

Review

Some physico-chemical aspects of hydroxylapatite

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A few significant physico-chemical aspects of hydroxylapatite, a biological material of extensive contemporary inter-disciplinary research, have been reviewed. Updated information on preparation techniques, characterization, isomorphous substitutions, and solubility equilibria is provided. Recent research trends and aspects which require further clarification have been emphasized.

1. Introduction

Hydroxylapatite (HA), $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, a double salt of tricalcium phosphate and calcium hydroxide, and an isomorph of naturally occurring fluorapatite, $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$, is a member of a large family of isomorphous substances. It is the principal inorganic constituent of human bones and teeth and consequently has become a topic of extensive biological and physico-chemical investigations.

While several reviews [1–37] are available dealing with different facets of investigations of HA, none of them appears to deal collectively with all the principal aspects. Being an area of intensive interdisciplinary research activity, the study of HA, has experienced rapid strides during the recent years. The present attempt is therefore intended to overcome the lacuna by providing a comprehensive review, updating information on principal aspects of HA, such as its formation conditions, preparation techniques of synthetic samples, characterization, heteroionic and isoionic substitutions leading to the formation of its solid solutions with a few of its isomorphs, its role in the incorporation of toxic ions in human skeletal and dental systems, and finally, biologically the most significant aspect, its solubility equilibria.

2. Biological significance

2.1. Hydroxylapatite and its isomorphs

It is a well-known fact that human bones and teeth [38] contain calcium and phosphorus. In addition, these elements occur in nature as fluorapatite, $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$, which is a member of an isomorphous series of minerals named [39] as “Apatites”, (“Deceivers” in Greek). The name is justified because of the difficulties involved in their identification due to their non-stoichiometric existence [40]. The names of some of the important members of this isomorphous series [41–50], together with their molecular formulae and lattice constants, are given in Table I.

Each member of the series can undergo a series of cationic and anionic isomorphous substitutions leading to the formation of the corresponding isomorphs or their solid solutions, depending upon the extent of substitution. It is evident that 100% substitution leads to the formation of an isomorph, while a partial replacement results in a solid solution [51].

The principal constituent of the inorganic part of human and animal bone and tooth, a bio-inorganic microcrystalline material [2], is shown to be predominantly HA [38], the latter thereby meriting a status as a subject of extensive physico-chemical and biological investigations. Despite significant structural and compositional differences between this biomaterial and the synthetic sample, the term HA is used in this presentation synonymously for both, for purposes of brevity. A knowledge of its location and role played in bone and tooth is a prerequisite for undertaking a meaningful series of investigations on it.

2.2. Presence in calcified tissues

Calcified tissues of the body can be divided into two groups known as mesodermal and ectodermal. While bone, dentine and cementum come under the first group, tooth enamel falls under the second category. The two groups differ in biological and chemical activity in addition to their compositional and ultrastructural disparities.

Bone is an important tissue of the body. Its biological and structural significance is extensive. Biologically, it acts as a reservoir of calcium, in addition to providing a seat for production of blood cells. Structurally, bone provides the architecture of the human body. It is HA which plays an important role in both biological and structural aspects of bone. The components of bone are classified as organic and inorganic. The organic constituents amounting to about 30% by weight, are made up of collagen, a cement substance and a cellular component. Collagen is a complex interwoven network of a fibrous protein and constitutes an

TABLE I Names, molecular formulae and lattice constants of a few principal members of the apatite series

No.	Name	Molecular formula	Lattice constants (nm)	
			a	c
1	Barium hydroxylapatite	Ba ₁₀ (PO ₄) ₆ (OH) ₂	1.019	0.770
2	Cadmium arsenic-chlorapatite	Cd ₁₀ (AsO ₄) ₆ Cl ₂	1.007	0.726
3	Cadmium hydroxylapatite	Cd ₁₀ (PO ₄) ₆ (OH) ₂	0.901	0.661
4	Cadmium chlorapatite	Cd ₁₀ (PO ₄) ₆ Cl ₂	0.962	0.649
5	Calcium hydroxylapatite	Ca ₁₀ (PO ₄) ₆ (OH) ₂	0.942	0.688
6	Carbonate apatite ^a	Ca ₁₀ (PO ₄) ₆ CO ₃	0.956	0.687
7	Chlorapatite	Ca ₁₀ (PO ₄) ₆ Cl ₂	0.963	0.678
8	Fermorite	Ca ₁₀ (AsO ₄) ₆ F ₂	0.975	0.692
9	Fluorapatite	Ca ₁₀ (PO ₄) ₆ F ₂	0.935	0.658
10	Hydroxylvanadinite	Ca ₁₀ (VO ₄) ₆ (OH) ₂	0.982	0.698
11	Lead hydroxylapatite	Pb ₁₀ (PO ₄) ₆ (OH) ₂	0.990	0.729
12	Magnesium apatite	Mg ₁₀ (PO ₄) ₆ (OH) ₂	0.930	0.689
13	Mimetite ^b	Pb ₁₀ (AsO ₄) ₆ Cl ₂	1.036	0.752
14	Pyromorphite ^b	Pb ₁₀ (PO ₄) ₆ (Cl) ₂	0.995	0.732
15	Strontium hydroxylapatite	Sr ₁₀ (PO ₄) ₆ (OH) ₂	0.976	0.728
16	Vanadinite	Pb ₁₀ (PO ₄) ₆ Cl ₂	1.047	0.743

^a Ambiguity exists regarding the molecular formula.

^b Also reported as members of the pyromorphite series.

extracellular matrix of bone, while the cement substance consists of a few hexoses linked with the protein. The cellular component, in turn, consists of cells designated as osteoblasts and osteocytes which are concerned, respectively, with dissolution, deposition and nourishment of bone. The inorganic part of bone consists of an amorphous and a crystalline phase, the former being tricalcium phosphate while the latter is HA. The amorphous phase [52] is predominant in younger bones and is partially transformed into a crystalline phase with age. About 40% by weight of an adult human bone was found to be HA. Mineral phase in bone is deposited within the collagen fibres as minute needles or platelets of dimensions 10–60 nm in length and 2–6 nm in width, such that their long axes line up with the fibres [53–55]. In addition, bone contains about 20% of water by weight, present mostly in the organic matrix and in traces in HA crystals.

Tooth consists of an outer part known as the crown, a neck which is surrounded by gum and one or more roots or fangs fitting into the sockets of the jaw-bones. The principal dental tissues are enamel, dentine and cementum. The crown is covered by enamel which rests on dentine and the latter occupies the bulk portion of tooth. The cementum helps in fixation of the tooth in the socket of the jaw-bones. As in the case of bone, the dental tissues are made up of inorganic and organic constituents. HA admixed principally with ions, such as magnesium and carbonate [38], was shown to be the inorganic constituent. The weight per cents of it in enamel, dentine and cementum amount to about 95, 75 and 35, respectively. Unlike other calcified tissues, which are in equilibrium with the internal fluids, enamel equilibrates with saliva and is thus a seat of action for locally administered prophylactic agents of dental caries [56, 57], a tooth decay caused by acidogenic bacteria. The enamel is constituted by crystals of HA of dimensions relatively bigger than those present in bones, their average length, width, and thickness reported [56, 58] as being

60, 100 and 35 nm, respectively. It is generally agreed that this tissue mainly consists of prisms or rods made up of dense clusters of crystals of HA. Bone, being a reservoir for body minerals, is chemically and biologically more active than enamel which is intended to protect the inner layers of a tooth.

3. Preparative techniques based on conditions of formation

3.1. The phase diagram of CaO–P₂O₅–H₂O system – conditions of formation of calcium phosphates

The precipitation of calcium phosphates is of considerable biological significance because the hard tissues of vertebrates contain them as the mineral constituents [59]. Among such phosphates those which are sparingly soluble and relatively more stable in an aqueous system deserve mention in the present context. A specific calcium phosphate phase is preferred in a particular tissue depending upon the prevalent conditions such as temperature, pH and chemical reactivity of the phase.

Studies on (i) inorganic constituents of human bones [60], (ii) utilization of phosphatic minerals by the plant kingdom [13], and (iii) geological aspects [61] of several naturally occurring phosphates, contributed to our present knowledge of calcium phosphates. Preliminary studies [43, 62, 63] intended to establish the conditions of existence of calcium phosphates were restricted to the solid-phase reactions of the binary system, CaO–P₂O₅. It was not, however, recognized that an imperceptible inclusion of traces of water elevates this binary system to a ternary one, CaO–P₂O₅–H₂O. Earlier investigations on this ternary system were restricted exclusively to chemical analysis [64], the results being unreliable due to the lack of proof of the homogeneity of the phases. The amorphous nature of the constituent phases accentuated the complications involved in their identification. These

considerations necessitated the application of a phase rule to the system and exposed the limitations of the exclusive use of chemical analysis for this purpose. It is well known that important prerequisites for the validity of the phase rule are the attainment of equilibrium and the existence of homogeneous phases separated by sharp boundaries. Fulfilment of these conditions in the $\text{CaO-P}_2\text{O}_5\text{-H}_2\text{O}$ system was found to be complicated owing to the slow attainment of equilibrium [65,66] and the amorphous nature of the products formed.

It is evident that a criterion for attainment of equilibrium in a precipitation reaction is the availability of identical results both from the sides of super-saturation and under-saturation. In addition, solid phases are characterized by a high degree of reactivity due to their large surface area. Further, they exist as colloidal suspensions causing additional complication in their filtration. The preliminary experimental data on the conditions for the existence of calcium phosphates are found to be repetitive, disorganized and contradictory, because the factors mentioned above were not given proper consideration. Cameron [67] and Bassett [68] independently carried out investigations on the applicability of the phase rule to the $\text{CaO-P}_2\text{O}_5\text{-H}_2\text{O}$ system and the results obtained at 25°C are incorporated in a phase diagram [69] given in Fig. 1. The abscissa and the ordinate of a given point on the phase diagram represent the weight per cent of phosphorus pentoxide and calcium oxide, respectively, the rest being water. The possible solid phases of the system are:

- (i) anhydrous monocalcium-phosphate, $\text{Ca}(\text{H}_2\text{PO}_4)_2$;
- (ii) monocalciumphosphate monohydrate, $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$;
- (iii) anhydrous dicalciumphosphate, CaHPO_4 ;
- (iv) dicalcium phosphate dihydrate, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$;
- (v) a crystalline precipitate of a variable composition with an X-ray diffraction pattern similar to that of an apatite.

All these phases could be identified through their distinct X-ray diffraction patterns supplemented by their chemical analyses. The blank regions of the phase diagram represent the solution phase while the solid phases are indicated by areas marked by convergent lines. Anhydrous monocalciumphosphate and its monohydrate exist as well-defined crystals in acidic regions of the phase diagram which correspond, evidently, to the compositional ranges having a high proportion [70] of phosphorus pentoxide. They can thus be obtained by cooling solutions of such compositions. The acidic region is also found to be favourable for the formation of dicalcium phosphate [70]. The aqueous solutions are heated to an optimum temperature for the precipitation of dicalcium phosphate as it follows a retrograde solubility. Dicalcium phosphate dihydrate exists over a limited range of experimental conditions, making its isolation difficult. Because a crystalline precipitate of variable composition exhibiting the X-ray diffraction pattern of naturally occurring apatites constitutes one of the phases

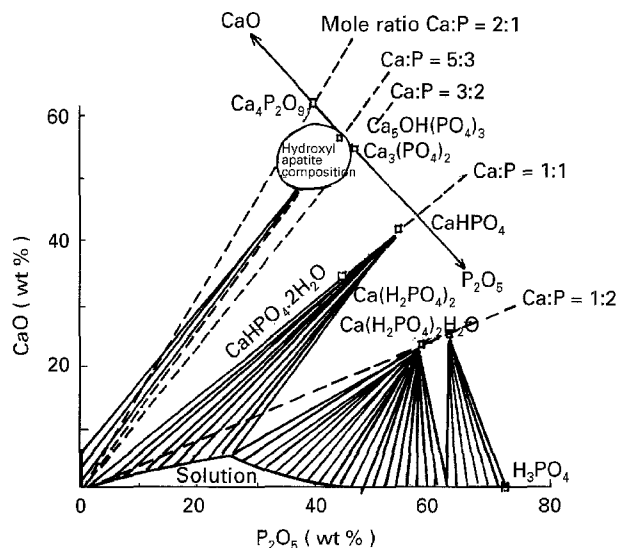


Figure 1 Phase diagram of the $\text{CaO-P}_2\text{O}_5\text{-H}_2\text{O}$ system at 25°C (after [69]).

of the diagram, the region of its existence is indicated by an elliptical area. This phase is given by the formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, and represented in Fig. 1 as $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ neglecting its variable composition and water content.

Most of the investigations done on calcium phosphates deal with HA phase, which is characterized by a variable composition and constitutes calcium phosphates having a gram atom ratio [59], Ca/P , ranging from $3/2$ to $4/2$; the limiting ratios correspond, respectively, to tricalcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$, and tetracalcium phosphate, $\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaO}$, as shown in the phase diagram. It is evident from the diagram that in the alkaline region which is characterized by a higher proportion of calcium oxide, the only solid phase capable of existence is that of HA. The phases stable in the acidic region become transformed into HA for compositional ranges indicated by an upward arrow in the diagram.

Some of these conclusions could be confirmed by Seuter [71] on the basis of studies on this phase diagram at a temperature higher than 800°C . The existence of an additional solid phase, called octacalcium phosphate, $\text{Ca}_8(\text{PO}_4)_6(\text{OH})_2$, in the neutral region of the ternary system, was suggested by Hayek *et al.* [72]. This could be demarcated from HA through its X-ray diffraction pattern and electron micrograph. However, it has not been indicated in the phase diagram as a separate phase. Its formation was substantiated by Chaikina and Nikol'skaya [73] on the basis of studies on this phase diagram at 25°C .

In addition to what has been mentioned in the present context involving the $\text{CaO-P}_2\text{O}_5\text{-H}_2\text{O}$ phase diagram, it is considered appropriate here to mention a few more phase diagrams, such as those of (i) $\text{Ca}(\text{OH})_2\text{-H}_2\text{O-H}_3\text{PO}_4$ [58, 19, 74] (ii) $\text{CaCO}_3\text{-phosphates of calcium}$ [75, 76], and (iii) $\text{Na}_3\text{PO}_4\text{-CaCl}_2$ [75, 77]. It is evident that in these cases also, as in the case of the $\text{CaO-P}_2\text{O}_5\text{-H}_2\text{O}$ phase diagram, the phases preferentially formed are dependent, among others, on the activities of calcium and phosphate ions and the initial pH.

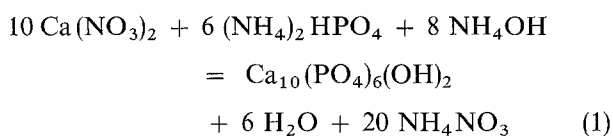
3.2. Preparative techniques

It was shown in the phase diagram of the CaO–P₂O₅–H₂O system that for a wide range of compositions, the formation of HA is favourable, as substantiated by X-ray diffraction patterns which are similar to those of naturally occurring apatites. Because such a phase is shown to be stable over a wide area in the phase diagram, the corresponding gram atom ratio, Ca/P, was found to be in the range, 1.5–2.0. These results facilitate the establishment of experimental parameters optimum for the formation of HA. It is evident [78] that HA is the stablest of the different calcium phosphates in weakly acidic, neutral and basic media. Consequently, hydrolysis of other calcium phosphates leads to its formation. Bassett [65] reported that precipitation of HA occurs from solutions of calcium oxide and phosphorus pentoxide of appropriate concentrations, which is in accordance with the suggestion offered by the phase diagram. Lorah *et al.* [79] investigated the time-dependence of the gram atom ratio, Ca/P, of the compounds monocalcium phosphate, Ca(H₂PO₄)₂, dicalcium phosphate, CaHPO₄, calcium pyrophosphate, Ca₂P₂O₇, and tricalcium phosphate, Ca₃(PO₄)₂, when treated with sodium hydroxide solution. They found that the gram atom ratio attained constancy, equal to that of naturally occurring apatite, within about 50 h, substantiating the statement that hydrolysis of calcium phosphate leads ultimately to the formation of HA. Schleede *et al.* [63] obtained similar results by refluxing tricalcium phosphate with a dilute solution of potassium hydroxide for about 7 h.

Isolation of HA from bones and teeth was found to be complicated, consequent upon changes sustained by the samples due to the techniques adopted. Synthetic samples, which can be prepared with a high degree of purity, are better-suited for the purposes of physico-chemical investigations. A survey of different methods of the formation of HA indicates that very few of them are suited for its preparation. Synthesis of apatites can be brought about by wet, dry and hydrothermal methods.

3.2.1. Wet methods

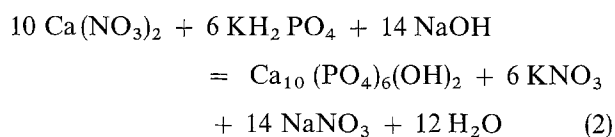
Among the available methods [35, 80–101] those based on precipitation from aqueous solutions are the most suited for preparation of appreciable quantities of apatites. A method suggested by Hayek and Stadlman [80] is widely used for the purpose, because of the simplicity of experimental operations, the accompanying high yields and purity of the samples. The method is based on the following equation



1600 ml of a solution containing 79 g diammonium hydrogen phosphate, maintained at a pH greater than 12 by addition of ammonium hydroxide, were dropped under constant stirring into 1200 ml of a solution containing 230 g calcium nitrate, Ca(NO₃)₂·4H₂O,

also maintained likewise at the same pH. The amounts of the reactants taken were intended to give a yield of about 100 g of the sample on the basis of the above equation. Based on the dissociation constants [81–83] of phosphoric acid, it can be shown that only the orthophosphate ions are predominant at the pH maintained, and thus the likely complications due to coprecipitation of acid phosphates can be avoided. The accompanying volatile ammonium salts were sublimed off by heating the filtered product to about 250 °C. Introducing minor alterations, O'Shea *et al.* [84] made use of this method to prepare samples of HA required for X-ray diffraction and spectral studies. With appropriate modifications, this method was extended to the preparation of a few more isomorphs of HA [85], the principal modifications being complexing of metal ions with appropriate ligands [86]. This method was subsequently modified by Collin [87] and Narasaraaju *et al.* [85], the most important among the modifications being the replacement of ammonium hydroxide by ethylene diamine.

Biji *et al.* [88] applied a modification of the method of Hayek and Stadlmann [80] for preparation of samples of solid solutions of calcium and barium hydroxylapatites spread over a limited compositional range extending up to 25% substitution of Ca²⁺ by Ba²⁺. They adopted solutions of the acetates of these metals and stoichiometric amounts of disodium hydrogen phosphate, the temperature of precipitation being 100 °C. No additional reagents were added to maintain the alkalinity of the precipitation medium. This method was extended for the preparation of a continuous series of solid solutions of cadmium hydroxylapatite and HA [88]. Mention should be made of another wet method proposed by Rathje [78, 89] known as "acidimetric precipitation", which is based on the following equation:



Convenient volumes of solutions of calcium nitrate and potassium dihydrogen phosphate containing the reactants in the proportions suggested by the above equation, were simultaneously dropped into boiling water. The medium was maintained pink to phenolphthalein by the addition of a solution of sodium hydroxide. By a judicious selection of appropriate dilutions and their rates of mixing, crystals upto about 50 μm in length could be obtained.

In another wet method, HA of a high order of purity could be obtained by Arnold [90] involving a simultaneous addition of ammoniacal solutions of calcium acetate and of ammonium phosphate to about 10 l of a mechanically stirred ammonium acetate solution, also maintained alkaline. These methods, which involve the precipitation of HA at high dilutions, have low yields and hence are unsuitable for a rapid preparation of appreciable quantities of the samples. Kani *et al.* [91] prepared samples of HA, similar to human dental enamel in crystallinity and gram atom ratio, Ca/P, through appropriate modification of the above

methods. Adopting a judiciously modified method of Hayek and Stadlmann [80], Narasaraju *et al.* [92] obtained HA and calcium arsenate apatite and a series of their solid solutions over the entire compositional range, the method being successful for the preparation of a similar series of compounds involving phosphate and vanadate apatites of lead [93] and arsenate apatites of barium [94]. During recent years [95, 96], several investigators adopted the wet methods mentioned above with minor alterations to prepare samples of HA required for specific purposes. An interesting recent wet method suggested by Meyer *et al.* [97] was based on the use of a reaction between solid calcium sulphate and highly alkaline solutions of sodium phosphate. This method was extended by Gupta *et al.* [98] for the preparation of calcium vanadate apatite and of its solid solutions with HA.

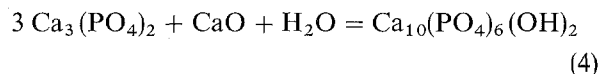
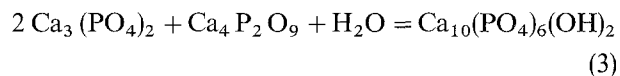
Samples of HA were obtained by Iino-Shinji *et al.* [99] by refluxing suspensions of calcium hydroxide with either mono-, di- or triphosphate of calcium, while the use of calcium carbonate in place of calcium hydroxide leads to the formation of carbonate apatite of calcium. The suspension medium consisted of mixtures of water and pentane, the constant temperature chosen being in the range, 30–200 °C. The homogeneity of the sample obtained was confirmed by X-ray diffraction. The method offered a simplified procedure for the preparation of HA and seems to be capable of being extended to the preparation of other apatites as well as their solid solutions. The manufacture of carbonate apatite was achieved by Kadoma [100] by a wet method involving hydrolysis of a mixture of dicalcium phosphate, its dihydrate and enough calcium carbonate to make the gram atom ratio of the mixture range from 1.5–1.67.

In order to bring about a wet method based on a direct interaction of Ca^{2+} with PO_4^{3-} , an aqueous solution of calcium hydroxide of appropriate concentration was mixed by Clark [102] with an aqueous solution of orthophosphoric acid containing a stoichiometric amount needed for the formation of HA. The resulting suspension was kept in contact with the mother liquor for increasing time intervals, following the accompanying changes in pH of the medium. Well-defined crystals of HA were obtained at pH values higher than 7.8 during increased reaction periods at high temperatures of the precipitation medium. Such a sample of HA was used by Clark [102] to establish its stoichiometric dissolution and to contradict the then prevalent concept of non-stoichiometric dissolution of HA as mentioned later in this presentation. A similar wet preparative method was suggested by Schleede *et al.* [63].

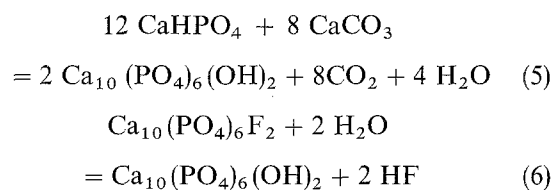
3.2.2. Dry methods

It is an established fact that an intimate heterogeneous mixture of appropriate solid ingredients when heated to an optimum temperature can lead to the formation of a desired lattice through solid state diffusion of the constituent ions. Trömel [103, 104] investigated the optimum conditions for the formation of HA through a solid-state reaction between tri- and tetracalcium

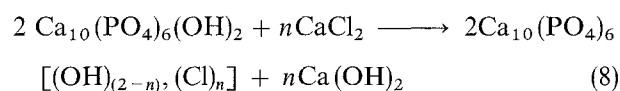
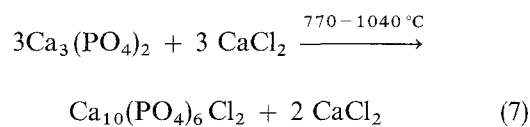
phosphates or alternately of tricalcium phosphate and calcium oxide. Solid mixtures of these ingredients in appropriate composition with a gram atom ratio, Ca/P, equal to 5/3, gave HA on heating for a few hours at 1050 °C in a current of moist air, as represented by the following equations



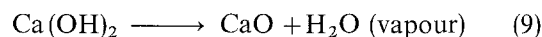
Narasaraju *et al.* [105] confirmed the utility of this method for preparing samples of HA of a high order of purity as investigated through X-ray, infrared, electron microscopic and chemical analyses. In addition, fusion of a sample of dicalcium phosphate, CaHPO_4 , and calcium carbonate or, alternatively, heating of a sample of finely divided naturally occurring fluorapatite [106] at about 1400 °C under a stream of moist air, resulted in the formation of HA



An extension of the above method to prepare chlorapatite and a series of its solid solutions with HA was reported by Rai *et al.* [107] to be possible, as shown below



where n ranges from 0–2



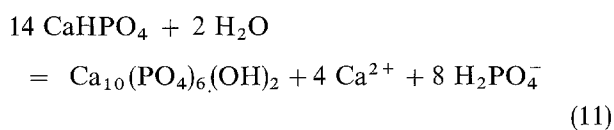
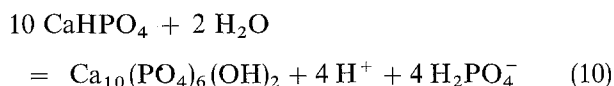
This method was extended by Narasaraju *et al.* [108] for preparation of arsenic chlorapatite, $\text{Ca}_{10}(\text{AsO}_4)_6\text{Cl}_2$, and its solid solutions with calcium arsenate apatite, $\text{Ca}_{10}(\text{AsO}_4)_6(\text{OH})_2$, using the latter and CaCl_2 as the starting materials.

A similar attempt to prepare solid solutions of HA of calcium and barium extending over compositional ranges of 60%–100% replacement of calcium by barium, was successfully carried out by Biji *et al.* [51], the temperature chosen for the solid-state reaction between appropriate intimate mixtures of the end-members being 1200 °C.

3.2.3. Hydrothermal methods

Hydrothermal methods [109], as the name implies, deal with the application of high temperatures to aqueous solutions to facilitate the precipitation of crystals of dimensions larger than those attainable using ordinary wet methods. Because the aqueous

precipitating medium at atmospheric pressure has its boiling point as the upper limiting temperature, heating under high pressure enables this limit to be exceeded. In an autoclave, the desired high pressure is produced by the vapour of the solvent of the precipitating medium, because the system is subjected to a high temperature in a sealed enclosure. The principal advantage of such methods has been to enhance considerably the crystallinity and purity of the product. Such sets of results were achieved by Hayek *et al.* [110] and Perloff and Posner [111] as substantiated by the formation of homogeneous crystals of HA, about 0.1 mm long in the form of hexagonal prisms. For this purpose, 2 g precipitated HA were heated at 380 °C in an autoclave for 24 h with 15 ml 2 M sodium hydroxide solution. The X-ray diffraction pattern of the sample was characterized by sharp peaks, as expected from its crystal dimensions. Young and Sudarshan [112] adopted a similar method to prepare a sample of strontium hydroxylapatite for crystallographic studies. Perloff and Posner [111] obtained HA by hydrolysis of dicalcium phosphate and the probable reactions involved are as follows



This process was brought about by heating 0.1 g dicalcium phosphate with 10 ml water at 300 °C for 10 days in a platinum-lined hydrothermal bomb and the product was found to be in the form of prismatic crystals. Another suitable method for preparation of single crystals of HA for purposes of X-ray and neutron diffractions and thermogravimetric investigations was suggested by Elliot and Young [113]. A crystal sphere of diameter 0.1 mm of synthetic chlorapatite when heated electrically on a piece of a platinum foil at about 1300 °C under a flow of steam at atmospheric pressure for 2 weeks, formed a single crystal of HA of a high order of purity. A further extension of hydrothermal methods for the preparation of HA was brought about independently by Akoi [114], Kazov *et al.* [115] and Yoshimura *et al.* [116].

4. Characterization

4.1. Chemical analysis

Quantitative separation of calcium and phosphate, which is a prerequisite for accurate chemical analysis of HA, requires special analytical procedures, the details of which have been worked out by Washburn and Shear [117]. Gravimetric [117, 118], complexometric [119] and spectrophotometric [120] techniques are usually employed for the accompanying determinations of these separated ions. Application of atomic absorption spectroscopy to determine the metal-ion concentration in apatites without a quantitative separation, was achieved for the first time by Hivo *et al.* [121]. These techniques [51, 88, 122–127] can be ex-

tended to quantitative analysis of other systems involving the isomorphs of HA as well as their solid solutions.

4.2. Structural aspects

The basic structural features [128–143] of apatite lattice were first worked out independently by de Jong [129], Mehmel [130], and Naray-Szabo [131]. de Jong showed for the first time, with the then relatively new technique of X-ray diffraction, that the mineral in bone bore a close resemblance to naturally occurring HA. In this pioneering X-ray diffraction study, de Jong also observed that the apatite crystals in bone were extremely minute and ill-defined. However, a detailed spatial arrangement of the constituent ions in the apatite structure was not firmly established. It was only after 25 years that Posner *et al.* [132] could arrive at these structural aspects from X-ray diffraction studies on synthetically prepared single crystals of HA, the findings being substantiated by Kay *et al.* [133] through neutron diffraction. These studies reveal the most striking feature of HA structure, namely, the hexagonal arrangement of Ca^{2+} and PO_4^{3-} about columns of monovalent OH^- . That animal bones and teeth contain HA [134] as an ingredient was proved by identification of X-ray diffraction patterns of the former with those of naturally occurring HA, as shown in Fig. 2.

HA, both of biological and synthetic origin, crystallizes in hexagonal $\text{P6}_3/m$ space group with lattice constants a and c equal to 0.942 and 0.688 nm, respectively. Details of the crystal structure of apatites were studied independently by Naray-Szabo [131] and Mehmel [130], and their conclusions were subsequently confirmed by Hendricks *et al.* [46]. The structure proposed by them was later modified by Beevers and McIntyre [135] and a few more refinements were suggested by Posner *et al.* [132]. Sudarshan [45] studied the structure of cadmium apatite and proved it to be iso-structural with naturally occurring fluorapatite. The lattice of HA is constituted

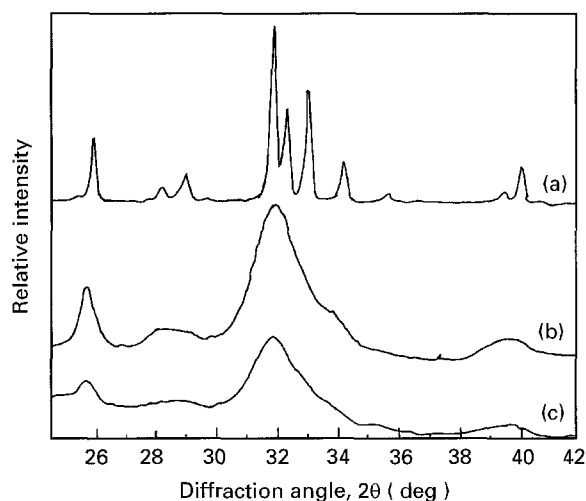


Figure 2. X-ray diffraction patterns of hydroxylapatite: (a) synthetic crystalline sample, (b) synthetic amorphous sample and (c) sample obtained from bone (after [134]).

by the ions, Ca^{2+} , PO_4^{3-} and OH^- , and their arrangement [52] in the unit cell is indicated in Fig. 3. The ions are distributed in two phases one over the other, such that each half is a mirror image of the other.

Out of a total of 14 calcium ions, six are located within the unit cell and thus belong to it entirely and the remaining eight peripheral ions are shared by adjacent unit cells, such that there is an allocation of four to each. Similarly, it can be shown that out of ten PO_4^{3-} groups, two situated inside and eight at the periphery, only six belong to each unit cell. These are made up of two which are situated inside and a total of four out of the eight peripheral ions. Likewise, two out of the eight OH^- groups represented in the figure belong to the unit cell. The number of ions present per unit cell of HA can thus be correlated with its molecular formula, accounting thereby for the basic chemical repeat unit in a three-dimensional symmetry pattern. Fig. 4 represents a cross-section of fluorapatite parallel to the c -axis and provides a further clarification [46] of the relative lattice positions of the atoms. It can be shown from the figure that O-Ca-O chains running parallel to the c -axis are joined together by phosphorus atoms constituting the inner lining of an elongated cavity accommodating F^- . It is evident that, depending upon ionic size and charge, other ions can replace F^- . In addition, every phosphorus atom is surrounded by four oxygen atoms forming a tetrahedron. The existence of cavities in the crystal structure accounts for the porosity and the consequent surface

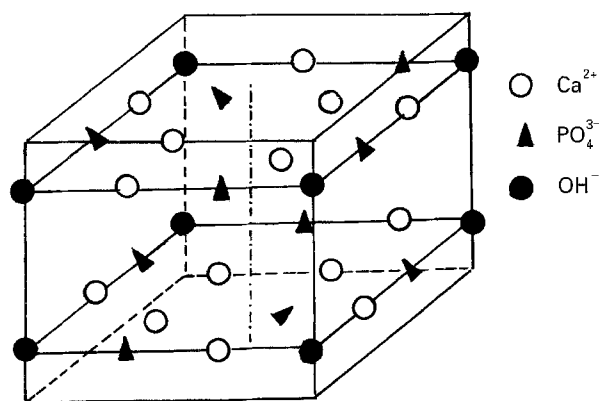


Figure 3 The unit cell perspective of hydroxylapatite (after [52]).

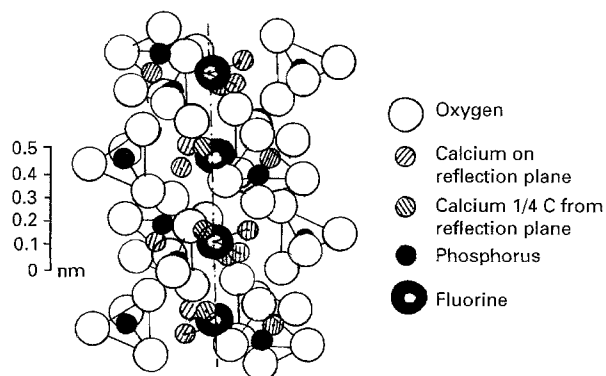


Figure 4 A cross-section of the apatite lattice parallel to the c -axis (after [46]).

activity of apatites. X-ray line-broadening experiments [55] using low-angle scattering revealed the apatites to be thin tabular hexagons elongated in the direction of the c -axis. A refinement of crystal structure of HA was suggested by Posner *et al.* [132] based on three-dimensional X-ray diffraction studies on single crystals. Bond lengths and atomic positions were determined more accurately than those already available. It was further shown that the PO_4^{3-} tetrahedra have P-O distances shorter than those reported earlier. In addition, calcium atoms situated around the hexagonal screw axis are shown to be coordinated to OH^- ions and oxygen atoms of the PO_4^{3-} tetrahedra. It was further shown that the calcium atoms among themselves constituted triangles, one over the other in the direction of the c -axis. Based on these refinements, the arrangement of the constituent atoms of HA is projected upon the basal plane of its structure, as shown in Fig. 5 [138].

Attempts to determine the orientation of the OH^- group in the crystal structure of HA were made by Kay *et al.* [133] through neutron and X-ray diffraction studies. They concluded that the OH^- groups occur in columns parallel to the c -axis and that these columns pass through the centres of the calcium triangles. The x , y and z coordinates of the constituents of the HA lattice are given in Table II. Depending upon their relative positions in the unit cell of HA, the

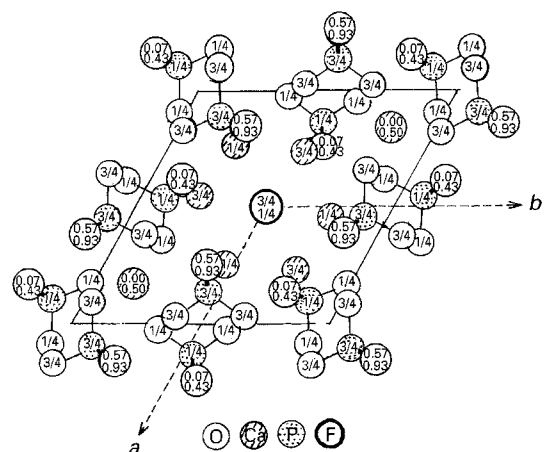


Figure 5 Arrangement of the constituent atoms of hydroxylapatite as projected upon the basal plane of its structure (the number in each circle represents the c -axis parameter perpendicular to the basal plane) (after [132]).

TABLE II Listing of position parameters for the constituent atoms of HA

Atom	No of atoms per unit cell	Position parameters		
		x	y	z
Ca_i	4	0.333	0.667	0.001
Ca_{ii}	6	0.246	0.993	0.250
P	6	0.400	0.369	0.250
O_i	6	0.329	0.484	0.250
O_{ii}	6	0.589	0.466	0.250
O_{iii}	12	0.348	0.259	0.073
OH	2	0.000	0.000	0.250

calcium and oxygen atoms are designated through the subscripts to their symbols.

A clarification of structure of HA with respect to incorporation of F^- in place of OH^- , was provided by Young [18] based on X-ray and neutron diffractions supplemented by spectroscopic studies. He could establish the existence of two prototype apatite structures, namely hexagonal and monoclinic. The hexagonal structure with space group $P6_3/m$ was the only one recommended for apatites until recently. It can now be shown that this structure is valid only for fluorapatite. It is stabilized in chlorapatite and HA, owing to their crystal imperfections. The hexagonal unit cell of fluorapatite contains two formula units. The structure can be explained as a column of F^- in the hexad axis parallel to the crystallographic c -axis surrounded at appropriate Ca–F distances by two oppositely directed triangles of Ca_{ii} atoms perpendicular to the axis and separated by a distance equal to $c/2$. Principal differences in the structures of the three occur in the vicinity of the hexad axis, which is a column of X, where $X = F^-, Cl^-$ or OH^- . With an increase in size, a dilation occurs in Ca_{ii} triangles which causes a transformation of hexagonal to monoclinic form. The monoclinic structure is first found in chlorapatite when it is free from crystal imperfections. This transformation is caused by a distortion produced by Cl^- in place of F^- in the hexagonal pattern. When a single crystal of chlorapatite is converted to HA at a high temperature, the monoclinic structure is retained by the latter. The dominant feature of monoclinic versus hexagonal structure is the ordering of X first in a column and then from column to column with glide planes replacing mirror planes. The change from hexagonal to monoclinic form is a consequence of attainment of a lower free energy. Consequently, the monoclinic form is more stable and hence less soluble. In addition, the monoclinic form leads to a tape-like morphology of its crystals instead of hexagonal prisms. Such a situation arises in tooth enamel. Synthetic chlorapatite exhibited the monoclinic form. However, if more than about 15% Cl^- is replaced by F^- , the hexagonal form returns.

Young and co-workers [136–139] showed that an expansion of O_{iii} triangles occurs when Cl^- is located in the cavity. This expansion tilts the attached phosphate groups, which creates a disorder which is propagated throughout the structure from one Cl^- to the one adjacent to it. This information is lost if Cl^- is absent in a particular position and the structure then returns to the hexagonal form. Consequently, for a substantial conversion to the monoclinic form, the population of Cl^- should be high. A particularly intriguing aspect of F^- substitution for Cl^- is such that a small amount of F^- substituting for Cl^- stabilizes the monoclinic phase by raising the phase transition temperature of the monoclinic to the hexagonal form. Incidentally, it explains the prophylactic action of F^- on tooth enamel, as mentioned later under OH^- – F^- isomorphous substitution, because the monoclinic form is less soluble. However, substitution of F^- for Cl^- exceeding about 15% has the contrary effect, and stabilizes the hexagonal phase. Because of

steric interaction between F^- and Cl^- , the latter moves in a direction less congenial for the monoclinic form. This form is found to be absent in human bone and tooth, within the permissible limits of sensitivity. When F^- substitutes for OH^- in HA, it tends to occupy sites on the hexad axis, as in fluorapatite. Such a location brings them close to the adjacent unsubstituted OH^- and consequently they become hydrogen-bonded. When a pair of OH^- ions on either side of F^- are both oriented in the same way, such that the OH^- direction is towards F^- , the monoclinic structure is exhibited. Alternately, if the orientation of a pair of OH^- ions on either side of F^- is such that OH^- directions oppose one another, the hexagonal structure is exhibited. In either case, F^- becomes hydrogen bonded to adjacent OH^- . The existence of such a hydrogen bonding is indicated by a perturbation of the O–H stretching frequency from 3572–3540 cm^{-1} [144]. An additional indication of the existence of hydrogen bonding is indicated by nuclear magnetic resonance studies which can demarcate the two situations mentioned above. Bonding on one side indicated an F–H distance equal to 0.211 nm, while bonding on both sides makes it 0.219 nm. These studies could also show that trace amounts of F^- substituting for OH^- in HA undergo dispersion in the columns without aggregating to form fluorapatite. Such a dispersion, coupled with the hydrogen bonding of F^- , can inhibit the diffusion of OH^- . These structural findings could throw light on the cariostatic and cariogenic aspects of F^- located in tooth enamel. Related aspects are mentioned later in the present publication in the context of $OH^- \rightleftharpoons F^-$ exchange on HA.

In the light of these structural considerations, it will be of interest to investigate the role of trace impurities such as F^- , Cl^- , CO_3^{2-} , and Mg^{2+} , in bringing about such a phase transformation. Recently, Elliot *et al.* [140] substantiated the findings of Young [136] by reporting that a sample, $Ca_{10}(PO_4)_6CO_3$, termed carbonate apatite, exhibits a pseudo-hexagonal symmetry with monoclinic space group $P_{2/b}$ with lattice parameters a and c being equal to 0.9557 and 0.6872 nm and b approximately equal to $2a$.

For purposes of characterization of samples of synthetic apatites, such refinements in the structure brought about by precise X-ray diffraction studies may not be of great relevance. In addition, as these structural refinements were carried out using samples obtained by dry methods, the divergence, if any, shown by precipitated samples of apatites from the hexagonal structure is not yet established.

The conclusions drawn from X-ray diffraction studies on the structural aspects of apatites can be supplemented by its electron microscopic pattern [36] which can confirm the homogeneity of the sample by proving the absence of extraneous phases. In addition, it provides information regarding the geometry and dimensions of individual crystals. A typical electron micrograph of strontium hydroxylapatite crystals given in Fig. 6, showing well-defined needle-shaped crystals, and proving the absence of extraneous phases, functions as a good illustration. Though X-ray

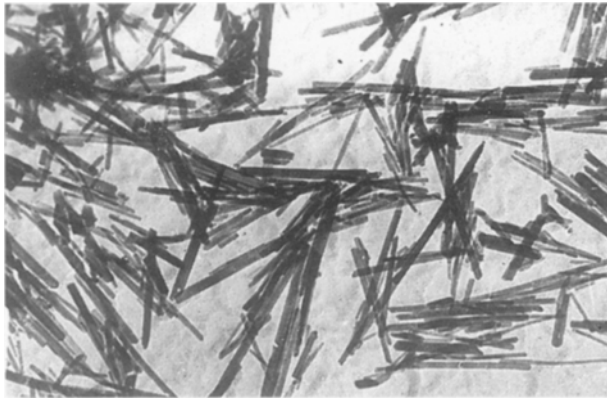


Figure 6 Electron micrograph of crystals of a synthetic sample of strontium hydroxylapatite, $\text{Sr}_{10}(\text{PO}_4)_6(\text{OH})_2$, $\times 40\,000$ (after [145]).

diffraction patterns of bone help in proving the presence of HA and determining its lattice constants, they do not permit one to see the shape of bone crystals nor to visualize directly the relationship these crystals hold to the other ingredients of bone. These aspects can be fulfilled by the electron microscopic investigations of bone, the corresponding *in vitro* studies being carried out by Hayek *et al.* [72]. Extensive *in vivo* electron microscopic studies carried out by Robinson [52] could throw light on the uniformity in size of the inorganic crystalline component and the spatial relationship existing between the collagen fibres, the cement substance and HA of bone.

5. Isomorphous substitution

An isomorphous substitution may be defined as a replacement of one ion by another in a crystal lattice without disrupting its crystal structure. Such substitutions are classified as isoionic [16] and heteroionic [146]. The former may be defined as a process by which ions from a solution phase exchange with identical ions of a solid phase in contact with it, the composition of the two phases being unaltered. In heteroionic substitution [146] an ion of a solid phase is displaced by a different ion from a solution in contact with it, thereby altering the composition of both the phases. A characteristic property of HA is its ability to undergo a series of iso- and heteroionic substitutions, involving both cations and anions, the criteria being the similarity of charge and proximity of sizes of the ions concerned. It was shown by Klement and Zureda [147] that bivalent metal ions with ionic radii of about 0.10 nm can form apatites, the most important of them being, Ca^{2+} , Ba^{2+} , Pb^{2+} and Cd^{2+} with ionic radii 0.099, 0.113, 0.135 and 0.097 nm, respectively. Among the anionic substitutions, the significant ones are replacement of OH^- by F^- [148], Cl^- [107] and I^- [149], and PO_4^{3-} by AsO_4^{3-} [92] and VO_4^{3-} [150], the ionic radii of these being 0.168, 0.132, 0.181, 0.216, 0.110, 0.118 and 0.122 nm, respectively.

In addition, a few miscellaneous substitutions are possible [151–153] involving replacement of an ion of

HA by another ion of different charge with appropriate compensation of the imbalance in charge through incorporation of an additional ion. Both iso- and heteroionic substitutions involving these ions are therefore possible. An explanation for the presence of CO_3^{2-} in bones and teeth is provided by such substitutions.

5.1. Isoionic substitutions

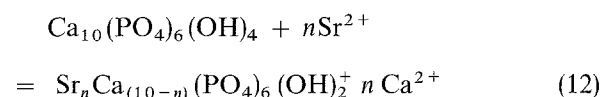
Isoionic substitutions of calcium and phosphate have been investigated extensively on synthetic HA, human bones and teeth [154, 155]. Such investigations are of significance in providing an explanation for the skeletal fixation of calcium and phosphorus and also for throwing light on the phenomenon of resorption, a process by which portions of bone during growth are dissolved and returned to the blood stream. Such substitutions were investigated by tracer techniques in which a slice of the labelled tissue is placed in contact with a photographic film which functions as a radiation detector.

5.2. Heteroionic substitutions

Based on their extensive biological significance, the following heteroionic substitutions are considered relevant and hence merit a mention in the present context: $\text{Ca}^{2+} \rightleftharpoons \text{Sr}^{2+}$, $\text{Ca}^{2+} \rightleftharpoons \text{Ba}^{2+}$, $\text{Ca}^{2+} \rightleftharpoons \text{Pb}^{2+}$, $\text{Ca}^{2+} \rightleftharpoons \text{Zn}^{2+}$, $\text{F}^- \rightleftharpoons \text{OH}^-$ and $\text{PO}_4^{3-} \rightleftharpoons \text{AsO}_4^{3-}$. The reversible sign indicates that each one of the substitutions can occur in either direction, depending upon the sample of apatite chosen as the seat for the occurrence of exchange.

5.2.1. Substitution of Ca^{2+} by Sr^{2+}

Substitutions of Ca^{2+} by Sr^{2+} on HA is one of the most important heteroionic substitutions, because it explains the mechanism of incorporation in the human skeletal system of β -active ^{90}Sr produced in atomic explosions. The substitution reaction is



solid solution of HA and
strontium hydroxylapatite

Owing to its long half-life period (about 28.5 years) ^{90}Sr can prove fatal, even when it is present in traces in the human skeletal system. Investigations on the substitution reactions are consequently supposed to be helpful in suggesting a possibility of removal of the incorporated strontium. The time-dependence of this substitution using β -active ^{89}Sr as a tracer was investigated by Ehret [156]. The substitution was achieved in solutions of strontium nitrate labelled with ^{89}Sr using synthetic samples of HA with different surface areas. Adsorption of strontium ions and their subsequent diffusion into the crystal interior, accompanied by simultaneous recrystallization of the

equilibrated HA, were shown to be the factors governing such a substitution.

Collin [157] showed, through coprecipitation, that a series of homogeneous solid solutions of HA and strontium hydroxylapatite over the entire compositional range could be formed, thereby confirming the occurrence of isomorphous substitution between these ions. These results were subsequently confirmed by Narasaraju and co-workers [145, 158]. Through X-ray diffraction studies, Khudolozhkin *et al.* [159] concluded that replacement of calcium by strontium or barium on the apatite lattice occurs preferentially on Ca_{ii} sites, in comparison with those of Ca_i . A substantiation of these results [160–162] was provided using infrared spectroscopy and thermoanalytical studies. Based on *in vivo* studies on isomorphous substitution on apatite crystals, Baud and Lee [163] showed that dimensions of the unit cell were dependent on the chemical composition: an alteration in the former offers evidence for the occurrence of substitution.

As mentioned earlier, the replacement of Ca^{2+} by Sr^{2+} on HA of human bones is of extensive biological importance. Commensurate with its contemporary significance, extensive work on this substitution has been reported [164–171] recently which deserves a brief mention in the present context. Roushdy *et al.* [164] investigated the levels of retention of ^{85}Sr and ^{89}Sr in rat femur. It was observed that the retention levels were inversely proportional to the amount of calcium in the diet. In addition, vitamin-D-deficient diet caused a decreased retention level of radio strontium. Dehos [165, 166] proved, through analysis of teeth of children of different age groups, that the uptake of ^{90}Sr was higher in the 15–25 year age group than that for the 25–55 age group. Based on the investigations on the eye lens of a rabbit, [167] it was found that the presence of Sr^{2+} reduced the subsequent replacement of Na^+ by K^+ . In addition, they found evidence to show that the Sr^{2+} present interacted with the cell surface of the lens.

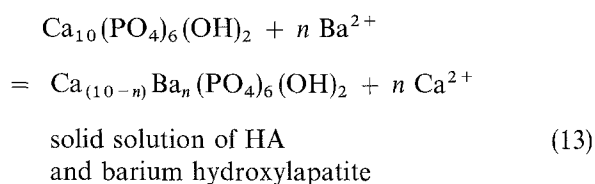
The uptake and turnover of ^{90}Sr in the human skeleton were investigated by Papworth and Vennart [168], who estimated the fraction of dietary intake of ^{90}Sr which reaches the skeleton, as well as its turnover. The age-dependence of these parameters was also investigated. It was suggested by Papworth and Vennart that these results could be used to predict future levels of ^{90}Sr in human bones from measurements of dietary levels of the radionuclide. Park [169] investigated the incorporation of ^{90}Sr in beagles, using the technique of radionuclide ingestion. The results could lead to equations for the retention and distribution of stabilized ^{90}Sr in skeletal components. Bang *et al.* [170] investigated the skeletal distribution of stable Sr^{2+} and its incorporation in bone mineral *in vivo*. They observed that bone mineralization in mice decreased with a strontium-high diet intake. In addition, they observed exchange of strontium for calcium in bone mineral, as substantiated by X-ray and infrared studies. Marie *et al.* [171] studied the dependence of bone metabolism in rats on oral doses of strontium. The serum and bone levels

of strontium were found to be proportional to the intake of the element.

5.2.2. Substitution of Ca^{2+} by Ba^{2+}

Because of the toxic effects of elemental barium and its soluble salts, the replacement of Ca^{2+} by Ba^{2+} on HA is another heteroionic substitution [105] of importance.

Analogous to strontium, β -active ^{140}Ba (half-life, 12.8 days), a product of atomic explosions, also becomes incorporated in the human skeletal system based on the following equation



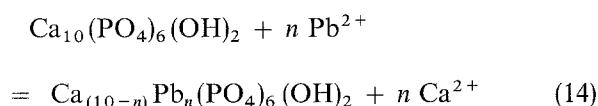
The relatively smaller half-life period of ^{140}Ba makes the radiation damage caused by it less toxic than that by ^{90}Sr . Unlike strontium, the non-active barium is also toxic and, consequently, studies on such a substitution are of importance, to explore the possibility of the elimination of the incorporated barium.

Detailed aspects of $Ca^{2+} \rightleftharpoons Ba^{2+}$ substitutions were investigated by Narasaraju *et al.* [105]. Adopting a thermal method, samples of HA of calcium and barium and a series of their solid solutions were prepared over the entire compositional range. The characterization of the sample was achieved by chemical, infrared, X-ray diffraction and electron microscopic analyses. It was shown through tracer techniques by Samachson *et al.* [172] that there is a preferential uptake of ^{133}Ba over ^{47}Ca and ^{85}Sr by human bones. In addition, Samachson and Schmidt [173] showed that traces of Zn^{2+} , when present in the equilibrating solution, increase the uptake of ^{85}Sr and ^{133}Ba . These results were explained on the basis of a supposition that Zn^{2+} distorts the surface of HA, thereby facilitating a replacement of Ca^{2+} by larger alkaline earth ions. The replacement of calcium by barium in the HA lattice by solid-state reactions at different temperatures, and also by precipitation from an aqueous system, have been investigated by X-ray diffraction and infrared absorption analyses. The products obtained by solid-state reactions at $1200^\circ C$ are solid solutions over a range of barium concentrations, 60–100 at %.

5.2.3. Substitution of Ca^{2+} by Pb^{2+}

“Lead poisoning” [174], also known as “plumbism”, is caused by inhalation of lead in the form of dust, or its absorption through the skin, the mechanism involved being attributed to isomorphous substitution of Ca^{2+} by Pb^{2+} on bone, leading to the formation of solid solutions [175] of HA and lead hydroxylapatite. Such a formation was shown by Müller [47] to be possible over the entire compositional range through coprecipitation in aqueous media, as substantiated by Narasaraju *et al.* [85] and also by Rao and Chickerur

[176]. The chemical equation involved is



solid solution of HA
and lead hydroxylapatite

It was shown recently that lead poisoning occurs through a coating of Mexican pottery, the damage being termed "Problems of Lead in Mexican Pottery" [177].

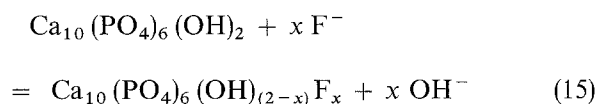
5.2.4. Substitution of Ca^{2+} by Zn^{2+}

Detailed investigations on the replacement of Ca^{2+} by Zn^{2+} on HA of bones were carried out by Samachson *et al.* [178–180]. Detailed radio-chemical studies on this exchange using solutions labelled with ^{65}Zn could prove the occurrence of 100% uptake of Zn^{2+} by HA within 5 min. Chelating agents, such as EDTA, were found to be considerably effective in hindering this process. An estimation of the amount of extra calcium which entered the solution during equilibration of HA with Zn^{2+} consequent upon an ion-to-ion replacement of Ca^{2+} , confirmed the occurrence of such an exchange. As in the case of other exchanges, this process was also found to be rapid initially, the subsequent process, controlled by diffusion of Zn^{2+} into the crystal lattice, being slow.

5.2.5. Substitution of OH^- by F^-

Neutron and X-ray diffraction studies carried out by Kay *et al.* [133] could confirm the earlier findings that OH^- groups are located in the cavities running parallel to the *c*-axis of the apatite lattice. It can be shown that, among others, ions of the type F^- , OH^- and Cl^- with ionic radii 0.132, 0.168 and 0.181 nm, respectively, fit into these cavities. In addition, the spherical symmetry of F^- makes it better suited for the purpose than OH^- . A replacement of OH^- by F^- , therefore, causes a contraction of the unit cell of HA [41,45,181]. The $\text{OH}^- \rightleftharpoons \text{F}^-$ exchange is of significance in explaining the occurrence of dental caries. It involves an attack of the inorganic phase of enamel by acidogenic bacteria existing in the vicinity of the enamel surface. Knappwost [56, 57, 182, 183] and co-workers, investigated the various aspects of this substitution and the role of F^- as a prophylactic in the occurrence of dental caries. He proved that tooth surface enriched with F^- was more resistant to caries. He suggested a mechanism for the caries-prophylactic action of fluorine. Deposition of fluorapatite layers on tooth surface resulting, *inter alia*, in alteration in the viscosity of saliva, was found to be caused by an oral daily dose of 1–5 mg fluorine. Fluorapatite being less soluble than HA, corrosion by acidogenic bacteria on tooth surface can be retarded by such a deposition, which was found to be preferentially formed on the affected regions of tooth surface. The substitution can be represented as

follows



solid solution of HA and
fluorapatite

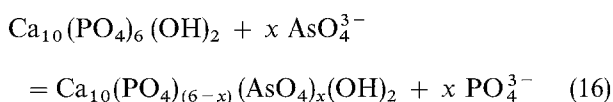
The product of this substitution is a solid solution of HA and fluorapatite, known as fluorhydroxylapatite. Calcium fluoride, formed simultaneously during the progress of this substitution, was found to interfere with the exchange reactions as shown by Liang and Higuchi [184], Stearns and Berndt [185] and Higuchi *et al.* [186]. A measure suggested for prevention of dental caries has been the application of appropriate amounts of F^- in some suitable form to the dental tissue. The process, known as fluoridation, has been in vogue for a few decades: 1 p.p.m. fluorine in drinking water was found to be an optimum level for caries control. A level higher than this was found to lead to mottled enamel and also to certain toxic effects.

Several mechanisms are suggested to explain the prophylactic action of fluorine in the occurrence of dental caries, the most important of which is based on an exchange of OH^- of HA of tooth by F^- , resulting in the formation of solid solutions of HA and fluorapatite. Narasaraju [148] showed that such solid solutions were less soluble than HA, thereby accounting for the caries-resistance imparted to the dental tissues by fluoridation. Results of recent investigation [187–192] on $\text{F}^- \rightleftharpoons \text{OH}^-$ exchange could substantiate these findings. The principal conditions favourable for the occurrence of $\text{F}^- \rightleftharpoons \text{OH}^-$ exchange on HA were shown by Narasaraju *et al.* [193] to be (i) an increase in concentration of F^- , (ii) a decrease in the pH of the exchange medium, and (iii) a reduction in the grain size of the sample of HA. The location of F^- present in traces in HA and a mechanism to explain the prophylactic action of fluorine in the occurrence of dental caries, were suggested by Young *et al.* [144] through nuclear magnetic resonance (NMR) studies. Further, the role played by the intermolecular distance in the formation of hydrogen bonding between OH^- and F^- ions in the apatite lattice, was explained by van der Lugt *et al.* [194]. NMR studies on $\text{F}^- \rightleftharpoons \text{OH}^-$ exchange on HA were further carried out by Lundin and Trofimov [195] and Knubovets and Gabudo [196]. Based on laser-Raman spectral studies, O'Shea *et al.* [84] showed that a halide substitution of OH^- on apatite is possible. The physico-chemical changes undergone by HA could substantiate the prophylactic action of fluorine in the occurrence of dental caries [197–199].

5.2.6. Substitution of PO_4^{3-} by AsO_4^{3-}

The toxic effects [200–202] of arsenic and its soluble salts are attributed to the replacement of PO_4^{3-} by AsO_4^{3-} on HA, which is another example of a hetero-ionic substitution [203]. The substitution reaction can

be represented as follows



solid solution of phosphate
and arsenate apatites of calcium

The existing literature confirms the occurrence of this substitution reaction [203, 204] spread over the entire compositional range and can be utilized to throw light on its mechanism to arrive at the possibility of removal of incorporated arsenic. In addition, it was shown by Rao [205] that such a substitution is possible on lead hydroxylapatite.

Arsenate is isoelectronic with phosphate, thereby facilitating $\text{PO}_4^{3-} \rightleftharpoons \text{AsO}_4^{3-}$ ion exchange on apatite, as well as in a few enzyme-catalysed reactions [194]. Lindgren *et al.* [206, 207] proved, from autoradiographic studies conducted on mice and hamsters, that PO_4^{3-} can be replaced by AsO_4^{3-} .

5.2.7. Miscellaneous substitutions

While systematic investigations were carried out by different workers on the heteroionic substitutions mentioned above, a few scattered results [208–217] on certain aspects of some other substitutions are available. The most important of these is that of CO_3^{2-} , accounting for its presence in human bone and tooth, which is of considerable biological significance [151, 152, 218–228]. In addition, CO_3^{2-} is shown to be incorporated into samples of HA prepared by wet methods [208] and its elimination is found to be very difficult. In the light of these considerations, a mention of the salient aspects of their exchange is considered appropriate in the present context. While the mechanism explaining the presence of CO_3^{2-} in human bone and tooth has been ambiguous for a long time [151, 152], considerable clarification was provided by recent investigations.

The weight per cent of CO_2 , surface-bound to bone mineral, has been shown [209] to be about 5, contributed by the presence of either CO_3^{2-} or HCO_3^- , although the evidence available is not unambiguous. A surface-limited heteroionic exchange of PO_4^{3-} by CO_3^{2-} with an appropriate substitution to attain charge balance is supposed to account for the reversibly bound CO_2 on synthetic HA. Neuman *et al.* [209] could substantiate this substitution by equilibrating a synthetic sample of HA with a solution containing CO_3^{2-} . The uptake was found to be rapid and attained saturation instantaneously. It was found to be proportional to the concentration of HCO_3^- in the equilibrated solution. On the other hand, Romo [210] proposed that carbonate apatite was formed by a replacement of OH^- of HA by CO_3^{2-} . The proportion of CO_3^{2-} in tooth enamel, amounting to about 4 wt %, is shown to be associated with cariogenicity. Young [18] emphasized the importance of the elucidation of structural location and the role of such a major and potentially active component of tooth enamel.

A considerable insight into the mechanism of incorporation of CO_3^{2-} into the apatite lattice was provided by Bonel and Montel [211], who showed that CO_3^{2-} exists on two distinct lattice sites of HA, designated A and B. A demarcation between substitutions of CO_3^{2-} on the two sites is possible through infrared studies with principal bands of CO_3^{2-} occurring at 884, 1465 and 1534 cm^{-1} for site A and at 864, 1430 and 1534 cm^{-1} for site B [212, 216]. Site B is presumed to be one of substitution of PO_4^{3-} by CO_3^{2-} . Such a substitution is shown to bring about a contraction of the lattice parameter a at a rate of 0.0006 nm per weight per cent of CO_3^{2-} . A further substantiation of these findings was provided by optical birefringence and X-ray diffraction studies [220].

On the other hand, location of CO_3^{2-} at site A is more conclusively shown, because it can be added or removed by heating HA at 1000 °C in an appropriate atmosphere. This causes an expansion of the lattice parameter, a , at a rate of 0.026 nm per weight per cent of CO_3^{2-} . Such a substitution is attributed to replacement of two OH^- groups by CO_3^{2-} , as reported by Bonel and co-workers [211, 212]. This observation is substantiated by neutron diffraction studies on enamel and HA. The substitution, $2 \text{OH}^- \rightleftharpoons \text{CO}_3^{2-}$ could be carried out reversibly on synthetic enamel and HA.

Based on recent solid-state ^{13}C and proton nuclear magnetic resonance studies, supplemented by Fourier transform–infrared investigations on carbonate-containing phosphates of enamel and precipitated carbonate apatite, Rey and co-workers [226–228] clarified the occurrence of CO_3^{2-} substitution on sites A and B, in addition to establishing several distinct environments to CO_3^{2-} in the apatite structure.

Radiochemical studies carried out by Knappwost [153] showed that $\text{PO}_4^{3-} \rightleftharpoons \text{SiO}_4^{4-}$ substitution was possible when it was accompanied by a compensation of the surplus negative charge through introduction of a univalent metal ion such as Na^+ , into the apatite lattice. Further studies on this substitution were carried out by Azimov *et al.* [229, 230] and by Gaude *et al.* [231].

Based on the proximity of ionic radii of Na^+ and H_3O^+ (0.095 and 0.100 nm, respectively) with that of Ca^{2+} (0.099 nm), evidence for a partial replacement of Ca^{2+} by Na^+ and H_3O^+ was shown by Neuman [13]. Meyer *et al.* [232] showed that Ca^{2+} – Eu^{2+} exchange is possible on HA. Fluor-chlor and hydroxylapatite of europium as well as a few solid solutions of some of these isomorphs were also prepared by those authors. The possibility of replacement of Ca^{2+} by Mn^{2+} , the latter being capable of occupying Ca_i and Ca_{ii} lattice positions [133], was proved by Gilinskaya and Scherbakova [233]. A substantiation of these results was provided by Dubrov *et al.* [234] through EPR studies which were extended to fluor- and chlorapatite by Vinnikov and Gugel [235].

6. Solubility equilibria

During the recent past, phenomena associated with dissolution of HA have attracted considerable attention

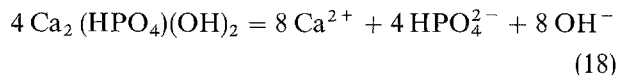
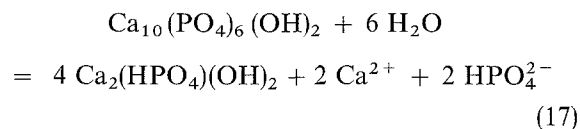
[13, 156, 236–251] because of their significance in diverse fields. Such studies are of importance to understand the physiology of bones and teeth from the point of view of calcification and resorption. In addition, consideration of the occurrence of dental caries and the prophylactic action of fluorine [57, 58] are based on the solubility of HA. Such solubility studies have an additional utility in soil chemistry to account for the mechanism of availability of phosphate-containing fertilizers to the plant kingdom. The interdisciplinary significance of solubility behaviour of HA explains its ability to command the attention of physical chemists, bone biologists, dentists, chemical engineers and geologists, to mention just a few. A survey of the literature on solubility of HA shows that the results can be classified under (i) non-stoichiometric, and (ii) stoichiometric dissolutions. The reported investigations, in general, were carried out with either synthetic or natural samples obtained from bones and teeth.

6.1. Non-stoichiometric dissolution

Extensive studies on the solubility of HA carried out independently by Levinskas and Neuman [236] and Rootare *et al.* [237] provided ample evidence to show that the sample exhibits a non-stoichiometric dissolution based on a divergence of the gram atom ratio, Ca/P, of its saturated aqueous solutions from the stoichiometric value of 1.67. The divergence was attributed to the formation of a surface coating on the solute, consequent upon its hydrolytic dissolution. While Rootare *et al.* [237] proved this surface coating to be a complex, $\text{Ca}_2(\text{HPO}_4)(\text{OH})_2$, others provided evidence that it is just one of the phases, dicalcium phosphate, CaHPO_4 [239, 240], calcium pyrophosphate, $\text{Ca}_2\text{P}_2\text{O}_7$ [241] and octa calcium phosphate, $\text{Ca}_3(\text{PO}_4)_2\text{CaHPO}_4$ [243].

Simulation of biological conditions and the maintenance of a constant ionic environment in the medium of dissolution were achieved by Levinskas and Neuman [236] through the use of a 0.165 M aqueous solution of sodium chloride as a solvent to study the solubility of HA. It was reported by them that for a given set of experimental conditions, the gram atom ratio, Ca/P, of its saturated solutions was different from that of the solute and that no reproducible values for its solubility product, K_{sp} , could be obtained. It was therefore supposed that the compound deviated from established laws of solubility. The solubility products of a number of commercial and laboratory-made samples of HA were determined by Rootare *et al.* [237]. The observed divergent values of the solubility product of HA were attributed to the dependence of solubility on solute to solvent ratio, defined by them as slurry density. Further, as shown by Levinskas and Neuman [236], the gram atom ratio, Ca/P, of the saturated solutions is not constant at the stoichiometric value of 1.67. Based on established physico-chemical principles, these investigators gave a convincing explanation to account for the results on solubility of HA. Their theoretical conclusions were subsequently substantiated by La Mer [238]. HA,

being the salt of a weak acid, undergoes hydrolysis in aqueous solutions, yielding a solid surface complex, $\text{Ca}_2(\text{HPO}_4)(\text{OH})_2$, which was found to dominate the solubility equilibria according to the following equations



Thus, it is evident that when Equation 17 predominates, the gram atom ratio, Ca/P, in the solution is unity, while it becomes 2 with the establishment of Equation 18. When both the equations contribute equally to the solution process, this ratio becomes 10/6 as expected for the stoichiometric dissolution. Complications involved in the accurate determination of individual activity coefficients of the ions involved in the systems employed, were supposed to be responsible for the non-reproducibility of K_{sp} of HA, as reported by Levinskas and Neuman [236] and substantiated later by La Mer. La Mer [238] pointed out that consequent upon its biological significance, an aqueous 0.165 M solution of sodium chloride, which is the solvent employed by Neuman, functions as a standard reference solvent in which all ion activity coefficients can be assumed to be unity. The problem of evaluating the individual ion activity coefficients involved in the solubility equilibria of HA can thus be avoided. The solubility of HA was shown by La Mer to respond precisely to the principles of solubility product on the basis of a recalculation of Neuman's data, based on the foregoing considerations. These studies were subsequently extended to natural samples of HA [239, 240].

The solubility of dental enamel with saliva as dissolution medium was investigated by Brudevold *et al.* [240]. According to them, dicalcium phosphate, CaHPO_4 , functions as a phase controlling the solubility because it gives rise to a constant K_{sp} in the pH range 4.5–7.5. In buffer solutions of pH range 3.5–6.0, the solubility of synthetic HA, dental enamel and a few more allied natural phosphatic minerals, were investigated by Francis [239]. The formation of a coating of dicalcium phosphate, CaHPO_4 , on the surface of these samples when equilibrated with acid buffers was shown through chemical analysis. Complex formation of the products of dissolution of HA with acetate, lactate and phosphate buffers, necessitated the application of a correction for the evaluation of K_{sp} of the samples. The deposition of dicalcium phosphate, CaHPO_4 , on the surface of HA was shown to be responsible for the change in composition of the solution during dissolution. Based on such considerations, the role played by parameters such as particle size, solute–solvent ratio, type and concentration of buffers and pH, in deciding the composition of a saturated solution of HA, was explained by Francis [239].

The role of a surface coating of calcium pyrophosphate, $\text{Ca}_2\text{P}_2\text{O}_7$, in controlling the solubility of HA

was investigated by Fleish *et al.* [241]. By extending the analogy to the bone processes, a mechanism was suggested by them for calcification and resorption.

Dissolution kinetics of synthetic samples of HA and strontium hydroxylapatite were investigated by Chickerur *et al.* [243] at 37 °C (biologically significant being the human body temperature), using G₄ sintered glass crucible for separating the colloidal component of the solute from the saturated solutions. A non-stoichiometric dissolution was reported. The process was found to obey first-order kinetics which could be explained on the basis of the surface-complex theory suggested by Rootare *et al.* [237]. Blitz *et al.* [243] studied the solubility equilibria of HA obtained from animal bones and teeth in order to characterize the phases likely to be formed as a result of the reactions occurring on the surface of the samples and also to study the role of such phases in governing the solubility. They concluded that octacalcium phosphate, Ca₃(PO₄)₂·CaHPO₄, formed during dissolution of HA, controlled its solubility. Bell *et al.* [74] investigated the solubility product of synthetic HA. While the value was found to be a constant within a pH range 4.6–9.7, an ionic strength range of 0.0003–1.03, and a range of solid-solution ratios of 0.06–5.6 g/200 ml, respectively, the dissolution was found to be non-stoichiometric. The formation of a surface layer on synthetic HA to control its solubility was suggested by them. However, the composition of this layer was not investigated.

6.2. Stoichiometric dissolution

The credit of proving, for the first time, that HA obeys the established laws of solubility goes to Clark [102]. He investigated such solubility equilibria over a wide range of experimental conditions, the samples used being obtained by mixing solutions of calcium hydroxide and orthophosphoric acid. He analysed the saturated solutions obtained from the mother liquor of the precipitate. In addition, the precipitate was subjected to conventional solubility equilibria to obtain the saturated solutions, which were also analysed. It was confirmed by both sets of investigations that HA exhibits stoichiometric dissolution, resulting in a definite K_{sp} at 25 °C, the pK_{sp} reported being 115.5. Using precipitated samples of HA, the solubility was investigated by Brady *et al.* [244] taking the solute in the form of a pellet mounted on an electrically operated stirrer fixed in a buffered dissolving medium containing an excess of potassium chloride to maintain a constant ionic environment. The activity coefficients of the dissolved species were found to be constant, and a stoichiometric dissolution was observed by them. Fassbender *et al.* [245, 246] could also substantiate such a stoichiometric dissolution. It was further shown by them that the observed dissolution rate was controlled by a combination of several consecutive partial reactions and was dependent on the pH and the surface area of the solute and independent of temperature in the range 15–35 °C.

A mathematical theory proposed by Zimmerman [247] on the basis of the data on the solubility of

enamel could establish the vulnerable pH range for the onset of dental caries. Based on a study of solubility isotherms of well-characterized samples of synthetic HA over a pH range 5.0–7.0, Moreno *et al.* [248] provided evidence for stoichiometric dissolution. The interfacial properties associated with solubility equilibria of HA, significant in the context of the phenomena occurring at the bone/body-fluid and tooth/saliva interfaces, were investigated by Chander and Feurstenan [249] through application of thermodynamics. They further studied the role played by the electrical double layer phenomenon in the solubility of apatites.

Wier *et al.* [250] extensively investigated the solubility behaviour of HA using commercial samples as solutes, after subjecting them to a detailed procedure of purification, consisting of either refluxing in contact with water or by auto claving. Systematic characterization of the samples was done through X-ray, petrographic and chemical analyses. The samples were equilibrated in media at pH values ranging from 4.3–6.7 at different slurry densities. The colloidal component of the solute present was removed by using specially prepared cells made of plexiglass and fitted with cellulose acetate dialysing membranes. Based on the results of chemical analyses of the saturated solutions, and calculating the activities of the ionic species present, they convincingly established stoichiometric dissolution of HA. The divergence observed in the results of solubility of HA was attributed by Wier *et al.* to the presence of traces of impurities which are eliminated on rigorous purification.

The dissolution kinetics and solubility studies of HA and chlorapatite and a series of their solid solutions, spread over the entire compositional range, were investigated by Narasaraju *et al.* [251] in buffered media extending over a pH range of 4.9–7.5 which could convincingly establish the occurrence of stoichiometric dissolution. The pK_{sp} values determined by them at 37 °C were found to be 110.6 and 115.4, respectively, for HA and chlorapatite. The calculation of pK_{sp} was done using the activity of PO₄³⁻ obtained by subdividing the measured phosphorus concentration into the different dissociation products of orthophosphoric acid, using its dissociation constants, pH of the dissolving medium, and the ionic product of water.

Valyashko *et al.* [251] investigated the influence of temperature on solubility of apatites and found that it decreased with increase in temperature, exhibiting what is known as retrograde solubility.

6.3. Miscellaneous aspects of solubility

A systematic survey of the existing literature on the solubility of apatites indicates that a major part of the results can be put under the above mentioned categories of non-stoichiometric and stoichiometric dissolutions. Over and above these aspects, a few significant scattered results [253–260] are available which merit a mention in the present context.

Bendict and Kanthak [253] found that the pH-dependence of solubility of dental enamel was similar to that of tricalcium phosphate, thereby establishing

compositional similarities between the two. Similar studies on dental enamel, coupled with those on synthetic HA, were carried out by Ericsson [254, 255] and the optimum pH range for solubility was calculated. The increased solubility of the samples in the presence of CO_3^{2-} , serum and saliva, was attributed to the formation of complexes involving Ca^{2+} . Further light was shed on the solubility of such complexes by Davies and Hoyle [256]. Studies on the pH-dependence of the solubility of HA of strontium and calcium and a series of their solid solutions distributed over the entire compositional range carried out by Narasaraju *et al.* [145] established an increased solubility with a decrease in pH. The salting-in effect of SO_4^{2-} on the solubility of synthetic HA at a series of pH values was established by Paunio and Makinen [257]. Studies on the kinetics of release of PO_4^{3-} , HPO_4^{2-} and CO_3^{2-} from powdered samples of normal human tooth enamel were carried out by Vogel *et al.* [258]. In addition, the influence of F^- on this dissolution in the acidic region was investigated by them. The solubility of HA, fluorapatite and few of their solid solutions, was investigated by Narasaraju [148] in the pH range 5.3–8.2. The solubilities calculated exclusively from the experimentally determined phosphate contents of the saturated solutions were found to decrease with an increase in pH, as well as with an increase in fluoride content of the samples. The significance of these results in the context of the prophylactic action of fluoride in the occurrence of dental caries, was emphasized.

Proof of anisotropic dissolution of a single crystal of HA was established by Jongebloed *et al.* [259] using citric and lactic acids as dissolving media. The dissolution parallel to the *c*-axis was found to be much faster than that perpendicular to it. Scanning electron microscopic patterns of the solute after attainment of saturation showed that the single crystal was twisted along the *c*-axis thereby confirming the existence of a screw dislocation parallel to it. Daculsi *et al.* [260] used high-resolution transmission electron microscopy to study the acid dissolutions of biological and synthetic apatite crystals and their dependence on crystal structure. According to them the dissolution starts at regions of dislocation and progresses preferentially across the *c*-axis. Savage [59] investigated the solubility of calcium phosphates by choosing the three-component system, $\text{Ca}(\text{OH})_2\text{-H}_2\text{O-H}_3\text{PO}_4$. The relative solubilities of different calcium phosphates of biological importance could thus be compared. He found that the solubilities increased in order of HA, β -tricalcium phosphate, octa-calcium phosphate, dicalcium phosphate dihydrate and mono-calcium phosphate. While HA was shown to be stable in media with pH values above 4.3, dicalcium phosphate dihydrate was found to be stable below this value. Savage further suggested that at a pH of 4.3, HA undergoes a conversion to dicalcium phosphate dihydrate, confirming stoichiometric dissolution.

Literature on the solubility of HA is characterized by divergence and mutual contradictions of the conclusions, as indicated above. When a critical assessment of the results is made, it is possible to shed light on the factors responsible for such disparities.

The observed divergence could be attributed to the fact that the earlier investigators ignored one or more of the following factors.

(i) An attempt to clarify the reported divergence in the gram atom ratio, Ca/P, of dicalcium phosphate solutions was made by Smith *et al.* [261] by dissolving a synthetic sample in hydrochloric acid at a pH of 4.5. The gram atom ratio was found to be higher than the stoichiometric value when 500 g solute were equilibrated in 1 l of the dissolving medium. A resuspension of the solute under identical conditions exhibited stoichiometric dissolution. Similar results were observed in the case of systems having lower slurry densities. It was established that the non-stoichiometric dissolution could be attributed to the higher proportion of surface impurities dissolved along with the solute. When the slurry density was low, the errors caused by surface impurities were less than the experimental errors associated with the microanalytical determinations of calcium and phosphorus and were not, therefore, perceptible.

(ii) Its low solubility and minute particle size enable HA to exist partially as a colloidal component in its aqueous solutions. This component should be separated before the solution is analysed for the determination of solubility [226].

(iii) Proof of the attainment of saturation by solutions of HA can be achieved only by obtaining identical values for the solubility determined from both the under-saturation and super-saturation sides [237]. The absence of such a proof leads to uncertainties.

(iv) The possibility of the existence of more than one solid phase [262] functioning as solutes in systems used for the determination of the solubility of HA is evident from the $\text{CaO-P}_2\text{O}_5\text{-H}_2\text{O}$ phase diagram.

(v) Impurities introduced by contamination [80, 263] by the adsorption of foreign ions, facilitated by the high surface area of the samples, contribute towards vitiating the solubility data.

(vi) Inaccuracies in the measurement of pH of the dissolving medium cause aberrations in the computed solubility data, because pH exerts a sensitive control over the solubility of apatites [102, 238].

(vii) Ingredients in the buffer combinations adopted for the solubility determination of HA exhibit a tendency to form soluble complexes [256] with calcium, thereby affecting the measured solubility.

(viii) HA, being the salt of a weak acid, undergoes hydrolysis in aqueous media, resulting in the precipitation of insoluble phases and thereby causing errors [237, 238] in the solubility determination.

(ix) Microdeterminations of Ca^{2+} , PO_4^{3-} and other ions when present together, as in apatite solutions, involve errors which are magnified in the computation of values of the solubility product, K_{sp} , of apatites, because of the high power to which the concentrations are to be raised.

7. Calcification

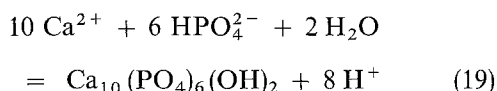
The physiology of human bone is governed by the phenomenon of calcification [264, 265] which involves an orderly precipitation of HA within the

organic matrix of bone. In view of the fact that a knowledge of solubility equilibria of HA plays an important role in understanding the mechanism of calcification [38], it is considered appropriate to mention a few aspects of it in the present context.

The results of *in vivo* and *in vitro* investigations of the solubility of HA facilitated an understanding of the mechanism of calcification. It has been established that the process occurs only at certain regions of the body known as sites of calcification. An essential prerequisite for the process to occur is the transport of assimilated calcium and phosphorus to these sites, which is brought about by body fluids. For the promotion of calcification, optimum local conditions should be prevalent at the sites of calcification. The mechanism for the establishment of these conditions was suggested by Robinson's scheme [38]. It involves a local increase in phosphate concentration brought about at the sites of calcification when a substrate of phosphate-ester is hydrolysed by an enzyme known as phosphatase, resulting in a deposition of HA. Studies on solubility phenomena of HA show that blood serum is supersaturated with respect to it, and its spontaneous precipitation is therefore expected. Because such a precipitation is found to take place only at the sites of calcification due to local action, as indicated by Robinson's scheme, it is supposed that the prevention of calcification in the non-calcifying regions is possibly due to chemical binding of calcium and/or phosphate. The theories suggested to explain the ability of only certain regions of the body to function as sites of calcification, are controversial.

It was shown [266] that about one-third of the calcium in blood is bound to a protein fraction through chelation, while the rest of it exists in the ionized form or as soluble complexes involving ions such as citrate and phosphate [256]. The phosphorus of blood serum exists principally as HPO_4^{2-} and to some extent as H_2PO_4^- and PO_4^{3-} . The composition of blood serum suggests that application of the principles of solubility product to account for the deposition of HA, is complicated. The calcium and phosphate concentrations of blood serum are higher than those corresponding to the solubility product of HA and approximate to those of saturated solutions of dicalcium phosphate. It was therefore supposed by Neuman [236] that the precipitation of dicalcium phosphate takes place initially, and HA which is the stablest of the calcium phosphates, is likely to be formed due to a subsequent hydrolysis.

Samachson [264, 265] provided a clear insight into the basic requirements of calcification. While he agrees with earlier theories as far as the role of HPO_4^{2-} as a prerequisite for calcification is concerned, he considers removal of H^+ as a factor essential for the process, as indicated by the following equation



It is evident from Equation 19 that deposition of HA is facilitated in the presence of a proton acceptor and when there is a local increase in pH at the sites

of calcification. The foregoing account shows that a plausible mechanism of calcification is possible only when a more comprehensible interpretation of the factors controlling the solubility of HA is available.

8. Calcium-deficient apatites

It is an established fact that synthetic calcium phosphates prepared by wet methods have X-ray diffraction patterns similar to HA. However, a deviation in stoichiometry was found in such samples, as indicated by fluctuations in the gram atom ratio, Ca/P, ranging from 1.33–1.65. Such compounds are defined as calcium-deficient apatites. These non-stoichiometric apatites are of biological importance, because the gram atom ratio, Ca/P, of bone is lower than the stoichiometric value of HA. They have an additional significance, because they are found to act as catalysts [267–270] in several organochemical reactions, such as dehydration and dehydrogenation of primary alcohols, leading to the formation of aldehydes and ketones, the catalytic activity of HA being proportional to the calcium deficiency [268] of the sample. Halogen-deficient cadmium haloapatite with the structure $\text{Cd}_5(\text{MO}_4)_3\text{X}$, where X represents chlorine, bromine and iodine, was studied by Wilson *et al.* [271]. Many theories [63, 272–274], the salient aspects of which have been presented here, have been proposed to explain the structure and composition of these compounds.

Cameron and Bell [272], Hodge *et al.* [273], Schleede [63] and Trömel and Moller [274] have independently advanced a theory based on the adsorption of HPO_4^{2-} to account for the non-stoichiometry of apatites. This theory was rejected by Posner and co-workers [275, 276] on the grounds that non-stoichiometry was not exclusively due to surface adsorption, because the measured surface areas of the samples were too low to account for the expected uptake of HPO_4^{2-} . The calcium deficiency was, instead, accounted for by Posner on the basis of a statistical model depicting the absence of Ca^{2+} from columnar positions of the apatite lattice, the charge balance being maintained by the introduction of two protons. Winand and Duyckaerts [277, 278] could explain the maintenance of charge balance through a compensation of two positive charges of each missing Ca^{2+} by addition of a proton and removal of a structural OH^- . Additional theories [279, 284] on non-stoichiometric apatites were advanced, among others, by Bett *et al.* [268], Brown *et al.* [282], Berry [283], Young [18], and Feenstra and De Bruyn [284].

9. Recent trends in apatite research

There have been several references available in recent literature [285–335] concerning the application of apatites in diverse fields. It is thought desirable to make a brief mention of them here in order to throw light on the contemporary trends in apatite research.

Apatites find an increasing application as catalysts [285] in various aspects of synthetic organic chemistry. On the industrial front they are being used as luminophosphors [286–291] and as starting materials

for the manufacture of several phosphatic fertilizers [292, 293].

A brief mention of clinical applications of HA is relevant in the present context, in the light of its role as a biological material of extensive contemporary interdisciplinary research. Aspects concerning skeletal and dental fluorosis merit a mention under clinical applications. While some aspects related to dental caries have been mentioned under the $F^- \rightleftharpoons OH^-$ exchange on HA, an attempt is made here to shed light on the trend in the clinical aspects concerning the incidence of these diseases. Fluorosis is a disease caused by excessive exposure to fluorine. While fluorine is one of the trace elements essential for life, it has been shown to be deleterious in large quantities. Fluoride, when given in large doses, has been shown to reduce collagen in bone [294], and has been found to lead to a pathological calcification [295]. Fluorosis is characterized by pains in the joints, stiffness of the spine, difficulty in walking, irregular bone deposition, narrowing of the spinal canal leading to a retardation of physical activity [296]. Dental fluorosis, also known as mottled enamel, is a dental aberration characterized by various degrees of discoloration of tooth [297, 298] making it brownish yellow. Studies of $F^- \rightleftharpoons OH^-$ exchange on HA of bone and tooth could shed light on the mechanism of the occurrence of these ailments, and their possible prevention and cure [299–302]. An additional clinical application of HA has been as a composite material in the form of apatite-coated ceramics for use as dental prosthesis [303–306] and as artificial bone [307–315]. Recent findings [316–319] confirming the presence of HA in kidney and prostrate stones as the principal constituent, considerably enhances the biological and physico-chemical significance of this compound. Investigations of its solubility are important in the context of a possible elimination of these stones by dissolution. In addition, the solubility of HA is significant in connection with the occurrence and cure of a few occupational diseases [320] caused by inhalation of dust containing, amongst others, toxic ingredients such as lead, cadmium, arsenic and vanadium. The mechanism of the occurrence of such pathological conditions is attributed to isomorphous substitutions on HA, as mentioned earlier.

As an ion exchanger, HA finds application as a column in chromatographic separation, its use being established in a few significant separations, such as those of mixtures of proteins [321–323]. Removal of toxic ions [324] such as F^- [193] in polluted water could be brought about by apatite columns. High surface areas of apatites, coupled with their wide-ranging ion exchanging ability, enable them to function as deodorants [325].

As the major constituents in fossil deposits, fission-track annealing of apatites finds application in assessment of geological time scales [326–329].

10. Conclusion

Extensive research activity witnessed during the recent past on various facets of HA establishes its inter-

disciplinary significance. The need to consolidate, into a single account, the available literature on apatites, scattered over several disciplines, motivated the compilation of the present review. It is hoped that such an updated comprehensive review will be helpful to those intending to investigate any particular aspect of apatites.

Acknowledgements

The authors are grateful to the University of Hamburg, Hamburg, Germany, the Banaras Hindu University, Varanasi, India, the North-Eastern Hill University, Shillong, India, and the Indian Institute of Technology, Madras, India, for providing access to the library facilities needed for the compilation of the review. One of us (TSBN) is grateful to a large group of research collaborators, especially Dr N. S. Chickerur, Dr R. P. Singh, Dr U. S. Rai, Dr V. L. N. Rao, Dr K. K. Rao, Dr B. K. Kapoor, Dr (Miss) P. Lahiri, Dr P. V. R. Rao, Dr S. K. Gupta and Dr (Mrs) Grace Skariah, for miscellaneous help in preparing the review. The authors thank Professor S. N. Bhat and Professor M. K. Chaudhury, former and present Heads, respectively, of the Department of Chemistry, North-Eastern Hill University, Shillong, India, for provision of technical assistance from the Department to facilitate the presentation of the review. One of us (DEP) thanks the authorities of Union Christian College, Shillong, India, for permission to write the review.

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Received 27 November 1994
and accepted 16 May 1995