www.sphinxsai.com

ChemTech

International Journal of ChemTech Research
CODEN( USA): IJCRGG ISSN : 0974-4290
Vol.2, No.2, pp 903-907, April-June 2010

# Hydroxyapatite Synthesis Methodologies: An Overview

**Amit Kumar Nayak***

**Seemanta Institute of Pharmaceutical Sciences, Jharpokharia, Mayurbhanj-757086. Orissa, India**

***Corres.author: amitkrnayak@yahoo.co.in,Tele: +91-9583131603.**

**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 teeth[1]. It possesses exceptional biocompatibility and unique bioactivity[2-4]. Naturally occurring HAp is hexagonal in structure with the chemical formula of one unit cell being $Ca_{10}(PO_4)_6(OH)_2$ [5]. The hydroxyl ion ($OH^-$) od 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 studies[6]. Because, synthetic HAp is thermodynamically stable at physiological pH[5] and osteoconductive, it has been widely used in hard tissue replacement and reconstruction applications, such as implant coatings[7], and bone substitues[8] etc. Its porous character also offers high binding affinity for a variety of pharmacological substances such as antibiotics, hormones, enzymes, antibody fragments, steroids etc[9-14]. 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 technique[15-17], sol-gel approach[18], hydrothermal technique[19], multiple emulsion technique[20], biomimetic deposition technique[21-22], electrodeposition technique[23] 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 cost[15].

This precipitation reaction for synthesis of HAp was first proposed by Yagai and Aoki, as indicated by Bouyer et al[24]. Calcium hydroxide [$Ca(OH)_2$] and orthophosphoric acid [$H_3PO_4$] were starting materials of this reaction (equation 1). The only byproduct of this reaction was water and the reaction involved no foreign elements.

$10\ Ca(OH)_2 + 6\ H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18\ H_2O$…….(1)

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 monocrystalline[25].

Santos et al[15] has stated another two reactions for the synthesis of HAp by precipitation technique. In one of them, ammonium phosphate [$(NH_4)_2.HPO_4$] and $Ca(OH)_2$ were starting materials (equation 2). Whereas in another reaction approaches, calcium hydrogen phosphate [$Ca(H_2PO_4)_2.H_2O$] 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.

$10\ Ca(OH)_2 + 6\ (NH_4)_2.HPO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6\ H_2O + 12\ NH_4OH$ …….(2)

$7\ Ca(OH)_2 + 3\ Ca(H_2PO_4)_2.H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 15\ H_2O$ …….(3) Synthetic HAp nanoparticles can be prepared by precipitation technique under stirring at room temperature and pH, 10, as stated by Manuel et al[17]. The $H_3PO_4$ was added to $Ca(OH)_2$ until Ca/P = 1.67 is maintained. Crystallization started after $NH_4OH$ addition. Crystal growth was allowed for 24 hours and sinteration performed at 1000°C for 1 hour.

Jarcho et al[1] has proposed an another possibility of precipitation method, which involves a wet chemical reaction of calcium nitrate [$Ca(NO_3)_2.4H_2O$], with $(NH_4)_2.HPO_4$. The grain size of the synthesized HAp by this reaction can be controlled by changing the reaction time and temperature[26]. Specifically to obtain HAp with grain sizes < 100 nm, the solution requires continuous stirring at room temperature for 24 hours[25].

Janackovic et al[27] modified based on the homogeneous precipitation technique using the following reaction (equation 4):

$Ca(EDTA)^{2-} + 3/5\ HPO4^{2-} + 2/5\ H_2O \rightarrow 1/10\ Ca_{10}(PO_4)_6(OH)_2 + HEDTA^{3-} + 1/5\ OH^-$……(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 parameters[28]. 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 materials[29-30]. 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* environment[31].

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 al[28] used $Ca(NO_3)_2.4H_2O$ and triethyl phosphate as calcium and phosphorous precursor respectively, when the stoicheometric Ca/P ratio 1.67 was maintained. The synthesized HAp powders were dried and calcinated at different temperatures up to 900°C. Brendel et al[32] synthesized HAp at low temperature (400°C) using $Ca(NO_3)_2.4H_2O$ and phenyl diclorophosphite [$C_6H_5PCl_2$] 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 al[33] developed a sol-gel route for synthesis of HAp using $Ca(NO_3)_2.4H_2O$ and phosphonoacetic acid [$HOOCCH_2PO(OH)_2$] 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 al[34] synthesized microcrystalline HAp powders from

calcium acetate and triethyl phosphate in water and ethanol medium. Haddow et al[35] used calcium acetate together with various phosphorous precursors, i.e. phosphoric acid [$H_3PO_4$], phosphorous pentoxide [$P_2O_5$] 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 technology[36] 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 solutions[36]. However, with the hydrothermal treatment, the Ca/P ratio for the precipitates improves with the increase in hydrothermal pressure or temperature[37].

Manafi et al[19] synthesized HAp by dissolving $CaHPO_4$. $2H_2O$/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:**

Kimura[20] 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 [$K_2HPO_4$] solution as an inner aqueous phase, benzene as an oil phase, and $Ca(NO_3)_2$. $4H_2O$ 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 advantages[20]. 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 al[22] synthesized biomimetic HAp from $Ca(NO_3)_2$. $4H_2O$ and $(NH_4)_2.HPO_4$, dissolved in SBF at 37°C.

**Table 1: Ion concentrations of SBF solutions[22]**

| 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 temperature[38-40]. 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 system[41] and induce osteogenic cell differentiation with subsequent bone-matrix apposition, which allows a strong bonding with bone[42].

Using this method, various porous implants can be coated with nanosized carbonated HAp biomimmetically by immerging implants in SBF[43]. The nature of the HAp coating, via its microstructure, its dissolution rate and its specific interactions with body fluids, can influence the osteogenecity 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 [$Ca^{2+}$] = 6.1 X $10^{-4}$ M, [$PO_4^{3-}$] = 3.6 X $10^{-4}$ M at physiological pH[23]. The precursors used for the electrodeposition of HAp coatings were $Ca(NO_3)_2$ and $NH_4H_2PO_4$. Sodium nitrate NaNO3 was used to improve the electrolytes ionic strength. Manso et al[44] 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.

**REFERENCES**


1. Jarcho M, Kay JF, Gumar KI, Doremus RH, Drobeck HP. Tissue, cellular and subcellular events at a bone ceramic hydroxyapatite interface. J Biosci Bioeng. 1977; 1: 79-92.
2. Bagambisa FB, Joos U. Preliminary studies on the phemomenological behaviors of osteoblasts cultured on hydroxyapatite ceramics. Biomaterials. 1990; 11: 50-56.
3. Wang M. Developing bioactive composite materials for tissue replacement. Biomaterials. 2003; 23: 2133-2151.
4. Chen QZ, Wong CT, Lu WW, Cheung KMC, Leong JCY, Luk KDK. Strengthening mechanisms of bone-bonding to crystalline hydroxyapatite *in-vivo*. Biomaterials. 2004; 25: 4243-4254.
5. Nath S, Basu B, Sinha A. A comparative study of conventional sintering with microwave sintering of hydroxyapatite synthesized by chemical route. Trends Biomater Artif Org. 2006; 19(2): 93-98.
6. Posner AS. The structure of bone apatite surfaces. J Biomed Mater Res. 1985; 19: 241-250.
7. Jaffe WL, Scott DF. Total hip arthroplasty with hydroxyapatite-coated prosthesis. J Bone Jt Surg. 1996; 78A: 1818-1934.
8. Oonishi H. Orthopaedic applications of hydroxyapatite. Biomaterials. 1991; 12: 171-178.
9. Netz DJA, Sepulveda P, Pandolfelli VC, Spadaro ACC, Alencastre JB, Bentley MVLB, Marchetti JM. Potential use of gelcasting hydroxyapatite porous ceramic as an implantable drug delivery system. Int J Pharm. 2001; 213: 117-125.
10. Zafirau W, Parker D, Billotte W, Bajpai PK. Development of ceramic device for the continuous local delivery of steroids. Biomed Sci Instrum. 1996; 32: 63-70.
11. Slosarczyk A, Szymura-Oleksiak J, Mycek B. The kinetics of pentoxifylline release from drug-loaded hydroxyapatite implants. Biomaterials. 2000; 21: 1215-1221.
12. Nayak AK, Bhattacharya A, Sen KK. Hydroxyapatite-antibiotic implantable minipellets for bacterial bone infections using precipitation technique: Preparation, characterization and in-vitro antibiotic release studies. J Pharm Res. 2010; 3(1): 53-59.
13. Pham HH, Luo P, Genin F, Dash AK. Synthesis and characterization of hydroxyapatite-ciprofloxacin delivery systems by precipitation and spray drying technique. AAPS PharmSci Tech. 2002; 3(1): article 1.
14. Abrams LB, Bajpai PK. Sustained release of heparin using hydroxyapatite. The 20 th Annual Meeting of the Society for Biomaterials. Boston, MA, USA. 1994; April 5-9: 165.
15. Santos MH, de Oliveira M, de Freitas Souza P, Mansur HS, Vasconcelos WL. Synthesis control and characterization of hydroxyapatite prepared by wet precipitation process. Mater Res. 2004; 7(4): 625-630.
16. Manuel CM, Ferraz MP, Monteiro FJ. Nanoapatite and microporous structures of hydroxyapatite. Proceeding of the 17 th European Society of Biomaterials. Barcelona, Spain. 2002: T 153.
17. Manuel CM, Ferraz MP, Monteiro FJ. Synthesis of hydroxyapatite and tri calcium phosphate nanoparticles. Preliminary Studies. Key Eng Mater. 2003; 240-242: 555-58.
18. Chai CS, Ben-Nissan B. Bioactive nanocrystalline sol-gel hydroxyapatite coatings. J Mater Sci: Mater Med. 1999; 10: 465-469.
19. Manafi SA, Joughehdoust S. Synthesis of hydroxyapatite nanostructure by hydrothermal condition for biomedical application. Iranian J Pharm Sci. 2009; 5(2): 89-94.
20. Kimura I. Synthesis of hydroxyapatite by interfacial reaction in a multiple emulsion. Res Lett Mater Sci. 2007; Article ID 71284: 1-4.
21. Tas AC. Synthesis of biomimetic Ca-hydroxyapatite powders at 37 degrees C in synthetic body fluids. Biomaterials. 2000; 21: 1429-1438.
22. Thamaraiselvi TV, Prabakaran K, Rajeswari S. Synthsis of hydroxyapatite that mimic bone mineralogy. Trends Biomater Artif Org. 2006; 19(2): 81-83.
23. Shikhanzadeh M. Direct formation of nanophase hydroxyapatite on cathodically polarized electrodes. J Mater Sci: Mater Med. 1998; 9: 67-72.
24. Bouyer E, Gitzhofer F, Boulos MI. Morphological study of hydroxyapatite nanocrystal suspension. J Mater Sci: Mater Med. 2000; 11: 523-531.
25. Ferraz MP, Monteiro FJ, Manuel CM. Hydroxyapatite nanoparticles: A review of preparation methodologies. J Appl Biomater Biomech. 2004; 2: 74-80.
26. Webster TJ, Ergun C, Doremus RH, Siegel RW, Bizios R. Enhanced functions of osteoblasts on

nanophase ceramics. Biomaterials. 2000; 21: 1803-1810.
27. Janackovic D, Petrovic-Prelevic I, Kostic-Gvozdenovic L, Petrovic R, Jokanovic V, Uskokovic D. Influence of synthesis parameters on the particle sizes of nanostructured calcium-hydroxyapatite. Key Eng Mater. 2001; 192-195: 203-206.
28. Balamurugan A, Michel J, Faure J, Benhayoune H, Wortham L, Sockalingum G, Banchet V, Bouthors S, Laurent-Maquin D, Balossier G. Synthesis and structural analysis of sol gel derived stoicheometric monophasic hydroxyapatite. Ceramics-Silikaty. 2006; 50(1): 27-31.
29. Masuda Y, Matubara K, Sakka S. S ynthesis of hydroxyapatite from metal alkoxides through sol gel technique. J Ceram Soc, Jpn. 1990; 98: 1266-1277.
30. Deptula A, Lada W, Olezak T, Borello A, Avani C, Dibartolomea A. Preparation of spherical powders of hydroxyapatite by sol gel processing. J Non-Cryst Solids. 1992; 147: 537-541.
31. Li P, de Groot K. Better bioactive ceramics through sol-gel process. J Sol-gel Sci Technol. 1994; 2: 797-801.
32. Brendel T, Engel A, Russel C. Hydroxyapatite coating by polymeric route. J Mater Sci: Mater Med 1992; 3: 175-179.
33. Takahashi H, Yashima M, Kakihana M, Yoshimura M. Synthesis of stoichiometric hydroxyapatite by a gel route from the aqueous solution of citric and phosphoneacetic acids. Eur J Solid State Inorg Chem. 1995; 32: 829-835.
34. Vijayalakshmi U, Rajeswari S. Preparation and characterization of microcrystalline hydroxyapatite using sol gel method. Trends Mater Artif Org. 2006; 19(2): 57-62.
35. Haddow DB, James PF, Van Noort R. Characterization of sol-gel surfaces for biomedical applications. J Mater Sci: Mater Med. 1996; 7: 255-260.
36. Suchanek WL, Riman RE. Hydrothermal synthesis of advanced ceramic powders. Adv Sci Technol. 2006; 45: 184-193.
37. Sadat-Shojai M. Preparation of hydroxyapatite nanoparticles: Comparison between hydrothermal and treatment processes and colloidal stability of produced nanoparticles in a dilute experimental dental adhesives. J Iranian Chem Soc. 2009; 6(2): 386-392.
38. Li P, Kangasniemi I, de Groot K, Kokubo T, Yli-Urpo AU. Apatite crystallization from metastable calcium phosphate solution on sol-gel prepared silica. J Non-Cryst Solids. 1994; 168: 281-286.
39. Li P, Kangasniemi I, de Groot K, Kokubo T. Bone- like hydroxyapatite induction by a gel-derived titania on a titanium substrate. J Am Ceram Soc. 1994; 77: 1307-1312.
40. van Blitterswijk CA, Grote JJ, Kuijpers W, Blok-van Hoek CJG, Daems WT. Bioreactions at tissue-hydoxyapatite interface. Biomaterials. 1985; 6: 243-51.
41. Loty C, Loty S, Kokubo T, Forest N, Sautier JM. Pre- fabricated biological apatite formation on a bioactive glass-ceramic promotes *in vitro* differentiation of fetal rat chondrocytes. In: Sedel L, Rey C eds. Bioceramics. Paris: Elsevier Science Publishers. 1997; 219-222.
42. Loty C, Sautier JM, Boulekbache H, Kokubo T, Kim HM, Forest N. *In vitro* bone formation on a bone-like apatite layer prepared by a biomimetic process on a bioactive glass-ceramic. J Biomed Mater Res. 2000; 49: 423-434.
43. Habibovic P, Barrére F, van Blitterswijk CA, de Groot K, Layrolle P. Biomimetic hydroxyapatite coating on metal implants. J Am Ceram Soc. 2002; 85: 517-522.
44. Manso M, Jimenez C, Morant C, Herrero P, Martinez-Duart JM. Electrodeposition of hydroxyapatite coatings in basic conditions. Biomaterials. 2000; 21: 1755-1761.

*****