The Egyptian Journal of Hospital Medicine (April 2014) Vol. 55, Page 129–136

Effect of Electron beam on Prepared HAP-Gel Composition

Naima A. El Gendy*,Tawfik M. S., and Asma M. Nour*****

Radiation Polymer Chemistry Department*, Radiation Health Research Department*, National Centre for Radiation Research and Technology (NCRRT) and Radioisotope Department, AEA, Egypt ***.

ABSTRACT

Background: Polyvinyl alcohol liquid PVAl was used as the organic carrier for Hydroxylapatite-gel (Hap-gel) composite. PVAl has the ability to form a nano- hydroxylapatite polyvinyl alcohol composite gel which has a wide range of uses in different environmental and medical applications. Prepared Hap-gel is known to have a very similar composition to human bone and is used as a substitute for bones in compound fractures and artificial dentures.

Matreia and Methods: In this work prepared HAP- gel was exposed to a high ionizing radiation electron beam (5 kilo Gray) and an aqueous solution containing aluminum ions (Al^{+}). Some investigations were done to illustrate the effect of radiation exposure and aluminum contamination on prepared Hap-gel.

Results: Energy dispersive X-ray analysis (EDx) showed that the electron beam used caused an obvious increase in the calcium ions (Ca^{++}) content of the prepared Hap-gel from 60% to 65.69 % with a prominent decrease in phosphorus ions (P^{+}) content from 40 % to 34.31 % in addition to an increase in the Ca/P ratio from 1.5 to 1.91. Exposure of the pre-irradiated Hap-gel samples to aluminium ions (Al^{+}) resulted in a noticeable decrease in Ca^{++} content from 65.69 atomic % to 32.14 % atomic % and a further noticeable decrease in P^{+} content from 34.31 % atomic % to 13 atomic % as well as an increase in the Ca/P ratio from 1.91 to 2.47. The levels for the original prepared Hap-gel were Ca^{++}; 60 atomic % and P^{+}; 40 atomic %. It was deduced that exposure of the Hap-gel to Al^{+} had a further damaging effect on the pre-irradiated Hap-gel composition in addition to the damaging effect that the electron beam used induced on the samples.

Conclusions: it could be concluded that electron beams and Al^{+} have an injurious effect on human bone tissue taking into consideration the similarity in composition between Hap-gel and bones. Therefore, this study could be beneficial in the field of osteoporosis research and assist the understanding of the effects of radiation such as that of electron beams and some pollutants such as aluminium present in running water on the health of human bone tissue.

Keywords: Hap-gel, Calcium, Phosphorus, Aluminium, Electron Beam.

Introduction

Osteoporosis is a systemic skeletal disease responsible for the high occurrence of fractures in older subjects, particularly in postmenopausal women. Its increasing incidence with population ageing and prolonged life expectancy raises the rates of linked morbidity, loss of independence, and mortality. Bone mineral density (BMD) and preceding fracture history are two main risk factors allied with osteoporosis such as the presence of prior fractures can predict future fractures [(1)]Osteoporosis is a common disease with a strong genetic component characterized by reduced bone mass, faults in the micro-architecture of bone tissue, and an increased risk of fragility fractures. Twin and family studies have shown high heritability of BMD and other factors related to fracture risk such as ultrasound properties of bone, skeletal geometry and bone remodeling.

Vulnerability to osteoporosis is ruled by many different genetic variants and their interaction with environmental factors such as diet and exercise [(2)].

Several epidemiological studies have shown a positive correlation between osteoporosis and low socioeconomic status. A

 DOI: 10.12816/0004497

study on Chinese populations showed a positive correlation between illiteracy and low socioeconomic status as a demographic factor and the high prevalence of osteoporosis and fractures among those populations [3].

There also has been a growing concern about the use of aluminum utensils which is an inherent practice in populations of low socioeconomic standard and the consequent release of aluminum ions in food and water consumed by populations using aluminum utensils during the cooking of such food especially with new utensils during the first time of cooking [4].The concentration of aluminium in natural water can vary prominently depending on various physicochemical and mineralogical factors. Dissolved aluminium concentrations in water with near-neutral pH values usually range from 0.001 to 0.05 mg/litre but rise to 0.5–1mg/liter in more acidic water or water rich in organic matter. At the extreme acidity of waters affected by acid mine drainage, dissolved aluminium concentrations of up to 90 mg/litre have been measured (WHO, 1997) [5].

Calcium phosphate ceramics such as hydroxyapatite (HAP) gel [Ca_{10} $(PO4)_6(OH)_2$] is one of the most effective biocompatible materials and is found to be a major component of bones. These materials are the most promising implant coating materials for orthopedic and dental applications due to their good biocompatibility [6]. The superior biocompatibility of calcium phosphates is contributed by their compositional resemblance with the bone mineral that has allowed them to be used [7].

The aim of the resent *in vitro* study was to investigate the effect of aluminum ions (a common water and food pollutant) on hydroxyapatite (HAP) gel samples pre-irradiated with an electron beam. The Hap-gel used in this study served as a model for human bone tissue when exposed to irradiation or aluminium ions.

Material and Methods

Chemicals:-

The chemical used were, polyvinyl alcohol 5% (PVA), Calcium nitrate, diammonium hydrogen phosphate (anhydrous), ammonium hydroxide, (0.1M); and aluminium chloride (0.05M) purchased from Merk, Germany.

The preparation and irradiation experiments:-

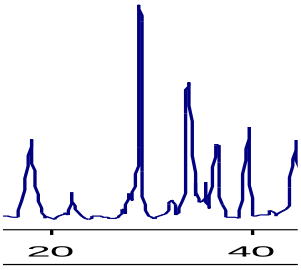
The preparation procedure followed in this study in order to create nano Hap-gel samples was essentially the same as that described by other authors using the wet precipitation technique [8]. Afterwards, the prepared gel samples were exposed to an electron beam at energy of 5 kilo Gray (kGy)/minute and the duration of exposure was 60 seconds per sample. Another experiment was carried out to assess the effect of aluminium ions (a water pollutant found in old water pipes or dissipated from cooking utensils) on the pre-irradiated Hap-gel. After treatment with the 5kGy electron beam, the Hap-gel samples were exposed to an aqueous aluminium chloride (0.05M) solution. Ten duplicate samples were used for each treatment.

Analysis:

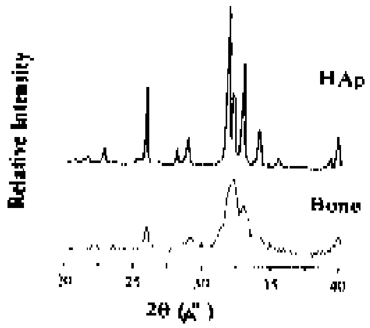
The morphology of hydroxyapatite gel (Hap-gel) was studied using a scanning electron microscopy unit 400(SEM; JEOL-JSM) supplied with energy dispersive X-ray analysis (EDX; Oxford) to detect the distribution of anions and cations quantitatively before and after irradiation by an electron beam and also after adding Al^+ ions. Crystallization of the formed structure was studied using an X-ray diffraction instrument (XRD). Hap-gel was precipitated on polyvinyl alcohol [8] then exposed to the electron beam at an energy of 5 kGy/min. for a minute and the changes were investigated by EDx .

RESULTS

Yoshioka [7] prepared Hap-gel and compared it to natural bone using an x- ray diffraction instrument. They found that its composition was similar to bone in its peaks. The Hap-gel prepared in this study was also found to be very similar to that prepared by Yoshioka. Figure 1 shows the similarity between the Hap-gel prepared in this study and the bone-like gel prepared by the author mentioned above.



Prepared Hap-gel



Hap-gel prepared by Yoshioka [7].

Fig (1): X Ray Diffraction for the prepared Hap-gel compared to that for natural bone.

The results showed a noticeable increase in the Ca^{++} content associated with a high decrease in the P^{+} ion content of the irradiated samples in comparison to the non-irradiated ones. These results are illustrated in table 1 as well as figures 1& 2.

Table (1): Hap-gel atomic % before and post-irradiation by a 5kGy electron beam.

Hap-gel	Elements	Atomic %
Before Irradiation.	Ca^{++}	60 %
	P^{+}	40 %
	Ca/P Ratio	1.50
After Irradiation with a 5kGy electron beam.	Ca^{++}	65.69 %
	P	34.31 %
	Ca/P Ratio	1.91
Percent change for Ca^{++}	9.4 %	
Percent change for P^{+}	14.2 %	
Percent change for Ca/P Ratio	27.3 %	

Each value represents the mean atomic % of 10 duplicate samples

Table (1) shows Hap-gel atomic composition and the prominent increase occurring in Ca^{++} content and the noticeable decrease in P^{+} content before and after irradiation using the electron beam accelerator.

Fig. (2): EDX for Hap-gel before irradiation.

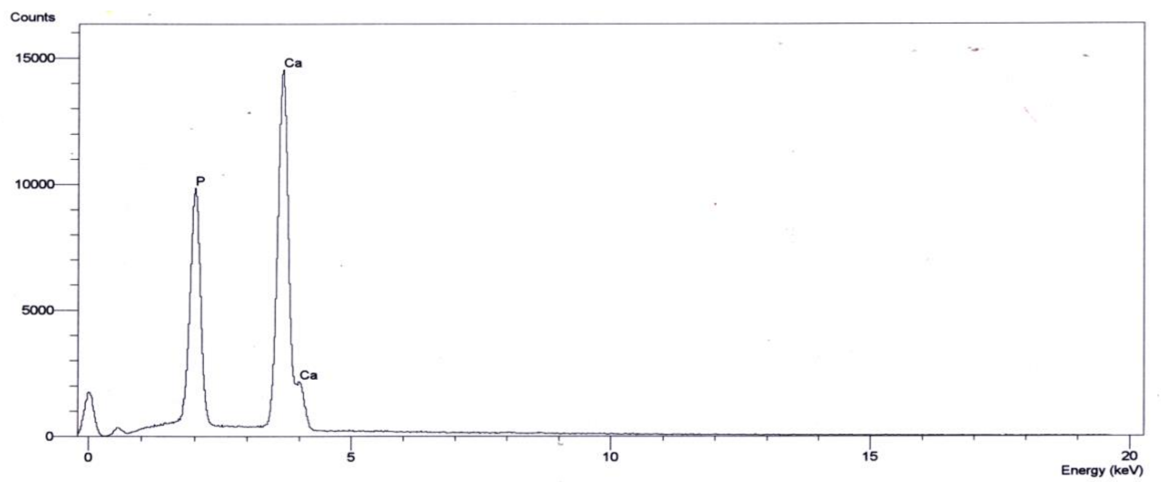


Fig (2) displays the atomic % of P^+ and Ca^{++} ions respectively in the prepared Hap-gel before irradiation (as detected by EDx).

Fig. (3) EDX for Hap-gel after irradiation.

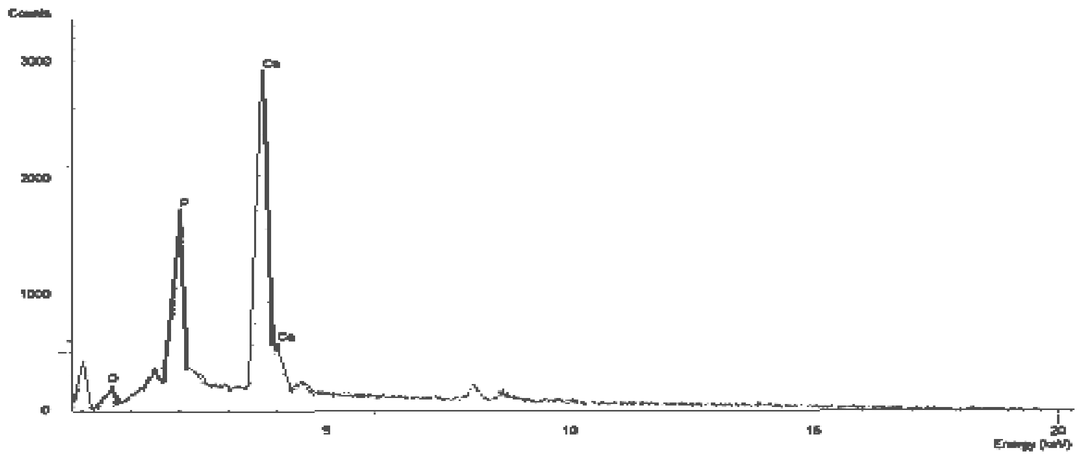


Fig (3) illustrates the decrease in atomic % of P^+ and Ca^{++} ions respectively in the prepared Hap-gel after irradiation by a 5kGy electron beam as detected by EDx.

The results also demonstrated that the composite was able to remove Al^+ ions from the added aqueous solution. Al^+ ions were absorbed by the Hap-gel composite and were found to cause a considerable decrease in the Ca^{++} and P^+ ion content of the pre-irradiated samples. The decrease in Ca^+ and P^+ ions content was found to be highly noticeable when the pre-irradiated samples exposed to Al^+ were compared to the non-exposed ones. These results are illustrated in table 2 and figures 4 &5.

Table 2: Pre-irradiated Hap-gel Ca^{++} and P^{+} atomic % before and after treatment with Al^{+}.

Hap-gel	Elements	Atomic %
Sample pre-irradiated with a 5kGy electron beam	Ca^{++}	65.69 %
	P^{+}	34.31 %
	Ca/P Ratio	1.91
After addition of Al^{+} solution (0.05M) to pre-irradiated samples.	Ca^{++}	32.14 %
	P^{+}	13 %
	Al^{+}	54.86 %
	Ca/P Ratio	2.47
Percent change for Ca^{++}	51.03 %	
Percent change for P^{+}	62.11 %	
Percent change for Ca/P	29.3 %	

Each value represents the mean atomic % of 10 duplicate samples

Table (2) demonstrates the substantial decrease in Ca^{++} and P^{+} Hap-gel atomic % after irradiation by a 5kGy electron beam and the subsequent addition of an aqueous aluminium solution (determined by EDx).

Fig. (4) Atomic % of AL^{+}, P^{+} and Ca^{++} respectively in Hap-gel after pretreatment with the electron beam followed by addition of Al^{+}.

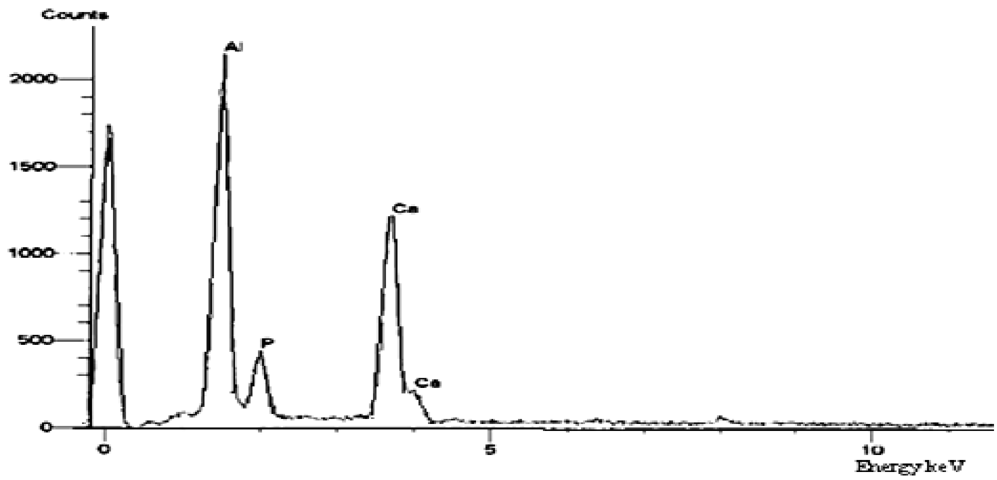


Fig (4) illustrates the obvious changes in atomic % of AL^{+}, P^{+} and Ca^{++} in the prepared Hap-gel after pretreatment with a 5kGy electron beam and the addition of Al^{+} ions in an aqueous solution.

Fig. (5) EDX for untreated Hap-gel compared to Hap-gel either treated with electron beams (5kGy) alone or combined with Al^{+} ions.

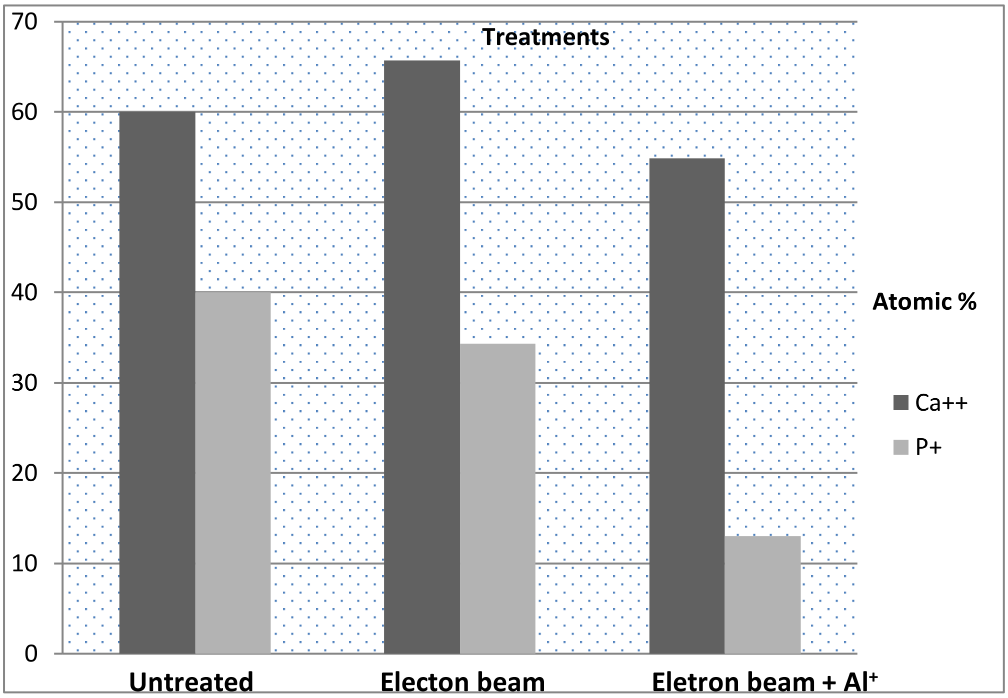
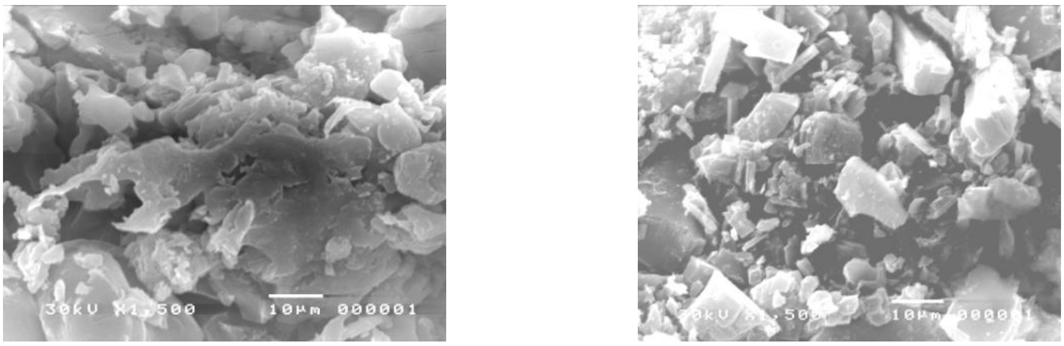


Figure (5) reveals the noticeable changes in the atomic % for Ca^{++} and P^{+} ions in Hap-gel samples exposed to all the treatments used

The SEM for Hap-gel exhibited a widespread damage occurring to the composite after exposure to the electron beam followed by the addition of Al^{+} ions. This damage is displayed in figure 6 .

Fig.(6) SEM for Hap-gel composite



Before uptake of Al^{+} After uptake of Al^{+}

Fig.(6) displays the SEM for Hap-gel highlighting the damage occurring to the prepared Hap-gel composite after pretreatment with a 5 kGy electron beam and the uptake of Al^{+} ions from the aqueous solution by the composite.

Discussion

Substantial research work has focused on the development of artificial bone and teeth that mimic natural bones and teeth and at the same time do not cause harm to human tissue. Synthetic ceramic materials based on calcium phosphate (CaP) chiefly in the composition of tricalcium phosphate [TCP-Ca3(PO4)2] and hydroxyapatite [HACa 10(PO4)6(OH)2] have extensively been investigated and clinically used. Biomaterials studies have concentrated on the production of these ceramics for 30 years for applications in the fields of orthopedics and dentistry [(8)].

The Hap-gel prepared in these experiments was found to be very similar to that prepared by Yoshioka [(7)] (as detected by XRD studies) who found a strong similarity between their prepared Hap-gel composite and natural bone.

Electron beam irradiation of the prepared Hap-gel material at an energy of 5 kGy resulted in a visible increase of the Ca^{++} content associated with a clear loss of the P^{+} ion content as well as an increase in the Ca/P ration as detected by EDx. This result is confirmed by a another study concerning the effect of electron beam irradiation on Hap-gel where exposure of the composite to an electron beam resulted in a considerable loss of the phosphorus content of the irradiated material linked with an increase in the Ca/P ratio resulting in its subsequent demineralization [(9)].

The most important effect of aluminum toxicity in the body occurs in the skeletal system of patients on dialysis for end-stage renal disease receiving excessive aluminum from the dialysate fluid and oral intake of aluminum hydroxide taken as a phosphate binder to prevent hyperphosphatemia. Parkinson [(10)] and Ward [(11)] first showed direct evidence that there is a linkage between aluminum and bone mineralization with increase in under-mineralized osteoid volume and increased bone aluminum content. Aluminum even in minute amounts crosses the dialyzing membrane causing gradual accumulation in patients undergoing dialysis. With the addition of orally administered aluminum containing compounds as phosphate binders in the gastrointestinal tract, the excessive aluminum is deposited in bone [(12-13)]. The same situation occurs in peptic ulcer disease patients with high aluminum ingestion from antiacids[13]. Alfrey [(12)] stated that aluminum loading of bone occurred when untreated water was used from community water sources with naturally occurring high aluminum content, or aluminum was used as a compound to purify water.

The present study indicated that Al^{+} ions caused a sizeable decrease in the calcium and phosphorous ion content of the pre-irradiated Hap-gel accompanied by a substantial increase in the Ca/P ratio causing extensive damage to its composition (as shown by SEM). AL^{+} ions had a more distinguished damaging effect on the composition of the bone-like material compared to exposure to the electron beam per se.

The present results are in agreement with those [(14-15)] who found that aluminium may inhibit bone formation; reducing mineralization and bone matrix formation. Other studies also established that aluminium may contribute to the production of reversible bone lesions by physically interfering with the formation of naturally occurring hydroxyapatite [(16)]. On the other hand, these results are contradictory to those of another study that concluded that no relationship existed between an increase in aluminium content of trabecular bone and the loss of bone mass or affection of its mineralization [(17)].

It could be concluded from our *in vitro* study that the hydroxyapatite portion of bone tissue including its phosphorus and calcium content is sensitive to electron beam irradiation and pollution by aluminium ions dissipated in water and food. Measures should therefore be taken to protect human bones from X-ray exposure or any food and water resources contaminated with pollutant elements such as aluminium.

REFERENCES

1) Cooper C (2010): Osteoporosis: disease severity and consequent fracture management. Osteoporosis Int., 2: 425-429.

2) Stuart HR and André GU (2010): Genetics of osteoporosis. Endocr Rev., 31(5): 629–662.

3) Keramat A, Khalilifard A, Adibi H, Chopra A, Kunjir V and Patwardhan B (2005): Association between demographic factors and osteoporosis in urban Iranian postmenopausal women. J Reprod Infertil., 6(1): 98-106.

4) Semwal AD, Padmashree A, Khan MA, Sharma GK and Bawa AS (2006): Leaching of aluminum from utensils during cooking of food. Sci. Food Agri., 86: 2425.

5) WHO (1997): Aluminium. Geneva, World Health Organization, International Program on Chemical Safety (Environmental Health Criteria 194).

6) Predoi D, Vatasescu-Balcan RA, Pasuk I, Trusca R and Costache M (2008): Calcium phosphate ceramics for biomedical applications. Journal of Optoelectronics and Advanced Materials, 10 (8): 2151 – 2155

7) Yoshioka M, Yoshida Y, Inoue S, Lambrechts P, Vanherle G, Nomura Y, Okazaki M, Shintani H, and Van Meerbeek B (2002): Adhesion/decalcification mechanisms of acid interactions with human hard tissues. J Biomed Mater Res., 59(1):56-62.

8) Santos MH, Oleveira M, Souza LM, Mansour HS and Vasconcelos WL (2004): Synthesis Control and Characterization of Hydroxyapatite Prepared by Wet Precipitation Process. Materials Research, 7(4): 625-630

9) Bilton M, Brown AP, and Milne SJ (2010): Sol-gel synthesis and characterisation of nano-scale hydroxyapatite. J. Phys. Conf. Ser, 241(1): 1-5.

10) Parkinson IS, Ward MK and Feest EG (1979): Fracturing dialysis osteoarthropathy and dialysis encephalopathy. An epidemiological survey. Lancet, 1: 407-409.

11) Ward MK, Feest EG and Ellis HA (1978): Osteomalacia dialysis osteodystrophy: evidence for waterborne etiological agent, probably aluminum. Lancet, 1:841-845.

12) Alfrey AC (1993): Aluminum toxicity in patients with chronic renal failure. Therapeutic Drug Monitoring, 15:595-597.

13) Sebes JI, Pinstein M and Massie JD (1984): Radiographic manifestations of aluminum induced bone disease. AJR, 142:424-426

14) Rodriguez M, Felsenfeld AJ and Llach F (1990): Aluminium administration in the rat separately affects the osteoblast and bone mineralization. J. Bone Miner. Res. 5:59-67.

15. Posner AS, Blumenthal NC and Hislop JS (1986): Model of aluminium-induced osteomalacia: Inhibition of apatite formation and growth. Kidney Int. 29:S17-S19.

16) Meyer JL and Thomas WC (1986): Aluminium and aluminium complexes: Effects on calcium phosphate precipitation. Kidney Int., 29:S20-S23.

17) Hellstrom, HO, Mjoberg, B, Mallmin, M, and Michaelsson K (2006): No association between aluminium content of trabecular bone and bone density, mass or size of the proximal femur in elderly men and women. BMC Musculoskelet Disord, 7: 69.