5.1. Introduction

Biology and Biophysics are linked up with chemistry. No physical method alone could establish the structure of biological complex molecules without chemical investigations. Chemistry solves these problems by studying the structure of substances. The biochemistry has almost taken up an independent path by studying the science concerned with the structure and properties of biological molecules, with the progress of chemical reactions in living organisms.

The union of biochemistry and biophysics is the molecular biology. Molecular biology deals with the physico-chemical and molecular interpretations of basic biological phenomena. At the same time organic chemistry turned to living matter on the basis of long experience of investigations of organic compounds. Thus arose bio-organic chemistry and bio-inorganic chemistry whose concern is the study of biological molecules containing metal atoms.

Biological molecules are built up of atoms of light elements C, H, O, N, P, S. Besides, the functionality of ions of alkali and alkaline earth metals (Na, K, Ca, and Mg) is universal in organisms. An equal important role is played by the small amounts of other metals like Fe, Zn etc.
As far as organic matter is concerned, the major portion of the integuments (skin, nail, hair, horn, hoof, feather, etc.) is made up of albuminoid proteins called keratins. These fibrous proteins are characterised by their very high insolubility in usual protein solvents, because of high sulphur content which is in the form of cystine. The keratin molecule consists of closely packed polypeptide chains, which are held together by the disulphide bond of cystine. To some extent, the solubility and digestibility of such proteins can be improved by grinding them into fine powder by mechanical means. This supports the resistance to solvents and enzymes being associated with the close packing of the chains. The eukeratins which contain the aminoacids histidine, lysine and arginine in addition to other aminoacids are found largely in horn, hoof, hair, nail etc. The ratio in which histidine, lysine and arginine present in eukeratins, are nearly 1:4:12 apart from 3 to 5 percent of sulphur. The pseudo keratins are found in skin and nerve tissues containing comparatively less percentage of sulphur.

The X-ray diffraction pattern of a crystal is unique, and it is only necessary to measure diffraction angles and intensities of the peaks of the diffractogram of the sample concerned and to compare the results with these from a sample of the pure substance. The difficulty with human nail arises from the fact that the lines are not properly present but the broad peaks are visible.

Klement (1929) and Tromel (1932) suggested that the X-ray diffraction pattern of bone salt must closely resemble that of hydroxyapatite. The surface area of inorganic crystallites is so large that absorption on their surfaces of carbonates and other ions could readily take place. Hendricks and Hill (1950, 1951) considered this aspect very carefully and came to the conclusion that the bone crystals consisted of
a mineral calcium phosphate, probably hydroxyapatite, with carbonate, citrate, sodium and magnesium ions absorbed on their surfaces.

Bell et al (1947) carried out a series of mechanical, chemical and X-ray diffraction tests on rat bones, concluded that there was no disturbance of the fundamental plan of ossification in rachitic bones.

The crystal morphology of calcium carbonate deposits in the molluscan shells was studied employing powder pattern X-ray crystallography by Bevelander and Benzer (1948) and Lutts et al (1960).

Louis Winand et al (1961), by combining data obtained from Infrared spectrometry, X-ray diffraction and chemical methods of investigation, have established the general formula of the apatite calcium phosphate series as

$$\text{Ca}_{10-x} \text{H}_x \text{(PO}_4\text{)}_6\text{(OH)}_{2-x}$$

Where the values of $x$ varies from 0 to 2

Cookson and Filling (1976) analysed the trace element distribution across the diameter of human hair by proton induced x-ray method.

Grympas (1976) determined the degree of crystallinity of human femoral cortical bone, from 16 to 83 years of age, by various X-ray and IR methods. The results showed that the crystallinity of bone material varies between 51 and 59%, with no significant change with age. Besides this, a new X-ray line was noted at $2\theta = 43^0$ in bone of age greater than 50 years.

Landis (1979) reviewed a number of investigations, carried out in his laboratory utilizing high resolution X-ray microanalysis and anhydrous method of specimen preparation.
Kennedy et al (1985) analysed the quantitative distribution of calcium in human skull tissue by scanning soft X-ray microscopic mapping.

Regulenkova et al (1986) carried out the X-ray diffraction studies of the collagen of marine invertebrates collected from different oceans. A similarly is found in the diffraction patterns of the collagen of invertebrates and vertebrates.

Annapuran et al (1989) carried out the X-ray diffraction studies for the molluscan shells collected from different sea shores (kakinada, vizag and Madras) of India. They determined the lattice parameters of Caco3 deposits in shells and also correlated these parameters with the composition of the environment.

Newton et al (1992) determined the bone composition by X-ray scattering technique. They also found the intensities of scattered radiations as a function of modules of scattering vector.

Astbury (1931) made X-ray studies on hair, nail, horn etc. and reported that these structures gave rise to submicroscopic elongated crystallites of keritans lying roughly parallel to the fiber axis.

Astbury and Woods (1933) carried out the X-ray studies on the structure on hair, wool and related fibers and also the molecular structure and elastic properties of keratins of hair. Several hundreds of X-ray photographs of mammalian hair were taken and they reported that stretched hair consists of the same chains in some regularly folded configuration.

Zertz et al (1970) determined the trace elements in hair by X-ray fluorescence method, while during the same year Robbins and Kelly (1970) studied the cosmetically altered hair for the amino acids.
Hirdy and Howard (1973) studied the biochemical variation of hair keratins from various racial groups employing different techniques like aminoacids analysis, acrylamide gel electrophoresis, x-ray diffraction studies and stress and strain analysis. Each of these techniques yielded identical results for all samples of man and non-human primates.

Kubo Hideo (1981) determined the trace element concentration, by using a simple method of x-ray fluorescence analysis in hair.

Pillay and Kins (1981) analysed human hair by proton induced x-ray emission and discussed the pattern recognition of trace elements composition.

Garson et al (2000) were observed three layers (characterized by different orientations of the keratin molecules) from the outer to the inner side of human nail by synchrotron X-ray microdiffraction. These layers are associated with the histological dorsal, intermediate and ventral plates. Using X-ray micro-diffraction, they showed that onychomycosis disrupts the keratin structure, probably during the synthesis phase.

Busson et al (1999) showed that, keratinous tissues play two major roles in the adaptation of vertebrates to their environment: a strong mechanical support and a chemical barrier. In order to determine whether these properties may originate from different zones in the tissues, microdiffraction experiments on the micrometre scale have been carried out on feather shaft, horse and human hair, and porcupine quill samples. The existence of several structural layers has been revealed in all the tissues, some corresponding to highly ordered α- or β-type keratin and the others to more or less amorphous keratin. The existence of lipid granules has also been evidenced, mainly in the outer layers. This study shows one of the possibilities which are now offered by third-generation
synchrotron sources for the structural microanalysis of biological tissues.

Meriem Er Rafik et al (2006) investigated the structure of the human hair follicle by means of X-ray microdiffraction. They conclude that the histology-based growth zones along the follicle are correlated to the fine architecture of the filaments deduced from X-ray microdiffraction. Their analysis reveals the existence of two major polymorph intermediate filament architectures.

John et al (1970) found that, a harlequin fetus was born to unrelated parents with a negative family history for any form of ichthyosis. The child was grossly deformed, showing thick plaques on the cutaneous surface, and survived only 48 hours. Pathological studies of the skin showed marked thickening of the stratum corneum, but no other distinctive changes. X-ray diffraction analysis of the horny layer revealed the presence of a cross-β fibrous protein rather than the usual - α protein.

A search of literature reveals that X-ray data on the male and female human nail is not available. Hence, an attempt has been made to identify the inorganic constituents, crystallinity, size distribution of human nail mineral by applying the technique of X-ray diffraction.

5.2. Material and Methods

For the study of x-ray diffraction of the human nail, male and female volunteers were selected. The finger nails are allowed to grow up to 12 weeks i.e. 84 days in female volunteers and nearly 11 weeks i.e. 75 days in male volunteers. The grown free edge nails (whitish grown part from the tip of nail) were cut smoothly and nail samples collected were washed and used to study the x-ray diffraction. For the XRD analysis, smooth
powder of both the specimens, were obtained by rubbing on file. The powder was collected in clean polythene packets for XRD analysis.

The quantitative X-ray analysis for the study of crystalline structure of human nail mineral is based on the measurement of relative intensities of X-ray reflections.

Bruker D8 Advance X-ray diffractometer was used with start $2^0$ and end $79.998^0$. The step time is 54.4 s. Anode with the Cu–WL1:1.5406 for the study of crystal structure. The X-ray diffractometer Bruker D8 Advance is originally a dedicated powder diffractometer.

**Powder diffractometer**

The instrument has an x ray source, which consists of anode, x ray tube and a target. The x ray source is connected to power supply with 40KV. The x ray beam emitted by source is collimated, frequency filtered and compressed. With the help of gobel mirror and V- groove (crystal) the beam is collimated and made monochrome. The V-groove is a germanium crystal which gives only cu-k$_{a1}$ beam. With the microprocessor controller, two goniometers with 100 mm and 200 mm circle concentric to the sample are fixed. The sample stage whose height can be adjustable capable of holding sample up to 50 mm thick and 20 mm diameter is fixed at the center of goniometer circle. The sample is placed on the sample stage, the x ray beam is incident on the sample and diffracted beam is received by the scintillation detector. In front of detector, computer control absorber is placed to attenuate the beam by 2 orders of magnitude.

The diffractometer is interfaced to a PC that controls the sample alignment and experiments. The software is able to run various types of
scans that can even be combined to a sequence and executed one after the other. A complete set of experiments can be performed on one sample fully automated. Data visualization during experiments as well as the processing afterwards is provided by adequate software tools including data treatment like merging, background correction etc. Diffractograms were taken for the analysis (Fig.5.1. and 5.2).

5.3. Results and Discussion

Many biominerals are complex composite materials constructed from a variety of inorganic and organic components. The organized array of amorphous or crystalline components in an organic matrix is under varying degrees of biological control during the process of biomineralisation.

In many organisms, the combination of nucleation and growth strategies give rise to composite materials with functionalized mechanical properties. The investigation of these materials has important industrial and biotechnological potential. The crystallographic properties of inorganic constituent and the role of the organic matrix may differ widely in different system and are classified into four types as:

1. Biocomposites like chiton teeth comprise randomly oriented crystals of heterogenous size distribution and structure. Their morphology is determined by physiochemical properties of the mineralization zone.

2. Biocomposites arise from matrix mediated process of site directed nucleation but with little control being exerted over crystal growth. Growth occurs along directions established by oriented nucleation and the absence of growth modulation results in a heterogenous
particle size distribution and morphologies that are characteristics of inorganic precipitates. For example, the avian egg shell is constructed from the oriented nucleation of calcite on the inner shell membrane.

3. This category is confined to biocomposites comprising amorphous mineral phases. In these materials the matrix is active in both nucleation and vectorial growth.

4. There are the most complex and highly organized biocomposites which combine matrix–mediated nucleation and vectorial growth regulation. These processes are coordinated spatially and temporally such that oriented composites are constructed. Crystal size, morphology, structure and growth orientation are precisely controlled. For example the nacreous inner surface of many molluse shells.

X-ray diffraction analysis is being extensively employed in the study of biological systems, especially in the geometrical description of their molecular components. The importance of X-ray diffraction analysis in the areas of DNA and proteins structure is well known. The method, however, has also been of considerable value in the study of mineralised tissue. Several examples can be cited to illustrate the important role of X-ray diffraction analysis played in the study of hard tissue. In the nineteenth century, the mineral of osseous tissue has been known to be a calcium phosphate. But in the year 1989, the X-ray diffraction analysis of horn and hoof showed that the presence of calcium phosphate crystal structure is amorphous in nature (Rama Rao, 1989).
The present study on X-ray diffraction analysis of human nail mineral is aimed to understand the following aspects.

1. Identification of inorganic constituent of human nail.
2. Crystallanity of human nail mineral and protein.
4. Preferential Orientation of crystallites.

The X-ray diffractograms of the human nail (Fig.5.1 and Fig.5.2) exhibit no characteristics peaks pertaining to inorganic materials. It appears that the presence of inorganic material in human finger nail, whether it is of male or female, is scanty, hence no question of identification of inorganic constituents in human nail. Also the evidence of the crystallinity, size distribution and preferential orientation of crystallite is ruled out.

However calcium, an element found in large quantities in bones and in proportional smaller quantities in nails (chapter 2). Although the nail is composed of calcium, mainly located on the surface of the nail plate, the potential benefit of supplemental calcium was questioned. Many patients do not consume adequate amounts of daily calcium; thus supplementation should be recommended to these patients.
Fig. 5.1. X-ray diffractogram of human nail for male
Fig. 5.2. X-ray diffractogram of human nail for female
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