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Modeling and optimization of the synthesis of oxygenated apatite by hydrolysis of dicalcium phosphate dihydrate (DCPD) using the Box-Behnken Design

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ABSTRACT

A Box-Behnken design was successfully applied to studying and optimizing preparation of oxygenated phosphocalcic apatite. At first, we have discussed the effects of three main process factors of preparation such as pH, temperature and duration of the reaction.Then, we have studied their interactions in order to increase the rate of oxygen insertion in the apatite channel. The quadratic model which is derived from the Taylor-Mac Lorain equation has contributed to optimizing the quantity of oxygeninserted. So, using graphical methodology based on isorespose curves, we have successfully introduced 4.5 % in weight of oxygen in the apatite channel.

Keywords: Oxygenated apatite, calcium phosphate compounds, Box-Behnken design, optimization.

INTRODUCTION

Some technological challenges in dentistry are to develop specialized materials with intrinsic antimicrobial properties. After the restoration of cavities or tooth defects, these materials release chemical species which contribute to limiting the proliferation of bacteria and microorganisms. The oxygenated phosphocalcic apatite is a very important compound that can cure and limit the proliferation of bacteria and micro-organisms at the site of the implantation [1]. These properties are due to the available oxygenated species in its channels such as molecular oxygen: O_2[1,2]. When this species is released in the environment of implantation, most of the anaerobes are destroyed due to their high sensitivity to oxygen [3].

C. Rey [4] was the first who has prepared oxygenated apatite. His method consists of a reaction of an anhydric β-tricalcium phosphate $Ca_3(PO_4)_2$ (β) in boiled oxygenated water. The doped quantity of oxygen is about 1.6 % in weight and it can be held into apatite structure under a heating process until 600 °C[5]. Another synthesis process used to prepare an oxygenated calcium phosphate compound that has apatite structure was described by Ledard*et al.*[6]. This product can be used against micro-organism developed at the implantation site. Beloufa*et al.* [7] have studied and optimized another way to prepare an oxygenated apatite with ratio Ca/P equal to 1.575. In their study they have proposed a mathematical model based on an experimented design, which helps to understand the effect of several factors that can improve immersion of oxygen into the apatite channel. M. Elgadi*et al.* [8] have proposed the reaction of phosphoric acid with calcium hydroxide or with calcium carbonate in oxygenated water. They have inserted 2.48% in weight of oxygen into the apatitic tunnel.

In our study, we have studied the effects of pH of reaction, temperature (T) and duration of reaction (Dr), on insertion of oxygen to an apatite tunnel. We tried to understand how to join together the three factors simultaneously in order to introduce a desired rate of oxygen into apatite, this by referring to iso-response plots.

MATERIALS AND METHODS

Preparation of oxygenated phosphocalcic apatite

The oxygenated apatite was prepared by several techniques [1,2, 4, 9-13]. We have used one technique which was invented by E.mejdoubi *et al.* [10]. This protocol was applied to each formulation cited below in matrix design (table 2).The method consists of the controlled hydrolysis of dicalcium phosphate dihydrate or brushite, in the presence of a hydrogen peroxide solution, which has a source of molecular oxygen.

Experimental study

The experimental strategy had chosen isthe methodology of design of experiments, this methodology identifies the most significant experimental factors, and possible interactions of these factors for a given operation. We have used the Box-Behnken design to explore the rate of oxygen inserted in phosphocalcicapatite. So,the response (% O_2) is a function of pH, temperature T and duration of reactionDr, having a second degree polynomial model, which can be written in coded variables as follows:

$$Y\ (\%O_2) = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X^2_1 + b_{22}X^2_2 + b_{33}X^2_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

The terms bi are the linear main effects, biiare the quadratic main effects andbijare the linear-bylinear interactions. These coefficients are determined using the ANOVA approach. All statistical analysis and graphs were done using N.E.M.R.O.D and JMP software.

The preliminary studieshave lead us to determinethe main factors and the limits of variation for each factor. The three factors with their ranges are grouped in Table 1.

Table 1: List of factors and their ranges for the experiments

Variables	Factors	Ranges Low level to high level
X1	x1= Ph	8 to 12
X2	x2= Dr (°C)	15 to 35
X3	x3= T (day)	1 to 3

We have realized 15 experimental trials using the experimented design of Box-Behnken strategy. The coded and uncoded design matrixwith the respective response valuesis shown in Table 2. The measurement of the percentage of oxygen integrated in the apatite was made by chemical analysis.In this protocol, we have attacked the oxygenated apatite with perchloric acid. Thus, when the tunnel of apatite is destroyed, the molecules of oxygen escape in gas form.So, it is practical to measure them by volumetric dosage. Finally, the quantity of oxygen is calculated from the volume of oxygen dosed.

RESULTS

Analysis of oxygenated apatite prepared

Allthe oxygenated apatite prepared wascontrolled using the Fourier-transformed infrared spectroscopy (FTIR). In figure 1, the spectrum “A” is obtained at ordinary temperature, and spectrum “B” is obtained after calcination at high temperature (900°C).The figure shows that it is a real phosphocalcic apatite.In fact, the spectrum is dominated by the typical PO_4 bands: asymmetric mode at 1021 and 1087 cm^{-1}, symmetric stretching mode at 962 cm^{-1} and bending mode at 599 and 562 cm^{-1}. Carbonate bands which are characteristic of air contamination (spectrum A) are also observed at 1550– 1350 cm^{-1}, 873 cm^{-1} and 712 cm^{-1}. The broad band of low intensity in the range 3000-3400 cm^{-1}(spectrum A) can be attributed to traces of water incorporated to the structure, together wit the very weak, broad band around 1640 cm^{-1} ofH–O–H bendingmode. These bands disappear after heating at high temperature (spectrum B). The characteristic band OH of phosphocalcic apatite appears at 3644 cm^{-1}.So finally, we can observe at 900°C, the small amount of beta-tricalcium phosphate (spectrum B). This shows that the oxygenated apatite prepared is non-steochiometric. Its decomposition at high temperature gives phosphocalcic apatite and beta-tricalcium phosphate.

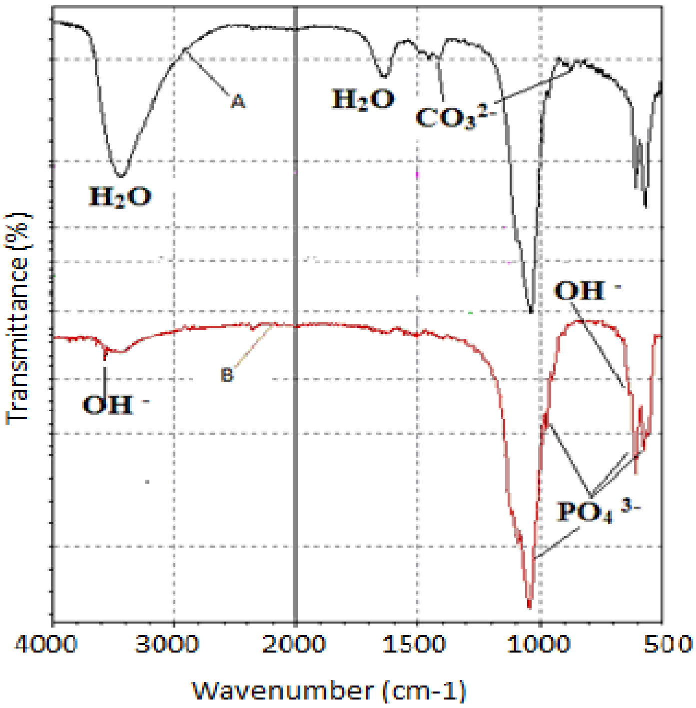

Figure 1. Fourier-transformed infrared spectroscopy of apatite prepared

Statistical study and interpretations

The construction of normal probability plot of residuals (diagram of Henry figure 2) shows that the normality assumptions are checked for all data. Thus, these data follow a normal distribution and their frequency has a Gaussian form.

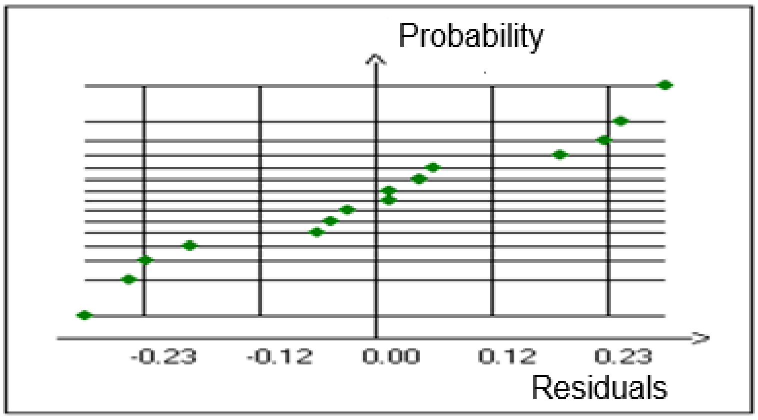

Figure 2. Diagram of Henry

Analysis of variance

The experiment value of Snedecor factor (Fexp) is equal to 24.15 (Table 3), and it is higher than the critical value which is equal to 10.15 ($F_{0.01}(9, 5) = 10.15$), with a significance level of 1 %. These involve that the regression is significant with a confidence level of 99 %. Then, it has good statistical characteristics, such as the R^2 and R^2 adjusted which are equal to 0.97 and 0.93 respectively. As a consequence, we can conclude that the changes in responses of oxygen inserted are due to variations of different factors. So, we can present all the results and the interpretation for this model.

Table 2. coded and uncoded Design matrix with response values

N	pH		Dm		Tr		$\%O_2$
	X1	X1	X2	x2	X3	x3	
1	-1	8	-1	1	0	25	0,81
2	-1	8	1	3	0	25	1,56
3	1	12	-1	1	0	25	0,89
4	1	12	1	3	0	25	3,24
5	0	10	-1	1	-1	15	0,74
6	0	10	-1	1	1	35	1,32
7	0	10	1	3	-1	15	2,39
8	0	10	1	3	1	35	4,66
9	-1	8	0	2	-1	15	1,49
10	1	12	0	2	-1	15	1,67
11	-1	8	0	2	1	35	2,39
12	1	12	0	2	1	35	4,03
13	0	10	0	2	0	25	3,13
14	0	10	0	2	0	25	3,17
15	0	10	0	2	0	25	3,17

Table 3. Analysis of variance

Source of variation	Sum of squares	Freedom	Mean square	Fexp	Signification test
Regression	20,39	9	22663	24 ,15	**
Residual	0,46	5	0,0938		
Total	20,86	14			

** Significant*

The analysis part involves the determination of significant main and interaction effects. The table 4 indicates that main effects Dr, T and pH andinteraction effects T*Dr and Dr*pH and square of effects pH and Dr, were considered to be real (or active). This is for a confidence level of 95 % (table 4). (For a confidence level chosen the coefficient is significant if its F_{exp} is higher than its F_{critic}). Moreover, it can be observed from the figure 3 (Pareto plot) thatthe duration of the reaction Drhas a strong effect on the preparation of apatite with a high rate of oxygen.

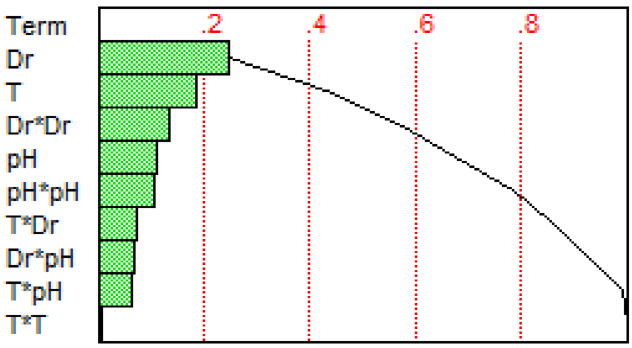

Figure 3. Pareto plot effects from the experiment

In order to determine the optimal percentage of oxygen inserted, main effects plots are constructed (figure 4). It can be observed that the three factors have a positive effect on “$\%O_2$ inserted”. When these are kept at high levels, the response takes a high value. This is very clear in the three-dimensional graph shown in figure6.The optimal for maximizing rate of oxygen inserted is:

pH: + 1 (high level), that means pH = 12
Dr: + 1 (high level), Dr = 3 days
T: + 1 (high level), T = 35.

Table 4. Estimated model coefficients and their significance from the experiment

Coefficients of model	Coefficients Value	Degrees of Freedom	Sum of squares	Fexp	Signification
b0	3,15	-	-	-	-
b1	0,4475	1	1,602	17,07	**
b2	1,0112	1	8,181	87,20	***
b3	0,7637	1	4,666	49,74	***
b11	-0,7070	1	1,846	19,67	**
b12	0,4	1	0,64	6,82	*
b22	-0,8245	1	2,51	26,76	**
b13	0,365	1	0,53	5,68	NS
b23	0,4225	1	0,71	7,61	*
b33	-0,0545	1	0,011	0,11	NS

****: significant at 0,1 % ($F_{0.001}(1, 5) = 47,18$).; **: significant at 1 % ($F_{0.01}(1, 5) = 16,25$).*
: significant at 5 % ($F_{0.05}(1, 5) = 6,60$).; **NS: not significant*

The table 4 shows that only two interactions are significant at 95% of confidence level. They are between T and Dr and between Dr and pH, so to analyze their interactions; we have decided to use interaction plots shown in figure 5.

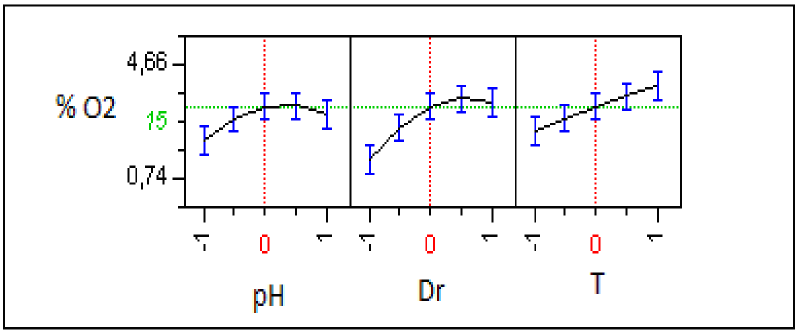

Figure 4. Main effects plot for % O_2 inserted

The figure indicates that there is a weak interaction between T and Dr, and between Dr and pH. However, it can be observed from the figure that the response of oxygen inserted in apatite is maximal when all factors are kept at a high level (+1). Thus for these values the response "% O_2 integrated" achieves 4.98 % in weight.
With an error less than 5 %, the equation of model predicted is:

$$Y = \%\ O_2 = 3{,}15 + 0{,}4475X_1 + 1{,}0112X_2 + 0{,}7637X_3 - 0{,}7070\ (X_1)^2 + 0{,}4\ (X_1{*}X_2) - 0{,}8245(X_2)^2 + 0{,}4225\ (X_2{*}X_3)$$

For the pH, we explained its positive effect on the response in that the pH increase promotes the formation of apatite. However, by further increasing the pH of the deoxygenated solution of hydrogen peroxide it may promote, and thus reduce the oxygen content. This is evidenced by the negative impact of the term square pH / pH on oxygen levels.

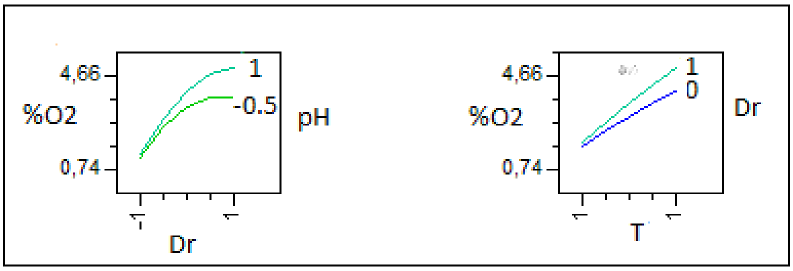

Figure 5. Interaction plots of (Dr, pH) and (T, Dr)

The positive effect of Ca / P on all the experimental field is chemically explained by the fact that the closer you get the atomic ratio of apatite is 1.67, the more it favors the precipitation of the latter. But exceeding the value Ca / P =

1.67 there is a risk to precipitate lime with the apatite phase. The temperature and duration of the reaction have a positive influence on the response. But above a certain limit, the two factors contribute to deoxygenation of hydrogen peroxide, which negatively affects the rate of oxygen in the oxygenated apatite.

From isoresponse plots (figure 7 and 8), we can find easily the optimal percentage of oxygen integrated to the phophocalcic apatite, according to different factors, with many possibilities between ranges of each factor. So, these presentations offer wide practical uses in dentistry without needing experimentation trials. The confirmatory trials were performed and we have obtained % O_2 equal to = 4.94% in weight foroptimum operational conditions: pH = 12, Dr = 3 days and T = 35°C.

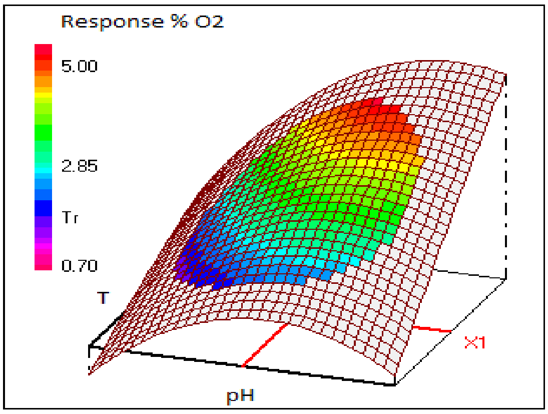

Figure 6. Variation of response %O_2 in (pH, T) plan, Dr is fixed at 3 days

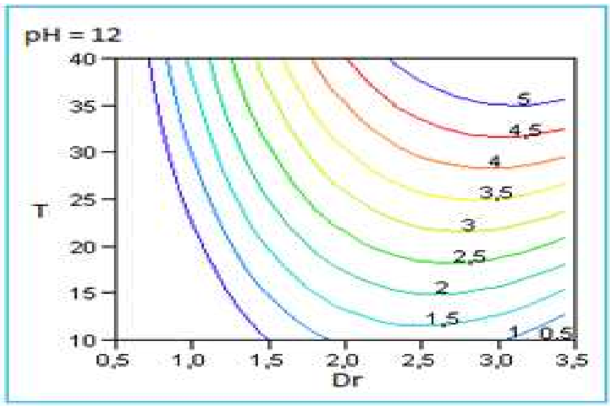

Figure 7.isoresponse plot of %O_2 in (Dr, T) plan with pH fixed at 12.

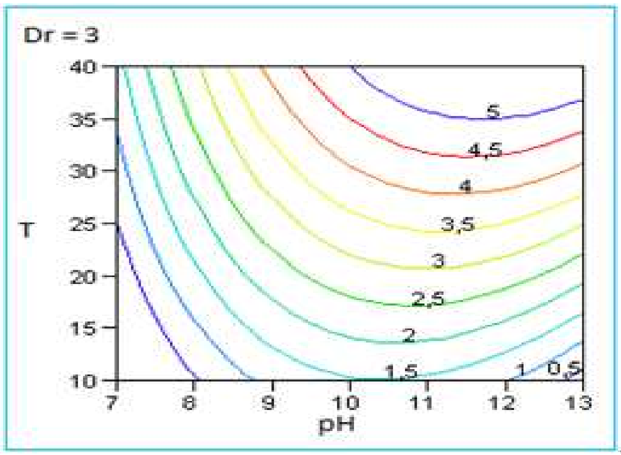

Figure 8.isoresponse plot of %O_2 in (ph, T) plan with Dr fixed at 3 days.

CONCLUSION

Methodology of experiments led to a better understanding of the chemical mechanisms. The progress of the reaction which is governed by a mathematical model is the result of chemical phenomena involved. The mathematical model actually reflects the chemical behavior of reactive with respect to different factors.

The results of the study have demonstrated significant improvement to the process of preparation. However, based on Box Behnken design we have established a mathematical model which describes the variation of the response "% O_2" according to synthesis factors. The maximum insertion of molecular oxygen in apatite is obtained where the three factors pH, Dr and T are kept at high levels.

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