

Effect of Surfactant on Formation of Hydroxyapatite under Reverse Micelle Condition

by

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Masters of Philosophy in Department of Chemistry



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Declaration

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 23/12/18

Signature of Supervisor

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Signature of Candidate

Dedicated
to
My parents for all the encouragement and support

Approval

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ABSTRACT

Hydroxyapatite, (HAp), $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is a naturally occurring material found in the inorganic component of human bone and enamel. The constituent elements of HAp are primarily calcium and phosphorous, with a stoichiometric calcium to phosphorous ratio is 1.667 capable of promoting intimate bone growth onto femoral implants. The performance, lifespan and quality of the resultant biological coating *in vivo* is largely dependent on the coating morphology, phase composition, particle size and crystallinity of the powders pre-coating application. The present study focused on preparing Hydroxyapatite nanoparticles (HAp-NPs) through chemical precipitation technique using mixed micelle core as a nanoreactor. Mixed micelle core was used to control morphology such as crystallinity, particle size, particle shape, particle size. Anionic (sodium dodecyl sulphate (SDS)) and cationic (Cetyl trimethyl ammonium bromide (CTAB)) surfactants were used to prepare the mixed micellar core. 18 : 2 ratio of propanol-1 : water system experimentally selected for the formation of reverse micelle for the afore mentioned surfactants. Compositions of both surfactants were varied to control the morphology of HAp-NPs under the same experimental condition. Several techniques such as FTIR, XRD, TGA and SEM were used to characterize the prepared HAp-NPs. Appearance of peaks at various position of the FTIR spectrum shows the P-O vibrations of PO_4^{3-} which are characteristics of hydroxyapatite. In addition, the diffraction pattern of the prepared HAp-NPs are in well agreement with the standard published by the International Centre for Diffraction Data. However, the diffractogram indicates the presence of crystalline as well as amorphous phase. No significant weight loss was observed for the prepared HAp-NPs. SEM result reveals significant morphological variation of the prepared HAp-NPs by changing the amount of two surfactants. Variation of the ratio of surfactants SDS and CTAB allow the variation in the morphology of the prepared particles.

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Abbreviation	Detail
KUET	Khulna University of Engineering & Technology
BCSIR	Bangladesh Council of Scientific and Industrial Research
HAp	Hydroxyapatite
NPs	Nanoparticles
HAp-NPs	Hydroxyapatite nanoparticles
SDS	Sodium Dodecyl Sulphate
CTAB	Cetyl trimethyl ammonium bromide
FTIR	Fourier Transform Infrared Spectroscopy
XRD	X-Ray Diffraction Analysis
TGA	Thermo-Gravimetric Analysis
SEM	Scanning Electron Microscopy
CMC	Critical Micelle Concentration
ATR	attenuated total reflection

CHAPTER 1

Introduction

1.1 General

Reverse micelles are thermodynamically stable assemblies of surfactant molecules organized around a core of water or dissolved solute that spontaneously form optically transparent solutions in low polar liquids. HAp-NPs was synthesized via wet chemical precipitation technique using binary reverse micellar assembly two surfactants SDS and CTAB where micelle core acted as nano-reactor. The ratio of these two surfactants were adjusted in order to evaluate the binary micellar assembly to control the HAp nano-particles of characteristic properties such as crystallinity, crystallite size, lattice parameters, particle size etc. FTIR, XRD, TGA and SEM techniques shall be adopted for the characterization of the synthesized HAp particles.

In this chapter, theory of surfactants, structural features of surfactant molecules for surface activity, classification of surfactants, micelles, inverse/reverse micelles, critical micelle concentration (CMC), shape and structure of micelles, structure of surfactant and CMC, specific conductance as well as conductance of electrolytes have been discussed. In addition, theoretical background of several techniques such as FTIR, XRD, SEM and TGA for the characterization of the synthesized HAp nanomaterials have been discussed.

1.2 Hydroxyapatite Calcium Phosphate

HAp is a calcium phosphate-based biomaterials. Over the years it has drawn a great attention due to their applications in various fields. The fields of materials science, biomedical engineering, nanotechnology has been advanced dramatically owing to the excellent bioactivity and biocompatibility properties of HAp [1, 2]. Biocompatibility of HAp is due to its similar chemical structure with native mineral component of bone and teeth [3, 4]. It is therefore considered an ideal material in the field of tissue engineering for substituting hard /tissues like bone and teeth. However, the applications of HAp nanomaterials in various fields are primarily related to their size and morphology [5, 6]. So, controlled synthesis has been found for application of HAp in drug delivery system, biological probes, bio-imaging and so on. Studies have found that HAp nanoparticles

with less than 100 nm sizes are more likely to be ingested and internalized by living cells [3]. HAp particle with spherical shape has high surface area and are preferred as biological ceramics [7]. In orthopedics and dentistry HAp nano-whiskers could be used to coat the surface of Ti substrates as reinforcing agents [8]. Therefore, it is imperative to design various shaped HAp nanoparticles under a given experimental condition.

1.3 Hydroxyapatite as Bone Material

Studies has been revealed the success of orthopedic and dental implants related to the structure of the bone-implant interface. Along with the structural feature the compositional and functional aspect of bone-implant interface should be analogous to that of human bone. The presence of Ca and P on bone surface ensured good cytocompatibility, enhanced bone contact and new bone apposition property [36]. HAp is rich in Ca ions. Cell adhesion, differentiation, mineralization and bone integration property of HAp coated implants can have improved dramatically compared to the uncoated ones [36]. Effectiveness of synthetic HAp for the aforementioned process are found Osteoconductive properties to contribute. Coated Ca ions on the implant surface are mainly responsible to stimulate osteogenic cells. Dissolution and reprecipitation of calcium ions favor those processes.

The favorable effect of calcium phosphate coatings on bone response may be explained through the hypothesis that dissolution and reprecipitation of calcium ions from the coating stimulates osteogenic cells to differentiate and deposit a mineralized matrix. It is therefore expected that the new bone will form directly on the surface of the HAp coating. HAp coatings with high degrees of crystallinity show low-dissolution rates *in vitro* and less resorption and more direct bone contact *in vivo*. However, HAp coatings with a high amorphous content result in rapid weakening and disintegration of the coating and often promote inflammatory responses in the surrounding tissue. Okamoto *et al.* [27] reported that a significantly higher number of cells adhered to HAp than to uncoated titanium. The crystallinity, composition, morphology and particle size of calcium phosphate effect its dissolution [26]. Synthetic HAp exhibits strong affinity to host hard tissue, promotes osseointegration and expedites the healing period required for titanium implants. It is known that human bone consists of roughly 70 % HAp mineral and an organic matrix consisting largely of collagen type I, which is the

principle collagen of skin and bone. Previous research has studied the coating of titanium alloy with collagen type I to investigate its beneficial effect in osseointegration [28]. It is demonstrated that the initial adhesion and spreading of osteoblasts have been accelerated. However, although collagen supports the adhesion of cells; enhanced bone mineral deposition still requires HAp.

The success or failure of HAp coated orthopedic implants; depends on the control and consequences of cell behavior post implantation [29]. Thus, the first and essential step for bone tissue-implant interface studies is *in vivo* tests using osteoblast cells due to the important role in which they play in the osseointegration of the implant. They have the ability to synthesize and produce extracellular matrix and to control its mineralization and thus regulate the ingrowth of bone to the implant. Cell lines and primary human cells are commonly used for *in vitro* tests. But, primary human cell culture system does not exhibit reproducible results on account of differences between donors. An immortalized cell line is relatively easier to maintain over a long period of time with many passages without any loss of phenotypic expression. MG-63 cell line may be used to study the interactions between cells and biomaterial and to study the biocompatibility of the biomaterial [29].

1.4 Techniques for Preparing Synthetic Hydroxyapatite

The various methodologies employed to produce synthetic HAp are discussed in this section to provide the necessary background information with respect to HAp powder production techniques used to date. It is important to note that each method is greatly dependent on their synthetic starter / raw materials [9]. Variation in process parameters such as powder morphology can induce microstructural and mechanical inconsistencies [10]. The production of HAp powders may be classified under three main categories:

- i. Wet Chemical Synthesis (such as Precipitation, Hydrothermal, Hydrolysis and Sol-Gel Techniques)
- ii. Dry Chemical Synthesis (such as solid-state reactions, mechanochemical synthesis)
- iii. Vapor Phase Reactions (such as spray and freeze-drying)

i) Wet Chemical Process

Wet chemical synthesis allows the attainment of inorganic particles, oxides or metals, eventually multicomponent phases, with controlled size and shape, high chemico-physical reactivity and very high purity control.

Hydrothermal Method

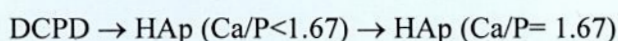
Hydrothermal method is one of the most common and effective synthetic routes to fabricate the nanomaterial with a variety of morphologies. In this method, the reactants are placed into an autoclave filled with water or organic compound to carry out the reaction under high temperature and pressure conditions. If the non-aqueous solvents are utilized as reaction medium, it is termed as solvothermal method; whereas, in case the preparation is carried out in the presence of water, it is known as hydrothermal process.

Hydroxyapatite Synthesis by Hydrothermal Method

HAp powders can be synthesized by hydrothermal processing in alcoholic media. HAp precursor slurry are prepared from $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and CaCO_3 which are well mixed in a pot mill using ZrO_2 balls in water for 24 h at 50 rpm. Various kinds of alcohols in different amounts were added to the HA precursor. After controlled to pH 10 by NH_4OH , this mixture is treated in an autoclave with stirring under hydrothermal conditions at 180°C for 5 h to crystallize.

Hydrolysis Method

The conversion of brushite, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (DCPD), into HAp can be carried out by hydrolysis on the basis of the following two stage scheme:



Reaction I corresponds to a structural change into the HA structure and Reaction II corresponds to a compositional increase in Ca/P ratio retaining the apatite structure. In reaction I DCPD is suspended and stirred with magnetic stirrer in distilled water by bubbling nitrogen gas. Reaction temperature and pH are kept within the ranges $40\text{--}80^\circ\text{C}$ and 3 to 13, respectively. The reaction pH are adjusted with ammonium hydroxide. Then sample are filtered washed with water and dried at 80°C . In reaction II HAp sample is treated at $30\text{ to }50^\circ\text{C}$ for 1 h alkaline solution containing $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ as a Ca^{2+} source for an increase in Ca/P ratio. The resulting sample is filtered, washed and dried as the same manner as in reaction I.

Sol–Gel Method

Sol–gel method, a wet chemical route is one of the well-established synthetic approaches to prepare novel metal oxide NPs as well as mixed oxide composites. This method has potential control over the textural and surface properties of the materials. Sol–gel method mainly undergoes in few steps to deliver the final metal oxide protocols and those are hydrolysis, condensation, and drying process. The formation of metal oxide involves different consecutive steps, initially the corresponding metal precursor undergoes rapid hydrolysis to produce the metal hydroxide solution, followed by immediate condensation which leads to the formation of three-dimensional gels. Afterward, obtained gel is subjected to drying process, and the resulting product is readily converted to Xerogel or Aerogel based on the mode of drying. The size of the nanoscale clusters, along with the morphology and microstructure of the final product, can be tailored by controlling the hydrolysis and condensation reactions.

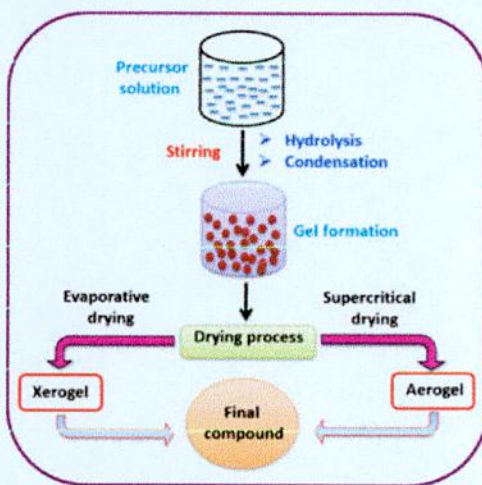


Figure 1.1: The Reaction Pathway for the Production of Metal Oxide Nanostructures in the Sol–Gel Method.

In case of amorphous calcium phosphate gel is obtained in ethanol from calcium ethoxide $[\text{Ca}(\text{OEt})_2]$ and H_3PO_4 solution. Calcium diethoxide solution is prepared by the reaction of calcium metal with ethanol for 4 h at 80 °C under nitrogen gas with stirring. H_3PO_4 dissolved in ethanol is added drop wise into the alkoxide solution at temperatures lower than 10 °C. A white ACP gel is slowly prepared and further aged at room temperature under flowing nitrogen for 24 h under constant stirring. Then the gel-precipitate is centrifuged and dried at 100 °C for at 8 h in an oven.

ii) Dry Chemical Process

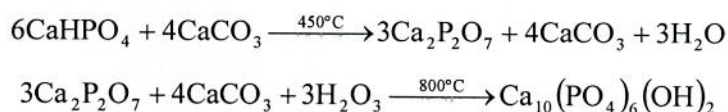
A dry media reaction or solid-state reaction or solventless reaction is a chemical reaction system in the absence of a solvent.

Solid State Reaction

The solid-state reaction route is the most widely used method for the preparation of polycrystalline solids from a mixture of solid starting materials. Solids do not react together at room temperature over normal time scales and it is necessary to heat them to much higher temperatures, often to 1000 - 1500 °C in order for the reaction to occur at an appreciable rate. The factors on which the feasibility and rate of a solid state reaction depend include, reaction conditions, structural properties of the reactants, surface area of the solids, their reactivity and the thermodynamic free energy change associated with the reaction

HAp Synthesis by Solid State Reaction

Solid state reactions usually give a stoichiometric and well-crystallized HAp powder. High temperatures and long heat treatment times are required. Sinterability of these powders is generally low. Dibasic calcium phosphate (DPC: CaHPO_4) and CaCO_3 as starting materials can be used to produce HAp powder by solid state reactions. These powders are mixed at about a ratio of 1.5 for a stoichiometric HAp powder and heat treated at temperatures up to 1000 °C for 2 h. Two separate reactions are determined with TGA and DTA analysis with these starting compounds. These reactions were observed to be:



Mechanochemical Synthesis

Mechanochemical processing is characterized by the repeated welding, deformation and fracture of the mixture of reactants. Chemical reactions occur at the interfaces of the nanometer-sized grains that are continuously re-generated during milling. As a consequence, chemical reactions, which would normally require high temperatures to occur due to separation of the reacting phases by the product phases, can occur at low temperatures in a ball mill without any need for external heating. The reactions may occur either in a steady state manner or self-propagating combusive manner. Solid-

state displacement reactions that occur in a combusive manner lead to micron-sized particles due to high temperature, whilst the reactions that occur in a steady state manner result in a nano-scale mixture of product phases.

HAp Synthesis by Mechanochemical Synthesis

The starting materials for this work are commercially available anhydrous calcium hydrogen phosphate (CaHPO_4) and calcium oxide (CaO). Appropriate amounts of the two starting materials at a molar ratio of 3 : 2 are mixed together in a conventional ball mill in ethanol with zirconia balls as the milling medium. The powder mixture is then loaded into an alumina vial of 37 mm in diameter and 40 mm in length, together with a stainless-steel ball of 12.7 mm in diameter. Mechanical activation is carried out in a high-energy shaker mill for various time periods.

iii) Vapor Phase Reactions

In general, reaction and synthesis are carried out at elevated temperatures and under a vacuum. Vacuum is needed to ensure a low concentration of growth species so as to promote diffusion-controlled subsequent growth. Grown nanoparticles are normally collected on a non-sticking substrate placed downstream at a relatively low temperature. Obviously only a small fraction of nanoparticles do settle on the substrate surface. Furthermore, the nanoparticles that settled on the substrate surface may not represent the true particle size distribution. It is also difficult to introduce stabilization mechanism during synthesis to prevent the formation of agglomerates. Despite the aforementioned challenges, it has been demonstrated that various nanoparticles can be synthesized by vapor phase reactions.

Since the present research utilizes the wet chemical precipitation method to synthesize pure Hap. Method is discussed in detail, with an overview of other possible synthetic routes for its synthesis. A comprehensive discussion of all of these techniques and the kinetics of precipitation are explained by Kehoe [14]. In this work, the advantages and disadvantages of each technique are considered, and compared against that of the wet chemical precipitation method. The main advantage associated to the wet process, is that its by-product consists primarily of water and the probability of contamination during processing is very low. Low processing costs has also been reported. Its disadvantage is that the resulting product can be greatly affected by even a slight difference in the reaction conditions. The dry process, on the other hand shows high

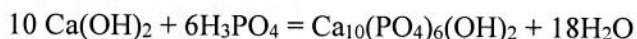
reproducibility in spite of the high risk of contamination during stages of milling and long heat treatment times [15].

1.4.1 Chemical Precipitation Route

In the precipitation route, HAp powders are obtained from a chemical reaction of inorganic oxide solutions [9]. The two most popular ways in precipitating HAp are described in the two reactions below [9, 16]:

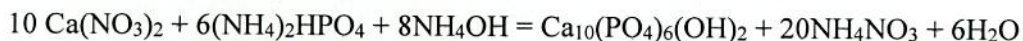
A. Reaction 1

The precipitation method for the reaction of orthophosphoric acid with calcium hydroxide:



B. Reaction 2

The precipitation method for the reaction of diammonium hydrogen phosphate with calcium nitrate:



Reaction 1 is simple, cheap, suitable for large scale industrial production and non-polluting by nature, but often leads to the production of non-stoichiometric HAp, which creates subsequent problems during sintering (thermal decomposition of non-stoichiometric HAp leads to TCP when the Ca/P molar ratio < 1.67), or to CaO when the Ca/P molar ratio > 1.67. In contrast, Reaction 2 is expensive to produce and polluting by nature, in that its by products must be removed. The quality of the initial reagents also play an important role in obtained high quality phase pure HAp. Synthesis, based on Reaction 1 has demonstrated that the purity and the properties of the chemicals and process parameters, such as, reagent addition flow rate, stirring rate, pH and reaction temperature all have a significant influence on the final quality of the HAp in relation to its crystallinity, crystallite size, morphology, particle size distribution, density and surface area. Another study has shown the effect of ripening time on crystallinity and sinterability and mechanical properties for dense HAp can be related to the initial reagent properties.

HAp synthesized using Reaction 2, have found that the reaction temperature, the reactant concentrations, rate of mixing the reactants and the residence time can affect the overall characteristics of the HAp produced [11]. The research also found that other phases can be produced with different reaction pH values.

HAp with a Ca/P molar ratio, ranging from 1.5 to 1.667 ($0 \leq x \leq 1$) using the conventional aqueous precipitation method from the addition of a diammonium phosphate solution ($(\text{NH}_4)_2\text{HPO}_4$) into a reactor containing a calcium nitrate solution was prepared by Raynaud *et al.* [10]. The device implemented was fully automated with the reactor placed in an argon atmosphere under dynamic flow in order to prevent any presence of CO_2 , which could result in carbonate apatite formation. In this study, pH and temperature of synthesis were the preponderant parameters for the control of the precipitate composition.

1.4.2 Surfactant Assisted Micelle/Reverse Micelle Route

Surfactant template systems offer the opportunity of exploring the complementarity geometric, electrostatic and stereo-chemical molecular/ionic interactions in an attempt to control the nucleation and growth of inorganic materials. [26] Shanthi and coworkers [27] reported the preparation HAp nanorods with ≈ 20 nm diameter and 100–120 nm length by co-precipitation at ambient temperature and pressure in the presence of a cationic surfactant.

The same research group has successfully prepared shell-like nano-HAp spheres of varied morphology, with uniform size (≈ 200 nm) using cetrimide as surfactant [28]. Saha and co-workers [29] synthesized HAp nano-powder by reverse micro-emulsion technique, using calcium nitrate and phosphoric acid as precursor salts dissolved in water. It was found that CTAB played a significant role in regulating the morphology of the nanoparticles. Liu *et al* [30] reported the synthesis of HAp-nano-fibres with diameter of 5–8 nm and length of 160–220 nm using the templates such as CTAB, polyethylene glycol (PEG) and cyclohexane. In a similar manner, Sun *et.al.*[31] reported the synthesis of single crystal HAp nanorods with diameter, 8–15 nm and length, 25–30 nm by using the quaternary reverse micro-emulsion (TX-100 + CTAB/*n*-butanol + *n*-Hexanol/cyclohexane/water) method. Another method like hydrothermal micro-emulsion was proposed by Kalita *et al.* [32] for the synthesis of nano-hydroxyapatite rods using CTAB as the surfactant and *n*-pentanol as the co-surfactant. However, the foresaid researchers neither used CTAB alone as a template nor optimized its concentration on the size and morphology of the as-synthesized HAp during synthesis.

Arami *et al* [33] reported the synthesis of hydroxyapatite nanostrips by using microwave heating method and CTAB as a template. But the effect of CTAB

concentration on phase purity, crystallinity and morphology of the as-synthesized HAp using this microwave heating method was not investigated. On the other hand, Yan *et al* [34] reported the synthesis of HAp nanorods using surfactants, CTAB and SDS under hydrothermal conditions at 150 °C. However, they failed to attempt the effect of surfactant concentration on morphology of the as-synthesized HAp. Salarian *et al* [35] also reported the synthesis of HAp nanorods by surfactant-assisted hydrothermal method. In their synthesis, CTAB acts as a soft template and PEG 400 acting as a co-template. They investigated mainly the effect of hydrothermal temperature alone on composition, size and morphology of as-synthesized HAp.

Motivated by the above approaches, in this thesis, attention has been focused on the investigation of effect of various binary reverse micellar assembly of surfactants (e.g., SDS and CTAB) on the structural growth of nano-hydroxyapatite using wet chemical precipitation method.

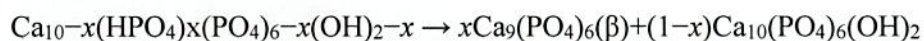
1.5 Process Variables Affecting the Chemical Precipitation Method

1.5.1 Initial Reactant Concentration

Direct HAp formation has been reported, for low reactant concentrations (in the 10^{-3} M range and at pH 7.4), where the solution was under saturated or slightly supersaturated with respect to the precursor phase [30, 31]. All the variance in results obtained for final HAp powder characteristics, define the complex role of chemical media in HAp formation; whose influence is evident not only through the super-saturation effect, but also through the nature and concentrations of ionic species. Difficulties encountered in preparing stoichiometric HAp from aqueous solution are caused by a high apatite affinity towards various impurities as well as the complex nature of the calcium phosphate system and the predominant role of kinetic parameters over thermodynamic ones during phase transformations [32].

Due to the low cost and simplicity of the wet chemical precipitation procedure, there is considerable interest in the HAp synthesis from aqueous solutions. However, most of the apatite's obtained from this method are reported as non-stoichiometric [32]. During heating, non-stoichiometric apatite's decompose and appear as either TCP or CaO. Sintering of pure stoichiometric HAp powder, however, yields pure dense HAp powders with superior mechanical properties, while sintering of nonstoichiometric HAp, lead to the presence of other phases, such as: CaO if the Ca/P molar ratio exceeds

1.67 and TCP if Ca/P is below 1.67. Decomposition of calcium deficient apatite's (Ca/P<1.67) in the temperature range 22–900 °C takes place according to the following chemical reaction [33]:



while in the reactant concentrations containing an excess of Ca(OH)₂ during thermal treatment; CaO is formed. A change in composition of HAp can decrease the strength of the final material, increase its dissolution rate, and consequently affect the rate and extent of biodegradation.

The substitution of phosphate ions PO₄³⁻ by hydrogen phosphate HPO₄²⁻ allows a continuous variation of the Ca/P atomic ratio between 9/6 and 10/6. This leads to calcium-deficient HAp (Ca-dHAp). Ca-dHAp powders can be precipitated from conventional wet chemical methods and decomposed into a mixture of HAp and tricalcium phosphate, Ca₃(PO₄)₂(TCP) by thermal treatment above 700 °C, dependent upon the initial reactant (that is, Ca²⁺) concentration. The resulting Ca/P molar ratio of the HAp powder is one of the most crucial parameters in determining its thermal stability. In general, HAp prepared at low pH is highly Ca-deficient (Ca/P = 1.58), while the wet mechanochemical route is also known to produce powders with Ca/P ratio of 1.65 [34].

Since precipitated anions (PO₄³⁻) are generated slowly in solutions containing the metal ion (Ca²⁺) during chemical precipitation; this process entails adding the phosphate solution drops to the calcium ion-containing solution. The hydroxyl ions originally present in the calcium hydroxide suspension are readily exhausted by the phosphoric acid under a very high addition rate, lowering pH in the bulk apart from the calcium hydroxide particles. It is important that the reactants have the correct molar ratio of calcium and phosphorous and if the pH level falls below 9 or 7, it leads to the production of calcium monophosphate and calcium dehydrates. They are more soluble in aqueous media and will resorb upon implantation

The control of pH is vital to the successful control of the chemical precipitation process. HAp, with a high crystallinity and with its primary particles in the submicron range and of finely divided precipitate was formed at a high pH in excess of 10 [5, 31]. Conn *et al.* [35] also confirm that the precipitation must be carried out at a pH above 10 in order to precipitate HAp powders with low bulk density and a high surface area. This research achieved this through implementing a two-stage reactor to produce HAp on a continuous basis with both stages under vigorous agitation. The phosphoric acid is

added in the first stage in a manner suitable to disperse the acid so that high local concentrations of acid are avoided. It is important to hold the pH in stage one between about 9.5 to about 11. A pH of 9.5-10 indicates that the reaction in stage one is approaching 100 % and the viscosity of the reaction mass approaches a maximum where agitation becomes less efficient. A pH above about 11 indicates that the extent of reaction is too low in stage one. Deviation from a pH of from about 9.5 to about 11, preferably from about 10 to about 11 and, more preferably, at about 10.5 indicates that either acid or lime flow is not correct and must be adjusted. Phosphoric acid delivery to the second stage is controlled in such a manner as to hold the pH at about 7.0 to about 7.4, preferably from about 7.2 to about 7.3.

Solids concentration also increases the collision frequency (since two particles are involved in each collision). pH has a very strong effect on the surface potential, for every compound there is a pH where the particle surface is electrically neutral (the isoelectric point). At this pH, particle-particle repulsion vanishes and agglomeration is highest [36]. A high ionic strength depresses the double layer, thus stimulating agglomeration. A high supersaturation decreases the time needed for binding particles together, thereby facilitating agglomeration [36].

1.5.2 Reaction Temperature

Temperature is found to affect predominantly the crystalline phase fraction, crystallite size and, as a consequence, specific surface area [33]. Controlling the reaction temperature during precipitation, Tampieri *et al.* [37] varied the degree of crystallinity (representing the fraction of completely crystallized phase) approximately from 20 to 80%. It is shown that increasing of precipitation temperature not only increases the crystallinity of the HAp powders but also induces an enhanced spherical morphology [32 - 33, 37 - 38]. A pure-phase HAp with the desired morphology could therefore be prepared by adjusting the reaction temperature and time [39].

The morphology of HAp crystals are strongly dependent on the temperature of the reaction [37, 39 - 40]. Increasing the precipitation temperature from 20-100°C induces a change in morphology from need-like precipitate to highly spheroidal precipitate, respectively, for chemical precipitation reaction (2), Equation 2-2 [37]. However, the morphological change with temperature follows the reverse order when HAp is precipitated via the chemical precipitation reaction (1), Equation 2-1. Instead, it was reported that the morphology of the HAp particles changed from spheroidal to needle-

like with increasing temperature [37]. In general, a needle-like morphology can be expected when the growth rate is faster and the aspect ratio decreases as the growth rate decreases. Thus, the growth rate of HAp should be expected to increase with increasing temperature when it is precipitated via chemical reaction (1), Equation 2-1 while decreasing with increasing temperature when precipitated via chemical reaction (2), Equation 2-2. An enhanced spherical morphology with larger particle size has elsewhere been reported with an increase in precipitation temperature. Kumar *et al.* [39] reinforces this evidence with Transmission Electron Microscopy graphs of HAp powders (synthesized via the wet chemical using $\text{Ca}(\text{OH})_2$ and H_3PO_4 revealed needle-shaped particles with a high aspect ratio at 40°C , which changed to spheroidal when the precipitation temperature was increased to 100°C . The changes in the morphology with temperature were analyzed taking into account the driving force for the HAp precipitation and the supersaturation level of Ca^{2+} and PO_4^{3-} ions with respect to HAp [41]. The analysis indicated that the supersaturation level of the reactants, especially the concentration of Ca^{2+} ions, played a predominant role on the precipitate morphology for this classical acid-base reaction. Lazic *et al.* [33] also prove the stoichiometry of the non-matured HAp varied significantly with reaction synthesis temperature, such that samples precipitated at 95°C demonstrated properties of thermally stable stoichiometric HAp. The crystals precipitated at 22°C had the form of tiny needles with an average length of 110 nm and width of 11 nm. Maturation (that is, ripening time) under conditions of precipitation does not significantly affect crystal size. The crystals however become thicker and aggregation more pronounced. Crystals precipitated at high temperature are well-rounded. For studies conducted by Lazic *et al.* [33] at reaction synthesis temperatures of 15°C , the particle size of HAp was less than 10 nm, and at 60°C it became an acicular crystal with a length of 100 nm. The aging / maturation time is required at a particular precipitation temperature in order to obtain stoichiometric HAp [42].

Synthetic HAp occurs in two structural forms, hexagonal and monoclinic, which have minor structural differences. The hexagonal HAp form is usually formed by precipitation from supersaturated solutions at 25°C to 100°C and the monoclinic form of HAp is primarily formed by heating the hexagonal form at 850°C in air and then cooling to room temperature [43]. The overall XRD patterns of hexagonal and monoclinic HAp are almost identical; however the pattern of monoclinic HAp has

additional weak lines whose intensities are less than 1 % of the strongest hexagonal HAp line. The main phase formed at room temperature was ACP, with minor amounts of HAp, whereas at 90 °C HAp crystallized. This behavior cannot be explained from the supersaturation in these experiments, since the solubility of HAp is higher at low temperatures [31].

At a constant reactant concentration, as the reaction temperature is increased, the particle size and aspect ratio is also reported to increase [40]. However, with this increase of reactant temperature, the HAp agglomerate size decreased and it was observed that larger agglomerates were formed for relatively smaller particles, such as those synthesized at 25 °C. Their sintering density also improved in spite of the decrease of the surface area with agglomerate size. Kothapalli *et al.* [40] also found that the density of the pre-sintered HAp increased with synthesis temperature in spite of the concentration of the reactants. The reactant temperature had more impact on the strength than the actual concentration. At a constant concentration, as the temperature increased, the flexural strength increased proportionally.

In the case of low temperature precipitation, stirring in the maturation stage has shown to play an essential role in obtaining required stoichiometry [33]. Also, raising the reaction temperature can also greatly shorten the reaction time for the formation of pure-phase HAp. Afshar *et al.* report a chemical precipitation reaction temperature of 40 °C is required to achieve a pure HAp product [44].

An investigation conducted by Conn *et al.* [35] reported that the temperature at which the chemical precipitation reaction is carried out has little effect on the product quality or on the efficiency of the process. Accordingly, temperatures from 35 to 90 °C were stated as satisfactory in this study. Lower temperatures and aging time can extensively improve the yield and reduce the cost of the reaction. On the other hand, however, these parameters can also lead to heterogeneous compositions and morphology, and a calcium deficiency in the final product [45].

1.5.3 Rate Reactant Addition

A slow and controlled addition of phosphoric acid in an alkaline environment is necessary (as it controls the pH level) in order to fabricate pure stoichiometric HAp [2, 5]. A slow reactant addition rate is found to yield pure, well-crystallised HAp, due to low supersaturation and an avoidance of local inhomogeneity [46]. A high addition rate of phosphoric acid or in excess, leads to the formation of calcium deficient precipitate

[2]. Afshar *et al.* [44] used extra acid solution to remove any extra calcium in the HA precipitate.

The rate of reactant addition affects the rate of HAp nucleation [5]. A slow addition rate ensures the absence of continuous nucleation. Addition times of 60 min or longer are found to result in a well crystallized HAp powder while an addition time of 30 min resulted in a biphasic mixture of HAp and ACP. For the latter case it is likely that the rate of addition of reactants was higher than the rate of their consumption due to HAp crystallization [31]. Therefore, the concentrations of calcium and phosphate ions in the solution built up during the addition period and eventually the solution became supersaturated with respect to ACP, which then formed preferentially. When the addition time was the longest (240 min), the crystallinity index was also the highest.

Large HAp powder particle sizes are obtained when the addition rate of acid is lowered [47]. The orders in which the reactants are added also play an important role in producing phase pure HAp. When the Ca solution is added to the P solution, Ca-HAp is produced at the beginning of the reaction and the final product can also be Ca-deficient, which is a common problem when employing wet methods of synthesis [48]. When P solution is added to a Ca solution, there exists a Ca-rich precipitate at the beginning, which progressively evolves into stoichiometric HAp towards the end of the reaction [48].

When the addition rate of the phosphoric acid is very high (2400 ml/min.), the synthesis reaction conversion decreases and there is some Ca(OH)_2 appears in the final product. When the acid addition rate is below 100 ml/min, the conversion rate of the reaction is 100% [49]. The addition rate of reactant, during the reaction synthesis therefore has a strong influence on the resulting composition. A higher addition rate of acid systematically results in an undesired increase of the Ca(OH)_2 content. This can be explained in terms of pH of the reaction media. When the acid is introduced at a very high addition rate, the pH of the reaction media decreases drastically (pH 7).

1.5.4 Stirring Speed

The chemical precipitation reaction requires mixing on a molecular level before actual precipitation of the crystallized product can proceed. The mixing procedure determines the final stoichiometry of HAp precipitate [48]. Since many precipitation processes are conducted at very high supersaturations, the characteristic reaction time can be of the same order of magnitude or even smaller than the mixing time [36]. When this situation

prevails, the course of the mixing process helps to determine the course of supersaturation. Guilletti *et al.* [36] described these mixing conditions to affect crystal shape, purity, crystallinity, and even the phases that are formed. When large crystals of high crystallinity and purity are desired, precipitation should be conducted at low supersaturation. Therefore, reactants should be added near areas of high mixing intensity, and as far as possible from each other, (that is, a good dispersion of the concentrated reactant with the reactor contents should be stimulated). If fine particles with a narrow size distribution are desired, intense mixing should provide rapid contact between concentrated reactants [36]. Mixing effects have to be considered in order to extrapolate correctly a pilot reactor to the industrial scale [50]. The hydrodynamic factors which govern mixing and the initial contact between the reagents play an essential role on morphology and on crystal size distribution [31].

The stirring speed should be high enough to prepare a good homogenous media for precipitation of HAp. Both Monetite and Brushite formation occurs by insufficient mixing of calcium solution as the orthophosphoric solution is added [45]. The PO_4^{3-} ion concentration builds up when it is not distributed homogeneously and a localized low pH is established. This part of the reaction vessel can be richer in PO_4^{3-} and provides conditions for the formation of soluble precipitates. A powerful and high-speed stirring device can be used to overcome this possibility [47]. Excellent agitation can be required to form a quality product [45], as poor agitation in the suspension contains entrapped unreacted $\text{Ca}(\text{OH})_2$. High local concentrations of phosphoric acid must be avoided in order to prevent the formation of dicalcium phosphate which is impossible to convert to the desired HAp [35].

Agglomeration is present in almost all precipitation processes and in some crystallization systems, particularly during start-up, where higher super saturations prevail [36]. When solid-liquid separation is critical, agglomeration is desired since it leads to larger, easy-to-filter particles. When products of high purity are required, agglomeration should be avoided since agglomerates trap impurities within their interstices [36]. The process of agglomeration starts with the transport of particles towards one another. When the particles have enough kinetic energy to overcome the repulsion due to their surface potential, they collide. If they remain in contact long enough for growth to take place and thereby bind the particles together; agglomerate is formed [36]. Thus, the variables can affect agglomeration during industrial precipitation. Mixing intensity improves the particle collision frequency and thus

agglomeration. However, a mixing intensity that is too high promotes particle disruption [36].

1.5.5 Ripening Time

During aging, the HAp crystals are subject to a process that includes dissolution and recrystallization. During this stage the smallest crystals disappear in favour of the larger ones, which grow further. As a consequence the total number of crystals is reduced and the surface roughness of the crystals is reduced. Both phenomena result in a decrease of the specific surface area [5].

HAp crystallinity can be largely dependent upon ripening or maturation time allowed after the precipitation reaction [37]. Both the crystallinity and crystallite size of the HAp powders are found to increase very rapidly at the early ripening stages and gradually levelled off as ripening continues. Tampieri *et al.* found similar results were obtained when the synthetic temperature was raised to 99 °C [37]. The degree of crystallinity of HAp powders increased from 20 – 70 % by increasing the maturation time to 24 h. Pang *et al.* [41] investigated the increase of crystallinity with the increase of the ripening time to observe a clear sharpening of the diffraction peaks (XRD) of the HAp powders. Both the crystallinity and crystallite size of the HAp powders increased very rapidly at the early ripening stages and gradually levelled off as ripening lasted. This profile of crystallinity increase with ripening time was also confirmed by FTIR analysis. The characteristic bands for HAp are exhibited in all the three patterns (1 h, 96 h and after calcination): 900–1200 cm^{-1} for phosphate stretching, 602 cm^{-1} for phosphate bending, 632 and 3571 cm^{-1} for hydroxyl vibrations [41]. The intensities of these two hydroxyl absorption bands and the band at 940 cm^{-1} for phosphate can be used as an indication of the HAp crystallinity. It is seen that the intensities of these three bands increase with the ripening time. In addition, the broad band at 2500–3700 cm^{-1} , which is a reflection of the combined water in HAp powders, also decreases with increasing the ripening time. The lower combined water content results from the higher crystallinity of HAp powder, implying the crystalline HAp powder is less hydrophilic than its amorphous counterpart.

Further evidence for the change of combined water with ripening time was provided by TGA measurements. The weight loss of HAp powders heated up to 750 °C is about 7.6 and 6% for 1 and 96 hrs ripening samples, respectively. The area of endothermic peak

on DTA curve shows the same result, too. The weight loss starts at about 100 °C and ends at about 500 °C based on the DTA curves. This weight loss comes from the elimination of combined water [41].

This crystalline behavior as a function of ripening time can be explained by the mechanism of crystal growth in solution. It is well known that precipitation of particles involves nucleation and growth from a supersaturated solution. Chemical precipitation often experiences high reaction-induced super saturation, leading to high nucleation rates [36]. In this case, a crystal nucleus usually has a rough surface due to the rapid production of insoluble materials. This rough surface provides energetically favorable conditions for crystal growth because the molecules adding on it has greater sticking probability. So the rough surface results in high growth rate. However, as the crystal continues to grow, its surface becomes smoother and as a result, the rate of crystal growth slows down. This is because the addition of molecules on the smooth surface has lower sticking probability.

Aging the suspension leads to the growth of the precipitate and changes the morphology to a more equiaxed status [47]. Leaving the suspension to settle after adding the acid solution causes completion of precipitation and more suitable properties as well as the process efficiency [47]. The thermodynamic stability of a solid compound arises from the ordered arrangement of its molecules in a crystal lattice in an infinite medium. For particles of finite size, the average particle stability is an average of its volume and surface contributions [36]. Since surfaces have a low stability, the smaller the particle, the lower its stability, (that is, the higher its solubility). This effect can be important for particles of 1 mm or less. The residual super saturation can slowly be consumed by larger particles. Aging can take days or even months, and in general, it helps to improve product quality as it results in larger crystal sizes and improved product crystallinity. Further XRD analyses have also confirmed that performing calcinations after HA precipitation can significantly increase the resultant crystallinity of the HAp precipitate [40].

The formation of synthetic HAp crystals from a highly supersaturated solutions proceeds via intermediate precursor phases, which have a transitory existence, such as TCP and OCP. Traces of precursors can be detected even after prolonged ripening times, therefore affecting the quality of the final HAp product. [5]

1.5.6 Atmospheric Environment

During the chemical precipitation route, adsorption of atmospheric CO₂ may occur, which in the form of CO₃²⁻ anion can be incorporated into the HAp crystal lattice, resulting in micro-stresses and distortion of the stoichiometric HAp lattice [51]. However, within Nitrogen (N₂) controlled environments, small percentages of CO₃²⁻ contamination was also found to arise as a consequence of subsequent sample handling steps. Filtered inert gas should be continuously injected to both the reactor and the acid solution container during the precipitation process [44]. The filter should contain a sufficient amount of carbon dioxide absorber (such as, KOH or N₂). The HAp powder can be very hospitable in the substitution of carbon dioxide in its crystal structure. CO₃²⁻ ions can substitute hydroxyls or phosphates sites and based on this substitution, type A and type B of carbonated HAp are, respectively, formed. So, in order to reach a pure HAp precipitate without any carbon dioxide contamination, it is effective to use controlled atmosphere during precipitation process. Filtered inert gas shall be continuously injected to the reactor and the acid solution container during the precipitation process. The filter should contain a sufficient amount of carbon dioxide absorber.

1.6 Surfactants

Surfactants are a class of compounds which reduce the surface tension of water by adsorbing at the gas-liquid interface. They are also known to reduce the interfacial tension between oil and water by *adsorbing* at the liquid-liquid interface. Many surfactants can also assemble in the bulk solution rather than at the gas-liquid interface. Soaps and detergents are classic examples of surfactants. Two examples of surfactants are given in Figure 1.2 and Figure 1.3.

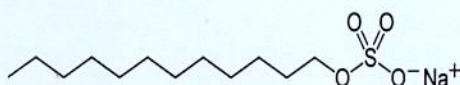


Figure 1.2: An example of anionic surfactant molecule (SDS).

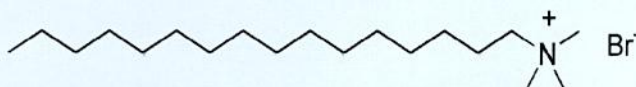


Figure 1.3: An example of cationic surfactant (CTAB)

The surfactant molecules can aggregate into larger sizes, and these aggregates are called micelles. The optimum concentration at which the surfactants begin to form micelles is known as the critical micelle concentration (CMC). Once the micelles are formed in water, the tails of the surfactant molecules form a core which can encapsulate an oil droplet, and their heads (ionic/polar) form an outer shell that maintains favorable contact with water. When surfactants assemble in oil, the aggregate is referred to as a reverse micelle. In a reverse micelle, the heads are in the core and the tails maintain contact with oil.

Surfactants are employed to increase the contact between two materials. Surface activity of surfactants are responsible in controlling the functions in many systems which include, among others, emulsification, detergency, foaming, wetting, lubrication, water repellences, waterproofing, spreading and dispersion, and stability of colloidal systems.

1.6.1 Structural Features of Surfactant molecules for Surface Activity

A molecule of surfactant possesses two part - (i) a hydrophobic group and (ii) a hydrophilic group. The hydrophobic group has a strong disliking for solvent (solvent hating), while hydrophilic group (solvent loving) has a strong attraction for the solvent as shown in Figure 1.4(a). As a result the surfactants are soluble in both organic solvents and water.

The hydrophilic end (head) of the molecule is generally shown by a circle. The hydrophobic end (tail) is represented by a long hydrocarbon. The tail may be depicted either as a straight line or a wavy tail (Fig. 1.4a). Surfactants tends to adsorb at the surface of oil droplets. The hydrophilic heads stick out into the water phase, while the hydrophobic tails stick into the oil phase as shown in Figure 1.4(b).

Schematic diagrams of the surfactants adsorbed on the surface of oil droplets are shown in Figure 1.4. Here, the hydrophilic heads are sticking out into the water phase, while the hydrophobic tails are sticking into the oil phase.

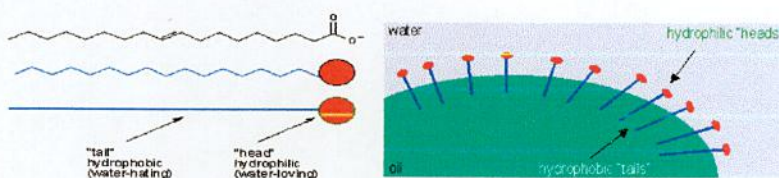


Figure 1.4: (a) Structure of a surfactant molecule, (b)

1.6.2 Classification of Surfactants

Surfactants are generally classified by the presence of formal charge in their heads. The head of an ionic surfactant carries a net charge. If the charge is negative, the surfactant is called anionic; if the charge is positive, it is called cationic. If a surfactant contains a head with two oppositely charged groups, it is termed zwitterionic. A non-ionic surfactant has no charge groups in its head. Examples of some commonly used surfactants are given below:

Ionic: Anionic (based on sulfate, sulfonate or carboxylate anions: Perfluorooctanoate, Perfluorooctanoate, Perfluorooctanesulfonate, SDS, ammonium lauryl sulfate, and other alkyl sulfate salts, Sodium laureth sulfate, also known as sodium lauryl ether sulfate, Alkyl benzene sulfonate, Soaps, or acid salts, Cationic (based on quaternary ammonium cations), CTAB and other alkyltrimethylammonium salts, Cetylpyridinium chloride, Polyethoxylated tallow amine, Benzalkonium chloride, Benzethonium chloride, Zwitterionic (amphoteric), Dodecyl betaine, Cocamidopropyl betaine, Coco amphi glycinate

Nonionic: Alkyl poly(ethylene oxide), Alkylphenol poly(ethylene oxide), Copolymers of poly(ethylene oxide) and poly(propylene oxide) (commercially called Poloxamers or Poloxamines), Alkyl polyglucosides, including: Octyl glucoside, Decyl maltoside, Fatty alcohols, Cetyl alcohol, Oleyl alcohol, Polysorbates: Tween 20, Tween 80, Dodecyl dimethylamine oxide

1.6.3 Micelles

A micelle is an aggregate of surfactant molecules dispersed in a liquid colloid. A typical micelle in aqueous solution forms an aggregate with the hydrophilic. "head" regions in contact with surrounding solvent, sequestering the hydrophobic tail regions in the micelle centre. This phase is caused by the insufficient packing issues of single tailed lipids in a bilayer. The difficulty filling all the volume of the interior of a bilayer, while accommodating the area per head group forced on the molecule by the hydration of the lipid head group leads to the formation of the micelle. This type of micelle is known as a normal phase micelle (oil-in-water micelle). Inverse micelles have the head groups at the centre with the tails extending out (water-in-oil micelle). Micelles are

approximately spherical in shape. Other shapes such as ellipsoids, cylinders, and bilayers are also possible. The shape and size of a micelle is a function of the molecular geometry of its surfactant molecules and solution conditions such as surfactant concentration, temperature, pH, and ionic strength. The process of forming micelle is known as micellization.

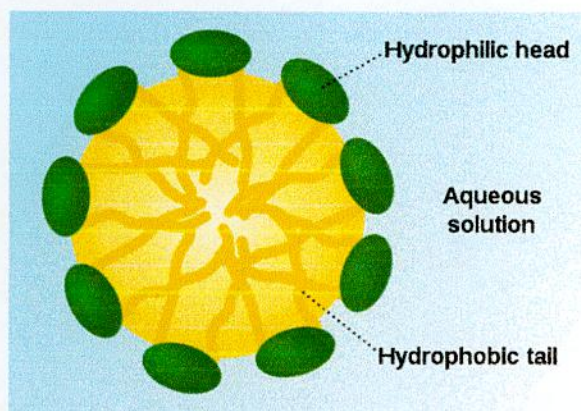


Figure 1.5: Micelle formation for surfactant molecule for oil in water phase medium.

1.6.4 Inverse/Reverse Micelles

In a non-polar solvent, it is the exposure of the hydrophilic head groups to the surrounding solvent that is energetically unfavorable, giving rise to a water-in-oil system. In this case the hydrophilic groups are sequestered in the micelle core and the hydrophobic groups extend away from the center. These inverse micelles are proportionally less likely to form on increasing head group charge, since hydrophilic sequestration would create highly unfavorable electrostatic interactions.

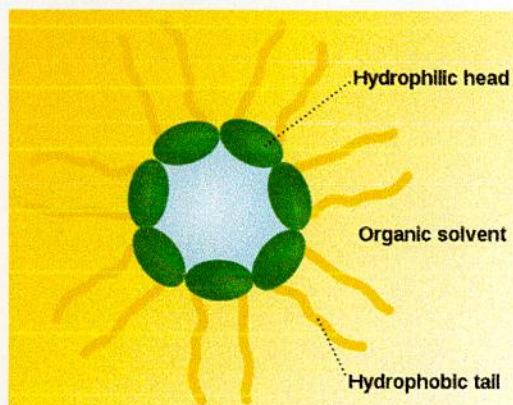


Figure 1.6: Formation of reverse micelle for surfactant molecule in water in oil phase

1.6.5 Critical Micelle Concentration

In chemistry, the CMC is defined as the concentration of surfactants above which micelles are spontaneously formed. Upon introduction of surfactants (or any surface active materials) into the system they will initially partition into the interface, reducing the system free energy by a) lowering the energy of the interface by removing the hydrophobic parts of the surfactant from contacts with water. Subsequently, when the surface coverage by the surfactants increases and the surface free energy (surface tension) has decreased, the surfactants start aggregating into micelles, thus again decreasing the system free energy by decreasing the contact area of hydrophobic parts of the surfactant with water. Upon reaching CMC, any further addition of surfactants will just increase the number of micelles (in the ideal case).

CMC is an important characteristic of a surfactant. Before reaching the CMC, the surface tension changes strongly with the concentration of the surfactant. After reaching the CMC, the surface tension stays more constant.

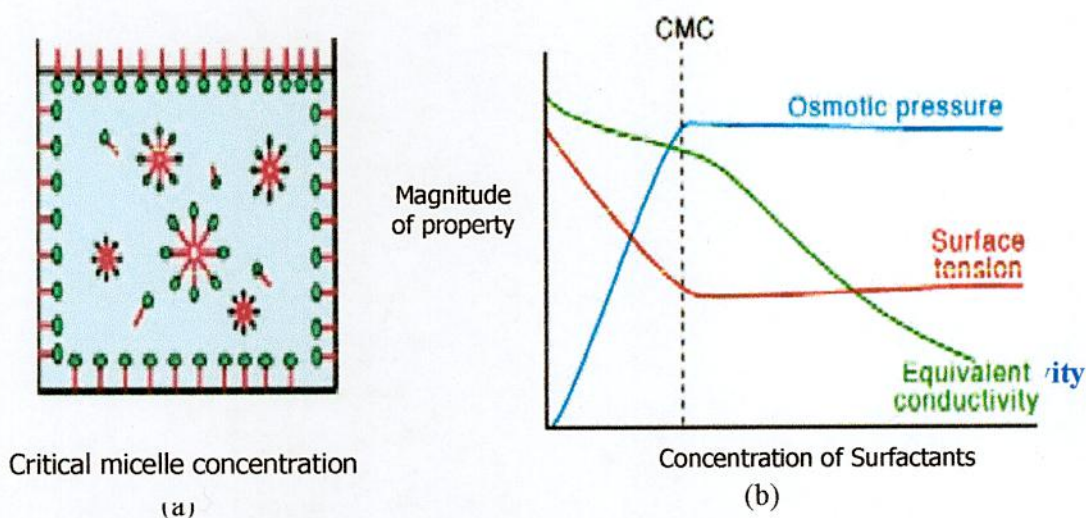


Figure 1.7: (a) Formation of micelle at CMC. (b) Schematic representation of the concentration dependence of conductivity of solution on micelle-forming amphiphiles

CMC is the concentration of surfactants in the bulk at which micelles start forming. The word bulk is important because surfactants partition between the bulk and interface and CMC is independent of interface and is therefore a characteristic of the surfactant molecule. In most of the situations like for e.g. in surface tension measurements or

conductivity measurements, the amount of surfactant at the interface is negligible compared to that in the bulk and CMC is approximated by the total concentration as is done in most of the textbooks.

There are important situations where interfacial areas are large and the amount of surfactant at the interface can't be neglected. For example if we take a solution of a surfactant above CMC and start introducing air bubbles at the bottom of the solution, these bubbles, as they rise to the surface, pull out the surfactants from the bulk to the top of the solution creating a foam column thus bringing down the concentration in bulk to below CMC. This is one of the easiest methods to remove surfactants from effluents (Foam Flotation). Thus in foams with sufficient interfacial area there will not be any micelles. Similar reasoning holds for emulsions.

The other situation arises in detergency. One initially starts off with concentrations greater than CMC in water and on adding fabric with large interfacial area and waiting for equilibrium, the surfactant concentration goes below CMC and no micelles are left. Therefore the solubilization plays a minor role in detergency. Removal of oily soil is by modification of the contact angles and release of oil in the form of emulsion.

1.7 Characterization Techniques

1.7.1 X-ray Diffraction

XRD is applied for identifying inorganic crystals, measuring degree of crystallinity, determining size of crystal and crystalline orientation. When x-ray enters to crystals which atoms line up regularly, it scatters due to the atoms. The X-ray diffracts when crystal's lattice spacing satisfies a specific law called Bragg's law. The formula of Bragg's law of X-ray diffraction is $2d\sin\theta = n\lambda$ ($n = 1, 2, 3, \dots$).

In this study, obtained substrates were identified with Bruker Advance D8 XRD. Spectra were collected within $10-70^\circ$ with $\text{CuK}\alpha 1$ radiation ($\text{CuK}\alpha 1 = 1.5418 \text{ \AA}$), current = 40 mA and voltage = 45 kV. The measurement was carried out with 0.01° per step.

1.7.2 Scanning Electron Microscopy

Scanning electron microscopy is a method to evaluate surface morphology. Brief mechanism of SEM is that photoelectrons such as secondary electrons and reflected electrons come out from a test piece when an electron beam radiates, and collecting information from the electrons. In this study, size and shape of HAp was investigated

with JSM-7401F (JEOL, Japan), JSM-7600F (JEOL, Japan), S-4700 (Hitachi Hi-Tech corporation, Japan), and Sirion (FEI, The United States). Samples were prepared on aluminum stands with conducting carbon tip coating. Images were captured at 2.0-5.0 kV.

1.7.3 Fourier Transform Infrared Spectroscopy

Fourier transform infrared is used for determining structure of organic compounds through measuring inherent absorbance from vibration which induces changes of dipole moments by irradiating infrared ray to substances.

In the current study, Attenuated Total Reflection Infrared (ATR-IR) was employed. Compared to KBr method, ATR method is superior in determining functional groups near surfaces. The absorbance in $400\text{--}4000\text{ cm}^{-1}$ was measured with IRTracer-100, Shimadzu Corporation, Japan.

1.7.5 Thermal Gravimetric Analysis

Thermogravimetric analysis is an analytical method to measure change of sample's weight continuously by thermobalance. Through the method, degradability and reactivity of inorganic or organic compounds are revealed. The variations in composite weight were measured between $30\text{--}800\text{ }^{\circ}\text{C}$ at the rate of temperature increase of $10\text{ }^{\circ}\text{C}/\text{min}$ under air atmosphere with TG/DTA STA 7200 Hitachi Corporation, Japan.

CHAPTER 2

Literature Review

Ceramic materials for orthopedic applications were introduced in the 1970s, where failures of the biomaterials in use then, such as steel, cobalt alloys and poly (methyl methacrylate), began to be detected. Hence, attention was directed to ceramic materials in an attempt to find good bone integration features. Ceramic materials used in surgery can either be bioactive. A bioactive material, is one which does not illicit a reaction with the surrounding physiological tissue upon implantation. A bioactive material, however, is one that allows a specific biological response at its interface, thus enabling the formation of links between the tissues and said material. [52]

Hydroxyapatite, (HAp), and other related calcium phosphate minerals are used extensively to improve the integration of femoral implants into the hip joint. HAp appears the most promising due to its outstanding biological properties such as a toxicity, lack of inflammatory response and absence of fibrous or immunological reactions [52]. HAp possesses identical chemical composition and high biocompatibility with natural bone [53].

The bioactive nature of HAp gives rise to a favorable reaction product, through a chemical transformation of its starting material to the desired final product. Subsequently, when HAp comes in contact with physiological fluids, a chemical reaction towards the production of newly formed bone takes place, in what is also termed an osteoconductive process [52]. Bioactivity is related to a modification of the surface of the material with formation of naturally induced; biologically equivalent HAp as a result of dissolution, precipitation and ion exchange reactions with the physiological environment. This ability to bond to bone tissue without the formation of a fibrous tissue layer at the bone - implant interface is extremely important since it guarantees the fixation and stabilization of implants and prostheses. The bone - implant interface mimics the type of interface that is formed when natural tissues repair themselves; it is dynamic in its nature, changes with time and requires a controlled degree of chemical reactivity of the ceramic [52]. Hence, the clinical advantage of HAp is due to its bioactive characteristics, which is dependent upon its synthetic structure such as chemical stoichiometry, crystallinity and surface morphology [52]. HAp has many crystallographic features that are similar to those of natural bone tissue; although they show insufficient mechanical reliability for medical applications where high loads are required [52].

Thermal spray processes have frequently been used to deposit functionally biomedical active coatings, such as HAp, onto prosthetic implants [54]. Kweh *et al.* [53] confirm from their investigation, that the quality of a HAp coating onto substrate is closely dependent on the overall attributes and characteristics of the synthesized powders. These attributes include particle size, particle-size distribution and particle morphology. These attributes help to determine the flow characteristics of the HAp particles in the powder-feeding systems and their subsequent melting behavior in the plasma jet. Consequently, the use of powder particles with spherical geometry and narrow size distributions so as to induce excellent heat transfer and consistent melting capabilities, increase the deposition efficiency and decrease coating porosity [53]. Spherical powders in general have better rheological properties than irregular powders and, thus, produce better coatings for femoral implants [55].

Several attempts to determine the exact composition of HAp via chemical analysis were undertaken in the first half of the 18th century. About a century later, the current concepts of synthesizing materials comprising of the various calcium phosphate crystal phases was introduced [56]. Most calcium phosphates are classified as biomaterials. This means that under physiological conditions they will dissolve. The benefit of calcium phosphate biomaterials is that the dissolution products can be readily assimilated by the human body.

Precipitation techniques can produce small particle size and high-purity HAp powders, when the precipitation system and conditions are well controlled to obtain a reproducible precipitate. The important precipitation process variables include solution concentration (Ca/P ratio), pH, acid addition rate, stirrer speed, temperature, reaction time and atmospheric condition as well [11]. The overall HAp-NPs production routes have been discussed by different researchers in different ways such as hydrothermal [11, 16, 17], hydrolysis [18], sol-gel [19], solid-state [20, 21], spray-drying [22] and freeze-drying [23] techniques, as well as other various novel synthesis routes [24] for preparing HAp using novel precursor material [25], are outlined in Kehoe [14].

Precipitation method of synthesizing HAp has been considered in this study. Precipitation reaction has been carried in the mixed micelle core of anionic and cationic surfactant. Reactions were done in water in oil systems. Various ratio of anionic and cationic surfactant was chosen to control the morphology of the HAp-NPs. Prepared HAp-NPs were characterized using a series of analytical experiments.

Chapter 3

Experimental

3.1 Overview

In this chapter, the experimental details of the total thesis work has been stated. At first, all the reagents and materials that were used in the experimental stage have been shown. Methodology of preparing HAp-NPs has been described in details. Finally the information regarding characterization of the prepared HAp-NPs has been given.

3.2 Reagents and materials

In this thesis reagents and materials used were divided into 3 parts: used in the i) precursor preparation ii) substrate material cleaning process and iii) cell assembly. The details of all the reagents and materials used in this thesis can be read in Table 3.2.1.

Table 3.2.1: Information of the used reagents and materials

Reagents and materials	Company
Cetyl trimethyl ammonium Bromide	Sigma Aldrich Chemie Gmbtt
Sodium dodecyl sulphate	Sigma Aldrich Chemie Gmbtt
Propanol-1	Qualikems Fine Chem Pvt. Ltd.
Ethanol	EMD Millipore Corporation
Calcium hydroxide, $\text{Ca}(\text{OH})_2$	Loba Chemie Pvt. Ltd.
Phosphoric acid, H_3PO_4	Merch Specialities Pvt. Ltd.
Ammonium hydroxide, NH_4OH	EMD Millipore Corporation
Ethanoic acid	EMD Millipore Corporation
Ammonium phosphate	Loba Chemie Pvt. Ltd.
Ammonium bi-phosphate	Merch Specialities Pvt. Ltd.

Distilled water prepared in Department of Chemistry, KUET, Khulna were used in all experiments

3.3 Characterization Methods and Equipment

Synthesis and chemical analysis and some characterization have been done in the Department of Chemistry, KUET, Khulna. In addition some other characterization of the synthesized materials have been performed in physics laboratory, KUET, Khulna and Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh. Numerous equipments were used during the research work and the list of them were given following table.

Table 3.3.1: Information of the instruments used in this experiment

Instrument name	Origin
Conductivity meter	CT 676, BOECO, Germany
pH meter	BT 675, BOECO, Germany
Centrifuge machine	EBA 21 Hettich Zentrifugen
Oven	Gemmy Industrial Corporation
FTIR spectrometer	IRTracer-100, Shimadzu Corporation, Japan
TGA	TG/DTA STA 7200 Hitachi Corporation, Japan
Analytical balance	ATY 224, Shimadzu Corporation, Japan
X-ray Diffraction	Bruker Advance D8 XRD, Germany
Scanning Electron microscopy	JEOL JSM-7600FJEOL, USA

3.4 Selection of composition of propanol-1 and water from conductance measurement

In the experiment a fixed concentration of 10×10^{-4} M SDS solution was prepared with different proportion of propanol-1 and water. The solution of different composition was taken in a beaker and the conductance of the solution was noted. The specific conductance obtained by multiplying the observed conductance with cell constant. Specific conductance of SDS solutions are listed in Table 3.4.1.

Table 3.4.1: Determination of specific conductance of 10×10^{-4} M SDS solution in various proportions of H₂O and propanol-1 at room temperature

Propanol-1 : H ₂ O	Mole fraction of	Cell constant	Conductance of SDS solution	Specific conductance of SDS solution $\times 10^{-5}$
20 : 0	1.000	1.0	31.0	3.10
18 : 2	0.729		37.0	3.70
16 : 4	0.545		36.5	3.65
14 : 6	0.411		40.2	4.02
12 : 8	0.310		45.3	4.53
10 : 10	0.230		51.5	5.15
8 : 12	0.166		55.5	5.55
6 : 14	0.114		64.8	6.48
4 : 16	0.069		67.9	6.79
2 : 18	0.031		79.6	7.96
0 : 20	0.000		81.8	8.18

The experimentally obtained data listed in Table 3.4.1 have shown that the conductance of 10×10^{-4} M SDS solution in various proportions of propanol-1 and H₂O increases with increase in the proportions of water. This is due to the less polar environment maintained at lower proportion of water. At relatively large proportion of propanol-1 the polar heads of the surfactant facing inward towards water as a result of electro-active repulsive interaction of water in oil phase. The confinement of water in the reverse micelle core exists at a certain proportion of propanol-1: H₂O system. In this study it has been maintained until 12:8 ratio of propanol-1: water system. The value has been attained from the intersection point of the two straight lines of the conductivity versus mole fraction of propanol-1. After that with increasing the amount of water a remarkable change in the orientation of polar head groups occurred. With increasing the proportion of water molecular reorientation of the polar head groups lead to the formation of micelle core. Thereby, an increase in the conductivity value has been examined with increasing the amount of water.

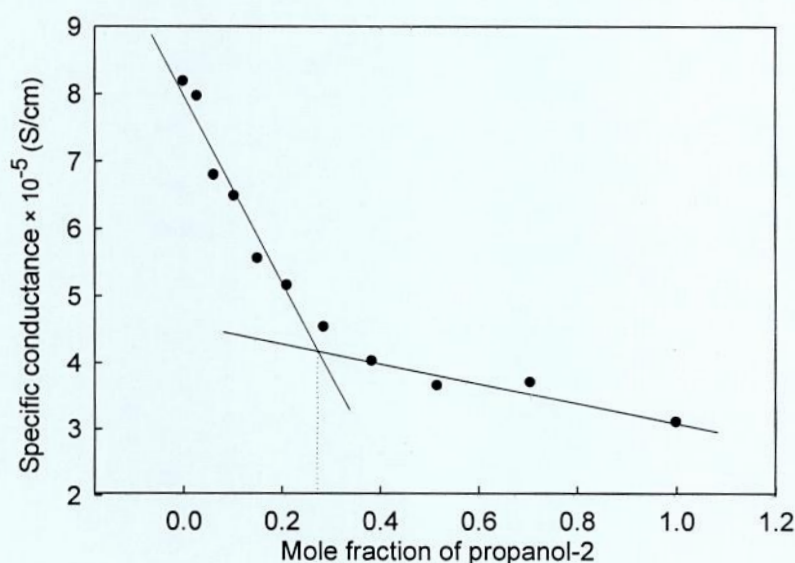


Figure 3.4.1: A plot of specific conductance of 10×10^{-4} M SDS vs mole fraction of propanol-1

The graph indicates that 10×10^{-4} M SDS solution can maintain reverse micelle assembly just after 16 : 4 ratio of propanol-1 : water system. Henceforth, the ratio after 16 : 4 of propanol : water system of SDS is supposed to be compatible for the formation of reverse micelle core. The reverse micelle formation region of 10×10^{-4} M CTAB in propanol-1 : water solution system was then determined. We followed the

aforementioned procedure to determine it. Specific conductance of 10×10^{-4} M CTAB solution in various ratio of propanol-1 : water system are listed in the following table 3.4.2.

Table 3.4.2: Determination of specific conductance of 10×10^{-4} M CTAB in various proportions of H₂O and propanol-1 solution system at room temperature

Propanol-1 : H ₂ O	Mole fraction of propanol-	Cell constant (cm ⁻¹)	Conductance of CTAB solution $\times 10^{-6}$ (S)	Specific conductance of CTAB solution $\times 10^{-6}$ (S/cm)
20 : 0	1	1	13.9	13.9
18 : 2	0.706		20.4	20.4
16 : 4	0.517		27.3	27.3
14 : 6	0.384		28.2	28.2
12 : 8	0.286		35.0	35.0
10 : 10	0.211		33.8	33.8
8 : 12	0.151		44.6	44.6
6 : 14	0.103		47.2	47.2
4 : 16	0.062		62.9	62.9
2 : 18	0.028		77.9	77.9
0 : 20	0.000		92.9	92.9

The data listed in Table 3.4.2 are plotted in the following Figure 3.4.2. Here, the theory for the difference in conductivity of 10×10^{-4} M CTAB solution at lower and higher mole fraction of propanol-1 is related to the orientation of the surfactant group.

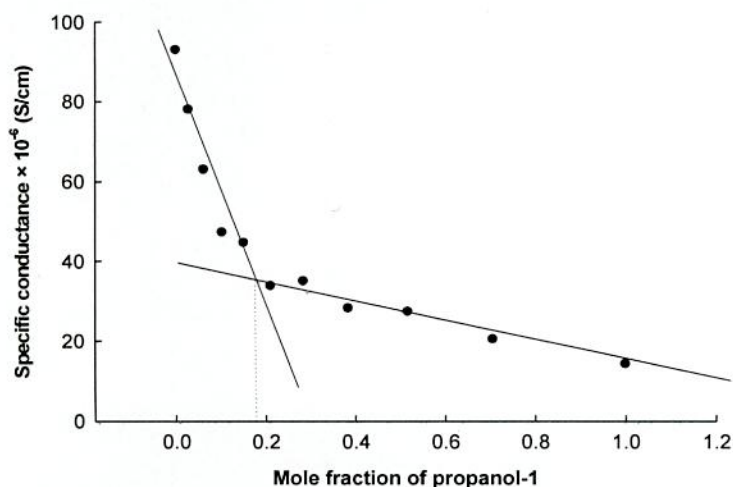


Figure 3.4.2: A plot of specific conductance of 10×10^{-4} M CTAB vs mole fraction of propanol-1

Orientation and re-orientation of the polar and non-polar groups occurred at lower and higher mole fraction of propanol-1. The details regarding this has been mentioned in case of SDS solution. We observed the same phenomena in case of CTAB also.

Figure 3.4.2 indicates that 10×10^{-4} M CTAB solution can maintain reverse micelle assembly just after 10 : 10 ratio of propanol-1: water system. Henceforth, the ratio after 10 : 10 of propanol-1 : water system of CTAB is compatible for the formation of reverse micelle core. From the above experiment a compatible ratio 18 : 2 of propanol-1: water system has been chosen to prepare HAp nanoparticles. In this study we used both anionic (SDS) and cationic (CTAB) surfactants to prepare mixed micelle core for the formation of HAp nanoparticles. Various mixed micelle core has been formed at 18 : 2 of propanol-1: water system by changing the amount of each surfactant. In preparing various mixed micelle core the composition of each surfactant have been chosen such that their summation turned out to be 1.2×10^{-4} mol. It is obvious that this concentration is slightly greater than the reverse micelle concentration of each surfactant. We chose it intentionally to form reverse micelle nano-core where positive and negative end of CTAB and SDS might have arranged in the following way.

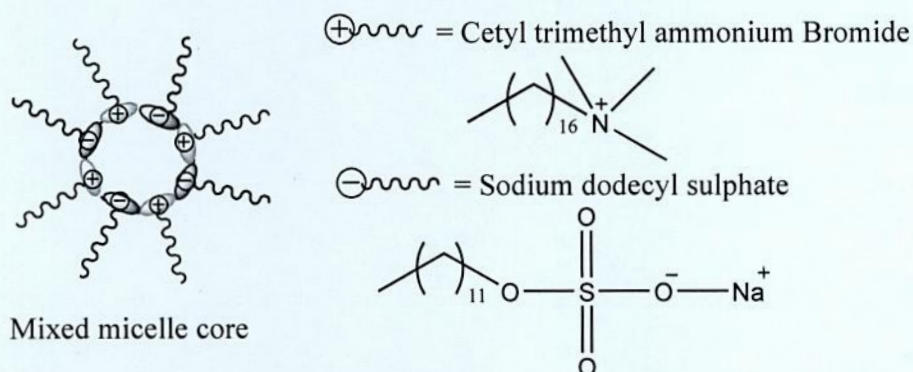


Figure 3.4.3: Mixed micelle arrangement of an anionic surfactant SDS and a cationic surfactant CTAB

3.5 Preparation of mixed reverse micelle core

The solution of different composition of SDS & CTAB such as 4 : 1, 3 : 2, 1 : 1, 2 : 3 and 1 : 4 has been prepared in 18:2 of propanol-1 : water system. Each of this mixed micelle core has been used as nano-reactor for preparing HAp-NPs. Various used composition of SDS and CTAB in 18:2 of propanol-1 : water system are presented in the Table 3.5.1.

Table 3.5.1: Composition of SDS and CTAB used to prepare the mixed micelle core

SDS : CTAB	Amount of SDS (mol)	Amount of CTAB (mol)	Total amount (mol)
4 : 1	0.96×10^{-4}	0.24×10^{-4}	1.20×10^{-3}
3 : 2	0.72×10^{-4}	0.48×10^{-4}	1.20×10^{-3}
2 : 3	0.48×10^{-4}	0.72×10^{-4}	1.20×10^{-3}
1 : 1	0.60×10^{-4}	0.60×10^{-4}	1.20×10^{-3}
1 : 4	0.24×10^{-4}	0.96×10^{-4}	1.20×10^{-3}

3.6 PREPARATION OF HAp POWDER

A stoichiometric amount of $\text{Ca}(\text{OH})_2$ and H_3PO_4 will be added separately in each of the mixed reverse micelle core solution to trap the inorganic raw materials in the micelle core. Then 10 mL of the prepared calcium hydroxide solution has been added to 6 mL phosphoric acid solution. Required amount of ammonia solution has been also added to this mixture to make pH 10-11. The solution is then aged at room temperature until precipitation stop. The resulting precipitate has been centrifuged, washed with water and ethyl alcohol respectively. Then it has been dried at 110 °C for 5 h to obtain white HAp-NPs.

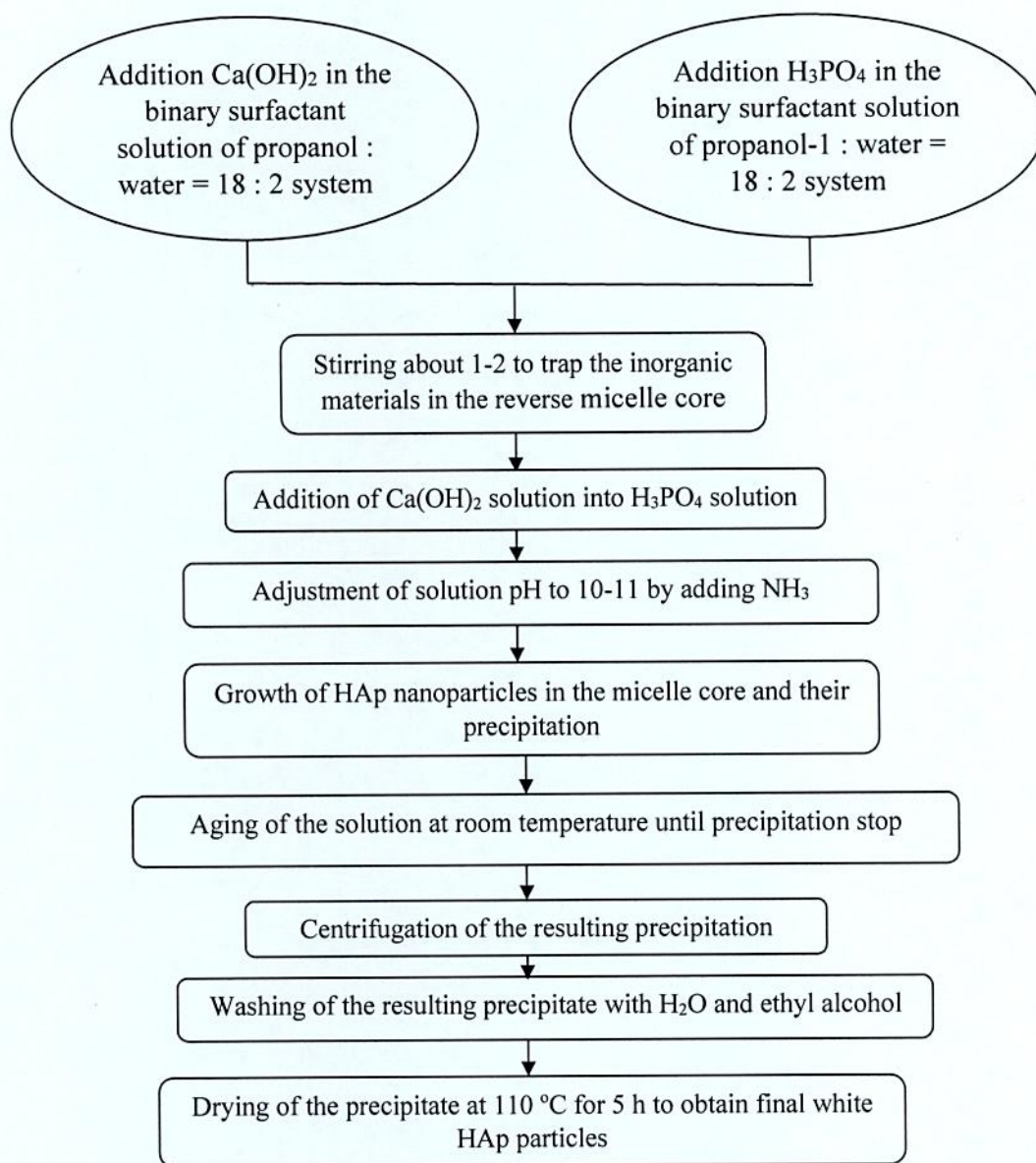


Figure 3.6.1: Flow chart of the HAp synthesis process

Reaction occur in this work:



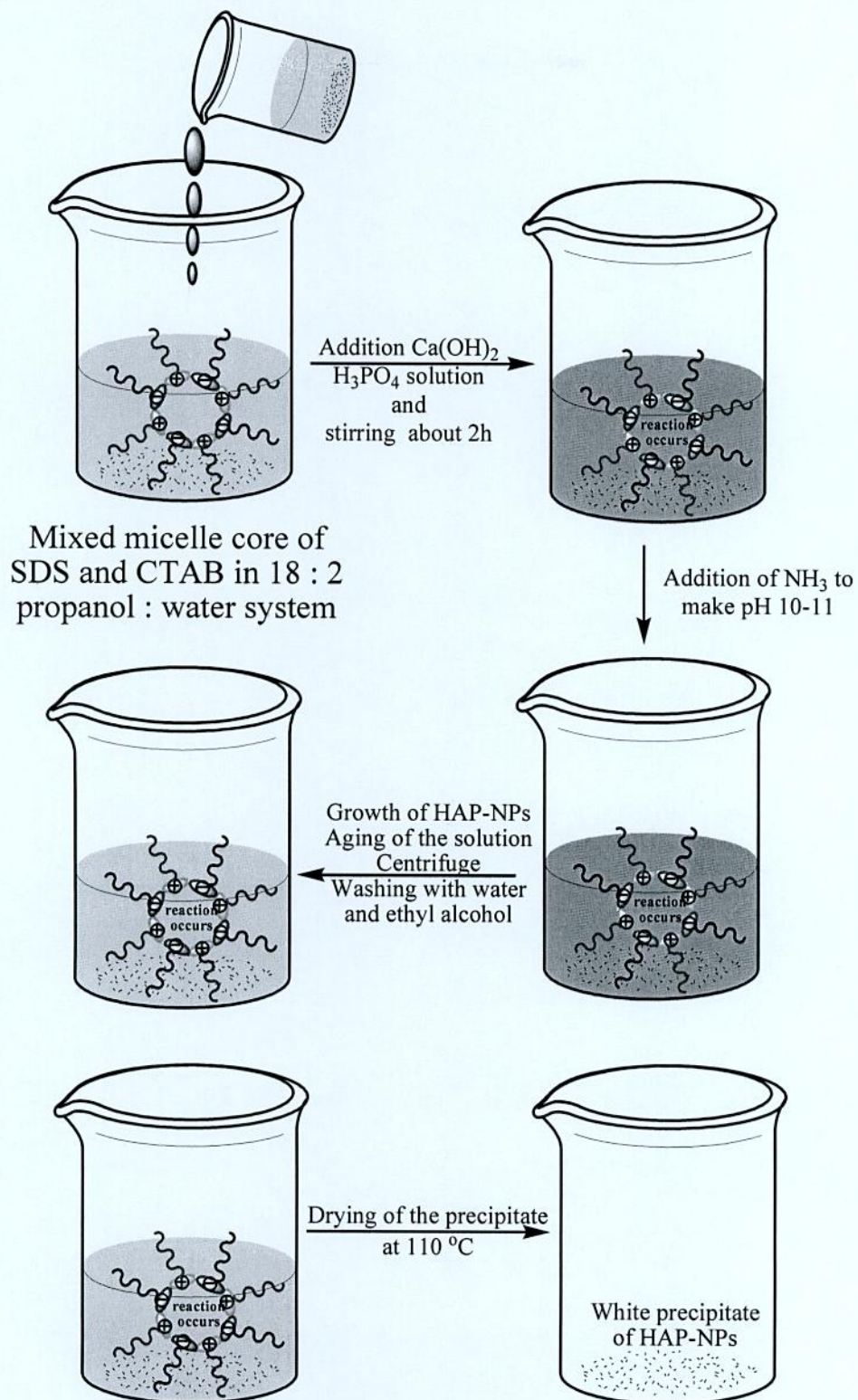


Figure 3.6.2: Different steps of preparing HAp-NPs

3.7 Characterization techniques:

3.7.1 Sample preparation for TGA

TGA experiments were performed for HAp-NPs prepared via mixed micelle core of 1:1, 1:4 & 3:2 ratio surfactants of SDS: CTAB. The weight loss of the sample was recorded by using TG/DTA STA 7200 Hitachi Corporation, Japan. The prepared HAp-NPs were placed in a TGA sample pan. Sample masses were confirmed with a precision balance. A computer based TGA program was used for obtaining TGA curve. The samples were held at room temperature under a flow of nitrogen gas to create inert environment for the sample and drive off gases such as oxygen, water vapor. Then the temperature was increased from room temperature to 800 °C at a rate of 10 °C/min. At the end of each experiment the temperature was held constant at 800 °C for ten minutes. After ten minutes the gas flow was switched off and waited until the temperature cooled down to room temperature.



Figure 3.7.1.1: Picture of a TGA machine

3.7.2 Functional group analysis using ATR

The attenuated total reflection (ATR) technique of FTIR is used to analyze the presence of functional groups of prepared HAp-NPs. Characterization was carried out using IRTracer-100 of Shimadzu corporation, Japan. Mainly P-O stretching vibration of PO_4^{3-} in the prepared hydroxyapatite nanoparticles were identified by using ATR technique. No sample preparation is required for this analysis. One kind of crystals knob attached with the FTIR machine is used to identify the presence of functional groups of the prepared sample. The crystal knob has been kept clean and scratch-free

for each of the experiment. Firstly we placed a sample of HAp-NPs on the top of a flat surface. Then we mounted and adjusted the knob above the sample surface. Adjustment between knob and the sample surface was done in such as a way that a consistent contact is achieved between them. Afterwards, a black gripper was used to cover the sample surface. A computer based FTIR program was used to identify the functional groups of HAp-NPs. The entire FTIR spectrum of the prepared sample has been recorded in the range of 500 to 4000 cm^{-1} .



Figure 3.7.2.1: Picture of a FTIR machine

3.7.3 Phase Identification using XRD

X-ray diffraction studies on the prepared samples were carried out using a high resolution Bruker Advance D8 XRD (Figure 3.7.3.1) diffractometer in Bragg-Brentano geometry, with a $\text{CuK}\alpha 1$ monochromated beam ($\lambda = 0.15406 \text{ \AA}$) produced at 40kV and 40mA. The sample holders has been loaded by simply pouring it with small amount of HAp-NPs. Then it was mounted on the diffractometer. The scanning range (2θ) was performed from 10-70°. This technique was used to assess the phases present in the prepared HAp-NPs.



Figure 3.7.3.1: Picture of a XRD machine

3.7.4 Sample Morphology

A JEOL JSM-7600F JEOL was used in this study to obtain the surface morphology of the prepared HAp-NPs. The surface morphology of the HAp powders were of interest as we used different composition of surfactants for preparing mixed micelle core. Henceforth, morphology of the prepared HAp-NPs provided a good indication about the effect of mixed micelle core. The HAp-NPs samples are non-conducting. Sample preparation for each HAp-NPs samples were carried out. The first stage involved suspending HAp-NPs samples in acetone. A conductive carbon tape was pasted on the alumina plate. Then the 2-3 drops of the sample was put on the conductive carbon tape. After that the sample was allowed to dry for a sufficient period of time. Any excess powder on the sample plate was eliminated using an air blower. Finally the sample plate was mounted on the sample chamber for SEM operation.

At the first attempt of operation a vacuum pump was used to achieve the level of vacuum required to produce a consistent electron beam. A finely focused electron beam between 10 – 25kV was irradiated on the sample under observation. A detector monitors the scattered electron (SE) signals and the brightness of the spot is controlled by an amplified version of the detected signal. The surfaces facing the light source appear bright while those facing away range from grey to black.



Figure 3.7.4.1: Picture of a scanning electron microscope machine

Chapter 4

Results and discussion

The present study is an attempt to know the effect of mixed micelle core of a cationic surfactant CTAB and an anionic surfactant SDS to control the morphology of HAp-NPs. A total of five HAp-NPs has been prepared in mixed micelle core of a cationic surfactant CTAB and an anionic surfactant SDS. Overall, five different ratio of SDS and CTAB has been chosen to prepare mixed micelle core. Each of the nano-core has been used as a nano-reactor to prepare the desired HAp-NPs. The as prepared HAp-NPs were then characterized by using FTIR, XRD, TG and SEM. The results of this study has been presented and discussed in this chapter.

4.1 FTIR data analysis of the prepared HAp-NPs using mixed micelle core of surfactants SDS and CTAB

FTIR spectroscopy has been employed to identify the different functional groups of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, (HAp) powder. The entire FTIR spectrum of the prepared sample has been recorded in the range of 500 to 4000 cm^{-1} . Spectrum for commercial HAp is presented in Figure 4.1. The appearance of peak at 561, 599, 628, 962, 1026 and 1087 cm^{-1} are due to the P-O stretching vibration of PO_4^{3-} in HAp. IR bands at 2331 and 2341 cm^{-1} attributed to the asymmetric and symmetric stretching vibrations for the presence of CO_2 molecule.

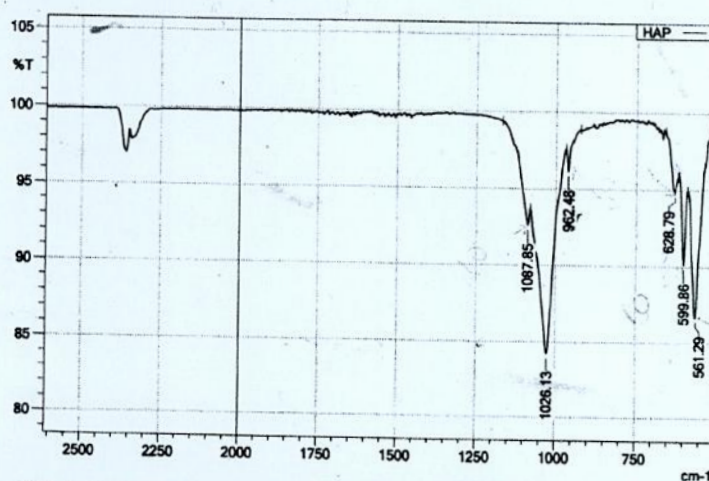


Figure 4.1.1: FT-IR spectrum of commercial HAp molecule.

The following table 4.1.1 listed the appearance of functional groups of commercial HAp-NPs from the FTIR-spectrum of Figure 4.1.1.

Table 4.1.1: Wave numbers (cm^{-1}) observed and tentative assignments in the spectrum of HAp

Wave numbers (cm^{-1})	Assignment
561.29	Vibration peaks of PO_4^{3-} group
599.86	
628.79	
962.48	Stretching mode of PO_4^{3-} group
1026.13	
1087.85	
2300-2500	Presence of CO_2

The FTIR-spectrum of the as prepared HAp-NPs using mixed micelle core of 1:1 ratio of SDS and CTAB have been shown in Figure 4.1.2.

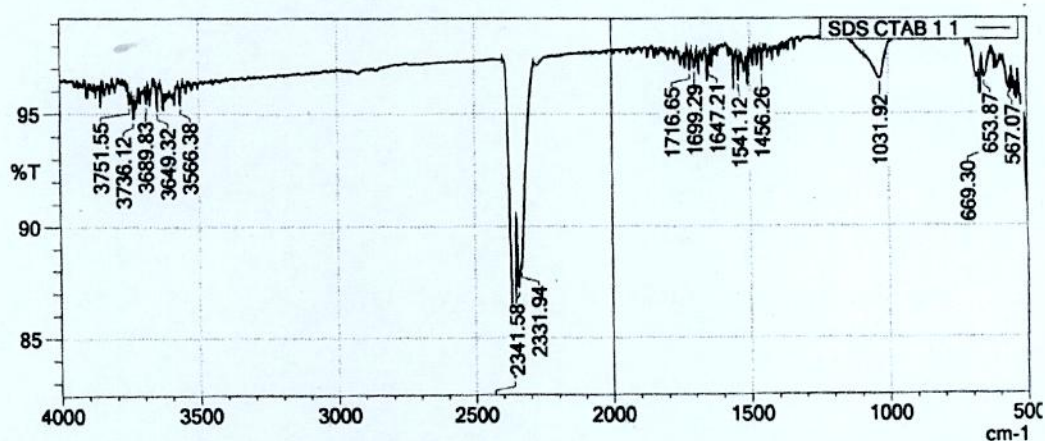


Figure 4.1.2: FT-IR spectrum of HAp-NPs prepared in 1:1 ratio of SDS and CTAB mixed micelle core

The appearance of functional groups in the FTIR-spectrum of the as prepared HAp-NPs from Figure 4.1.2 are listed in the following table 4.1.2.

Table 4.1.2: Wave numbers (cm^{-1}) observed and tentative assignments in the spectrum of HAp

Wave numbers (cm^{-1})	Assignment
567.07	Vibration peaks of PO_4^{3-} group
653.87	
669.30	
1031.92	Stretching mode of PO_4^{3-} group
1456.26 to 1716.65	Adsorbed water
2331.94	Presence of CO_2
2341.58	
3649.32 to 3751.55	Adsorbed water
3566.38	-OH group vibration

The FTIR-spectrum of the as prepared HAp-NPs using mixed micelle core of 1:4 ratio of SDS and CTAB are presented in the following Figure 4.1.3.

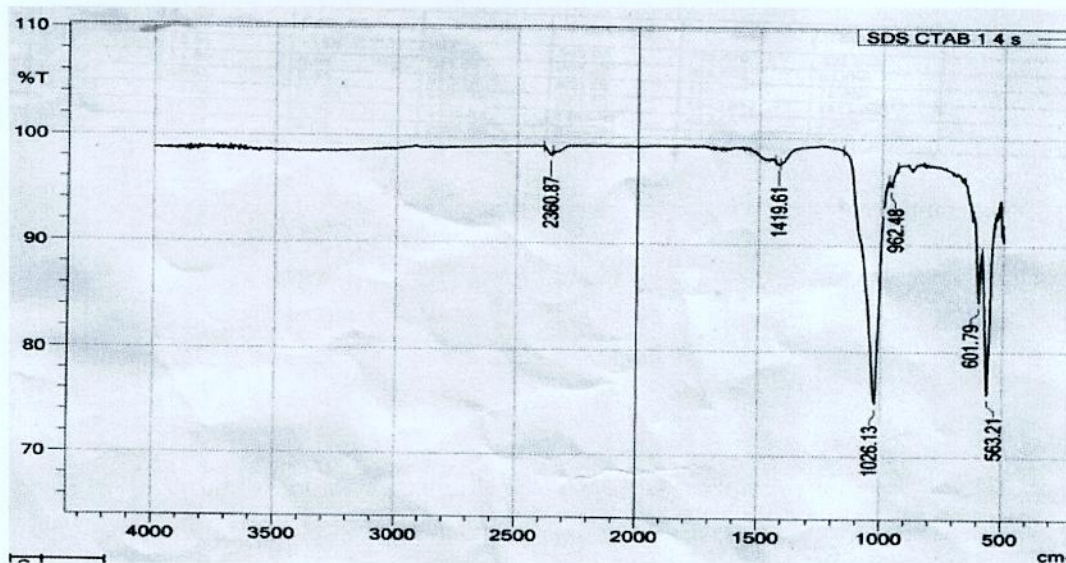


Figure 4.1.3: FT-IR spectrum of HAp-NPs prepared in 1:4 ratio of SDS and CTAB mixed micelle core

The presence of functional groups has been assigned from Figure 4.1.3 and are listed in the following table 4.1.3.

Table 4.1.3: Wave numbers (cm^{-1}) observed and tentative assignments in the spectrum of HAp

Wave numbers (cm^{-1})	Assignment
563.21	Vibration peaks of PO_4^{3-} group
601.79	
962.48	Stretching mode of PO_4^{3-} group
1026.13	
1429.61	Adsorbed water
2360.87	Presence of CO_2

The FTIR-spectrum of the as prepared HAp nanoparticles (HAp-NPs) using mixed micelle core of ratio 4:1 ratio of SDS and CTAB are presented in Figure 4.1.4.

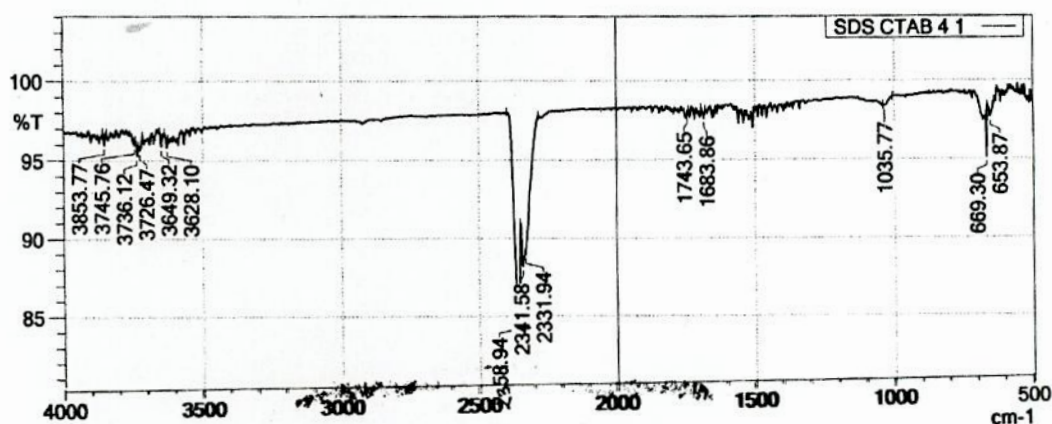


Figure 4.1.4: FT-IR spectrum of HAp-NPs prepared in 1:1 ratio of SDS and CTAB mixed micelle core

The following table 4.1.4 contains information about the appearance of functional groups of the as prepared HAp-NPs from Figure 4.1.4.

Table 4.1.4: Wave numbers (cm⁻¹) and tentative assignments in the spectrum of HAp

Wave numbers(cm ⁻¹)	Assignment
653.87	Vibration peaks of PO ₄ ³⁻ group
669.30	
1035.77	Stretching mode of PO ₄ ³⁻ group
1683.86 to 1743.65	Adsorbed water
2331.94 to 2558.94	Presence of CO ₂
3628.10 to 3853.77	Adsorbed water

The FTIR-spectrum of the as prepared HAp nanoparticles (HAp-NPs) using mixed micelle core of ratio 2:3 of SDS and CTAB are presented in Figure 4.1.5.

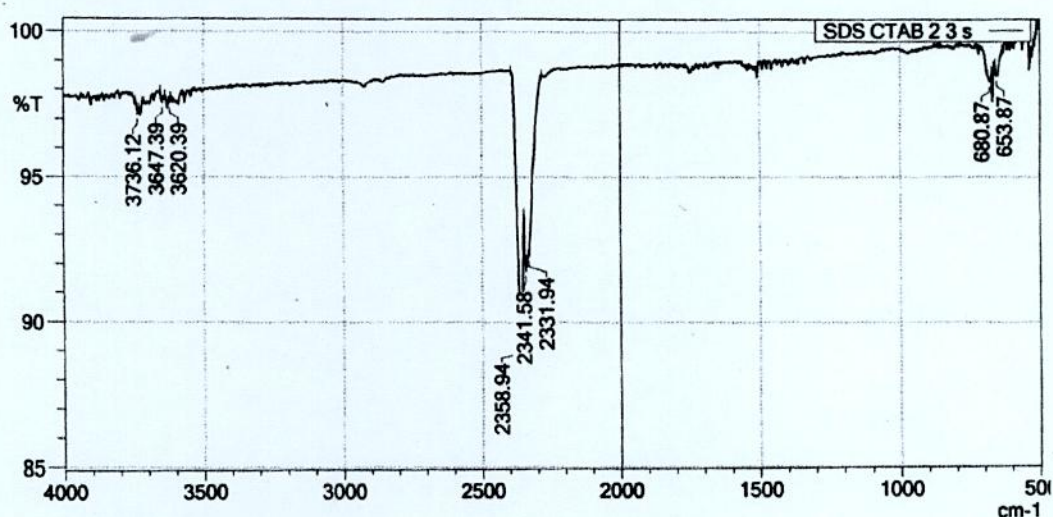


Figure 4.1.5: FT-IR spectrum of HAp-NPs prepared in 2:3 ratio of SDS and CTAB
The following table 4.1.5 provides information of functional group presence from the FTIR-spectrum of Figure 4.1.5.

Table 4.1.5: Wave numbers (cm⁻¹) observed and tentative assignments in the spectrum of HAp

Wave numbers(cm ⁻¹)	Assignment
653.87	Vibration peaks of PO ₄ ³⁻ group
680.87	
Peak around 1200	Stretching mode of PO ₄ ³⁻ group
2331.94 to 2358.94	Presence of CO ₂
3620.39 to 3736.12	Adsorbed water

The FTIR-spectrum of the as prepared HAp nanoparticles (HAp-NPs) using mixed micelle core of ratio 3:2 of SDS and CTAB are given in Figure 4.1.6.

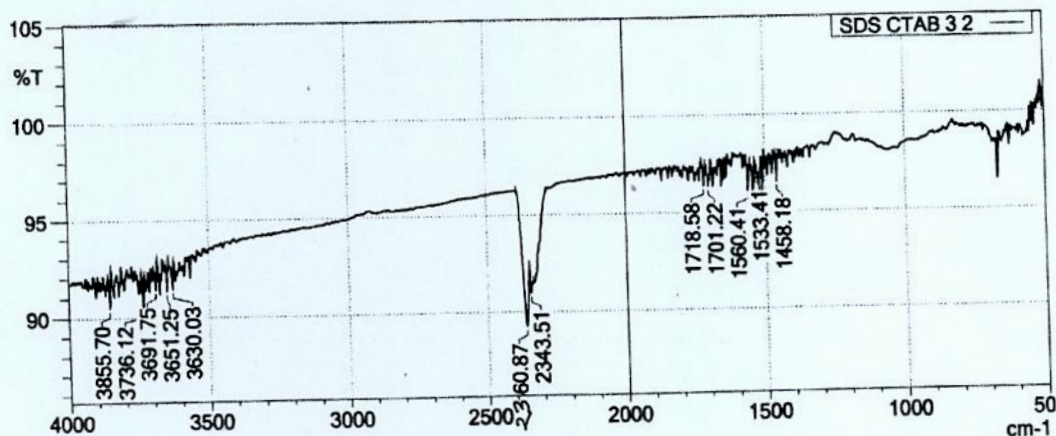


Figure 4.1.6: FT-IR spectrum of HAp-NPs prepared in 1:1 ratio of SDS and CTAB

The table 4.1.6 below shows the appearance of functional group as prepared HAp nanoparticles (HAp-NPs) from the FTIR-spectrum of Figure 4.1.6.

Table 4.1.6: Wave numbers (cm^{-1}) observed and tentative assignments in the spectrum of HAp

Wave numbers(cm^{-1})	Assignment
Peak around 550 to 680	Vibration peaks of PO_4^{3-} group
Broad peak around 900 to 1200	Stretching mode of PO_4^{3-} group
1458.18 to 1718.58	Adsorbed water
2343.51 to 2360.87	Presence of CO_2
3630.03 to 3855.70	Adsorbed water

Figure 4.1.2 to figure 4.1.6 confirmed the presence of different functional groups of the prepared HAp-NPs using different ratio of a cationic surfactant CTAB and an anionic surfactant SDS. The range of figure in the aforementioned line show bands that is assigned to the presence of PO_4^{3-} groups. Broad bands presence from 1400 to 1800 cm^{-1} and 3600 to 4000 cm^{-1} suggested the existence of adsorbed water. The peak assigned from 900 to 1200 cm^{-1} are related to the vibration of PO_4^{3-} groups. Presence of peaks in

the region of 900 to 1200 cm^{-1} are related to the stretching mode of PO_4^{3-} group. In addition, appearance of peaks from 500 to 700 cm^{-1} are due to the vibration of PO_4^{3-} group. However, all the FTIR spectrum of the prepared HAp-NPs shows peak between 2300 to 2500 cm^{-1} . Those peaks are assigned for the CO_2 from air or water. From the aforementioned results we concluded that HAp-NPs were obtained via the mixed micelle core of various ratio of a cationic surfactant CTAB and an anionic surfactant SDS.

4.2 XRD data analysis of the prepared HAp-NPs

Phase identification of the prepared HAp-NPs were performed with reference to the JCPDS, Card No. 9-432. This database published by the International Centre for Diffraction Data (ICDD). The list of major peaks for Standards (JCPDS) files for HAp are given in the following Tables 4.2.1.

Table 4.2.1: Major peaks for standards HAp JCPDS - International Centre for Diffraction Data (1998)

2θ ($^\circ$) ICDD	(h k l)
25.879	(0 0 2)
31.773	(2 1 1)
32.196	(1 1 2)
32.902	(3 0 0)
34.048	(2 0 2)
39.818	(3 1 0)
46.711	(2 2 2)
49.468	(2 1 3)
50.493	(3 2 1)
53.143	(0 0 4)

The following figures show the XRD patterns for the prepared HAp-NPs by using mixed micelle core of SDS and CTAB surfactants. XRD spectrum of the as prepared HAp-NPs are presented from figure 4.2.1 to Figure 4.2.5. Investigation on the obtained diffractogram with ICDD card-00-009-0432 revealed that the pattern did not differ significantly from each other. However, the difference in peak broadening and peak intensity among the diffractogram indicate differences in crystallinity and crystallite size of the prepared HAp-NPs.

The XRD-spectrum of the as prepared HAp-NPs using mixed micelle core of SDS and CTAB at ratio 1: 4 are given in Figure 4.2.1.

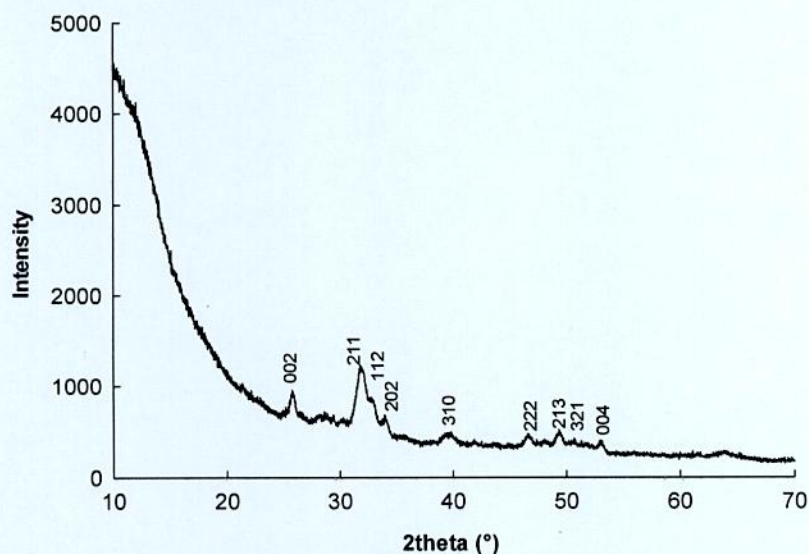


Figure 4.2.1: XRD spectrum of HAp-NPs prepared by using mixed micelle core of SDS: CTAB surfactants at ratio 1: 4.

The table 4.2.2 below shows the appearance of planes at various position of the prepared HAp-NPs from Figure 4.2.1.

Table 4.2.2: List of important peaks of the prepared HAp-NPs via mixed micelle core of SDS and CTAB surfactants at ratio 1 : 4

2θ (°)	(h k l)
25.504	(0 0 2)
31.664	(2 1 1)
32.992	(3 0 0)
34.000	(2 0 2)
39.504	(3 1 0)
46.656	(2 2 2)
49.504	(2 1 3)
50.656	(3 2 1)
53.168	(0 0 4)

The XRD-spectrum of the as prepared HAp-NPs using mixed micelle core at ratio 4: 1 of SDS and CTAB are given in Figure 4.2.2.

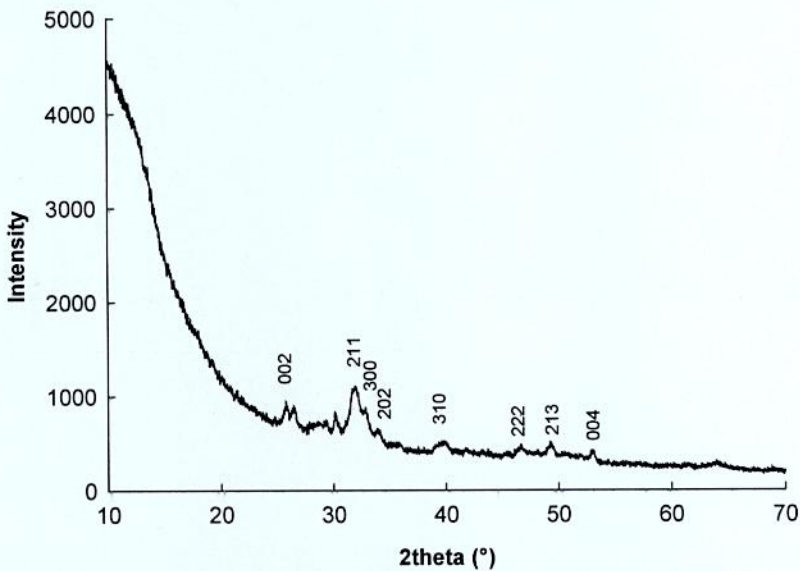


Figure 4.2.2: XRD spectrum of HAp-NPs prepared by using mixed micelle core of surfactants SDS: CTAB at ratio 4 : 1

The following table 4.2.3 provides the presence of planes at various position of the prepared HAp-NPs from Figure 4.2.2.

Table 4.2.3: List of important peaks of the prepared HAp-NPs via mixed micelle core of SDS and CTAB surfactants at 4:1 ratio

2θ (°)	(h k l)
25.744	(0 0 2)
31.628	(2 1 1)
32.872	(3 0 0)
34.252	(2 0 2)
39.628	(3 1 0)
46.624	(2 2 2)
49.372	(2 1 3)
53.128	(0 0 4)

The XRD-spectrum of the as prepared HAp-NPs using mixed micelle core of SDS and CTAB at ratio 2: 3 are given in Figure 4.2.3

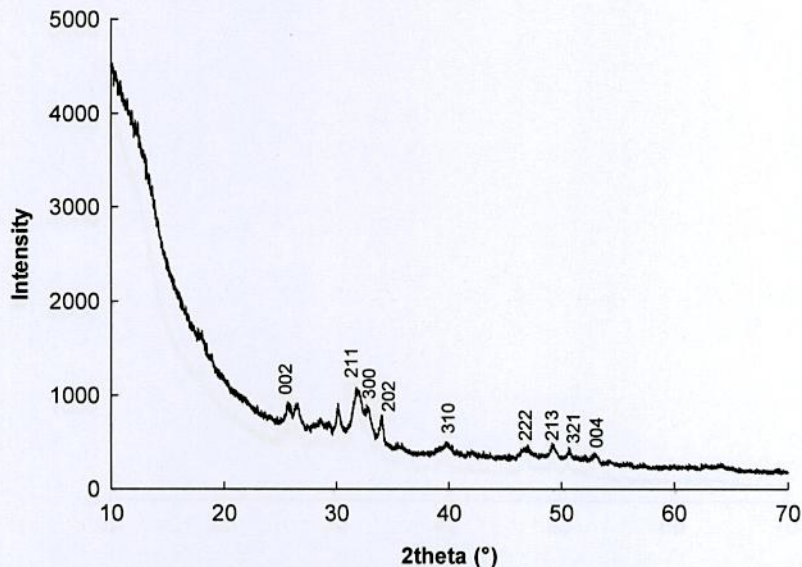


Figure 4.2.3: XRD spectrum of HAp-NPs prepared by using mixed micelle core surfactants SDS and CTAB at 2 : 3 ratio

The following table 4.2.4 provides the presence of planes at various position of the prepared HAp-NPs from Figure 4.2.3.

Table 4.2.4: List of important peaks of the prepared HAp-NPs via mixed micelle core of surfactants SDS and CTAB at 2 :3 ratio

2θ (°)	(h k l)
25.504	(0 0 2)
31.624	(2 1 1)
32.620	(3 0 0)
34.094	(2 0 2)
39.868	(3 1 0)
46.744	(2 2 2)
49.252	(2 1 3)
50.752	(3 2 1)
52.996	(0 0 4)

The XRD-spectrum of the as prepared HAp-NPs using mixed micelle core of SDS and CTAB at ratio 1: 1 are given in Figure 4.2.5.

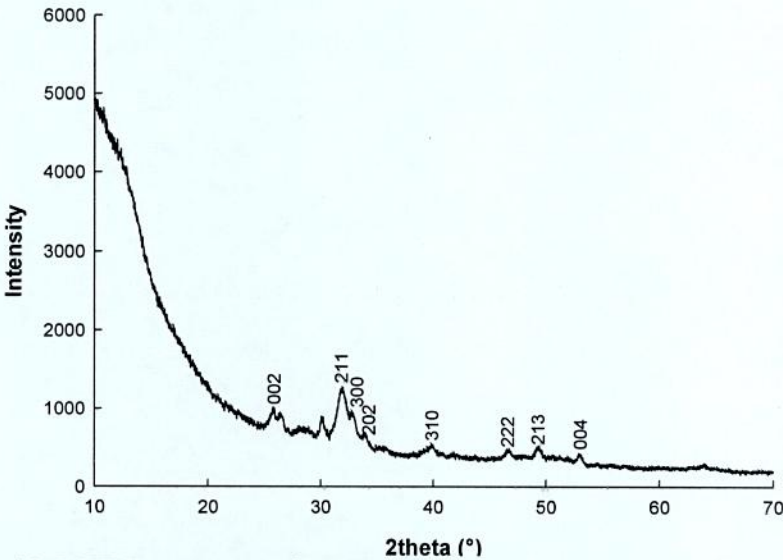


Figure 4.2.5: XRD spectrum of HAp-NPs prepared by using mixed micelle core of surfactants SDS: CTAB at 1 : 1 ratio.

The following table 4.2.6 provides the presence of planes at various position of the prepared HAp-NPs from Figure 4.2.5.

Table 4.2.6: List of important peaks of the prepared HAp-NPs via mixed micelle core of SDS and CTAB surfactants at 1 :1 ratio

2θ (°)	(h k l)
25.756	(0 0 2)
31.876	(2 1 1)
32.752	(3 0 0)
33.940	(2 0 2)
39.880	(3 1 0)
46.624	(2 2 2)
49.252	(2 1 3)
53.008	(0 0 4)

XRD patterns of the synthesized HAp-NPs shows similar phase to the database of JCPDS, Card No. 9-432. Identified position of the planes has almost similar position with HAp from the XRD pattern of JCPDS. Some characteristics peaks of the prepared HAp-

NPs from Figure 4.2.1 to 4.2.5 evidenced the formation of a single phase HAp. However, the broad diffraction peaks within the 2θ values of $10-70^\circ$ indicate the presence of amorphous phase. In other words it suggests the formation of small crystalline particles.

4.3 Thermal Stability Analysis of Prepared HAp-NPs by TG

Thermal stability of CaP reinforced composites primarily depends on the thermal stability of CaP particles and important in developing various CaP- reinforced compounds [57]. The thermogram of the as prepared HAp-NPs using mixed micelle core of SDS and CTAB at ratio 1: 1 are given in Figure 4.3.1.

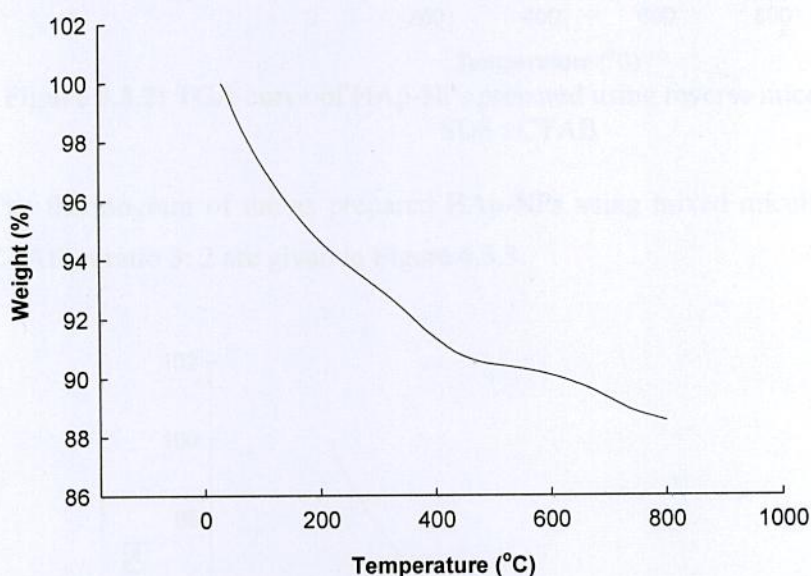
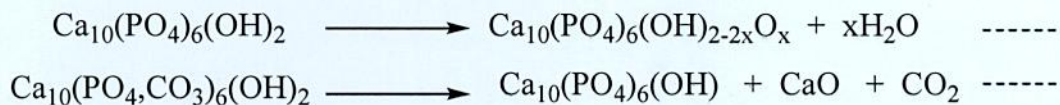


Figure 4.3.1: Thermogravimetric curve of HAp-NPs prepared in 1: 1 SDS : CTAB

The thermogram of the as prepared HAp-NPs using mixed micelle core of SDS and CTAB at ratio 1: 4 are given in Figure 4.3.2.

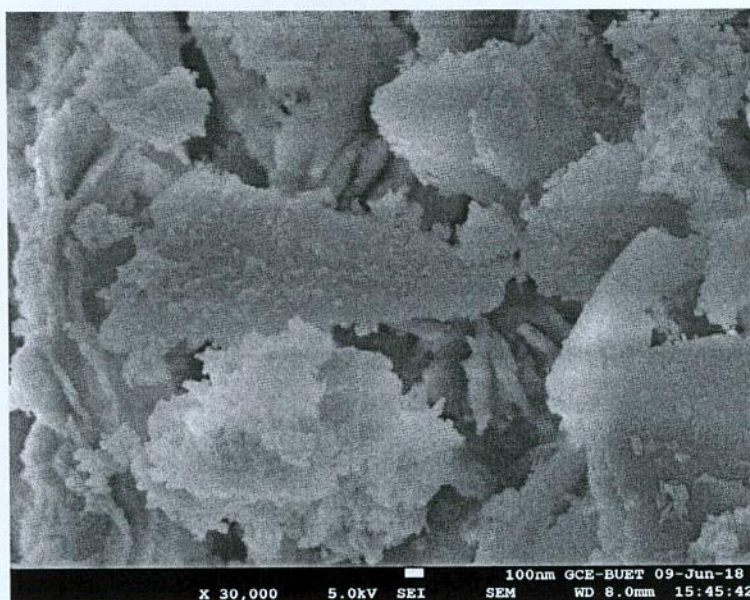
thermal stability. The weight loss between 25 and 800 °C temperature for 1 : 1 ratio of SDS : CTAB is 11.452 %. This value for 1 : 4 is 10.667 % and for 3 : 2 is 9.014 %. For all the three samples the mass loss between 25 to 800 °C is around 10%. The mass loss is associated to the elimination of carbonates and combined water. Initial mass loss is related to the release of physically adsorbed water molecules. Afterwards a partial dehydroxylation and/or decarboxylation of HAp-NPs might have happened. The reaction associated with mass loss can be represented by the following equations.



The weight loss for HAp-NPs prepared in 1 : 1 and 1: 4 ratio of SDS : CTAB is higher weight loss than for 3: 2. Thus the HAp-NPs prepared in 1 : 1 and 1: 4 ratio of SDS : CTAB is slightly less stable than in 3 : 2 ratio. It is also worth to mention that the particles prepared in 3 : 2 ratio has slightly good stability to maintain stoichiometric ratio compare to 1 : 1 and 1: 4 ratio of SDS : CTAB.

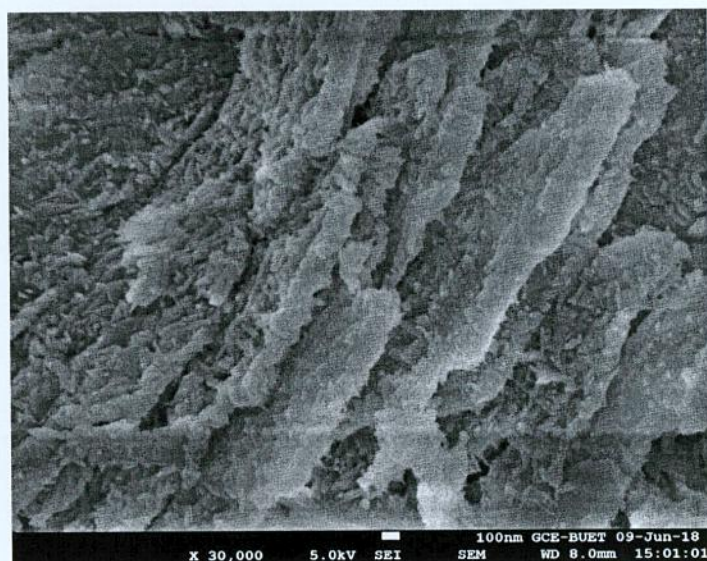
4.4 Morphology Analysis of Prepared HAp-NPs by SEM

The morphologies of mixed micelle core assisted HAp-NPs are presented in the following Figure 4.4.1 to 4.4.5. This result reveals morphological variation of the prepared HAp-NPs by changing the amount of two surfactants. Variation of the ratio of surfactant amount SDS and CTAB allow the variation in the morphology of the prepared particles. Each of the mixed micelle core formed at a certain ratio of SDS and CTAB in 18:2 of propanol-1 : water system has been able to produce a specific morphology. The details morphology of the prepared HAp-NPs using five different ratio of SDS and CTAB presented and discussed below.



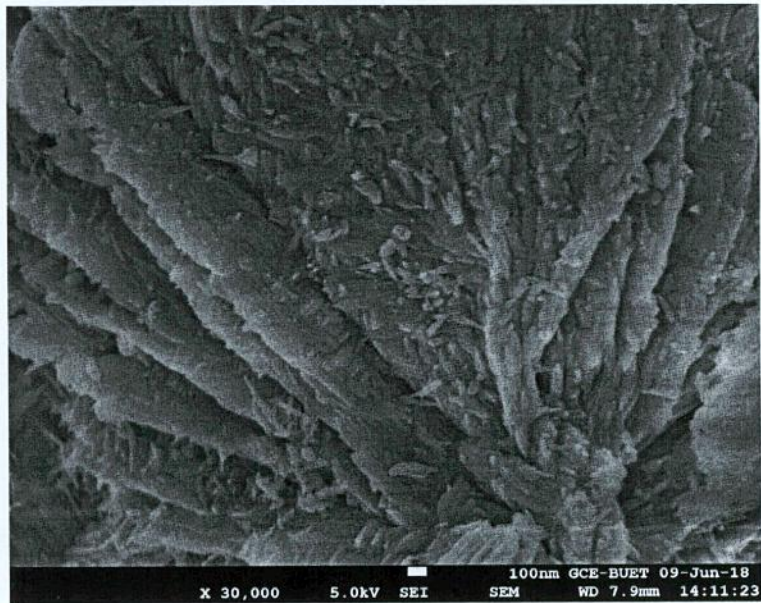
SDS : CTAB = 4 : 1

Figure 4.4.1: SEM image of the prepared HAp at a 4 : 1 ratio of SDS and CTAB



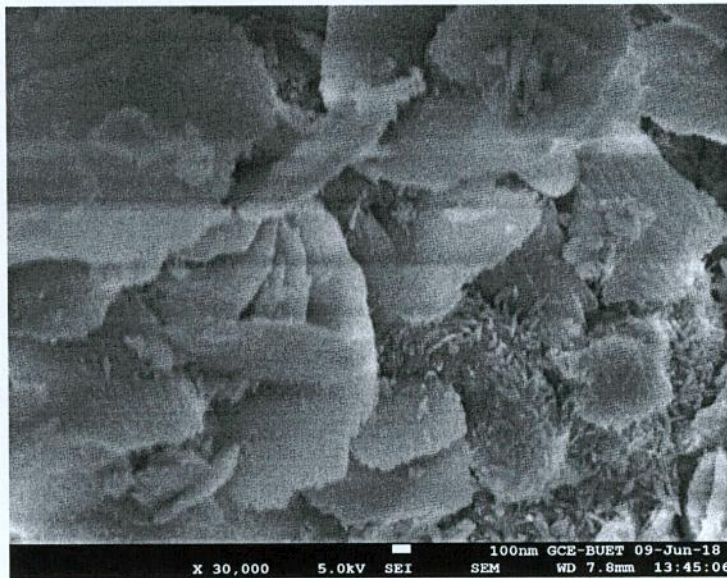
SDS : CTAB = 3 : 2

Figure 4.4.2: SEM image of the prepared HAp at a 3 : 2 ratio of SDS and CTAB



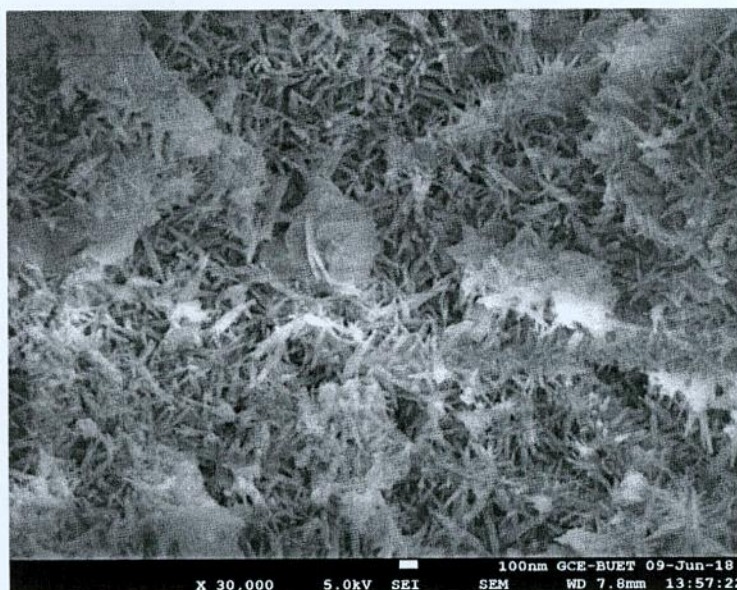
SDS : CTAB = 2 : 3

Figure 4.4.3: SEM image of the prepared HAp at a 2 : 3 ratio of SDS and CTAB



SDS : CTAB = 1 : 1

Figure 4.4.4: SEM image of the prepared HAp at a 1 : 1 ratio of SDS and CTAB



SDS : CTAB = 1 : 4

Figure 4.4.5: SEM image of the prepared HAP at a 1 : 4 ratio of SDS and CTAB

Figure 4.4.1 to 4.4.5 show the SEM images of the as-prepared HAP nanoparticles at different ratio of SDS and CTAB. The shape of the particles resulting from 4 : 1 ratio of SDS and CTAB is spherical. The spherical shape is also observed for 1: 1 ratio of SDS and CTAB. However, the HAp-NPs prepared by using 1 :4 ratio has needle like shape. Surprisingly, plate like sheets are observed for both 2: 3 and 3: 2 ratio of SDS and CTAB. Nevertheless, it is observed that the surfaces of the prepared HAp-NPs are not smooth. They have rough surface. It might have resulted from the agglomeration of the HAp nanoparticles. The agglomeration of the nanoparticles could have occurred due to Ostwald ripening. Finally, based on the obtained SEM images it is clear that the HAp nano-particles formed at various ratio of SDS and CTAB has various morphology. Thus it can be stated from the above result that the different shaped HAp particle would be prepared by changing the molar ratio of surfactant SDS and CTAB.

Chapter 5

Conclusion

HAp-NPs biomaterials of immense applications were prepared by chemical precipitation techniques. Mixed micellar core was used as nano reactor to control morphologies such as crystallinity, particle size, particle shape, particle size. Anionic SDS and cationic CTAB surfactants were used to prepare the mixed micellar core. 18 : 2 ratio of propanol-1 : water system experimentally was carefully chosen for reverse micelle formation of the aforementioned surfactants. Peaks at 561, 599, 628, 962, 1026 and 1087 cm^{-1} are due to the P-O stretching vibration confirm the presence of PO_4^{3-} in HAp-NPs. Diffraction pattern of the prepared HAp-NPs are in well agreement with the standard published by the International Centre for Diffraction Data. Diffractogram also indicate the presence of crystalline as well as amorphous phase. No significant weight loss was detected for the HAp over the measured temperature range (25 - 800 $^{\circ}\text{C}$), indicating its high thermal stability. SEM result reveals significant morphological variation of the prepared HAp-NPs by changing the amount of two surfactants.

References

1. Liao, C. J., Lin, F. H., Chen, K. S. and Sun, J. S., 1999, "Thermal decomposition and reconstitution of hydroxyapatite in air atmosphere", *Biomaterials*, Vol. 20, pp. 1807-1813
2. Jarcho, M., 1981, "Calcium phosphate ceramics as hard tissue prosthetics", *Clin. Orthop. Relat. R.* Vol. 57, pp. 259-278
3. Cai, Y. R., Liu, Y. K., Yan, W. Q., Hu, Q. H., Tao, J. H., Zhang, M., Shi, Z. L. and Tang, R. K., 2007, "Role of hydroxyapatite nanoparticle size in bone cell proliferation", *J. Mater. Chem.*, Vol. 17, pp. 3780-3787
4. Dorozhkin, S. V., 2010, "Bioceramics of calcium orthophosphates". *Biomaterials*, Vol. 31, 1465-1485
5. Ketaki, D. M., Monsoor, S., Sutapa, R. R. and Meenal, K., 2016, "Self-Activated Fluorescent Hydroxyapatite Nanoparticles: A Promising Agent for Bioimaging and Biolabeling ", *ACS Biomater. Sci. Eng.*, Vol. 2, 1257–1264
6. Koirala, M. B., Nguyen, T. D., Pitchaimani, A., Choi, S. O. and Aryal, S., 2015, "Synthesis and Characterization of Biomimetic Hydroxyapatite Nanoconstruct Using Chemical Gradient across Lipid Bilayer", *ACS Appl. Mater. Interfaces* , 27382-27390
7. Ma, Y., Zhang, J., Guo, S., Shi, J., Du, W., Wang, Z., Ye, L. and Gu, W., 2016, "Biomimetic mineralization of nano-sized, needle-like hydroxyapatite with ultrahigh capacity for lysozyme adsorption", *Mater. Sci. Eng. C*, Vol. 68, 551-556
8. Teshima, K., Wagata, H., Sakurai, K., Enomoto, H., Mori, S., Yubuta, K., Shishido, T. and Oishi, S., 2012, "High-Quality Ultralong Hydroxyapatite Nanowhiskers Grown Directly on Titanium Surfaces by Novel Low-Temperature Flux Coating Method", *Cryst. Growth Des.* Vol. 12, 4890-4896
9. Zhang, H., 2005, "Mechanochemical–Hydrothermal Synthesis and Characterization of Fluoridated Hydroxyapatite" *Materials Research Bulletin*, Vol. 40, pp. 1326 – 1334
10. Hu, J., 2001, "Production and Analysis of Hydroxyapatite from Australian corals via hydrothermal process", *Journal of Materials Science Letters*, Vol. 20, pp. 85 – 87, 2001

11. Bogdanoviciene, I., 2006, "Calcium Hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ Ceramics Prepared by Aqueous Sol–Gel Processing", *Materials Research Bulletin*, Vol. 41, pp. 1754 – 1762
12. Morgan, H., 2000, "Preparation and Characterization of Monoclinic Hydroxyapatite and its Precipitated Carbonate Apatite Intermediate", *Biomaterials*, Vol. 21, Issue 6, pp. 617 – 627
13. Jha, L. J., 1997, "Preparation and Characterization of Fluoride-Substituted Apatite's", *Journal of Materials Science: Materials in Medicine*, Vol. 8, pp. 185 – 191
14. Chow, L. C., 2004, "Properties of Nanostructured Hydroxyapatite Prepared by a Spray Drying Technique", *Journal of Research of the National Institute of Standards and Technology*, Vol. 109, pp. 543 – 551
15. McGrath, P. J. and Laine, R. M., 1992, "Theoretical Process Development for Freeze-Drying Spray-Frozen Aerosols", *Journal of American Ceramic Society*, Vol. 75, pp. 1223-1228
16. Lin, K., 2007, "Hydrothermal Micro-emulsion Synthesis of Stoichiometric Single Crystal Hydroxyapatite Nanorods with Mono-Dispersion and Narrow-Size Distribution", *Materials Letters*, Vol. 61, pp. 1683 – 1687
17. Rivera, E. M., 1999, "Synthesis of Hydroxyapatite from Eggshells", *Materials Letters*, Vol. 41, pp. 128 – 134
18. Shanthi, P. M. S. L., Ashok, T., Balasubramanian, A., Riyasdeen, and M. A. Akbarsha, 2009, *Mater. Let.* Vol. 63, pp. 2123 -2125
19. Shanthi, P. M. S. L. Mangalaraja, R. V., Uthirakumar, A. P., Velmathi, S., Balasubramanian, T. and Ashok, M., 2010, *J. Coll. Interf. Sci.*, Vol. 39, pp 350-353
20. Saha, S. K., Banerjee, A., Banerjee, S. and Bose, S., 2009, *Mater. Sci. Eng. C*, Vol. 29, pp. 2294- 2301
21. Klug, H. P. and Alexander, L. E., 1959, Wiley, New York
22. Liu, Y., Wang, W., Zhan, Y., Zheng, C. and Wang, G., 2002, *Mater. Lett.* 56496
23. Sun, Y., Guo, G., Wang, Z. and Guo, H., 2006, *Ceram. Inter.*, Vol. 32, pp. 951-954
24. Kalita, S. J., Bhardwaj, A. and Bhatt, H. A., 2007, *Mater. Sci. Eng. C*, Vol. 27, pp. 441-449
25. Arami, H., Mohajerani, M. and Mazloumi, M., 2009, *J. Alloys Compd.*, Vol. 469, pp. 391-394

26. Yan, L., Li, Y., Deng, Z. X., Zhuang, J. and Sun, X., 2001, *Inter. J. Inorg. Mater.*, Vol. 3, pp. 633-637
27. Salarian, M., Solati-Hashjin, M., Sara, S. S., Goudarzi, A., Salarian, R. and Nemati, A., 2009, *Mater. Sci. Poland*, Vol. 27, pp. 961-971
28. Zhu, X., 2005, "Nano Hydroxyapatite/Collagen, Nano Hydroxyapatite and Anodic Oxides on Titanium – Preparation, Characterization and Biological Response", Inaugural Dissertation, Medical Faculty, Eberhard-Karls-University
29. Okamoto, K., 1998, "RGD Peptides Regulate the Specific Adhesion Scheme of Osteoblast to Hydroxyapatite but not to Titanium", *Journal of Dental Research*, Vol. 77, pp. 481 – 487
30. Jallot, E., 2005, "Nanoscale Physico-Chemical Reactions at Bio-ceramics-Bone Tissues Interfaces, Handbook of Nanostructured Biomaterials and their Applications in Nanobiotechnolog", pp. 495 – 509
31. Jallot, E., 2005, "Nanoscale Physico-Chemical Reactions at Bioceramics-Bone Tissues Interfacesl, Handbook of Nanostructured Biomaterials and their Applications in Nanobiotechnolog", pp. 495 – 509
32. Boskey, A. L. and Posner, A. S., 1976, "Formation of Hydroxyapatite at Low Supersaturation", *Journal of Physical Chemistry*, Vol. 80, pp. 41 – 45
33. Seckler, M. M. 1999, "Influence of Process Conditions on Hydroxyapatite Crystallinity Obtained by Direct Crystallization", *Materials Research*, Vol. 2(2), pp. 59 – 62
34. Lazic, S., 1996, "Properties of Hydroxyapatite Crystallized from High Temperature Alkaline Solutions", *Journal of Crystal Growth*, Vol. 165, pp. 124 – 128
35. Lazic, S., 2001, "The effect of Temperature on the Properties of Hydroxyapatite Precipitated from Calcium Hydroxide and Phosphoric Acid", *Thermochimica Acta*, Vol. 374, pp. 13 – 22
36. Mostafa, N. Y., 2005, "Characterization, Thermal Stability and Sintering of Hydroxyapatite Powders Prepared by Different Routes", *Materials Chemistry and Physics*, Vol. 94(2 – 3), pp. 333 – 341
37. Conn, J. F., 198), "Process for Producing Hydroxyapatite", Patent Number: US 4,324,772

38. Giulietti, M., 2001, "Industrial Crystallization and Precipitation from Solutions: State of the Technique", *Brazilian Journal of Chemical Engineering*, Vol. 15(4), pp. 423 – 440
39. Tampieri, A., 2000, "Characteristics of Synthetic Hydroxyapatites and Attempts to Improve their Thermal Stability", *Materials Chemistry and Physics*, Vol. 64, pp. 54 – 61
40. Kumar, T. S. S., 2000, "Synthesis of Carbonated Calcium Phosphate Ceramics using Microwave Irradiation", *Biomaterials*, Vol. 21, pp. 1623 – 1629
41. Kumar, R., 2004, "Temperature Driven Morphological Changes of Chemically Precipitated Hydroxyapatite Nanoparticles", *Langmuir*, Vol. 20, pp. 5196 – 5200
42. Kothapalli, C., 2004, "Influence of Temperature and Concentration on the Sintering Behavior and Mechanical Properties of Hydroxyapatite", *Acta Materialia*, Vol. 52, pp. 5655 – 5663
43. Pang, Y. X., 2003, "Influence of Temperature, Ripening Time and Calcination on the Morphology and Crystallinity of Hydroxyapatite Nanoparticles", *Journal of the European Ceramic Society*, Vol. 23, pp. 1697 – 1704
44. Liu, C., 2001, "Kinetics of Hydroxyapatite Precipitation at pH 10 to 11", *Biomaterials*, Vol. 22, pp. 301 – 306
45. Markovic, M., 2004, "Preparation and Comprehensive Characterization of a Calcium Hydroxyapatite Reference Material", *Journal of Research of the National Institute of Standards and Technology*, Vol. 109, pp. 553 – 568
46. Afshar, A., 2003, "Some Important Factors in the Wet Precipitation of Hydroxyapatite", *Materials and Design*, Vol. 24, pp. 197 – 202
47. Smiciklas, I., 2005, "Experimental Design Approach in the Synthesis of Hydroxyapatite by Neutralization Method", *Separation and Purification Technology*, Vol. 44(2), pp. 97 – 102
48. Knowles, J. C., 2000, "Characterization of the Rheological Properties and Zeta-Potential of a Range of Hydroxyapatite Powders", *Biomaterials*, Vol. 21, pp. 1387 – 1392
49. Saeri, M. R. 2003, "The Wet Precipitation Process of Hydroxyapatite", *Materials Letters*, Vol. 57, pp. 4064 – 4069

50. Bernard. L. 2000, "Modelling of the Dissolution of Calcium Hydroxide in the Preparation of Hydroxyapatite by Neutralization", *Chemical Engineering Sciences*, Vol. 55, pp. 5683 – 5692
51. Bouyer, E., 2000, "Morphological Study of Hydroxyapatite Nanocrystal Suspension", *Journal of Materials Science: Materials in Medicine*, Vol. 11, pp. 523 – 531
52. Liu, J., 2003, "The Influence of pH and Temperature on the Morphology of Hydroxyapatite Synthesized by Hydrothermal Method", *Ceramics International*, Vol. 29, pp. 629 – 633
53. Raynaud, S., 2002, "Calcium Phosphate Apatite's with Variable Ca/P Atomic Ratio, Synthesis, Characterization and Thermal Stability of Powders", *Biomaterials*, Vol. 23, pp. 1065 – 1072
54. Cheang, P. and Khor, K. A., 1996, "Addressing Processing Problems Associated with Plasma Coating of Hydroxyapatite Coatings", *Biomaterials*, Vol. 17, pp. 537 – 544
55. Suchanek, W. and Yoshimura, M., 1998, "Processing and Properties of Hydroxyapatite-Based Biomaterials for use as Hard Tissue Replacement Implants", *Journal of Materials Research*, Vol. 13, pp. 94 – 114
56. Bernard. L., 1999, "Preparation of Hydroxyapatite by Neutralization at Low Temperature - Influence of Purity of the Raw Material", *Powder Technology*, Vol. 103, pp. 19 – 25
57. Zhang, H. and Zhang, M., 2011, "Phase and thermal stability of hydroxyapatite whiskers precipitated using amine additives", *Ceramics International*, Vol. 37, pp. 279–286