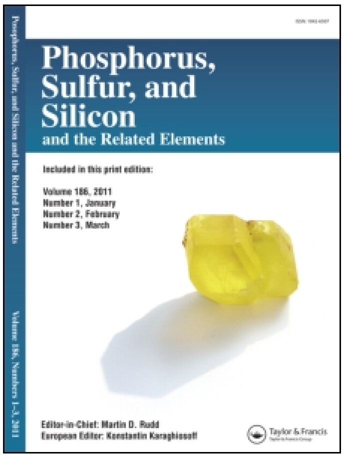

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Advanced Statistical Optimization of Parameters of Synthesis Process of Oxygenated Carbonated Apatite

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The synthesis process of oxygenated carbonated apatite was optimized by an advanced statistical planning of experiments. Full factorial design of 24 experiments was used to find the effects of five principal parameters: pH of the reaction medium, atomic ratio Ca/P of the reagents, concentration of the calcium solution ([Ca^{2+}]), temperature of the reaction medium (T) and duration of the reaction (D), with fixing the H_2O_2 composition at 30% and stirring to 600 turns/min. Studied responses were the atomic ratio Ca/P, % O_2, % O_2^{2-} and % CO_3^{2-}. Optimum synthesis parameters were found to be pH = 7.38, Ca/P = 1.647, [Ca^{2+}] = 0.636 M, T = 40°C and D = 1 h. The prediction responses were Ca/P = 1.575, % O_2 = 0.76, % O_2^{2-} = 0.50 and % CO_3^{2-} = 1.84. The actual experimental results were in agreement with the prediction.

Keywords Optimisation; oxygenated carbonated apatites; statistical analysis; synthesis

INTRODUCTION

Calcium hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is well known as the primary constituent of bone and teeth of animal organisms.[1,2] Many papers have been published about the use of materials based on calcium phosphate apatite as bone substitutes in medical and dental treatments. Specially, clinical dental applications include the maintenance of periodontal defects,[3,4] the implantation into tooth extraction sockets to

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conserve alveolar ridge height,[5,6] and the increase of a deficient alveolar ridge to improve denture support and stability.[7–10]

Phosphocalcic oxygenated apatites are among the most promising calcium phosphate apatites because of their antiseptic properties, which make them able of limiting the proliferation of microorganisms at the site of implantation.[11] These properties are due to the oxygenated species (peroxide ions: O_2^{2-} and/or molecular oxygen: O_2) contained in the channels of the apatite structure.[11–13] These species are liberated in the living environment either by progressive dissolution of the material, or by chemical exchange with the living environment.[11]

Several methods of chemical synthesis have been developed to prepare phosphocalcic oxygenated apatite powders using various types of calcium and phosphorus sources.[11–15]

The difficulty of most of the conventional syntheses used is to obtain well defined solids, which means a solid with a given Ca/P ratio, % O_2 and % O_2^{2-}[13,15]: factors governing the precipitation, such as pH, temperature, and Ca/P ratio of the reagents, are usually not precisely controlled.

With the aim of developing a more oxygenated apatite, partially carbonated in site B, with speed of dissolution adaptable to that of the osseous neoformation and allowing a progressive diffusion of oxygenated species, the purpose of the investigations described in this paper is to modelize and optimize the synthesis of this apatite by applying the experimental design methodology. The relationship between the atomic ratio Ca/P (Ca/P $\approx$ 1.575),[16] % O_2, % O_2^{2-}, and % CO_3^{2-} responses and the variables governing the precipitation (pH of reaction medium, atomic ratio Ca/P of the reagents, concentration of the calcium solution ($[Ca^{2+}]$), temperature of reaction medium (T) and duration of reaction (D)) was determined by a second degree polynomial function in a set of experiments according to a fractional central composite design.[17]

EXPERIMENTAL

The preparation of phosphocalcic oxygenated carbonated apatite was performed by precipitation reaction with calcium and phosphate solutions:

- The calcium suspension was prepared by adding calcium carbonate ($CaCO_3$) in 250 mL of oxygenated water (30%).
- The phosphate solution was prepared by adding phosphoric acid (84%) in 250 mL of oxygenated water (30%).

The method of synthesis consists in putting the calcium solution into a reactor maintained at the temperature of synthesis. The pH was adjusted by manual addition of NH_4OH solution (d = 0.92). Then the orthophosphoric acid solution was poured into the reactor all at once. The reacting medium was kept under agitation for the duration of the reaction at the pH of synthesis. At the end the suspension was vacuum-filtered, washed with distilled water, and dried in a desiccator.

X-ray diffraction analysis was carried out by means of a SEIFERT XRD 3000 P using CuK radiation.

For infrared absorption analysis, 1 mg of the powdered sample was carefully mixed with 300 mg of KBr and pelletized under vacuum. The pallets were analysed using a Perkin Elmer 1600 FTIR spectrophotometer.

Calcium, phosphorus and the content of the oxygenated species were determined by wet chemical methods. Calcium was titrated by complexometry methods,[18] the phosphorus content was analysed by colorimetry,[19] molecular oxygen was determined by measuring the volume displaced during the acid dissolution of the powder,[11] and the peroxide ions were titrated by manganometry methods.[20]

STATISTICAL ANALYSIS

Experimental analysis is frequently performed in agriculture, biology and chemistry[21] to study the empirical relationships between one or more measured responses and a number of variables. In this part of the paper, we discuss the principles governing the construction and analysis of a central composite design in which responses (y) are the atomic ratio (Ca/P), % O_2, % O_2^{2-}, and % CO_3^{2-} of the obtained solid and the variables x_j are the pH, the Ca/Pratio of the reagents, $[Ca^{2+}]$, T and D, hereafter called respectively x_1, x_2, x_3, x_4, and x_5. This study was carried out using JMP software.[22]

Table I shows the central composite design presented according to the standard order; the values of the coded variables X_j are dimensionless. The values of the natural variables are summarized in Table II. The 24 experiments to be run are of orthogonal design (which means that the coefficients do not change when any model parameter changes). They are the following (Table I): (i) The first 16 experiments belonged to a 2^{5-1} factorial fractional design; the $\pm$ 1 coded values X_j were obtained by calculating:

$$x_j = (x_j - \bar{x}\ j)/\Delta x \quad (1)$$

The additional variable, X_5, is confounded (i.e., confused with the product $X_1X_2X_3X_4$).

TABLE I Matrix and Results of Experimental Design

Logical order	Coded units of variables					Responses			
	pH	Ca/P	$[Ca^{2+}]$	T	D	Ca/P	% O_2	% O_2^{2-}	% CO_3^{2-}
1	−1	−1	−1	−1	1	1.349	0.10	1.22	1.95
2	−1	−1	−1	1	−1	1.289	0.28	1.20	1.80
3	−1	−1	1	−1	−1	1.341	0.40	0.74	2.36
4	−1	−1	1	1	1	1.434	0.04	0.96	1.97
5	−1	1	−1	−1	−1	1.370	0.25	0.87	2.18
6	−1	1	−1	1	1	1.409	0.03	1.14	2.00
7	−1	1	1	−1	1	1.567	0.02	0.81	2.07
8	−1	1	1	1	−1	1.527	0.11	1.19	2.04
9	1	−1	−1	−1	−1	1.436	0.31	0.35	2.55
10	1	−1	−1	1	1	1.524	0.36	1.15	1.87
11	1	−1	1	−1	1	1.580	0.06	0.62	2.44
12	1	−1	1	1	−1	1.525	0.09	0.93	2.03
13	1	1	−1	−1	1	1.420	0.16	0.73	2.59
14	1	1	−1	1	−1	1.580	0.59	1.12	1.63
15	1	1	1	−1	−1	1.565	0.70	0.40	1.77
16	1	1	1	1	1	1.605	0.50	0.80	1.89
17	−1.3408	0	0	−1	−1	1.370	0.30	0.75	1.87
18	1.3408	0	0	−1	−1	1.577	0.42	0.28	1.96
19	0	−1.3408	0	−1	1	1.511	0.25	0.88	2.15
20	0	1.3408	0	−1	1	1.546	0.34	0.77	2.24
21	0	0	−1.3408	1	−1	1.470	0.48	1.12	1.91
22	0	0	1.3408	1	−1	1.528	0.23	1.00	2.13
23	0	0	0	1	1	1.540	0.27	0.84	1.75
24	0	0	0	1	1	1.560	0.27	0.83	1.74

(ii) The next 6 experiments were the points on the 6 axes, at a distance $\pm\alpha$ from the center.
(iii) In this center, the two last experiments were realized. The distance α was calculated to have vectors of square variables X_j^2 that are

TABLE II Experimental Field and Coded Variables

		Coded variables X_1, X_2, X_3, X_4, X_5				
Natural variables	Units	−1.3408	−1	0	1	1.3408
x_1 = pH	/	6	6.25	7	7.75	8
x_2 = Ca/P	/	1.33	1.373	1.5	1.627	1.67
$x_3 = [Ca^{2+}]$	$mol.l^{-1}$	0.516	0.533	0.582	0.631	0.648
x_4 = T	°C	—	40	—	80	—
x_5 = D	h	—	1	—	4	—

The coded variables $X_j = \pm 1$ are obtained using the equation: $X_j = (x_j - \bar{x}_j)/\Delta x$.

mutually orthogonal: in the present design α is equal to:

$$\alpha = \sqrt{\frac{\sqrt{24 \times 16} - 16}{24}} = 1.3408. \qquad (2)$$

However, the 24 values of X_j^2 presented a sum different from zero and the mean value was 0.8164; therefore the variable X_j^2 was replaced by the centered variable $U_j^2 = X_j^2 - 0.8$. Consequently, the estimated model was:

$$\hat{y} = b_0 + \sum_{j=1}^{5} b_j x_j + \sum_{j=1}^{5} \sum_{j'=1 j'=j}^{5} b_{jj'} x_j x_{j'} + \sum_{j=1}^{3} b_{jj} U_j^2 \qquad (3)$$

Let $b_u X_u$ be the general term of y; the 19 terms generally used for the construction of the model must be mutually orthogonal 2 by 2, and the normal equation gives the b_u coefficients with the least squares method:

$$b_u = \frac{Y_u}{\sum_{i=1}^{n} X_{iu}^2} \quad \text{where} \quad Y_u = \sum X_{iu} y_i' \qquad (4)$$

Where X_{iu} and y_i are the X_u and y values for the ith experiment; Y_u is named contrast.

Table I shows the experimental data for each response. The 19 terms are easily calculated by substituting the data values in the expressions for the least squares estimates of the coefficients (Tables III–VI). The models adapted to the responses are written:

-for Ca/P response:

$$\widehat{Ca/P} = 1.54 + 0.06 X_1 + \ldots\ldots\ldots\ldots - 0.01 X_5$$
$$-0.02 X_1^2 + \ldots\ldots\ldots\ldots - 0.03 X_3^2$$
$$-0.02 X_1 X_2 + \ldots\ldots\ldots\ldots - 0.01 X_4 X_5. \qquad (5)$$

-for % O_2 response:

$$\widehat{\%O_2} = 0.35 + 0.1 X_1 + \ldots\ldots\ldots\ldots\ldots - 0.1 X_5$$
$$-0.1 X_1^2 + \ldots\ldots\ldots\ldots\ldots - 0.03 X_3^2$$
$$+0.1 X_1 X_2 + \ldots\ldots\ldots\ldots\ldots + 0.07 X_4 X_5. \qquad (6)$$

TABLE III Comparison of Experimental and Estimated Results

	Ca/P			% O_2			% O_2^{2-}			% CO_3^{2-}		
Or.	Ca/P_{ex}	Ca/P_{es}	$e10^3$	$\%O_{ex2}$	$\%O_{2es}$	$E10^2$	$\%O_2^{2-}$ ex	$\%O_2^{2-}$ es	$e10^2$	$\%CO_3^{2-}$ ex	$\%CO_3^{2-}$ es	$e10^2$
1	1.349	1.347	2	0.10	0.10	0	1.22	1.21	1	1.95	1.94	1
2	1.289	1.292	−3	0.28	0.31	−3	1.20	1.20	0	1.80	1.80	0
3	1.341	1.336	5	0.40	0.41	−1	0.74	0.73	1	2.36	2.35	1
4	1.434	1.431	3	0.04	0.02	2	0.96	0.96	0	1.97	1.97	0
5	1.370	1.368	2	0.25	0.27	−2	0.87	0.86	1	2.18	2.18	0
6	1.409	1.408	1	0.03	0.02	1	1.14	1.14	0	2.00	2.01	−1
7	1.567	1.571	−4	0.02	0.05	−3	0.81	0.82	−1	2.07	2.08	−1
8	1.527	1.526	1	0.11	0.11	0	1.19	1.19	0	2.04	2.03	1
9	1.436	1.446	−10	0.31	0.32	−1	0.35	0.37	−2	2.55	2.55	0
10	1.524	1.517	7	0.36	0.34	2	1.15	1.14	1	1.87	1.86	1
11	1.580	1.583	−3	0.06	0.07	−1	0.62	0.62	0	2.44	2.43	1
12	1.525	1.531	−6	0.09	0.10	−1	0.93	0.94	1	2.03	2.03	0
13	0.420	1.428	−8	0.16	0.18	−2	0.73	0.75	−2	2.59	2.60	−1
14	1.580	1.585	−5	0.59	0.61	−2	1.12	1.13	−1	1.63	1.63	0
15	1.565	1.576	−11	0.70	0.72	−2	0.40	0.42	−2	1.77	1.78	−1
16	1.605	1.599	6	0.50	0.48	2	0.80	0.71	1	1.89	1.89	0
17	1.370	1.377	−7	0.30	0.27	3	0.75	0.77	−2	1.87	1.88	−1
18	1.577	1.556	21	0.42	0.39	3	0.28	0.24	4	1.96	1.95	1
19	1.511	1.510	1	0.25	0.24	1	0.88	0.89	−1	2.15	2.17	−2
20	1.546	1.534	12	0.34	0.29	5	0.77	0.74	3	2.24	2.22	2
21	1.470	1.462	8	0.48	0.43	5	1.12	1.11	1	1.91	1.91	0
22	1.528	1.523	5	0.23	0.22	1	1.00	0.99	−1	2.13	2.13	0
23	1.540	1.558	−18	0.27	0.31	−4	0.84	0.85	−1	1.75	1.75	0
24	1.560	1.558	2	0.27	0.31	−4	0.83	0.85	−2	1.74	1.75	−1

ex: Experimental; es: estimated; e: residue = $y_{exp} - y_{es}$.

TABLE IV Estimated Values of the Model Coefficients Associated with the Ca/P Ratio Response

Coefficient (b_u)	Estimated value	Degree of freedom (ν_u)	Sum of squares (SC_{bu})	Value of F_{exp}	Significance
B_0	1.5408165	—	—	—	—
b_1	0.0616454	1	0.07022922	222.4083	****
b_2	0.0362675	1	0.02430807	76.9810	***
b_3	0.047678	1	0.04200989	133.0408	****
b_4	0.0146956	1	0.00380094	12.0372	**
b_5	0.0140706	1	0.00348451	11.0351	**
b_{12}	−0.022188	1	0.00787656	24.9442	***
b_{13}	−0.008563	1	0.00117306	3.7150	NS
b_{14}	0.0102296	1	0.00193389	6.1244	NS
b_{15}	−0.015395	1	0.00438025	13.8718	**
b_{23}	0.0126875	1	0.00257556	8.1565	**
b_{24}	0.0073575	1	0.00100042	3.1682	NS
b_{25}	−0.020108	1	0.00747196	23.6629	***
b_{34}	−0.012072	1	0.00269326	8.5293	**
b_{35}	0.012822	1	0.00303830	9.6220	**
b_{45}	−0.011429	1	0.00229912	7.2811	**
b_{11}	−0.018858	1	0.00168593	5.3392	NS
b_{22}	−0.016633	1	0.00131157	4.1536	NS
b_{33}	−0.033738	1	0.00539600	17.0886	***

****: Very significant; ***: significant on a level of 0.1% ($F_{0.001}(1.5) = 47.18$); **: significant on a level of 1% ($F_{0.01}(1.5) = 16.26$); NS: nonsignificant

-for % O_2^{2-} response:

$$\hat{\%\, O_2^{2-}} = 0.73 - 0.12\,X_1 + \ldots\ldots\ldots\ldots\ldots + 0.04X_5 \\ +0.04\,X_2^1 + \ldots\ldots\ldots\ldots\ldots + 0.05\,X_3^2 \\ +0.01\,X_1X_2 + \ldots\ldots\ldots\ldots\ldots - 0.09\,X_4X_5. \quad (7)$$

-for % CO_3^{2-} response:

$$\hat{\%CO_3^{2-}} = 1,88 + 0.03\,X_1 + \ldots\ldots\ldots\ldots + 0.03\,X_5 \\ -0.06\,X_1^2 + \ldots\ldots\ldots\ldots + 0.18\,X_3^2 \\ 0.08X_1X_2 + \ldots\ldots\ldots\ldots - 0.09\,X_4X_5. \quad (8)$$

From these equations, it is possible to compute the estimated values (y) and the corresponding residuals $e_i = y_i - y_i$ (Table II). Estimates of the experimental error variance (s_r^2) are obtained by dividing the residual sum of squares $\sum_i e_i^2$ by ν (number of degrees of freedom = number of experiments minus number in the model, i.e., 24–19 = 5)

TABLE V Estimated Values of the Model Coefficients Associated with % O_2 Response

Coefficient (b_u)	Estimated Value	Degree of freedom (ν_u)	Sum of squares (SC_{bu})	Value of F_{exp}	Significance
b_0	0.3475436	—	—	—	—
b_1	0.0965519	1	0.17228136	60.0720	* * *
b_2	0.048666	1	0.04376917	15.2617	**
b_3	−0.01295	1	0.00309927	1.0807	NS
b_4	−0.00815	1	0.00116903	0.4076	NS
b_5	−0.0994	1	0.17389553	60.6349	* * *
b_{12}	0.09625	1	0.14822500	51.6839	* * *
b_{13}	0.00125	1	0.00002500	0.0087	NS
b_{14}	0.0384481	1	0.02731904	9.5258	* * *
b_{15}	0.0146981	1	0.00399243	1.3921	NS
b_{23}	0.0475	1	0.03610000	12.5875	* * *
b_{24}	−0.05795	1	0.00144221	0.5029	NS
b_{25}	0.008834	1	0.00942577	3.2866	NS
b_{34}	−0.05795	1	0.06206174	21.6400	* * *
b_{35}	0.0092	1	0.00156422	0.5454	NS
b_{45}	0.0656001	1	0.07573985	26.4094	* * *
b_{11}	−0.105855	1	0.05311868	18.5217	* * *
b_{22}	0.0415524	1	0.00818504	2.8540	NS
b_{33}	−0.026589	1	0.00335133	1.1686	NS

***: Significant on a level of 0.1% ($F_{0.001}(1.5) = 47.18$); **: significant on a level of 1% ($F_{0.01}(1.5) = 16.26$) NS: nonsignificant.

(Table VIII):

$$s_{r^2 Ca/P} = 0.158 \times 10^{-2}/5 = 0.316 \times 10^{-3}; \quad (9)$$

$$s_{r^2_{\%} O2} = 1.434 \times 10^{-2}/5 = 2.868 \times 10^{-3}; \quad (10)$$

$$s_{r^2 \% O22\_} = 0.639 \times 10^{-2}/5 = 1.278 \times 10^{-3}; \text{ and} \quad (11)$$

$$s_{r^2 \% CO32\_} = 0.217 \times 10^{-2}/5 = 0.435 \times 10^{-3}. \quad (12)$$

Estimated variances of coefficients s^2_{bi} given in Table VIII were therefore calculated by the following formula:

$$s^2_{bu} = S^2_r / \sum_i X^2_{iu}. \quad (13)$$

The significance of effects may be estimated by comparing the values of the ratio b^2_i/s^2_{bi} to a critical value ($F_{0.001}(18.5) = 25.65$) or ($F_{0.01}(18.5) = 9.635$) of the F distribution [23] at respectively a 99.9% or 99% level of confidence with 18 and 5 degrees of freedom.

For the Ca/P response, it appears that only the main effects: pH, Ca/Pratio of the reagents, [Ca^{2+}], T and D and the interactions

TABLE VI Estimated Value of the Model Coefficients Associated with the % O_2^{2-} Response

Coefficient (b_u)	Estimated Value	Degree of freedom (ν_u)	Sum of squares (SC_{bu})	Value of F_{exp}	Significance
b_0	0.7326789	—	—	—	—
b_1	−0.121458	1	0.27262699	213.2455	****
b_2	−0.003405	1	0.00021421	0.1676	NS
b_3	−0.083343	1	0.12836749	100.4075	***
b_4	0.1690512	1	0.50298226	393.4266	****
b_5	0.0365512	1	0.02351363	18.3921	***
b_{12}	0.006875	1	0.00075625	0.5915	NS
b_{13}	0.008125	1	0.00105625	0.8262	NS
b_{14}	0.060208	1	0.06699236	52.4006	***
b_{15}	0.017708	1	0.00579505	4.5328	NS
b_{23}	0.000625	1	0.00000625	0.0049	NS
b_{24}	0.0046546	1	0.00040038	0.3132	NS
b_{25}	−0.048405	1	0.04330013	33.8688	***
b_{34}	−0.008343	1	0.00128637	1.0062	NS
b_{35}	−0.047907	1	0.04241442	33.1760	***
b_{45}	−0.090949	1	0.14558275	113.8731	***
b_{11}	0.038167	1	0.00690566	5.4015	NS
b_{22}	0.0687609	1	0.02241360	17.5316	***
b_{33}	0.0520733	1	0.01285462	10.0547	**

****: Very significant; ***: significant on a level of 0.1% ($F_{0.001}(1.5) = 47.18$); **: significant on a level of 1% ($F_{0.01}(1.5) = 16.26$); NS: nonsignificant.

Ca/P-pH, $[Ca^{2+}]$-Ca/P, $[Ca^{2+}]$-$[Ca^{2+}]$, T-$[Ca^{2+}]$, D-pH, D-Ca/P, D-$[Ca^{2+}]$ and D-T are significant (Table IV).

Best fitting Ca/P is then conveniently written as follows:

$$\begin{aligned}Ca/P = 1.54 &+ 0.06\,X_1 + 0.04\,X_2 + 0.05\,X_3 + 0.01\,X_4 + 0.01\,X_5\\ &- 0.03\,X_3^2\\ &- 0.02\,X_1X_2 - 0.02\,X_1\,X_5 + 0.01\,X_2X_3\\ &- 0.02\,X_2X_5 - 0.01\,X_3\,X_4 + 0.01\,X_3X_5 - 0.01\,X_4X_5. \quad (14)\end{aligned}$$

For the % O_2 response, it appears that only the main effects: pH, Ca/P ratio of the reagents and D and the interactions pH-pH, Ca/P-pH, $[Ca^{2+}]$-Ca/P, T-pH, T-$[Ca^{2+}]$ and D-T are significant (Table V).

Best fitting % O_2 is then conveniently written as follows:

$$\begin{aligned}\widehat{\%O_2^-} = 0.35 &+ 0.1\,X_1 + 0.05\,X_2 - 0.1\,X_5\\ &- 0.1\,X_1^2\end{aligned}$$

$$+ 0.1\,X_1X_2 + 0.04\,X_1X_4 + 0.05\,X_2X_3 - 0.06\,X_3X_4 + 0.0\,7X_4X_5. \quad (15)$$

For the % O_2^{2-} response, it appears that only the main effects: pH, $[Ca^{2+}]$, T and D and the interactions Ca/P-Ca/P, $[Ca^{2+}]$-$[Ca^{2+}]$, T-pH, D-Ca/P, D-$[Ca^{2+}]$ and D-T are significant (Table VI).

Best fitting % O_2^{2-} is then conveniently written as follows:

$$\widehat{\%O_2^{2-}} = 0.73 - 0.12\,X_1 - 0.08\,X_2 + 0.17\,X_4 + 0.04\,X_5 + 0.07\,X_2^2 + 0.05\,X_3^2 + 0.06\,X_1\,X_4 - 0.05\,X_2\,X_5 - 0.05\,X_3\,X_5 - 0.09X_4\,X_5. \quad (16)$$

For the % CO_3^{2-} response, it appears that only the main effects: pH, Ca/Pratio of the reagents, T et D and the interactions pH-pH, pH-Ca/P, pH-$[Ca^{2+}]$, pH-T, pH-D, Ca/P-Ca/P, Ca/P-$[Ca^{2+}]$, Ca/P-T, Ca/P-D, $[Ca^{2+}]$-$[Ca^{2+}]$ and $[Ca^{2+}]$-T are significant (Table VII).

Best fitting % CO_3^{2-} is then conveniently written as follows:

$$\widehat{\%CO_3^{2-}} = 1,88 - 0.03\,X_1 - 0.05\,X_2 - 0.17\,X_4 + 0.03\,X_5 - 0.06\,X_1^2 + 0.07\,X_2^2 + 0.18\,X_3^2 - 0.08\,X_1\,X_2 - 0.06\,X_1\,X_3 - 0.08\,X_1\,X_4 + 0.07\,X_1\,X_5 - 0.08\,X_2\,X_3 + 0.03\,X_2\,X_4 + 0.09\,X_2\,X_5 + 0.08\,X_3\,X_4. \quad (17)$$

RESULTS AND DISCUSSION

The geometrical representation of the Ca/P response (Figure 1) or the % O_2 response (Figure 2) in the diagram of pH and concentration of calcium ions ($[Ca^{2+}]$), for values of Ca/P = 1.647 (X_2 = 1.1592), T = 40°C (X_4 = –1) and D = 1 h (X_5 = –1), shows that when $[Ca^{2+}]$ and the pH increase simultaneously or when $[Ca^{2+}]$ increases and the pH remains unchanged or conversely, the Ca/P ratio or % O_2 of the precipitate increases until obtaining an optimum, which remains unchanged in the experimental field and equalizes 1.575 for the Ca/P response and 0.77% for the % O_2 response.

The geometrical representation of the% O_2^{2-}response (Figure 3) in the same diagram shows that when the $[Ca^{2+}]$ and the pH increase simultaneously or when the pH increases and the $[Ca^{2+}]$ remains unchanged, the % O_2^{2-} of the precipitate decreases.

TABLE VII Estimated Values of the Model Coefficients Associated with % CO_2^{3-} Response

Coefficient (b_u)	Estimated Value	Degree of freedom (ν_u)	Sum of squares (SC_{bu})	Value of F_{exp}	Significance
b_0	1.884639	—	—	—	—
b_1	0.0263171	1	0.01279943	29.4329	**
b_2	−0.045998	1	0.03910224	89.9174	**
b_3	−0.000229	1	0.00000097	0.0022	NS
b_4	−0.167621	1	0.49450560	1137.138	****
b_5	0.0261293	1	0.01201636	27.6321	**
b_{12}	−0.07625	1	0.09302500	213.9151	****
b_{13}	−0.06375	1	0.06502500	149.5279	****
b_{14}	−0.075067	1	0.10413940	239.4732	****
b_{15}	0.0736829	1	0.10033452	230.7237	****
b_{23}	−0.07875	1	0.09922500	228.1723	****
b_{24}	0.0322484	1	0.01921908	44.1951	**
b_{25}	0.0940016	1	0.16330027	375.5163	****
b_{34}	0.0785205	1	0.11394178	262.0142	****
b_{35}	−0.004771	1	0.00042058	0.9672	NS
b_{45}	−0.090949	1	0.00009964	0.2291	NS
b_{11}	−0.063384	1	0.01904521	43.7953	**
b_{22}	0.0659449	1	0.02061533	47.4059	***
b_{33}	0.1841485	1	0.16075516	369.6637	****

****: Very significant; ***: significant on a level of 0.1% ($F_{0.001}(1.5) = 47.18$); **: significant on a level of 1% ($F_{0.01}(1.5) = 16.26$); NS: nonsignificant.

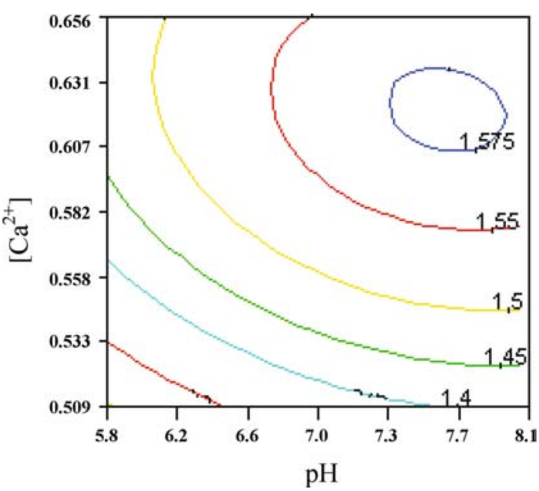

FIGURE 1 Response function contour lines of Ca/P with Ca/P = 1.647, T = 40°C, and D = 1 h.

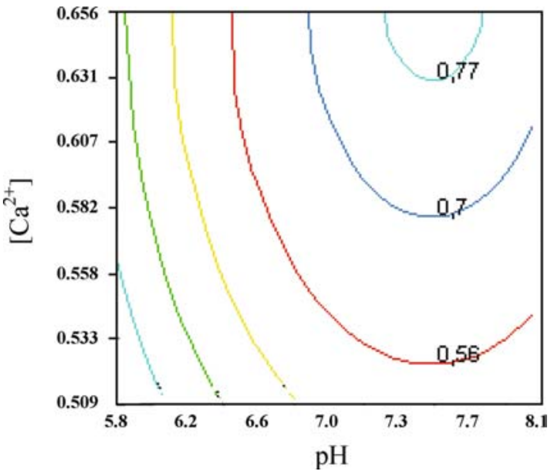

FIGURE 2 Response function contour lines of % O_2 with Ca/P = 1.647, T = 40°C, and D = 1 h.

From the first two representations (Figures 1 and 2), we deduce the limits of the values of the operational parameters allowing the synthesis of an apatite with a Ca/P ratio equal to 1,575 and having the maximum of oxygen. The obtained results appear in Table IX.

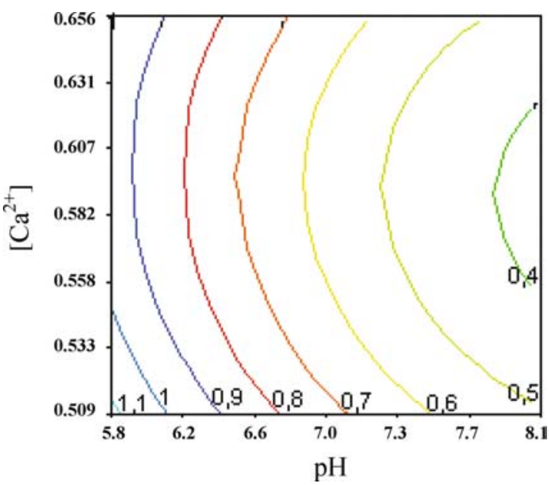

FIGURE 3 Response function contour lines of % O_2^{2-} with Ca/P= 1.647, T = 40°C, and D = 1 h.

TABLE VIII Table of Analysis Regression for Ca/P ratio, % O_2, % O_2^{2-}, and % CO_3^{2-} Responses

Response	Source of variation	Sum of squares	Degree of freedom	Mean square	Ratio	Significance
Ca/P	Regression	0.19155412	18	0.010642	33.7017	* * *
	Residue	0.00157884	5	0.000316	—	—
	Total	0.19313296	23	—	—	—
% O_2	Regression	0.74319376	18	0.041289	14.3967	**
	Residue	0.01433957	5	0.002868	—	—
	Total	0.75753333	23	—	—	—
% O_2^{2-}	Regression	1.6048577	18	0.089159	69.7389	* * *
	Residue	0.0063923	5	0.001278	—	—
	Total	1.6112500	23	—	—	—
% CO_3^{2-}	Regression	1.4943215	18	0.083018	190.9033	* * **
	Residue	0.0021743	5	0.000435	—	—
	Total	1.4964958	23	—	—	—

F_{exp}: estimated value of Snedecor, ****: very significant, ***: significant on a level of 99.9%, **: significant on a level of 99%.

These results show that for the factors pH and [Ca^{2+}] a range of the experimental values can be defined. However, we can't give to these factors an unspecified value even if they are inside the definite range. In order to limit the intersection field of the factors pH and [Ca^{2+}] corresponding to the optima of the two responses Ca/P and % O_2, we superimposed the two representations of the contour lines of these responses. The obtained results are represented in Table X.

However, according to Figure 3, we may find it better to work at low pH in order to obtain a high percentage of O_2^{2-}ions. Consequently, the pH will be fixed at value 7.38 ($X_1 = 0.5$). It thus remains to determine the optimal concentration of calcium ions. Indeed, while varying the concentration of the calcium solution from 0.620 M ($X_3 = 0.77$) to 0.636 M ($X_3 = 1{,}1$), this results in several experimental conditions in

TABLE IX Optimal Fields of the Apatite Synthesis Parameters

Variables	Experimental field (Ca/P)	Experimental field (% O_2)
pH	[7.38 ; 7.99]	[7.20 ; 7.89]
Ca/P	1.647	1.647
[Ca^{2+}] (M)	[0.606 ; 0.636]	[0.620 ; 0.656]*
T (°C)	40	40
D (h)	1	1

*: Value outside of the experimental field (Table II).

TABLE X Optimal Field of the Apatite Synthesis Parameters

Variables	Experimental field
pH	[7.38 ; 7.89]
Ca/P	1.647
$[Ca^{2+}]$ (M)	[0.620 ; 0.636]
T (°C)	40
D (h)	1

TABLE XI Optimum Conditions for the Synthesis of Apatite

Variables	Optimum condition
pH	7.38
Ca/P	1.647
$[Ca^{2+}]$ (M)	0.636
T (°C)	40
D (h)	1

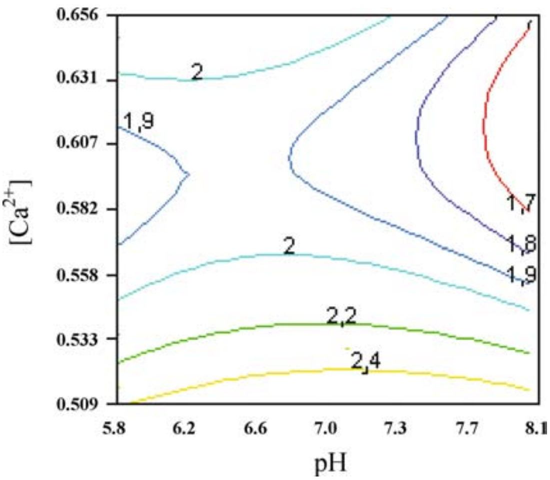

FIGURE 4 Response function contour lines of % CO_3^{2-} with Ca/P = 1.647, T = 40°C, and D = 1 h.

TABLE XII Estimated Responses Under the Optimum Conditions for the Synthesis of Apatite

Estimated responses	Results
Ca/P	1,575
% O_2	0,77
% O_2^{2-}	0,52
% CO_3^{2-}	1,87

the synthesis of apatite. We noted that the % O_2^{2-} (Figure 3) and the % CO_3^{2-} (Figure 4) increase with increasing the concentration of calcium ions. Therefore, the concentration of calcium ions will be fixed at its maximum value. From there the optimum conditions for the synthesis of the apatite with the characteristics mentioned above are gathered in Table XI.

Under these conditions, values of the Ca/P, % O_2, % O_2^{2-} and % CO_3^{2-} estimated responses appear in Table XII. These values correspond in the studied experimental field to a more oxygenated apatite partially carbonated in site B with Ca/P speed of dissolution adaptable to that of the osseous neoformation and allowing a progressive diffusion of oxygenated species.[16]

The experimental checking under the optimal conditions pH = 7.38 (X_1 = 0.5), Ca/P = 1.647 (X_2 = 1.15), [Ca^{2+}] = 0.636 M (X_3 = 1.1), T = 40°C (X_4 = -1), and D = 1 h (X_5 = -1) of synthesis shows that the obtained product is a phosphocalcic oxygenated apatite of atomic ratio Ca/P, % O_2, % O_2^{2-}, and % CO_3^{2-} equal respectively to 1.578 ≈ 1.575, 0.77, 0.52, and 1.83 and the chemical formula is:

$$Ca_{8,05}(PO_4)_{4,7}(HPO_4)_{0,4}(CO_3)_{0,9}(OH)_{0,88}\left(O_2^{2-}\right)_{0,16}(O_2)_{0,23} \qquad (18)$$

CONCLUSION

The precipitation of a phosphocalcic oxygenated apatite, partially carbonated in site B, was studied using a fractional central composite design. Response equations for the atomic ratio Ca/P, % O_2, % O_2^{2-}, and % CO_3^{2-} of the obtained precipitate were established. From these equations, it is possible to predict the optimal conditions to obtain a phosphocalcic oxygenated carbonated apatite with Ca/P = 1.578, % O_2 = 0.77, % O_2^{2-} = 0.52, and % CO_3^{2-} = 1.83 and the chemical formula is:

$$Ca_{8,05}(PO_4)_{4,7}(HPO_4)_{0,4}(CO_3)_{0,9}(OH)_{0,88}\left(O_2^{2-}\right)_{0,16}(O_2)_{0,23} \qquad (19)$$

Nomenclature

b_i = Coefficient of the polynomial model
e_i = Residual of the ith experiment: $e_i = y_i – y_i$
k = Either pH of reaction medium, atomic ratio Ca/Pof the reagents, concentration of the calcium solution ($[Ca^{2+}]$), temperature of reaction medium (T) and duration of the reaction (D)
s^2_{bi} = Estimated variance of coefficient b_i
s^2_r = Residual variance: $s^2_r = \sum_i e^2_i$
U^2_k = Transformed variable U^2 for element k
x_j = Natural variable x for element k, and $\bar{x}_j$ its mean value, i.e., either pH, Ca/Pof the reagents, $[Ca^{2+}]$, T, and D
X_j = Coded variable X for element k
y_i = Response for the ith experiment
$\check{y}_i$ = Estimated response for the ith experiment
α = Distance from the center of the design
Δx = Difference of variable of x from $\bar{x}$
ν = Number of degrees of freedom = number of experiments minus the number of coefficients in the model

REFERENCES

[1] H. Chaair, I. Mansouri, and S. Nadir, *Phosphorus, Sulfur, and Silicon*, **170,** 247 (2001).
[2] O. Britel, M. Hamad, H. Chaair, S. Belouafa, K. Digua, and B. Sallek, *Phosphorus, Sulfur and Silicon*, **179,** 1857 (2004).
[3] A. N. Cranin, G. P. Tobin, and J. Gelbman, *Compend. Contin. Educ. Dent.*, **8,** 254 (1987).
[4] A. Ogilvie, R. M. Frank, E. P. Benque, M. Gineste, M. Heughebaert, and J. Hemmerle. *J. Periodont. Res.*, **22,** 270 (1987).
[5] H. W. Denissen and K. de Groot, *J. Prosthet. Dent.*, **42,** 551 (1979).
[6] P. Scheer and P. J. Boyne, *J. Am. Dent. Assoc.*, **114,** 594 (1987).
[7] S. S. Rothstein, D. A. Paris, and M. P. Zacek, *J. Oral Maxillofac. Surg.*, **42,** 224 (1984).
[8] J. N. Kent, J. H. Quinn, M. F. Zide, I. M. Finger, M. Jarcho, and S. S. Rothstein, *J Oral Maxillofac. Surg.*, **44,** 597 (1986).
[9] A. N. Cranin, G. P. Tobin, and J. Gelbman, *Compend. Contin. Educ. Dent.*, **8,** 334 (1987).
[10] H. D. Larsen, I. M. Finger, L. R. Guerra, and J. N. Kent, *J. Prosthet. Dent.*, **49,** 461 (1987).
[11] C. Ledard, E. Benque, J. L. Lacout, and C. Rey, U.S. Patent Nr. 5 141 561 (1992).
[12] D. R. Simpson, *Amer. Miner.*, **53,** 432 (1968).
[13] C. Rey, Thèse, Toulouse (1984).
[14] S. Belouafa, H. Chaair, K. Digua, B. Sallek, and H. Mountacer, *Phosphorus, Sulfur, and Silicon*, **180,** 2679 (2005).
[15] S. Belouafa, H. Chaair, K. Digua, H. Oudadesse, B. Sallek, and H. Mountacer, *Phosphorus, Sulfur, and Silicon*, **181,** 337 (2006).
[16] D. D. Lee, C. Rey, and M. Aiolova, U.S. Patent Nr. 5 683 461 (1997).

[17] G. Sado et M. C. Sado, Les plans d'expériences: De l'expérimentation à l'assurance qualité. AFNOR, Paris (1991).

[18] J. L. Meyer and E. D. Eanes, *Calcified Tissue Res.*, **23,** 259 (1977).

[19] A. Gee and V. R. Dietz, *Anal. Chem.*, **25,** 1320 (1954).

[20] J. C. Trombe and G. Montel, *J. Inorg. Nucl. Chem.*, **40,** 15 (1978).

[21] G. E. P. Box, W. G. Hunter, and J. S. Hunter, *Statistics for Experimenters: An Introduction to Design, Data Analysis and Model Building* (Wiley. NewYork, 1978).

[22] SAS Institute, *JMP Statistic and Graphics Guide and User's Guide* (SAS, 1995), Version 3.1.

[23] G. E. P. Box and N. R. Draper, *Empirical Model-Building and Response Surfaces* (Wiley, NewYork, 1987).