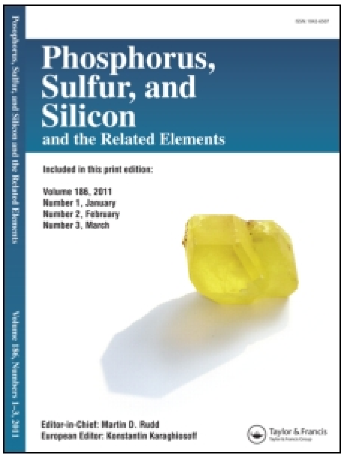

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STATISTICAL ANALYSIS OF CALCIUM PHOSPHATES PREPARATION

H. Chaair[a]; I. Mansouri[b]; M. Heughebaert[c]; S. Nadir[d]
[a] Laboratoire de Génie des Proédés, Faculté des Sciences et Techniques Mohammedia, Mohammedia [b] Laboratoire de Chiniie des Solides, Faculté des Sciences, [c] Laboratoire des Matériaux, Equipe de Physico-Chimie des solides, Toulouse, France [d] Laboratoire de Chimie-Physique des Matériaux, Ecole Hassania, Oasis, Casablanca, Morocco

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STATISTICAL ANALYSIS OF CALCIUM PHOSPHATES PREPARATION

H. CHAAIR[a]*, I. MANSOURI[b], M. HEUGHEBAERT[d] and S. NADIR[c]

[a]Laboratoire de Génie des Procédés, Faculté des Sciences et Techniques Mohammedia, B.P. 146, Mohammedia 20650, [b]Laboratoire de Chimie des Solides, Faculté des Sciences, Ain Chock, B.P. 5366 and [c]Laboratoire de Chimie-Physique des Matériaux, Ecole Hassania, des Travaux Publics, Km 7, Route d'El Jadida, B.P. 8108 Oasis, Casablanca, Morocco and [d]Laboratoire des Matériaux, Equipe de Physico-Chimie des solides, 38 rue des 36 Ponts, 31400 Toulouse, France

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Calcium phosphate was obtained by a semicontinuous wet process using the conventional double decomposition method between an aqueous calcium nitate solution, $Ca(NO_3)_2$, and an aqueous ammonium phosphate solution, $(NH_4)_2HPO_4$. To check the effet of certain variables on the reaction, a fractional central composite design was set up taking five variables into account: pH, $(Ca/P)_{reagents}$, concentration of the calcium nitrate solution ($[Ca^{2+}]$), temperature (T) and duration of precipitation (D). The limiting factors of precipitation for apatitic tricalcium phosphate are discussed

Keywords: Calcium Phosphates; Semicontinuous; Process; Statistical Analysis; Experimental Parameter Effect

INTRODUCTION

Calcium phosphates are used as bioceramics for prosthetic applications[1–5]. They are mainly based on hydroxyapatite [HAP; $Ca_{10}(PO_4)_6(OH)_2$] and β-tricalcium phosphate [β-TCP; $Ca_3(PO_4)_2$]. The difficulty with most of the conventional precipitation methods used is in obtaining well defined and reproducible solids, i.e. a solid with a given Ca/P ratio[6–8]. Factors governing the precipitation, such as pH, temperature, $(Ca/P)_{reagents}$, duration of precipitation, are not usually precisely controlled; consequently, the

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* Corresponding Author: Tel **212(3)31 47 08; Fax **212(3)31 53 53; E-mail: chaair@uh2m.ac.ma

solid precipitated is, in fact, a mixture of various calcium phosphates that, after heating to 900°C in air, leads to a solid with approximately the desired Ca/P ratio.

The purpose of this paper is to optimize the semicontinuous synthesis of tricalcium phosphate by looking for a possible optimum in the response surface representing the relationship between the atomic Ca/P ratio (Ca/P $\approx$ 1.50) of the precipitate, and the variables governing precipitation reaction.

In this work the relationship between the atomic ratio of the precipitate and five quantitative variables, i.e. pH, atomic ratio of the reagents [$(Ca/P)_{reagents}$], concentration of the calcium solution ($[Ca^{2+}]$), temperature (T), and duration of precipitation (D), were determined by a parabolique model for a set of experiments according to a fractional central composite design[9,10].

EXPERIMENTAL

The precipitates were obtained by a wet process using a conventional double decomposition method between a calcium solution, $Ca(NO_3)_2$, and an ammonium phosphate solution, $(NH_4)_2HPO_4$[11]. A schematic diagram of the apparatus is shown in Figure 1. A 8 dm^3 reactor was maintained at constant temperature, both reagents were introduced at the same constant flow rate and the pH was adjusted to a constant value with a pH-stat, which controlled the addition of base (or acid) as necessary. The precipitate obtained in the reactor was filtered, washed with deionized water, air-dried at 80 °C and heated to 900 °C under air. In this process different parameters of synthesis were controlled precisely.

The resulting material was studied by X-ray diffraction, infrared (IR) spectroscopy, and chemical analysis. X-ray diffraction patterns were recorded at room temperature with cobalt-Kα 1 radiation (λ = 1.78892 Å) and a Seeman-Bohlin camera, the presence of impurities was checked by comparison with the American Society for Testing and Materials (ASTM) file [12]. Precise Bragg angles for the samples were measured with respect to lines of NaCl, which was used as internal standard [13]. IR spectra were recorded with a Perkin-Elmer FTIR 1600 spectrophotometer using pellets

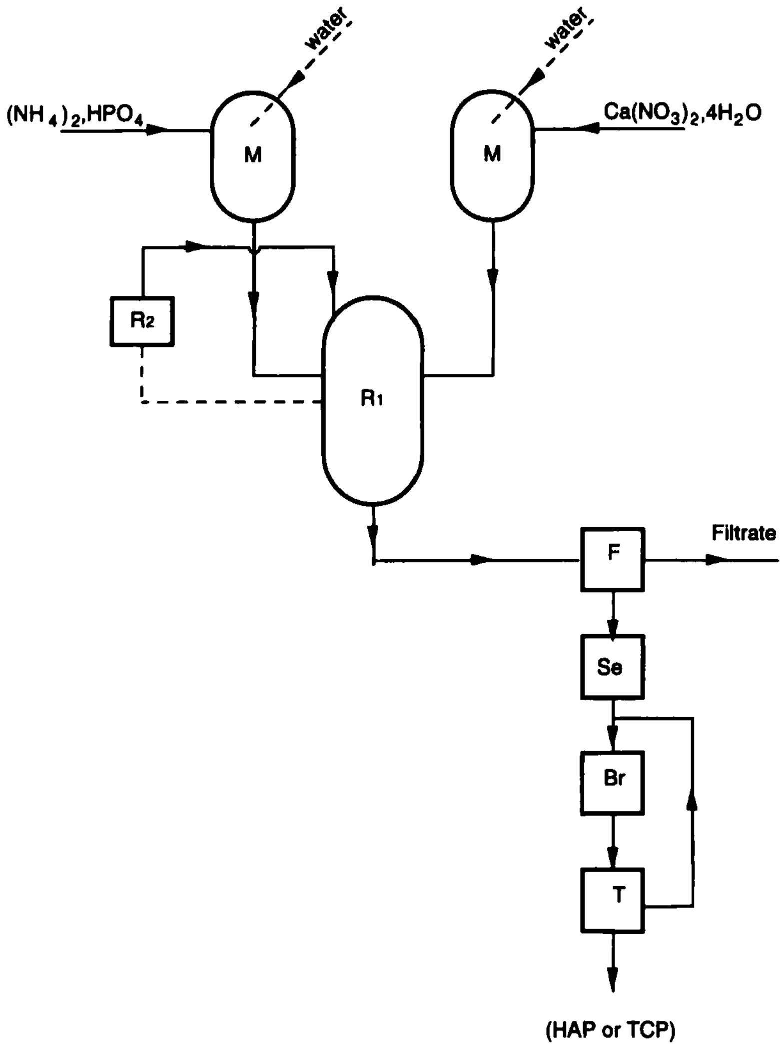

FIGURE 1 Block diagram of the semicontinuous synthesis of calcium phosphate. R_1 = reactor; M = stirrer; R_2 = pH-stat; F = filtration system; Se = dryer; Br = grinder; T = sieve

consisting of 1 mg powder in 300 mg KBr. The phosphorus content was analysed by the colorimetric method described by Gee and Deitz[14]. The relative error for phosphorus determination was 0.1%. Calcium was determined by atomic absorption[15], the relative error was 0.3 %.

TABLE I The fractional central composite design presented according to the standard order

der	*coded units of variable*					*$(Ca/P)_{whs}$ exp y_i*	*$(Ca/P)_{whs}$ cal $\hat{y}_i$*	*residues*
logical	X_1	X_2	X_3	X_4	X_5			
1	−1	−1	−1	−1	+1	1.419	1.459	− 4
2	+1	−1	−1	−1	−1	1.475	1.475	+ 0
3	−1	+1	−1	−1	−1	1.446	1.444	+ 0
4	+1	+1	−1	−1	+1	1.469	1.470	− 0
5	−1	−1	+1	−1	−1	1.435	1.440	− 0
6	+1	−1	+1	−1	+1	1.446	1.454	− 0
7	−1	+1	+1	−1	+1	1.461	1.467	− 0
8	+1	+1	+1	−1	−1	1.516	1.518	− 0
9	−1	−1	−1	+1	−1	1.459	1.456	+ 0
10	+1	−1	−1	+1	+1	1.533	1.533	+ 0
11	−1	+1	−1	+1	+1	1.481	1.479	+ 0
12	+1	+1	−1	+1	−1	1.536	1.530	+ 0
13	−1	−1	+1	+1	+1	1.424	1.429	− 0
14	+1	−1	+1	+1	−1	1.484	1.485	− 0
15	−1	+1	+1	+1	−1	1.470	1.470	+ 0

der	*coded units of variable*					*(Ca/P)$_{whs}$ exp y_i*	*(Ca/P)$_{whs}$ cal $\hat{y}_i$*	*residues*
logical	X_1	X_2	X_3	X_4	X_5			
16	+1	+1	+1	+1	+1	1.546	1.548	− 02
17	−1.72	0	0	0	0	1.445	1.439	+ 06
18	+1.72	0	0	0	0	1.529	1.526	+ 03
19	0	−1.72	0	0	0	1.467	1.458	+ 09
20	0	+1.72	0	0	0	1.506	1.507	− 01
21	0	0	−1.72	0	0	1.475	1.482	− 07
22	0	0	+1.72	0	0	1.498	1.483	+ 15
23	0	0	0	−1.72	0	1.469	1.457	+ 12
24	0	0	0	+1.72	0	1.505	1.509	− 04
25	0	0	0	0	−1.72	1.481	1.485	− 04
26	0	0	0	0	+1.72	1.493	1.481	+ 12
27	0	0	0	0	0	1.490	1.492	− 02
28	0	0	0	0	0	1.490	1.492	− 02
29	0	0	0	0	0	1.485	1.492	− 07
30	0	0	0	0	0	1.485	1.492	− 07

STATISTICAL ANALYSIS

Statistical analysis is frequently performed in agriculture, biology and chemistry [16] to study the empirical relationships between one or more measured responses and a number of variables. This part of the paper discusses the principles governing the construction and analysis of a fractional central composite design in which the response (y) is the atomic Ca/P ratio of the washed solid obtained, heated to 900 °C in the air [$(Ca/P)_{whs}$], and the variables x_j are: pH, $(Ca/P)_{reactants}$, [Ca^{2+}], T, and D, hereafter called respectively x_1, x_2, x_3, x_4, and x_5.

TABLE II Factorial levels used to optimize apatitic-TCP

natural variables(x_j)	*coded variables $X_1, X_2, X_3, X_4, X_5^a$*				
	− 1.7188	*−1*	*0*	*1*	*1.7188*
x_1 = pH	5.39	5.75	6.25	6.75	7.00
$x_2 = (Ca/P)_{reagents}$	1.45	1.47	1.50	1.53	1.55
$x_3 = [Ca^{2+}]$ / mol l^{-1}	0.82	1.00	1.25	1.50	1.68
x_4 = T/°C	58.2	60.0	62.5	65.0	66.8
x_5 = D/h	2.28	3.00	4.00	5.00	5.72

a. $X_1 = (x_1 - 6.25)/0.50$; $X_2 = (x_2 - 1.50)/0.03$; $X_3 = (x_3 - 1.25)/0.25$; $X_4 = (x_4 - 62.5)/2.50$; and $X_5 = (x_5 - 4.00)/1.00$.

Table I shows the fractional central composite design presented according to the standard order; the values of the coded variable X_j are dimensionless. The values of the natural variables are summarized in Table II. The 30 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 belong to a 2^{5-1} factorial fractional design; the ± 1 coded values X_j were obtained by calculating:

$$X_j = (x_j - \bar{x}_j)/\Delta x$$

The additional variable, x_5, is conformed (i.e. confused) with the product $x_1x_2x_3x_4$. (ii) The next 10 experiments were the points on the six axes, at a distance ± α from the centre. (iii) In this centre the four last experiments of the table were performed. The distance α was calculated to have vectors of square variables X_j^2 that are mutually orthogonal: in the present design, which consisted of five variables belonging to 2^{5-1} and four experiments in the centre, we have $\alpha = 1{,}7188$.

But the 30 values of X_j^2 presented a sum different from zero, and the mean 0.73; therefore the variable X_j^2 was replaced by the centred variable $U_j^2 = X_j^2 - 0.73$, Consequently the estimated model was:

$$\hat{y} = b_0 + \sum_{j=1}^{5} b_j X_j + \sum_{j=1}^{5} \sum_{j'=1\ j'\neq j}^{5} b_{jj'} X_j X_{j'} + \sum_{j=1}^{5} b_{jj} U_j^2$$

Let $b_u X_u$ be the general term of; the 21 terms (1 constant + 5 variables j + 10 interactions jj′ + 5 squared variables jj = 21) 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:

$$b_u = \frac{Y_u}{\sum_{i=1}^{n} X_{iu}^2} \quad \text{where} \quad Y_u = \sum_{i=1}^{n} X_{iu} y_i,$$

X_{iu} and y_i being the X_u and y values for the i^{th} experiment; Y_u is named contrast.

RESULTS

Table I shows the experimental data for each atomic ratio of precipitate washed and heated at 900 °C (yi). The 21 terms are easily calculated by substituting data values in the expressions for the least squares estimates of the coefficients (Table III). The fitted response surface is; if expressed in real variables:

$$\begin{aligned} \left(\widehat{\frac{Ca}{P}}\right)\text{whs}\ .10^3 = 1492 + 25\,X_1 &+ \cdots\cdots\cdots\cdots - 1\,X_5 \\ +1\,X_1X_2 &+ \cdots\cdots\cdots\cdots + 7\,X_4X_5 \\ -3\,X_1^2 &+ \cdots\cdots\cdots\cdots - 3\,X_5^2 \qquad (1) \end{aligned}$$

From this Equation, it is possible to compute estimated values $(\hat{y}_i)$ and the corresponding residuals $e_i = y_i - \hat{y}_i$ (Table I). An estimate of the variance of the experimental error (s_r^2) was obtained by dividing the residual sum of squares $\sum_i e_i^2$ (Table I), by v (number of degrees of freedom = number of experiments minus number in the model, i.e. 30 – 21 = 9) [see Table IV]:

$$s_r^2 = (0.001)/9 = 1.11 \times 10^{-4} \quad \text{(Table IV)}$$

TABLE III Analysis of variable effect

	coefficient (b_u)	*variance (S^2_{bu}) × 10^6*	*F-value (b^2_u/S^2_{bu})*	*significance*
b_0	1.492	–	–	–
b_1	0.025	5.07	123.27	***
b_2	0.014	5.07	38.66	***
b_3	0.0002	5.07	0.005	NS
b_4	0.015	5.07	44.38	***
b_5	– 0.001	5.07	0.20	NS
b_{12}	0.001	6.94	0.14	NS
b_{13}	– 0.0004	6.94	0.02	NS
b_{14}	0.008	6.94	9.22	*
b_{15}	0.0005	6.94	0.04	NS
b_{23}	0.01	6.94	14.41	**
b_{24}	0.001	6.94	0.14	NS
b_{25}	0.001	6.94	0.14	NS
b_{34}	– 0.008	6.94	9.22	*
b_{35}	– 0.001	6.94	0.14	NS
b_{45}	0.007	6.94	7.06	*
b_{11}	– 0.003	3.32	2.71	NS
b_{22}	– 0.003	3.32	2.71	NS
b_{33}	– 0.003	3.32	2.71	NS
b_{44}	– 0.003	3.32	2.71	NS
b_{55}	– 0.003	3.32	2.71	NS

***: significant at a level of 0.1% ($F_{0.001}(1,9) = 22.86$); **: significant at a level of 1% ($F_{0.01}(1,9) = 10.56$); *: significant at a level of 5% ($F_{0.05}(1,9) = 5.12$); and NS: non-significant.

TABLE IV Regression variance analysis for model ratio Ca/P of the solid washed, air-dried and heated to 900 °C

source of variation	*sum of squares*	*degrees of freedom*	*mean square*	$F_{exp}{}^{a}$	S^{b}
regression	0.029	20	1.0×10^{-3}	12.299	c
residue	0.001	9	1.1×10^{-4}	–	–
sum	0.03	29	–	–	–

a. F_{exp}: Snedecor factor.
b. Significance test.
c. Significant at a level of 0.1% ($F_{0.001}(20,9) = 8.90$)[16].

The estimated variances of coefficients $s_{bu}{}^2$ given in Table III were therefore calculated by the following formulae:

$$s_{bu}^2 = \frac{S_r^2}{\sum_i X_{iu}^2}$$

The significance of the effects can be estimated by comparing the values of the ratio $b_u{}^2/s_{bu}{}^2$ to a critical value, $F_{0.95}(1,9) = 5.12$, of the F distribution [17], at a 95% level of confidence with 1 and 9 degrees of freedom. According to the results shown in Table III. It appears that only the main effects: pH, $(Ca/P)_{reagents}$ and T, and the interactions pH-T, $(Ca/P)_{reagents}$-$[Ca^{2+}]$, $[Ca^{2+}]$-T and T-D are significant.

The best fitting response function is then conveniently written as follows:

$$\left(\frac{\widehat{Ca}}{P}\right)_{whs}\times10^3 = \underset{(\pm2)}{1492+25\,X_1} + \underset{(\pm2)}{14\,X_2} + \underset{(\pm2)}{15\,X_4} + \underset{(\pm3)}{8\,X_1X_4} + \underset{(\pm3)}{10\,X_2X_3} - \underset{(\pm3)}{8\,X_3X_4} + \underset{(\pm3)}{7\,X_4X_5} \qquad (2)$$

The quantities between brackets below the coefficients represent standard deviations (table III); for example:

$$S_{bj} = \sqrt{5.07.10^{-6}} = 2.25\times10^{-3} \approx 2.10^{-3}$$
$$S_{bjj'} = \sqrt{6.94.10^{-6}} = 2.63\times10^{-3} \approx 3\times10^{-3}$$

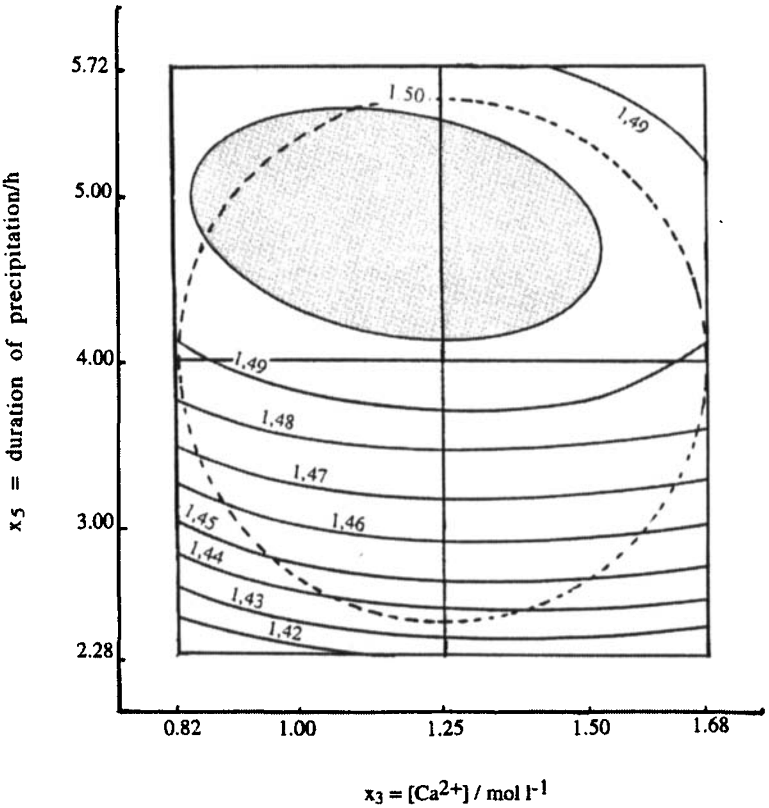

FIGURE 2 Response function contour lines [eqn. (1)], x_1 = pH = 6.25; $x_2 = (Ca/P)_{reagents} = 1.50$; and x_4 = T = 65 °C. Broken line shows the experimental range

DISCUSSION

The investigation of Equation (2) showed that, if $X_1 = 0$, $X_2 = 0$, $X_3 = +1$, $X_4 = +1$ and $X_5 = 0$, $(\widehat{Ca/P})whs = 1.499 \simeq 1.50$. This value corresponds to $(\widehat{Ca/P})whs = 1.50$ of β-TCP. It has been shown [11] that the most efficient physical methods of characterization are X-ray diffraction and IR spectroscopy, both techniques allow the determination of the Ca/P ratio β-TCP with an error of the order of ± 0.003. These characterization techniques, when applied to the sample obtained under our conditions confirmed the high purity of the β-TCP obtained.

A geometrical representation of this response [18], which is dependent on the calcium concentration of the reagents and the duration of the precipitation for constant values of pH, $(Ca/P)_{reagents}$ and T, shows a set of line contours (Figure 2). When the calcium concentration of the $Ca(NO_3)_2$ solution and the duration of the precipitation increase together, or when the duration of the precipitation increases, the calcium concentration of the $Ca(NO_3)_2$ solution remaining unchanged, then the Ca/P ratio of the precipitate increases up to 1.50 (shadowed domain).

CONCLUSION

The precipitation of apatitique tricalcium orthophosphate, has been studied using a fractional central composite design. The response equation for the atomic ratio Ca/P of the precipitate obtained was established. From this equation, it was possible to forecast the optimal conditions to obtain β -TCP [$Ca_3(PO_4)_2$; Ca/P = 1.50], from apatitic tricalcium orthophosphate [$Ca_9(HPO_4)(PO_4)_5(OH)$; Ca/P = 1.50], after heating to 900°C, and also to determine the Ca/P ratio of the precipitates.

NOMENCLATURE

$(Ca/P)_{whs}$ = Ca/P ratio of washed heated solid.

b_u = coefficient of the polynomial model.

e_i = residual of the i^{th} experiment, $e_i = y_i - \hat{y}_i$

${s_{bu}}^2$ = estimated variance of coefficient b_u

${s_r}^2$ = residual variance, $s_r^2 = \sum_i e_i^2/n$

${U_j}^2$ = transformed variable U^2 for element j

x_j = natural variable x for element j, and $\bar{x}_j$ its mean, i.e. either pH, $(Ca/P)_{reagents}$, $[Ca^{2+}]$, temperature (T), and duration of precipitation (D).

X_j = coded variable × for element j.

y_i = measured response for the i^{th} experiment.

$\hat{y}_i$ = calculated response for the i^{th} experiment.

α = distance from the centre of the design.

Δx = difference between × and $\bar{x}$.

ν = number of degrees of freedom = number of experiments minus the number of coefficients in the model.

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