

Hydroxyapatite Synthesis Methodologies: An Overview

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ABSTRACT: Hydroxyapatite (HAp) is the emerging most bioceramic, which is widely used in various biomedical applications, mainly in orthopedics and dentistry due to its close similarities with inorganic mineral component of bone and teeth. Synthetic HAp is known to be similar to naturally occurring HAp on the basis of crystallographic and chemical studies. Several methodologies have been investigated and developed for the synthesis of HAp. But, new economic and versatile methods for HAp synthesis are of interest due to its immense importance and wide utilization in biomedical applications. This review presents various well known methodologies for HAp synthesis like precipitation technique, sol-gel approach, hydrothermal technique, multiple emulsion technique, biomimetic deposition technique, electrodeposition technique etc.

Key words: Hydroxyapatite; Bioceramic; Precipitation technique; Sol-gel approach; Biomimetic deposition

INTRODUCTION

During past few decades, considerable research efforts have been directed towards the synthesis of various bioceramics for biomedical applications. Among different classes of bioceramics, hydroxyapatite (HAp) is the emerging most bioceramic, which is widely used in various biomedical applications, mainly in orthopedics and dentistry. HAp has close similarities with inorganic mineral component of bone and teeth¹. It possesses exceptional biocompatibility and unique bioactivity²⁻⁴. Naturally occurring HAp is hexagonal in structure with the chemical formula of one unit cell being $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ⁵. The hydroxyl ion (OH^-) of it can be replaced by F^- , Cl^- , CO_3^{2-} , etc in the collagen fiber matrix.

Synthetic HAp is known to be similar to naturally occurring HAp on the basis of crystallographic and chemical studies⁶. Because, synthetic HAp is thermodynamically stable at physiological pH⁵ and osteoconductive, it has been widely used in hard tissue replacement and reconstruction applications, such as implant coatings⁷, and bone substitutes⁸ etc. Its porous character also

offers high binding affinity for a variety of pharmacological substances such as antibiotics, hormones, enzymes, antibody fragments, steroids etc⁹⁻¹⁴. This has opened the potential for using synthetic HAp to deliver pharmacological substances in many clinical applications with sustained release capacity for the treatment of osteomyelitis, osteoporosis, osseous cancers etc in which local delivery is effective with the need to fill defects in the skeleton. New economic and versatile methods for HAp synthesis are of interest due to the importance of this material for various biomedical applications. The current communication deals with various methodologies for the preparation of synthetic HAp with optimum properties closer to those of living hard tissues like bone and teeth, aiming at better and more effective biomedical applications.

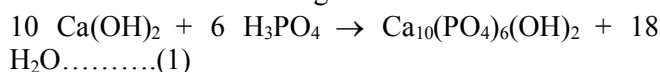
METHODS FOR HAp SYNTHESIS

Several methods have been utilized for the synthesis of HAp include precipitation technique¹⁵⁻¹⁷, sol-gel approach¹⁸, hydrothermal technique¹⁹, multiple emulsion technique²⁰, biomimetic deposition technique²¹⁻²², electrodeposition technique²³ etc.

Precipitation technique:

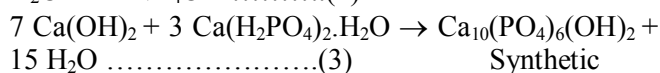
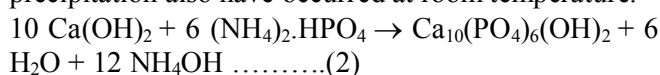
The most popular and widely researched technique for synthesis of HAp is precipitation technique. This technique is also called as wet precipitation or chemical precipitation or aqueous precipitation. This technique is chosen widely to synthesize HAp in contrast to other techniques. Because, relatively large amount of HAp can be produced by precipitation technique in absence of organic solvents at a reasonable cost¹⁵.

This precipitation reaction for synthesis of HAp was first proposed by Yagai and Aoki, as indicated by Bouyer et al²⁴. Calcium hydroxide [Ca(OH)₂] and orthophosphoric acid [H₃PO₄] were starting materials of this reaction (equation 1). The only byproduct of this reaction was water and the reaction involved no foreign elements.



The size, shape and surface area of the HAp particles obtained by this reaction are very sensitive to the orthophosphoric acid addition rate and the reaction temperature. The orthophosphoric acid addition rate is strongly linked to the pH obtained at the end of the synthesis, and also to the suspension stabilization. The reaction temperature determines whether the synthetic HAp crystals are monocrystalline or polycrystalline. HAp particles synthesized at low temperature (< 60°C) are monocrystalline²⁵.

Santos et al¹⁵ has stated another two reactions for the synthesis of HAp by precipitation technique. In one of them, ammonium phosphate [(NH₄)₂.HPO₄] and Ca(OH)₂ were starting materials (equation 2). Whereas in another reaction approaches, calcium hydrogen phosphate [Ca(H₂PO₄)₂.H₂O] and Ca(OH)₂ were starting materials (equation 3). The pH was monitored in both cases, but not corrected. In the first reaction, the temperature of HAp synthesis was maintained at 40°C and in the second, synthesis was done at room temperature. A higher temperature was used to enhance the reaction kinetics of HAp formation and to improve Ca(OH)₂ dissolution, although the HAp precipitation also have occurred at room temperature.

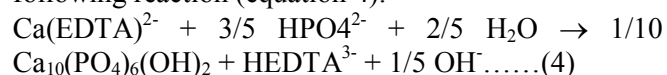


Synthetic HAp nanoparticles can be prepared by precipitation technique under stirring at room temperature and pH, 10, as stated by Manuel et al¹⁷. The H₃PO₄ was added to Ca(OH)₂ until Ca/P = 1.67 is maintained. Crystallization started after NH₄OH addition. Crystal growth was allowed for 24 hours and sinteration performed at 1000°C for 1 hour.

Jarcho et al¹ has proposed an another possibility of precipitation method, which involves a

wet chemical reaction of calcium nitrate [Ca(NO₃)₂.4H₂O], with (NH₄)₂.HPO₄. The grain size of the synthesized HAp by this reaction can be controlled by changing the reaction time and temperature²⁶. Specifically to obtain HAp with grain sizes < 100 nm, the solution requires continuous stirring at room temperature for 24 hours²⁵.

Janackovic et al²⁷ modified based on the homogeneous precipitation technique using the following reaction (equation 4):



The modification developed constituted of the addition of urea for precipitation instead of NaOH, which led to more homogenous precipitation and further transformation to HAp due to pH changes for urea hydrolysis. The reaction temperature varied between 125°C and 160°C.

Sol-gel approach:

The sol-gel approach is an effective method for the synthesis of nanophasic HAp, due to the possibility of a strict control of process parameters²⁸. This method offers a molecular level mixing of the calcium and phosphorous, which is capable of improving the chemical of resulting HAp to a significant extent. Only limited attempts have been reported on the sol-gel processing for the HAp materials²⁹⁻³⁰. It has been reported that the HAp materials synthesized by sol-gel process are efficient to improve the contact and stability at the artificial/natural bone interfaces in both *in vitro* and *in vivo* environment³¹.

A number of calcium and phosphorous precursor combinations were employed for sol-gel synthesis of HAp. Again, chemical activity and the temperature required to form the apatite structure depends largely on the chemical nature of the precursors. Balamurugan et al²⁸ used Ca(NO₃)₂.4H₂O and triethyl phosphate as calcium and phosphorous precursor respectively, when the stoichiometric Ca/P ratio 1.67 was maintained. The synthesized HAp powders were dried and calcinated at different temperatures up to 900°C. Brendel et al³² synthesized HAp at low temperature (400°C) using Ca(NO₃)₂.4H₂O and phenyl dichlorophosphite [C₆H₅PCl₂] as precursors. But, the resulting HAp had low purity and poor crystallinity. Further increase in temperature up to 900°C resulted in a pure, well-crystallized HAp phase. Takahashi et al³³ developed a sol-gel route for synthesis of HAp using Ca(NO₃)₂.4H₂O and phosphonoacetic acid [HOOCCH₂PO(OH)₂] in an aqueous solution at 700°C. The crystallinity was improved with the increasing of temperature up to 1100°C. In an another approach, Vijayalakshmi et al³⁴ synthesized microcrystalline HAp powders from

calcium acetate and triethyl phosphate in water and ethanol medium. Haddow et al³⁵ used calcium acetate together with various phosphorous precursors, i.e. phosphoric acid [H₃PO₄], phosphorous pentoxide [P₂O₅] and triethyl phosphite for HAp coating. Among them, the HAp coating using calcium acetate and triethyl phosphate showed the best result. The temperature required to form an apatite phase was > 600°C

Hydrothermal technique:

In the 20th century, the hydrothermal technique for synthesis of materials was clearly identified as an important technology³⁶ and using this technology various ceramic materials including HAp can be synthesized. Hydrothermal synthesis is a process that utilizes single or heterogeneous phase reactions in aqueous at elevated temperature (T > 25°C) and pressure (P > 100 kPa) to crystallize ceramic materials directly from solutions³⁶. However, with the hydrothermal treatment, the Ca/P ratio for the precipitates improves with the increase in hydrothermal pressure or temperature³⁷.

Manafi et al¹⁹ synthesized HAp by dissolving CaHPO₄. 2H₂O/NaOH/distilled water, followed by adding 2-3 gm cetyl trimethyl ammonium bromide (CTAB). The hydrothermal synthesis was conducted at 150°C for 2 hours in an electric oven.

Multiple emulsion technique:

Kimura²⁰ developed an alternative approaches for HAp synthesis by interfacial reaction in a multiple emulsion. The multiple emulsion was a w/o/w emulsion, made of dipotassium hydrogen phosphate [K₂HPO₄] solution as an inner aqueous phase, benzene as an oil phase, and Ca(NO₃)₂. 4H₂O as an outer aqueous phase. The interfacial reaction was carried out at 323 K for 24 hours. The crystalline phase was varied with an initial pH of the inner aqueous phase, and a single HAp was synthesized at an initial pH of 12. The synthesized products were composed of porous microspheres of less than 3 μm in size. This method has some advantages²⁰. A common stirred tank is sufficient to be used as reactor, and therefore, any special apparatus is unnecessary. Low synthesis temperature around room temperature are usable.

Biomimetic deposition technique:

Metastable synthetic body fluid (SBF) with an organic salt composition similar to that of human body fluid (blood plasma), facilitate the spontaneous nucleation and growth of nanosized, carbonated and 'bone-mimic' HAp at physiological pH and temperature. Thamaraiselvi et al²² synthesized biomimetic HAp from Ca(NO₃)₂. 4H₂O and (NH₄)₂.HPO₄, dissolved in SBF at 37°C.

Table 1: Ion concentrations of SBF solutions²²

Ions	Concentration, mM
Na ⁺	142.00
Cl ⁻	125.00
HCO ₃ ⁻	27.00
K ⁺	5.00
Ca ²⁺	2.50
HPO ₄ ²⁻	1.00
SO ₂ ²⁻	0.50
Mg ²⁺	1.50

The SBF is prepared according to the chemical composition of human body fluid, with various ion concentrations nearly similar to the inorganic constituent of human blood plasma. The ion concentrations of SBF solution is given in Table 1. Metastable SBF has been proven to generate the growth of carbonated and 'bone-mimetic' apatite on various orthopaedic and dental biomaterials like silica, titania, bioglass etc at physiological pH and temperature³⁸⁻⁴⁰. The formation of apatite layer by this biomimetic deposition process on several orthopaedic and dental biomaterials was proven to promote *in vitro* cell differentiation in mineralized chondrocyte cell culture system⁴¹ and induce osteogenic cell differentiation with subsequent bone-matrix apposition, which allows a strong bonding with bone⁴².

Using this method, various porous implants can be coated with nanosized carbonated HAp biomimetically by immersing implants in SBF⁴³. The nature of the HAp coating, via its microstructure, its dissolution rate and its specific interactions with body fluids, can influence the osteogenicity of the coating as well as the bone remodeling process.

Electrodeposition technique:

Ultrafine-grained, nanophase HAp coating can be synthesized by electrodeposition from dilute electrolytes [Ca²⁺] = 6.1 X 10⁻⁴ M, [PO₄³⁻] = 3.6 X 10⁻⁴ M at physiological pH²³. The precursors used for the electrodeposition of HAp coatings were Ca(NO₃)₂ and NH₄H₂PO₄. Sodium nitrate NaNO₃ was used to improve the electrolytes ionic strength. Manso et al⁴⁴ investigated the growth of HAp coating induced by constant anodic voltages (2-4 V) in an alkaline electrolyte.

CONCLUSION

It can be concluded that HAp can be synthesized by various methods like precipitation technique, sol-gel approach, hydrothermal technique, multiple emulsion technique, biomimetic deposition

technique, electrodeposition technique etc. Scientists and researchers are engaged in solving various challenges related with synthesis HAp with optimum properties for the use in various biomedical

applications. We expect that, with continuous research efforts they will develop various novel and economic synthesis methodologies of HAp for the near future.

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