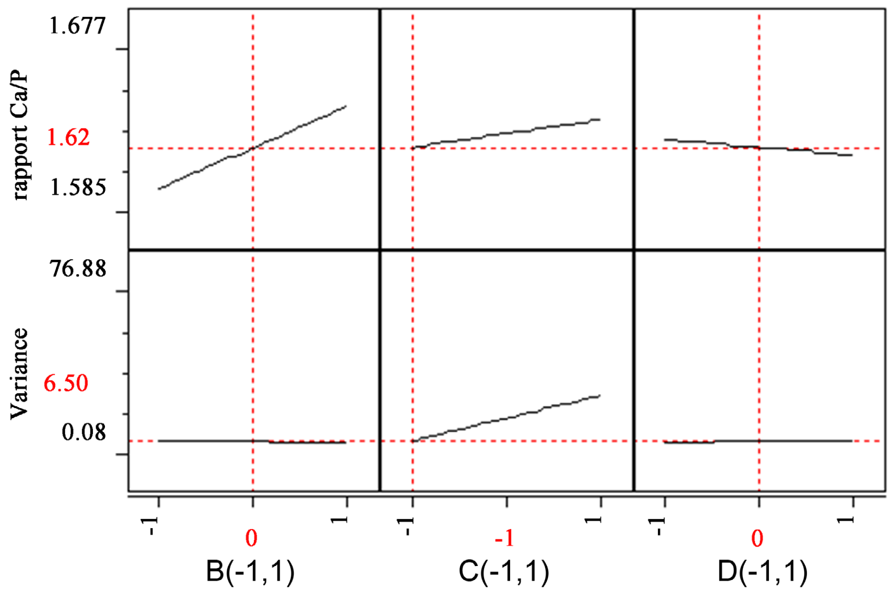

Figure 6. Profiles of the effects of the different factors on both the ratio Ca/P and the variance for $C = -1$.

Third case: when $D = 1$

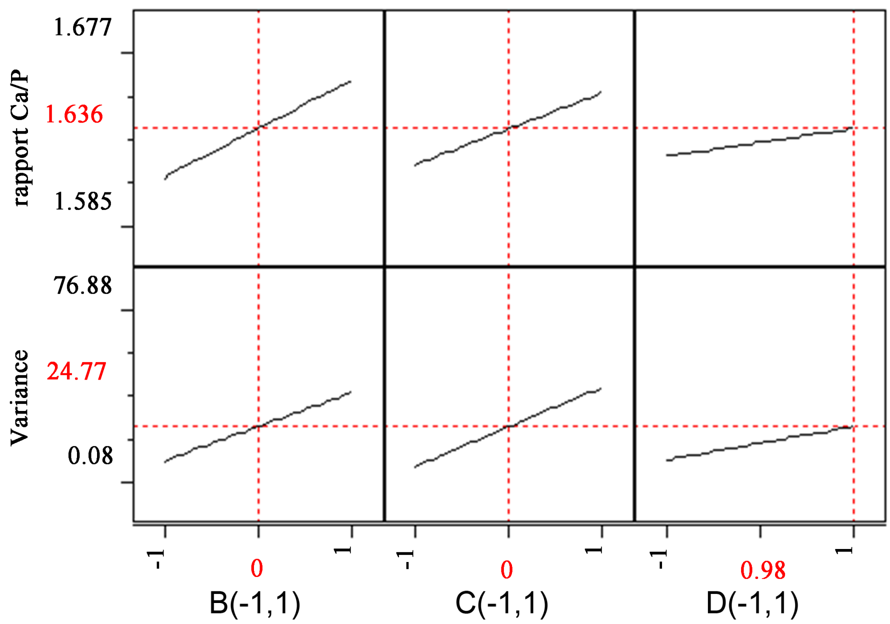

Figure 7. Profiles of the effects of the different factors on the ratio Ca/P and the variance for $D = 1$.

Looking at **Figures 5-7**, it appears that the first two cases give better results, as the dispersion is very low with a Ca/P ratio very close to 1.61. At this point, the first case is preferred because the effects of the other factors were very weak, which contributes to the stability of the apatitic phosphate synthesis. Furthermore, this combination for the low factor B offers several solutions for the synthesis of calcium carbonate phosphate with a Ca/P ratio = 1.61.

4. Conclusions

Taken together, these findings suggest that by using the Taguchi method for the optimization of apatitic calcium carbonate phosphate synthesis, it is possible to achieve a synthesis that is insensitive to uncontrolled temperature variations. At a low level of the mass of calcium as a reagent, the robust model offers several configurations for the synthesis of the apatitic phosphate with a Ca/P ratio equal to 1.61. Moreover, the effect of other factors is reduced, increasing the robustness of the process. The pH is a very low impact factor. In addition to this, the proposed model is simple with only three factors and very efficient statistical indicators.

The results reported here confirm that there is no need to perform an optimization of the signal/noise function proposed in Taguchi's approach for the loss of quality function. This is due to the double optimization that offers no ambiguity to find a configuration suitable for the robust synthesis of calcium carbonate phosphate. The design of experiments with the Taguchi approach offers an effective tool for better understanding this type of problem, as temperature is a difficult factor to control in chemical synthesis. Overall, the configurations presented in this study are diverse and independent of random temperature variations, which offer a new insight into the optimization of the synthesis process.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Shojaei, S., Shojaei, S. and Pirkamali, M. (2019) Application of Box-Behnken Design Approach for Removal of Acid Black 26 from Aqueous Solution Using Zeolite: Modeling, Optimization, and Study of Interactive Variables. *Water Conservation Science and Engineering*, **4**, 13-19. https://doi.org/10.1007/s41101-019-00064-7

[2] Pourabadeh, A., Baharinikoo, L., Shojaei, S., Mehdizadeh, B., Davoodabadi Farahani, M. and Shojaei, S. (2020) Experimental Design and Modelling of Removal of Dyes Using Nano-Zero-Valent Iron: A Simultaneous Model. *International Journal of Environmental Analytical Chemistry*, **100**, 1707-1719. https://doi.org/10.1080/03067319.2019.1657855

[3] Shojaei, S., Shojaei, S., Band, S.S., Kazemzadeh Farizhandi, A.A., Ghoroqi, M. and Mosavi, A. (2021) Application of Taguchi Method and Response Surface Methodology into the Removal of Malachite Green and Auramine-O by NaX Nanozeolites. *Scientific Reports*, **11**, Article No. 16054. https://doi.org/10.1038/s41598-021-95649-5

[4] Shojaei, S., Shojaei, S., Nouri, A. and Baharinikoo, L. (2021) Application of Chemometrics for Modeling and Optimization of Ultrasound-Assisted Dispersive Liquid-Liquid Microextraction for the Simultaneous Determination of Dyes. *NPJ Clean Water*, **4**, Article No. 23. https://doi.org/10.1038/s41545-021-00113-6

[5] Carrillo-Cedillo, E.G., Rodríguez-Avila, J.A., Arredondo-Soto, K.C. and Cornejo-Bravo, J.M. (2010) Design of Experiments for Chemical, Pharmaceutical, Food, and Industrial Applications. IGI Global, Hershey. https://doi.org/10.4018/978-1-7998-1518-1

[6] Xia, Y.F., Jiang, C.J. and Zhang, J.N. (2021) Optimization of Carbamazepine-Succinic

Acid Cocrystal Preparation Using Quality by Design Approach. *Open Access Library Journal*, **8**, e7074. https://doi.org/10.4236/oalib.1107074

[7] Belouafa, S., Chaair, H., Chroqui, W., Digua, K., Britel, O. and Essaadani, A. (2006) Central Composite Design and Optimization by Response Analysis of β-Tricalcium Phosphate Elaboration. *Phosphorus, Sulfur, and Silicon and the Related Elements*, **181**, 779-786. https://doi.org/10.1080/10426500500271816

[8] Tampieri, A., Celotti, G. and Landi, E. (2005) From Biomimetic Apatites to Biologically Inspired Composites. *Analytical and Bioanalytical Chemistry*, **381**, 568-576. https://doi.org/10.1007/s00216-004-2943-0

[9] Osman, M.B., Diallo-Garcia, S., Herledan, V., Brouri, D., Yoshioka, T., Kubo, J., Millot, Y. and Costentin, G. (2015) Discrimination of Surface and Bulk Structure of Crystalline Hydroxyapatite Nanoparticles by NMR. *The Journal of Physical Chemistry C*, **119**, 23008-23020. https://doi.org/10.1021/acs.jpcc.5b08732

[10] Legeros, R.Z. (1993) Biodegradation and Bioresorption of Calcium Phosphate Ceramics. *Clinical Materials*, **14**, 65-88. https://doi.org/10.1016/0267-6605(93)90049-D

[11] Ribeiro, G.B.M., Trommer, R.M., Dos Santos, L.A. and Bergmann, C.P. (2011) Novel Method to Produce β-TCP Scaffolds. *Materials Letters*, **65**, 275-277. https://doi.org/10.1016/j.matlet.2010.09.066

[12] Ducheyne, P., Radin, S. and King, L. (1993) The Effect of Calcium Phosphate Ceramic Composition and Structure on *in Vitro* Behavior. I. Dissolution. *Journal of Biomedical Materials Research*, **27**, 25-34. https://doi.org/10.1002/jbm.820270105

[13] Kwon, S.-H., Jun, Y.-K., Hong, S.-H. and Kim, H.-E. (2003) Synthesis and Dissolution Behavior of β-TCP and HA/β-TCP Composite Powders. *Journal of the European Ceramic Society*, **23**, 1039-1045. https://doi.org/10.1016/S0955-2219(02)00263-7

[14] Jarcho, M. (1981) Calcium Phosphate Ceramics as Hard Tissue Prosthetics. *Clinical Orthopaedics and Related Research*, **157**, 259-278. https://doi.org/10.1097/00003086-198106000-00037

[15] Dos Santos, L.A., Carrodéguas, R.G., Rogero, S.O., Higa, O.Z., Boschi, A.O. and de Arruda, A.C.F. (2002) α-Tricalcium Phosphate Cement: "*In Vitro*" Cytotoxicity. *Biomaterials*, **23**, 2035-2042. https://doi.org/10.1016/S0142-9612(01)00333-7

[16] Brasseur, H. (2010) Etude du phosphate tricalcique hydraté et de l'hydroxylapatite de synthèse par voie humide. *Bulletin des Sociétés Chimiques Belges*, **62**, 383-400. https://doi.org/10.1002/bscb.19530620705

[17] Montgomery, D.C. and Runger, G.C. (2018) Applied Statistics and Probability for Engineers. 7th Edition, Wiley, Hoboken.

[18] Taguchi, G. (1987) System of Experimental Design: Engineering Methods to Optimize Quality and Minimize Costs. Tome I et II. UNIPUB/Kraus International Publications, American Supplier Institute, Dearborn.

[19] Sergio, F., Adriana, C., Thaise, B., Ariana, L., Laiana, S. and Walter, S. (2017) Robustness Evaluation in Analytical Methods Optimized Using Experimental Designs. *Microchemical Journal*, **131**, 163-169. https://doi.org/10.1016/j.microc.2016.12.004

[20] Dagnelie, P. (2011) Statistique théorique et appliquée—Tome 2: Inférence statistique à une et à deux dimensions. De Boeck, Bruxelles.

[21] Goodarzi, E., Ziaei, M. and Hosseinipour, E.Z. (2014) Introduction to Optimization Analysis in Hydrosystem Engineering. Springer, Berlin, 111-148. https://doi.org/10.1007/978-3-319-04400-2