Research Article

Application of Statistical Experimental Design and Surface Plot Technique to Optimize Oxygenated Apatite Synthesis

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This work deals with oxygenated apatite synthesis from calcium chloride and phosphoric acid using a wet precipitation method. An experimental design is applied to precise the influence of the synthesis 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)) on the chemical composition (Ca/P molar ratio, % O_2 , and % O_2^{2-}). An empirical model was developed and validated by applying the ANOVA analysis incorporating the interaction effects of all parameters and optimized using the response surface methodology. A reproducible synthesis of more oxygenated apatite with speed of dissolution adaptable to that of the osseous neoformation and allowing a progressive diffusion of oxygenated species (Ca/P = 1.575) is attained.

1. Introduction

Oxygenated apatites based on calcium phosphate have a wide range of potential applications for bone and dental substitutes because of their antibacterial properties, which make them able to limit the proliferation of microorganisms at the site of implantation [1–3]. These properties are due to the oxygenated species (molecular oxygen: O_2 , and peroxide ions: O_2^{2-}) contained in the channels of the apatite structure [1–3]. These species are liberated in the living environment either by progressive dissolution of the material or by chemical exchange with the living environment [1].

In the last years, various batch precipitation methods oxygenated apatites have been developed [1–3]. The aim of this work is to obtain a more oxygenated apatite with the speed of dissolution adaptable to that of the osseous neoformation and allowing a progressive diffusion of oxygenated species (Ca/P = 1.575) [4]. The difficulty is the factors governing the precipitation that are usually not precisely controlled [5, 6] (e.g., 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)).

On this basis, our work is concerned to modelize and optimize this synthesis process by applying the experimental design and response surface methodologies. These methodologies are essential in engineering to improve the performance of a manufacturing process. They are widely employed in the development of new processes. The application of experimental design and response surface techniques during the initial phase of process development can lead to improved process performance, less variability, shorter development time, and thus, reduced overall cost [7].

2. Materials and Methods

2.1. Powder Synthesis. The preparation of phosphocalcic oxygenated apatite was performed by the precipitation reaction with calcium and phosphate solutions. The calcium solution was prepared by adding calcium chloride (CaCl_2) 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, dried in a desiccator and analysed.

2.2. Powder Characterization. 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 pelletised under vacuum. The pellets were analysed using a PerkinElmer 1600 FTIR spectrophotometer. Calcium, phosphorus, and the content of the oxygenated species were determined by wet chemical methods. Calcium was titrated by complexometry methods [8], the phosphorus content was analysed by colorimetry [9], molecular oxygen was determined by measuring the volume displaced during the acid dissolution of the powder [1], and the peroxide ions were titrated by manganometry methods [10].

2.3. Statistical Experiment Design. A central composite design, with two replicates at the center point, six experiments on the six axes, and sixteen experiments belonging to a 2^{5-1} factorial fractional design, was employed to evaluate the influence of the synthesis 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)) on the chemical composition (Ca/P molar ratio, % O_2 , and % O_2^{2-}).

The statistical data treatment was accomplished through the JMP statistical software [11] in experimental design modules. This program uses the one-way ANOVA table to analyze statistical data. ANOVA involves calculations of the some square (SS), degree of freedom (DF), and media square (MS) for the main effects, interactions, and residual part. Test F (media square of effects/media square of residual) and significance factor (P) are used to evaluate the significant effects. If the F value for a given effect is higher than the F value in the table, this effect is a significant one. Usually, the F table used for significance level equals 0.05. When the significance factor (P) is used to evaluate the effects significance, this value must be lower than the significance level, so lower than 0.05 [12, 13].

The independent variables interval used in this work is shown in Table 1. They were selected based on a previous study of this synthesis process.

3. Results and Discussion

The influence of the synthesis variables, on the composition of the powders, evaluated through the experiment design can be seen in Table 2. The results in the form of analysis of variance (ANOVA) are given in Table 3. The significance factor (P) values for all dependent variables were expressed as function of independent ones (Tables 4–6). The dependent variable has a significant influence when the significance factor (P) is lower than 0.05. Thus, the regression model

TABLE 1: Experimental range and level of independent variables.

Variables	Units	Range and levels				
		−1.3408	−1	0	1	1.3408
$x_1 = \text{pH}$	—	6	6.25	7	7.75	8
$x_2 = \text{Ca/P}$	—	1.33	1.373	1.5	1.627	1.67
$x_3 = [\text{Ca}^{2+}]$	mol.l ^{−1}	0.516	0.533	0.582	0.631	0.648
$x_4 = \text{T}$	°C	—	40	—	80	—
$x_5 = \text{D}$	h	—	1	—	4	—

The variables were coded according to the following equation: $X_j = (x_j - \bar{x}_j)/\Delta x$; T: temperature; D: duration of the reaction.

TABLE 2: Experimental design with results.

Logical order	Coded units of variables					Responses		
	pH	Ca/P	[Ca ²⁺]	T	D	Ca/P	% O_2	% O_2^{2-}
1	−1	−1	−1	−1	1	1.359	1.18	1.52
2	−1	−1	−1	1	−1	1.300	1.13	2.94
3	−1	−1	1	−1	−1	1.355	1.15	1.15
4	−1	−1	1	1	1	1.452	1.18	2.89
5	−1	1	−1	−1	−1	1.392	0.96	1.34
6	−1	1	−1	1	1	1.430	1.18	2.46
7	−1	1	1	−1	1	1.579	1.18	1.22
8	−1	1	1	1	−1	1.543	1.12	1.23
9	1	−1	−1	−1	−1	1.450	1.18	1.42
10	1	−1	−1	1	1	1.541	1.15	1.45
11	1	−1	1	−1	1	1.586	1.21	0.76
12	1	−1	1	1	−1	1.540	1.13	0.96
13	1	1	−1	−1	1	1.433	1.15	1.38
14	1	1	−1	1	−1	1.595	1.24	2.28
15	1	1	1	−1	−1	1.580	1.37	0.51
16	1	1	1	1	1	1.624	1.18	0.45
17	−1.3408	0	0	−1	−1	1.440	1.18	0.84
18	1.3408	0	0	−1	−1	1.610	1.40	1.23
19	0	−1.3408	0	−1	1	1.551	1.16	0.72
20	0	1.3408	0	−1	1	1.584	1.15	0.99
21	0	0	−1.3408	1	−1	1.487	1.21	3.01
22	0	0	1.3408	1	−1	1.541	1.12	1.48
23	0	0	0	1	1	1.600	1.21	1.64
24	0	0	0	1	1	1.590	1.21	1.63

$\Delta\text{Ca} = 0.005$, $\Delta\text{P} = 0.005$, $\Delta(\% \text{O}_2) = 0.02$, $\Delta(\% \text{O}_2^{2-}) = 0.01$.

equations relating responses and synthesis parameters were developed and given in the following equations:

$$\begin{aligned}
 \widehat{\text{Ca/P}} &= 1.59 + 0.06X_1 + 0.04X_2 + 0.05X_3 + 0.01X_4 \\
 &\quad + 0.01X_5 - 0.02X_2^2 - 0.06X_3^2 - 0.02X_1X_2 \\
 &\quad + 0.01X_1X_4 - 0.01X_1X_5 - 0.02X_2X_5 + 0.01X_3X_5, \\
 \widehat{\% \text{O}_2} &= 1.23 + 0.03X_1 + 0.02X_3 - 0.05X_2^2 + 0.03X_1X_2 \\
 &\quad - 0.02X_1X_4 - 0.04X_1X_5 - 0.04X_3X_5, \\
 \widehat{\% \text{O}_2^{2-}} &= 0.73 - 0.12X_1 - 0.08X_2 + 0.17X_4 + 0.04X_5 \\
 &\quad + 0.04X_1^2 + 0.05X_3^2 + 0.06X_1X_4 + 0.02X_1X_5 \\
 &\quad - 0.05X_2X_5 - 0.05X_3X_5 - 0.09X_4X_5.
 \end{aligned} \tag{1}$$

The proposed model equations were validated. The results revealed that the predicted as well as the experimental values corresponded well with each other (Table 7).

TABLE 3: ANOVA for full quadratic models.

Response	Source	SS	DF	MS	F value	Significance
Ca/P	Regression	0.19852890	18	0.011029	27.3600	***
	Residue	0.00201560	5	0.000403	—	—
	Total	0.20054450	23	—	—	—
% O ₂	Regression	0.15219041	18	0.008455	10.2974	**
	Residue	0.00410543	5	0.000821	—	—
	Total	0.15629583	23	—	—	—
% O ₂ ²⁻	Regression	12.365827	18	0.686990	34.9235	***
	Residue	0.098356	5	0.019671	—	—
	Total	12.464183	23	—	—	—

***Significant on a level of 99.9%; **significant on a level of 99%.

TABLE 4: Model coefficient estimated by multiple linear of Ca/P ratio response.

Factor	Estimated coefficient	DF	SS	F _{exp} value	P value
Intercept	1.5920365	—	—	—	<0.0001
X ₁	0.0593194	1	0.06502936	161.3150	<0.0001
X ₂	0.0376153	1	0.02614846	64.8651	0.0005
X ₃	0.0468771	1	0.04061039	100.7401	0.0002
X ₄	0.0149504	1	0.00393388	9.7586	0.0261
X ₅	0.0123254	1	0.00267373	6.6326	0.0497
X ₁ X ₂	-0.022688	1	0.00823556	20.4295	0.0063
X ₁ X ₃	-0.008563	1	0.00117306	2.9100	0.1487
X ₁ X ₄	0.0125556	1	0.00291335	7.2270	0.0434
X ₁ X ₅	-0.013819	1	0.00352934	8.7550	0.0316
X ₂ X ₃	0.0120625	1	0.00232806	5.7751	0.0614
X ₂ X ₄	0.0072597	1	0.00097398	2.4161	0.1808
X ₂ X ₅	-0.02051	1	0.00777381	19.2841	0.0071
X ₃ X ₄	-0.011373	1	0.00239035	5.9296	0.0590
X ₃ X ₅	0.0128729	1	0.00306247	7.5969	0.0400
X ₄ X ₅	-0.010175	1	0.00182201	4.5198	0.0868
X ₁ X ₁	-0.022998	1	0.00250741	6.2200	0.0549
X ₂ X ₂	-0.024389	1	0.00281981	6.9949	0.0457
X ₃ X ₃	-0.057069	1	0.01543930	38.2995	0.0016

TABLE 5: Model coefficient estimated by multiple linear of % O₂ response.

Factor	Estimated coefficient	DF	SS	F _{exp} value	P value
Intercept	1.2253689	-	-	-	<0.0001
X ₁	0.0318049	1	0.01869415	22.7676	0.0050
X ₂	0.0059718	1	0.00065905	0.8027	0.4114
X ₃	0.0174539	1	0.00562993	6.8567	0.0472
X ₄	-0.007055	1	0.00087599	1.0669	0.3490
X ₅	0.0054451	1	0.00052182	0.6355	0.4615
X ₁ X ₂	0.029375	1	0.01380625	16.8146	0.0093
X ₁ X ₃	-0.000625	1	0.00000625	0.0076	0.9339
X ₁ X ₄	-0.020555	1	0.00780817	9.5096	0.0274
X ₁ X ₅	-0.035555	1	0.02336234	28.4530	0.0031
X ₂ X ₃	0.018125	1	0.00525625	6.4016	0.0525
X ₂ X ₄	0.0102782	1	0.00195233	2.3777	0.1837
X ₂ X ₅	-0.006528	1	0.00078761	0.9592	0.3724
X ₃ X ₄	-0.037546	1	0.02605225	31.7290	0.0024
X ₃ X ₅	-0.006204	1	0.00071130	0.8663	0.3947
X ₄ X ₅	-0.002055	1	0.00007432	0.0905	0.7756
X ₁ X ₁	0.0307836	1	0.00449227	5.4711	0.0665
X ₂ X ₂	-0.052654	1	0.01314306	16.0069	0.0103
X ₃ X ₃	-0.033185	1	0.00522065	6.3582	0.0531

TABLE 6: Model coefficient estimated by multiple linear of % O₂²⁻ response.

Factor	Estimated coefficient	DF	SS	F _{exp} value	P value
Intercept	1.3719791	—	—	—	<0.0001
X ₁	-0.328742	1	1.9972229	101.5298	0.0002
X ₂	-0.118524	1	0.2596133	13.1976	0.0150
X ₃	-0.345284	1	2.2032808	112.0049	0.0001
X ₄	0.3279447	1	1.8928547	96.2242	0.0002
X ₅	0.0116947	1	0.0024071	0.1224	0.7407
X ₁ X ₂	0.1425	1	0.3249000	16.5165	0.0097
X ₁ X ₃	-0.13	1	0.2704000	13.7459	0.0139
X ₁ X ₄	-0.218758	1	0.8843915	44.9585	0.0011
X ₁ X ₅	-0.177508	1	0.5823078	29.6019	0.0028
X ₂ X ₃	-0.155	1	0.3844000	19.5412	0.0069
X ₂ X ₄	-0.092784	1	0.2194726	11.1570	0.0205
X ₂ X ₅	0.0202263	1	0.0075605	0.3843	0.5624
X ₃ X ₄	-0.092784	1	0.1590980	8.0878	0.0361
X ₃ X ₅	0.1590342	1	0.4674095	23.7610	0.0046
X ₄ X ₅	-0.045805	1	0.0369273	1.8772	0.2290
X ₁ X ₁	0.0127027	1	0.0007649	0.0389	0.8514
X ₂ X ₂	-0.151392	1	0.1086509	5.5233	0.0655
X ₃ X ₃	0.2699695	1	0.3455077	17.5641	0.0086

TABLE 7: Validation of the experimental design.

Order	Ca/ P_{ex}	Ca/ P_{es}	e 10^3	% O_{2ex}	% O_{2es}	e 10^2	% O_{2ex}^{2-}	% O_{2es}^{2-}	e 10^{-2}
1	1.351	1.356	-5	1.10	1.10	0	1.42	1.36	6
2	1.293	1.300	-7	1.05	1.07	-2	2.74	2.74	0
3	1.347	1.353	-6	1.07	1.07	0	1.07	1.02	5
4	1.444	1.437	7	1.10	1.09	1	2.69	2.68	1
5	1.384	1.391	-7	0.89	0.91	-2	1.25	1.22	3
6	1.422	1.416	6	1.10	1.10	0	2.29	2.31	-2
7	1.570	1.576	-6	1.10	1.11	-1	1.14	1.19	-5
8	1.534	1.538	-4	1.04	1.03	1	1.15	1.14	1
9	1.442	1.446	-4	1.10	1.10	0	1.32	1.36	-4
10	1.532	1.526	6	1.07	1.07	0	1.35	1.29	6
11	1.580	1.585	-5	1.13	1.13	0	0.71	0.68	3
12	1.531	1.535	-4	1.05	1.04	1	0.89	0.95	-6
13	1.425	1.430	-5	1.07	1.07	0	1.29	1.38	-9
14	1.586	1.592	-6	1.16	1.17	-1	2.13	2.12	10
15	1.571	1.575	-4	1.28	1.28	0	0.48	0.57	-9
16	1.615	1.609	6	1.10	1.09	1	0.42	0.41	1
17	1.420	1.407	12	1.10	1.08	2	1.47	0.86	-8
18	1.570	1.562	8	1.30	1.30	0	0.57	1.04	13
19	1.529	1.519	9	1.08	1.08	0	0.67	0.76	-9
20	1.552	1.541	11	1.07	1.05	2	0.92	0.78	13
21	1.478	1.465	13	1.13	1.10	3	2.81	2.82	-1
22	1.533	1.525	8	1.04	1.06	-2	1.48	1.32	6
23	1.570	1.572	-2	1.13	1.14	-1	1.53	1.55	-2
24	1.550	1.572	-22	1.13	1.14	-1	1.52	1.55	-3

$$e = y_{exp} - \hat{y}_{cal}$$

The dependent variable equations represent the surface responses as function of only those factors that have had significant influence on it.

The response surface contour plots to estimate the Ca/P and % O_2 responses over independent variables Ca/P_r and $[Ca^{2+}]$ for values of pH=7.87 ($X_1=1.1592$), $T=40^\circ C$ ($X_4=-1$), and $D=1h$ ($X_5=-1$) are shown in Figures 1 and 2. The contour plots given in figures show that when $[Ca^{2+}]$ and Ca/P_r increase simultaneously or when $[Ca^{2+}]$ increases and the Ca/P_r remains unchanged or conversely, the Ca/P ratio and % O_2 of the precipitate increase until obtaining an optimum value, which remain unchanged in the experimental field and equalize to 1.6 for the Ca/P response and 1.40% for the % O_2 response.

By analyzing the response surface contour plots of Ca/P and % O_2 , we note that the formation of an oxygenated apatite with Ca/P equal to 1.575 and the most oxygenated (% $O_{2(max)}=1.40\%$ in the experimental field) can be done only in the arc intersection of the contour plot where the Ca/P equals 1.575 with the isoresponse surface of the % O_2 optimum. This field corresponds to a concentration of calcium ions $[Ca^{2+}]=0.631$ ($X_3=1$) and Ca/P_r ratio between 1.513 ($X_2=0.1$) and 1.622 ($X_2=0.96$). However, in this field, we see that the % O_2^{2-} falls by moving into the higher ratios of Ca/P_r (Figure 3). So, it was an interest in working with a minimal Ca/P_r. Therefore, the optimal conditions for obtaining an oxygenated apatite, with the above criteria, are as follows: pH=7.87, Ca/P_r = 1.513, $[Ca^{2+}]=0.631$ M, $T=40^\circ C$, and $D=1$ h. Under these conditions, the values of the predicted responses; Ca/P, % O_2 , and % O_2^{2-} , for the oxygenated apatite

FIGURE 1: Isoresponse contour plot for Ca/P with pH=7.87, $T=40^\circ C$, and $D=1$ h. FIGURE 2: Isoresponse contour plot for % O_2 with pH=7.87, $T=40^\circ C$, and $D=1$ h.

synthesis from calcium chloride and phosphoric acid are, respectively, as follows: 1.575, 1.40%, and 0.79%.

The experimental verification in the optimum conditions of synthesis gave a phosphocalcic oxygenated apatite with Ca/P ratio, % O_2 , and % O_2^{2-} equal to $1.577 \approx 1.575$, 1.39%, and 0.77%, respectively. X-Ray diffraction analysis (Figure 4) indicates that this product is an amorphous apatite, similar to that of the mineral part of the bone [14], since it was prepared under temperature and pH conditions close to biomimetic conditions and for only one hour. The infrared spectrum of this product (Figure 5) reveals only the band characteristic of nonstoichiometric apatites (attributable to PO_4^{3-} and HPO_4^{2-} ions) more the band characteristics of the presence of water [14].

FIGURE 3: Isoresponse contour plot for % O_2^{2-} with $pH = 7.87$, $T = 40^\circ C$, and $D = 1$ h.

FIGURE 4: X-ray diffraction diagram of the obtained precipitate under optimum conditions.

FIGURE 5: Infrared spectrum of the obtained precipitate under optimum conditions.

4. Conclusion

This work points out the variability of the chemical composition (Ca/P molar ratio, % O_2 , and % O_2^{2-}) of phosphocalcic oxygenated apatite precipitated from aqueous media ($CaCl_2$, H_3PO_4 , H_2O_2 , and NH_4OH) with the

experimental synthesis conditions (pH , Ca/P_r , $[Ca^{2+}]$, T , and D). A central composite design was successfully employed for the experimental design and analysis of results, and the graphic response surfaces and contour plots were used to locate the optimum conditions ($pH = 7.87$, $Ca/P_r = 1.513$, $[Ca^{2+}] = 0.631$ M, $T = 40^\circ C$, and $D = 1$ h) of the synthesis of

phosphocalcic oxygenated apatite with Ca/P ratio, % O₂, and % O₂²⁻ equal to 1.577 $\approx$ 1.575, 1.39%, and 0.77%, respectively.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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