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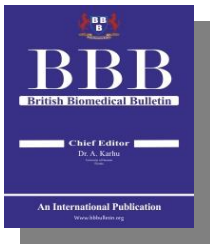

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In-vitro Aluminium Uptake by the Bone and its Effect on Bone Mineral

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A B S T R A C T

In-vitro aluminium uptake by the bone and its effect on bone mineral has been studied at different Al^{3+} concentrations viz., 500, 1000, 1500, 2000, 2500 and 3000 ppm and for different time periods viz., 30 and 60 days. Leg bone of goat was used for the study. Results revealed that Al uptake by the bone is concentration dependent as well as time dependent. In 30 days, the aluminium uptake varied from 0.66 to 3.25 mg/g, and in 60 days, it varied from 1.19 to 5.48 mg/g, depending on Al^{3+} concentration (500 to 3000ppm) of the milieu. X-ray diffraction studies of experimental (Al exposed) and control (without Al exposure) samples of bone powders reveal a change in position (2Θ) and d-spacings of the peaks of calcium hydroxylapatite mineral of the bone by Al exposure. X-ray diffraction pattern of synthetic calcium hydroxylapatite, prepared in the absence and presence of Al^{3+} also revealed similar changes brought about by aluminium. The infrared (FTIR) spectra of experimental and control bone powders revealed a change in the position of symmetric and asymmetric P-O stretching vibrations of phosphate bands by Al exposure. Similar changes in P-O bands were also observed on comparing the infrared spectra of synthetic calcium hydroxylapatite prepared in the absence and presence of aluminium.
Thus, it seems aluminium, uptaken by the bone, disturbs the mode of phosphate bonding in the calcium hydroxylapatite mineral of the bone and thus, distorts its usual crystal structure. This might be the chemical mechanism behind aluminium osteotoxicity.

Introduction

Aluminium is the third most abundant element in the earth's crust. Despite abundance, aluminium does not have any useful biological function. It has been rather proved to be toxic to human body[1-17]. Aluminium has been implicated as a potential neurotoxic factor in different pathological conditions[6,7]. Aluminium may enter into the body through food, water or airborne dust particles. Recent studies indicate that most individuals, on an average, consume about 1-10 mg Al/day from different natural sources. The extensive use of aluminium cookware and food packaging material, as well as use of aluminium salts in food additives and some drugs, provide other potential sources of aluminium ingestion.

Aluminium toxicity has also been implicated in osteoporosis and osteomalacia. Faulty mineralization of calcium hydroxylapatite over the bone matrix might be the factor. Aluminium either interferes in functioning of osteoblastic activity or it might be directly replacing the ca^{2+} in the apatite crystals and thus causing defect crystal structure of hydroxylapatite. The osteomalacia due to aluminium toxicity has been attributed to the localization of aluminium along the calcification front[18-20]. In cases of Al-related osteomalacia bone turnover is decreased so that typically there is minimal active production of matrix by "plump" osteoblasts and minimal osteoblastic bone resorption. Both of these features reflect the suppression of parathyroid hormone (PTH) secretion either directly by Al or else indirectly by the effect of hypercalcaemia[21]. The development of hypercalcaemia is promoted by a number of factors including the presence of aluminium at the calcification front where it blocks the uptake of calcium into bone. Aluminium might also be inhibiting the enzymes involved in mineralization such as alkaline phosphatase[22]. The exact mechanism by which aluminium causes defective mineralization is yet to be clearly established. The Al uptake by the bone has also not yet been well quantified.

With the above views in mind, we have presently studied the *in-vitro* aluminium uptake by the bone at different Al^{3+} concentrations and time periods. Effect of Al uptake on the bone mineral has also been studied through spectral data.

Materials and Method

All chemicals used were of A.R. (Analytical Reagent) quality. All solutions were prepared in distilled water. Al^{3+} solutions of 500 ppm, 1000 ppm, 1500 ppm,2000 ppm,2500 ppm and 3000 ppm were prepared separately, using aluminium sulphate, $Al_2(SO_4)_3.16H_2O$.

The leg bone of goat was procured from the local slaughter house. The bone was washed with distilled water and cut into small pieces. The bone pieces were separately weighed out. Next, the weighed bone pieces were placed separately in conical flasks containing 100 ml of Al^{3+} solution of different concentrations. The work was carried out in two sets. In set-I, the bone pieces were left in Al^{3+} solutions for 30 days, and in the set-II, the bone were left in the solutions for 60 days. One control set each, in both the experiments (30 days and 60 days), were also set up. The various experimental sets were as follows:

Set-I (30 days)

I. 100 ml distilled water and bone (control set).
II. 100 ml 500 ppm Al^{3+} solution and bone.
III. 100 ml 1000 ppm Al^{3+} solution and bone.
IV. 100 ml 1500 ppm Al^{3+} solution and bone.

V. 100 ml 2000 ppm Al^{3+} solution and bone.
VI. 100 ml 2500 ppm Al^{3+} solution and bone.
VII. 100 ml 3000 ppm Al^{3+} solution and bone.

Set-II (60 days)

I. 100 ml distilled water and bone (control set).
II. 100 ml 500 ppm Al^{3+} solution and bone.
III. 100 ml 1000 ppm Al^{3+} solution and bone.
IV. 100 ml 1500 ppm Al^{3+} solution and bone.
V. 100 ml 2000 ppm Al^{3+} solution and bone.
VI. 100 ml 2500 ppm Al^{3+} solution and bone.
VII. 100 ml 3000 ppm Al^{3+} solution and bone.

After the experimental period (30 days or 60 days, as the case may be), the bone were removed from the solutions, washed well with distilled water and dried at room temperature.

The bone pieces were then powdered for analytical and spectral studies.

Estimation of Aluminium

A known weight of bone powder was decomposed with 10 ml of conc. HNO_3 and evaporated to dryness. The dry residue was extracted with distilled water to a known volume (100 ml) into a measuring flask. Aluminium in the solution was estimated spectrophotometrically using Eriochrome Cyanine R reagent[23].

Preparation of Calcium hydroxylapatite

50 ml of calcium acetate solution (0.05M) was added drop wise from a burette into a 50 ml solution of ammonium dihydrogen phosphate (0.03M) taken in a beaker, maintaining the pH between 7.2 and 7.4. A 0.05M NaOH solution was added drop wise to maintain the pH during precipitation. CO_2-free N_2 gas was bubbled through the reaction mixture to eliminate the possibility of formation of carbonate apatite. The reaction mixture was refluxed for 4 hours and then left overnight (well covered) at room temperature. The precipitate was filtered and washed with CO_2-free distilled water till the washings were free from ammonium salts. The precipitate was dried at 100°C and preserved over fused calcium chloride.

Preparation of Calcium hydroxylapatite in the presence of $Al^{3}+$

35 ml calcium acetate solution (0.05M) and 15 ml Al^{3+} solution (0.05M) solution were added drop wise from separate burettes into a 50 ml solution of ammonium dihydrogen phosphate (0.03M), maintaining the pH between 7.2 and 7.4. A 0.05M NaOH solution was added drop wise to maintain the pH during precipitation. CO_2-free N_2 gas was bubbled through the reaction mixture to eliminate the possibility of formation of carbonate apatite. The reaction mixture was refluxed for 4 hours and then left overnight (well covered) at room temperature. The precipitate was filtered and washed with CO_2-free distilled water till the washings were free from ammonium salts. The precipitate was dried at 100° C and preserved over fused calcium chloride.

X-ray diffraction Spectra

X-ray diffraction spectra of the samples of bone powders (experimental and control sets) as well as those of synthesized calcium hydroxylapatite samples were recorded on a Diffractometer system XPERT-PRO and type 0000000011023505.

FTIR Spectra

FTIR spectra of the samples of bone powders (experimental and control sets) as

well as those of synthesized calcium hydroxylapatite samples were recorded in KBr phase in the range of 4000-450 cm^{-1}, on a Excalibur HE3600 Infrared Spectrophotometer.

Results and Discussion

In-vitro aluminium uptake by the bone, exposed to different Al^{3+} concentrations and time periods is recorded in Table-1. The X-ray diffraction spectra of some samples of bone powders (experimental and control sets) and of synthetic hydroxylapatite samples are shown in fig. 1 to 4 .The infrared (FTIR) spectra of some samples of bone powders (experimental and control sets) and of synthetic calcium hydroxylapatite samples are shown in fig. 5 to 8.

A study of Table-1 reveals that the *in-vitro* uptake of aluminium from its aqueous solution by the bone is both, time dependant and concentration dependant. The uptake of aluminium by the bone has been found to be from 0.66 to 3.25 mg/g in 30 days depending on the concentration of Al^{3+}. At a low concentration of 500 ppm, the Al uptake was 0.66 mg/g; whereas at 3000 ppm the uptake was 3.25 mg/g. A six fold increase in Al^{3+} concentration has led to a five fold (appx.) increase in Al uptake. In the experiment of 60 days, it is seen that at 500 ppm Al^{3+}, the uptake is 1.19 mg/g. At 3000 ppm Al^{3+}, the total uptake in 60 days has been found to be 5.48 mg/g. It looks that at a given concentration the doubling of time period exposure almost doubles Al uptake also. Thus it is clear that Al uptake is more or less proportional to exposure time. It looks, even at 30 days exposure the bone is not completely saturated with respect to Al. If the exposure is continued beyond 30 days, the uptake is also continued. This means aluminium has enough space/sites on the bone where it can be absorbed/lodged, and the absorption is a slow process. Indirectly, it leads to an understanding that probably aluminium has something to do with the principal mineral of the bone (calcium hydroxylapatite) in the process of its uptake. Possibly aluminium replaces calcium from its site in the apatite crystal. Such a situation would result in aluminium doped calcium hydroxylapatite crystal in the bone and this aluminium doped mineral would definitely be not as strong and thick as pure apatite mineral. This is because Al^{3+} has a smaller radii (0.50 A°) compared to Ca^{2+} (0.99 A°). The bonding of Al-phosphate would also be different from that of Ca-phosphate. The aluminium doped apatite crystal is expected to be very much distorted as compared to that of pure calcium hydroxylapatite. The result of this distortion might lead to osteoporosis and probably osteomalacia also.

X-ray diffraction studies

A study of X-ray diffraction patterns of bone exposed to 3000 ppm for 60 days and its corresponding control (exposure to only water, without Al^{3+}) suggest that there is definite crystal distortion in Al exposed bone as compared to that of control. A comparative study of the x-ray diffraction pattern of laboratory synthesized calcium hydroxylapatite in the absence and presence of aluminium also suggest that aluminium leads to distortion of hexagonal lattice pattern of hydroxylapatite.

The relative intensity of the peaks at a given position (2Θ) were found to vary widely. The corresponding d-spacings have also been found to differ between Al exposed and non-exposed (control) bones. Similar variation of relative intensity of peaks at different positions and corresponding d-spacings were found between the diffraction patterns of pure synthetic calcium hydroxylapatite and aluminium doped calcium hydroxylapatite (calcium hydroxylapatite synthesized in

presence of Al^{3+}). All these suggest encroachment of aluminium into the crystal lattice of calcium hydroxylapatite mineral of the bone.

Infrared studies

Bone is a complex material consisting of inorganic and organic compounds. The inorganic portion is mainly calcium hydroxylapatite mineral. The organic portion consists of a number of proteins and other compounds. Collagen is the principal matrix protein of the bone. The infrared spectra of bone, particularly in the finger print region, would be much complex with a number of peaks and bands. Interpretation and assignment of bands would rather be difficult. Interpretation of spectral changes of bone upon interaction with external ions would also be quite difficult. Presently, a comparative study of bands in the infrared spectra of aluminium treated bone samples and the corresponding untreated (control) samples show that two bands at 3085 and 870 cm^{-1} in the control sample are missing in the spectra of treated sample. On the other hand, a new band at 2400 cm^{-1} appears in the treated sample. This suggest that Al^{3+} has definitely interacted with the bone and brought about some structural changes. Probably Al^{3+} has interacted with the apatite mineral of the bone and disturbed the vibrations of phosphate bonds. The symmetric P-O stretching vibration would be Raman active in an ideal tetrahedral symmetry of ionic phosphates. However, in a non-equivalent force field, this band becomes infrared active[24], occurring at ~ 900 cm^{-1}. In a complex material like bone, the P-O symmetric stretch could be expected to be IR active because of possible covalent interactions of phosphate. Presently, the P-O symmetric stretch showed as a split band with two components at 922 and 870 cm^{-1} in the bone (control set). The split might be due to the coordinated nature of phosphate in the bone[24]. In the spectra of Al treated bone, one of the component at 870 went missing while the 2nd component at 922 shifted up by 4 cm^{-1} and showed at 926 (much farther from 900 cm^{-1}). This suggest that phosphate is still coordinated in the aluminium treated bone but the Al treatment has affected the phosphate bands to some extent. This indirectly suggest that it the apatite part of the bone where Al attacks and distorts the apatite microcrystal structure in the bone. The asymmetric P-O stretching vibration usually appears at around 1100 cm^{-1} in the phosphates[25,26]. This band is likely to be shifted and/or split up due to additional interaction. Presently in the infrared spectra of bone (control), the asymmetric P-O stretching vibration appears as two bands at 1385 and 1260. Upon Al^{3+} treatment the 1385 component went missing suggesting some changes in the mode of P-O vibrations. All these facts (observations) suggest that aluminium has interacted with the apatite mineral of the bone and distorted its structure. Such distortion is probably the cause of aluminium induced porocity of the bone (osteoporosis). The aluminium- apatite interaction is also perhaps the cause of aluminium induced osteomalacia.

A comparative study of the infrared spectra of synthesized calcium hydroxylapatite in the absence and presence of aluminium also suggest that the phosphate vibrations (symmetric and asymmetric P-O stretch) have been affected as a result of interaction of Al with the apatite. The symmetric P-O stretch occurring at 869 in the pure apatite has showed up as two bands at 870 and 927 cm^{-1} in the spectra of aluminium- interacted apatite (the sample of apatite synthesized in the presence of Al^{3+}). The asymmetric P-O stretch occurring at 1114 and the 1217 in the pure calcium hydroxylapatite was also found to undergo change upon interaction with

aluminium. The band at 1114 went missing and 1217 band shifted down to 1215. These changes in asymmetric P-O stretch bands upon Al- interaction with the apatite further suggest that aluminium, when present in the milieu, distorts the bondings of phosphate in calcium hydroxylapatite. Such changes in P-O bonding of the apatite mineral of the bone, and resulting structural distortion of the mineral, might be the chemical mechanism behind aluminium osteotoxicity.

Conclusion

Our present studies suggest that *in-vitro* aluminium uptake by the bone depends on the concentrations of Al^{3+} of the milieu as well as on the time period of exposure. The Al uptake was found to vary from 0.66 to 3.25 mg/g in 30 days, and 1.19 to 5.48 mg/g in 60 days, depending upon Al^{3+} concentration (500 to 3000ppm) of the milieu. As evidenced by x-ray diffraction and infrared spectral studies, the aluminium ions seem to distort the crystal structure of calcium hydroxylapatite mineral of the bone, probably, by changing the mode of phosphate bonding in the mineral. This might be the chemical mechanism behind aluminium osteotoxicity.

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Table-1. *In-vitro* Aluminium uptake by the bone

S. No.	Al^{3+} Concentration (ppm)	Al-uptake (mg/g)	
		In 30 Days	In 60 Days
1.	Blank	0.00	0.00
2.	500	0.66	1.19
3.	1000	1.27	3.58
4.	1500	2.16	4.50
5.	2000	2.28	4.59
6.	2500	2.85	5.37
7.	3000	3.25	5.48

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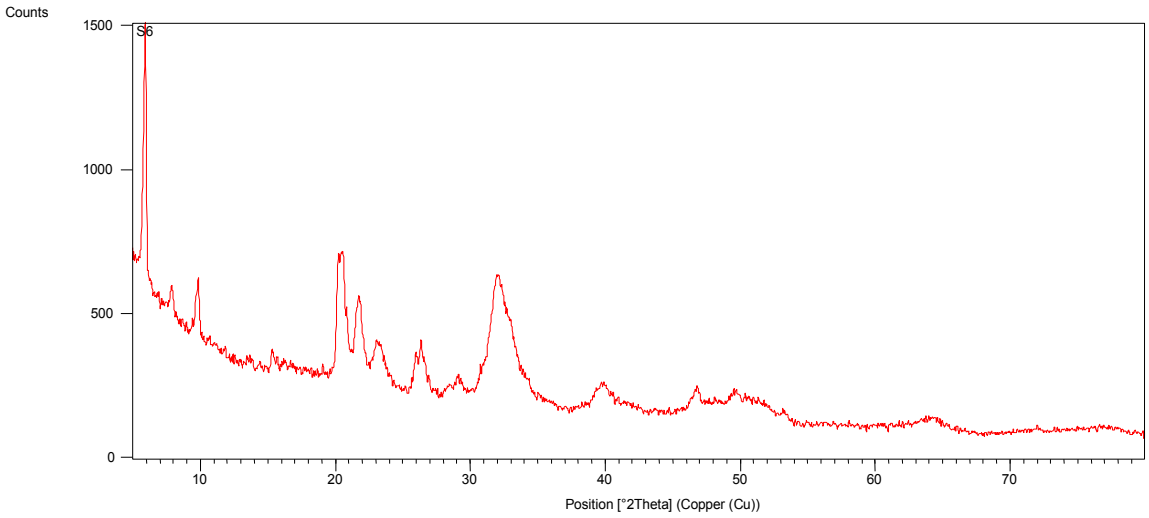

Figure 1. X-ray diffraction spectra of bone sample (control)

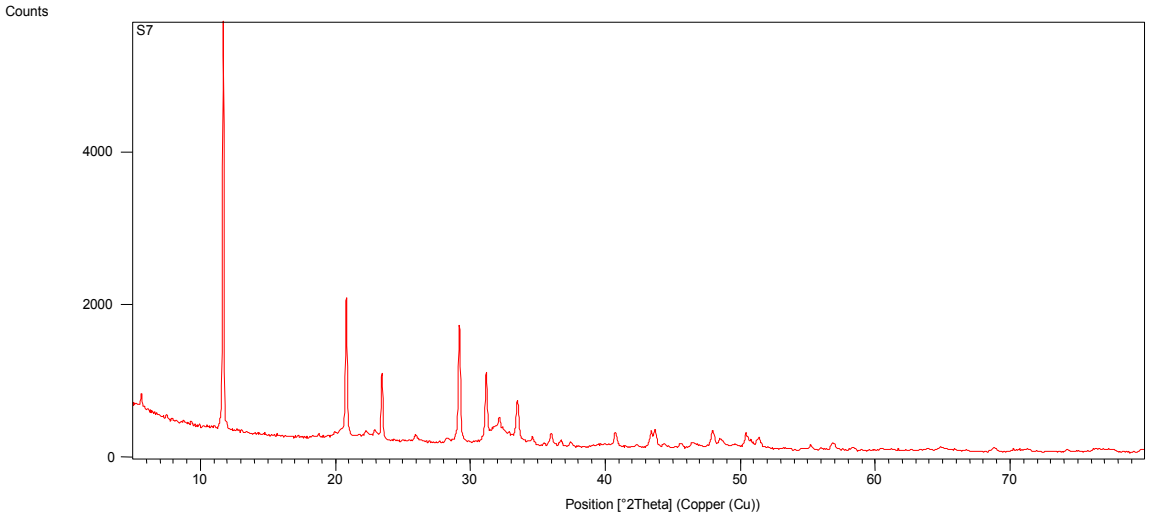

Figure 2. X-ray diffraction spectra of bone sample exposed to Al^{3+} (3000 ppm) for 60 days

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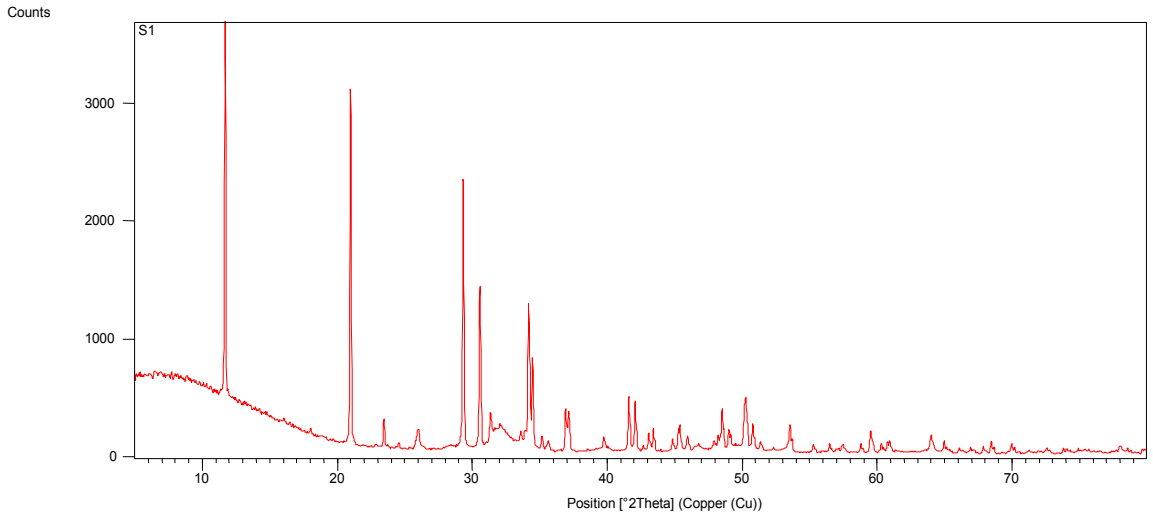

Figure 3. X-ray diffraction spectra calcium hydroxylapatite

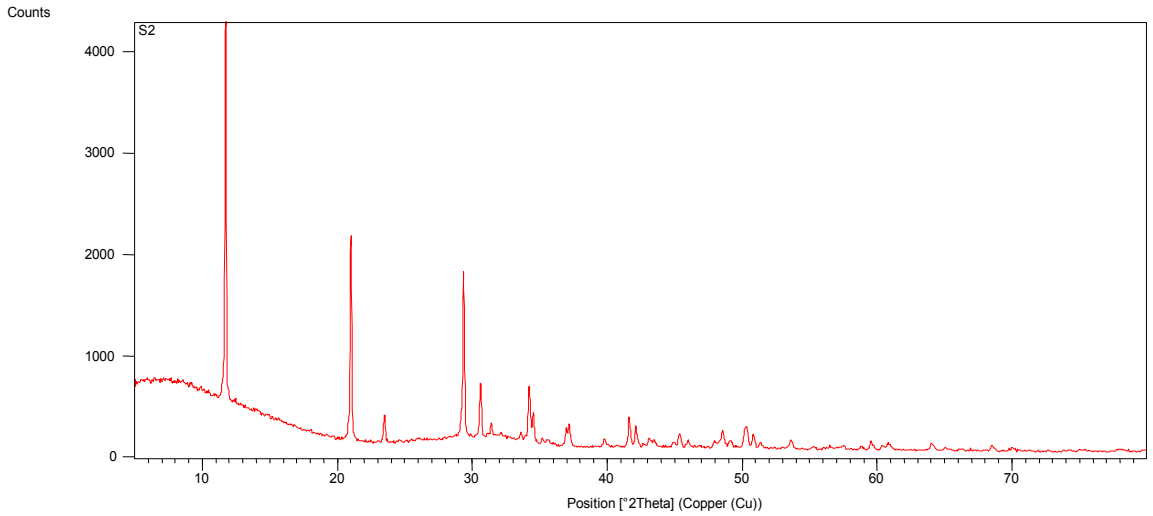

Figure 4. X-ray diffraction spectra of calcium hydroxylapatite prepared in the presence of Al^{3+}

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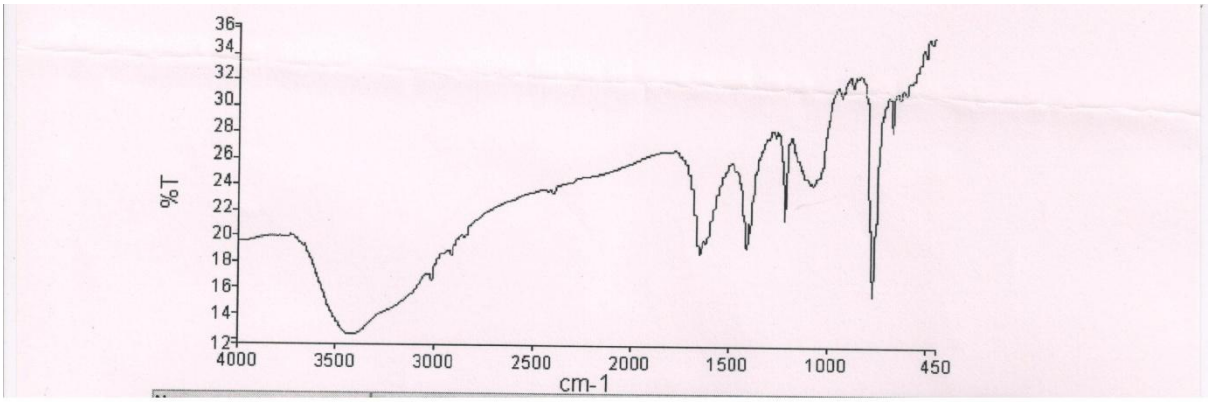

Figure 5. Infrared spectra of bone sample (control)

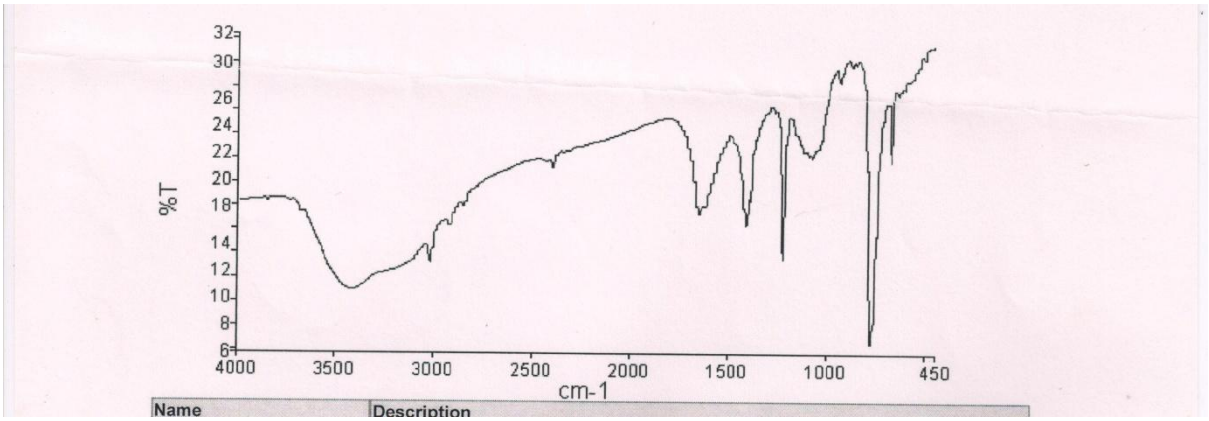

Figure 6. Infrared spectra of bone sample exposed to Al^{3+} (3000 ppm) for 60 days

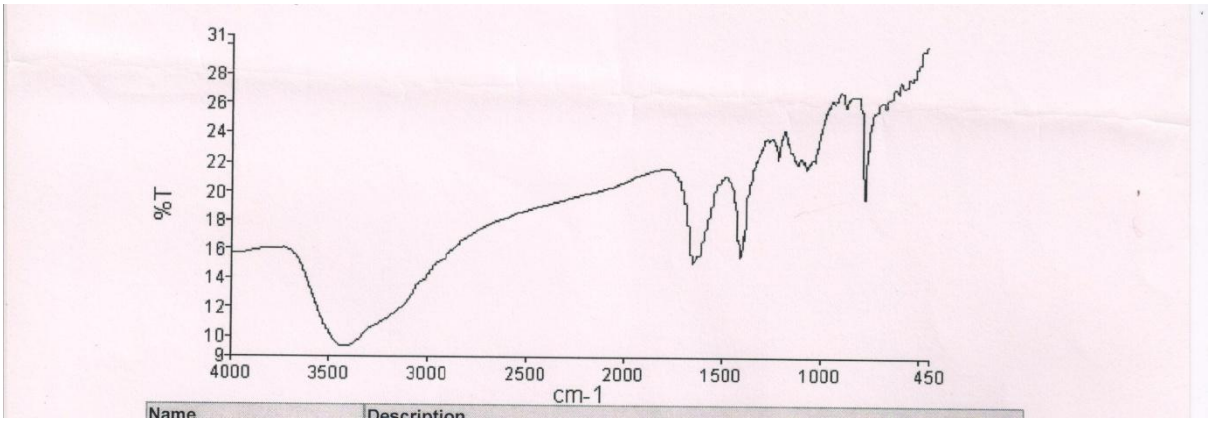

Figure 7. Infrared spectra of calcium hydroxylapatite

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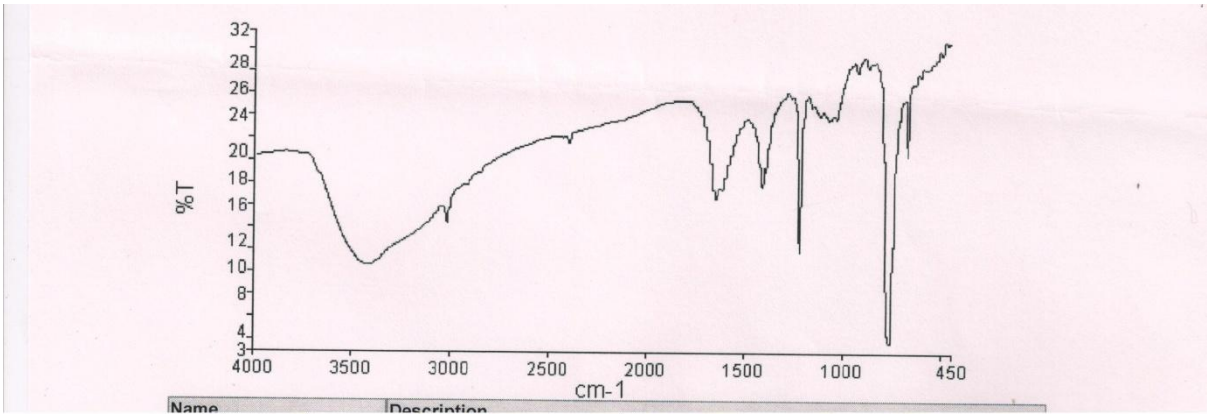

Figure 8. Infrared spectra of calcium hydroxylapatite prepared in the presence of Al^{3+}