

# Mineral Content and Physicochemical Properties in Female Rats Bone During Growing Stage

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## 1. Introduction

The atomic absorption spectrophotometry (AAS) is a technique extensively used for trace and ultra trace analysis of organic and inorganic materials. AAS is a novel method that determines in liquid samples the presence of metals such as: Ca, Fe, Cu, Al, Pb, Zn, and Cd from different sources. The determination of minerals is important in environmental and biological studies as well as in the clinical practice. The determination of mineral content is a key to understand changes in some metabolism that conduct to diseases as result of the increase or decrease of mineral components in diet and consequently, to develop new models in the field of animal and human nutrition.

In the case of biological samples including plant leaves, fruits, vegetables, organic vegetables i.e. nopal (*Opuntia ficus indica*), Rodríguez-García et al., (2007) reported that the major mineral components in this cactus was as follows: Calcium, Magnesium, Sodium, Manganese, Iron, Zinc, Potassium, and minor mineral components were: Lithium, Vanadium, Cobalt, Arsenic, Selenium, Cadmium, Thallium (Hernández-Urbiola et al., 2010, 2011). AAS has been used also to analyze muscle tissue, blood, urine, hair, bones, among others (Martínez-Flores et al., 2002; Christian, 1972). In most cases complex nature of biological materials requires dry ashing followed by wet digestion with oxidizing acids, i.e. HNO<sub>3</sub> and HCl<sub>4</sub>.

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The use of AAS spectroscopy to the study of nutrimental components in food specially Ca has been reported by Rojas-Molina et al., (2009), Palacios-Fonseca et al., (2009). Cornejo-Villegas et al., (2010), analyzed calcium content in instant corn flours obtained during traditional thermo-alkaline-treatment of corn kernels (nixtamalization), as well as, commercial corn flours and corn flours supplemented with a vegetable i.e. Nopal. AAS technique is an excellent tool to determine anti-nutrimental compounds that compromise the bioavailability of some important minerals such Ca in diet. Contreras-Padilla et al., (2011) use the AAS to study anti-nutrimental components in fresh nopal and nopal powders as a function of the maturity stage. They found that calcium oxalates decreases as the maturity stage increases, while the total Ca increases. This fact could be an indicative of a better bio-availability or bio-accessibility of Ca in diet. The determination of minerals as nutrimental factors in food have also been an important issue in the development of new products and to establish physicochemical criteria for food processing, i.e. Rojas-Molina et al., (2007) and Gutiérrez-Cortez et al., (2010) reported that Ca content in nixtamalized corn kernels is associated with gelatinization of the starch granules. Additionally, Galvan-Ruiz et al. (2007) used AAS to study the incorporation of Ca as a food additive in food.

In Biological studies AAS has been used to determine changes in minerals content, Brem et al., (2004) studied mineral changes in blood and ash bone, as well as the bone mineral density in three different regions of the body, and the minerals related to the osseous tissue activity. This study was carried out in order to evaluate the osteoporosis type I, that is associated with vertebral compression and hip fracture on ovariectomized rats in contrast with normal rats. They analyzed different ways to ameliorate the health alterations. Findings showed that the bones of ovariectomized rats had significant decreases in Ca, P, and Mn respect to the control group, but no differences were found in the case of Cu and Mg between these groups. They also reported significant differences in bone mineral density in ovariectomized rats compared to the control group, after 6 and 9 months of treatment.

On the other hand, for inorganic materials as is the case of bones; the AAS has been used to determine their mineral contents. This technique have been used in order to analyze the most important bone minerals as Ca, Mg and Zn in human studies, and Ca and Mg contents in animals (Ma et al., 2000). In the field of nutrition it is well known the importance of the Ca/P ratio for the bone formation. Ca is determined using AAS, where P has been determined colorimetrically (Nowicka et al., 2006). The human skeleton develops through infancy, childhood and adolescence and the peak bone mass is achieved during maturity. High bone mass density (BMD) at skeletal maturity is the best protection against age-related bone loss and fractures, the loss of BMD conduce to develop osteoporosis (Heaney, 1993). This degenerative disease is associated with a decrease of minerals in bone. Fractures occur when skeleton is exposed to minimal or moderate trauma (Matkovic, 1991).

Several researches have demonstrated that Ca and P play an important role in skeletal mineralization and also on peak bone mass (Masuyama et al., 2003; Matkovic & Ilich, 1993). Both minerals are present in hydroxyapatite (HAp), this component  $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$  constitutes seventy percent of bone. Collagen and the inorganic mineral HAp, combine, providing hardness and mechanical strength to the bone (Smith et al., 1983). From a nutrimental point of view, an adequate intake of Ca in the human diet during growth is a critical factor for acquiring and maintaining an adequate bone mass, the regulation of body weight; protect organism against high blood pressure and certain types of cancer (Rodríguez-Rodríguez et al., 2010). Ščančar et al., (2000), studied the mineral content in

human bone from the iliac crest, using AAS. The concentration of Al, Cu, Fe, and Ca was reported for normal individuals. They found that microwave method for digestion procedure was appropriate technique for dissolution of bone biopsy samples and will be an excellent method for evaluated trace elements in bone samples from dialysis patients.

Martinez-Flores et al., (2002) found that the femurs of rats fed with diets based on corn (tortilla), and with a Ca/P ratio at 1.1, were higher in length, and they were heavier, thicker than bones of rats fed with a Ca/P less than 1. Mexican diets are high in P with Ca/P ratio higher than recommend for an optimal bone development. Bean and tortillas are the primary source of energy and minerals in the typical Mexican diet. Wyatt et al., (2000), studied the effect of different Ca/P ratios in Mexican diets on the growth and composition of rat femurs; they found that levels of dietary Ca affect bone mass and Ca bone deposition to a much greater extent than dietary P levels.

Environmental applications of AAS are also important because these are close related to the health in individual and community. The determination of contaminants in water, air, and soil are fundamental in order to improve treatments or to resolve problems that affect the human and animal health. On the other hand, in the case of cultivars the soil mineral content is the most important condition to ensure a well grown and development of plants.

## 2. Bone mineral content

The amount of minerals in a human bone is an important parameter which is of interest for many reasons. The measurement of this parameter could be used, for example, to diagnose and follow the treatment of various demineralizing diseases, such as osteoporosis and osteomalacia. The skeleton of the human body is a complex matrix that suffers changes along life; these changes are related to the incorporation of several minerals into the matrix and this is a kinetic process that is affected by factors such as hormones, lifestyle, nutrition amount others. Peak bone mass in humans is achieved after sexual maturity and is then maintained for two decades. Thereafter, the mass of virtually all bones declines until death. Thus, it has been established that Ca deposition in bone in the growing stage contributes to the prevention of generated bone diseases (Takahara et al., 2000). The bone mineral composition consists of Ca and P in amorphous and crystalline fractions as a major components, as well as Ma, Na, Zn, K, as minor elements. According to Blokhuis et al., (2000), mineral components are composed of approximately 30% amorphous calcium phosphates and around 70% fine crystalline HAp  $[Ca_{10}(PO_4)_6(OH)_2]$ .

The bone is a matrix constituted by minerals immerse in a collagen matrix, both determine its physical and functional properties. There are several methods to evaluate the mineral content in a bone sample. Direct evaluation through AAS and inductively couple plasma (ICP), and an indirect manner by using single or dual X-ray diffraction and computer tomography, to obtain the bone mineral density and the bone mineral content that involves in both cases the use a calibration sample or a matrix. In the second case, it is possible to obtain an average information about the density and mineral content by in a semi quantitatively manner (Bonnick, 2010). By using direct methods, it is possible to obtain quantitative information regarding to each one of the bone minerals components, while using indirect methods the information is close related the optical absorption of the bone tissues. The measurement of BMD has been performed during the last decade, and different technologies have been used for this purpose. These technologies include: computer

tomography (CT) scans (Langton et al., (2009), ultrasound (Higuti & Adamowski (2002), and dual energy x-ray absorptiometry (Norcross & Van Loan, 2004).

Bone loss could induce osteopenia or osteoporosis; this loss may be due to increased bone resorption and decreased bone formation. Osteoporosis with a decrease in bone mass is widely recognized as a major public health problem. Pharmacological and nutritional factors may have the potential to prevent bone loss with increasing age. Nutritional factors may be especially important in the prevention of osteoporosis, although this is poorly understood (Ma et al., 2000). Several studies have been done to establish the role of minerals in bone. Bone stores 99% of the body's Ca, and calcium salts are responsible for the hardness of bones. Mg is not only a critical ion in mammals as a cofactor for many enzymes of the energy extraction system and protein synthesis pathways, but it is also necessary for bone formation. Thus, Mg might also play an important role in bone structure or the hardness of bone (Takahara et al., 2000). Zn, an essential trace element, has been demonstrated to have a potent stimulatory effect on bone formation and an inhibitory effect on bone resorption. Zn can stimulate protein synthesis in osteoblastic cells in vitro by activating aminoacyl tRNA synthetase. The oral administration of a Zn compound can prevent bone loss in ovariectomized rats, an animal model of osteoporosis. (Ma et al., 2000).

HAp is a crystalline compound constituted of three molecules of calcium phosphate and one molecule of calcium hydroxide. HAp concentrate is the matrix of Ca from bone protein found in the unprocessed bone. This natural substance contains about 14% collagen protein and 4% other proteins and small amino acids (especially hydroxyproline, glycine and glutamic acid). Ca comprises between 24-30% of the hydroxyapatite matrix and together with several minerals (Zn, K, silicon manganese, and Fe) is a group active bio-available Ca (Gómez et al., 2004). The HAp and dibasic calcium phosphate are the only calcium phosphate phases that are chemically stable to temperature and pH of the human body (37 °C and about 7, respectively). The mineral part of bone that support nearly all the organic and the mechanical load (collagen) acts as a bonding material, which also absorbs shock, providing flexibility to the bones; Additionally to mechanical functions, bones have an essential role in metabolic activity as mineral reservoirs that is able to absorb and release ions.

Skeletal allow mobility of the body and protect internal organs. While the properties of bone tissue and the proportions of the constituent minerals vary with the different parts of the skeleton. Bone is specialized tissue that changes along the life. The bones, teeth, cells, fat, and natural polymers such as: polysaccharides, collagen, and polyphosphates are composed by minerals. It can be considered that bones contain about two thirds of inorganic material and a third of portion of organic matter. Bone tissue is composed of a mineral phase of 69% of its total weight, 9% water and 22% of an organic matrix, which in turn is composed mainly of collagen (90-96%). In terms of structure, bones can be considered as a dispersion of mineral particles (bio-minerals) embedded in an organic matrix, which forms the contiguous phase. Bioapatite  $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})]$  is considered the major component in the mineralized part of mammalian bone, is a calcium-phosphate biomineral with a structure that closely resembles HAp (Meneghini, 2003).

Bone mineral composition depends of several factors such as: age, nutritional condition, diseases and habits. These factors make difficult to establish the composition and structure of HAp (Meneghini, 2003). HAp is able to store and release minerals as calcium, phosphorous and several other ions such Na, K, Mg, F,  $\text{CO}_3$  and OH. For this reason HAp is an important mineral reservoir for the metabolic activity of the organism. Atomic absorption

technique combined with other techniques such as ICP and UV-VIS are adequate methods to determine mineral contents in bones. Ca is the most important mineral in HAp because > 90% of the this element in the human body is deposited as calcium-phosphate within the skeleton and teeth; the apatite crystals are one of the major constituents in bone and other mineralized vertebrate tissues; their presence, which accounts for about 65 % of weight bone, provides most of the stiffness and strength of bone. With the normal increase of apatite deposition during tissue aging and maturation, bone mechanical properties increase greatly. The physical character of bone, including the morphology, dimensions, and distribution of its composite apatite crystals, affect its mechanical properties (Su et al., 2003).

### 3. Biological studies

The rat constitutes a well established model for study the bone health research and the preclinical evaluation of agents used in the prevention or treatment of osteoporosis (Frost & Jee, 1992; Ke et al., 2001). Consequently, Ca and P content as majority minerals in rat bones constitutes and adequate tool to evaluate bone formation and development during growing stage and to correlate mineral content with peak bone mass.

#### 3.1 Animal model

Fifty four female Wistar rats, 21 days old, and  $50 \pm 4.3$  g of weight, from the bioterium of the Neurobiology Institute, UNAM, Queretaro, Qro., Mexico were fed with a control diet (AIN 93G, Reeves et al., 1993 ) and water, and fed *ad libitum* during 8 weeks. The rats were individually placed in stainless steel cages under controlled temperature ( $25^{\circ}\text{C} \pm 1$ ) and light conditions (12:12 h light-dark cycle), and the body weigh was weekly verified. The rats were cared for in compliance with the "principles of Laboratory Animal Care" formulated by the National Institutes of Health (National Institutes of Health publication no. 96-23, revised 2003) and the protocol was approved by the Ethics committee of the Universidad Nacional Autónoma of México.

#### 3.2 Experimental protocol

Six female rats were sacrificed each week by carbon dioxide aspiration. The left and right femoral bones were dissected and introducing in paraformaldehyde solution (4%) in order to eliminate the adhering bone tissue; after that, they were introducing in a buffer solution to eliminate paraformaldehyde excess and finally in distilled water in order to eliminate the residual buffer and soft tissues were removed. Following, bones were dried at  $90^{\circ}\text{C}$  for 12 hours (Farris & Griffith, 1949). The length and weight of the dry femurs were measured according to Gómez-Aldapa et al., (1999) methodology. Subsequent bones were charred in order to obtain a sample without organic component eliminated at  $550^{\circ}\text{C}$  for 4 h, immediately powders were passed for a sieve US 60 ( $250 \mu\text{m}$ ) to carry out ray diffraction experiments and also to AAS measure.

#### 3.3 Physical development of rats

The rats in this study were maintained under controlled environmental conditions: temperature, light cycles, water consumption. The physical development of rats is related to the changes in the skeletal and muscle, which depends drastically on four factors: genetic, feeding conditions, mechanical (living conditions as physical activity), and hormonal.

(Fernández-Tresguerres et al., 2010). However, changes in one of these parameters have important effects in the development and maintenance of the bone.

Figure 1 shows the increases of the weight for female rats from 3 to 11 weeks that corresponds to the called self- accelerating phase. According to the data showed in this figure the changes in body weight of the rats in this period is around 600%. This curve exhibits two different behaviors: for the first 4 weeks, the growth rate is bigger than for the final 4 weeks. After the week 11 according to Tamaki et al., (1995) the rats are in the adult phase in which their organs and functions reach the state call adult phase. This curve represents only the self-accelerating phase and is important because this can be considered as a normal reference curve for future clinical or biological studies. This accelerating phase is the period where individuals are more sensitive to the adverse effects that influence their future development for long-time. Although, the individual survives, some physiological adaptations have to appear in the adult phase, and one of them could be directly related to the bone formation and mineralization.

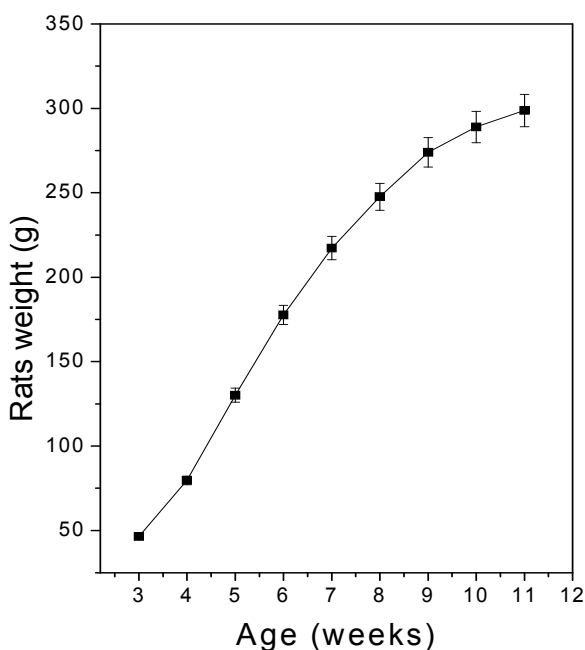


Fig. 1. Body weight for female rats as a function of the age.

A very important aspect related to the rats development, are the hormonal changes where thyroid hormone and parathormone, calcitonin, growth hormone, estrogen, progesterone, and calcitriol are indispensable for the bone development and osseous remodeling (Bogden et al 2008). In order to study the changes in the inorganic-organic bone matrix, the weight and the length of the femur (right-left) were obtained. As was mentioned, bone is a matrix composed by fat, protein, and minerals. It means that changes in these constituents affect the final physicochemical composition of the bones.

Figure 2.a and b shows the femur weight and length of female rats during the growth stage. As can be seen, these physical characteristics have the same trend found in the case of total weight. It is important to remark, that a change in the slope of this curve occurs between the 7<sup>th</sup> and 8<sup>th</sup> week. The environmental conditions, as well as feeding and exercise or physical activity, and hormonal changes are fundamental to explain this behavior. At the beginning of the growing, the rats exhibit a mayor physical activity due to the dimension of the cage (Martin, 2000). On the other hand, the hormonal activity in the case of male and female rats plays an important role in their physicochemical development that includes muscle and bones. In female rats, estrogen is one of the major regulating factors responsible for bone formation and maintenance and the loss of this hormone after menopause often leads to bone loss in the case of human (McMillan, 2007).

### 3.4 Evaluation of mineral content in femurs

Ca, Na, K, and Mg content in the femurs rats were determined according to the dry-ashing procedure A.O.A.C. 968.08 (1998), using lanthanum chloride. The concentration was measured with a double beam atomic absorption spectrometer (Analyst 300 Perkin Elmer, USA) equipped with a deuterium lamp, background corrector and a hollow cathode lamp. The equipment was operated with 12 psi of dry air, 70 psi of acetylene, a 422.7 nm flame, a 10 mA. lamp current, and a 0.7 nm slit width.

In order to have a precise determination of minerals content in the rats bones as a function of the age, a certified minerals bone ash was used as calibration standard. The standard of bone ash used in this experiment was certified by the National Institute of Standards & Technology (NIST), with Standard reference Material 1400. This standard reference material (SRM) is intended for use in evaluating analytical methods for the determination of selected major, minor, and trace elements in bone and in material of a similar matrix.

The certified concentrations of the constituent elements are shown in table 1. These concentrations are based on the results of a definitive analytical method or the agreement of results by at least two dependent analytical methods. In this table, it was included the mineral content obtained for the same sample using AAS in our laboratory. Our results are in good agreement with those reported by NIST.

ELEMENT	CONCENTRATION (wt. Percent)	AAS CONCENTRATION (wt. Percent)
Calcium	38.18 ± 0.13	37.70± 0.11
Phosphorous	17.91 ± 0.19	18.02 ± 1.29*
Magnesium	0.684 ± 0.013	0.638 ±0.021
Iron	0.066 ± 0.003	N/A
Potassium	0.0186 ± 0.001	0.0195 ±0.002
Strontium	0.0249 ± 0.001	N/A

Table 1. Certified concentrations of constituent elements for NIST bone ash sample, and experimental values obtained using AAS. \*Phosphorous value was obtained by UV-VIS. These concentrations are based on the results of a definitive analytical method or the agreement of results by at least two dependent analytical methods. Additionally Phosphorous in femurs was determined according to the A.O.A.C official methods 985.35 (2000).

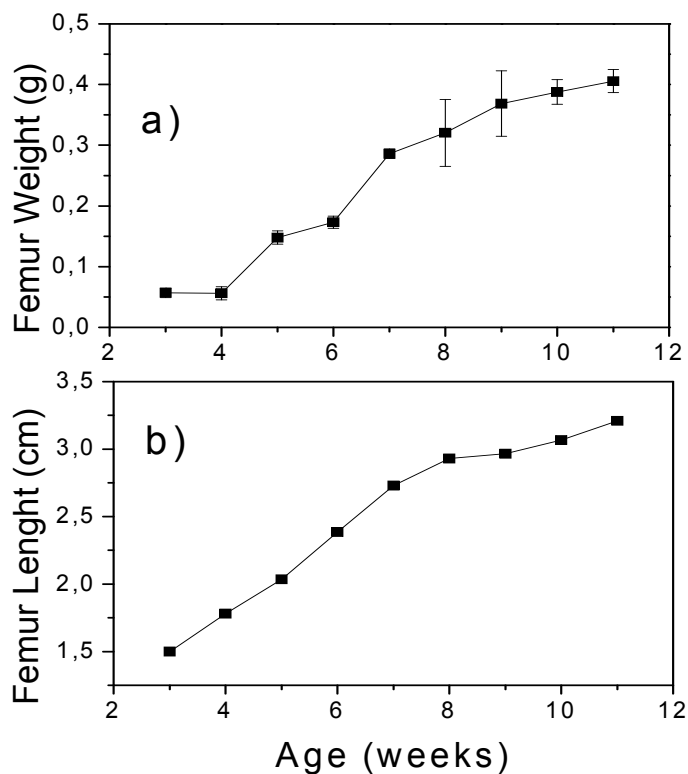


Fig. 2. a. Femur and b. Femur length for female rats as a function of the age.

Bone is a composite formed by inorganic and organic compounds. The organic material can be eliminated during the calcination process and the ash is composed only by minerals such as Ca, P, Mg, Na, P, among others. Figure 3 shows the ash content in the femurs as a function of the age. The increase in the ash content along the growth period is due to the decreases of the organic content (proteins) and the incorporation of new minerals into the bone matrix. Tamaki & Uchiyama (1995) found that the total weight of the rats has an abrupt increase until the 10<sup>th</sup> week of age. It is important to mention, that the growth of the living body depends on the development of the skeletal system, tissues, and organs.



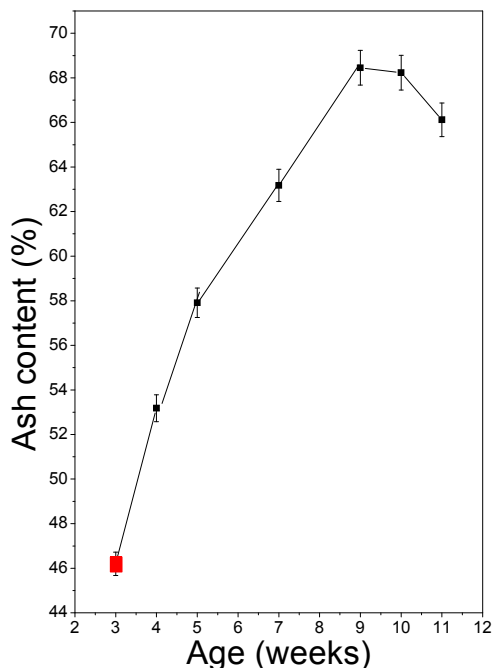


Fig. 3. Ash content in femurs of female rats as a function of the age.

The mineral content of femurs was obtained for rats between 3 and 11 weeks, but is important to know that the first data, for 3 weeks, corresponds to 21 day of life (red square in Figure 3) and at this time, rats were at weaning. Figure 4.a shows the Ca content in ash as a function of the age. During the period from 4<sup>th</sup> to 7<sup>th</sup> week, an increment in the Ca content is evident, while for the 7<sup>th</sup> to 11<sup>th</sup> week it has small changes.

Figure 4.b shows the P content as a function of the age during the acceleration phase. After weaning a reduction of P is evident, due to changes in the diet, and increases during the 7<sup>th</sup> and 10<sup>th</sup> weeks is present. This result is related to the formation of amorphous and crystalline compound of the bone as will be explained in the X-ray section as well as hormonal fluctuations. The bone mineral phase is composed mainly of microscopic crystals of calcium phosphates, in which the HAp  $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ , is the main component. Other mineral phases that are present in bone are dicalcium phosphate ( $\text{Ca}_2\text{P}_2\text{O}_7$ ), dibasic calcium phosphate ( $\text{CaHPO}_4$ ), and some amorphous phases of calcium phosphate. There are also ions such as: citrate ( $\text{C}_6\text{H}_5\text{O}_7^{4-}$ ), carbonate ( $\text{CO}_3^{2-}$ ), fluoride ( $\text{F}^-$ ) and hydroxyl ions ( $\text{OH}^-$ ), which can lead to subtle differences in bone microstructure. There are also some impurities such as Mg and Na with Cl and Fe traces.

Several researches employing biological models describe that, even if the corporal Ca concentration is adequate and the daily Ca intake covers recommendations, bones

demineralization takes place if the  $\text{Ca}^{2+}/\text{P}$  ratio in diet is not adequate (Anderson & Draper, 1972). Nnakwe & Kies (1985) reported that rats fed with rich P diets showed high risk of bone fracture. Similarly, Spencer et al., (1988) showed that high P content in diet has a negative effect in Ca deposition in bones. Calvo & Park (1996) observed that P modifies significantly the Ca metabolism. These authors detected that the  $\text{Ca}^{2+}/\text{P}$  ratio has a greater effect on bone mass formation and Ca deposition than the Ca content itself. According to Figure 5, the Ca/P ratio increases from 1.75 to 2.2; it is important to remember that there are amorphous compounds and ectopic Ca in the bone. The optimal Ca/P ratio for HAp formation, about 1.664, was reported by Benhayoune et al., (2001).

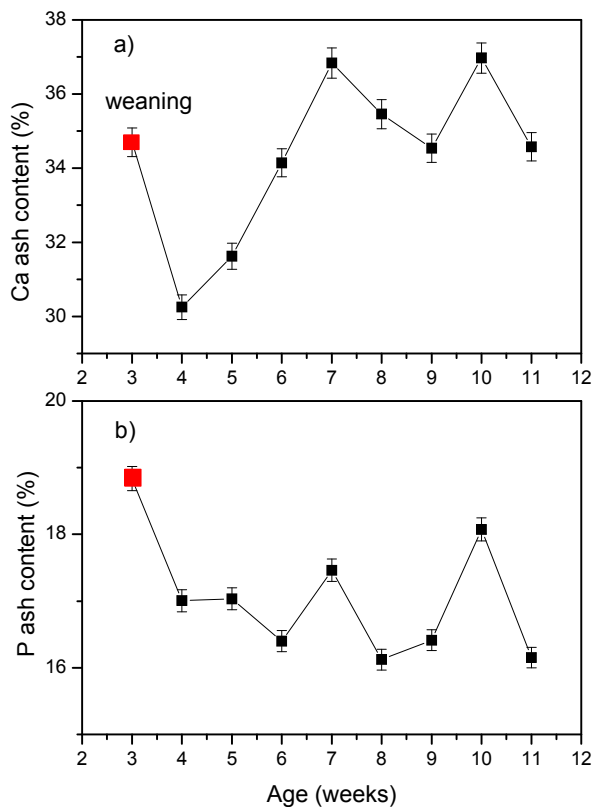


Fig. 4.a. Calcium and b. Phosphorous content in ash as a function of the age.

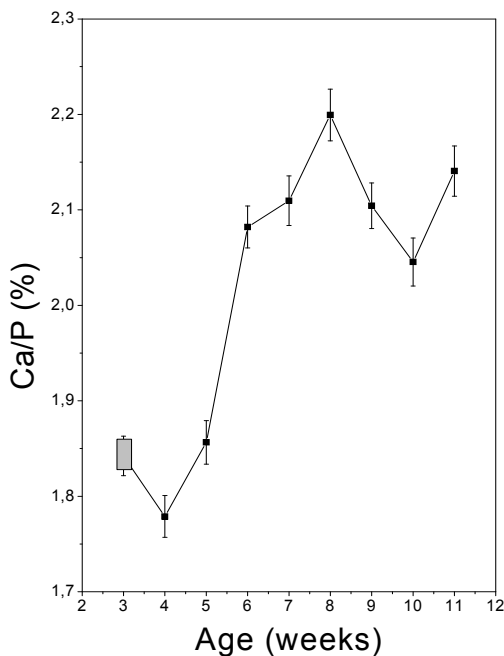


Fig. 5. Ca/P ratio for femur ash of female rats as a function of the age.

Figure 6 shows the changes in the ash mineral content as a function of the age decreases in the mineral content from the 3 to 4 weeks corresponds to the change in the diet. It is important to remark that the 10<sup>th</sup> week shows strong changes in K, Na and Mg. This increase is close related to those observed in the ash content, but as can be seen, when these minerals increase, a reduction in Ca and P is observed. At this point Boskey (2005) reported that there are minerals substitutions in HAp structure.

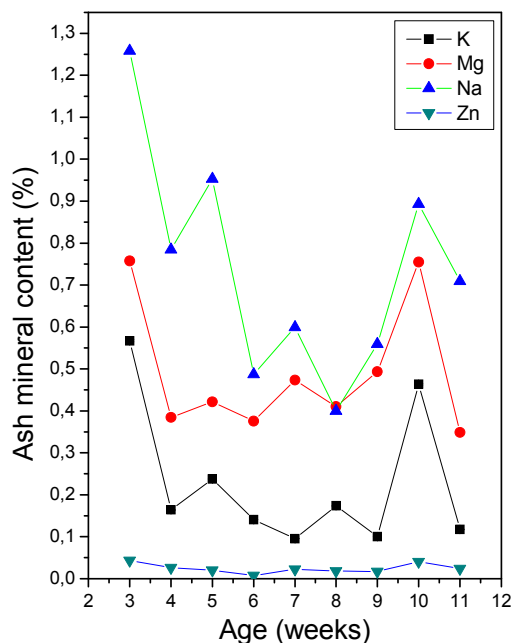


Fig. 6. Mineral content in femur of female rats as a function of the age.

### 3.5 Evaluation of the structural composition in bones

The study of the crystalline structures present in ash bones was obtained using the method described by Rodríguez et al., (1995), using X-ray diffraction. The ash bone was ground to a fine powder passing through a 150- $\mu\text{m}$  mesh screen. The powder samples were densely packed into an aluminum frame. X-ray diffraction patterns of the samples were carried out by using a diffractometer (Siemens D5000) operating at 35 Kv, and 15 mA, Cu  $K_{\alpha}$  radiation. Data was collected from 4 to 30° on 2 $\Theta$  scale with a step size of 0.05°.

Changes in the amorphous and crystalline structures of the bone tissues are attributed to the bone forming process and mineral changes during the growth stage. At this point Bandyopadhyay-Ghosh (2008) reported that the osteoblast release Ca, Mg and Phosphate ions, which chemical combine and harden within the matrix (collagen) into several compounds mainly HAp and phosphates.

The configurational crystalline order, gives important information regarding the synthesis of bone formation which is closely related to the diet and especially to the Ca/P ratio. Many of the biological and mechanical functions of the bone are determined by the composition and crystal structure of the bone. It is widely known that the bone mineral crystals (compositionally and structurally similar to the synthetic mineral calcium hydroxyapatite) are nano-crystalline, highly disordered, compositionally non stoichiometry and labile, and exists as a composite mineral embedded in the collagen organic matrix. Using the data obtained in Figures 4 and 5, it is clear that at the beginning of the development the femur

rats are deficient in Ca, while in the case of P no significant changes were found. This means that for the structural formation of crystalline phase the amount of minerals available plays the most important role.

Figure 7 shows the X-ray diffraction patterns of the bone at the beginning and at the end of the growing stage, in this figure it was included bone ash that was used in this experiments as reference values for minerals, According to Figure 7, there are structural and compositional changes in the inorganic matrix as a function of the age, that are evident in bone ash from rats to 3<sup>th</sup> and 11<sup>th</sup> weeks. The bone ash used as standard reveals the existence of one phase identified as Hydroxyapatite. On the other hand, the X-ray diffraction patterns obtained from femurs at several ages, exhibit two different crystalline structures: Calcium phosphate hydrated at the earlier growing stage and Hydroxyapatite at the end of the growth stage. These crystalline phases were identified using Powder Diffraction Files PDF# 09-0432 and PDF# 41-0483. It means that the formation of bio-hydroxyapatite as the main inorganic component of bone in rats is formed after the 7<sup>th</sup> week.

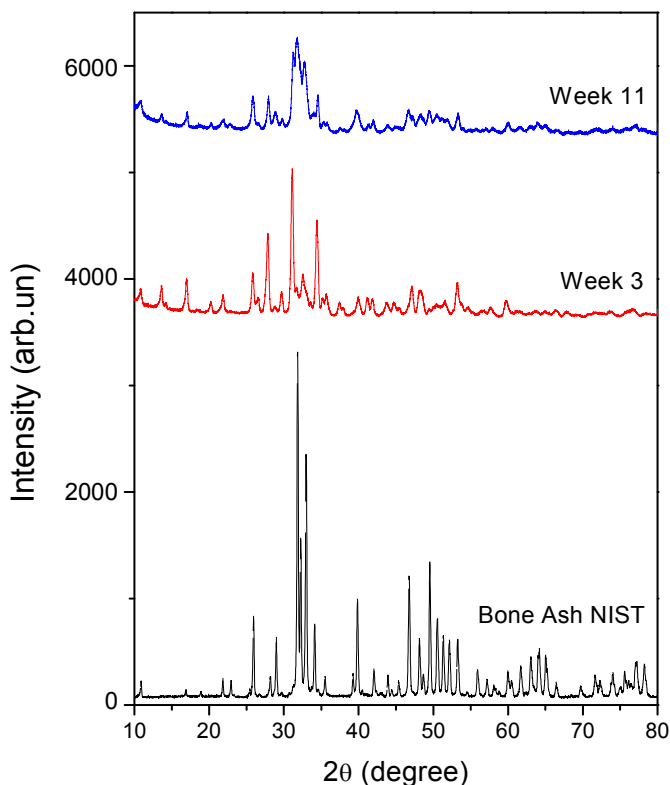


Fig. 7. X-ray diffraction patterns of bone ash for 3 and 11 weeks for female rats, as well as the bone ash used as standard (NIST).

In figure 8 the continuous lines correspond to the complete identification of Calcium phosphate hydrated (PDF# 41-0483), as can be seen, the transformation of this structure to bio HAp can be detected by studying the decrease of its peak intensity as the rat age increase, an important point is that until the end of the growing process this crystalline structure does not disappear. It means that this is a phase present during the growing stage. A careful examination of Figure 8 shows that Hydroxyapatite phase increases as age increases. The structural development is close related to the hormonal changes. An abrupt change in the crystalline structures between these two phases is evident at the 5<sup>th</sup> week, in which thin peaks are observed in comparison to the other X-ray diffraction patterns. By direct inspection of Figure 5 for the Ca/P ratio, is clear that at this age the amount of Ca and P are forming mainly crystalline structures as was mentioned before, but after the 6<sup>th</sup> weeks increase in the Ca is evident. The remaining elements and the excess of Ca and P allow the formation of amorphous structures.

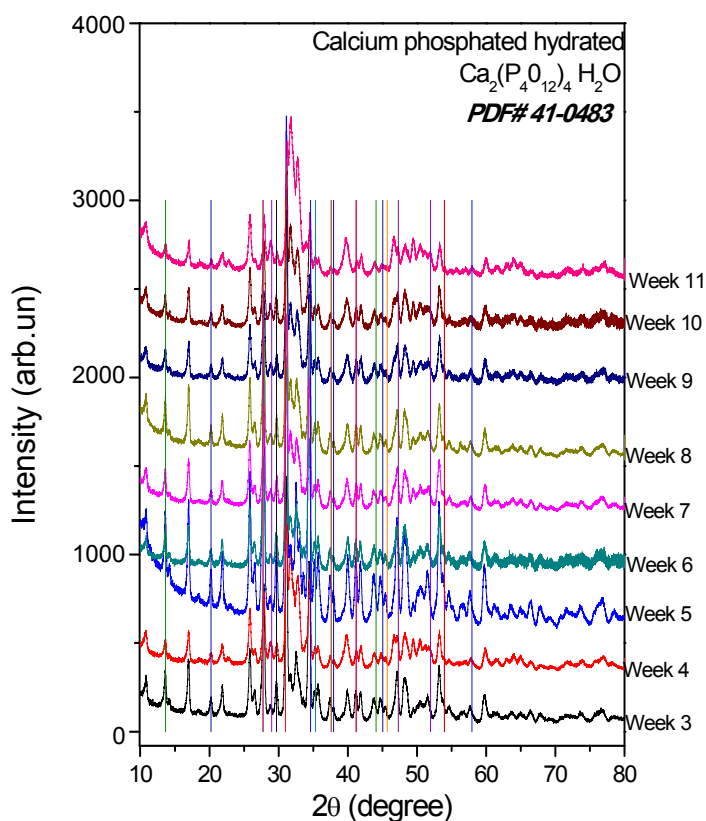


Fig. 8. X-ray diffraction patterns of bone ash from 3<sup>th</sup> to 11<sup>th</sup> weeks.

Taking into account the results of Figure 6 and the HAp showed in Figure 7, we could conclude that the main structure of the bone corresponds to bio-Hydroxyapatite, where some ions substitutions are present. It means that Na, K; Mg and other minerals were

incorporated into the HAp structure and also into de amorphous materials. Additionally, by inspection of the figure 7, the quality of the bio-hydroxyapatite from rats ash bones is smaller than bone ash from NIST, likely due to the minerals present in the organic part that were not eliminated in the calcined process. Possible, the NIST sample is calcinated after removing the fat and protein matrix that contain minerals. The structural conformation of the bone has strong implication on the mechanical and functional properties of it. The structural conformation of the bone is an amazing kinetic process that takes place at body temperature. It is well known that for the crystallization of HAp is required a temperature close to 700°C and the formation of the bone is still an unresolved and open problem for future researches.

Figure 9, shows the X-ray diffraction patterns of bone ash corresponding to different ages of the rats. In this case, the continuous lines correspond to the identification of HAp (PDF 09-0432). X-ray patterns confirm that if the rat age increases, the minerals content into the bone begin to form HAp. This fact is important; because the new bone formation influences the mechanical properties of the bone that can be reflected in more breaking force, which is an excellent indicative to prevent bone fractures.

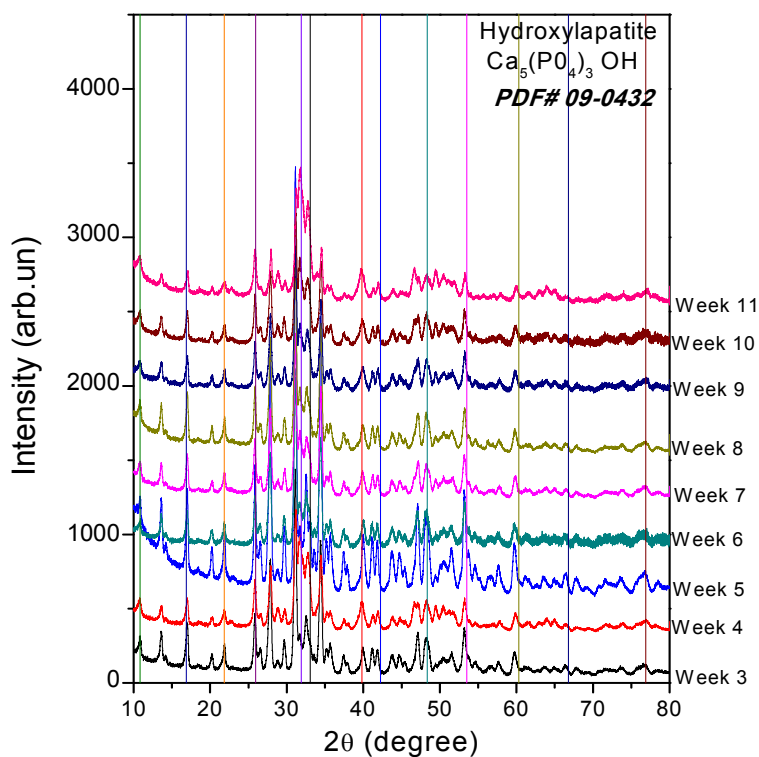


Fig. 9. X-ray diffraction patterns of bone ash for female rats.

#### 4. Conclusion

In the case of biological studies, AAS is an excellent tool that allows a quantitative determination of mineral content in bones and other materials. The physical development depends on four factors: genetic, feeding conditions, mechanical, and hormonal. These factors are responsible and determine the performance of all structures of the rat in adult phase. During acceleration phase the alteration of any of the aforementioned factors have strong effects on the rat develop. Nevertheless, during this phase these factors can be modified according to the experimental model proposed for any study.

The ash content shows an increase as a function of the age, during the acceleration phase, being this behavior the normal develop of the rats. This current data indicate that the organic material into the bone matrix decreases in the same manner, and it is confirmed by the determination of Ca, P, Mg, and K. The Ca/P ratio in bone is bigger than 1.67 in all cases, but this increases with the age, indicating that part of these minerals are conforming the bio-Hydroxyapatite and the excess of minerals are amorphous compound based in these minerals. The analysis of X-ray diffraction patterns of bone ashes showed that during this period, two crystalline phases are present: Calcium phosphate hydrated at the earlier growing stage and bio-Hydroxyapatite during the whole stage.

These results are very important for the biological models, because these constitute the normal curves for growing, weight, femur length in female rats. Additionally, for the first time the normal curves for ashes, Calcium, Phosphorus, Sodium, Zinc, Potassium, and Magnesium for female rats bones during growing were obtained.

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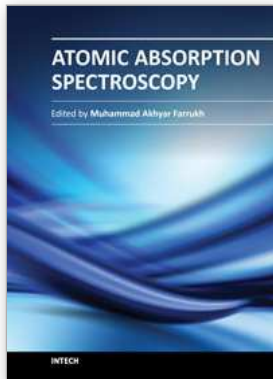


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## **Atomic Absorption Spectroscopy**

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Atomic Absorption Spectroscopy is an analytical technique used for the qualitative and quantitative determination of the elements present in different samples like food, nanomaterials, biomaterials, forensics, and industrial wastes. The main aim of this book is to cover all major topics which are required to equip scholars with the recent advancement in this field. The book is divided into 12 chapters with an emphasis on specific topics. The first two chapters introduce the reader to the subject, its history, basic principles, instrumentation and sample preparation. Chapter 3 deals with the elemental profiling, functions, biochemistry and potential toxicity of metals, along with comparative techniques. Chapter 4 discusses the importance of sample preparation techniques with the focus on microextraction techniques. Keeping in view the importance of nanomaterials and refractory materials, chapters 5 and 6 highlight the ways to characterize these materials by using AAS. The interference effects between elements are explained in chapter 7. The characterizations of metals in food and biological samples have been given in chapters 8-11. Chapter 12 examines carbon capture and mineral storage with the analysis of metal contents.

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