CHAPTER I

BIOMINERALIZATION

1.1 INTRODUCTION

Crystals play major role in biology. Most of the living beings form crystals. In human body, there are many minerals, which are present in dissolved form. The body fluids contain minerals at various levels of saturation. When the body fluids get supersaturated with minerals, crystallization takes place. These crystals are beneficial and they produce pathological effects on humans. The major beneficial role of mineralization is formation of bones and teeth, which consists of oriented micro crystals of hydroxyapatite. Our sense of balance and acceleration is dependent upon small calcium carbonate (calcite) crystals present in the inner ear. The pathological effects result in the crystal deposition diseases.

The increasing incidence of crystal deposition diseases, such as heart disease, gout, gallstones, urinary stones etc., among an increased population has resulted in an extensive research on the above. Gallstones consist mainly of cholesterol with some quantities of calcium phosphates and calcium carbonates. The clusters of small crystals formed in the urinary system give rise to urinary (renal) calculi, which consist primarily of different forms of calcium, such as calcium oxalates, calcium phosphates etc.

Biomineralization is the process by which living forms influence the precipitation of mineral materials. The process creates heterogeneous accumulations, composites composed of biologic (or organic) and inorganic compounds, with inhomogeneous distributions that reflect the environment in which they form. The hunt for survival in dynamic and extreme environments has provided the organisms with unique capabilities to produce inorganic minerals with interesting properties known as biomineralization. Biominerals perform a variety of roles in organisms, the most important being support, defence and feeding. Furthermore, having formed under controlled conditions, biomineral phases often have properties such as shape, size, crystallinity, isotopic and trace.

Organisms ranging from bacteria to humans have been using the biomineralization process for at least 3500 million years. The first book on biomineralization was published in 1924 in German by W.J. Schmidt (Schmidt 1924), and the subject has continued to intrigue a dedicated community of scientists for many years. Until the early 1980s the
field was known as “calcification,” reflecting the predominance of biologically formed calcium-containing minerals. As more and more biogenic minerals were discovered that contained other cations, the field became “biomineralization.” Thus, biomineralization is the process by which living organisms produce minerals, often to harden or stiffen existing tissues. Such tissues are called mineralized tissues. Organic and inorganic biominerals crystallize in the human body elegantly, in symmetry with an ultra structure.

(i) Biologically induced mineralization

Minerals that are formed by biologically induced mineralization (BIM) processes generally nucleate and grow extracellularly as a result of metabolic activity of the organism and subsequent chemical reactions involving metabolic by-products. In many cases, the organisms secrete one or more metabolic products that react with ions or compounds in the environment resulting in the subsequent deposition of mineral particles (Fig.1.1). Thus, biologically induced mineralization is seemingly unintended and uncontrolled consequence of metabolic activities. The minerals that form are often characterized by poor crystallinity, broad particle-size distributions and lack of specific crystal morphologies. In addition, the lack of control over mineral formation often results in poor mineral specificity and/or the inclusion of impurities in the mineral lattice.

![Schematic diagram of biologically induced mineralization](image)

**Fig 1.1 Schematic diagram of biologically induced mineralization**
The biologically induced mineralization process is commonly associated with various bacterial activities and with epicellular mineralization in marine environments, occasionally leading to the complete encrustation of organisms that sink subsequently and form sediments [1, 2]. Biologically induced mineralization is, in essence, equivalent to inorganic mineralization under the same environmental conditions, and the minerals are therefore likely to have crystallochemical features that are generally indistinguishable from minerals produced by inorganic chemical reactions.

(ii) Biologically controlled mineralization

In ‘biologically controlled’ mineralization (BCM), minerals are usually deposited on or within organic matrices or vesicles (Fig. 1.2) within the cell, allowing the organism to exert a significant degree of control over the nucleation and growth of the minerals and thus over the composition, size, habit, and intracellular location of the minerals [3, 4]. These BCM mineral particles are structurally well-ordered with a narrow size distribution and species and also consistent crystal habits. Because of these features, BCM processes are thought to be under metabolic and genetic control. Since intra-vesicular conditions (e.g., pH, Eh) are controlled by the organism, mineral formation is not as sensitive to external environmental parameters as in BIM.

![Diagram of biologically controlled mineralization](image)

Fig 1.2 Biologically controlled mineralization
(iii) Biologically uncontrolled mineralization

The uncontrolled ‘pathological’ crystallization resulting in painful or even life threatening conditions such as formation of kidney stones, the development of gout, the formation of gallstones, and the deposition of plaque on the walls of the arteries. Crystal deposition disease is the result of a complex sequence of events that give rise to disease due to blocking of ducts or by hardening or weakening of the flexible tissue.

The mechanism of the formation of these structures is not well understood due to the complex nature of both their internal structure and the heterogeneous environment in which they form; the urine, synovial fluid, bile, and blood are comprised of numerous cells, macromolecules, inorganic salts, and organic molecules. The thesis has been focussed on the common pathological crystallization, i.e., kidney stones.

1.2 URINARY CALCULI

The kidneys serve several homeostatic functions, including the regulation of blood pressure, electrolytes, and acid-base balance in addition to acting as the body’s natural filter for removal of waste products from the body in the form of urine. Sometimes, the waste products are not dissolved and they stay behind in solid form in the kidney. Such crystals or lumps of waste products are referred to as Kidney stones. The size of the kidney stone varies from small, medium to large. The stones found in the kidney are either brown or yellow in color. Some stones may be smooth and brittle while some of them are hard.

In some cases, inorganic and/or organic components normally found in the kidneys may initiate the process of kidney stone formation, beginning with the nucleation of specific components, and increased residence time leading to the subsequent aggregation or growth of heterogeneous components.

Urolithiasis, i.e. the formation of stones or calculi in the urinary tract, is a painful condition affecting some 10% of the population in industrialized countries. For decades, urolithiasis has arguably been the most intensive research sector of clinical and fundamental investigations for the cause, prevention and treatment of crystal deposition diseases in human. However, it appears that a real breakthrough in this area is lacking as yet.
General conditions that contribute to stone formation include high concentration of salts in urine, retention of these salts and crystals, pH, infection, and a decrease in the body’s natural inhibitors of crystal formation. The general reasons for the stone formation include mainly dietary preferences, water quality, climate, genetics, disorders, and metabolic imbalance.

Kidney stone has a symptom of the most unbearable pain in the lower back just below the ribs spreading around the front of the abdomen and frequently extending into the groin area. The pain may come in waves as the stone tries to move through the tubes between the tubes and the bladder. The condition causes the individual severe discomfort and pain, and can lead to renal failure if left untreated [5]. Calculi contain mainly oxalate, phosphate, and uric acid crystals. The formation of stones in the urinary tract includes the bladder, ureters, urethra and kidneys (Fig. 1.3).

![Diagram of the human urinary system](image)

**Fig 1.3** Main components of the human urinary system and probable sites of calculus formation
Urinary stones are located in the kidneys, and only a small percentage is lodged in the urinary bladder and urethra. The composition of kidney stones can be classified into two parts. The first part is represented by organic matrix containing mainly proteins, lipids, carbohydrates, and cellular components. The term matrix refers to a group of macromolecules comprised of proteins, polysaccharides or glycoproteins that assemble to form a three-dimensional framework. The other part is biomineral component. There are three main types of kidney stones, according to the occurrence of biominerals [6]: oxalate stones which contain salts of oxalic acid; phosphate stones which contain salts of orthophosphoric acid; and urate stones which mostly consist of uric acid and its salts. Other minor phases in urolithiasis are cystine, xanthine, calcium carbonate, silicon dioxide, or calcium sulfate dihydrate. Several factors are responsible for the formation of urolithiasis.

The understanding of these processes is important for the treatment and prevention of urinary stones. Calculus composition depends upon the underlying cause that leads to their precipitation. For this reason it is particularly critical to know exactly which kind of stone is present in order to consider the best treatment, and also to guide prognosis and preventative measures.

1.3 TYPES OF STONES

Renal stones can be formed into various morphologies, depending upon the growth environment and the compounds present in the stone. Urinary calculi may occur where there is a high level of calcium, oxalate or uric acid in the urine; a lack of citrate in the urine (which is an inhibitor); or insufficient water in the kidneys to dissolve waste products. The compositions of the most common types are comprised of forms of calcium oxalate, magnesium phosphate, uric acid, and cystine. Table 1.1 shows the different compositions of urinary calculi, their mineral name and chemical formulae.

Approximately 70% of all kidney stones are composed of calcium oxalates either alone or mixed with calcium phosphates [7]. Other inorganic phases such as struvite are also detected to a certain extent (Fig.1.4). Calcium phosphates which forms a minor fraction is usually present as apatite (hydroxyapatite or carbonate apatite) sometimes as brushite (CaHPO₄·2H₂O) and rarely as whitlockite (Ca₃(PO₄)₂), Hydroxyapatite (Ca₅(PO₄)₃(OH)), is present in stones formed in sterile urine and carbonate apatite (with a varying content of carbonate ions), in stones associated with infection.
### Table 1.1 Common human urinary calculi and their major constituent(s)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Mineral Name</th>
<th>Chemical Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium oxalate monohydrate (COM)</td>
<td>Whewellite</td>
<td>CaC₂O₄.H₂O</td>
</tr>
<tr>
<td>Calcium oxalate dihydrate (COD)</td>
<td>Weddellite</td>
<td>CaC₂O₄.2H₂O</td>
</tr>
<tr>
<td>Magnesium ammonium phosphate hexahydrate (MAP)</td>
<td>Struvite</td>
<td>MgNH₄PO₄.6H₂O</td>
</tr>
<tr>
<td>Calcium phosphate, Carbonate form</td>
<td>Carbonate apatite</td>
<td>Ca₁₀(PO₄)₃(OH)₃(OH)₂</td>
</tr>
<tr>
<td>Calcium phosphate, Hydroxyl form</td>
<td>Hydroxyapatite</td>
<td>Ca₁₀(PO₄)₆(OH)₂</td>
</tr>
<tr>
<td>Calcium hydrogen phosphate dihydrate</td>
<td>Brushite</td>
<td>CaHPO₄.2H₂O</td>
</tr>
<tr>
<td>Octacalcium phosphate</td>
<td></td>
<td>Ca₃H₂(PO₄)₆·5H₂O</td>
</tr>
<tr>
<td>Tricalcium phosphate</td>
<td>Whitlockite</td>
<td>Ca₃(PO₄)₂</td>
</tr>
<tr>
<td>Magnesium hydrogen phosphate trihydrate</td>
<td>Newberryite</td>
<td>MgHPO₄.3H₂O</td>
</tr>
<tr>
<td>Uric acid</td>
<td>N/A</td>
<td>C₅H₄N₄O₃</td>
</tr>
<tr>
<td>Sodium acid urate</td>
<td></td>
<td>NaHC₅H₂O₃N₄.H₂O</td>
</tr>
<tr>
<td>Ammonium acid urate</td>
<td></td>
<td>NH₄H.C₅H₂O₃N₄.H₂O</td>
</tr>
<tr>
<td>Cystine</td>
<td>N/A</td>
<td>SCH₂CH(NH₂)COOH₂</td>
</tr>
</tbody>
</table>

**Figure 1.4 Composition of urinary stones.**
1.3.1 Calcium stones

Calcium stones are the most common variety accounting for about 75% of all episodes. The chemical composition will vary slightly from occurrence to occurrence but generally has a matrix comprised of calcium, phosphate and oxalate. These are always seen in acid urine (pH less than 6.5).

(i) Calcium oxalate stones

Calcium oxalate stones are the most frequent urinary stones of all. 70–75% of all stones contain calcium oxalate. Men are afflicted two times more frequently than women; people aged 30–50 years run the highest risk. Calcium oxalate stones develop as a multifactorial process in which an imbalance between crystallization-driving and -inhibiting forces plays a fundamental role. Dietary factors might be of great importance in stone development. Inborn errors of metabolism as well as acquired metabolic disorders are important contributing factors. So are most certainly also subepithelial calcifications (Randall’s plaques), and it is of note that a large fraction of calcium oxalate-containing stones also contain calcium phosphate. In nature, calcium oxalate exists in three different hydration states: the monoclinic calcium oxalate monohydrate (COM, Fig.1.5), which is thermodynamically the most stable phase at room temperature, the tetragonal calcium oxalate dihydrate (COD, Fig.1.6) and the triclinic calcium oxalate trihydrate (COT, CaC₂O₄.3H₂O caoxite).

![Calcium oxalate monohydrate stones](image)

Fig 1.5 Calcium oxalate monohydrate stones
(ii) **Calcium phosphate**

Phosphate stones are smooth, irregular, fine grained and soft in structure. In neutral or alkaline urine, hydroxyapatite stones are formed whereas brushite stones are formed in acidic urine. The most important mineral in the human body is apatite, the principal mineral constituent of bones and teeth. In urinary calculi two varieties occur: hydroxyl-apatite, \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \), and carbonate-apatite, in which \( \text{CO}_3\text{OH} \) groups replace \( \text{PO}_4 \) in amounts up to 5 percent by weight. Carbonate-apatite is more abundant in calculi than hydroxyapatite, but their habits are the same. It is associated with most other stone-forming materials, often serving as a center for deposition of subsequent compounds. Apatite, brushite, whitlokite, and octacalcium phosphate are some of the \( \text{CaP} \) crystals observed in human urinary calculi [8]. The occurrence rate of apatite, 4-10%; brushite, 2-6%; and octacalcium phosphate, less than 1% [9]. Among several calcium phosphate stones present, the main concentration of the thesis is on ‘brushite’ or CHPD.

(a) **Calcium hydrogen phosphate dihydrate**

Calcium hydrogen phosphate dihydrate (brushite, CHPD, \( \text{CaHPO}_4 \cdot 2\text{H}_2\text{O} \)) is much less common than apatite in calculi, but still occurs regularly in the acidic conditions. Flat, bladed, colorless-to-yellow monoclinic crystals occur in calculi as radiating aggregates giving the surfaces of stones a rough, nodular texture (Fig.1.7). Brushite is the initial phase of calcium salts formed by spontaneous precipitation or in an organic matrix in a normal acid urinary environment (pH less than 6.9).
A requirement for the crystallization of brushite is hypercalciuria. Moreover, urinary pH must be greater than 6. Below this pH the limited dissociation of phosphonate leaves an insufficient amount of divalent phosphate ion to increase brushite saturation. Clinically, it is harder to treat brushite stone patients as compared to apatite stone formers. The incidence of calcium phosphate (CaP) stone disease has increased over the last three decades; specifically, brushite stones have been diagnosed and treated more frequently than in previous years.

Brushite is a unique form of CaP, which in certain patients can form into large symptomatic stones. Treatment of brushite stones can be difficult since the stones are resistant to shock wave and ultrasonic lithotripsy, and often require ballistic fragmentation. Patients suffering from brushite stone disease are less likely to be rendered stone free after surgical intervention and often experience stone recurrence despite maximal medical intervention. Once a person has formed a stone the opportunity for recurrent stone formation approaches 65 to 100%.

![Image of brushite stones](image1.png)

**Fig 1.7 Calcium hydrogen phosphate dihydrate stones**

Apart from the urinary calculi it plays a number of rolls in biomineralization. It is formed in guano and phosphorite deposits, soil and human calculi. It is a precursor to the formation of bones and teeth. Hence, its study is important for the understanding of biomineralization phenomena. It also has interesting industrial applications as an intermediate in phosphate fertilizer production and in pharmaceutical applications as a food additive and a component of toothpaste [10]. It is relatively soluble in simulated physiological solutions. Therefore, when they are used as bone substitute it can dissolve and possibly provide increased levels of calcium and phosphate ions near the tissue-implant. Brushite coatings are cheap and applicable at room temperature. As long as
particles are in nanoscale range it is not that much important to prepare the particles in exactly the same crystalline form as in bone since they are not going to be used as intact building blocks for bone [11].

The major advantage of brushite cements is the higher solubility of secondary protonated calcium phosphates under physiological conditions, such that brushite is dissolved at the implantation site more rapidly than hydroxyapatite materials. This property has been demonstrated in various in vitro [12, 13] and in vivo studies [14, 17]. Brushite cement is replaced by new bone and the cement-new bone composite is shown to have similar or even better mechanical properties than normal bone within 16 weeks after implantation in rabbits [18]. In brushite cement, resorption is closely followed by the new bone formation. Apart from biological applications, the calcium phosphate mineral provides the world’s main supply for phosphorous to make agricultural fertilizers and various phosphorous containing chemicals.

(b) Hydroxyapatite

Hydroxyapatite (HA, Ca_{10}(PO_{4})_{6}(OH)_{2}) kidney stones are a form of calcium phosphate stones. Apatite is the most widespread mineral in calculi. It is associated with most other stone-forming materials, often serving as a centre for deposition of subsequent compounds. The first of two important habits for urinary apatite is soft, crumbly, fine grained, white, yellow, or light brown powder (Fig.1.8). The second habit is massive, glassy, yellow, brown, or black material.
Commonly, the urine pH is high and the urinary calcium is low. It has been proposed that HA forms the nidus of all calcium-based stones, serving as a site for heterogeneous nucleation [19]. Consequently, it is important to understand the factors controlling HA crystallization (especially those that may prevent or limit HA formation), for the design of effective preventive measures against nephrolithiasis. Hydroxyapatite calculi decreases with age in both men and women, but they are predominant in women [20].

(c) Calcium hydrogen phosphate

Calcium hydrogen phosphate (Monetite, CaHPO₄) was first reported by Rhamy (1974). It is reported that the monetite is formed in human beings due to very low urinary pH (4.13-5.08), naturally buffered urines, and high osmolality [21]. Monetite in the calculi was laminated, gray-to-brown, finely granular material associated with oxalates and apatite. Beck's monetite calculi also displayed lathlike triclinic crystals, and an additional associated with brushite.

(d) Carbonate apatite

Carbonate-apatite (Ca₁₀(PO₄)₂CO₃) calculi is more abundant in calculi than hydroxyl apatite, but their habits are the same. Fig.1.9 shows the carbonate apatite stone. Infection is not a prerequisite for the formation of carbonate apatite stones, but infective conditions favour carbonate apatite formation. They occur directly at pH values greater than 7.0 or via phase transformation from brushite. The following reaction equations demonstrate the formation of carbonate apatite. Urea is hydrolysed in the presence of urease

\[
\text{H₂N-CO-NH₂} \xrightarrow{\text{urease}} \text{H₂O} \quad \text{CO₂ + 2NH₃}
\]

Ammonia and carbon dioxide hydrolyse to ammonium ions and bicarbonate. Binding with available cations produces carbonate apatite

\[
\text{CO}_3^{2-} + 10\text{Ca}^{2+} + 6\text{PO}_4^{3-} \quad \text{pH} \geq 6.8 \quad \text{pH} < 6.8 \quad \text{Ca}_{10} (\text{PO}_4)_6\text{CO}_3
\]
(e) Octacalcium phosphate

Octacalcium phosphate (OCP, Ca₈H₂(PO₄)₆.5H₂O) has structural similarities with calcium-OH-apatite (HA), while differing in their Ca/P ratios (1.33 for OCP and 1.67 for HA). OCP crystallizes in the triclinic system. The naturally occurring kidney stones of octacalcium phosphate are shown in Fig.1.10.

(f) Tricalcium phosphate

Tricalcium phosphate (Whitlockite, Ca₁₈Mg₂H₂(PO₄)₁₄) is very rarely found in the urinary system. It is a calcium phosphate with small amounts of magnesium. The mineral is a brown, hackly-fracturing material, and it commonly forms multiple small stones in the prostate. Whitlockite is unstable in the urinary system. Fig.1.11 shows the naturally occurring kidney stones of tricalcium phosphate.
1.3.2 Uric acid

Uric acid stone formation (5% to 10%) is usually found in males and is associated with a chronically low urinary pH (less than 5.5). They are smooth, round, yellow-orange and nearly radiographically transparent unless mixed with calcium crystals or struvite (Fig.1.12). Diets high in purines, especially those containing meats and fish, result in hyperuricosuria and in combination with low urine volume and low urinary pH, can aggravate uric acid stone formation. Higher incidences are seen in patients with myeloproliferative disorders or cancer patients on cytotoxic drugs [22]. Uric acid stones are not visible on x-ray due to the lack of calcium in the matrix.

1.3.3 Cystine

Cystine stone formation (1 to 2%) is due to an inborn error of metabolism resulting in excess intestinal and renal tubule absorption. The stones are greenish- yellow, flecked with shiny crystallites, and are moderately radio-opaque with a rounded
appearance (Fig. 1.13). High pH levels are needed (> 7.5) in order for cystine stones to form, with the process being independent of crystal inhibitors. Despite treatment, high recurrence rates are often seen. People who are homozygous for cystinuria excrete more than 600 mg per day of insoluble cystine, so cystine stones should be suspected in patients with a history of childhood stones or a strong family history. Cystinuria is the cause of 1–2% of stones observed in adults and 10% of those occurring in children. More than half the stones in cystinuria are of mixed composition, and many patients have associated physiological problems such as hypercalciuria (19% of patients), hyperuricosuria (22%), and hypocitraturia (44%) [23].

![Fig 1.13 Cystine stones](image)

### 1.3.4 Magnesium ammonium phosphate hexahydrate (MAP)

Magnesium ammonium phosphate hexahydrate (struvite, MgNH₄PO₄·6H₂O) is a separate kind of urinary stones that are related to urinary tract infection [24]. Struvite kidney stones are sometimes called triple phosphate stones because of their combination of magnesium, ammonium and calcium/phosphate. This often occurs in persons who experience infections of the urinary system. For this reason they are most common in women than men [25, 26]. They are most often associated with infections of the urinary tract. Struvite stones are also found in dogs and house cats that are infected with ammonia producing organisms. It was reported that struvite stones are more common in the children of Europe than in the USA [27].

It accounts for 10-15% of renal stones and can grow rapidly widened in a jagged or branch-shaped structure (Fig.1.14) called a "staghorn" with urea-splitting infections
caused by several strains of bacteria. They are mainly the microorganisms from the Proteus species which are isolated in the case of 70% of the bacteria-induced urinary stones [28]. The danger with struvite stone formation is that they can form and enlarge rapidly resulting in obstruction of the renal calyx.

For struvite to form, the urine must contain ammonium and trivalent phosphate ions at the same time. The renal tubule only makes ammonium when the organism is excreting an acid load, however trivalent phosphate is almost not present in acid urine. Thus, under normal physiological conditions struvite may not precipitate. For struvite to form, conditions which are non-physiological are required and these are created by urease-secreting bacteria. Urease is an enzyme splitting urea into carbon dioxide (CO₂) and ammonia (NH₃):

\[ \text{H}_2\text{N}-\text{CO}-\text{NH}_2 \xrightarrow{\text{urease}} \text{CO}_2 + 2\text{NH}_3 \]

Ammonia then mixes with water to produce ammonium hydroxide and under these alkaline conditions, carbonic acid moves toward bicarbonate and carbonate ions.

\[ 2\text{NH}_3 + 2\text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + 2\text{OH}^- \]

Generated ammonia increases urinary pH, which leads to elevation of the concentration of the NH₄⁺, CO₃²⁻ and PO₄³⁻ ions. These ions together with the ions of calcium and magnesium present in the urine lead to the crystallization of struvite, according to the following reaction:

\[ \text{Mg}^{2+} + \text{NH}_4^+ + \text{PO}_4^{3-} + 6\text{H}_2\text{O} \xrightarrow{\text{pH}7.2} \text{MgNH}_4\text{PO}_4, 6\text{H}_2\text{O} \]

Struvite stone may grow very rapidly and may involve the entire renal pelvis and calyces, which may lead to the blockage of the urinary tract.
Struvite is a phase deposited along the pipelines of the wastewater treatment and sewage sludge purification plants, causing the undesired clogging of pipes [29]. On the other hand, intentional and controlled precipitation of struvite is practiced in the landfills and waste water treatment plants to reduce the environmentally harmful free ammonium and phosphorus presence [30, 31]. Struvite may have the potential of being used as a fertilizer, as well [32]. Magnesium phosphate cements (MPC), which form crystalline struvite upon setting, were developed as bone substitute biomaterial or bioceramic [33, 35]. Ibasco et al [36] reported that struvite-coated titanium samples exhibited better biocompatibility with the osteoblastic cells, in vitro, than pure titanium.

1.3.4.1 Magnesium hydrogen phosphate trihydrate

Magnesium hydrogen phosphate trihydrate, also known as Newberyite (MgHOP₄·3H₂O) is a very rare crystalline component of urinary calculi was first found in 1956 [37]. He found that a single crystal of struvite became contaminated on the surface by newberyite, and it seems likely that the newberyite in urinary calculi is formed by the decay of struvite. When it occurs, it often occurs as tiny isolated globular crystals on the surfaces of apatite-struvite stones. This probably reflects an alteration of struvite to newberyite, or perhaps a change of conditions to more acidic solutions. Newberyite may be associated with infections of the bacterium _Proteus_.

1.4 NEED FOR IN VITRO STUDIES

_In vitro_ crystallisation systems are widely used for different purposes in urolithiasis research. Since stone formation is a process that includes crystal
nucleation, growth and agglomeration, the different systems are aimed at investigation of all or at least some of these partial events. Basically, they are intended to improve the understanding of the physicochemical and/or biochemical processes underlying urinary stone formation. The study of effects of well-defined chemical substances (e.g. citrate, Mg, chondroitin sulfate) or various less-defined substrates isolated from human urine (e.g. macro-molecular fractions) on crystallisation parameters have often been carried out. For the prevention of occurrence of the urinary calculi and for the non surgical management of the urinary calculi, the in vitro growth inhibition study is of prime importance. In this point of view, the role of trace elements has been discussed below.

1.5 TRACE ELEMENTS

Trace elements are inorganic substances found in human blood in the order of micrograms. These elements are incorporated into the structures of proteins, enzymes, and complex carbohydrates. They take part in biochemical reactions together with enzymes. Zinc (Zn) and copper (Cu) are especially active in metabolic and biochemical processes in the recovery phase of wounds. It is known that the trace elements are necessary for the continuous systematic functioning of the immune system. Magnesium (Mg) is required for the synthesis of all proteins, nucleic acids, nucleotides, lipids, and carbohydrates, as also for the contraction of muscles. Iron (Fe), apart from its presence in all body cells, plays a role in the oxygenation of tissues as it is incorporated in the haeme structure of haemoglobin. Burns, a pathology characterized by injury and loss of the organism's largest organ, is a condition in which there is an increase in certain metabolic processes, such as tissue repair, wound healing, microcirculation, and oxygenation.

1.5.1 Effect of trace elements on urolithiasis

Trace elements play an important role in the complex metabolic pathways in human system and their deficiency or in excess may cause disease. Trace elements have an effect on the crystallization of urinary stones. i.e. some metal ions may adsorb on the crystal surface and get concentrated within the lattice of the crystal. These substances typically exert their effect at micro molar concentrations. Once adsorbed on the surface of the crystal, they may alter or inhibit crystal dissolution.
In the crystal growth experiments, the following inhibitory effects may be observed, in general: (i) no nucleation (giving only a powdery mass), (ii) reduction in the number and size of the crystals, (iii) reduction in the total mass of the crystals formed, (iv) change in the morphology of the crystals and (v) change in the crystalline quality (good to poor quality).

The promotary effects that may be observed are the following: (i) increase in the number and size of the crystals and (ii) increase in the total mass of the crystals formed.

The main concept of the calculus formation is a deficiency of inhibitors of nucleation, aggregation and growth of urinary crystals. Certain chemicals that are present in the urine can act as inhibitors or promoters of calculi formation. Among promoters are the ions which are the part of calculi like calcium, oxalate, urate and phosphate. Compounds known to inhibit include pyrophosphate, citrate, magnesium and some macromolecules. The inhibitors may play an important role in the regulation of the urinary stone formation.

Substitution of trace elements, such as Mg, Sr, Zn, K, Ag, Cu, Li, F and Cl ions, into the structure of calcium phosphates is the subject of widespread investigation nowadays, because of their impending role in the biological process [38]. Consequence of these substitutions is changes in its properties like lattice parameters, crystallinity, morphology, solubility etc., without significant change in the symmetry. To have a detailed knowledge of the mechanisms involved in the process of biomineralization, the strength of the calculi, the ageing time for the full growth of the calculi, the inhibitory and promotary effects of different dopants must be obtained. In vitro studies draw close parallels with growth in the biological system. The next chapter deals with the crystal growth methods.