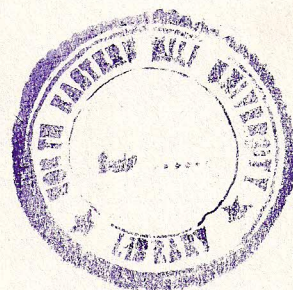


STUDIES ON
PREPARATION AND SOME PHYSICO-CHEMICAL ASPECTS
OF SOLID SOLUTIONS OF PHOSPHATE AND VANADATE
APATITES OF LEAD

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DEPARTMENT OF CHEMISTRY
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A THESIS
SUBMITTED IN FULFILMENT OF
THE REQUIREMENTS OF
THE DEGREE OF
DOCTOR OF PHILOSOPHY



To



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I certify that the thesis entitled "Studies on Preparation and some Physico-chemical Aspects of Solid Solutions of Phosphate and Vanadate Apatites of Lead" submitted by Mr. P.V. Ramana Rao for the degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by him under my supervision. He has been duly registered and the thesis presented is worthy of being considered for the Award of the Ph.D. Degree. This work has not been submitted for any Degree of any other University.

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TSBN:VTJ/-

The first part of the book is devoted to a general survey of the history of the subject. It begins with a discussion of the early work of the chemists, and then proceeds to a more detailed account of the progress of the science up to the present time. The second part of the book is devoted to a description of the various methods which have been employed for the determination of the composition of organic compounds. It begins with a description of the elementary analysis, and then proceeds to a more detailed account of the various methods which have been employed for the determination of the composition of organic compounds. The third part of the book is devoted to a description of the various methods which have been employed for the determination of the composition of organic compounds. It begins with a description of the elementary analysis, and then proceeds to a more detailed account of the various methods which have been employed for the determination of the composition of organic compounds.

P R E F A C E

The object of this book is to provide a general survey of the history of the subject, and to describe the various methods which have been employed for the determination of the composition of organic compounds. It is intended for the use of students of chemistry, and of those who are interested in the history of the science. The book is divided into three parts. The first part is devoted to a general survey of the history of the subject. The second part is devoted to a description of the various methods which have been employed for the determination of the composition of organic compounds. The third part is devoted to a description of the various methods which have been employed for the determination of the composition of organic compounds.

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PREFACE

Calcium hydroxylapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, the principal inorganic constituent of human bones and teeth, belongs to a large family of isomorphous substances known as 'Apatites'. It is capable of undergoing several cationic and anionic substitutions without disrupting the crystal structure. Such substitutions are of significance both from biological and physico-chemical points of view and constitute the basis for formation of solid solutions among these isomorphs leading to the incorporation of several toxic ions into human skeletal system. Two among such incorporations are replacement of calcium by lead and of phosphate by vanadate, both the replacing elements being toxic. The present work was therefore intended to investigate the physico-chemical aspects of these substitutions.

A prerequisite for such a series of investigations was to arrive at an optimum set of experimental conditions to prepare samples of phosphate and vanadate apatites and a series of their solid solutions spread over the entire compositional range and to characterize them through sophisticated physico-chemical techniques. In spite of the fact that phosphate and vanadate apatites of lead were prepared by earlier investigators the availability of an identical set of experimental conditions for the preparation of both these samples has been lacking. It is evident that

such a set of conditions suitable for the end-members can be extended to the preparation of their solid solutions.

The physiology of bone and tooth is governed by calcification and resorption which are respectively the dissolution and deposition of calcium hydroxylapatite at the bone / body fluid and tooth / saliva interfaces. It is evident that any attempt in the direction of removal of the incorporated toxic ions is based on a knowledge of the solubility phenomena of the samples under simulated biological conditions. While the solubility of lead phosphate apatite has earlier been investigated that of lead vanadate apatite and its solid solutions with the former was not undertaken and therefore motivated the present investigations. In spite of the fact that studies on apatites are scattered over several disciplines, a physical chemist is considered to be better suited for investigating the aspects mentioned above providing thereby an additional justification for undertaking the present studies.

The thesis has been divided into three Sections. Section 1 has been an attempt at a judicious compilation of information about calcium hydroxylapatite based on the earlier investigations carried out in diverse branches of research, the physico-chemical aspects being naturally given more emphasis. The experimental work carried out by the author has been included in Sections 2 and 3. Each

Section has been presented in the conventional pattern of a research publication. The preparation of lead phosphate apatite, lead vanadate apatite and their solid solutions along with the confirmation of their homogeneity through chemical, X-ray, electronmicrographic, infrared and thermogravimetric analyses have been included in Section 2. Section 3 is constituted by the details of investigations on the solubility equilibria of samples.

A consolidated list of books and journals consulted has been given at the end of the thesis to facilitate easy reference. References pertaining to each Section have not been separately listed to avoid duplication. For such of the references, the originals of which could not be consulted due to non-availability, the particulars about their 'Chemical Abstracts' have also been given. The journals have been abbreviated based on the pattern followed in 'Chemical Titles' published by the American Chemical Society.

I express my gratitude and indebtedness to Dr.T.S.B. Narasaraju, M.Sc., Ph.D. (Hamburg), Professor of Chemistry and Head of the Department of Chemistry, North-Eastern Hill University, Shillong, for suggesting the problem and for guidance. I am grateful to Dr. Y.S.T. Rao, Reader, Department of Physics, North-Eastern Hill University,

Shillong, for offering a constructive criticism. I am also thankful to the staff of the Department of Chemistry, North-Eastern Hill University, Shillong, for cooperation throughout the progress of this work. Thanks are also due to GSI, Shillong, in general, and to Dr. Kamal Nandi and Sri Rampratap, in particular, for the X-ray diffraction patterns. Help extended by Prof. C. Suryanarayana, Department of Metallurgical Engineering, Banaras Hindu University, Varanasi, for recording the electronmicrographs of the samples is also gratefully acknowledged. I am grateful to Sri Lakshminarayana and Sri V. Krishna Murty for preparation of the diagrams included in the thesis. I thank Sri S.K. Gupta, Lecturer, Shillong College, Shillong, for his kind cooperation throughout the progress of the work. I place on record my indebtedness to the authorities of Lady Keane Girls' College, Shillong, in general, and to the Principal of the College, Miss. A. Marbaniang, in particular, for permitting me to carry out part-time research while holding a teaching assignment in the college.

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3rd July, 1984.

LIST OF ABBREVIATIONS

CaHA : $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, Calcium Hydroxylapatite

or

Calcium Phosphate apatite

LPA : $\text{Pb}_{10}(\text{PO}_4)_6(\text{OH})_2$, Lead phosphate apatite

LVA : $\text{Pb}_{10}(\text{VO}_4)_6(\text{OH})_2$, Lead vanadate apatite

LPVA: $\text{Pb}_{10}(\text{PO}_4)_x(\text{VO}_4)_{6-x}(\text{OH})_2$ Solid solution of Lead phosphate apatite and Lead vanadate apatite.
($6 > x > 0$)

K_{ip} : Ionic product of samples in their saturated solutions.

K_{sp} : Activity solubility product.

N : Avogadro's Number (6.023×10^{23}).

$p^{K_{ip}}$: $-\log K_{ip}$

$p^{K_{sp}}$: $-\log K_{sp}$

p^{K_w} : $p^H + p^{OH}$

Slurry density : Weight of solute per unit volume of the medium of dissolution (g/ml).

V_{uc} : Unit cell volume ($\text{\AA}^3$).

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SECTION I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

1.1 Classification of Apatites

Calcium and phosphorus exist in human and animal bones and teeth¹. They also occur in nature principally as fluorapatite, $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$, which is a member of an isomorphous series of minerals named by Werner² as "Apatites" ("Deceivers" in Greek). This name is justified because of the difficulties involved in their identification due to their non-stoichiometric existence. Some of the important members of the series³⁻¹¹ and their lattice constants are given in Table 1-1.

Table 1-1

Name, molecular formula and lattice constants of a few principal members of Apatite series:

S. No.	Name	Molecular formula	Lattice constants (Å)	
			a	c
1	Barium hydroxyl-apatite	$\text{Ba}_{10}(\text{PO}_4)_6(\text{OH})_2$	10.19	7.70
2	Cadmium " "	$\text{Cd}_{10}(\text{PO}_4)_6(\text{OH})_2$	9.01	6.61
3	Calcium " "	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	9.42	6.88
4	Chlorapatite	$\text{Ca}_{10}(\text{PO}_4)_6\text{Cl}_2$	9.63	6.78
5	Fermorite	$\text{Ca}_{10}(\text{AsO}_4)_6\text{F}_2$	9.75	6.92
6	Fluorapatite	$\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$	9.37	6.88
7	Hydroxyl-vanadinite	$\text{Ca}_{10}(\text{VO}_4)_6(\text{OH})_2$	9.82	6.98
8	Lead hydroxyl-apatite	$\text{Pb}_{10}(\text{PO}_4)_6(\text{OH})_2$	9.90	7.29
9	Mimetite*	$\text{Pb}_{10}(\text{AsO}_4)_6\text{Cl}_2$	10.36	7.52
10	Pyromorphite*	$\text{Pb}_{10}(\text{PO}_4)_6\text{Cl}_2$	9.95	7.32
11	Strontium hydroxylapatite	$\text{Sr}_{10}(\text{PO}_4)_6(\text{OH})_2$	9.76	7.28
12	Vanadinite	$\text{Pb}_{10}(\text{VO}_4)_6\text{Cl}_2$	10.47	7.43
13	Cadmium Chlorapatite	$\text{Cd}_{10}(\text{PO}_4)_6\text{Cl}_2$	9.62	6.49
14	Calcium arsenic chlorapatite	$\text{Ca}_{10}(\text{AsO}_4)_6\text{Cl}_2$	10.07	7.26
15	Magnesium Apatite	$\text{Mg}_{10}(\text{PO}_4)_6(\text{OH})_2$	9.30	6.89

* Also reported as members of Pyromorphite series.

Each member of the series can undergo a series of cationic and anionic isomorphous substitutions leading to the formation of the corresponding isomorphs. A biologically important example of such a substitution is provided by the replacement of F^- by OH^- on fluorapatite resulting in the formation of hydroxylapatite, $Ca_{10}(PO_4)_6(OH)_2$. This compound is shown^{1,12} to be the principal inorganic constituent of both human and animal bones and teeth and has, therefore, been the subject of extensive physico-chemical and biological investigations. Consequently a knowledge of its location and its role played in bones and teeth described below, is a pre requisite for undertaking a meaningful series of investigations on it.

1.2 Location and Role Played in Bones and Teeth:

Bone is a dense, hard and highly specialised tissue consisting of organic and inorganic constituents. The former, amounting to about 30 per cent by weight, is made up of collagen, a cement substance and a cellular component. Collagen is a complex interwoven net-work of a fibrous protein and constitutes an extra cellular 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, osteoclasts and osteocytes

which are concerned respectively with dissolution, deposition and nourishment of bone. The inorganic part of the bone consists of an amorphous and a crystalline phase, the former being tricalcium phosphate while the latter is hydroxylapatite. The amorphous phase is predominant¹³ in younger bone and gets partially transformed into the crystalline phase with age. About 40 per cent by weight of an adult human bone was found to be hydroxylapatite the crystals of which are oriented in the collagen fibres with their long axes parallel to the fibre direction. This view has been confirmed recently by Ascenzi, A., Bonucci, E. et.al.¹⁴ through x-ray diffraction studies contrary to an earlier finding¹³ showing their presence in cement. Intimate morphological and chemical relationships exist between collagen and these crystals, the former being a nucleating agent for the precipitation of the latter.¹⁵ The low angle X-ray scattering studies by Engström and Finean¹⁶ suggested the average length and breadth of these crystals to be about 200 and 65 Å respectively. In addition to the constituting elements of hydroxylapatite, sodium, Potassium, magnesium, chloride, carbonate, fluoride and trace amounts of iron, copper, manganese and zinc are the usual inorganic ions present in the above and were shown¹⁷ to be located in the hydration layer at the bone-body fluid interface. In addition, bone contains about 20 per cent of water by weight¹³, present mostly in the organic matrix and in traces in hydroxylapatite crystals.

Bone plays a significant role in the metabolism and storage of calcium, phosphorus and other inorganic ions present in the human system. In addition, its mineral phase was shown¹³ to be the seat of action for the removal of several toxic ions such as lead, cadmium, barium and arsenic from the body fluids. The strength and rigidity of all higher forms of life can be attributed to the individual bones and their joints which together constitute the skeletal system.

Tooth consists of an outer part known as crown, a neck which is surrounded by gum and one or more roots or fangs fitting into the sockets of the Jaw-bone. The main dental tissues are enamel, dentine and cementum. The crown is coated by enamel which rests on dentine and the latter occupies the bulk portion of tooth. Cementum helps in the fixation of tooth in the socket of the jaw-bone. As in the case of bone, the dental tissues are made up of inorganic and organic constituents. Hydroxylapatite admixed principally with ions such as magnesium and carbonate was shown¹ to be the inorganic constituent. The weight per cents. of it in enamel, dentine and cementum were found to be about 95, 75 and 35 respectively. The crystals of hydroxylapatite of bone, dentine and cementum were found to be similar in size according to their morphological similarities, while those of enamel were found to be bigger and arranged at right angles to the tooth surface. Recent X-ray diffraction studies¹⁸ on powdered enamel samples

indicated that the formation of crystals of hydroxylapatite of enamel located on human deciduous teeth was less perfect than that of the permanent teeth. Unlike other calcified tissues which are in equilibrium with the internal fluids, enamel equilibrates with saliva and is thus a seat of action of locally administered prophylactic agents of dental caries,^{19,20} a tooth decay caused by acidogenic bacteria.

1.3 Stability ranges of calcium salts of ortho phosphoric acid - $\text{CaO-P}_2\text{O}_5\text{-H}_2\text{O}$ Phase Diagram

Studies on (i) inorganic constituents of human bones,²¹ (ii) utilisation of phosphatic minerals by plant kingdom²² and (iii) geological aspects²³ of several naturally occurring phosphates contributed to our present knowledge of the phosphates of calcium. Preliminary studies intended to establish²⁴⁻²⁶ the conditions of existence of phosphates of calcium were restricted to the solid phase reactions of the binary system, $\text{CaO-P}_2\text{O}_5$. It was not, however, recognised that an imperceptible inclusion of traces of water elevates this binary system to a ternary one, $\text{CaO-P}_2\text{O}_5\text{-H}_2\text{O}$. Earlier investigations on this ternary system were restricted exclusively to chemical analysis, the results being unreliable due to absence of a proof of homogeneity of the phases. The amorphous nature of the

constituent phases further complicated their identification. These considerations necessitated the application of phase rule to the system and exposed the limitations of the exclusive use of chemical analysis²⁷ for the purpose. It is well known that important prerequisites for the validity of 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 were found to be complicated due to the slow attainment of equilibrium^{28,29} and the amorphous nature of the precipitates formed.

A criterion of attainment of equilibrium in a precipitation reaction is the availability of identical results both from the sides of supersaturation and undersaturation. In addition, solid phases are characterised by a high degree of reactivity due to their large surface areas. Further, they exist as colloidal suspensions causing additional complication in their filtration. The preliminary experimental data on the conditions of existence of calcium phosphates became repetitive, disorganised and contradictory since the factors mentioned above were not given proper considerations. Cameron³⁰ and Bassett³¹ independently carried out investigations on the applicability of Phase Rule to $\text{CaO-P}_2\text{O}_5\text{-H}_2\text{O}$ system and the results obtained at 25°C are incorporated in the phase

diagram³² given in Fig.1.1 The abscissa and the ordinate of a given point on the phase diagram represent the weight per cents. 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) monocalcium phosphate monohydrate, $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$
- (iii) anhydrous dicalcium phosphate, CaHPO_4
- (iv) dicalcium phosphate dihydrate, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and
- (v) a crystalline precipitate of 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 monocalcium phosphate 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 of phosphorus pentoxide; they can thus be obtained³³ by cooling solutions of such compositions. The acidic region is also found to be favourable for the formation of dicalcium phosphate.³³ The aqueous acidic

Fig. 1.1 Phase Diagram of $\text{CaO-P}_2\text{O}_5\text{-H}_2\text{O}$
System at 25°C .

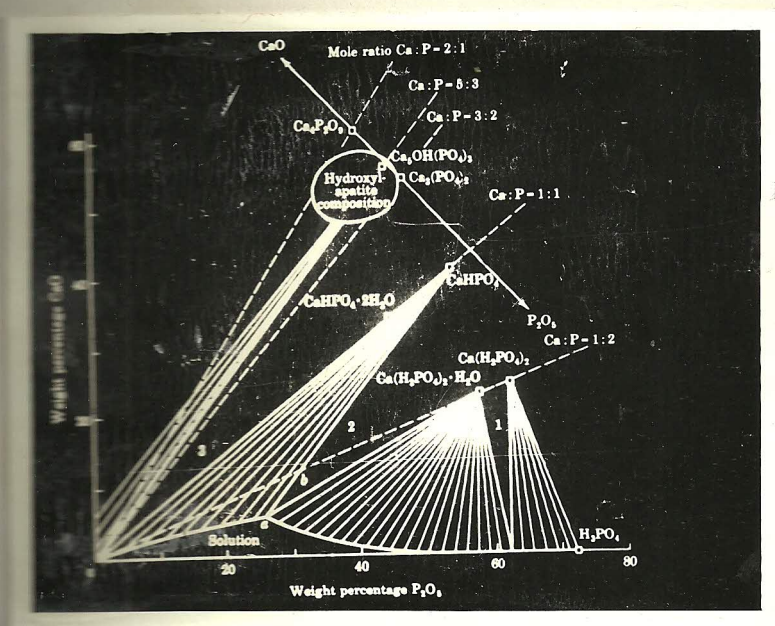


FIG. 1.1

solutions are to be 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. Since a crystalline precipitate of variable composition exhibiting the X-ray diffraction pattern of naturally occurring apatites constitutes one of the phases of the diagram the region of its existence is indicated by an elliptical area. This phase is given the formula, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, and represented in Fig. 1-1 as $\text{Ca}_5(\text{PO}_4)_3\text{OH}$, neglecting its variable composition and water content.

Most of the investigations done on the calcium phosphates deal with the hydroxylapatite phase which is characterised by a variable composition and constitutes phosphates of calcium having a g atom ratio²¹ ranging from 3/2 to 4/2; the limiting ratios correspond respectively to tricalcium phosphate (TCP), $\text{Ca}_3(\text{PO}_4)_2$, and tetracalcium phosphate, $\text{Ca}_4(\text{PO}_4)_3\text{OH}$, as shown in the phase diagram. It is evident from the diagram that in the alkaline region which is characterised by a higher proportion of calcium oxide, the only solid phase capable of existence is that of hydroxylapatite. The phases stable in the acidic region get transformed into hydroxylapatite for compositional

ranges indicated by an upward arrow in the diagram. Some of these conclusions could be confirmed by Seuter³⁴ on the basis of studies on this phase diagram at temperatures higher than 800°C.

The existence of an additional solid phase called octacalcium phosphate (OCP), $\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaHPO}_4$, in the neutral region of the ternary system was suggested by Hayek et.al.³⁵ OCP could be demarcated from hydroxylapatite through its X-ray diffraction pattern and electron micrographs. However, OCP has not been indicated in the phase diagram as a separate phase. Formation of OCP could be substantiated by Chaikina³⁶ et.al; on the basis of recent studies on this phase diagram at 25°C.

1.4 Preparative Techniques Based on Conditions of Formation

It was shown in the phase diagram of $\text{CaO-P}_2\text{O}_5\text{-H}_2\text{O}$ system, that for a wide range of compositions calcium phosphates can be shown to be hydroxylapatites based on X-ray diffraction patterns which are similar to those of naturally occurring apatites. Since such a phase is shown to be stable over a wide area in the phase diagram, the corresponding g atom ratio, Ca/P was found to range from 1.5 to 2.0. These results facilitate the establishment

of experimental parameters optimum for the formation of hydroxylapatite. It is evident³⁷ that hydroxylapatite is the stablest of the different calcium phosphates in weakly acidic, neutral and basic media. Consequently, hydrolysis of other calcium phosphates leads to the formation of hydroxylapatite.

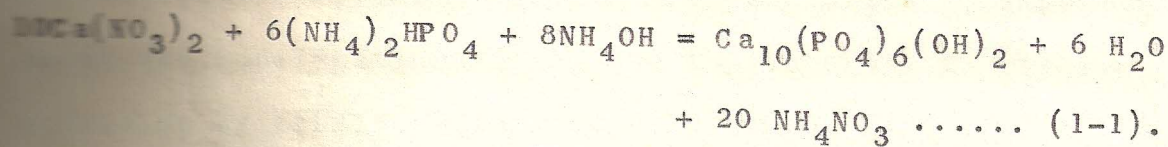
Basset²⁸ reported the precipitation of hydroxylapatite from solutions of calcium oxide and phosphorus pentoxide of appropriate concentrations which is in accordance with the suggestion offered by the phase diagram. Lorah et.al.³⁸ investigated the time-dependence of gatom ratio, Ca/P, of the compounds, calcium monophosphate, $\text{Ca}(\text{H}_2\text{PO}_4)_2$, calcium diphosphate, CaHPO_4 , calcium pyrophosphate, $\text{Ca}_2\text{P}_2\text{O}_7$, and TCP, $\text{Ca}_3(\text{PO}_4)_2$, when treated with sodium hydroxide solution. They found that the ratio attained constancy equal to that of naturally occurring apatite within about 50 hours substantiating the statement that hydrolysis of calcium phosphates leads to the formation of hydroxylapatite. Schleede et.al.²⁶ obtained similar results by refluxing TCP with a dilute solution of Potassium hydroxide for about 7 hours.

Isolation of hydroxylapatite from bones and teeth is found to be complicated, the techniques adopted reflecting a change on the sample. Synthetic samples which

can be prepared with a high degree of purity are better suited for purposes of physicochemical investigations. A survey of different methods of formation of hydroxyl-apatite indicates that very few of them are suited for its preparation. Synthesis of apatites can be brought about by wet, dry and hydrothermal methods.

1.4.1 Wet Methods

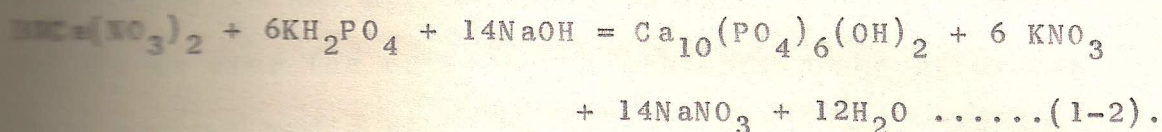
Among the available methods those based on precipitation from aqueous solutions are the most suited for preparation of appreciable quantities of apatites. A method suggested by Hayek and Stadlmann³⁹ is widely used for the purpose because of the simplicity of experimental operations, the accompanying high yield and purity of the samples. This method is based on the following equation:-



1600 ml of a solution containing 79 g of ammonium hydrogen phosphate maintained at pH greater than 12 by the addition of ammonium hydroxide were dropped under constant stirring into 1200 ml of a solution containing 230 g of calcium nitrate, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, also maintained

like-wise 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 dissociation⁴⁰⁻⁴² of phosphoric acid, it can be shown that only the orthophosphate ions are predominant at the pH maintained and thus likely complications due to co-precipitation 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.⁴³ made use of this method to prepare samples of hydroxylapatite desired for X-ray diffraction and spectral studies. With appropriate modifications, this method could be extended to the preparation of a few more isomorphs of hydroxylapatite⁴⁴, the principal modification being the complexing⁴⁵ the metal ions with appropriate ligands. This method was subsequently modified by Collin⁴⁶ and Narasaraaju⁴⁴, important among the modifications being the replacement of ammonium hydroxide by ethylene-diamine. The consumption of a large volume of ammonium hydroxide to attain a high alkalinity desired for the precipitation as well as its unpleasant smell may thus be avoided. A mention may be made of another wet method proposed by Rathje^{37,47} 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 rates of mixing of the solutions, crystals upto about 50 μ in length could be obtained.

By another wet method hydroxylapatite of a high order of purity could be obtained by Arnold⁴⁸ involving a simultaneous addition of ammoniacal solutions of calcium acetate and of ammonium phosphate to about 10 litres of a mechanically stirred ammonium acetate solution also maintained alkaline. These methods which involve the precipitation of hydroxylapatite at high dilutions have low yields and hence are unsuitable for a rapid preparation of appreciable quantities of the sample. Kani et.al.⁴⁹ could prepare samples of hydroxylapatite similar to human dental enamel in crystallinity and g atom ratio, Ca/P, through appropriate modification of the above methods.

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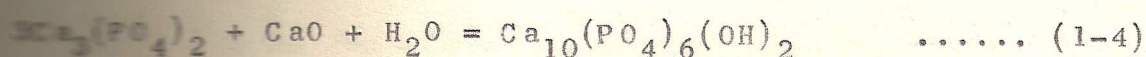
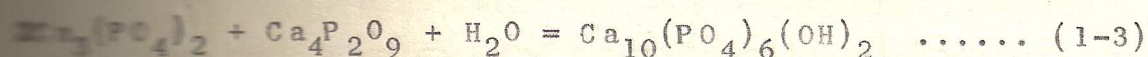


Narasaraju and Rai⁵⁰ obtained hydroxylapatites of phosphorus and arsenate and a series of their solid solutions over the entire compositional range by precipitation from aqueous media at 37°C introducing appropriate modifications of the method suggested by Hayek and Stadlemann.³⁷ During the recent past several investigators^{51,52} adopted the wet methods mentioned above with minor alterations to prepare samples of hydroxylapatites intended for specific purposes. An interesting recent **wet** method suggested by Mayer et.al.⁵³ was based on the use of a reaction between solid calcium sulphate and highly alkaline solutions of sodium phosphate. This method could be extended⁵⁴ recently for the preparation of calcium vanadateapatite and of its solid solutions with hydroxylapatite.

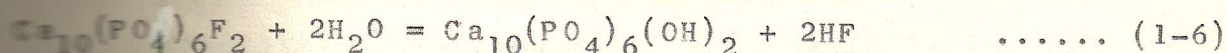
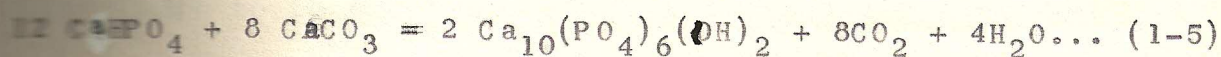
2.4.2 Dry Methods

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^{55,56} investigated the optimum conditions for the formation of hydroxylapatite through a solid state reaction between TCP and tetracalcium phosphate, $\text{Ca}_3(\text{PO}_4)_2\text{CaO}$, or calcium oxide solid mixtures of

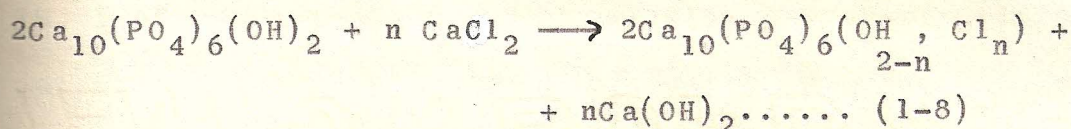
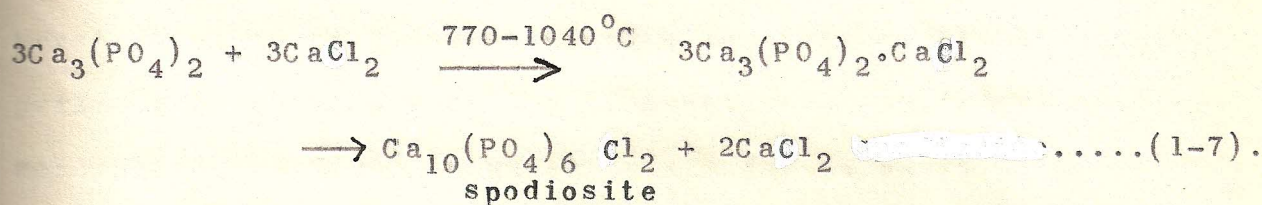
These ingredients in appropriate compositions with a given ratio, Ca/P, equal to 5/3 gave hydroxylapatite on heating for a few hours at 1050°C in a current of moist air as represented by the following equation:-



Narasaraju et.al.⁵⁷ confirmed the utility of this method for preparing samples of hydroxylapatite of a high order of purity as investigated through X-ray, i.r., 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 fluorapatite⁵⁸ at about 1400°C under a stream of moist air resulted in the formation of hydroxylapatite:-



An extension of the above methods to prepare chlorapatite and a series of its solid solutions with hydroxylapatite was brought about by Narasaraju and Rao⁵⁹ as shown below:-



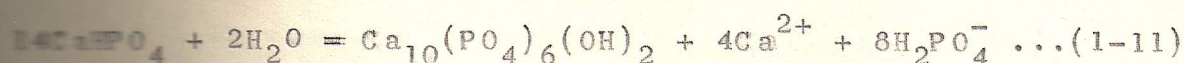
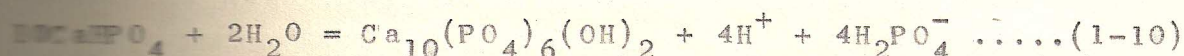
where n ranges from 0 to 2.



1.4.3 Hydrothermal Methods

Hydrothermal methods⁶⁰, as the name implies, deal with the application of high temperatures to aqueous solutions to facilitate the precipitation of crystals of dimensions bigger than those attainable using ordinary wet methods. Since an 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 since 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.⁶¹ and by Perloff and Posner⁶² as substantiated by formation of Homogeneous

crystals of about 0.1 mm in length in the form of hexagonal prisms. For this purpose 2 g of precipitated hydroxylapatite were heated at 380°C in an autoclave for 24 hours with 15 ml of 2M sodium hydroxide solution. The X-ray diffraction pattern of the sample was characterised by sharp peaks as expected from its crystal dimensions. Young and Sudarsanan⁶³ adopted a similar method to prepare a sample of strontium hydroxylapatite for crystallographic studies. Perloff and Foster⁶² obtained hydroxylapatite by the hydrolysis of dicalcium phosphate and the probable reactions involved are the following:-



This process was brought about by heating 0.1g of dicalcium phosphate with 10 ml of 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 the preparation of single crystals of hydroxylapatite for purposes of X-ray, neutron diffraction and thermodynamic investigations was suggested by Elliot and Young⁶⁴. A crystal sphere of 0.1 mm in diameter of synthetic chlorapatite when heated electrically on a piece of platinum foil at about 1300°C under steam at

atmospheric pressure for 2 weeks formed a single crystal of hydroxylapatite of a high order of purity. A further extension of hydrothermal methods for preparation of hydroxylapatite was brought about independently by Aoki and Hideki⁶⁵ and Kazov et.al.⁶⁶

1.5 Chemical Analysis

Quantitative separation of calcium and phosphate, which is a prerequisite for an accurate chemical analysis of hydroxylapatite needs special analytical procedures, the details of which have been worked out by Washburn and Shear⁶⁷.

Gravimetric⁶⁷, complexometric⁶⁸ and spectrophotometric⁶⁹ techniques are usually employed for the accompanying determinations of these separated ions. Applications of atomic absorption spectroscopy to determine the metal ion concentration in apatites without a quantitative separation was brought about by Hivo et.al.⁷⁰

1.6 Structural Aspects

That animal bones and teeth contain hydroxylapatite as an ingredient was proved⁷¹ by the identity of X-ray diffraction patterns of the former with those of the naturally occurring hydroxylapatites as shown in Fig. 1-2(a). In addition,

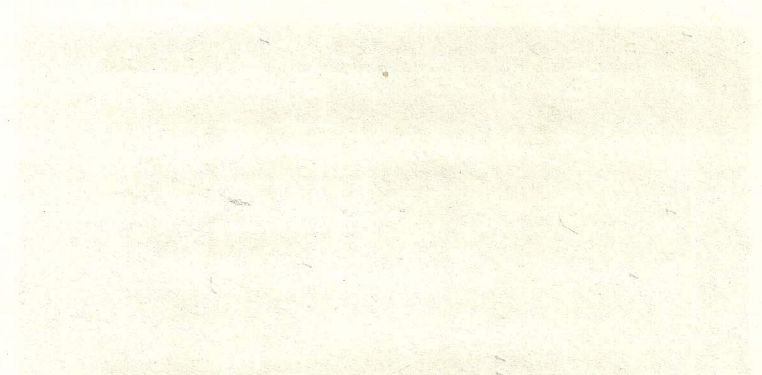
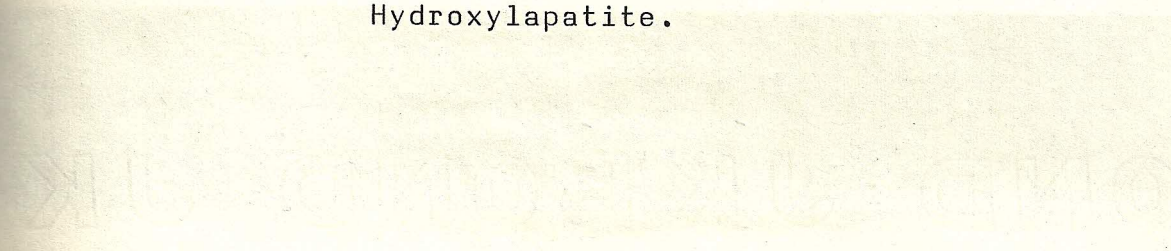
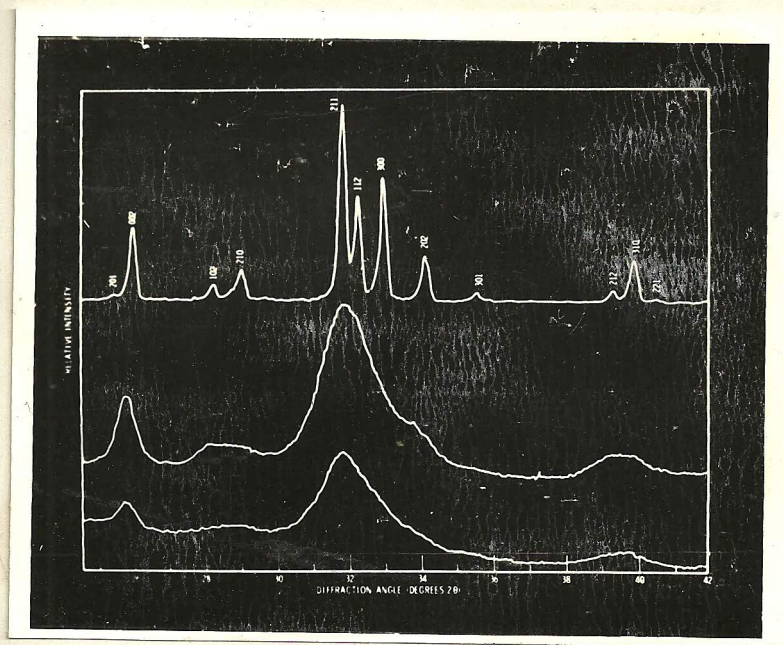


Fig. 1.2 (a) X-ray Diffraction Patterns of

- (i) Crystalline Synthetic Hydroxylapatite
- (ii) Amorphous Synthetic Hydroxylapatite
- (iii) Hydroxylapatite Obtained from Bone

1.2 (b) A Representative Debye-Scherrer Powder
Pattern of Crystalline Synthetic
Hydroxylapatite.





(i)

(ii)

(iii)

FIG. 1.2 (a)

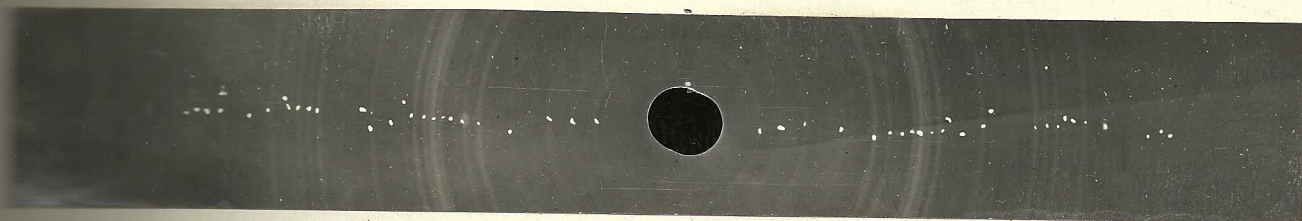


FIG. 1.2 (b)

a representative Debye-Scherrer powder pattern of synthetic hydroxylapatite is given in Fig. 1-2(b), since it serves as a reliable means of characterizing the synthetic samples.

Hydroxylapatites, both of biological and synthetic origin, crystallise in hexagonal $P6_3/m$ space group with lattice constants, 'a' and 'c', equal to 9.42 Å and 6.88 Å respectively. Nary-Szabo⁷² and Mehmel⁷³ studied independently the details of the crystal structure of apatites and their findings were subsequently confirmed by Hendricks et.al.²⁴ Later Beevers and Mc Intyre⁷⁴ modified the structure proposed earlier and a few more refinements were subsequently made by Posner et.al.⁷⁵

The ions, Ca^{2+} , PO_4^{3-} and OH^- (also sometimes referred to as the corresponding atoms or group of atoms) constitute the lattice of hydroxylapatite, their spacing¹³ in the unit cell being indicated in Fig. 1-3. The ions are distributed in two planes, one over the other, such that each half is a mirror image of the other. It is evident that the whole volume of the unit cell is closely packed with the constituent ions and only for purposes of clarification of their relative positions, they are represented wide apart from one another in the figure. Out of a total of fourteen calcium ions, six are located within the unit cell and thus belong to it entirely and the remaining eight peripheral

Fig. 1.3 The Unit Cell Perspective of
Hydroxylapatite.

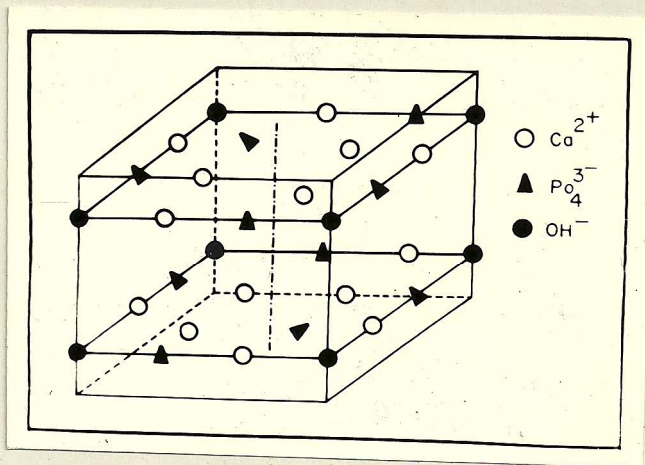


FIG.1.3

ions are shared by adjacent unit cells such that there is an allocation of four per each. Similarly, it can be shown that out of ten phosphate groups, two situated inside and eight at the periphery, only six belong to each unit cell, these being the two situated inside and the four out of the eight peripheral ions. Likewise two out of eight hydroxyl groups represented in the figure belong to the unit cell. The number of ions present per unit cell of hydroxylapatite can thus be correlated with its molecular formula accounting thereby for the basic chemical repeat unit in the three dimensional symmetry pattern.

Fig. 1-4(A) and (B) represent cross-sections of hydroxylapatite lattice parallel to the c-axis and provide a further clarification²⁴ of the relative lattice-positions of the atoms. It can be shown from the figure that O-Ca-O chains exist running parallel to the c-axis. The chains are joined by phosphorus atoms constituting the inner lining of an elongated cavity which runs parallel to the c-axis and accommodates the F^-/OH^- ions. It is evident that depending upon ionic size and charge, other ions can replace F^- ions. 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 hence the surface activity of apatites. X-ray line-broadening experiments¹⁴ using low angle scattering revealed

Fig. 1.4(a) A Cross-Section of the Apatite
Lattice Parallel to the c-axis.

Fig. 1.4(b) Apatite Lattice Viewed from
the Plane 001
as Reported by
Beevers and Mc Intyre.

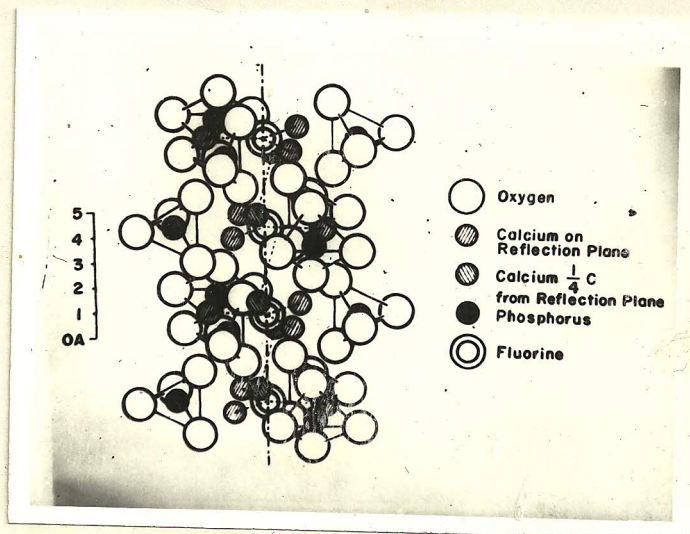


FIG. 1.4 (a)

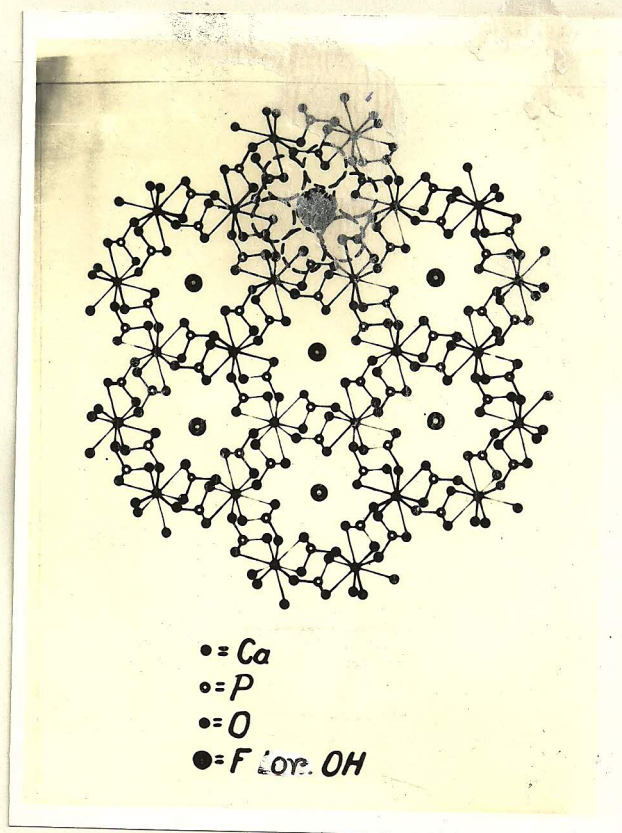


FIG. 1.4 (b)

the apatites to be thin tabular hexagons elongated in the direction of the c-axis.

As mentioned earlier, Posner et.al.⁷⁵ suggested a refinement of the crystal structure of hydroxylapatite based on three dimensional X-ray diffraction studies on single-crystals. In addition, determination of the bond-lengths and the atomic positions was made with an accuracy higher than that attained earlier. It was shown that the phosphate-tetrahedra have P-O distances shorter than those reported earlier. Further, the calcium atoms situated around the hexagonal screw-axis were shown to be co-ordinated to the hydroxyl ions and oxygen atoms of the phosphate-tetrahedra. The calcium atoms among themselves were found to constitute triangles, one over the other, in the direction of the c-axis. On the basis of these refinements, the arrangement of the constituent atoms of hydroxylapatite as projected upon the basal plane of its structure is shown in Fig. 1-5.

Kay et.al.⁷⁶ used neutron and X-ray diffraction techniques to determine the orientation of hydroxyl groups in the crystal structure of hydroxylapatite. Their studies revealed that the hydroxylgroups occur in columns parallel to the c-axis as reported by earlier workers^{72,74,75} and that these columns pass through the centres of the calcium triangles. The x, y and z co-ordinates of the constituents

Fig. 1.5 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).

of the hydroxylapatite lattice are given in Table 1-2. On the basis of their relative positions in the unit cell of hydroxylapatite, the calcium and oxygen atoms are designated through the subscripts given to their symbols.

Table 1-2.

Listing of Position-Parameters for the Constituent Atoms of Hydroxylapatite.⁷⁶

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

Young and his co-workers⁷⁷⁻⁸⁰ carried out extensive work on the crystal structure of apatites during the recent past, the samples used being obtained exclusively by thermal methods. As mentioned earlier, apatites are normally expected

to be hexagonal belonging to the space group $P6_3/m$. They could establish that a synthetic sample of chlorapatite was pseudohexagonal belonging to the monoclinic space group, $P2_1/b$ with 'a' and 'c' being respectively $9.628 \text{ \AA}$ and $6.764 \text{ \AA}$. It is evident that for a monoclinic system lattice parameter 'b' is equal to $2a$. While the monoclinic and hexagonal structures are very similar, the former is characterised by an ordered arrangement of the Cl^- ions in chlorapatite resulting in the transformation of the mirror plane of the hexagonal structure to a glide plane in the monoclinic structure with an accompanying doubling of one of the cell dimensions. Subsequent structural analysis of single-crystals of hydroxylapatite by these workers confirmed the analogy between this compound and chlorapatite regarding the monoclinic structure, the corresponding lattice parameters being $9.4214 \text{ \AA}$ and $6.8814 \text{ \AA}$. The single-crystal of hydroxylapatite used for these investigations was obtained by the conversion of that of chlorapatite by heating in steam at $1200^\circ C$. However, the significance of the monoclinic space group for biological considerations is yet to be proved. In addition, the optimum range of temperatures required for the phase transformation from monoclinic to hexagonal form is yet to be investigated to know whether the

monoclinic form exists under in vivo conditions. It will be of interest to investigate the role of trace-impurities like F^- , Cl^- , CO_3^{2-} and Mg^{2+} ions in bringing about such a phase transformation. Recently Elliot, Bonel and Trömbé⁸¹ substantiated the findings of Young by reporting that a sample, $Ca_{10}(PO_4)_6CO_3$, termed as carbonateapatite, exhibits a pseudohexagonal symmetry with monoclinic space group, P_b , with lattice parameters 'a' and 'c' being equal 9.557 Å and 6.872 Å and b approximately equal to 2a.

For purposes of characterisation 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 electronmicroscopic patterns which can confirm the homogeneity of the samples by proving the absence of extraneous phases. In addition, they provide information regarding the geometry and dimensions of its individual crystals. Though X-ray diffraction patterns of bone help in

proving the presence of hydroxylapatite and determining its lattice constants, they do not permit one to see the shape of bone crystals or to visualise directly the relationship these crystals hold to the other ingredients of bone. These aspects can be fulfilled by electron microscopic investigations of bone, the corresponding in vitro studies being carried out by Hayek et.al.³⁵

Extensive in vivo electron microscopic studies carried out by Robinson¹³ could throw light on the uniformity in size of the inorganic crystalline component and the spatial relationship existing between the Collagen fibres, cement substance and calcium hydroxylapatite in bone.

1.7 Isomorphus Substitution of Biological Significance

By the term isomorphous substitution is meant a replacement of one ion by another in a crystal lattice without disrupting the geometry of the crystal. This can be classified as isoionic and heteroionic substitutions. While isoionic substitution is 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, heteroionic substitution involves a replacement by a different ion present in a solution in contact with the solid phase, changing thereby

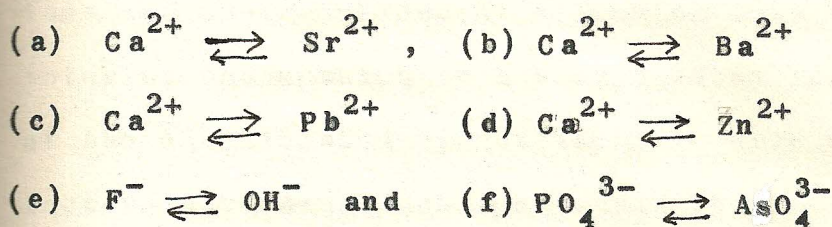
The composition of both the phases. Hydroxylapatite is unique in that it can undergo a series of iso- and heteroionic substitutions⁸² involving both cations and anions, the criteria being similarity in charge and size of the ions concerned. Clement and Zureda⁸³ showed that metal ions with ionic radii of about $1.0 \text{ \AA}$ can form apatites, the most important among them being Ca^{2+} , Sr^{2+} , Ba^{2+} , Pb^{2+} and Cd^{2+} with ionic radii 0.99, 1.13, 1.35, 1.20 and $0.97 \text{ \AA}$ respectively.

1.7.1 Isoionic Substitutions

Isoionic substitutions of calcium and phosphate have been investigated extensively on synthetic hydroxylapatite, human bones and teeth^{84,85}. Such studies are not only significant in providing an explanation for the skeletal fixation of calcium and phosphorus but also throw light on the phenomenon of resorption, a process by which portions of bone during growth are dissolved and returned to blood stream. Such substitutions were investigated by tracer technique¹ in which a slice of the labelled tissue is placed in contact with a photographic film which functions as a radiation detector.

1.7.2 Heteroionic Substitutions

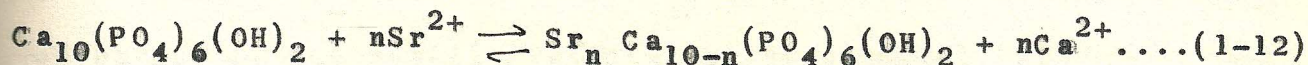
Among heteroionic substitutions the following are considered relevant by virtue of extensive biological significance associated with them:-



and merit a mention in the present context.

(a) Ca^{2+} - Sr^{2+} Substitution.

Ca^{2+} - Sr^{2+} substitution on hydroxylapatite is one of the most important heteroionic substitutions since it explains the mechanism of incorporation in the human skeletal system of β -active Sr-90 produced in atomic explosions. This can be represented as follows:-



Solid solution of hydroxylapatite
of calcium and strontium

Incorporation of Sr-90 can prove fatal, because of its long half-life period (about 20 years), even when it is present in traces. Hence studies on this substitution reaction are supposed to be helpful in suggesting a possibility of removal of the incorporated strontium. Knappwost and Ehret⁸⁶ investigated the kinetics of this substitution using β -active Sr-89 as a tracer. Synthetic samples of hydroxylapatite of different surface areas were equilibrated with solutions of strontium nitrate labelled with Sr-89. Adsorption of strontium

ions and their subsequent diffusion into the crystal interior accompanied by a simultaneous recrystallisation of the equilibrated hydroxylapatite were shown to be the factors governing such a substitution.

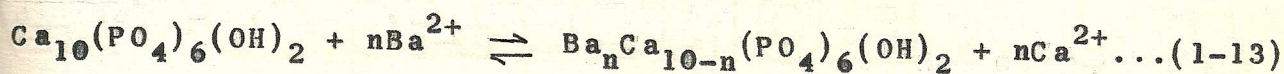
Collin⁸⁷ showed through coprecipitation that a series of homogeneous solid solutions of hydroxylapatite of calcium and strontium over the entire compositional range could be formed confirming thereby the occurrence of isomorphous substitution between these ions. These results were subsequently confirmed by Narasimharaju et.al.^{88,89}

X-ray diffraction studies by Khudolozhkin et.al.⁹⁰ could prove that for replacement of calcium by strontium or barium on the apatite lattice, sites of Ca(II) are preferred to those of Ca (I). A substantiation of these results was provided by Trombe et al.⁹¹⁻⁹³ using i.r. spectroscopy and thermoanalytical studies. Based on in vivo studies on isomorphous substitutions on apatite crystals, Baud⁹⁴ could show that the dimensions of the unit-cell were dependent on the chemical composition; the alteration in the former offers an evidence for the occurrence of substitution.

(b) Ca^{2+} - Ba^{2+} Substitution

Because of the toxic effects of elemental barium

and its soluble salts, replacement of Ca^{2+} by Ba^{2+} on hydroxylapatite is another heteroionic substitution⁵⁷ of importance. As in the case of strontium, β -active Ba-140 (half-life, 12.8 days), a product of atomic explosions, gets incorporated in the human skeletal system based on the following equation:-



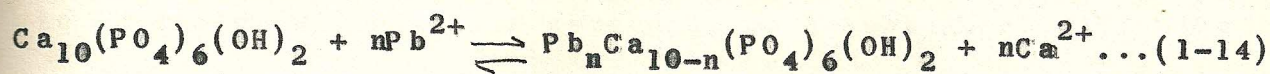
Solid solution of hydroxylapatite of calcium and barium.

It is evident that radiation damage caused by Ba-140 is much less than that by Sr-90 consequent upon the relatively smaller half-life period of the former. Unlike strontium, the non-active barium is toxic and so the studies on such a substitution are of importance to explore a possibility of elimination of the incorporated barium. Narasaraju et al.⁵⁷ made investigations in detail, on the various aspects of Ca^{2+} - Ba^{2+} substitution. Samples of hydroxylapatites of calcium and barium and a series of their solid solutions were prepared by a thermal method over the entire compositional range. The samples could be characterised by chemical, i.e., X-ray diffraction and electronmicroscopic analyses. It could be shown through tracer technique by Samachson et al.⁹⁵ that there is a preferential uptake of Ba-133 over Ca-47 and Sr-85 on human bones. In addition, it was shown by Samachson

and Schmitz⁹⁶ that traces of Zn^{2+} when present in the equilibrating solution increase the uptake of Sr-85 and Ba-133. These results were explained on the basis of the supposition that Zn^{2+} distorts the surface of hydroxylapatite facilitating a replacement of Ca^{2+} by larger alkaline earth ions.

(c) Ca^{2+} - Pb^{2+} Substitution

Inhalation of lead in the form of dust or its adsorption through skin leads to "lead-poisoning"⁹⁷ (also known as "plumbism"). The mechanism of this process can be attributed to isomorphous substitution of Ca^{2+} by Pb^{2+} on bone. As envisaged by Klement⁹⁸ lead-poisoning occurs due to formation of solid solutions of hydroxylapatites of calcium and lead on bones. Formation of such a series of solid solutions over the entire compositional range by coprecipitation in aqueous media was reported by Müller⁶ and later substantiated by Narasaraaju et al.⁴⁴ and also by Rao and Chickerur⁹⁹. The chemical reaction involved may be represented as follows:-



Solid solution of hydroxylapatites
of calcium and lead.

A famous recent example of lead-poisoning has been the toxic

effect of lead present in the coating of pottery in Mexico, the damage being termed as "Problem of lead in Mexican pottery"¹⁰⁰ Consequent upon the contemporary significance of this exchange a few more aspects of the latter constituted the subject matter of the investigations reported in the present work.

(d) $\text{Ca}^{2+} - \text{Zn}^{2+}$ Substitution

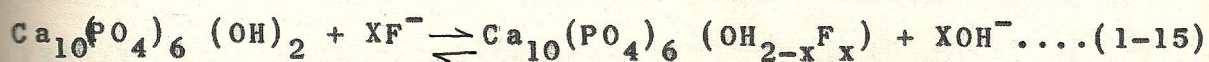
Samachson et al.¹⁰¹⁻¹⁰³ made extensive investigations on the replacement of Ca^{2+} by Zn^{2+} on the hydroxylapatite of bones. Based on detailed radio-chemical studies on this exchange using solutions labelled with Zn-65 it could be confirmed that the uptake of Zn^{2+} by calcium hydroxylapatite could be 100 per cent. within five minutes. Further, this process was found to be considerably reduced by chelating agents like E.D.T.A. Proof for the occurrence of exchange was provided by estimation of the amount of extra calcium which entered the solution during equilibration resulting in an ion to ion replacement of lattice- Ca^{2+} by Zn^{2+} present in the equilibrating solution. The exchange which was found to be rapid initially was dependent on a subsequent diffusion of Zn^{2+} ions into the crystal lattice.

(e) $\text{F}^- - \text{OH}^-$ Substitution

Through neutron - and X-ray diffraction studies,

Kay et al.⁷⁶ could confirm the earlier findings that the OH^- groups are located in the cavities running parallel to the C-axis of the apatite lattice. Based on the dimensions of these cavities, it can be shown that, among others, ions of the type F^- , OH^- and Cl^- with ionic radii 1.32, 1.68 and 1.81 Å respectively fit into them. Further, F^- ion due to its spherical symmetry is better suited for the purpose than OH^- ion. A replacement of OH^- by F^- brings about a contraction of the unit cell of hydroxylapatite as expected from such a spherical symmetry.^{3,5,104} The $\text{OH}^- - \text{F}^-$ exchange is of significance in explaining the occurrence of dental caries which involves the attack of the apatitic inorganic phase of enamel by acidogenic bacteria existing in the vicinity of the enamel surface. Various aspects of this substitution and the role of F^- ion as a prophylactic in the occurrence of dental caries have been extensively investigated by Knappwost^{19,20,105,106}. He proved that the tooth surface enriched with F^- ions was more resistant to caries. Through experimental investigations he could suggest a convincing mechanism of the caries - prophylactic action of fluorine. An oral dose of 1-5 mg of fluorine taken daily was found to accelerate the deposition of fluorapatite layers on the tooth surface resulting, inter alia, in the accompanying alteration in the viscosity of saliva. Such a deposition which was found to be favoured

on the effected regions of the tooth surface could retard further corrosion by acidogenic bacteria, fluorapatite being less soluble than hydroxylapatite. These conclusions could be drawn from the kinetic studies of this substitution which can be represented as follows:-



Solid solution of hydroxylapatite
and fluorapatite.

The product of this substitution is a solid solution of hydroxylapatite and fluorapatite known as fluorhydroxylapatite. Liang and Higuchi¹⁰⁷, Stearns and Berndt¹⁰⁸ and Higuchi et al.¹⁰⁹ investigated the interference caused by the co-precipitation of calcium fluoride during the progress of this substitution reaction. Based on the results of the kinetic studies of this substitution, the most important measure suggested for the prevention of the dental caries has been the application of F^- ions in some suitable form to the dental tissue. This process known as fluoridation¹ has been in vogue since a few decades.

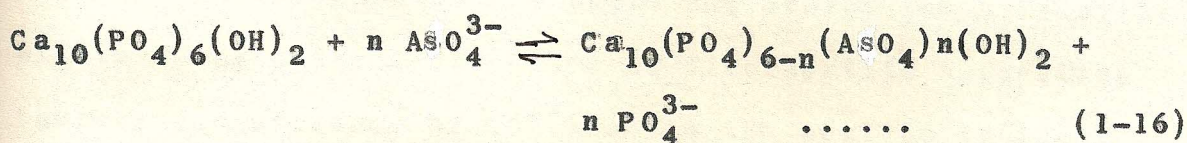
1 ppm of fluoride in drinking water was found to be an optimal level for caries control; a level higher than this was found to lead to mottled enamel and also to certain toxic effects. Several mechanisms are suggested to explain the prophylactic action of fluorine in the

occurrence of dental caries, the most important among which is based on the exchange of OH^- ion of hydroxylapatite of tooth by F^- ion resulting in the formation of fluorhydroxylapatite. It was shown by Narasaraju¹¹⁰ that fluorhydroxylapatite is less soluble than hydroxylapatite accounting thereby for the caries - resistance imparted to the dental tissues by fluoridation. These conclusions could be substantiated by results of recent investigations¹¹¹⁻¹¹⁶ on $\text{F}^- - \text{OH}^-$ exchange. Narasaraju et al.¹¹⁷ could establish the conditions favourable for the occurrence of $\text{OH}^- - \text{F}^-$ exchange on hydroxylapatite, the principal among them being (i) an increase in the concentration of F^- ion, (ii) a decrease in pH of the medium of exchange and (iii) a reduction in the grain size of the sample of hydroxylapatite. Through NMR studies, Young et al.¹¹⁸ could investigate the location of F^- ions present in traces in hydroxylapatite and a mechanism was suggested to explain the prophylactic action of fluorine in the occurrence of dental caries. Vander Lugt et al.¹¹⁹ carried out similar studies and established the role played by internuclear distances in the formation of hydrogen bonding between OH^- and F^- in the apatite lattice. Subsequent NMR studies on $\text{F}^- - \text{OH}^-$ exchange on hydroxylapatite were carried out by Lundin et al.¹²⁰ and Knubovets et al.¹²¹ That a halide substitution of OH^- on apatite lattice is possible was

proved by O'Shea et al.⁴³ Based on Laser-Raman spectral studies, attaining an accuracy as low as five per cent. Further studies on the prophylactic action of fluorine in the occurrence of dental caries were made by Fetkowska-Mielink and Krystina,¹²² Aoba, Takaaki et al.¹²³ and Freund et al.¹²⁴ based on physico-chemical changes undergone by amorphous and apatitic calcium phosphate.

(f) PO_4^{3-} - AsO_4^{3-} Substitution

Another example of a heteroionic substitution¹²⁵ of importance is replacement of PO_4^{3-} by AsO_4^{3-} on hydroxylapatite, accounting for the toxic effects of arsenic¹²⁶ and its soluble salts.¹²⁷ The substitution reaction can be represented as follows:-



Solid solution of phosphorus
and arsenic hydroxylapatite

While the existing literature confirms the occurrence of this substitution reaction,^{125,128} spread over the entire compositional range, the utility of a knowledge of the mechanism of this substitution for the removal of incorporated arsenic is yet to be investigated. Evidence for the occurrence of such a substitution reaction on lead hydroxylapatite could be provided by Rao.¹²⁹

(g) Miscellaneous Substitutions

In addition to the detailed investigations on heteroionic substitution on hydroxylapatite described above, scattered results on some aspects of a few more miscellaneous substitutions are available.

Though the presence of CO_3^{2-} ion in human bones is established, the concept that it exists due to heteroionic substitution is still controvertial.^{130,131} Studies on the possibility of a substitution reaction of hydroxylapatite involving^{132,133} CO_3^{2-} ion are, therefore, of importance. Extensive X-ray studies of a mineral, francolite, $(\text{Ca}, \text{Mg}, \text{Na}, \text{K})_{10} \text{[P,C]}_{10} \text{O}_{4-7} \text{F}_6 (\text{F}, \text{OH})_2$ which is a carbonate-containing fluorapatite, indicated the absence of free calcium carbonate and proved the similarity of its X-ray pattern with that of fluorapatite suggesting thereby the presence of CO_3^{2-} ion in the apatite lattice. Additional evidence in favour of this observation was provided by Le Geros et al.¹³⁴ and Bonel and Montel.¹³⁵⁻¹³⁷ However, solubility studies of francolite indicated a preferential dissolution of CO_3^{2-} ion which can be attributed to its free existence, providing thereby evidence against the CO_3^{2-} substitution.¹³⁸ Whether CO_3^{2-} ion exists as a separate submicroscopic phase or as a substituent in apatite lattice is yet to be unambiguously clarified.

Knappwost showed through radio-chemical studies,¹³⁹ that PO_4^{3-} - SiO_4^{4-} substitution was possible when it is accompanied by a compensation of the surplus negative charge through introduction of a univalent metal ion like Na^+ into the apatite lattice. Some aspects of this substitution were investigated by Azimove et al.^{140,141} and by Gaude et al.¹⁴² Neuman showed that monovalent ions like Na^+ and H_3O^+ (ionic radii, 0.95 and 1.00 Å respectively) can displace Ca^{2+} ion (ionic radius, 0.99 Å) partially from the apatite lattice, charge compensation being brought about by appropriate depletion of some of the boundary ions.

The occurrence of Ca^{2+} - Eu^{2+} substitution on hydroxylapatite could recently be established by Mayer et al.¹⁴³ They could prepare fluor-, chlor- and hydroxylapatites of europium as well as a few solid solutions of some of these isomorphs. Gilinskaya and Shcherbakova¹⁴⁴ could provide evidence for the replacement of Ca^{2+} by Mn^{2+} ions, the latter being capable of occupying both Ca(I) and Ca(II) lattice positions.⁷⁶ These results were confirmed by Dubrov et al.¹⁴⁵ through EPR studies. Vinnikov and Gugel¹⁴⁶ also carried out similar exchange studies on fluor and chlorapatites. Synthesis of oxyapatites of composition, $\text{M}_4\text{Nd}_6(\text{SiO}_4)_4(\text{EO}_4)_2\text{O}_2$, where $\text{M} = \text{Ca}, \text{Sr}$ and

E = P, As and V was accomplished by Fedorov et al.¹⁴⁷, the characterisation of the samples being done by X-ray diffraction studies. Mayer et al.¹⁴⁸ carried out similar investigations in the system $\text{Ln}_x \text{M}_{10-2x} \text{Na}_x (\text{PO}_4)_6 \text{F}_2$ where Ln = La, Pr, Nd, Sm, Eu, Dy, Er, Lu, Y and M = Ca, Sr and Ba.

Evidence for $\text{Ca}^{2+} - \text{Fe}^{2+}$ substitution on hydroxylapatite over a limited compositional range was provided, through Mössbauer studies by Khudolozhkin et al.¹⁴⁹, a substantiation of these results by chemical analysis being done by Rao.¹⁵⁰ Through equilibration studies of hydroxylapatite with solutions containing Ni^{2+} and Cu^{2+} ions, Misra et al.¹⁵¹ could provide evidence for the replacement of Ca^{2+} by Ni^{2+} and Cu^{2+} ions on the apatite lattice. Narasaraju et al.¹⁵² investigated $\text{Ca}^{2+} - \text{Co}^{2+}$ and $\text{Ca}^{2+} - \text{Cd}^{2+}$ exchanges on hydroxylapatite. Band, Besse et al.^{153,154} reported the existence of a series of compounds having the general formula $\text{Ba}_{10}(\text{ReO}_5)_6 \cdot x \cdot 2$, (where x = Br, I) named by them as 'New rheniumapatites' while Schiff, Francosis, Alain et al.¹⁵⁵ reported the formation of a series of apatites containing S^{2-} and SO_4^{2-} ions. Berak et al.¹⁵⁶ could prepare strontium oxyapatite.

1.8 Studies on Solubility

1.8.1 Significance

During the recent past phenomena associated with dissolution of $^{21,86,157-162}$ hydroxylapatite have attracted considerable attention because of their significance in diverse fields. An understanding of the physiology of human bones and teeth from the point of view of calcification and resorption is possible through studies on the solubilities of hydroxylapatite. Further, such studies constitute the basis of the different aspects concerning the occurrence of dental caries and the prophylactic action of fluorine.^{19,20} In addition, it is evident that the availability of phosphatic fertilizers to plant kingdom can be explained through the solubility behaviour of hydroxylapatite, the latter being the stablest phase of calcium phosphates in aqueous media.

It is, therefore, no wonder that the solubility phenomena of hydroxylapatite have attained inter disciplinary significance and have been demanding the attention of physical chemists, bone biologists, dentists, chemical engineers and geologists, to mention only a few. A survey of literature on solubility of hydroxylapatite 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.

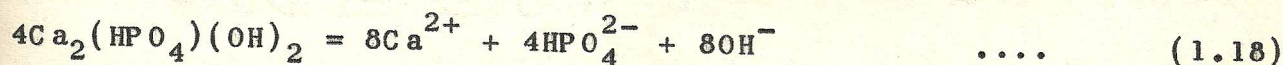
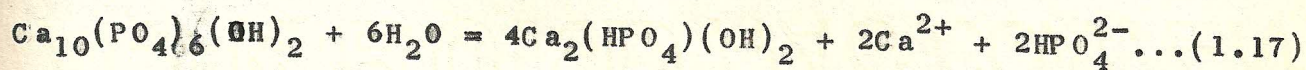
1.8.2 Non-stoichiometric Dissolution

Extensive studies on solubility of hydroxylapatite carried out independently by Neuman and Co-workers¹⁵⁷ and Rootare et.al.¹⁵⁹ could provide ample evidence to show that the sample exhibits a non-stoichiometric dissolution on the basis of a divergence of Ca/P, g atom ratio, of its saturated aqueous solutions from the stoichiometric value of 1.67 . The divergence from stoichiometric behaviour was attributed to the formation of a surface coating on the solute consequent upon its hydrolytic dissolution. While Rootare et al.¹⁵⁹ could prove this surface coating to be a complex, $\text{Ca}_2(\text{HPO}_4)(\text{OH})_2$,¹⁶³⁻¹⁶⁵ others could provide evidence that it is either calcium monohydrogen phosphate, CaHPO_4 , or calcium pyrophosphate, $\text{Ca}_2\text{P}_2\text{O}_7$, or octacalcium phosphate, $\text{Ca}_4\text{H}(\text{PO}_4)_3$.

In order to simulate biological conditions and to maintain a constant ionic environment in the medium of dissolution, Levinskas and Neuman¹⁵⁷, used 0.165M aqueous solutions of sodium chloride as solvent to study the

solubility of hydroxylapatite. They reported that for a given set of experimental conditions, (i) the g atom ratio, Ca/P, of the saturated solution was different from that of the solute and that (ii) no reproducible values for the solubility product, K_{sp} , could be obtained. It was therefore supposed that the compound deviated from the established laws of solubility.

The solubility products of a number of commercial and laboratory-made samples of hydroxylapatite were determined by Rootare et al.¹⁵⁹ They observed that the values obtained were divergent and dependent upon the solute to solvent ratio. Further, as shown by Lavinskas and Neuman¹⁵⁷, the g atom ratio, Ca/P, of solutions is not constant at the stoichiometric value of 1.67. These investigators gave a convincing explanation to account for the observed behaviour from the point of view of established physico-chemical principles. Their theoretical considerations were subsequently substantiated by La Mer.¹⁶⁰ Hydroxylapatite being the salt of a weak acid, undergoes hydrolysis in aqueous solutions yielding a solid surface complex, $Ca_2(HPO_4)(OH)_2$, which was found to dominate the solubility equilibria according to the following equations:-



Thus, it is evident that when equation (1. 17) predominates the g atom ratio, Ca/P , in the solution is unity while it becomes 2 with the establishment of equation (1. 18). When both the equations contribute equally to the solution process this ratio becomes $10/6$ as expected for the stoichiometric dissolution of hydroxylapatite.

The non-reproducibility of the K_{sp} of hydroxylapatite as reported by Lavinskas and Neuman¹⁵⁷ was attributed by La Mer¹⁶⁰ to the complications involved in the accurate determination of individual ion activity coefficients for the systems employed. It was pointed out by La Mer that by virtue of its biological significance an aqueous 0.165 M solution of sodium chloride which is the solvent employed by Neuman, can be considered as a standard solvent of reference in which all ion activity coefficients can be assumed to be unity. Based on that assumption the complicated problem of evaluating the individual ion activity coefficients involved in the solubility equilibria of hydroxylapatite can be avoided. It was shown by La Mer that Neuman's data, when recalculated on the basis of the foregoing considerations, indicated that the solubility of hydroxylapatite responded precisely to the principles of solubility product.

Details of similar studies carried out with natural samples were also available in the literature. Brudevold et al.¹⁶³ investigated the solubility of dental enamel with saliva as a medium of dissolution. They found that among the possible solid phases, calcium monohydrogen phosphate, CaHPO_4 , was found to give a constant K_{sp} in the pH range, 4.5 - 7.5. Francis¹⁶¹ investigated the solubility of synthetic hydroxylapatite, dental enamel and a few more allied natural phosphatic minerals in solutions buffered to a pH range, 3.5 - 6.0. He could show through chemical analysis the formation of a coating of CaHPO_4 on the surface of those equilibrated with acid buffers. He also emphasized the significance of corrections which are to be applied for the evaluation of K_{sp} of the samples taking into account the complexing of the products of dissolution in acetate, lactate and phosphate buffers. The change in the composition of the solution observed during dissolution of hydroxylapatite could be explained on the basis of deposition of CaHPO_4 on its surface. Based on such theoretical considerations, Francis explained the role played by the parameters such as particle size, solute to solvent ratio, type and concentration of buffers and pH in deciding the composition of hydroxylapatite solution.

Fleisch et al.¹⁶⁴ investigated the role of a surface coating of calcium pyrophosphate, $\text{Ca}_2\text{P}_2\text{O}_7$, in controlling the solubility of hydroxylapatite. By extending the analogy to the

bone processes a mechanism was suggested for the calcification and resorption.

Dissolution kinetic studies of synthetic samples of hydroxylapatites of calcium and strontium were investigated by Chickerur et al.¹⁶⁶ at 370°C using IG4 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.¹⁵⁹

Blitz et al.¹⁶⁵ studied the solubility equilibria of hydroxylapatite obtained from animal bones and teeth in order to characterise 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 of the sample. They concluded that octacalcium phosphate, $\text{Ca}_4\text{H}(\text{PO}_4)_3$, formed during dissolution of hydroxylapatite controlled its solubility.

1.8.3 Stoichiometric Dissolution

The credit of proving for the first time that hydroxylapatite obeys the established laws of solubility

goes to Clark.¹⁵⁸ He investigated the solubility equilibria of hydroxylapatite 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 precipitate. In addition, the precipitate was subjected to the conventional solubility equilibria to obtain the saturated solution which was also analysed. It could be confirmed from both the sets of investigations that hydroxylapatite exhibits stoichiometric dissolution resulting in a definite K_{sp} at 25°C, the pK_{sp} reported being 115.5

Using precipitated samples of hydroxylapatite, its solubility was investigated by Bardy et al.¹⁶⁷ taking the solute in the form of a pellet mounted on an electrically operated stirrer fixed in a buffered dissolving medium containing excess of potassium chloride to maintain a constant ionic environment so that the activity coefficients of the dissolved species remained constant. A stoichiometric dissolution was observed by them. Another set of investigations substantiating stoichiometric dissolution of hydroxylapatite were considered by Fassbender et al.^{168,169} In addition they observed that the dissolution rate was controlled by a combination of several consecutive partial reactions and was dependent on the pH and surface area of the solute and

independent of the temperature in the range 15-35°C. Zimmermann could propose a mathematical theory on the basis of the data on the solubility of enamel, thereby suggesting the pH range vulnerable for the onset of dental caries. The influence of temperature on solubility of apatites was investigated by Valyashko et al.¹⁷⁰ and it was found that the solubility decreased with increasing temperature.

Solubility isotherms of well-characterised samples of synthetic hydroxylapatite were investigated by Moreno et al.¹⁷¹ in the pH range, 5.0 - 7.0, and a stoichiometric dissolution was reported. Chander and Fuerstenau¹⁷² used thermodynamics to study the interfacial properties and equilibria in the apatite - aqueous solution system. They observed that electrical double layer properties play an important role in the solubility of apatite. Systematic investigations on the solubility of hydroxylapatite were carried out by Wier et al.,¹⁶² using commercial samples subjected to a rigorous purification either by refluxing for 24 hours in contact with water or by auto-claving in presence of 1N ammonium chloride solution for 50 days at 120°C under a pressure of 1.06 kg/cm², the wash-liquid being periodically replaced. Systematic characterization of the samples was done through X-ray, petrographic and

chemical analyses. The samples were equilibrated in media with pH values ranging from 4.3 - 6.7 and choosing 0.1, 0.5, 1.0 and 10.0 g of the solute per 100 ml of the solvent. The colloidal component of the solute present in the solutions was removed by using specially prepared cells made of plexiglas and fitted with cellulose acetate dialyzing membranes. Based on the results of chemical analyses of the saturated solutions and calculating the activities of the ionic species present, they could convincingly establish a stoichiometric dissolution of hydroxylapatite, the observed average pK_{sp} being 116.5. Divergence observed in the results of solubility of hydroxylapatite was attributed to the presence of trace impurities which get eliminated on rigorous purification.

Narasaraju and Rao¹⁷³ undertook the dissolution kinetics and solubility studies of hydroxylapatite, chlorapatite and a series of their solid solutions spread over the entire compositional range in buffered media extending over a pH range, 4.9 - 7.5. They could convincingly establish a stoichiometric dissolution of hydroxylapatite, chlorapatite and their solid solutions, the corresponding pK_{sp} values at 37°C being 110.6 for hydroxylapatite and 115.4 for chlorapatite.

1.3.4 Miscellaneous Aspects of solubility

While the bulk of the results on solubility of hydroxylapatite can be put under the abovementioned categories of non-stoichiometric and stoichiometric dissolutions, a few significant scattered results can be shown under the category of miscellaneous aspects of solubility of hydroxylapatite which involve both stoichiometric and non-stoichiometric dissolutions. The principal aspects coming under such a category are the dependence of solubility of hydroxylapatite on pH of the dissolving medium and on its crystal structure.

Among the earlier systematic investigations, mention may be made of the observations of Benedict and Kanthak¹⁷⁴. They noticed that the pH dependence of solubility of dental enamel was similar to that of tricalcium phosphate indicating compositional similarities between the two.

Ericsson^{175,176} investigated the pH dependence of the solubility of synthetic hydroxylapatite as well as of dental enamel. The solubility of hydroxylapatite and its optimum pH range could be calculated by him. According to him the increased solubility in the presence of CO_3^{2-} , serum and saliva is due to the formation of complexes

involving Ca^{2+} ions. Davies and Hoyle¹⁷⁷ carried out further investigations on the solubility of such complexes.

Narasaraju et al.⁸⁹ studied the pH-dependence of the solubility of hydroxylapatites of strontium and calcium and a series of their solid solutions distributed over the entire compositional range, the amount of the dissolved apatite being calculated exclusively from the phosphate concentration of the solution. An increase of solubility with a decrease in pH was reported. Paunio and Makinen¹⁷⁸ could establish an enhancement in solubility of tooth material and synthetic hydroxylapatite at a series of pH values by the presence of sulphate ions.

Dissolution kinetic studies were also made by Voegel and Garnier et al.¹⁷⁹ on the release of PO_4^{3-} , HPO_4^{2-} and CO_3^{2-} from powdered samples of human normal tooth enamel supposed to contain CO_3^{2-} ion and on the influence of F^- ion on it in buffered solutions spread over the acidic region.

The solubility of hydroxylapatite, fluorapatite and a few of their solid solutions was investigated by Narasaraju¹¹⁰ 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 prophylactic action of fluorine in the occurrence of dental caries was emphasized. Proof for anisotropic dissolution of a single crystal of hydroxylapatite was conclusively established by Jongebloed et al.¹⁸⁰, using citric and lactic acids as dissolving media. The dissolution parallel to c-axis was found to be much faster than that perpendicular to it. Scanning electronmicroscopic patterns of the solute after attainment of saturation could show that the single crystal was twisted along c-axis confirming thereby the existence of a screw dislocation parallel to it.

Daculsi, Kerbel and Kerbel¹⁸¹ used high resolution transmission electronmicroscopy to study the acid dissolution of biological and synthetic apatite crystals and their dependence on the lattice pattern. According to them the dissolution starts at the regions of dislocation and is preferential across the 'c' axis due to the likely existence of dislocations.

1.8.5 Explanation for Divergence in solubility

The foregoing account shows that results on the solubility of hydroxylapatite are characterized by divergence and mutual contradictions among themselves. An exhaustive survey of the literature on the subject enables one to make

a critical assessment of the results to throw light on the factors responsible for such disparities.

Smith et al.¹⁸² made attempts to clarify the reported divergence in the g atom ratio, Ca/P, of hydroxylapatite solutions by dissolving synthetic samples in dilute hydrochloric acid at a pH equal to 4.5. When 500 g of the solute were equilibrated in 1 litre of the dissolving medium, the ratio was found to be higher than the stoichiometric value. However, when the solute was resuspended under identical conditions, stoichiometric dissolution could be observed. Similar results could be arrived at when the amount of the solute taken was less than 8 g per litre of the same dissolving medium. The non-stoichiometric dissolution was therefore attributed to the higher proportion of surface impurities dissolved along with hydroxylapatite when the amount of the solute taken for a given volume of the solvent was large. It was concluded by them that when the solute/solvent ratio was low the non-stoichiometric dissolution caused by surface impurities was less than the experimental errors associated with the micro-analytical determinations of calcium and phosphorus and was therefore not perceptible.

In addition, the role played by errors involved in the micro-determination of Ca^{2+} , PO_4^{3-} and other ions in

the case of solid solutions of apatite in causing divergence of the results is not to be ignored. The fact that the K_{sp} values of the supposed surface coatings such as CaHPO_4 , $\text{Ca}_2\text{HPO}_4(\text{OH})_2$ and $\text{Ca}_4\text{H}(\text{PO}_4)_3$ appear to remain constant is an evidence to substantiate this argument. It is evident that in all such cases, the concentrations / activities of the species such as Ca^{2+} , HPO_4^{2-} , H_2PO_4^- and PO_4^{3-} are raised only to relatively lower powers for K_{sp} calculation resulting in a minimization in the errors associated with the determination of such ions. On the other hand when the calculations are extended to evaluation of K_{sp} of hydroxylapatite these errors get magnified.

1.9 Mechanism of Calcification

Calcification,^{183,184} which involves an orderly precipitation of hydroxylapatite within the organic matrix of bone, governs the physiology of human bone. In the light of the fact that a knowledge of the solubility equilibria of hydroxylapatite plays an important role in understanding the mechanism of calcification, it has been considered appropriate to mention a few aspects of it in the present context.

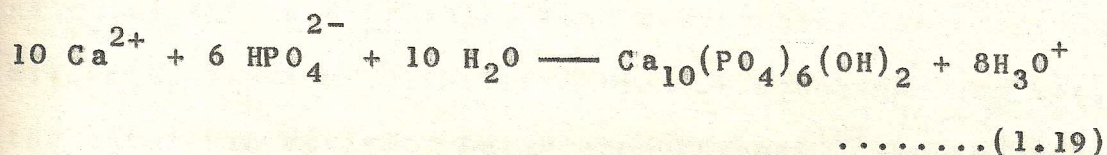
The results of in vitro and in vivo studies on the solubility of hydroxylapatite facilitated the understanding of the mechanism of calcification. It has been established that calcification occurs only at certain regions of the body known as sites of calcification¹. An important prerequisite for the process to occur is the transport of the assimilated calcium and phosphorus to such sites which is brought about by the body - fluids. For the promotion of calcification, optimum environmental conditions should be prevalent at these sites. The mechanism of establishment of these conditions was suggested by Robison's scheme according to which a local increase in phosphate - concentration is brought about at the sites of calcification when a substrate of phosphate ester is hydrolysed by an enzyme known as phosphatase resulting thereby in the deposition of hydroxylapatite.

It is found from a knowledge of solubility behaviour of hydroxylapatite that blood serum is supersaturated with respect to it and its spontaneous precipitation is therefore expected. Since such a precipitation is found to take place only at the sites of calcification due to local action as mentioned by the Robison's scheme, it is supposed that the prevention of calcification in the non-calcifying regions is due to a

possible chemical binding of calcium and/ or phosphate, the details of which are yet to be clarified. The theories suggested to explain the ability of only certain regions of the body to function as sites of calcification are controversial.

From physiological considerations, it can be shown, that in addition to calcium and phosphorus blood serum contains ions such as sodium, carbonate, magnesium and citrate. Further, about one-third of this calcium is shown to be bound to protein-fraction¹⁸⁵ through chelation while the rest of it exists in the ionised form or as soluble complexes of ions such as citrate and phosphate.¹⁷⁶ The phosphorus of blood serum exists mainly as HPO_4^{2-} and to some extent as H_2PO_4^- and PO_4^{3-} . The composition of blood serum suggests that it is complicated to apply the principles of solubility product to account for the deposition of hydroxylapatite. Its calcium and phosphate concentrations are higher than those corresponding to the solubility product of hydroxylapatite and approximate to those of saturated solutions of dicalcium phosphate. It was therefore supposed by Neuman that precipitation of dicalcium phosphate takes place initially. Hydroxylapatite, which is the stablest among the calcium phosphates, is likely to be

formed due to a subsequent hydrolysis of dicalcium phosphate . Samachson^{183,184} could provide 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 the removal of H_3O^+ ion as a factor essential for the process as indicated by the following equation:-



From the equation, it is evident that the deposition of hydroxylapatite is favoured when there is presence of proton-acceptors and 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 comprehensive interpretation of factors controlling the solubility of hydroxylapatite is available.

1.10 Non-stoichiometric Apatites

Synthetic basic calcium phosphates obtained by wet methods have the x-ray diffraction patterns similar to hydroxylapatite but often depart from it in stoichiometry by exhibiting a g atom ratio, Ca/p, ranging from 1.33 to 1.67 . Such compounds, known as calcium deficient or

non-stoichiometric apatites, are of biological significance since the g atom ratio, Ca/p, of bone is lower than that corresponding to the stoichiometric value. Such a flexibility in the permissible values of Ca/p g atom ratio is responsible for the role of bone-hydroxylapatite as a buffer in controlling the composition of blood. These non-stoichiometric apatites have an additional importance by virtue of their role as catalysts in dehydration and dehydrogenation of primary alcohols, leading to the formation of aldehydes and ketones,¹⁸⁶ the catalytic activity being proportional¹⁸⁷ to the calcium deficiency of the sample. Wilson, Sundarsanam and Young¹⁸⁸ investigated the halogen deficient cadmium haloapatites with the structure $\text{Cd}_5 (\text{MO}_4)_{3.3} \text{X}$ (where X=Cl, Br or I). Many theories^{189,199,205} have been put forward to explain the structure and composition of these compounds. Cameron,¹⁸⁹ Hodge,¹⁹⁰ Schleede¹⁹¹ and Trömel et al.¹⁹² have advanced independently a theory based on the adsorption of HPO_4^{2-} ions to account for the non-stoichiometry of apatites. But Posner^{193,194} rejected this theory on the basis that non-stoichiometry was not exclusively due to surface adsorption since the measured surface areas of the samples were too low to account for the expected uptake of HPO_4^{2-} ions. Instead, the calcium deficiency was accounted for by Posner on the basis of a statistical model depicting the

absence of Ca^{2+} ions from the columnar positions of the apatite lattice, the charge - balance being maintained by the introduction of two protons. Winand et al.,^{195,196} could explain the maintenance of charge-balance through a compensation of the two positive charges of each missing Ca^{2+} ion by an addition of Proton and removal of a structural OH^- ion. The suggestion of Brown et al.,¹⁹⁷ that the calcium deficiency is caused by an epitaxial inter-growth of octacalcium phosphate, OCP, ($\text{Ca/p} = 1.33$) and stoichiometric hydroxylapatite ($\text{Ca/p} = 1.67$), was rejected by Berry¹⁹⁸ based on infrared and thermoanalytical evidence for the absence of OCP. Kuhl and Nebergall¹⁹⁹ tried to account for the existence of calcium deficient apatites on the basis of introduction of HPO_4^{2-} ions into the apatite lattice, accompanied by a partial removal of OH^- ions. Based on the recent literature²⁰⁰⁻²⁰⁴ Young²⁰⁵ suggested that occurrence of a deficiency of calcium hydroxide in hydroxylapatite is responsible for the nonstoichiometry. According to Young a cation-deficient apatite lattice attains a charge-balance by replacement of OH^- by Na^+ .

The precipitation of calcium phosphates was studied recently by Feenstra and De Bruyn²⁰⁶ by a method which allowed super-saturation to build up under well-defined conditions.

They investigated the stage preceding the growth of non-stoichiometric apatite crystals through a growth-study as a function of pH.

1.11 Inter-disciplinary significance

The trend of recent investigations²⁰⁷⁻²¹³ in the field of apatites establishes convincingly their interdisciplinary significance. In a brief review like the present one, only a few significant aspects other than those given earlier merit a mention.

Apatites find an increasing application as catalysts²⁰⁷ in synthesis of organic compounds such as sugars and their phosphoric esters, as luminophosphors²⁰⁸⁻²¹³ and as starting materials for several phosphatic fertilizers.^{214,215} The observation that kidney and prostrate²¹⁶⁻²¹⁹ stones contain hydroxylapatite further enhances the biological and physico-chemical significance of this compound since its solubility behaviour can throw light on their possible elimination by dissolution.

Similar studies on apatites play a role in preventing diseases caused by inha-lation²²⁰ of dust containing apatites. Another significant role of apatites in biology is the utility of apatite-coated ceramics as dental prosthesis²²¹ and artificial bones.²²²

Though extensive work^{1,21,30,31,157-162,173} was carried out during the recent past on various aspects of apatites in different disciplines, a researcher in the field suffers from the absence of an up-to-date reviews containing a consolidated and systematic account of apatites. The present review has been an attempt to compensate for this lacuna.