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Statistical Studies of the Physicochemical Analytic Results of a Series of Synthetic Calcium Hydroxyapatite Containing Carbonate and Sodium

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Abstract

The objective of this study is to present a simple method of statistical calculation that allowed us to determine the relationship between the different data obtained from the characterization of the synthetic carbonated apatites containing sodium, in order to find the fundamental substitution mechanism(s) for incorporation of Na^+ and CO_3^{2-} and to establish the general formula. For that, a series of hydroxyapatites containing carbonate and sodium (Na-CO_3HAps) has been obtained by the precipitation method. All the compounds were characterized by infrared spectra (IR), powder X-ray diffraction (PXRD) and elemental analysis. The statistical treatment of the experiment result allows us to determine the relationship between one variable and the change in the other and to found the fundamental substitution mechanism(s) for incorporation of Na^+ and CO_3^{2-}. Analysis of variance (ANOVA) allows us to test the models proposed.

Keywords

Carbonated Calcium Hydroxyapatite Containing Sodium Na-CO_3HAps, Statistical Studies, Multiple Linear Regression, Analysis of Variance (ANOVA)

1. Introduction

Carbonated calcium hydroxyapatite containing sodium and/or potassium, magnesium etc. is the most important mineral compound in human dental, enamel and bone [1] [2]. We can also found this type of apatite in sedimen-

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tary phosphate apatite [3]. Consequently, these materials have necessitated studies of the effects of ions, such as CO_3^{2-} Na^+, Mg^{2+}, on the physicochemical properties and especially the mode of CO_3^{2-} Na^+ incorporation in apatite structure. But, the complexity of biological and sedimentary phosphates apatite has returned these studies very difficultly.

For this reason, multiple techniques have been used to prepare these products, with wet chemical methods and solid-state reactions [4]-[14]. Carbonate incorporation in synthetic carbonated calcium hydroxyapatites "CO_3 HAp" has been classified as either type A or type B depending on the mode of CO_3^{2-} substitution: CO_3^{2-} for OH^- type A substitution and CO_3^{2-} for PO_4^{3-} type B substitution [10] [11].

In the precipitation of "CO_3HAps", different products are obtained if the calcium solution is added to the phosphate plus carbonate solution "direct" rather than vice versa "inverse" apatite [6] [8] [12]-[14]. All these products are B-Apatite, but it seems that the maximum possible carbonate content was greater in the "direct" apatite compared with "inverse" apatite [13]. Na-free B-type "CO_3HAps" has been prepared with a maximum CO_3^{2-} content between 10 wt% at 14 wt%. In the presence of ions such as (Na^+, K^+ or Mg^{2+}), we have a coupled substitution. With Na^+ for Ca^{2+}, B-type apatite "Na-CO_3HAps" contains up to 22 wt% CO_3^{2-} [14].

Based on some studies, such as EPR and IR spectroscopy, X-ray and neutron diffraction, chemical analyses, infrared and Raman spectroscopy, the crystallite size, chemical composition and physicochemical nature of synthetic apatite have been determinated. But there have been multiple studies, speculations and controversy about the mechanism(s) by which CO_3^{2-} and alkali metal(s) are incorporated in the apatite lattice [15]-[20]. According to De Maeyer *et al.*, the fundamental substitution mechanisms for the incorporation of Na^+ and CO_3^{2-} in hydroxyl apatite "HAp" can be described using six basic processes [21].

1) $Ca^{2+} + PO_4^{3-} + OH^- \leftrightarrow V^{Ca} + CO_3^{2-} + V^{OH}$
2) $Ca^{2+} + 2PO_4^{3-} \leftrightarrow V^{Ca} + 2CO_3^{2-}$
3) $Ca^{2+} + PO_4^{3-} \leftrightarrow CO_3^{2-} + Na^+$
4) $2Ca^{2+} + OH^- \leftrightarrow V^{OH} + Na^+$
5) $PO_4^{3-} \leftrightarrow CO_3^{2-} + OH^-$
6) $2OH^- \leftrightarrow V^{OH} + CO_3^{2-}$

where V^X is the vacancy on a regular apatite lattice site occupied by X.

Moreover, these authors propose a coupling of different fundamental mechanisms in a fixed proportion, leading to the definition of apparently new mechanisms. The composition of B-type of Na-CO_3HAps is determined by the occurrence of one or more of these fundamental mechanisms. Thus, these authors suggest that the stoichiometry would be given by:

$$Ca_{10-(a+b+d+c+e)}Na_{(c+d)}\left(PO_4\right)_{6-(a+2b+c+d+e)}\left(CO_3\right)_{(a+2b+c+d+e)}\left(OH\right)_{2-(a+d)+e}$$

where *a*, *b*, *c*, *d* and *e* are the contribution per unit cell of basic substitutions (I to V) resulting in the fundamental substitution mechanisms for incorporation of Na^+ and CO_3^{2-} in the crystal lattice of hydroxyapatite "HAp".

The aim of the present study is to present a statistical method which allows us to determine the relationship between the obtained values and experimental conditions and to estimate the change in one variable from the given increase or decrease in another. The analysis of variance (ANOVA) allows us to test the mathematical model. Finally, the statistical method allows us to find the fundamental substitution mechanism(s) for incorporation of Na^+ and CO_3^{2-}. So, the stoichiometry of the solid can be described as a function of the contributions of each mechanism to the composition of the unit cell.

2. Experimental Procedure

2.1. Preparation of Na-CO_3HAps

The preparation method is described in detail elsewhere [12] [13], In brief, The precipitated Na-CO_3HAps were prepared by dripping calcium solution 0.03 M ($Ca(NO_3)_2{\cdot}4H_2O$) into a phosphate solution 0.008 M of ($Na_2HPO_4{\cdot}12H_2O$) which contains also different concentrations of (Na_2CO_3) such as the molar ratio *R*:

$0 < R = n_{CO_3^{2-}} / n_{PO_4^{3-}} \le 60$. So that, the concentrations of $\left[CO_3^{2-}\right]$ and $[Na^+]$ were varied in the hydrolysis solution: $0.098\ \text{M} \le \left[Na^+\right] \le 0.998\ \text{M}$, $0.04\ \text{M} \le \left[CO_3^{2-}\right] \le 0.49\ \text{M}$. Then, the concentration ratio $\left[Na^+\right]/\left[CO_3^{2-}\right] \cong 2$.

After hydrolysis which took 3 h, the precipitates were filtered, washed abundantly with hot distilled water (95˚C), dried for 12 h at 70˚C and then heated at 400˚C in air for 24 h in order to eliminate inter- and intra-crystalline water.

2.2. Characterization

2.2.1. Infrared Spectroscopy and X-Ray Diffraction

Na-CO_3HAps samples are characterized by IR absorption spectroscopy and X-ray diffraction. In infra-Red, we use pellets of absorption. They are prepared using the usual KBr disk technique. It consists in mixing 1 mg of powder of a sample of Na-CO_3HAp with 300 mg KBr then pressed at 6 psi. The pellets so prepared were then scanned on a Shimadzu IR spectrometer in the range (4000 - 400) cm^{-1}.

Powder X-ray diffraction analysis for the Na-CO_3HAps samples were carried out using an X-ray diffractometer MRD with a generator (40 kV and 40 mA). After indexation of the full pattern, cell parameters were refined using the program "WINCELL"

2.2.2. Chemical Analysis and Density Measurements

The dehydrated samples were subjected to a chemical analysis. The phosphorus content of precipitates is determined by colorimetry after complexation with vanado-molybdate [22]. The sodium and calcium content are obtained by atomic absorption spectroscopy and the carbonate content was determined by coulometry method (release of CO_2 by dissolution in acid). The hydroxide content % OH was calculated on the basis of electro neutrality. Density of the solids was measured by a flotation method [13].

3. Results

3.1. Physical Analysis

The infra-Red spectrums presented in **Figure 1** show that they are typical of apatite containing B-type carbonate [13]. The assignment of absorption bands was made according to the studies [12] [13]. IR analyses of the samples show the effect of increasing CO_3^{2-} and Na^+ on the spectral properties of apatites. However, increasing CO_3^{2-} and Na^+ contents caused increasing of the intensities of the CO_3^{2-} absorption bands at 1420 - 1460 cm^{-1} (ν_3 C–O) and 872 cm^{-1} (ν_2 C–O) and decreasing in the resolution of PO_4^{3-} (ν_3 P–OH) absorption band at 1032 cm^{-1}, The shoulder observed at 740 cm^{-1} is attributed to the ν_L mode of OH^- close neighbors to Na^+ ions [13].

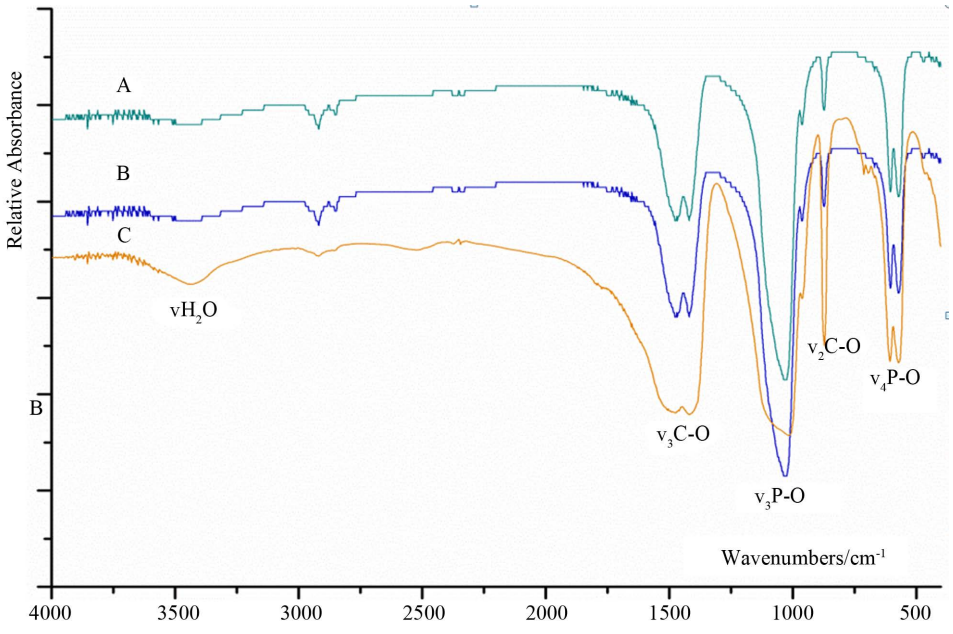

Figure 1. Absorption Spectra of Na-CO_3Haps obtained from solutions with: (A) R = 5, (B) 15 AND (C) 25.

In **Figure 2** we presented the X-ray diffraction patterns. The peaks are relatively sharp and well resolved and can all attributed to the hexagonal crystal form of hydroxyapatite, but some shifts of peaks position can be observed, reflecting a change in unit cell dimensions due to incorporation of CO_3^{2-} and Na^+. This allows us to consider our samples as pure well-crystallized phases of apatite type. The results of dimensions "*a*" and "*c*" and the volume of the hexagonal apatitic unit cell obtained from X-Ray analysis as well as the densities of the compounds allow determining the molar weight (Mw). The results are grouped together in **Table 1**.

3.2. Chemical Results

The compositions of samples in Weight % determined by chemical analysis are displayed in **Table 2**. The results of quantitative analysis of phosphorus, calcium and sodium were determined with standards deviations 0.17; 0.03 and 0.09 respectively and the amount of CO_3 was determined on relative uncertainty 2%.

The results of the chemical and physical analysis (**Table 1** and **Table 2**) allowed us to calculate the number of each ion X per unit cell, n_x according to:

$$n_x = \frac{\%X \cdot M}{100 \cdot M_x}, \quad (1)$$

where M ($\rho \cdot V_{cell} \cdot N$) is the molar mass, V_{cell} is the unit cell volume, M_x is the atomic or ionic mass of X and N Avogadro's constant. The amount of OH ions was calculated taking into account the electroneutrality of each compound. **Table 3** gathers the calculation results.

4. Discussion

As in the case of the reference [23], the last column of **Table 3** shows that the sum of the number of phosphate and carbonate per unit cell $O_p = n_{PO_4} + n_{CO_3}$ is approximately equals to 6 with experimental error as well as the XRD and IR spectra clearly demonstrate that the samples "Na-CO_3HAps" of this study are pure B-type carbonated apatites, this indicates that CO_3^{2-} substitutes for PO_4^{3-} on a 1:1 basis which agrees with the fundamental substitution mechanisms for B-type CO_3^{2-} [21] [23]-[25]. On the basis of the observation, the real number of each ion per unit cell can be obtained using the following equation:

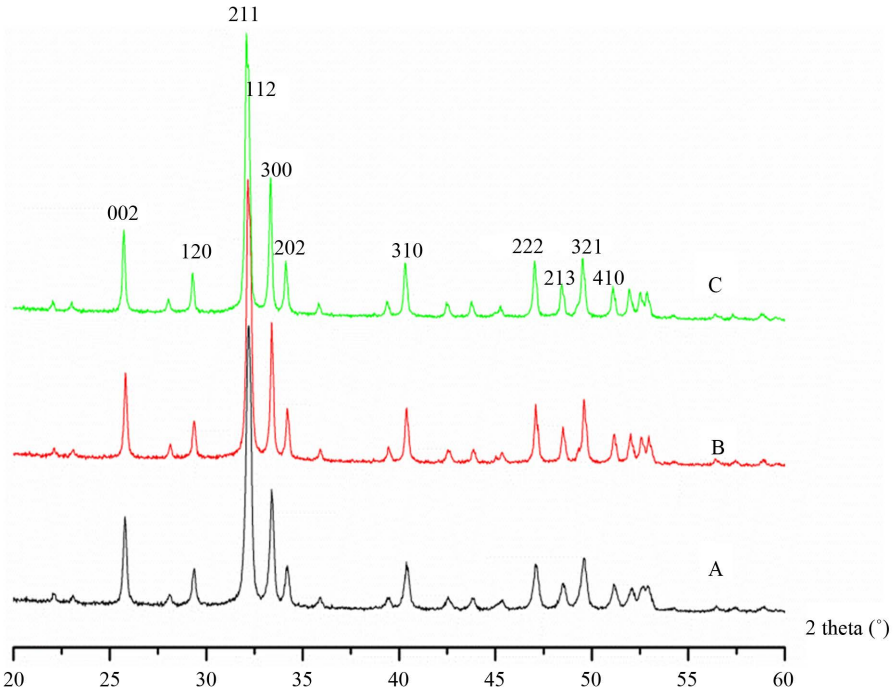

Figure 2. XRD patterns of synthetic apatites obtained from solutions with: (A) R = 5, (B) 15, (C)25.

Table 1. Values of lattice parameters "*a*", "*c*" and volume molar; density and weight (Mw) of the prepared samples.

Lattice parameters					
Apatites	density	a-Axis (Å)	c-Axis (Å)	Volume/Å^3	M/g mol^{-1}
(1) R = 5	2.79	9.3550 ± 0,0017	6.9221 ± 0,0011	524.63	881.5
(2) R = 10	2.78	9.3784 ± 0.0013	6.9276 ± 0.0009	527.68	883.5
(3) R = 15	2.79	9.3262 ± 0.0004	6.9003 ± 0.0003	519.77	873.4
(4) R = 20	2.77	9.3584 ± 0.0015	6.9440 ± 0.0010	526.55	878.5
(5) R = 25	2.79	9.3375 ± 0.0015	6.9318 ± 0.0010	523.41	879.5
(6) R = 30	2.71	9.3236 ± 0.0017	6.9363 ± 0.0011	522.19	852.3
(7) R = 35	2.72	9.2854 ± 0.0012	6.9148 ± 0.0008	516.32	845.9
(8) R = 40	2.73	9.3059 ± 0.0013	6.9241 ± 0.0009	519.29	853.8
(9) R = 45	2.80	9.3001 ± 0.0009	6.9163 ± 0.0006	518.06	874.3
(10) R = 50	2.72	9.3008 ± 0.0016	6.9202 ± 0.0011	518.42	849.3
(11) R= 55	2.76	9.2710 ± 0.0015	6.9230 ± 0.0012	515.32	856.6
(12) R = 60	2.77	9.2691 ± 0.0005	6.9216 ± 0.0010	515.01	859.2

Table 2. Chemical composition (weight %) and total mass balance Σ% of Na-CO_3HAps prepared from solutions with $0 \prec R = n_{CO_3^{2-}}/n_{PO_4^{3-}} \leq 60/1$.

Composition %w						
Apatites	Ca^{2+}	Na^+	PO_4^{3-}	CO_3^{2-}	OH^-	Σ%
(1) R = 5	37.71	3.47	46.62	10.47	3.65	101.93
(2) R = 10	37.53	3.95	45.87	12.63	3.04	103.03
(3) R = 15	37.02	4.70	44.71	13.36	3.36	103.16
(4) R = 20	37.24	4.62	42.97	14.67	3.69	103.19
(5) R = 25	36.74	4.90	41.36	15.26	4.00	102.26
(6) R = 30	35.75	5.74	39.15	17.98	3.43	102.05
(7) R = 35	35.85	5.38	39.54	17.71	3.18	101.67
(8) R = 40	35.31	5.40	37.66	18.56	3.27	100.21
(9) R = 45	33.95	6.03	35.65	19.25	3.26	98.15
(10) R = 50	34.72	6.12	35.21	21.39	3.01	100.45
(11) R= 55	34.33	7.30	36.14	22.44	2.46	102.66
(12) R = 60	34.70	6.68	33.89	23.71	2.80	101.77

$$n_X = \frac{\%X}{M_X}\frac{6}{\left(\%P/M_P + \%CO_3/M_{CO_3}\right)} \quad (2)$$

Similarly, we have calculated the number of vacancies on Ca^{2+} and OH^- lattice sites respectively:

$$n_{V^{Ca}} = 10 - n_{Ca} - n_{Na} \quad (3)$$

and

$$n_{V^{OH}} = 2 - n_{OH} \quad (4)$$

The results of these calculations are summarized in **Table 4**. The errors in **Table 4** were estimated by means of error propagation theory.

Table 3. Unit cell composition of Na-CO_3AHps calculated on the basis of the chemical composition and by means of Equation (1).

	Composition					
Sample	n_{Ca}	n_{Na}	n_{PO_4}	n_{CO_3}	n_{OH}	O_P
(1)	8.609	1.687	4.327	1.538	2.081	5.86
(2)	8.359	1.933	4.265	1.859	1.354	6.12
(3)	8.451	2.266	4.112	1.945	2.105	6.06
(4)	8.371	2.266	3.977	2.150	1.988	6.13
(5)	8.095	2.388	3.829	2.237	1.875	6.07
(6)	7.880	2.726	3.510	2.552	1.995	6.06
(7)	7.865	2.533	3.523	2.498	1.882	6.02
(8)	7.485	2.561	3.387	2.643	1.486	6.03
(9)	7.410	2.813	3.277	2.801	1.959	6.04
(10)	7.198	2.881	3.146	3.025	1.063	6.17
(11)	7.360	3.341	3.262	3.207	1.748	6.47
(12)	7.443	3.077	3.060	3.390	1.830	6.45

Table 4. Unit cell compositions of Na-CO_3Aaps calculated on the basis of the chemical composition and by means of following Equation (2).

	Composition of apatite						
sample	$n_{Ca^{2+}}$	n_{Na^+}	$n_{PO_4^{3-}}$	$n_{CO_3^{2-}}$	n_{OH^-}	$n_{V^{Ca}}$	$n_{V^{OH}}$
(1)	8.502 ± 0.073	1.361 ± 0.078	4.426 ± 0.052	1.573 ± 0.037	1.939 ± 0.239	0.137 ± 0.108	0.061 ± 0.239
(2)	8.118 ± 0.073	1.488 ± 0.077	4.178 ± 0.051	1.821 ± 0.044	1.547 ± 0.241	0.393 ± 0.106	0.452 ± 0.241
(3)	8.008 ± 0.073	1.768 ± 0.077	4.073 ± 0.051	1.926 ± 0.046	1.712 ± 0.245	0.223 ± 0.108	0.288 ± 0.245
(4)	8.017 ± 0.075	1.730 ± 0.077	3.895 ± 0.051	2.105 ± 0.051	1.868 ± 0.255	0.253 ± 0.110	0.131 ± 0.255
(5)	7.990 ± 0.079	1.854 ± 0.078	3.787 ± 0.051	2.212± 0.054	2.048 ± 0.261	0.155 ± 0.113	-0.048 ± 0.261
(6)	7.534 ± 0.082	2.105 ± 0.077	3.474 ± 0.051	2.526 ± 0.063	1.699 ± 0.270	0.361 ± 0.114	0.301 ± 0.270
(7)	7.559 ± 0.083	1.973 ± 0.077	3.510 ± 0.051	2.489 ± 0.062	1.581 ± 0.268	0.468 ± 0.113	0.419 ± 0.268
(8)	7.504 ± 0.083	2.000± 0.077	3.370± 0.051	2.629 ± 0.066	1.638 ± 0.276	0.496 ± 0.116	0.362 ± 0.276
(9)	7.315 ± 0.086	2.260 ± 0.079	3.235 ± 0.051	2.763 ± 0.070	1.655 ± 0.283	0.425 ± 0.118	0.344 ± 0.283
(10)	7.16 1 ± 0.087	2.196 ± 0.077	3.059 ± 0.050	2.941 ± 0.075	1.460 ± 0.286	0.642 ± 0.117	0.539 ± 0.286
(11)	6.826 ± 0.088	2.524 ± 0.076	3.025 ± 0.049	2.974 ± 0.075	1.151 ± 0.281	0.650 ± 0.114	0.849 ± 0.281
(12)	6.922 ± 0.090	2.316 ± 0.076	2.847 ± 0.048	3.153 ± 0.081	1.314 ± 0.293	0.761 ± 0.108	0.686 ± 0.293

Comparing our experiment data with the data available in the literature [24]-[26], we can found that the presents results are in agreement with those of reference [25], though, this series was prepared by hydrolysis of monetite in solution with varied CO_3^{2-} and alkali metal Na^+ concentration.

So, it may be said in the present study, that the fundamental substitution mechanisms (I, III and IV) could account for the incorporation of CO_3^{2-} and Na^+ in the HAp lattice. If *a*, *c* and *d* are the contributions of mechanisms I, III and IV respectively, thus,

$$a = n_{V^{Ca}} \tag{5}$$

$$c = n_{CO_3} - a \tag{6}$$

and

$$d = n_{Na} - c \tag{7}$$

and the generic formula has the following expression:

$$Ca_{10-(a+d+c)}Na_{(c+d)}(PO_4)_{6-(a+c+d)}(CO_3)_{(a+c+d)}(OH)_{2-(a+d)}$$

5. Statistical Studies

The objective of this study is to construct a mathematical model which allows us to estimate the change in *Y* the dependent variable from a given increase or decrease in *X* the independent variable and to determine which mechanism(s) is related with experimental conditions.

The mathematical model is expressed as:

$$Y_i = \beta_0 + \Sigma_1^j \beta_j X_{j,i} + \varepsilon_i \tag{8}$$

where ε_i is the random variable drawn from $N(0, \sigma^2)$, β_0 and β_j are the estimated regression coefficients. The model assumes that their deviation ε from the line is normally distributed with means 0 and constant variances σ^2.

Least square method [26] allows calculating the regression and correlation coefficients, the variance of the β parameters and to test the null hypothesis H_0: $\beta_j = 0$ and their significances level. The analysis of variance for the linear regression or the F test allows us to be confident that at least one of X-variable contributes to the regression. The theoretical basis of these calculations is given in references [12] [26].

5.1. Multiple Linear Regression of $Y = c/a$ Crystallographic Parameters Ratio on $X_1 = \%CO_3^{2-}$ and $X_2 = \%$ Na^+ the % Weightions in the Hexagonal Apatite Lattice

In attempts to disentangle and to measure the effects of the insertion of CO_3^{2-} and Na^+ ions on the hexagonal apatite lattice dimensions, we have constructed a multiple linear regression on two *X*-variables where, $X_1 = \%CO_3^{2-}$ and $X_2 = \%Na^+$ (data **Table 2**) and *Y* is the estimate ratio of the hexagonal lattice dimensions (data **Table 1**). The results of the calculation values of the different correlations coefficients, the estimated values of β_0, β_1 and β_2, the variances and t-test of β_1 and β_2 are summarized in **Table 5(a)**. The analysis of the variance for the linear regression or the F-test is given in **Table 5(b)**.

The multiple linear regression (**Table 5(a)**) indicates that the ratio of the hexagonal lattice dimensions of Na-CO_3Aps "*c*/*a*" vary linearly with the carbonate and sodium content according to:

$$c/a = 0.730 + 3.23\times10^{-4}\left(\%CO_3^{2-}\right) + 1.25\times10^{-3}\left(\%Na^+\right) \tag{9}$$

The analysis of variance (**Table 5(b)**/ANOVA) shows further that F-test = 100.5 is higher than criterion F(5%; 2.9) = 4.26. This allowed us to rejet H_0: $\beta_1 = \beta_2 = 0$ and affirm that at least one of the predictors is linearly associated to the response.

T-test of of the estimated regression coefficients β_1 and β_2 has shown that β_2 and β_1 are significant at 70% level.

5.2. Multiple Linear Regression of $Y = n_{CO_3}/n_P$ or n_{Na}/n_P the Molar Ratio in Na-CO_3HAps Solid on the Concentration of Na^+: $X_{1,i}$ and the Concentration of CO_3^{2-}: $X_{2,i}$ in Aqueous Solution

To understand the influence of the experimental conditions on the composition of these apatites (data **Table 4**), we conducted a multiple linear regression [22] on two *X*-variables where, X_1 and X_2 are the Na^+ and CO_3^{2-} ions

Table 5. calculation for fitting a multiple linear regression analysis of the estimated $Y = c/a$ the ratio of the lattice parameters of Na-CO_3HApson carbonate and sodium contents X_1 = wt% CO_3, X_2 = wt% Na. (a) Calculation of regression, correlation coefficients and variances, (b) F-test: Analysis of Variance.

(a)

Coefficients of correlation		Coefficients of regression		Variances		t-test	
R^2	0.953						
R_{x_1Y}	0.972	β_0	0.730				
R_{x_2Y}	0.973	β_1	3.1×10^{-4}	$v(\beta_1)$	8.46×10^{-8}	$t(\beta_1)$	1.11
$R_{x_2x_1}$	0.985	β_2	1.25×10^{-3}	$v(\beta_2)$	9.77×10^{-7}	$t(\beta_2)$	1.26

(b)

Source of variation	DF	Sum of Squares	Mean Square	Fobs
Regression	2	21.85×10^{-5}	10.9×10^{-5}	100.54
Deviations	10	1.08×10^{-5}	1.08×10^{-6}	
Total	12	22.9×10^{-5}	1.91×10^{-6}	

concentrations in solution and Y is the estimates molar ratios of n_{CO_3}/n_P and n_{Na}/n_P in the solids. The values of the different correlations coefficients, the estimated values of β_0, β_1 and β_2, their variances and the F-test or the test of the variance are summarized in **Table 6** and **Table 7**.

The multiple linear regression (**Table 6(a)**) indicates that the molar CO_3/P ratio of the Na-CO_3HAps vary linearly with the concentrations of carbonate and sodium in solution according to:

$$n_{CO_3}/n_P = 1.49 \times 10^{-4} - 32.96 \cdot \left[CO_3^{2-}\right] + 17.28 \cdot \left[Na^+\right] \quad (10)$$

The result of the F-test (**Table 6(b)**) F = 635.5 and the individual t-test of the β's **Table 6** b for β_1, t = −7.82. For β_2, t = 8.26 confirm that the molar CO_3/P ratio of Na-CO_3HAps solid is significantly depends on both concentrations of carbonate $\left[CO_3^{2-}\right] = X_1$ and sodium $[Na^+] = X_2$ in aqueous solution. However, constantly we have $\left[Na^+\right] \cong 2\left[CO_3^{2-}\right]$, thus the expression in brackets $\left(32.96 \cdot \left[CO_3^{2-}\right] + 17.28 \cdot \left[Na^+\right]\right)$, hardly varied, thus, CO_3/P molar ratio is constant. This shows that for $\left[CO_3^{2-}\right]/\left[Na^+\right]$ concentration ratio in solution constant the increase on molar CO_3^{2-} in solid is equal to the decrease on molar PO_4^{3-}. Thus, the molar ratio in the solid is not significantly influenced by the increase of Na^+ and CO_3^{2-} ions in solution. This result is in a good agreement with the results in literature [24].

The multiple linear regression (**Table 7(a)**) indicates that the molar Na/P ratio of Na-CO_3HAps vary linearly with the carbonate content according to*:*

$$n_{Na}/n_P = 0.198 - 0.249 \cdot \left[Na^+\right] + 1.85 \cdot \left[CO_3^{2-}\right]. \quad (11)$$

An analysis of variance (**Table 7(b)**/ANOVA), further shows that F-test = 43.5 is higher than criterion F(1%, 2.10) = 7.56, this allowed us to rejet H_0: $\beta_1 = \beta_2 = 0$ and affirm that at least one of the predictors is linearly associated to the response. T-test of the regression coefficients β_1 and β_2 has shown for β_1, t = −0.45 signifiant at 30% level. For β_2, t = 1.67 signifiant at 80% level.

5.3. Multiple Linear Regression of the Estimated Molar Content of Calcium $Y = n_{Ca^{2+}}$ on $X_1 = n_{Na^+}$ and $X_2 = n_{CO_3^{2-}}$ the Molar Contents of Sodium and Carbonate in the Solid

The examination of **Table 4** showed too that n_{Ca} molar content of the solid decreases when n_{CO_3} and n_{Na} incorporate increase. To determine the relationship between these three variables given in **Table 4**, a multiple linear regression analysis was undertaken. The results are summarized in **Table 8**.

From **Table 8**, the multiple linear regression analysis shows that the relationship between these quantities is given by equation:

Table 6. Calculation for fitting a multiple linear regression of the estimated $Y_i = n_{CO_3}/n_P$ molar ratio in Na-CO_3HAps solid on X_1 = [Na^+] and $X_2 = \left[CO_3^{2-}\right]$ the concentrations in solution. (a) Calculation of regression and correlation coefficients and variances, (b) Values of F-test: Analysis of Variance.

(a)

Coefficient of Correlation		Coefficients of Regression		Variances		t-test	
R^2	0.99						
R_{X_1Y}	0.969	β_0	0,00014				
R_{X_2Y}	0.972	β_1	−32.96	$v(\beta_1)$	17.72	$t(\beta_1)$	−7.82
$R_{X_2X_1}$	0.999	β_2	17.28	$v(\beta_2)$	4.37	$t(\beta_2)$	8.26

(b)

Source of Variation	DF	Sum of Squares	Mean Square	Fobs
Regression	2	1.0799	0.5399	635.6
Deviations	10	$8.49.10^{-3}$	$8.79.10^{-4}$	
Total	12	1.0884	0.0907	

Table 7. Calculation for fitting a multiple linear regression analysis of the estimated $Y = n_{Na}/n_P$ molar ratio in Na-CO_3HAps solid on X_1 = [Na^+] and $X_2 = \left[CO_3^{2-}\right]$ the concentrations in solution. (a) Calculation of regression and correlation coefficients and variances, (b) Values of F-test: Analysis of Variance.

(a)

Coefficients of correlation		Coefficients of regression		Variances		t-test	
R^2	0.897						
R_{X_1Y}	0.935	β_0	0.198				
R_{X_2Y}	0.948	β_1	−0.249	$v(\beta_1)$	0.30	$t(\beta_1)$	−0.45
$R_{X_2X_1}$	0.992	β_2	1.854	$v(\beta_2)$	1.23	$t(\beta_2)$	1.67

(b)

Source of variation	DF	Sum of Squares	Mean Square	F_{obs}
Regression	2	0.5618	0.2809	43.56
Deviations	10	0.0645	0.00645	
Total	12	0.626	0.0522	

$$n_{Ca} = 10.07 - 0.383 \cdot \left(n_{Na^+}\right) - 0.698\left(n_{CO_3^{2-}}\right) \quad (12)$$

From the intercept of the following equation it can seen that, within experimental error, a carbonate-free apatite $\left(n_{CO_3} = 0\right)$ contains 10 Ca^{2+} ions per unit cell. The expression in brackets $\left(-0.383 n_{Na^+} - 0.698 \cdot n_{CO_3^{2-}}\right)$ shows that the number of Ca^{2+} ions in the unit cell decreases with increasing of Na^+ and CO_3^{2-} numbers for about one Ca^{2+} ion.

The analysis of variance (**Table 8(b)**), further shows that F-test = 379.4 is higher than criterion F(5%, 2.9) = 4.26, this allowed us to rejet H_0: $\beta_1 = \beta_2 = 0$ and affirm that at least one of the predictors is linearly associated to

the response. T-tests of the regression coefficients b_1 and b_2 show that the value of: β_1 is signifiant at 80% level and for β_2 is signifiant at 90% level.

5.4. Multiple Linear Regression of $Y = a$, c or d the Contributions of Mechanisms I, III and IV on $X_1 = [Na^+]$ and $X_2 = \left[CO_3^{2-}\right]$ the Concentrations in Solution

For determine which mechanism(s) is related with experimental conditions, we have undertaken a statistical analysis [26] of the values of a, c and d the contribution of the mechanisms I, III and IV (**Table 9**) calculated according to the following Equations (5)-(7) as a function of Na^+ and CO_3^{2-} concentrations in aqueous solution. Results of these calculations are summarized in **Tables 10-12**.

Table 8. Calculation for fitting a multiple linear regression of the estimated $Y_i = n_{Ca^{2+}}$ the molar number of calcium as a function of carbonate and sodium contents $X_{1,i} = n_{Na}$ and $X_{2,i} = n_{CO_3}$ in the solid Na-CO_3Haps (a) Calculation of regression, correlation coefficients and variances, (b) F-test: Analysis of Variance.

(a)

Coefficient of Correlation		Coefficients of Regression		Variances		t-test	
R^2	0.987						
R_{X_1Y}	−0.985	β_0	10.06				
R_{X_2Y}	−0.992	β_1	−0.383	$v(\beta_1)$	0.066	$t(\beta_1)$	−1.49
$R_{X_2X_1}$	0.983	β_2	−0.698	$v(\beta_2)$	0.039	$t(\beta_2)$	−3.52

(b)

Source of Variation	DF	Sum of Squares	Mean Square	Fobs
Regression	2	8.033	4.016	379.4
Deviations	10	0.10586	0.01058	
Total	12	8.1386	0.6782	

Table 9. Values of (a, c and d) the estimated contribution of the mechanism I, II and IV.

Sample	$\left[CO_3^{2-}\right]$	$[Na^+]$	a	c	d
0	0	0	0	0	0
1	0.098	0.041	0.137	1.437	−0.076
2	0.180	0.082	0.393	1.429	0.060
3	0.262	0.123	0.223	1.704	0.065
4	0.344	0.164	0.253	1.852	−0.122
5	0.426	0.205	0.155	2.058	−0.203
6	0.507	0.245	0.361	2.165	−0.060
7	0.590	0.287	0.468	2.023	−0.048
8	0.671	0.327	0.497	2.133	−0.134
9	0.753	0.368	0.425	2.340	−0.080
10	0.834	0.409	0.642	2.299	−0.103
11	0.916	0.450	0.650	2.325	0.199
12	0.998	0.491	0.761	2.392	−0.076

From **Table 10**, the multiple linear regression analysis shows that the relationship between these quantities is given by equation:

$$Y_i = a = 5.68\times10^{-5} + 5.92\cdot\left[\mathrm{Na}^+\right] - 10.16\cdot\left[\mathrm{CO}_3^{2-}\right] \quad (12)$$

An analysis of variance (**Table 10(b)**/ANOVA), further shows that F-test = 25.36 is higher than criterion F (5%, 2.9) = 4.26, this allowed us to rejet: is linearly H_0: $\beta_1 = \beta_2 = 0$ and affirm that at least one of the predictors associated to the response. The individuals t-test of the regression show that for β_1, t = 0.79 signifiant at 60% level and for β_2, t = −0.70 signifiant at 50% level.

Table 10. Calculation for fitting a multiple linear regression of the estimated $Y_i = \left(a = n_{V^{Ca}}\right)$ the contribution of the mechanism I on the concentration of Na^+: $X_{1,i}$ and the concentration of CO_3^{2-}: $X_{2,i}$ in aqueous solution, (a) Calculation of regression and correlation coefficients and variances, (b) Values of F-test: Analysis of Variance.

(a)

Coefficient of Correlation		Coefficients of Regression		Variances		t-test	
R^2	0.835						
R_{X_1Y}	0.909	β_0	5.68×10^{-5}				
R_{X_2Y}	0.908	β_1	5.92	$v(\beta_1)$	51.35	$t(\beta_1)$	0.79
$R_{X_2X_1}$	0.999	β_2	−10.16	$v(\beta_2)$	208.2	$t(\beta_2)$	−0.70

(b)

Source of Variation	DF	Sum of Squares	Mean Square	Fobs
Regression	2	0.506	0.253	25.36
Deviations	10	0.0998	0.010	
Total	12	0.606	0.050	

Table 11. Calculation for fitting a multiple linear regression of the estimated $Y_i = \left(c = n_{CO_3} - n_{V^{Ca}}\right)$ the contribution of the mechanism III on the concentration of Na^+: $X_{1,i}$ and the concentration of CO_3^{2-}: $X_{2,i}$ in aqueous solution, (a) Calculation of regression and correlation coefficients and variances, (b) Values of F-test: Analysis of Variance.

(a)

Coefficients of Correlations		Coefficients of Regression		Variances		t-test	
R^2	0.970						
R_{X1Y}	0.825	β_0	7.61×10^{-4}				
R_{X2Y}	0.818	β_1	87.85	$v(\beta_1)$	76.93	$t(\beta_1)$	10.01
R_{X2X1}	0.999	β_2	−173.54	$v(\beta_2)$	311.83	$t(\beta_2)$	−9.83

(b)

Source of Variation	DF	Sum of Squares	Mean Square	Fobs
Regression	2	4.856	2.428	163.6
Deviations	10	0.1484	0.0148	
Total	12	5.004	0.417	

From **Table 11**, the multiple linear regression analysis shows that the relationship between these quantities is given by equation:

$$Y_i = c = 11.1\times10^{-4} + 87.83\cdot\left[\mathrm{Na}^+\right] - 173.5\cdot\left[\mathrm{CO}_3^{2-}\right] \quad (13)$$

An analysis of variance (**Table 11(b)**/ANOVA), further shows that F-test = 163.55 is higher than criterion F(5%, 2.9) = 4.26, this allowed us to rejet H_0: $\beta_1 = \beta_2 = 0$ and affirm that at least one of the predictors is linearly associated to the response. The individual t-tests on the regression coefficients β_1 and β_2 and, Hence, for β_1, t = 10, for β_2, t = −9.8 t(β_1) and t(β_2) are signifiants at P > 99.9% level.

From **Table 12**, the multiple linear regression analysis shows that the relationship between these quantities is given by equation:

$$Y_i = d = 2.59\times10^{-4} - 3.44\cdot\left[\mathrm{Na}^+\right] + 6.92\cdot\left[\mathrm{CO}_3^{2-}\right] \quad (14)$$

An analysis of variance (**Table 12(b)**/ANOVA), further shows that F-test = 0.10 is lower than criterion F(5%, 2.9) = 4.26, this test affirm that mechanism IV is unrelated to experiment conditions. The result of the present study provide that mechanisms I and III are the main in incorporation of Na^+ and CO_3^{2-} in HAp.

5.5. Multiple Linear Regression Analysis for the Determination of Formula of Unit Cell

The determination of unit cell has been realized by a multiple linear regression between the variables $Y = n_{Ca}$ and $X_1 = a$, $X_2 = c$, $X_3 = d$. Least square allows calculating the regression and correlation coefficients. The sample regression (prediction equation) is:

$\hat{Y} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$, where $\beta_0, \beta_1, \beta_2, \beta_3$ are the estimated regression coefficients. These have been calculated from the values of correlation coefficients, variance and covariance according to the method of Scherrer [27]. The results of calculations are given in **Table 13**. To testing the utility of the model, we conduct the F-test according to:

$$F = \frac{(n-m-1)\times R^2}{m\times(1-R^2)}, \quad (15)$$

where n is sample size, m is number of parameters and $(n-m-1)$ is degree of freedom.

From **Table 13**, the multiple linear regression analysis shows that the relationship between these quantities is given by equation:

$$Y_i = n_{Ca} = 10 - 0.989\cdot a - 1.00\cdot c - 1.00\cdot d \quad (16)$$

Table 12. Calculation for fitting a multiple linear regression of the estimated $Y = d = n_{Na} - c$ the contribution of the mechanism IV on the concentration of Na^+: $X_{1,i}$ and the concentration of CO_3^{2-}: $X_{2,i}$ in aqueous solution, (a) Calculation of regression and correlation coefficients and variances, (b) Values of F-test: Analysis of Variance.

(a)

Coefficient of Correlation		Coefficients of Regression		Variances		t-test	
R^2	0.018						
R_{X1Y}	−0.009	β_0	-22×10^{-4}				
R_{X2Y}	−0.007	β_1	−3.46	$v(\beta_1)$	62.4	$t(\beta_1)$	−0.44
R_{X2X1}	0.999	β_2	6.97	$v(\beta_2)$	253.2	$t(\beta_2)$	0.44

(b)

Source of Variation	DF	Sum of Squares	Mean of Squares	Fobs
Regression	2	0.0025	0.0012	0.10
Deviations	10	0.1279	0.0127	
Total	12	0.1304	0.0109	

Table 13. Calculation for fitting a multiple linear regression of the estimated $Y = n_{Ca} = Y$ in Na-CO_3HAps on the contribution of mechanism I a: X_{1i}, contribution of mechanism III c: X_{2i} contribution of mechanism IV d: X_{3i}.

Coefficient of Correlation		Coefficients of Regression		Variances		Co-variances	
R^2	0.998	β_0	10.00	$var(Y)$	0.626	$Cov(X_1,Y)$	−0.149
R_{X_1Y}	−0.875	β_1	−0.989	$v(X_1)$	0.047	$Cov(X_2,Y)$	−0.473
R_{X_2Y}	−0.965	β_2	−1.00	$v(X_2)$	0.385	$Cov(X_3,Y)$	−0.003
R_{X_3Y}	−0.038	β_3	−1.00	$v(X_3)$	0.010	$Cov(X_1X_2)$	0.099
$R_{X_2X_1}$	0.741					$Cov(X_1X_3)$	0.004
$R_{X_2X_3}$	−0.17					$Cov(X_2X_3)$	−0.010
$R_{X_3X_1}$	0.17						

An analysis of variance (ANOVA), further shows that F-test = 2018.9 is higher than criterion F(5%, 2.9) = 4.26, this allowed us to rejet H_0: $\beta_1 = \beta_2 = \beta_3 = 0$ and affirm that at least one of the predictors is linearly associated to the response.

for testing the regression coefficients β_1, β_2 and β_3 and discovering with variable(s) is related to estimate $Y = n_{Ca}$, we conduct on the one hand individual t-tests on the β's, Hence, for β_1, t = −5.28, for β_2, t = −28.3 and for β_3, t = −1.2 t(β_1) and t(β_2) are signifiants at P > 99.9% level. Thus, the molar n_{Ca} of Na-CO_3Aps solid is significantly depends on both a and c contributions of mechanisms I and III. The general formula can be written as follows:

$$Ca_{10-(a+c)}Na_{(c)}\left(PO_4\right)_{6-(a+c)}\left(CO_3\right)_{(a+c)}\left(OH\right)_{(2-a)}$$

Also, structural study for two samples obtained under comparable conditions [28] has been investigated extensively by physicochemical analysis and by Rietveld method refinements. The results of unit cell content calculated from the occupations of the atomic sites and the data of chemical composition $\left(n_{CO_3^{2-}} \cong 3n_{Na^+}\right)$ may be represented by the following ideal substitution scheme and corresponding solid solution :

$$2Ca^{2+} + 3PO_4^{3-} \leftrightarrow V^{Ca} + 3CO_3^{2-} + Na^+$$

(The sum of mechanism II and III)

$$Ca_{10-2/3x}Na_{x/3}V_{x/3}\left(PO_4\right)_{6-x}\left(CO_3\right)_x\left(OH\right)_2, \text{ with } x = 1.5 \text{ (I) and } 2.4 \text{ (II)}.$$

These results show that we cannot consider unique and well defined substitution mechanisms resulting in apatites, especially for homogeneous precipitation methods in aqueous solutions, because the lack of control of the reaction parameters as well as incomplete analyses of the solids could result in erroneous interpretations of the substitution mechanism.

On the other hand El Feki *et al.* [28] confirm that no vacancies of OH^- are observed by Rietveld refinements. But, small fraction of vacancies is undetectable by this method and was ignored in the structure refinements.

6. Conclusion

The different statistical analyses present in this review mainly focused on original and new approaches of the knowledge of the substitutions mechanisms. However, in biological calcifications, part of lattice ions of Hap are substituted to considerable extent ions. Consequently, these substitutions have an important influence on several processes (the growth, the dissolution, the mineralization and the demineralization processes. In order to derive the fundamental thermodynamic properties of the solid which determine the course of these processes, the stoichiometry of the apatite and especially of the mechanisms by which CO_3^{2-} and Na^+ are incorporated in the lattice must be known.

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