CHAPTER 1

INTRODUCTION

1.1 BIOMINERALIZATION

Biomineralization is the process by which the living organisms produce minerals, often to harden or stiffen existing tissues. It is a frequently used term in nanotechnology, astrobiology, geology and medicine. In this process living organism provides a chemical environment that controls the nucleation and growth of unique mineral phases. Teeth, bones, kidney stones, skeletons of algae, mussels and magnetotactic bacteria are all examples of biomineralization. Cellular processes enable to control both spatial and temporal domain in such a way that hierarchical composite structures could be built, which increase the toughness and durability of the material, for load-bearing materials such as bones, teeth, mollusk shells etc (Pokroy 2004). Many biological minerals contain calcium phosphate as a major component. In biological systems, calcium phosphate mineralization has frequently been suggested as proceeding through precursor phases such as amorphous calcium phosphate (ACP) or octacalcium phosphate (OCP) before transformation to the thermodynamically more stable hydroxyapatite (HAp) (Nancollas 1992). The hard tissues are biocomposites and incorporate both structural macromolecules (lipids, proteins and polysaccharides) and inorganic minerals (Ciftcioglu et al 2006). The cells of mineralized tissue and extracellular organic matrices play important roles in initiating and controlling in vivo mineral deposition.
1.2 BIOMATERIALS

Materials which can interact with or can have connections with living tissues or biological fluids are called biomaterials. Biomaterials are expected to perform the functions of the natural organ or tissue in very aggressive environment, due to the pH variation (1-9) of body fluids in various tissues. Biomaterials in the form of implants (sutures, bone plates, joint replacements, ligaments, vascular grafts, heart valves, intraocular lenses, dental implants etc) and medical devices (pacemakers, biosensors, artificial hearts, blood tubes etc) are widely used to replace and/or restore the function of degenerated tissues or organs, to assist in healing, improve function, correct abnormalities and improve the quality of life of the patients. The various materials used in biomedical applications may be grouped into metals, ceramics, polymers and composites (Ramakrishna 2001).

1.2.1 Metals

Materials that exhibit metallic bonding in the solid state are metals. Mixtures of different metals are called alloys. Tissue replacement with metals and metal alloys is achieved due to the similar physical properties of the tissue and reactions of the tissue to the implant and of the implant to the tissues. The main considerations in metals and alloys for biomedical applications are biocompatibility, appropriate mechanical properties and corrosion resistance. Metals and metal alloys (stainless steel, titanium, cobalt-chromium, platinum) find applications in dental implants, orthopedic load bearing and fixation devices due to the advantages such as high impact strength, high resistance to wear, ductile and absorption of high strain energy (Bhat 2007). Even though these materials are bioinert there may be a chance to release cytotoxic metal ions to the surrounding tissues during their long-term implantation.
1.2.2 Polymers

Polymers are large molecules made up by the repetition of small, simple chemical units termed as monomers. The chains are branched and interconnected to form three-dimensional networks. The selection of polymers for biomedical use (dermatology, ophthalmology, pharmacy) mainly depends upon their chemical and physical properties. The stability and lifetime of polymers in long-term implantation depends not only on the chemical structure, but also on the conditions under which they are utilized. Degradable and non-degradable polymers are used in controlled drug delivery, scaffolds for tissue engineering, wound dressing, cosmetic skin masks and protective clothing (Kumbar 2008 and Ratner 1996). The required properties of polymeric biomaterials are similar to other biomaterials like biocompatibility, sterilizability, adequate mechanical and physical properties. Polymers are classified into two groups such as degradable and non-degradable polymers.

1.2.2.1 Degradable Polymers

Degradable polymers have the capability of being decomposed chemically or biologically. Degradable polymeric implant need not be removed surgically once it is no longer needed. Degradable polymers such as poly(glycolic acid) (PGA), copolymers of PGA, polydioxanone (PDS) and poly(lactic acid) (PLA) are used as sutures, drug delivery devices, temporary scaffolds etc.

Biodegradable polymers are more attractive with increasing applications in pharmaceutical, medical and biomedical engineering. Biodegradable polymers are not only limited to the release of drugs, peptides or proteins to specific target site, but also extended to medical devices, wound dressing as well as for fabricating scaffold in tissue engineering.
Biodegradable polymers can be either natural or synthetic. The commonly used biodegradable polymers which were used in drug delivery viz., natural polymers such as collagen, albumin and gelatin are protein based polymers, whereas agarose, alginate, carrageenan, hyaluronic acid, dextran, chitosan and cyclodextrins are polysaccharides (Mishra et al 2008).

1.2.2.2 Non Degradable Polymers

The non-degradable polymers which were most widely used in biomedical field in various forms are poly(methyl methacrylate) (PMMA), polyethylene (PE) in high density and very high density molecular weight, poly(tetrafluoroethylene) (PTFE) in microporous (Gore-Tex), poly(vinylchloride) (PVC) and poly(dimethyl siloxane) (PDMS). The polymers which were used for various applications are intraocular lenses, hard contact lenses, bone cement, fixation of articular prostheses and dentures, tubing for drains and catheters, acetabular component in artificial hips, as vascular grafts, for making tube in blood transfusion, feeding and dialysis as catheter and drainage tubing, insulation for pacemaker leads, prostheses such as finger joint, blood vessels, heart valves, breast implants, outer ears, chin and nose implants (Jagur-Grodzinski et al 1999). Polyacrylamide gel is extensively used in ophthalmic surgery, drug treatment, facial depressions, lip enhancement and breast-augmentation (Christensen 2008 and Qiao 2005).

1.2.3 Ceramics

A ceramic is an inorganic, non-metallic solid prepared by the action of heat and subsequent cooling. Ceramic materials may have a crystalline or partly crystalline structure, or may be amorphous (Ex. glass). Ceramics, glasses and glass ceramics include a broad range of inorganic/nonmetallic compositions. In the medical industry these materials have been essential for
eye glasses, diagnostic instruments, chemical ware, thermometers, tissue culture flask and fiber optics for endoscopy. Ceramics are also widely used in dentistry as restorative materials such as in gold-porcelain crowns, glass-filled ionomer cements and dentures. The bioceramics are classified as inert and bioactive. Alumina, zirconia, silicone nitrides and carbons are inert bioceramics. Bioactive ceramics are further classified as resorbable and non resorbable. The non resorbable bioactive materials obtain a specific biological response between the tissues and the material. Resorbable bioactive ceramics degrade gradually over a period of time and are replaced by the natural host tissues (Ratner 1996).

1.2.3.1 Calcium Phosphates

Calcium phosphate is the well known biomaterial which has both beneficial and pathological effects on human body. The beneficial parts fall into the categories of bioactive and resorbable materials (Matthew et al 2003). There are varieties of compounds in the calcium phosphate family; many of them are used in the biomedical applications. The calcium phosphates which exist in various forms are listed in Table 1.1. Calcium phosphate biomaterials have been widely used in medical field in the form of powders, granules, dense porous blocks and various composites (Armentano et al 2010).

<table>
<thead>
<tr>
<th>Phase</th>
<th>Formula</th>
<th>Structure</th>
<th>Ca/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicalcium phosphate (DCP)</td>
<td>CaHPO₄</td>
<td>Triclinic</td>
<td>1.00</td>
</tr>
<tr>
<td>Dicalcium phosphate dihydrate (DCPD)</td>
<td>CaHPO₄·2H₂O</td>
<td>Monoclinic</td>
<td>1.00</td>
</tr>
<tr>
<td>Calcium pyrophosphate (CPP)</td>
<td>Ca₃P₂O₇</td>
<td>Orthorhombic</td>
<td>1.00</td>
</tr>
<tr>
<td>Tricalcium phosphate (TCP)</td>
<td>Ca₃(PO₄)₂</td>
<td>Hexagonal</td>
<td>1.50</td>
</tr>
<tr>
<td>Octa calcium phosphate (OCP)</td>
<td>2Ca₄(PO₄)₃·5H₂O</td>
<td>Triclinic</td>
<td>1.33</td>
</tr>
<tr>
<td>Hydroxyapatite (HAp)</td>
<td>Ca₁₀(PO₄)₆(OH)₂</td>
<td>Hexagonal</td>
<td>1.67</td>
</tr>
<tr>
<td>Tetracalcium phosphate (TTCP)</td>
<td>Ca₄O(PO₄)₂</td>
<td>Monoclinic</td>
<td>2.00</td>
</tr>
</tbody>
</table>
1.2.3.2 Hydroxyapatite

Bone is a natural composite of calcium phosphate crystals in a collagenous matrix. The most used calcium phosphate in implant fabrication is hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$, HAp), which is most similar to the mineral component of bone and dentine. The HAp crystals are nanometer sized, with an average length of 50-75 nm and width 25-30 nm, which was regularly aligned along the collagen fibers (Soichiro et al 2002). The lattice constant of HAp are a=0.95 nm and c=0.68 nm, with hexagonal structure and its space group was P6$_3$/m which shows that the unit cell will be arranged along the c-axis. This confirmed a preferred orientation that gives rise to an oriented growth along the c-axis and a needle like morphology. HAp exhibits properties such as biocompatibility, bioactivity, osteoconductivity, direct bonding to bone etc (Vallet-Regi et al 2004).

HAp is the emerging and most promising biomaterial for biomedical applications in orthopedics and dentistry. HAp is thermodynamically stable at physiological pH. Its porous character offers high binding affinity for a variety of pharmacological substances such as antibiotics, hormones, enzymes, antibody fragments etc. In addition, the porous nature of HAp has ability to promote much faster tissue growth into the pores (Nayak 2010). It was realized that dimension and morphology of pores are crucial factors for an excellent osteointegration. Minimum pore size required to enable ingrowth of the surrounding bone together with blood supply is about 100-150 µm for macropores and pores at 50 µm is adequate for osteoconduction (Sopyan 2007).

HAp may be employed in forms such as powders, porous blocks or beads to fill bone defects or voids. These forms may arise when large sections of bone have had to be removed (eg. bone cancers) or when bone augmentations are required (eg. Maxillofacial reconstructions or dental
applications). The bone filler will provide a scaffold and encourage the rapid filling of the void by naturally forming bone and provides an alternative to bone grafts. HAp with various morphologies and surface properties have also been investigated as drug carriers for the delivery of a variety of pharmaceutical molecules because of their biocompatible, osteoconductive, non-toxic and non inflammatory properties (Yang 2008). Applications of HAp include femoral plugs in total hip replacement and HAp coating on metal components for cementless fixation (Oonishi 1991).

1.2.3.3 Tricalcium Phosphate (TCP, Ca₃(PO₄)₂)

TCP crystals are preferred over HAp due to their excellent resorbability, which allows their dissolution in the surrounding body fluid and eventual replacement by body tissues (Viswanath 2008). TCP exhibits two polymorphic forms (α and β). Both α- and β-TCP are obtained by heat treating HAp above 800 °C. When compared with α-TCP, β-TCP is not soluble. β-TCP is stable up to 1200 °C and α-TCP was obtained above 1200 °C. The transition temperature of β-TCP to α-TCP is ~1150 °C. α-TCP is biodegradable and it is used in bone voids. β-TCP is bioresorbable because of its greater solubility than HAp in physiological environments (Chow 2009). β-TCP has successfully been used to correct periodontal defects and to augment bony contours. When TCP and Tetracalcium phosphate (TTCP, Ca₄O(PO₄)₂) are implanted, they gradually degrade, being totally replaced by the host tissue (Adamopoulos 2007). TCP has been used as a ceramic bone substitute material in the orthopedic field, craniofacial surgery and also as a filling material in reconstructive surgery (Handschel 2002). Biphasic calcium phosphate is a synthetically prepared hybrid of HAp and β-TCP that possesses a greater resorption rate than HAp alone. Biphasic composites cannot be used for load-bearing applications due to their poor mechanical properties. The α- and β-TCP find application in temporary scaffolds and in bone cements.
1.2.3.4 **Calcium Pyrophosphate (CPP, Ca$_2$P$_2$O$_7$)**

Calcium pyrophosphate is commonly used as a mild abrasive agent in toothpastes. CPP can be used as a coating material for bone and teeth implants. Deposition of calcium pyrophosphate dihydrate (CPPD) in articular joints causes an arthritics condition called pseudogout. CPPD crystals preferentially deposit within fibrocartilage and are the most common cause of cartilage calcification (Osano 2003). Pyrophosphate arthropathy is a common joint disease in older people. It is due to the deposition of pyrophosphate dihydrate in peripheral joints in the hands, feet, knees, elbows, hips, shoulders and wrists (Dieppe and Calvert 1983).

1.2.3.5 **Dicalcium Phosphate Dihydrate (DCPD, CaHPO$_4$.2H$_2$O)**

Dicalcium phosphate dihydrate (Brushite) plays an important role in the biological mineralization process. DCPD is a stable phase under acidic conditions and it may play a significant role in the formation of pathological calcifications like urinary calculi, dental calculi etc (Wefel and Harless 1987). DCPD is proved to be one of the precursors for synthesizing HAp and it is being used as coating material for bone and teeth implants. DCPD find applications in pharmaceutical, an intermediate in phosphate fertilizer production, food additive and a component of tooth paste.

1.2.3.6 **Substituted HAp**

The major constituent of bones and teeth are composed of HAp, a non-stoichiometric compound with the ability to accept compositional variations in its sub-lattices. Besides calcium, phosphate and carbonate, mineral bone contains many inorganic compounds such as sodium, fluoride, chloride, magnesium, strontium, zinc, copper and iron. The OH$^-$ site of HAp was also occupied by F$^-$ or Cl$^-$. Anionic complexes, such as AsO$_4^{3-}$, SO$_4^{2-}$,
CO$_3^{2-}$, SiO$_4^{4-}$ can replace PO$_4^{3-}$ and a large number of metal cations, such as K$^+$, Na$^+$, Mn$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Co$^{2+}$, Zn$^{2+}$, Sr$^{2+}$, Ba$^{2+}$, Pb$^{2+}$, Cd$^{2+}$, Y$^{3+}$ and trivalent ions of rare earth elements can substitute for Ca$^{2+}$ (in trace concentrations).

The toxic metal and semi-metal ions such as Pb$^{2+}$ and As$^{5+}$ can be incorporated easily into the apatite structure have clinical ramifications for bones and teeth (Wopenka 2005). Sr-HAp enhanced the proliferation and differentiation of osteoblasts. In addition Sr-HAp might be used as a template to grow new bone and they are proposed for the treatment of osteoporosis. Europium doped apatite is also used as a biological probe because of their stable luminescence (Doat 2003). Silicon is essential to the growth and development of biological tissue such as bone, teeth and some invertebrates. Various metal cations like Mg$^{2+}$, Zn$^{2+}$, La$^{3+}$, Y$^{3+}$, In$^{3+}$ and Bi$^{3+}$ doped with HAp enhance orthopaedic and dental applications (Webster 2004). HAp obtained by sol-gel method with various iron concentrations, could be used for the hyperthermia treatment of bone tumors. Silver (Ag) was strongly active against bacteria and therefore Ag doped HAp could be considered as an antimicrobial biomaterial which could be used in implant to avoid infections. Zn$^{2+}$ ion into HAp, is a potential dopant to enhance the mechanical strength, antimicrobial property and osteointegration.

1.3 SINTERING

Sintering extend to be of great importance to produce bioceramics with required properties. The effect of sintering on the properties of bioceramics revealed a correlation between temperature and time lead to increase the density, porosity, grain size, chemical composition and strength of the scaffolds. Sintering below 1000 °C results in initial particle coalescence, no densification and a significant loss of surface area or porosity. Sintering at higher temperatures results with exaggerated grain growth and decomposition because HAp becomes unstable at temperature
Dense, sintered HAp has many bone replacement applications and are used for applications such as repair of bone defects in dental and orthopedic sites, immediate tooth replacement, augmentation of alveolar ridges, pulp capping material and maxillofacial reconstruction etc (Shiny 2000).

1.4 IN VITRO STUDIES

1.4.1 In vitro Drug Release

Nowadays, nanomaterials have been applied in many medical and biological fields such as clinical diagnosis, drug delivery and fluorescent markers in vitro and in vivo. Among these applications, drug delivery technology can bring both commercial and therapeutic values to health care products. Local delivery of antibiotics has been successfully used to cure or reduce the risk of infections during orthopaedic surgery. The most widely used materials for the delivery of antibiotics are based on biodegradable polymers and HAp (Xu 2008).

The effectiveness of orthopaedic implants can be increased through designing substrates to allow for the slow release of therapeutic drugs, thereby minimizing the induced inflammatory, possible infection response, and increasing the function of the implanted devices. A method of improving treatment is the use of sustained released systems such as natural and synthetic polymer and bioceramic implants. The drug need to cross a porous layer is dependent upon the parameters such as, solubility of drug in the solution and possible physical and chemical interactions with the surface of the delivery device. Site specific drug delivery will help to avoid chronic bone infections, such as osteomyelitis where limited blood circulation results in poor antibiotic distribution at the infection site making this condition difficult
to treat (Melville 2008). The amount of drug incorporated into the HAp matrix strongly dependent upon the solvent, pH and concentration of drug.

The core shell usually helps to enhance and possibly reduce the effect of the initial burst effect. Microparticles and nanoparticles based on core shell structures or polymeric micelles are advantageous in terms of their long circulation in the body in addition to drug solubility, stability and high level of drug encapsulation. The advantage of core shell microspheres is that both hydrophilic and hydrophobic drugs can be incorporated (Babu 2006). Amoxicillin is penicillin like antibiotic used to treat infections caused by bacteria such as pneumonia, bronchitis, venereal disease (VD) and ear, lung, nose, urinary tract and skin infections. It is used before surgery or dental related works to prevent infection (Vallet-Regi 2004).

5-Fluorouracil (5FU) is an antineoplastic drug used extensively in cancer chemotheraphy and is an antimetabolite which is, used to prevent the subsequent scarring following trabeculectomy and to improve the prognosis for long-term retinal reattachment. It is an acidic, water soluble, hydrophilic drug and an antineoplastic agent used in the chemotheraphy for the treatment of solid tumours (Santos 2009).

1.4.2 Antibacterial Activity

Bacterial infection after implantation is a significant rising complication. Even though the use of perioperative antimicrobial prophylaxis and laminar flow operating rooms, the infection rates associated with prosthetic joints range between 1 to 9 % depending on the type of implant. Therefore infection associated implant failures occur and there will be a need for second surgery in the case of total hip replacements. Thus, efficient prevention of implant associated infections to avoid surgical revisions, expensive and long hospital stays that increased the patient’s morbidity more.
To treat and prevent infections associated with orthopedic implants is to deliver antibiotics in a controlled manner at the site of implantation in order to administer high local doses without exceeding the systemic toxicity of drugs. The capacity of certain bacterial strains to develop resistance against antibiotics has aroused an increasing interest for the controlled delivery of other antibacterial agents with a broader activity and low incidence of resistance (Diaz 2009).

Studies on antibiotic coated implants such as the use of minocycline/rifampin have been shown to be more effective in reducing bacterial adhesion when compared to silver sulfadiazine-chlorhexidine coated implants. The co-sputtered Ag-HAp surface would not only enhance tissue compatibility, but also promote the inhibition of bacterial adhesion on the implant surface. Staphylococcus epidermidis (S. epidermidis) and Staphylococcus aureus (S. aureus) have been reported to be the most important pathogens in biomaterial associated infections (Chen 2006).

Metals like zinc, silver etc are used as an alternative strategy to prevent the formation of adhesive bacterial films. Silver containing antimicrobial biomaterials consist of either elemental silver or Ag⁺ (silver salts or silver complexes) incorporated into organic (polymers) or inorganic (bioglasses and HAp) matrices. Silver doped silica thin films are used as an antibacterial glass due to the excellent antibacterial performance against (Escherichia coli) E. coli and S. aureus (Jeon 2003). The antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin and vancomycin increase in the presence of Ag-nano particles against S. aureus and E. coli strains (Shahverdi 2007).

1.4.3 In vitro Bioactivity

Bioactivity is defined as the property of the material to develop a direct adherent and strong bonding with the bone tissue. An ideal biomaterial
for bone regeneration is expected to be not only bioactive but resorbable, that is eventually replaced by newly formed bone. The ability to cause the formation of apatite from a supersaturated solution has been widely used to imply the bioactivity of an implant in vivo (Pan 2010). Factors which influence bioactivity are composition, crystallinity, morphology, porosity and specific surface area. The denser materials tend to have lower bioresorbability due to their smaller surface area (Kokubo 1996). The mechanism of apatite formation on CaP in SBF is due to the partial dissolution of CaP. When CaP is immersed in SBF both dissolution and precipitation occurs and Ca\(^{2+}\), HPO\(_2\)\(^{2-}\) and PO\(_4\)\(^{3-}\) are continuously released. This release has an effect on the pH of the solution and these ions increase the supersaturation of SBF resulting in the formation of apatite layer on the surface of the bioceramic material (Cortes 2005). The enrichment of Ca and P ions in the environment promote the bone mineralization that enhances the bone formation.

### 1.4.4 Biocompatibility Test (Haemolysis)

Biocompatibility refers essentially to the compatibility of materials with the biological systems. Since it is rarely possible to find a fully biocompatible material, it is necessary to identify the materials, which are physiologically tolerable. There are number of standard methods for testing the biocompatibility of materials. In the current trend of biocompatibility studies, aspects of both biosafety and biofunctionality are considered. Biosafety tests such as tests on cytotoxicity and mutagenesis or carcinogenesis are aimed at excluding the severe harmful effects of biomaterials on organisms (Chowdhury 2004). The haemolysis count is an important parameter in testing the biocompatibility of materials. Haemolysis indicates premature destruction of red blood cells when they come in contact with water or other foreign elements (Chowdhury 2007). The accepted norm is that if the haemolysis percentage is less than 20, the test material is taken as
haemocompatible and if it is less than 5 the material is highly haemocompatible.

1.5 CRYSTAL GROWTH

1.5.1 Gel Method

Gel method is useful for growing crystals or materials which are slightly soluble in water, but it cannot be grown from vapour or melt. There is an increasing interest in the crystallization of biomolecules using gel as the growth medium. In gel method chemical reaction is achieved by diffusion. The cooling of a sol can bring about the gelling process, by chemical reaction, by the addition of precipitating agents or incompatible solvents. Gels can also be formed by the reaction of two chemical reagents. The time of gelling depends upon the pH, density and temperature etc of the gel and the type of the gelling agent (Dhanaraj 2010).

The materials which are ordinarily called gel include silica gel (usually sodium meta silicate solution), agar (a carbohydrate polymer derived from sea weed), gelatin (a substance closely related to proteins), a variety of oleates and stearates, polyvinyl alcohol, various hydroxides in water and even water insoluble tetra ethoxy silane in the presence of electrolytes and co-solvents (eg. methanol) or surface active agents (Henisch 1998).

1.5.2 Agarose Gel

Agarose, a natural polysaccharide obtained from red algae, is an alternating copolymer of 1, 3-linked β-D-galactose and 1, 4-linked 3, 6-anhydro-α-L-galactose, substituted at irregular intervals with sulfate esters, methyl esters, and/or pyruvate residues. The linear agarose molecules aggregate in dilute solution and in the sol phase forming large fiber bundles and microgel domains held together by non covalent hydrogen bonds.
(Stellwagen 1995 and Bao 2010). In the case of agarose gels, pore formation is a physical process, resulting from a shift in conformation of the molecules composing it.

The powered form of agarose is composed of loose, random coils of polysaccharide. When mixed with water, melted and cooled, however, these random coils adopt a more orderly helical conformation. Each polysaccharide molecule participates in forming a number of double helical structures with other polysaccharide molecules, resulting in a tangled mass of molecules (gel), with the spaces between helices acting as pores. Pore size is not particularly regular, however, average pore size can be controlled. The greater the concentration of agarose in solution to start with, the greater will be the number of helices formed per unit of space and therefore the average pore size will be smaller.

During the last few decades, various forms of the agarose based systems have been developed for the applications in pharmaceutical industries and medical research. The major drawbacks of agarose are that it shows significantly low cell adhesiveness and cell proliferation, as it does not contain any moieties associated with cellular adhesion and adsorption of cell adhesives proteins (Sakai 2007).

1.5.3 Gelatin Gel

Gelatin is a natural polymer derived from collagen, and has been used for medical applications such as wound dressings, as scaffolds for tissue engineering and absorbent pads during surgery (Roman et al 2008). In addition, it has been widely used as a space filler because it can be handled easily especially in oral surgery, neurosurgery and orthopaedic surgery (Zhang et al 2009). Gelatin has some individual importance as a polymeric product widely utilized in the manufacture of various articles and materials.
Gelatin has the ability to form thermally reversible networks. Below the sol-gel transition temperature, part of the protein coils gives rise to triple helices reminiscent of the native collagen and protein solution turns into gel. Physical protein gels may be stabilized by the further addition of covalent bonds due to transglutaminase reaction. This enzyme catalyzed intra- and intermolecular cross-linking of some proteins, including gelatin, by N-(γ-L-glutamyl)-L-lysine side chain bridges (Giraudier 2004).

1.5.4 Polyacrylamide Gel

A polymer gel is defined as a cross-linked polymer network, with infinite viscosity, swollen in a liquid medium. Crosslinks in the gel may form chemically, which are thermo-set, or physically, which are thermoreversible. Polymerization is the formation of long, repeating organic polymer chains. Photopolymerization is an alternative method that can be used to polymerize acrylamide gels. Cross linked polyacrylamide gels are formed from the polymerization of acrylamide monomer in the presence of smaller amounts of N, N, N’, N’-methylenbisacrylamide. Bisacrylamide is essentially to acrylamide molecules linked by a methylene group, and is used as a cross-linking agent. Acrylamide monomer is polymerized in a head-to-tail fashion into long chains and occasionally a bisacrylamide molecule is built into the growing chain, thus introducing a second site for chain extension. The polymerization of acrylamide is an example of free radical catalysis. Diethoxyacetophenone is initiated by the UV light (350 nm) which produce free radical and initiate the reaction to form the polyacrylamide gel (Wilson and Walker 2006). The formation of polyacrylamide gel from acrylamide and bisacrylamide was as shown in Figure 1.1.
\[
\text{CH}_2=\text{CHCONH}_2 + \text{CH}_2(\text{NHCOHC}=\text{CH})_2
\]

Acrylamide \quad N,N,N',N'-\text{methylenebisacrylamide}

\[
\text{UV photopolymerization} \quad \text{Diethoxyacetophenone}
\]

Figure 1.1 The formation of polyacrylamide gel from acrylamide and bisacrylamide.

Polyacrylamide is a relatively nontoxic and non-degradable biomaterial, which is extensively used in ophthalmic surgery, drug treatment, facial depressions, lip enhancement, breast-augmentation, water purification, cosmetic products and in food packing (Christensen et al 2008 and Qiao et al 2005). It is a transparent and water based stable polymer with high viscoelastic bulking agent. In soft tissue augmentation, it is used as excellent tissue filler and it is fixed to the host tissue by thin strands of a fibrous network. Polyacrylamide gels have been used as substrates for fibroblasts, macrophages, epithelial cells and spinal cord neurons among many other cell types. Polyacrylamide hydrogels have applications in drag reduction agents,
thickening agents, cutting fluids, soil stabilizers, soaps, textiles and enhanced oil recovery. These gels are used in polyacrylamide gel electrophoresis (PAGE) as a porous supporting medium through which molecules migrate due to an electric field. In the medical field, the versatility of polyacrylamide gel provides vascular applications, reconstructive surgery and even bone implants.

1.6 SOL-GEL PROCESS

Sol-gel is a useful process of self-assembly for the synthesis of nanoparticles and it involves the fabrication of glass-like or ceramic materials through the hydrolysis and condensation of suitable metal alkoxides. Sol-gel process is the name given to any process, which involves a solution or sol that undergoes a sol-gel transition. Colloids are suspensions with molecules of 20-100 µm in diameter in a solvent. The colloid that is suspended in a liquid is the sol and the suspension that keeps its shape is the gel. Thus sol-gels are suspensions of colloids in liquid that keep their shape. The sol-gel process involves the evolution of networks through the formation of a colloidal suspension and gelation of the sol to form a network in continuous liquid phase. The precursors for synthesizing these colloids normally consist of ions of a metal, but also sometimes of other elements surrounded by various reactive species ie the ligands (Adamopoulos 2007).

The sol-gel formation occurs in four stages a) hydrolysis b) condensation and polymerization of monomers to form particles c) growth of the particles d) agglomeration of the particles followed by the formation of networks that extend throughout the liquid medium resulting in thickening, which forms a gel. Metal alkoxides are compounds in which the metals are bonded to a hydrocarbon moiety through oxygen, eg: NaOCH₃, Mg(OC₂H₅)₂, Al(OC₃H₇)₃. They may be considered as either derivatives of alcohols or metal hydroxides or inorganic acids. The most important reagent in the sol-gel
process is a hydrolysable organometallic that is called a metal alkoxide, \( M(OR)_x \), where (OR) is an alkoxy group. Hydrolysis occurs when the metal alkoxides and water are mixed in a material solvent (Collinson 2002).

### 1.6.1 Advantages of Sol-Gel Process

Sol-gel process involves the use of liquid solutions as mixtures of raw materials. The reactants are so well mixed in the solution that they are likely to be equally well mixed at the molecular level when the gel is formed. Controlled heating of the porous gel will give porous ceramics and porous non-crystalline solids with ultra fine pores. Impregnation of other pores with organic and inorganic materials results in unique composites. Modification of the structure of metal-organic precursors such as those organic groups that remain after gelation yields unique new polymers. Sol-gel process is well known for its potential of high purity, molecular mixing, chemical homogeneity, control of stoichiometry and reduction of densification temperature (Zarzycki 1997 and Adamopoulos 2007).

### 1.6.2 Disadvantages of Sol-Gel Process

Very large shrinkage associated with the gelation process and the drying of the gels, the presence of large concentrations of pores and the removal of undesirable residues like hydroxyls and organics.

### 1.6.3 Applications of Sol-Gel Process

Materials in various configurations (films, fibers, monoliths, powders) can be readily made. Thin films are most often utilized in chemical sensor applications due to the short pathlength for diffusion. Bulk monoliths have been frequently used in spectroscopic measurements due to the longer optical pathlength. High surface area powders are useful in catalysis
applications. Reagents can be readily incorporated in a stable host matrix by simply adding them to the sol prior to its gelation. The matrix actually stabilizes the entrapped reagent from photodegradation or caustic solution environments. The materials are chemically, photochemically, and electrochemically stable. They are also optically transparent, which can be used for optical characterization (Collinson 2002).

1.7 SCOPE OF THE WORK

The crystal deposition in human tissues associated with the formation and growth of bones and teeth. The synthesis of HAp by sol-gel method and the effect of sintering yield HAp, α-CPP and β-TCP find applications in biomedical field. Fabrication of degradable scaffolds using gels and HAp, the synthesis of HAp in the polymer matrix by mineralization and the effect of dopants in photopolymerized polyacrylamide gel is also studied. Extensive research work is still in need to understand the factors that influence the formation and growth of biocrystals. In vitro investigations such as bioactivity, drug release, haemocompatibility etc attract much attention in connection with the potential use of bioceramics as a bone replacement material.

The thesis broadly comprises the following:

The pure and lanthanum doped HAp nanorods was synthesized and the effect of sintering was investigated. The prepared samples were characterized by XRD, FT-IR, hardness, SEM, TEM and BET. In vitro drug loading/release, bioactivity, dissolution and antibacterial were also tested.

The degradable scaffolds and scaffolds embedded with HAp were prepared and characterized by XRD, FT-IR, TGA, UTM and SEM and EDX.
In vitro studies such as swelling, drug loading/release, haemolysis, dissolution and antibacterial were also tested.

Mineralization of nano HAp in photopolymerized polyacrylamide gel matrix was prepared and dried by air and freeze drier. The polymer composites were characterized using XRD, FT-IR and SEM. In vitro bioactivity, swelling, drug loading/release and antibacterial were also tested.

The effect of dopants on the synthesis of nano HAp in photopolymerized polyacrylamide gel matrix was prepared, freeze dried and characterized by XRD, FT-IR, TG/DTA, SEM and EDX. The in vitro studies such as swelling, drug loading/release and antibacterial were also tested.