Effect of Ca/P Ratio on Biotype Hydroxyapatite Characterization

Abdul-Salam Khashan, Their Latif, Ismael Khalil and Sherin Abdul-Muhsin

Directorate of Materials Science, Ministry of Science and Technology
Al-Mustansiriya University, Ministry of Higher Education and Scientific Research

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ABSTRACT

The parameters required for the preparation of stiochiometric hydroxyapatite (HAP/ Ca_{10}(PO_4)_6(OH)_2) with biological Ca/P ratio 1.67 was carried out by using wet chemical method which refers to the drop wise addition of (NH_4)_2HPO_4 to Ca (NO_3)_2 solution.

The effect of intrinsic factors like those that pH, reaction temperature, ageing, digestion time and sintering temperature on the synthesis of HAP powders were studied and then the powder were characterized by analytical and spectral techniques. The method is indicating the formation of crystalline HAP with no major decomposition peaks and absence of impurities by close control of the all process parameters.

INTRODUCTION

Hydroxyapatite Ca_{10}(PO_4)_6(OH)_2 (HAP) is the most versatile material used for implantation purposes owing to their similarity with natural bone mineral and its ability to bond to bone. These materials characterized by a certain solubility, which provokes the surrounding bone or tissue to form direct bonding to the implant [1, 2].

The solubility leads to gradual degradation and resorption by the surrounding tissue, which stimulates the bone to grow on the material and through its pores, and in some cases; it believed to cause total transformation of the material into living bone [3]. This bonding is able to transfer shear and tensile stress along the interface that could be an advantage in anchoring the implants and reducing the stress peaks in the bone. The main restriction of these materials lies
in their low strengths so that they can use as bulk materials only for low loaded devices [4].

The dominant requirements connected with the development of HAP coatings on metallic implants are preparation of stiochiometric powder material with required chemical and phase composition established by their chemical identity (Ca/P ratio 1.67) and by close crystallographical affinity with bone tissue and their deposition as coatings without the presence of non-stiochiometric phases of the powder [5,6].

The objective of the present study is to provide a novel and simple process for the preparation of hydroxyapatite powder suitable to use in biomedical applications with exactly Ca/P ratio suitable to use in bio fields.

**MATERIAL AND METHODS**

*Synthesis of Hydroxyapatite (HAP)*

Hydroxyapatite is generally synthesised by wet chemical method, which involves the addition of phosphate reagent to a solution of calcium ions in one of two main methods (method I: Ca (OH)₂ – H₃PO₄ [7]), and method– II: Ca (NO₃)₂ – (NH₄)₂HPO₄, which has been currently administrated below.

A solution of analytical reagent grade calcium nitrate (1M) adjusted to pH 11 with concentrated NH₄(OH)₂ in 45 ml of degassed distilled water and diluted to 90 ml. A solution of diammonium hydrogen phosphate, 0.6 M in 75 ml distilled water brought to pH 11 with 37.5 ml of aqueous NH₃ and diluted to 160 ml. The Ca(NO₃)₂.4H₂O solution was vigorously stirred at room temperature and the phosphate solution ((NH₄)₂HPO₄) was added drop wise for 2 hours to produce a milky white semi gelatinous precipitate which was then stirred for 20-30 hours.

The changes in the pH of the reaction system during HAP synthesis maintained with aqueous NH₃ at pH 11 using the pH meter. It left for ageing for 24 hours, followed by stirring for 30 minutes, then filtered, washed thoroughly with double distilled water and dried in an air oven at 110 °C for 3 hours. Sintering of the precipitate carried out in a muffle furnace. The samples initially kept at a temperature of 240 °C for 1 hour to remove the traces of ammonium nitrate that could be present and then raised to 900 °C for the same time and cooled in the furnace after sintering.
RESULTS AND DISCUSSION

Wet Chemical Method

Hydroxyapatite is generally synthesised by wet chemical method, which involves the addition of phosphate reagent to a solution of calcium ions and occurs by a mechanism, which is interracially controlled [1,4]. The reactants dissolve incongruently and HAP over grows on these particles and its conversion eventually becomes diffusionally controlled. The rate of formation of the products is dependent on the solution chemistry and reaction temperature. However, stiochiometric HAP may only obtained in small quantities after using time consuming (several months) and tedious processes. It is a great challenge to prepare large amounts of HAP by precipitation with in a few hours or days. Hence, the methods listed below explore the possibilities to over come these drawbacks to synthesis stiochiometric hydroxyapatite (Ca/P ratio 1.67) devoid of other calcium phosphate phases.

Hydroxyapatite with varying Ca/P ratio from 1.28 to 2.01 is synthesised by wet chemical methods under varying experimental conditions. The physiochemical parameters of HAP give in table 1. The number with prefix C indicates the sample identity numbers. The Ca/P molar ratios reported in this table obtained after sintering the powder.

<table>
<thead>
<tr>
<th>Sample Identity no.</th>
<th>Strength of Solution (conc.)</th>
<th>Ageing Time (hr)</th>
<th>Addition Rate ML/min</th>
<th>Ca/P molar Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Ca(NO₂)₂ 1.0 (NH₄)₂HPO₄ 0.6</td>
<td>24</td>
<td>1.10</td>
<td>1.61</td>
</tr>
<tr>
<td>C2</td>
<td></td>
<td>24</td>
<td>1.80</td>
<td>1.48</td>
</tr>
<tr>
<td>C3</td>
<td></td>
<td>24</td>
<td>1.30</td>
<td>1.67</td>
</tr>
<tr>
<td>C4</td>
<td></td>
<td>30</td>
<td>1.30</td>
<td>1.78</td>
</tr>
<tr>
<td>C5</td>
<td></td>
<td>24</td>
<td>1.30</td>
<td>2.01</td>
</tr>
<tr>
<td>C6</td>
<td></td>
<td>16</td>
<td>1.30</td>
<td>1.28</td>
</tr>
</tbody>
</table>

Ca/P Stiochiometric Ratio

A change in the stoichiometry of hydroxyapatite (Ca/P 1.67) primarily influences its structure, properties and hence its nature of application in implantation and oral surgery [8, 9]. HAP with Ca/P ratio of 1.67 is non-resorbable while α and β- TCP and other nonstoichiometric CaP phases are resorbable when implanted in the human body.

\[ 5 \text{Ca(OH)₂} + 3\text{H₃PO₄} \rightarrow \text{Ca₅(PO₄)₃OH} + 9\text{H₂O} \]
The large amount of water produced leads to a decrease in pH of the reaction system on completion of the reaction. These factors bring complexity in controlling the reaction conditions to prepare the powder of desired characteristics [4].

The Ca/P ratio for the powders prepared using Ca(NO$_3$)$_2$ under NH$_3$ alkaline conditions varied from 1.28 to 2.01. During the sintering process, the amorphous products formed initially were converted to crystalline HAP, which is in agreement with the results of Berry and Eanes et al. The decreases in Ca/P ratio in samples is, due to the formation of Ca$^{2+}$ deficient apatite and decrease in pH. The apparently low Ca/P ratios obtained is due to the existence of absorbed phosphate ions on the precipitated solids and at low pH, where HPO$_4^{2-}$ ions are more stable than PO$_4^{3-}$ ions in the neighborhood of hydroxide ions, the non-stiochiometric HAP is obtained.

When HAP precipitated from a basic aqueous solution, three main solid phases formed, ACP, Octa calcium phosphate and HAP ascending in thermodynamic order and descending on the time scale. This is in agreement with well-published theory of on crystal growth [6], that "growth units (ions) constantly bombarded the crystal surface and are attracted by electrical charges of the ions on the surface. As the water molecule leaves the ion, it passes through an activated state and the activation energy (Gibbs) is several times the thermal energy ($kT$). Therefore, an adsorption layer form and constantly renewed. The ions hydrated in solution and give of some of their hydration water molecules in order to get closer to the ions already integrated on to the surface. Therefore, the adsorption rate may be controlled by the partial dehydration of the cations or by partial penetration through the hydration layer of the crystal [10]". This may explain why ACP is to precipitate first, because the formation of a solid containing the hydration sphere, like the ions in solution requires much lesser energy than Ea (energy barrier). The total time required for amorphous to crystalline transformation increases with increasing pH. The stirring rate, slurry concentration and particle size altered the induction time but these factors did not change the transformation half-life. The conversion started sooner in the presence of fast stirring, smaller amorphous particles and/or greater concentration of the amorphous particles. The increased rate of conversion with increased water to solid ratio reported as up to 400:1 further illustrate the necessity for water in this conversion [11].

The theory of the process of conversion of amorphous to crystalline product states that solution consisting of stable and crystalline nucleus alone result in the subsequent growth
proliferation of the material. An induction period where no formation of crystalline material observed is a characteristic feature of such reactions. It is during this period that a critical supersaturating attained subsequent to the formation of the primary nuclei. In the ACP to HAP transformation, the induction period includes additionally the time required for the amorphous calcium phosphate to dissolve and is a function of all factors related to the solubility of ACP. Thus, the induction time is decreased by using smaller ACP particles, higher ACP slurry concentration, aqueous rather than non aqueous solutions. Lower pH and higher temperature all of which decrease the time required for some critical degree of supersaturating to reach [9].

The conversion of amorphous precursor phase to HAP has shown to be autocatalytic, while the rate of conversion at any given temperature depends on the pH of the mediating solution [12, 13]. In hydroxylated amorphous phase, the hydroxide ions provide more charge carriers per unit weight and therefore movement of anions for crystallization will occur more readily [8].

In dilute solutions where only one phase seen, the growth of HAP appears to occur by a ripening process after a brief period during which nucleation and growth probably take place simultaneously. In addition, in this system, it is seem that the number of particles decreases as a function of time suggesting that the smaller more soluble particles dissolve and redeposit on larger particles. Again, this reinforces the view that a ripening process is taking place.

The lack of systematic deviation of Ca/P from the expected ratio for hydroxyapatite of 1.67 indicates that during the course of the experiment only one phase is being formed and that the precipitated phase has the Ca/P ratio expected for HAP. The rate of addition, pH, and the ripening processes play a critical role in the formation of stoichiometric HAP. It is during the ripening process that the ACP formed initially converted to crystalline HAP [2, 5].

The existence of phosphate species and its effects on stoichiometry

The rates of crystallization initially surge during the first few minutes of the reaction, which might be due to the rapid reconditioning of the seed surface from that of the saturated suspension to the higher supersaturated solution concentration. The decrease in the rate, with the extension of reaction is not due to the available surface area for growth, but it could attribute to a surface maturation phenomenon in which the number of available growth sites decrease to a steady state value under conditions of sustained supersaturation. In general, apatites have a relatively high specific
surface area as compared to many sparingly soluble salts. When normalized with respect to this parameter HAP seen to have a relatively slow growth and a high sensitivity to the presence of molecules, which may adsorb to the surface [15].

When the precipitating conditions improperly maintained in the apatite systems, various factors contribute to induce non-stoichiometry, which summarized by [14]. From the experimental results presented in table 1, the non stoichiometry in the samples are attributed to high addition rates of phosphate solutions or low Ca/P ratios, temperature and also by the presence of mono hydrogen orthophosphate HPO$_4^{2-}$.

In the pH interval between 7.21 and 12.3, predominate species is HPO$_4^{2-}$. The range of the stability of the different phosphate species vs. pH is given in figure 1. The pH is necessarily to maintain at 9 to 11, because when the pH is around 10 the predominant species is HPO$_4^{2-}$, which also be inferred from figure 1. Hence, at pH 10, the rate of formation of stiochiometric HAP is high due to increased amount of OH$^-$ and HPO$_4^{2-}$ species. At higher pH above 10.5, PO$_4^{3-}$ and NH$_4$ are predominant when compared with other phosphate species while at pH lower than 8, HPO$_4^{2-}$ species is dominant.

The calcium phosphates decrease in solubility in the order CaHPO$_4$.2H$_2$O (brucite) > CaHPO$_4$ (monetite) > Ca$_8$H$_2$PO$_4$.5H$_2$O (octacalcium phosphate) > β- Ca$_3$(PO$_4$)$_2$ (β- tricalcium phosphate) > Ca$_5$(PO$_4$)$_3$OH (hydroxyapatite) [1, 14].

Initially at pH between 9.5 and 10.5, the solution supersaturated with respect to HAP and the precipitation takes place. From figure 1 it can be observed that in the pH range between 9 and 10.5 the predominant species together with HPO$_4^{2-}$, (can be HCO$_3^-$ or CO$_3^{2-}$ as traces with starting material) consequently, both of them can precipitate together with HAP.
Fig- 1: Distribution of phosphate species as a function of pH [3].

Characterization of HAP
XRD analysis

X-ray diffraction studies of the powder samples carried out only for the samples, which supplemented by their chemical analysis of near stiochiometric HAP. Figure 2 shows the XRD patterns recorded for stiochiometric HAP sample. The peaks indexed based on the JCPDS file card numbers for the various CaP phases that could exist in HAP (JCPDS file card no. 9-432) [16].

The XRD patterns of HAP sintered at 900 °C given in (fig.2) did not contain any peak other than those of HAP. The CaP ratio measured as 1.6 ± 0.01, which is very close to the Ca/P stiochiometric ratio (1.67) of perfect HAP.

Fig -2: XRD pattern of HAP Prepared
Morphological Studies

The morphology of the HAP powders indicates that it is composed of spheroid and angular agglomerates with wider particle size distributions. The micrograph shows the presence of nearly spherical agglomerates of (1-10 µm) in diameter.

The formation of a regular crystal structure observed that could group in crystal colonies of different morphologies with some entanglement. It is also possible to observe the presence of some sintered polyhedrons (pentagon dodecahedrons) stacks in the micrograph [15, 17].

The HAP powders indicated as a single phase by XRD investigations had a pore free microstructure. Figure 3 showed the micrograph of HAP powder synthesized by wet chemical method.

![SEM Micrographs of HAP Powder Prepared](image)

CONCLUSIONS

Nanostructure calcium phosphate materials are required for biotype HAP applications to identified as potentially useful materials for a number of biomedical applications including scaffolds for tissue engineering and as carrier for non-viral gene delivery.

The results of present work indicate that the nature of the reagents, pH of solutions and ageing time influences the composition of the final product. The reproducibility of the process achieved by close control of these parameters.

HAP with Ca/P ratio of (1.67) is non-resorbable while α and β-TCP and other nonstoichiometry CaP phases are resorbable when implanted in the human body.

A change in the stiochiometric of hydroxyapatite Ca/P (1.67) primarily influences its structure, properties and its nature of application in implantation and oral surgery.

The pH of 10 required to maintain for all the system II powders, as the state of HPO$_4^{2-}$ species is pH dependent. A slow rate of addition of phosphate solution was found to be at 1.3 -1.50 ml/min, accompanied
an appropriate ripening procedure is essential to obtain stoichiometric HAP.

During the ageing process, the amorphous phases formed converted to crystalline HAP. Sintering of the precipitates at 900°C causes densification and increases the mechanical strength of the precipitates.

REFERENCES