INTRODUCTION
1.1 THE APATITES

Over a century and a half ago, the name apatite was given to a group of minerals. This name is derived from a Greek word which means "deception". Because it was often confused with other minerals such as aquamarine, amethyst and olivine. The history of the investigations up to the present day concerned with this mineral show that the name was well chosen, as many persons who have studied the apatite minerals have surely been deceived by their findings.

The composition and structure of the mineral component aroused the interest of physical scientists more than a century ago. In 1862 Hopper-Seyler analysed the mineral elements of bone and teeth and was impressed by the analogy which existed between the composition of the tissues and that of mineralogic crystals known as apatites. He formulated the hypothesis that the bone mineral has an apatitic structure. The general formula for apatites is
$\text{M}_{10}\text{(RO}_4\text{)}_6\text{X}_2$

which contains 42 atoms.

where $\text{M} = \text{Ca, Pb, Na, K, Sr, Mn, Zn, Cd, Mg, Fe}^{n}$, $\text{Al}$, and the rare earths (especially $\text{Ce}$) and

$\text{X} = \text{F, OH, Cl, Br and}$

$\text{RO}_4^- = \text{PO}_4^-, \text{AsO}_4^-, \text{VO}_4^-, \text{SO}_4^-, \text{and SiO}_4^-$.

Calcium and phosphorus occur in nature as mineral Fluorapatite, $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$, which is an important member of Apatite Family. In addition, bones and teeth contain these elements in the form of Hydroxylapatite (Hydroxyapatite), $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ as the major crystalline inorganic constituent [2]. Some of the principal members of the series are given in Table 1 [3-6].

**TABLE 1. Principal Members of apatite family.**

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Formula</th>
<th>Symbol</th>
</tr>
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<tbody>
<tr>
<td>Hydroxylapatite</td>
<td>$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$</td>
<td>H$_{\text{ap}}$</td>
</tr>
<tr>
<td>Fluorapatite</td>
<td>$\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$</td>
<td>F$_{\text{ap}}$</td>
</tr>
<tr>
<td>Chlorapatite</td>
<td>$\text{Ca}_{10}(\text{PO}_4)_6\text{Cl}_2$</td>
<td>Cl$_{\text{ap}}$</td>
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Hydroxylapatite, $3Ca_3(PO_4)_2Ca(OH)_2$, $Ca_{10}(PO_4)_{6}(OH)_2$, the product of $F^- \rightarrow OH^-$ substitution in fluorapatite is the most important of the series. Each member undergoes a series of isomorphous cationic and anionic substitution reactions leading to the formation of some of the other members and hence, it has recently been the subject of extensive physico-chemical and biological investigations [7]. Knowledge of its location and significance in bones and teeth is consequently a prerequisite for carrying out meaningful investigations in this field.

1.2 HYDROXYLAPATITE IN BONE AND TOOTH MORPHOLOGY

(a) Organic Portion of Mineral Tissue

The four mineralised tissues in the mammalian body are bone, cementum, dentin and enamel. The last three comprising the hard dental tissues. Each of these is composed of an organic and inorganic fraction which are so intimately mixed that the electron - microscope is needed to resolve the fractions for observation. Fig.1-A and Fig.1-B are optical photomicrographs of a molar tooth section and
a bone section, respectively, which show the detailed morphology of these tissues. Fig.1-A is a comparison between the constituent of bone, cementum, dentin and enamel.

The organic portion of bone has two major fractions, the collagen and the ground substance. The protein, collagen, is characterised physically by its fibrillous nature and chemically by its high content of pyrrolidine amino acids and its low content of aromatic amino acids.
Fig. 1-A Photograph of a longitudinal section of a human molar, enamel, cementum, dentin bone.

Fig. 1-B Photograph of a section of normal human bone.
The collagen fibers act as a latticework which is filled in by the bone mineral, the ground substance and the reticulin. In addition, there is the interstitial fluid which makes the bridge between the solid substance in bone and blood system in the animal. The ground substance, very similar chemically to the fluid, is an amorphous solid which contains mainly mucopolysaccharides. The small portion of the bone solid associated with ground substance is called the reticulin. It is a fibrous material, a hybrid, combining a polysaccharide with a protein.

In the enamel, keratin is substituted for collagen as it is in other external organs such as skin. The enamel equilibrates with saliva rather than with the internal fluids which are in equilibrium with the other calcified tissue.

(b) Inorganic Portion of Mineral Tissue.

Bone, a mineralised connective tissue, not only act as a supportive frame work for the body but enters into metabolic interfractions with it. It is composed of organic, inorganic and aqueous fractions. The inorganic part is composed of primarily crystalline hydroxylapatite amounting to about 35
percent by weight. The hardness and physical strength of bone results from decomposition of hexagonal needles of hydroxylapatite within the organic matrix and the process is known as calcification. The low angle X-ray studies of Engstrom and Finean [8] suggested the average length and breadth of these crystals to be about 220 Å and 65 Å respectively. These crystals are arranged in a periodic pattern around the collagen fibers with their longer axes oriented in the fiber direction.

(C) The Inorganic Composition of Teeth.

Tooth consists of an outer part known as crown a neck which is surrounded by gum and one or more roots or fangs fitting in to the sockets of the jaw bone. The crown is covered by enamel which consists of hexagonal prisms of hydroxylapatite arranged at right angles to the tooth surface. About 95 percent by weight of enamel is constituted by these crystals. The enamel rests on dentin which occupies the bulk portion of tooth. The cementum helps the fixation of tooth in the sockets of jaw bone. The weight percent of the inorganic fraction was shown to be approximately 75 and 35 in dentin and cementum respectively. The hydroxylapatite crystal of bone
dentin and cementum are found to be similar in size in accordance with their morphological similarities while those of enamel are found to be bigger than those found in bone. Unlike the other calcified tissues which are in equilibrium with the internal fluids, enamel which forms the tooth surface equilibrates with saliva and is thus a set of action of locally administered prophylactic agents of dental caries. The causes of caries can be divided into two general categories which are not necessarily independent.

(i) The first theory of tooth decay is the acidogenic theory proposed by Miller [9]. This view of dental carries stresses the role of acids in destroying dental tissue.

(ii) The second broad theory of dental carries is the proteolytic theory which emphasizes the primary destruction of dental protein as a precursor to the removal of mineral matter [10].
TABLE 2. Comparison of Normal Mineralized Tissues of the Body.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Bone</th>
<th>Cementum</th>
<th>Dentin</th>
<th>Enamel</th>
</tr>
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<tr>
<td><strong>Inorganic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Wt.% (approx)</td>
<td>35%</td>
<td>35%</td>
<td>75%</td>
<td>95%</td>
</tr>
<tr>
<td>2. Character</td>
<td>Apatite, Carbonate, adsorbed ions</td>
<td>Apatite, Carbonate, adsorbed ions</td>
<td>Apatite, Carbonate, adsorbed ions</td>
<td>Apatite, Carbonate, adsorbed ions</td>
</tr>
<tr>
<td><strong>Organic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Fibrous Protein Frame work</td>
<td>Collagen</td>
<td>Collagen</td>
<td>Collagen</td>
<td>Keratin</td>
</tr>
<tr>
<td>2. Ground substance</td>
<td>Mucopoly-saccharide</td>
<td>Mucopoly-saccharide</td>
<td>Mucopoly-saccharide</td>
<td>Mucopoly-saccharide</td>
</tr>
</tbody>
</table>
1.3 AQUEOUS PHASE DIAGRAM

Studies on the inorganic constituent of bone, the utilisation of phosphatic minerals in plant and the minerological aspects of several naturally occurring phosphates contributed to our present knowledge of the phosphates of calcium.

The preliminary investigations\textsuperscript{11-13} to establish the conditions of existence of phosphates of calcium were restricted to the solid phase reactions of the binary system, CaO - P\textsubscript{2}O\textsubscript{5}; it was not however, known that the presence of traces of water resulted in the establishment of a ternary system, CaO - P\textsubscript{2}O\textsubscript{5} - H\textsubscript{2}O.

The earlier information about the ternary system which was based exclusively on the chemical analysis was found to be unreliable since it was not known whether the solid phase consist of a single substance, or a solid solution of two or more substances. Further, the amorphous nature of the solid phase complicated its identification. These considerations emphasized the inadequacy of chemical analyses\textsuperscript{14} and the utility of the application of the phase rule for the purpose. The later application of
CaO - P₂O₅ - H₂O phase diagram to establish the conditions of existence of phosphates of calcium became complicated due to

(i) the slow attainment of equilibrium \(^{15,16}\)
(ii) the amorphous nature of the precipitates and
(iii) the reactivity of the solid phase consequent upon the size of the solute particles which existed in the range of colloidal dimensions.

Since these factors were not given proper consideration, the preliminary experimental data on the conditions of existence of calcium phosphates became repetitious, disorganised and contradictory.

Investigations on the applicability of phase rule to CaO-P₂O₅-H₂O system carried out independently by Bassett\(^{17}\) and Cameron\(^{18}\) and the results obtained at 25°C are incorporated in the phase diagram\(^{19}\) given in Fig.2. The abscissa and the ordinate of a given point on the diagram represent the weight percent of phosphorus pentoxide and calcium oxide respectively, the rest being that of water. The possible phases of the system, in addition to the solution, are
(i) anhydrous monocalcium phosphate, $\text{Ca}(\text{H}_2\text{PO}_4)_2$ 
(ii) monocalcium phosphate monohydrate, $\text{Ca}(\text{H}_2\text{PO}_4)_2\text{H}_2\text{O}$ 
(iii) anhydrous dicalcium phosphate, $\text{CaHPO}_4$
(iv) dicalcium phosphate dihydrate, $\text{CaHPO}_4.2\text{H}_2\text{O}$ and
(v) a crystalline precipitate of variable compositions, shown as $[\text{Ca}_5(\text{PO}_4)_3\text{OH}]$ in the diagram with x-ray pattern similar to that of an apatite.

The blank region of the phase diagram represents solution phase while the solid phases are indicated by areas marked by convergent lines. All these phases could be identified through their distinct X-ray patterns supplemented by their chemical analyses.
Fig. 2. Phase diagram of $\text{CaO} - P_2O_5 - H_2O$ system at $25^\circ\text{C}$. 
Anhydrous monocalcium phosphate and its monohydrate exist as well defined crystals in the compositional range having a higher proportion of phosphorous pentoxide. These compositional ranges constitute the acidic region of the phase diagram. Weak, acidic or neutral region is found to be favourable for the formation of dicalcium phosphate. Dicalcium phosphate dihydrate was found to exist over a limited range of experimental conditions and hence its isolation was difficult. A crystalline precipitate of variable composition exhibiting the X-ray pattern of the naturally occurring apatites is found to occur in distinctly alkaline region of the phase diagram having higher proportion of calcium oxide. The region of its existence is indicated by a circular area in the phase diagram. The formula suggested for the phase is \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \) (represented in Fig.2 as \([\text{Ca}_5-(\text{PO}_4)_3\text{OH}]\)).

Hayek, et al. suggested the existence of an additional solid phase called octacalcium phosphate (OCP) \( \text{Ca}_4\text{H}(\text{PO}_4)_3 \), near the neutral region of the system with Ca/P g. atom ratio of 4/3. The crystal of OCP can be demarcated from those of hydroxylapatite through its X-ray pattern and electromicrographs. The
crystals of OCP are found to be tabular while those of hydroxyapatite are cylindrical or needle shaped. No separate mention of OCP has been made in the phase diagram since this was considered to be a part of the solid phase of the composition of hydroxyapatite. The limiting Ca/P g. atom ratio for hydroxyapatite phase are 3/2 and 4/2 corresponding to tricalcium phosphate (TCP), $\text{Ca}_3(\text{PO}_4)_2$, and tetracalcium phosphate, $\text{Ca}_4\text{P}_2\text{O}_9$, as shown in the phase diagram.

1.4 METHODS OF FORMATION OF HYDROXYLAPATITE

It was shown by Warrington$^{22}$ that hydroxyapatite was the stablest of the different calcium phosphates in weakly acidic, neutral and basic media. Thus the hydrolysis of the other calcium phosphate leads to the formation of hydroxyapatite. Lorah, et. al.$^{23}$ boiled calcium phosphates of the type $\text{CaH}_4(\text{PO}_4)_2$, $\text{CaHPO}_4$, $\text{Ca}_2\text{P}_2\text{O}_7$ and $\text{Ca}_3(\text{PO}_4)_2$ with dilute sodium hydroxide solution (0.06 to 0.5 N) and determined the time dependence of their calcium to phosphorous g. atom ratios. It was found that the ratio attained a constant value equal to that of naturally occurring apatites within about 50 h. Similar results were obtained by schleede, et.al.$^{13}$ by
refluxing 30g. of TCP with 500 ml of 0.5 M potassium hydroxide for 67 h.

In addition to the methods of formation of hydroxylapatite in aqueous media, attempts were made by Tromer\textsuperscript{24,25} to prepare hydroxylapatite through solid state interactions by heating a mixture of tri and tetra calcium phosphates, Ca\textsubscript{3}(PO\textsubscript{4})\textsubscript{2} and Ca\textsubscript{4}P\textsubscript{2}O\textsubscript{7} amounting to a CaO/P\textsubscript{2}O\textsubscript{3} value of 10/3 for few hours of 1100 °C in a current of moist air. The reaction is represented by the following equation:

$$2\text{Ca}_3\text{(PO}_4\text{)}_2 + \text{Ca}_4\text{P}_2\text{O}_7 + \text{H}_2\text{O} \rightleftharpoons \text{Ca}_{10}\text{(PO}_4\text{)}_6\text{(OH)_2}$$

Fusion of dicalcium phosphate, CaHPO\textsubscript{4} and calcium carbonate at about 1400 °C under a stream of moist air resulted in the formation of hydroxylapatite as observed by Schleede, et al.\textsuperscript{26}. 
Fig. 3 (a) Electronmicrographs\textsuperscript{21} of synthetic hydroxylapatite.

Fig. 3 (b) Electronmicrographs\textsuperscript{21} of Octacalcium phosphate
1.5 CHEMICALLY PRECIPITATED HYDROXYPATITES

The key to the nature of bone mineral is the study of hydroxylapatites precipitated from solution under conditions similar to physiological in calcium and phosphate content, pH and temperature. Hydroxylapatites prepared in this way consist of needle-like crystals which are so small that they are only visible in the electron microscope.

For purposes of physico-chemical investigations synthetic samples of high degree purity are better suited than the natural hydroxylapatite isolated from bones and tooth. A survey of the different methods of the formation of hydroxylapatite describe earlier indicates that very few of them are suited for its preparation.

A method suggested by Hayek and Stadlmann\textsuperscript{27} which was subsequently modified by Collin\textsuperscript{28} is widely used since

(i) the degree of purity of the sample obtained is high
(ii) the yield is about 80 percent of the stoichiometric amount, and
(iii) the experimental operations are simple.
The method is based on the following equation:

$$10 \text{Ca(NO}_3\text{)}_2 + 6(\text{NH}_4\text{)}_2\text{HPO}_4 + 8 \text{aq.NH}_3 = \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 6\text{H}_2\text{O} + 20\text{NH}_4\text{NO}_3$$

1600 ml of a solution containing 79 g of diammonium hydrogen phosphate maintained at a pH of about 12 by the addition of liquor ammonia, were dropped under constant stirring into 1200 ml of a solution containing 230 g of calcium nitrate Ca(NO$_3$)$_2$.4H$_2$O, also maintained at the same pH. The precipitation was brought about in carbon dioxide free atmosphere. The amount of the reactants taken were intended to give an yield of about 100 g of hydroxylapatite as indicated by the above equation. The precipitation was boiled with mother liquor for about 10 min. to improve its homogeneity and crystallinity. The filtered product was heated to 250°C to volatilise the admixed ammonium salts. Collin$^{29}$ used ethylene diamine instead of liquor ammonia to maintain the required pH.

Among the rest of the methods mention may be made of those of Rathje$^{30,31}$ and Arnold$^{32}$. Rathje's method, called by him as "acidimetric precipitation", is based on the following equation:
Convenient volumes of solutions of calcium nitrate, \( \text{Ca(NO}_3\text{)}_2 \cdot 4\text{H}_2\text{O} \) and potassium dihydrogen phosphate containing the reactants in the proportions suggested by the above equation were simultaneously dropped into boiling water. The medium of precipitation was maintained pink to phenolphthalein by addition of sodium hydroxide solution. By selection of appropriate dilutions and the rates of addition of solutions, crystals up to 50 in length could be prepared.

Arnold\(^{32}\) could prepare a sample of high purity by simultaneous addition of calculated quantities of solutions of calcium acetate and ammonium phosphate containing an excess of ammonium hydroxide to 10 litres of mechanically stirred carbon dioxide free ammonium acetate solution. The medium of precipitation was maintained alkaline.

Production of pure single crystals which are of about 0.1mm in length constitutes an important aspect of investigation on the precipitation of hydroxylapatite. This was achieved by Hayek et al.\(^{33}\) and later by Perloff and Posner\(^{34}\) through hydrothermal synthesis. Hayek could get homogeneous single crystals.
in the form of hexagonal prisms by heating about 2 g of precipitated hydroxylapatite at 380 °C in autoclaves for 24 h with 15 ml of 2 M sodium hydroxide solution. The X-ray diagram of the sample was characterised by sharp peaks as expected from its crystal dimensions.

The method of Perloff and Posner is based on the hydrolysis of dicalcium phosphate and the probable reactions involved are represented as follows:

$$10 \text{CaHPO}_4 + 2 \text{H}_2\text{O} \xrightarrow{300°C} \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 4\text{H}^+ + 4\text{H}_2\text{PO}_4^-$$

$$14\text{CaHPO}_4 + 2\text{H}_2\text{O} \xrightarrow{300°C} \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 4\text{Ca}^{2+} + 8\text{H}_2\text{PO}_4^-$$

The process was brought about by heating 0.1 g of dicalcium phosphate with about 10 ml of water at 300°C for 10 days in platinum-lined hydrothermal bomb and the product was found to be in the form of this prismatic crystals.

1.6 APATITES DEFICIENT IN DIVALENT CATIONS

Calcified tissue mineral as well as certain finely divided synthetic and natural calcium phosphates, have X-ray diffraction patterns similar to hydroxylapatite but often depart from its stoichiometry by exhibiting low \( \frac{\text{Ca}}{\text{P}} \) molar ratios. The explanations to account for this effect can be divided into two general categories:
(i) The low ratios are due to excess $\text{HPO}_4^{-2}$ groups adsorbed on the surface of stoichiometric hydroxyapatite crystals.

(ii) Calcium ions are missing in some way, either from the surface or from the interior of the crystals.

Some workers explained this apparent lack of stoichiometry by the existence of adsorbed phosphate, in some form, in the proportion needed to lower the molar $\frac{\text{Ca}}{\text{P}}$ ratio from the ideal 10/6 to the observed value which is usually about 9/6. Neuman from surface exchange studies, found that the difference in $\frac{\text{Ca}}{\text{P}}$ ratio can not be explained in terms of surface composition alone. Also the surface area of some synthetic apatites are too low to account for their non-stoichiometry by surface adsorption. Finally, Gee and Dietz reported less pyrophosphate formed upon gentle ignition of calcium deficient apatites than they predicted on the basis of surface adsorbed $\text{HPO}_4^{-2}$ or $\text{H}_2\text{PO}_4^{-}$ on structurally intact hydroxyapatite. These results seem to rule out the adsorption viewpoint.

Without phosphate adsorption explanations for the existence of non-stoichiometric apatites on the basis of deficiencies in calcium ions can generally be divided into three groups:
(1) The calcium ions are missing from the crystals surface with replacement by hydronium ions \((H_3O^+)\) to preserve charge neutrality.

Neuman and Neuman\(^7\) suggested that since the surface of the bone apatite crystals is such a great part of the solid, the Ca/P ratio is lowered by this surface non-stoichiometry. In addition, apatite crystals in bone would have to be smaller than observed (i.e. have higher surface areas) in order to lower Ca/P to 9/6 by surface substitution of \(H_3O^+\) for the missing calcium and still maintain at least 4.52% calcium content on the surface.

(2) Recent work has provided X-ray diffraction and index of refraction evidence for the existence of calcium-deficient apatites.

The calcium ions were shown to be absent statistically from certain positions not only from the surface but throughout the synthetic apatite crystals.

(3) Electrical neutrality is maintained in the crystal by the addition of hydrogen bonding \((2H^+\) for each missing \(Ca^{2+}\)) between orthophosphate oxygen. A general formula for apatite may be written for
\[ M_{10-x}H_{2x}(PO_4)_{6}(OH)_2 \]

where M would be any divalent cation such as Ca\(^{2+}\), Pb\(^{2+}\), Mg\(^{2+}\) etc.) and H the sum of the monovalent cations such as H\(^{+}\) and Na\(^{+}\)

where P\(_4\) and OH can be substituted by the usual ions,

x = values ranging from 0 to 2.

Experiments with synthetic apatites deficient in calcium show that these compound react chemically with calcium in solution, possibly releasing hydrogen from the apatite structure.

Other experiments have shown that the lactic acid solubility of both synthetic apatites and rat molar enamel (with low Ca/P) can be reduced by treatment with calcium acetate solution. The density of pure hydroxylapatite is higher than the density of a low Ca/P apatite and in addition it has been demonstrated that low calcium apatites are most reactive than perfect apatites. Finally low \( \frac{Ca}{P} \) apatites when heated to 900°C will revert to \( Ca_3(PO_4)_2 \) whereas stoichiometric hydroxylapatite under the same treatment remains unchanged. Perhaps this difference in reactivity of low calcium phosphate mineral, coupled with the surface properties of bone crystals defines the behavior of mineralized tissue with respect to body fluids.
1.7 WATER CONTENT AND THERMAL DEHYDRATION

The samples of hydroxylapatite obtained by precipitation in aqueous media were reported to certain varying amounts of water. Schleede et al. suggested the presence of water of crystallisation by their dehydration studies. Later Hayek et al. showed that the lattice dimensions of hydroxylapatite remained unchanged on heating to 900°C indicating that the water content was due to adsorption. Chickerur et al. convincingly established that water present was adsorbed in nature, by application of thermogravimetric, x-ray, infrared and electronmicroscopic techniques.

Thermal dehydration of synthetic hydroxylapatite by Chickerur and Narasaraju indicated that at about 900°C the entire water other than that due to hydroxyl groups was lost. Loss of constitutional water resulting in the decomposition of hydroxylapatite to form tricalcium phosphate, Ca$_3$(PO$_4$)$_2$ and tetracalcium phosphate, Ca$_2$P$_2$O$_9$ were found to take place in the range 1200-1500°C. The electron paramagnetic resonance spectral study of Backer and Marino and the recent thermographical study of Chiranjeevi Rao and Chickerur indicated that the water in hydroxylapatite was lost in three discrete steps.
corresponding to the loss of adsorbed and associated water and liberation of hydroxyl groups.

1.8 CHEMICAL ANALYSES

The chemical analyses of hydroxylapatite is difficult due to the interference of phosphate in the determination of alkaline earth. Washburn and Sheer\textsuperscript{42} have established a quantitative procedure for their separation and determination. Chickerur and Narasaraju\textsuperscript{43} have worked out a method of separation and determination of calcium, strontium and phosphate in the solid solutions of hydroxylapatites of calcium and strontium. Similar gravimetric separations have been worked out recently by Narasaraju et al.\textsuperscript{44-45} and the elements present in hydroxylapatite were subsequently determined complexometrically. Among other methods known, the complexometric procedures for the determination of calcium and phosphate in hydroxylapatite developed by Singh et al.\textsuperscript{46} and calcium in calcium hydroxylapatites worked out by Chiranjeevi Rao and Patel\textsuperscript{47} and calcium, strontium or barium and magnesium or lead or zinc worked out by Patel, et al.\textsuperscript{48} are important.
The crystals of hydroxylapatite belong to the P6$_3$/m space group of the hexagonal class with lattice constant 'a' and 'c' equal to 9.43 Å and 6.88 Å respectively. The formula \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \) represents the unit cell which is the basic chemical repeat unit in the three dimensional symmetry pattern. The relative positions of the contributing ions in the unit cell are shown in Fig. 4. It is evident from the figure that the whole volume of the unit cell is closely packed with the constituent ions. However, their relative positions are represented wide apart to one another for the purpose of clarity. The ions present on the edges are shared by four unit cells, one on each other. Taking this as a consideration out of ten calcium ions, six are located within the unit cell while four are present on four different edges. Thus six calcium ions exclusively belong to a particular unit cell while four others are shared mutually with other unit cells. Similarly, two phosphate ions are located within and four on four edges. Through similar considerations it can be shown that each unit cell of hydroxylapatite contains two hydroxyl groups.
A cross section of the apatite lattice parallel to the 'c' axis is provided in Fig. 5. The details of the crystal structure of apatites were studied independently by Naray - Szabo$^{50}$ and Mehmel$^{51}$ and their conclusions were subsequently confirmed by Hendricks et al.$^{11,52}$. The structure proposed by them was later modified by Beevers and Mc Intyre$^{53}$ that the calcium atoms in hydroxylapatite are linked to one another through oxygen atoms forming chains which run parallel to the c - axis. Each calcium atom along with the three fold axis is surrounded by 9 oxygen atoms, six out of which are nearer to it. The Ca-O-Ca chains are joined together by phosphorus atoms constituting a cavity which runs parallel to the c - axis. Every phosphorus atom is surrounded by four oxygen atoms forming a tetrahedron.
Fig. 4. The unit cell perspective of hydroxylapatite
Fig. 5. A cross section of the apatite lattice parallel to the c – axis.
The existence of cavities in the crystal structure accounts for the porosity of apatites. Key, et al. proved through neutron and X-ray diffractions that the hydroxyl groups are located in these cavities.

The refinement of crystals structure was suggested by Posner et al. based on the three dimensional X-ray diffraction studies on single crystals of synthetic hydroxylapatite. Fig. 6 illustrates an X-ray diffraction pattern of a sample of powdered enamel compared with a pattern of powdered synthetic hydroxylapatite. In the figure, the abscissa is the diffraction angle and the ordinate is the relative intensity of the diffracted beam. The bond length and the atomic position were more accurately determined. It was further shown that the phosphate tetrahedra have P-O distances shorter than those reported earlier. In addition, there are calcium atoms situated around the hexagonal screw axis which are shown to be co-ordinated to the hydroxyl ions and six oxygen atoms of the phosphate tetrahedra. It was also shown that the calcium atoms among themselves constituted triangles one over the other in the direction of the c-axis.

Attempts to determine the orientation of hydroxyl groups in the crystal structure of
hydroxylapatite were made by Kay et al.\textsuperscript{54} by neutron diffraction studies. They concluded that the hydroxyl groups occur in columns parallel to the c-axis and these columns pass through the centres of the calcium triangles.
Fig. 6. X-ray diffraction powdered patterns of human enamel and synthetic hydroxylapatite (alternatively known as hydroxyapatite).
Fig. 7 X-ray diffraction patterns taken with copper K$_\alpha$ radiation.

1. The top two patterns illustrate the difference between ACP (upper pattern) and well-crystallized HA (with peaks indexed).

2. The two lower patterns are from 100% crystalline synthetic HA with small crystal size comparable to bone apatite and from powdered human femur diphysis (bottom pattern).
The arrangement of the constituent atoms of the hydroxylapatite as projected upon the basal plane of its structure is shown in Fig. 8. The position parameters for the constituent atoms of hydroxylapatite lattice are listed in Table-2.
TABLE 2. Listing of position parameters for the constituent atoms of hydroxylapatite.

<table>
<thead>
<tr>
<th>Atom</th>
<th>No. of atoms per unit cell</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
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</thead>
<tbody>
<tr>
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<td>0.667</td>
<td>0.001</td>
</tr>
<tr>
<td>Ca_2</td>
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<td>0.993</td>
<td>0.250</td>
</tr>
<tr>
<td>P</td>
<td>6</td>
<td>0.400</td>
<td>0.369</td>
<td>0.250</td>
</tr>
<tr>
<td>O_1</td>
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</tr>
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<td>O_2</td>
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<td>0.000</td>
<td>0.000</td>
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</tbody>
</table>
Fig. 8 The arrangement of the constituent atoms of hydroxylapatite as projected upon the basal plane of its structure. The number in each atom represent the c-axis parameter, perpendicular to the basal plane.
1.10 INFRARED SPECTRA

Growing interest in the physico-chemical properties of materials with the apatite structure has stimulated work on the IR absorption spectra of such compounds. The degree of splitting of certain IR absorption bands, and their breadth and shape give useful information about the internal structure and atomic ordering in such solid materials. For calcium phosphate, the most intense bands associated with vibrations of the $\text{PO}_4^{3-}$ group that one normally sees are the triply degenerate antisymmetric bending mode at 550-600 cm$^{-1}$ ($\gamma_4$) and triply degenerate antisymmetric stretching mode at 1000-1100 cm$^{-1}$ ($\gamma_3$). For ACP, the $\gamma_4$ band at 550-600 cm$^{-1}$ appears as a single broad band, while in HA, this degenerate mode is split into bands of unequal intensity by the apatite crystal field. Earlier investigators assumed that the crystal field around each $\text{PO}_4^{3-}$ group in the amorphous material is spherically symmetric, and therefore does not lift the degeneracy of the $\gamma_4$ mode. This difference in appearance of the $\gamma_4$ for ACP and crystalline calcium phosphate is the basis of the IR methods for determining the percent crystallinity in mechanical mixtures of ACP and HA whose spectra are illustrated in Fig. 9. Such information is useful concerning the structure and composition of both biological and synthetic hydroxyapatite.
Fig. 9  IR spectra (KBr disks) of 100% ACP (upper) 
ACP-HA mixture (middle) and 100% HA (lower).
IR spectroscopy is also useful in studying so called Ca-deficient HA's. In general, the low Ca/P ratios of these apatites have been explained on the basis of missing Ca ions in their crystalline networks. An IR study of Ca-deficient HA by Berry (1967) found no absorption bands due to octacalcium phosphate. For both synthetic HA, and well deproteinized and ground bone samples, the KBr pellet method gives very good IR spectra with minimal background scattering. Bone samples with considerable organic matrix yield cloudy KBr pellets that generate poor IR spectra. For such materials, the Nujol IR bands do not interfere with the experimentally observed IR absorption bands of the apatite phase.

The IR spectra of various apatites of the type

$$|M_5(RO_4)_3X| \text{ or } M_{10}(RO_4)_6 X_2$$

where $M = Ca^{2+}, Sr^{2+}, Ba^{2+}, Mg^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}$, and $X = OH^{-}$ or $F^{-}$ or $Cl^{-}$ or $Br^{-}$

and $RO_4 = PO_4^{3-}, AsO_4^{3-}, VO_4^{3-}, SO_4^{2-}, SiO_4^{4-}, CO_3^{2-}$, etc.

were recorded by Klee and Engel\textsuperscript{57}. Their investigation encompasses the internal vibrations of the phosphate groups. The dependence of wave numbers of the observed bands on the proportion of ions $M$ and $X$ was studied. Termine and Posner\textsuperscript{58} made infrared determinations of the percentage of crystallinity of calcim
apatite during precipitation. The infrared spectra of both minerological and synthetic chloroapatite, \((\text{Ca}_{10}(\text{PO}_4)_6\text{Cl}_2)\) were reported by Bhatnagar\(^{59}\), and the frequencies over the range \(4000-200\ \text{cm}^{-1}\) were assigned. The absorption bands at 635 and 3575 cm\(^{-1}\) are due to the replacement of the hydroxyl ions in hydroxylapatite by chloride ions. Similar spectral studies of chloroapatite were also undertaken by Bhatnagar\(^{40}\). The effect of anion substitution in lead apatite structure was investigated by Bhatnagar\(^{61}\) using infrared spectral techniques. The formation of homogeneous solid solutions of calcium-lead hydroxylapatite was indicated on the basis of similar studies of Chickerur and Chiranjeevirao\(^{62}\). The effect of carbonate ion substitution in the apatite lattice on the vibrations of \(\text{PO}_4^{3-}\) groups was investigated by Chickerur and Patel\(^{63}\).

1.11 ISOMORPHIC SUBSTITUTION

The isomorphic substitution theory is not new and like the absorption theory, has been extended to remarkable limits. In most cases, single specific substitutions have been considered and almost no attempt has been made to investigate the substitution of divalent cations into the inorganic crystalline phase of bone and synthetic hydroxylapatite.
Substitution of ions in the crystalline solid hydroxylapatite by similar ions in solution with no net change in the composition of the two phases, is called isoionic substitution. It is also capable of undergoing heteroionic substitution which is resulted due to substitution of an ion in the crystal by different ions of the solution without disrupting the general symmetry. Hydroxylapatite is characterised by its ability to undergo a series of both cationic and anionic substitutions. It has been shown that strontium can replace calcium in hydroxylapatite, in synthetic crystals as well as in mineral apatite in vivo. In view of the analogy between apatite and mineral tissue it is of interest to know whether cobalt, copper, zinc cadmium and mercuric can substitute in the pure apatite structure. It was shown by Klement\(^5\) that divalent metal ions with ionic radii $\sim 1.0$ Å can form apatites. The most important among such are Ca\(^{2+}\) (0.99 Å), Sr\(^{2+}\) (1.13 Å), Cu\(^{2+}\) (0.72 Å). Consequently, mutual isomorphous substitution between pairs of such ions is possible. In addition, anionic substitution leading to the replacement of OH\(^-\) by F\(^-\) or Cl\(^-\) or Br\(^-\) and (PO\(_4\))\(^3-\) by (AsO\(_4\))\(^3-\) or (VO\(_4\))\(^3-\) or (SiO\(_4\))\(^4-\) in hydroxylapatite established that the apatite structure is not restricted only to the CaO - P\(_2\)O\(_5\) - H\(_2\)O system\(^6\).
1.12 HETEROIONIC SUBSTITUTIONS

(i) $\text{OH}^- \xrightarrow{} X^- \text{ substitution}$

(where $X = F^-$ or $\text{Cl}^-$ or $\text{Br}^-$ or $I^-$)

From crystal structure considerations, the $\text{OH}^- \xrightarrow{} F^-$ exchange in hydroxylapatite is most favourable. It is shown that the closeness of the ionic radii of $F^- = 1.32 \, \text{Å}$ and $\text{OH}^- = 1.68 \, \text{Å}$ and their spherical symmetry favour the substitution. The various aspects of the substitution and the role of fluoridation occurring in dental caries, the corrosion of tooth by acidogenic bacteria have been extensively investigated by Knappwost. He suggested a convincing mechanism for the prophylactic action of fluorine. An oral dose of 1 to 5 mg of fluorine taken daily was found to accelerate the formation of apatite layers on the tooth surface and the accompanying alternation in the viscosity of saliva. Besides, the kinetics of the following isomorphous substitution reaction resulting in solid solution of hydroxylapatite and fluorapatite was extensively studied by Knappwost:

$$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + X\text{F}^- \xrightarrow{} \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{2-x}\text{F}_x + x\text{OH}^-$$
It is shown by Narasaraju\textsuperscript{69} that the solubility of such solid solutions\textsuperscript{70} diminished with the proportion of fluorapatite. Based on these considerations, fluoridation, which is the addition of inaqueous soluble fluoride to drinking water is supposed to enhance caries resistance. The optimum level of fluoridation is found to be 1 ppm. Isomorphous substitution of hydroxyl ions by fluoride ions brings about a contraction of the unit cell.\textsuperscript{70} Indications have been obtained that regular continuous solid solutions of hydroxylapatite and fluorapatites are formed.

The details of $\text{OH}^- \rightarrow \text{Cl}^-$ substitution in hydroxylapatite have not been investigated even though there remains the possibility that $\text{Cl}^-$ may be substituted within apatite structure resulting in chlorohydroxylapatite.\textsuperscript{59} Chloroapatite is isomorphous with hydroxylapatite and the recent work of Duff\textsuperscript{71} on the effect of chloride substitution into hydroxylapatite and the resulting alterations in solution chemistry adds further knowledge to $\text{OH}^- \rightarrow \text{Cl}^-$ exchange in hydroxylapatite.

Little work appears to have been carried out on bromoapatite and its solid solutions with hydroxylapatite, though the concept of the discontinuous solid
solutions exists in bromo-hydroxylapatite system. There is no mention of possibility of OH$^-$ $\rightarrow$ I$^-$ exchange in hydroxylapatite.

(ii) $\text{PO}_3^{3-} \xrightarrow{4} \text{AsO}_3^{3-} \xrightarrow{4}$

As arsenic is widely distributed in nature, poisoning from arsenic is very common.

Arsenic commonly occurs in insecticides, fungicides & herbicides. Among its compounds, those of As(III) are the most toxic.

As(III) exerts its toxic action by attacking - SH groups of an enzyme, thereby inhibiting enzyme action.

The enzymes which generate cellular energy in the citric acid cycle are adversely affected. The inhibitory action is based on inactivation of pyruvate dehydrogenase by complexation with As(III), whereby the generation of ATP (adenosine triphosphate) is prevented.

By virtue of its chemical similarity to P, As interferes with some biochemical processes involving P. This is observed in the biochemical generation of the key energy yielding substance, ATP.
Arsenic is used extensively in industry for dyeing, glass manufacture, artificial flower making, taxidermy and paper making. It is used therapeutically for a variety of purposes. Quite a large number of trivalent arsenic compounds or arsephenamines, such as salvarsan, sulpharsenol, etc. and similar pentavalent compounds such as soamin or atoxyl, stavarsal, carbarsone etc. have been synthesized for treatment of syphilis and other diseases. They are extensively used in medicine and many cases of severe arsenic poisoning have been known to occur some of which having ended fatally. They have thus introduced new problems in toxicological analysis. It is sold as a constituent of vermin-killers and fly papers. In medicine and fly papers, the arsenic usually exists in the form of sodium or potassium arsenate, since arsenious acid is soluble in alkaline solutions. Arsenic in the form of a soluble sodium arsenate is a common constituent of weed killers and cases of poisoning by weed killers have been occurred. It is an occasional ingredient of insecticide solutions used for spraying fruit trees.

Among trade processes of food manufacturer which utilize chemicals, the hydrolysis or inversion of starch to sugar by commercial sulphuric acid has a place. The acid made from iron pyrites, which is invariably contaminated with arsenic, contain the
poison, thus transmit it to finished food product. Beer
and confectionary which may contain invert sugar have
been responsible for arsenical poisoning. Baking powder
"improved" with acid calcium phosphate may derive
arsenic from the same source. The varnish used to
lacquer tin plate containers and to polish sweets has
also yielded the poison. Arsenic may reach apples too
when its solution is employed as an insecticide in
orchards.

\[
\text{(iii) } \text{PO}_4^{3-} \rightarrow \text{CO}_3^{2-} \text{ substitution}
\]

Since the presence of carbonate in human
bones is established, the possibility of its
substitution reactions of hydroxylapatite involving
carbonate ions are of importance. Extensive X-ray
studies of a mineral Francolite which is carbonate
containing fluorapatite indicates the absence of free
carbonate phase. Additional evidence in favour of this
observation is provided by Geros, et al.\(^7\). The recent
work of Termine and Posner\(^7\) indicates the possibility
of presence of carbonate in hydroxylapatite of bones as
adsorption phenomena. Hayek\(^7\) studied synthetic salts of
hydroxylapatite and proposed the possibility of
replacement of \(\text{PO}_4^{3-}\) by \(\text{CO}_3^{2-}\). The infrared spectral
studies of synthetic carbonate containing hydroxylapa-
tite by Chickerur and Patel\(^7\) reveals the presence of
carbonate as a substituent in the apatite lattice.
The localization and metabolic control of many trace elements by the skeletoon has been known for a number of years. Recently, the number of cationic radio-active emitters found to be preferentially incorporated in bone has increased and of these, strontium, because it has many characteristics and chemical properties similar to calcium and is probably metabolised and incorporated in the same fashion, has received particular attention. This study was undertaken in order to determine the manner in which strontium is incorporated in the same fashion as calcium and is probably metabolised and incorporated in the same fashion, has received particular attention. This study was undertaken in order to determine the manner in which strontium is incorporated into inorganic crystal phase of bone. In the apatite structure several isomorphous substitutions are known to occur and in some cases a continuous series of solid solutions have been demonstrated to exist.

\[ \text{Ca}^{2+} \leftrightarrow \text{Sr}^{2+} \] substitution

in hydroxylapatite is important since it explains the mechanism of incorporation of \( \beta \)-active Sr-90 of atomic debria into the human skeletal system. Knappwost and Ehret\textsuperscript{78} investigated the kinetics of this substitution
reaction using $\beta^-$-active Sr-90 as tracer. The replacement of calcium on the surface of the hydroxylapatite could be clearly demarcated from that occurring in the crystal interior. X-ray and electronmicrographic investigations and the solubility equilibria of the solid solutions of CaHA and SrHA were extensively investigated by Chickerur et al.$^{79,80}$.

$\text{(v) \hspace{1cm} Ca}^{2+} \rightarrow \text{Cu}^{2+}$ substitution.

Copper plays an important role in the bone formation. Copper deficiency is accompanied by impaired growth, weight loss, coavening & depigmentation of hair, decreased reproductive capacity & milk production. A bone disorder is caused by a copper-deficient diet. Wilsons disease (hepatolenticular degeneration) and "knee-knock" are the manifestation of abnormal copper metabolism in man and characterised by abnormally large accumulation of copper in liver, brain & bones.

A serious health hazard that threatens the modern world is environmental pollution. In recent years, environmental pollution due to some poisonous divalent cations has become a national & international problem. Several trace elements are toxic at high concentration but some are highly toxic even at lower concentration. The heavy metals like copper, lead,
mercury, cadmium etc. are toxic. The toxic chemicals are discharged by industries into air, water & soil. They get into the human food chain from environment. Once they enter our biological system they disturb the biochemical processes, leading in some cases to fatal results. They have a great affinity for sulphur and attack sulphur bonds in enzymes, thus immobilizing the latter & also incorporate into the skeletal system. The incorporation of Sr$^{2+}$ (1.13 Å), Cu$^{2+}$ (0.72 Å) and AsO$_4^{3-}$ (2.48 Å) into human skeletal system is not thoroughly investigated as is evident from the literature and hence the present work has been undertaken in order to determine the manner in which divalent cations are incorporated into the bone mineral.

1.13 ISOIONIC SUBSTITUTION

Isoionic substitution of calcium and phosphate were studied in detail both the synthetic hydroxylapatite and with human bones and teeth$^{85,86}$, such studies are helpful in understanding the phenomena of resorption, a process by which portions of bone during growth are dissolved and returned to the bloodstream. These investigations are carried out by tracer technique and radio autography, a process in which a slice of the labelled tissue is placed in contact with a photographic film which functions as radiation detector.
1.14 SOLUBILITY

The solubility of bone mineral merits extensive discussion because of its important physiological implications. Certainly, the blood levels of calcium, phosphate, bicarbonate, sodium and other ions must be determined to some extent by the study of the bone mineral. Also, historically, the calcification process in bone formation has almost universally been assumed to involve an orderly precipitation of bone mineral within a high organised organic matrix. This assumption has provided the basis for most theories of calcification including the classical phosphates theory of Robinson. For precipitation to occur, it is necessary that some solubility product to exceeded and it has been the search for this \( K_{sp} \) that has led many investigators to study the solubility behaviour of bone and basic calcium phosphate. In addition, considerations of the occurrence of dental caries and the protective action of fluorine are based on the information about the solubility of hydroxylapatite. Such solubility studies have additional utility in soil chemistry to account for the action of phosphate containing fertilizers. Consequently, solubility phenomena of hydroxylapatite have been investigated in details and possible explanations of the mechanism of calcification was suggested through the results of solubility.
In spite of the fact that extensive investigations in this field have been carried out, no coherence in the resulting data could be observed, this can be attributed among other things to the divergence in the experimental conditions adopted for the purpose. For accurate determination of the solubility of hydroxylapatite which amounts to a few milligrams per litre at ordinary temperature, it should not be overlooked that it is colloiddally dispersed in its aqueous solution. Thus the colloidal part is to be separated before an apatite solution is analysed.

Lenvinskas and Neuman\textsuperscript{87} studied the solubility of the synthetic hydroxylapatite with a 0.165M. Solution of sodium chloride as solvent to simulate the biological conditions and to have a constant ionic environment. Aqueous NaCl (0.165 M) should be taken as a standard solvent or reference in which all activity coefficients are assigned a value of unity, thus postponing the difficult and sometimes impossible problem of extrapolating the data to pure water as the solvent of reference. It was reported by them for a given set of experimental conditions.

(i) Calcium to phosphorus ratio in the solution was different from that of the solute and
(ii) no reproducible value for the solubility product of hydroxylapatite could be determined.

Thus, hydroxylapatite seemed to depart from the established laws of solubility. Rootara et al. explained these observations on the basis of some physico chemical principles. It was shown that hydroxylapatite being a salt of weak acid undergoes hydrolysis in aqueous solution yielding a solid state surface complex, having the formula $\text{Ca}_2(\text{HPO}_4)(\text{OH})_2$, which was found to determine the solubility equilibria according to the following equation:

$$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 6 \text{H}_2\text{O} =$$

$$4[\text{Ca}_2(\text{HPO}_4)(\text{OH})_2] + 2\text{Ca}^{2+}$$

$$+ \text{HPO}_4^{2-} \quad \ldots \quad (1)$$

$$4[\text{Ca}_2(\text{HPO}_4)(\text{OH})_2] = 8 \text{Ca}^{2+}$$

$$+ 4\text{HPO}_4^{2-} + 8 \text{OH}^- \quad \ldots \quad (2)$$

It is evident that when the equation (1) predominates, the g.atom ratio of calcium to phosphorus in the solution is unity while the predominance of equation (2) makes the ratio equal to two. When both the reactions (1) and (2) contribute equally to the solution process, this ratio becomes $10/6$ which is the value expected for the stoichiometric dissolution of hydroxylapatite. The non-reproducibility of solubility product of hydroxylapatite as reported by Levenskas and
in the accurate determination of individual ion activity coefficients for the system employed. It was supported by La Mer\textsuperscript{89} that by virtue of its biological importance an aqueous 0.165 M NaCl which is the solvent employed by Neuman, be considered as standard solvent of reference in which all ion activity coefficients can be assumed to be unity. Neuman's data when recalculated on the basis of the foregoing consideration indicated that the solubility of hydroxylapatite responded precisely to the principles of the solubility product. The solubility of synthetic apatite can be changed by the substitution of certain ions in the crystal interior. The isomorphous substitution of fluoride for hydroxyl in the apatite structure has been noted to reduce the solubility of apatite\textsuperscript{89}. The solubility of calcium-deficient hydroxylapatite in weak lactic acid was shown to be greater than the solubility of a calcium treated "deficient" hydroxylapatite. It is quite possible that mineral tissue crystals are deficient in calcium and that their solubilities will change with the filling of the calcium deficiencies through treatment or even aging.