XANES AND FTIR STUDY ON DRIED AND CALCINED BONES

by

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ABSTRACT

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The animal bones are common archeological finds and are helpful in understanding the human past. The bones undergo transformation when they are calcined. The cooked and cremated bones undergo different transformation, because of their difference in their temperatures.

Various techniques are employed to characterize the bone. In this study, XANES and FTIR are used to characterize the dried and calcined bones of different species. The calcined bones are prepared at two different temperatures, which helps in understanding the difference between the cooked and cremated bones.

The objective of this research is to comprehend the chemical property of the bone material across different species and across different calcination conditions.

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CHAPTER 1

INTRODUCTION

The characterization of bones is important in different fields like, archaeology, paleoanthropology, paleopathology, etc. The fragmented bones are usually discarded in bone studies, since it lacks the morphological features that are used to differentiate the species of animals. The characterization of the bone is important in finding whether the chemical property of bone sample is different or same across the different species. The bone is composed of organic and inorganic components. Identifying the change in the bone property between dried and calcined bones can be used to determine the origin of the fire. To differentiate the cooked and charred bones, it is important to understand of the chemical change in the bone component.

The bone samples of different species were chosen to identify the species specific chemical property of the bones. The sample bones were from sheep, deer, bovine, chicken, chamoix and pig. To determine the heat induced changes, the bones are calcined. The fresh bones were dried and a set of dried bone samples of sheep, deer, bovine and chicken are prepared. The bones of sheep, deer, bovine, chicken, chamoix and pig are calcined at 400^{o}C and 700^{o}C. These temperatures will corresponds to the half and full combustion of the bone samples, respectively. The information obtained from the calcined bones may help to understand the charred and boiled archaeological bones. The temperature and the species are the variables in this study, to understand the heat dependent and species specific chemistry of the bones.

The X-ray diffraction (XRD) is sensitive to crystalline structure and is employed to identify the mineral structure of the bone. The X-ray absorption of near edge structure (XANES) spectroscopy is element specific and it gives the information about the surrounding atoms. This

helps in understanding the presence of compounds, its structure and the local environment. These techniques provide information about the inorganic species but not the organic component of the bone. The Fourier Infra-red (FTIR) spectroscopy is sensitive to the different functional groups corresponds in the organic and inorganic component of the bone. This technique provides the change in organic and inorganic content across the species and at different conditions.

The information gathered from XRD, XANES and FTIR was used to characterize both dried bone and as well as the effect of calcination on both the organic and inorganic constituents of bone.

CHAPTER 2

BACKGROUND

2.1 Bones

2.1.1.1 Composition of bone

Bone is the main structural component of human beings and other vertebrates. It protects the vital organs, helps in movement, serves as mineral storage and gives shape to the body. The bones are composed of inorganic and organic components and they account for about 65 wt% and 35wt% of bone, respectively, see Figure 2.1. The organic portion of the bone is made up of proteins, mostly of collagen type I. About 20 wt% of bone is composed of collagen, which is second largest to the hydroxyapatite in quantity. There are other types of non-collagen fibrils present in the bone material. The non-collagenous materials play a pivotal role in attracting the hydroxyapatite (HA) crystals and attaching it to the organic collagen matrix. The non-collagenous proteins has an affinity towards the HA crystals, and very scarce in quantity; they account for 3 – 5 wt% of bone. The bone mineral component is mostly composed of HA and other elements like sodium, magnesium, etc in trace quantity. The HA accounts for 60 wt% of bone and it is the primary component of the entire bone [1].

The bone mineral is analogous to the naturally available ceramic hydroxyapatite. The chemical formula for the HA is $Ca_{10}(PO_4)_6(OH)_2$, and the actual bone mineral has some impurities added to the structure of this ceramic compound. Often, the OH^-, PO_4^{3-} are replaced by other compounds like carbonate, etc. This results in formation of carbonated hydroxyapatite. The calcium can also be replaced by elements like, strontium or magnesium. These replacements increase the imperfection in the apatite and it makes the mineral to be more soluble. These substitutions results in reduction of size of the crystal. The fluoride ion occupying

the OH^- site is an exception to the above statement. In this case, the mineral is less soluble and their crystal size is comparatively larger.

Inorganic phase	wt%
Hydroxyapatite	~60
Carbonate	~4
Citrate	~0.9
Sodium	~0.7
Magnesium	~0.5
Other traces: Cl^-, F^-, K^+ Sr^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}	

Organic phase	wt%
Collagen	~20
Water	~9
Non-collagenous proteins (osteocalcin, osteonectin, osteopontin, thrombospondin, morphogenetic proteins, sialoprotein, serum proteins)	~3
Other traces: Polysaccharides, lipids, cytokines	
Primary bone cells: osteoblasts, osteocytes, osteoclasts.	

Figure 2.1 The composition of bone [1]

The hard tissues are macroscopically composed of two different structures, namely, cortical and cancellous bones, shown in Figure 2.2. They differ in their density and porosity, but their chemistry remains the same. The cortical is the dense outer region of bone, while, the cancellous bone is the inner less dense region. The cancellous bones are sometimes called as trabecullar or spongy bones.

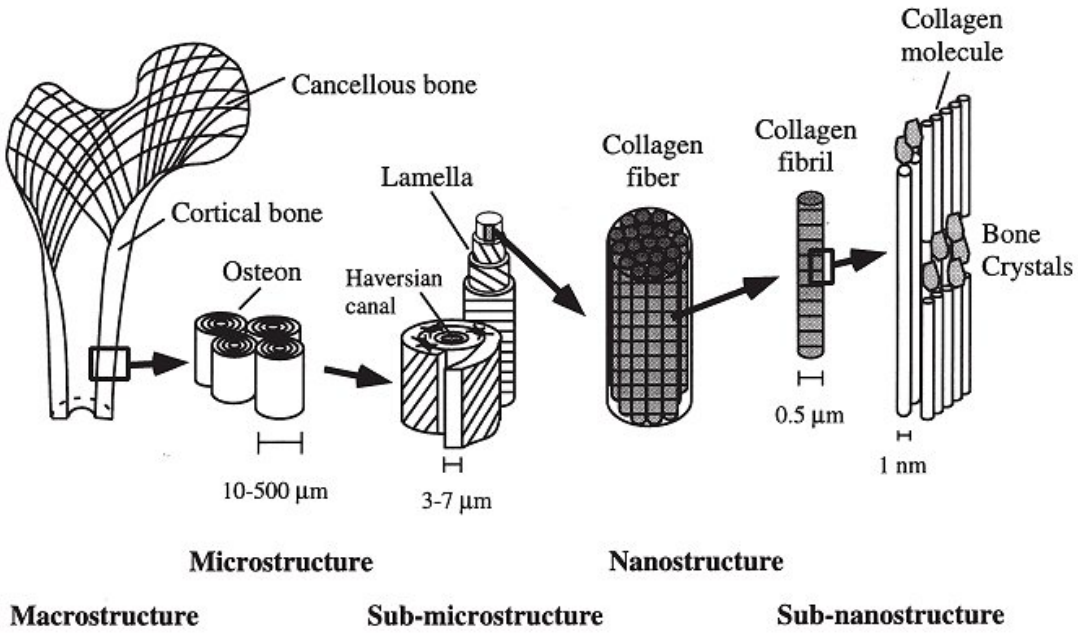


Figure 2.2 The structure of bone [2]

Microscopically the bone is made up of osteons. Each osteon consists of a Haversian canal and a lamella. Each osteons are few hundred microns in diameter. The nanostructure of bone has two main components, collagen fibrils and HA crystals. The bone crystals are attached to the end of the each collagen fibrils. These organic and inorganic components are the building blocks of the bone material [2].

2.1.2 Bone types

The bones are categorized into different types depending upon its size, function, formation and density. Depending on its size it is divided into long bones, short bones and flat bones. The long bones are the cylindrical bones, like, femur, tibia, etc. Femur is the longest bone in the entire human skeleton. The short bones are cylindrical short bones, like, carpus, tarsus, etc., and the flat bones are bones of shoulder, pelvis and skull. On the basis of function, it is divided into two types, namely, axial and appendicular. There are 80 axial bones in an adult human body and their function is to protect the primary organs. The axial bones are flat bones,

like, skull and ribs, which protects the vital organs brain and heart, respectively. The appendicular bones are short and long bones, which helps in weight bearing, locomotion, and balance. There are 126 appendicular bones in the skeleton system of an adult human body. The bone can form in two different ways, endochondral ossification or intra-membranous formation. In endochondral ossification, the cartilage is formed from the bone cells, and they later developed into calcified bone. On the other hand, in intra-membranous the bone is directly formed from the bone cells, without the formation of the cartilages [3].

2.1.3 Bone cells

There are three major bone cells, which involves in the formation, maintenance and healing of bones. They are osteoblasts, osteoclasts and osteocytes. The osteoblast cells are 15 to 20 micron in the longest dimension, and they are responsible for the formation of new bones. They secrete the organic materials of the bone, thus forming the matrix for the formation of the bone material. They synthesize collagen and non-collagen proteins, and hence the collagen matrix is formed along with the non-collagen protein. The non-collagen proteins have an affinity towards HA, which is present in the body fluids. These bone cells are present on the surface the bone, where the new bones are to be built. The osteocytes are about 20 to 60 micron in its longest dimension. They are present deep inside the bone material and are connected to other osteocytes and osteoblast cells on the surface of bone.

The osteoclast cells are 20 to 100 microns in its longest dimension and are responsible for the resorbtion of bones. The old or damaged bones are eaten away by the osteoclast cells. The resorbtion is carried out by the acids secreted by the osteoclast cells. These cells usually found on the surface of the damaged or old bones.

2.1.4 Bone remodeling

The bone remodeling is a process carried out by all the bone cells as a part of maintenance and healing. The old bones are replaced quite often with new ones. In case of

facture or damage, the bone remodeling helps in the replacement of damaged bones with new bones. Cancellous bones are remodeled more frequently than the cortical bones, and hence, the cancellous bones are younger than the cancellous bones.

The osteoclast bone cells are responsible for the resorbtion of bone. This process will take few weeks to complete. The osteoclast pre-cursor cells are converted into osteoclast cells and form on the surface of the old or damaged bones. These bone cells then resorb the bone material and leaves a cavity. The osteoblast then comes in to this area on the surface on the bone, where the bones are resorbed. The osteoblast secretes organic matrix of collagen and non-collagen proteins. This forms the matrix for the bone composite. By attracting the HA crystals from the body fluids, the bone formation completes on the resorbed surface. This process of formation of new bone will take few months to complete [3].

2.2 Archaeological bones

The archaeology is the study of past from the materials that are excavated. The most common excavated artifacts are pottery, tools, coins, and bones. The human and animal bones are equally important in gaining the knowledge of the past. The animal bones are helpful in understanding its life and existence, and it also helps in understanding the life style and history of humans.

2.2.1 Identifying and calcifying the bone

The size and shape of the bones are used to identify the species of the animal, by comparing with the modern animals. Further, the shape of the bone is used to identify the part in their respective skeleton system. The bone joints, long bones, skulls, pelvis, for instance, have characteristic features, which are straightforward in identification. The vertebrates share a very similar skeleton system with skull, ribs, limbs, etc. but, they have pronounced differences to identify their respective species. For instance, the fish and the mammals have ribs and skulls, but their shape and relative sizes are very different. The teeth of herbivores are flat, to help

them in grinding the leaves. On the other hand, the carnivores have sharp teeth to tear the flesh of its prey. These differences in their shape of teeth are used to identify the species.

The mandible and long bones are very useful in determining the age of the specimen when it was died. The formation of different types of teeth is age dependent. The wear on the surface of the teeth is proportional to the age of that individual. This age related differences are used to identify the age of the fossil bone specimens. The development and the fusing of the long bone with the cartilage are also used to determine the age. Few types of bones like, pelvis are used to identify the sex of the specimen. In some animals the anatomical features are different for male and female; for instance, the antlers of deer are longer for the male counterpart [4].

2.2.2 Human history from animal bones

The seasons of the environment have an effect on the bone growth, the spring and early summer helps in dramatic growth, because of the availability of plenty of foods. On the other hand, the winter has less food; hence, the animal won't get the required nutrients and are reflected in the less or no bone growth. This helps to decipher the duration of the seasons and the nature of that location. Nutrient related pathologies are also identified by the bone specimens.

The human past can also be explained with the animal bone specimens. The butchery mark represented that the animals meats consumed by the humans. The butchery marks are used to determine the tool used to cut open the meat. The information about the material used as tool can be obtained from those butchery markings. The tools like, flint, metals are used by the pre-historic humans. This also helps in understanding their knowledge in tool making and its related technologies [4].

The presence of more than one set of animal bone of same species from the same sediment layer indicates that those animals are possibly domesticated. Mostly, the old domesticated animals are killed for their meat and hence, their age can be used to support

these conclusions. The presence of bone remains of random species could possibly indicate the hunting process. The bones of same species of animals with different age group could possibly from hunting as well.

The burnt bones indicate the knowledge of fire handling by the human. The burned bones are also used to identify the age and sex of the animal, which in turn, helps to predict the hunting or domestication practices. The temperature of the calcined bones, possibly indicate the availability and/or usage of fire source.

The pre-historic humans used bones as tools for hunting, digging, etc. The stones and bones are the hardest materials known to early humans. The flint stones are most widely used stone tool, because of its sharp edges. The bones are used as tools where these stone are not available. Also, the bones are abundant and they have access to all the bones from the animals they hunt. The deer antlers are also used as tools in pre-historic times. The burial rituals can also be analyzed from the bones. Pet animals like cats, could be buried with the human body. Some culture use large urns to bury their descendants, some cultures mummify their bodies, etc, and are also be helpful in understanding their life style and culture. The early decorative items are made out of the bones of animals and ivories. These decorative items will be used to understand their sophisticated knowledge in carving and craft making.

2.3 XANES and FTIR introduction

In the past mass spectrometry, XRD and FTIR are widely used to study the bone and other apatites [5]. There are research on the bones and its implants using the nuclear magnetic resonance (NMR) technique [6]. The XRD and FTIR are very promising and easy technique in characterizing the bones [7]. The effect of heating of the bones are also performed using XRD and FTIR techniques [8] [9] [10] [11], and also the similar apatite crystals are studied [12]. The isotope study of bones are also performed using XRD and FTIR techniques [13]

The applications of XANES in the field of forensic science are reviewed [14] and the requirements are not very different from the archaeology. The different edges are studied by different researchers, the calcium L edge [15] [16] and calcium K edge [17] [18] are helpful in identifying the nature of calcium and its structure. The phosphorous L edge [19] and K edge [18][19] are employed to determine the phosphorous structure in bone. Calcium and phosphorous edges are studied, since; the bone mineral is made of calcium phosphate apatite. The other edges like silicon K and L edge [20] are also used to study the bone implant materials like Bioglass, etc. The manganese k edge [21] used to find the presence of unusual color on the bone surface. The effect of osteoporosis and other age related changes are also studied [18].

2.3.1 XANES study

The X-ray Absorption Near Edge Structure (XANES) spectroscopy helps in identifying the chemistry of the individual elements, by using the edge feature. The edge reveals the surrounding information of the element. The spectral variation depends on the electronic configuration and the electronic structure. The structure and the local surrounding information can be obtained [14].

The excitation of the electron to the conduction band gives rise to the absorption of X-ray and are acquired by the XANES spectroscopy. The detailed calcium L-edge XANES study is available in the literature, along with the comparison between the carbonate rich HA and pure HA [15]. The local electronic structure and the chemistry of the bone samples can be studied with XANES spectroscopy. It claims to be a new technique to study the K^{+}, Ca^{2+}, Sc^{3+} and Ti^{4+} ions [6] [19].

The XANES spectroscopy is employed in studying other artificial apatites like hydroxyapatite and also bio activity study of new biomaterials. The Bioglass45S5 and HA biomaterials are studied with the XANES and the bioceramic composition are determined [22]. The phosphorous is an important mineral in agriculture and the XANES study is performed in the L edge and the K edge energy levels. The phosphorous K edge and L edge XANES are

used for the identification of the species and quantifying the amount of phosphorous in the sample. The P L edge XANES spectra have a very distinct feature with different organic phosphates, which is not so straight forward in P K edge.

The standard spectra are compared with the standards and to find the species of the bone sample in the P K edge XANES study [18]. The study helps in finding presence of strontium to understand the bone disorder related to the decrease in strontium concentration. The origin of the presence of color in a bone is analyzed by the XANES spectra and by comparing the manganese K edge XANES spectra of the standards, the origin of the color is determined to be manganese and is substituted in the PO4 3- of the bone apatite [21].

2.3.2 XRD study

The X-Ray Diffraction (XRD) spectra show increase in crystallinity of the bone mineral with respect to heating. The dried or unheated bone samples are poorly crystalline and they becomes more crystalline when heated [8].Also, the overlapping of the diffraction peaks are attributed to the fact that the bone samples are poorly crystalline [11] [12] [5].

2.3.3 FTIR study

The Fourier Transformation Infrared (FTIR) spectroscopy is sensitive to the different molecular bond vibrations, like, stretching, bending, etc [5,9,23]. The absorption of the incident IR rays depends on the configuration of the atom and the presence of the functional groups in the sample [24]. The change in bond angle and length can be easily determined by FTIR spectroscopy. FTIR is sensitive to all the functional group in the organic and inorganic content of the bone. The mineral and the different proteins are identified and hence the collagen quantity can also be determined [25].

The presence of carbonate and the different type of carbonate that can substitute the bone mineral are well studied. The carbonate ions replacing the OH- sites in HA are called type A carbonate apatite and if the carbonate ion replaces the phosphate sites, they are called type

B carbonate apatite. The carbonate ion can replace both of these monovalent and trivalent anionic sites and they are called type AB carbonate apatite [26] [27] .

The phosphate vibrations arise from the inorganic part of the bone identified by the FTIR studies. They can be divided in to v_1, v_3 and v_4 vibrations. The v_3 phosphate vibrations are thoroughly studied and are explained in the literature [27] and the $v_4$phosophate is explained in [26].

CHAPTER 3

CHARACTERIZATION OF DRIED AND CALCINED BONES BY XANES

3.1 Introduction

The XANES spectroscopy helps in identifying the chemistry of the individual elements, by using the edge feature. The edge reveals the surrounding information of the element. The spectral variation depends on the electronic configuration and the electronic structure. The structure and the local surrounding information can be obtained.

3.2 Experimental procedure

The XANES spectra are used to characterize the chemical structure of the dried and calcined bones, and are obtained at Canadian Light Source (CLS), Saskatoon, Canada. The spectra for silicon, sulfur, phosphorous and chloride can be obtained using a plane grating monochromator (PGM) beamline. The phosphorous L edge FY XANES spectra are obtained for the model compounds and the bones samples in the region 132 – 155 eV with a step size of 0.1 eV. The calcium L edge TEY and FY spectra are obtained using the spherical grating monochromator (SGM) beamline. The calcium L edge TEY and FY spectra are obtained in the region 346 – 358 eV with a step size of 0.1 eV. The oxygen K edge peaks falls in the region of 527 – 552 eV and hence, it can be obtained with the SGM beamline along with the calcium L edge TEY and FY spectra. TEY and FY spectra of oxygen K edge is obtained from 527 – 552 eV with a step size of 0.15 eV. The Soft X-ray beamline for the micro-characterization of materials (SXRMB) are used to acquire the phosphorous and calcium K edge TEY in the region of 2140 – 2190 eV and 4000 – 4130 eV, respectively. The step size for phosphorous and calcium K edge TEY are 0.25 and 0.3 eV. All the XANES spectra are plotted using Originpro software after subtracting their respective background. The background is manually selected, without causing any change to the actual spectra.

The X-ray powder diffraction is carried in the D5000 instrument at Characterization Center for Materials and Biology (CCMB), University of Texas at Arlington. The XRD spectra are obtained in the range from 20^{o} to 90^{o} with a step size of 0.02^{o}. The XRD spectra are then normalized from 1 to 0, with the maximum value being 1.0 arbitrary units and the lowest being 0.0. To determine the phase in the bone samples calcined at 700^{o} C, the deer bone sample calcined at 700^{o} C spectrum is obtained and are compared with the obtained standard spectra. The model compounds used are sintered and unsintered HA, alpha TCP, and sintered and unsintered beta TCP.

3.2.1 Sample preparation

The recovered fresh bones are cut into small fragments and are preserved in the freezer. After removing the attached soft parts from the bone samples, they are further broken into small fragments of about few centimeters in dimension. They are then put into the test tubes and are mixed with water to remove the still attached soft tissues. The jet of compressed air is blown in to dry the fresh samples. The plastic dryers are employed to remove the liquids present in the bone sample. Then the samples are placed in the vacuum chamber, and the air is removed to create vacuum inside the chamber. The temperature is maintained at 20^{o}C -25^{o}C and the sample is placed inside the vacuum for about 5 days. Thus the dried bone samples are prepared, except for the deer dried bones. The deer dried bone samples are dried naturally for about 6 months. The deer bone sample is the only exception, which are dried under natural conditions, which are similar to the formation of archaeology dried bone specimens.

The calcined bone samples are prepared by heating the dried bone samples in an oven, which has a maximum capability of 1800^{o}C. The bone samples are placed in the oven and the temperature of the oven is increased slowly to reach 400^{o}C. It temperature is increased slowly from room temperature to 400^{o}C in about 40 minutes and the temperature is maintained for one hour, in air. The temperature is brought to room temperature after decreasing the temperature from 400^{o}C to 22^{o}C for about an hour. Thus the first set of calcined bones are prepared, which

are calcined at 400^{o}C. The same process is repeated for preparing the other set of calcined bones, by changing the temperature to 700^{o}C and maintained if for one hour in air. Thus the bone samples calcined at 700^{o}C are prepared. These samples were prepared at The University of Trento, Italy [10].

3.3 Results and discussion – X-ray Powder Diffraction

The X-ray powder diffraction (XRD) is performed to identify the phase of the bone mineral in the bone sample calcined at 700^{o}C. The XRD spectra are obtained with a 5000D instrument. The sintered beta TCP is prepared by sintering commercially available beta TCP standard from Alpha Acer at 1100^{o}C for about 2 hours. The spectrum of sintered and unsintered beta TCP, along with sintered and unsintered hydroxyapatite, and alpha TCP standards are used for the identification of the bone mineral.

The XRD spectra of the deer bone sample calcined at 700^{o}C and other sintered and unsintered standards are shown in Figure 3.1 on page 31. The spectrum of the deer bone calcined at 700^{o}C has diffraction peaks at 31.67^{o}, 32.14^{o}, and 32.8^{o}. The broadening and the overlapping of the diffraction peaks are due to the low crystal symmetry and crystal size of the bone mineral [11] [7].

The peak positions and its relative intensities are used for the identification of phase of unknown samples in XRD. The other diffraction peaks of spectra of bone samples are at 25.83 ^{o}, 33.9^{o}, 39.7^{o}, 46.6^{o} and 49.3^{o}, and series of low intensity peaks are present in the region of 20^{o} – 25^{o}, 26^{o} – 28^{o}, 60^{o} – 65^{o} and 67^{o} – 80^{o}. The spectrum of the bone sample calcined at 700^{o}C is compared with the standards to find the phase of the bone mineral. There are many unresolved peaks, when it is compared with the alpha TCP standard, except for one weak peak at 46.6^{o}. The primary peaks of the bone samples calcined at 700^{o} C are absent in the alpha TCP spectrum. Since, the alpha TCP standard spectrum has primary peaks at 30.72^{o}, 34.26^{o}, 34.56^{o} and 22.88^{o}. So, it is less likely to have alpha TCP in the bone samples.

The comparison of the spectra of sintered HA and sintered beta TCP show that they do not have any common set of primary peaks. The sintered HA has its primary peaks at 31.85°, 32.98° and 32.27° (listed in the order of intensity of the peak). On the other hand, the sintered beta TCP has its peaks at 31.03°, 34.31° and 27.25°. They do have a common peak at 25.83°, which is present in all the sintered and unsintered HA and beta TCP standards. Even though, it's not a perfect match, some of the weaker peaks in the spectra of sintered HA, which are at 46.6° and 53.2° are close to the sintered beta TCP peaks at 46.9° and 52.29° respectively. All other peaks in their respective peaks are a complete mismatch to each other.

The XRD spectra of unsintered HA and unsintered beta TCP standards looks very similar, with their primary peaks at 31.67°, 32.14°, 25.88°, 32.8° and 33.9°, see Figure 3.2 on page 32. The perfect match of these primary peaks makes it hard to differentiate between them. The relative intensity between the peaks at 31.67° and 25.83° and the shape of the broad peaks at 39.7° for the two standards are different. When the spectrum of deer bone samples calcined at 700° C is compared with the spectra of the standards, it is clear that the sintered beta TCP and alpha TCP are not a match, and hence, they less likely to present in the bone mineral. But, the unsintered beta TCP is a near perfect match with the spectra of bone sample. It is possible to conclude that the bone mineral does not have beta TCP in a sintered from. Since, the spectra of unsintered HA is also a near perfect match to the spectrum of bone sample calcined at 700° C, the bone mineral could possibly composed of HA and beta TCP. The weaker peaks in the spectrum of the bone samples are also match with that of unsintered HA and unsintered beta TCP standards. The sintered HA has its primary peaks shifted towards its higher degree, when compared with the spectra of the deer bone sample calcined at 700° C. Hence, it is possible to say, that the bone mineral is made up of hydroxyapatite and beta TCP in its unsintered form.

3.4 Results and discussion – XANES

3.4.1 Phosphorous L edge FY

3.4.1.1 Phosphorous L-edge FY of model compounds

The phosphorous L-edge XANES spectra has been used extensively in the past to fingerprint the nature of phosphate compounds in bone [22] [19]. With the phosphorus compounds mainly coordinated with Ca as the cationic species, all the examined model compounds were Ca based. They include pure hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, $CaHPO_4$, $CaHPO_4\ H_2O$, $Ca_2P_2O_7$, beta Tricalcium phosphate (beta TCP or β-$Ca_3(PO_4)_2$), and alpha Tricalcium phosphate (alpha TCP or α-$Ca_3(PO_4)_2$). All the model compounds have a distinctive feature which will be used to identify the composition of the bone samples, see Figure 3.3. The white line for pure HA is at 139.70 eV and is not the same for all the other compounds except beta TCP. $CaHPO_4$ and $CaHPO_4\ H_2O$ have their white line peaks at 139.25 eV and 139.44 eV, respectively. On the other hand Ca_2P_2O7 and alpha TCP have their main white lines at 138.87 eV and 138.60 eV, respectively. The first pre-edge peak are close to 137.20 eV for $CaHPO_4$, $CaHPO_4\ H_2O$, $Ca_2P_2O_7$ and beta TCP white that for pure HA is shifted towards high energy at 138.04 eV and alpha TCP is shifted towards low energy at 136.76 eV. $CaHPO_4$ H O has a very dominant second pre-edge peak at 138.32 eV, which is less obvious in the case of pure HA (at 139.0 eV) and $Ca_2P_2O_7$ (at 138.20 eV) and is nearly absent in $CaHPO_4$, beta TCP and alpha TCP. Interestingly, there is a third pre-edge peak for the beta TCP which corresponds to the second pre-edge peak position of pure HA at 139.00 eV. The first post-edge peak which is present in the pure HA is absent in other model compounds. The shoulder between 140.5 eV to 143.00 eV is almost a flat line parallel to the x-axis for pure HA and beta TCP, is the prominent feature for the less soluble phosphate. On the other hand, the rest of the model compounds are very soluble, since they possess a slanting shoulder. The spectra acquired for the model compounds were compared to those that were acquired from the dried and calcined samples.

3.4.1.2 Phosphorous L-edge FY of dried bone samples

The Figure 3.4 shows the XANES spectrum for dried bones of deer, bovine and chicken. All three spectra looks similar, but for one distinctive difference seen in the case of the chicken sample where the relative height between the main peak and the third pre-edge peak is different for chicken when compared to bovine or deer. The presence of a third pre-edge peak indicates the presence of beta TCP in the dried bone samples. A small peak between second and third pre-edge peaks in dried bovine spectrum at 138.5 eV and between first and second pre-edge peaks in dried deer spectrum at 136.9 eV are not to be confused as additional peaks, but they are purely the noise in the signal. The structure of the spectrum is close to the beta TCP when compared to the spectra of all the model compounds. The positions of all the absorption peaks are a perfect match to the beta TCP standard. First pre-edge peak position is at 137.15 eV (137.2 eV for beta TCP), second pre-edge peak position is at 138.18 eV (138.15 for beta TCP), the third pre-edge peak position is at 138.9 eV (139.0 eV for beta TCP) and the primary peak position is at 139.6 eV (139.7 eV for beta TCP). However, bone being made up of mixture of phosphates contains in addition to beta TCP small amounts of other trace compounds of phosphates of calcium.

3.4.1.3 Phosphorous L-edge FY of bone samples calcined at 400°C

The pre-edge peak positions at 137.2 eV, 138.2 eV and 139.0 eV and the primary peak position at 139.8 eV in all the 400°C calcined samples as shown in Figure 3.5 corresponds to the beta TCP. Except for the post edge peak at 143.34 eV that likely corresponds to the pure HA, which has a post-edge peak at 143.55 eV. Other than this, there is little difference between the dried and the 400°C samples. It is possible to say that 400°C samples the in addition to beta TCP small amounts of other trace compounds of phosphates of calcium.

3.4.1.4 Phosphorous L-edge FY of bone samples calcined at 700°C

Figure 3.6 shows the P L-edge spectra of all the bone samples that were calcined at 700°C for 1 hour. The 700°C spectra appear to match the spectra of pure HA with just two pre-

edge peaks. The absence of a third pre-edge peak associated with beta TCP indicates that is the calcined sample is not entirely made of beta TCP at 700^{o}C. The quantity of HA in 700°C samples is much larger compared to the bone calcined at 400°C which was primarily composed of beta-TCP. The post-edge peak position at 139.4 eV is the characteristic of pure HA.

Shape of the spectrum looks likes HA but the peak positions matches with alpha TCP. The XRD results can be used to eliminate the possibility of presence of alpha TCP in the bone samples and are shown in the Figure 3.2. The Chamoix bone that was calcined at 700°C sample posses a flatter post-edge shoulder compared to the other bones that were calcined at 700^{o}C. This indicates that the Chamoix has more stable phosphate than any other 700^{o}C samples.

3.4.2 Calcium L-edge TEY

3.4.2.1 Calcium L-edge TEY of Model Compounds

The calcium L-edge TEY XANES spectra of all the model compounds are shown in the Figure 3.7, with all their primary peaks at 350.09 eV and 353.40 eV. The carbonate spectrum has a presence of stronger peaks at 348.8 eV and 352.23 eV, than any other standard spectrum. The CaO has a very similar feature to that of carbonate, but they are shifted towards the lower energies at 348.7 eV and 352.11 eV, respectively. The pre-edge peaks at 347.87 eV, 348.46 eV and 348.98 eV are present in pure HA, beta TCP and alpha TCP. But, the peak at 348.46 eV is dominant for the alpha TCP than that of pure HA and beta TCP. There is no other distinguishing feature to differentiate beta TCP from pure HA standard. These model compounds are compared with the dried bone, calcined bones at 400^{o}C and 700^{o}C samples. The previous studies of bone on calcium L-edge XANES spectra are available [15] [16].

3.4.2.2 Calcium L-edge TEY of Dried Bone samples

All the calcium L-edge TEY XANES spectra of Dried Bone samples are identical to each other and are shown in Figure 3.8. The spectra looks more close to beta TCP and pure HA with its peaks matches perfectly with their standard spectra. The peak at 352.33 eV for the

spectra of dried bones is a match to beta TCP, pure HA and carbonates standards. It is little stronger than pure HA or beta TCP peak, hence, it should be coming from the carbonate peak. The presence of carbonate in the dried bone sample surface than the calcined bone samples are already clearly shown by the oxygen K edge spectra. Hence, it is possible to conclude that the dried bone samples are primarily made up of beta TCP or pure HA and carbonate.

3.4.2.3 Calcium L-edge TEY of bone samples calcined at 400^{o}C

The pre-edge peaks at 347.9 eV and 349.0 eV are present in all the calcium L-edge TEY XANES spectra of bone samples calcined at 400^{o}C, shown in Figure 3.9 and are a perfect match to the beta TCP and pure HA. The primary absorption peaks at 350.10 eV and 353.39 eV in all the calcined samples are not of much help in identifying the chemistry, because, all the model compounds have their peak position at the same energy level. The peak 352.35 eV is coming primarily from the beta TCP and pure HA, and a small contribution from the carbonate is undeniable. This shows the carbonate content in the bone samples calcined at 400^{o}C is less than the surface of the dried bone samples. This is also supported by the decrease in the carbonate content in the calcined bones than dried bone samples by the oxygen K-edge spectra.

3.4.2.4 Calcium L-edge TEY of bone samples calcined at 700^{o}C

All the calcium L-edge TEY XANES spectra of bone samples calcined at 700^{o}C have the pre-edge peaks at 347.9 eV and 349.0 eV, which corresponds to the pre-edge peaks of the beta TCP and pure HA. The spectra of all the different species of bone samples calcined at 700^{o}C are very similar to each other, see Figure 3.10. The peak at 352.35 eV matches with beta TCP, pure HA and carbonate, but, the carbonate peaks are more dominant than the other two standards. Since, the peak for the calcined samples are not predominant, the possibility of that peak corresponds to the carbonate is very less. The overall feature and peak positions of the bone samples calcined at 700^{o}C spectra looks more likely to have beta TCP and pure HA than the any other model compounds.

3.4.3 Calcium L-edge FY

3.4.3.1 Calcium L-edge FY of Model Compounds

The calcium model compounds that are possibly present in any bone sample are examined and their Calcium L-edge FY XANES spectra are shown in Figure 3.11. The white line at 350.09 eV, and the absorption peaks at 352.33 eV and 353.40 eV are common for all the model compounds. The peak at 352.33 eV in the spectra of most of the model compounds is shifted towards the lower energy for CaO and is at 352.10 eV. Since, the shift is as low as 0.23 eV, it still can possibly be considered as the same peak for other standards. The pre-edge peaks are the real differentiators in the calcium L-edge FY spectra of model compounds. The two prominent pre-edge peaks at 347.8 eV and 348.90 eV are present only in pure HA, beta TCP and alpha TCP standards. The pre-edge peak at 347.80 eV is absent for carbonate and CaO standards, and the other pre-edge peak at 348.90 eV is broader for CaO. The model compounds, pure HA and beta TCP are indistinguishable.

3.4.3.2 Calcium L-edge FY of Dried Bone samples

The presence of pre-edge peaks at 347.8 eV and 348.90 eV in the calcium L-edge FY XANES spectra for all the dried bone samples matches with pure HA, beta TCP and alpha TCP, than any other model compounds. The absorption peak at 352.23 eV for the dried samples could be coming from the carbonate, since that peak is present in spectra of all the model compounds, it is hard to conclude. The rest of the dried bone spectra, see Figure 3.12, look very similar to pure HA and beta TCP. It is possible to conclude that all the dried bone samples are primarily composed of pure HA or beta TCP.

3.4.3.3 Calcium L-edge FY of Bone samples calcined at 400^{o}C

The white lines of calcium L-edge FY XANES spectra of all the bone samples calcined at 400^{o}C are at 530.09 eV, shown in Figure 3.13, matches with all the standards. The pre-edge peaks at 347.8 eV and 349.0 eV corresponds to the pre-edge peaks of pure HA, alpha TCP and beta TCP standards, which are at 347.8 eV and 348.90 eV, respectively. The absence of sharp

absorption peak at 352.33 eV in the spectra of all the dried bone samples can be used to eliminate the presence of alpha TCP in the bone chemistry. Hence, it is possible to conclude that the main chemistry of the dried bone samples is either coming from pure HA or beta TCP. The only exceptional among the dried bones is the chicken dried bone sample, with its presence of shoulder at 352.5 eV and absence of peak at 352.3 eV, which does not matches with any of the standards.

3.4.3.4 Calcium L-edge FY of Bone samples calcined at 700^{o}C

The XANES spectra of all the bone samples calcined at 700^{o}C are very similar to the beta TCP and pure HA standards, with their pre-edge peaks at 347.81 eV and 348.9 eV. These bone samples calcined at 700^{o}C are identical to each other, on the other hand, the chicken bones calcined at 400^{o}C is significantly different from all the other bone samples calcined at 400^{o}C, see Figure 3.14 and Figure 3.13. It is possible to say that the bone samples calcined at 700^{o}C are primarily composed of HA or beta TCP.

3.4.4 Oxygen K-edge TEY

3.4.4.1 Oxygen K-edge TEY of Model Compounds

All the model compounds pure HA, beta and alpha TCP, CaO and $CaCO_3$ have very distinctive spectra, see Figure 3.15. Even though, spectrum of HA, beta and alpha TCP appear very similar, there are some subtle differences. The second peak around 540 eV for alpha TCP is higher than the first peak at 537 eV. This is the only difference that can be used to differentiate between alpha and beta TCP. The peak at 534.84 eV for pure HA spectrum is the differentiator peak, which helps in differentiating it from the other phosphates. The Tricalcium phosphates also have a pre-edge peak that has a larger separation from the primary peak, when compared to the separation between the pre-edge of HA and its white line. The carbonate spectrum is a very clear and significantly different from the other spectra, and has completely different features. The first peak is at 534.46 eV, second peak at 537.83 eV, third peak at 541.18 eV and last peak at 545.63 eV. The CaO spectrum is similar to the carbonate in the

primary and the third post edge peak structure and its positions. But, the second and third post-edge peaks that are at 536.78 eV and 540.5 eV, respectively, are completely different in its shape and positions. This clearly shows distinctive characteristics for phosphates and non-phosphates.

3.4.4.2 Oxygen K-edge TEY of Dried Bone samples

All the dried samples have a very common structural feature, except some intensity variations, see Figure 3.16. The Chicken sample has a pre-edge position at 531.60 eV, which is not present in all the other samples. This peak position possibly indicates that the Chicken dried sample has some more beta-TCP than other species, because that peak corresponds to the beta TCP's peak at 531.85 eV.

The overall spectrum of all the samples looks more likely to have carbonate in it. And it is not in the purest form and it also have some beta TCP or HA too. Since the spectrum of beta TCP and HA are identical it is very hard to differentiate them. The final conclusion can be made by comparing all the results from other beam lines. The dominant peak in all the dried samples is the carbonate peak. The second half (537 eV to552 eV) is not at all like the carbonate standard, this is because the beta TCP or HA is also present and this co-existence shifts the primary peak to the left. The second half is the superimposition of both HA or beta TCP and carbonate.

The first two dominant peaks at 534.46 eV and 537.83 eV of carbonate standards are shifted slightly to the left of the standard $CaCO_3$ in the case of the dried samples and are located at 532.92 eV and 535.55 eV, respectively. It is clear from the TEY spectra that the dominant chemistry of the Ca is in the form of carbonate near the surface. However, the broad post edge structure after the first two peaks is similar to what is seen in HA and beta-TCP. Hence, it can be hypothesized that a mixture of carbonates and phosphates are present at the surface of the bone in the dried samples. The reason for the shift of the carbonate peaks can be hypothesized based on the fact that in bone the carbonate and phosphates are blended

together and are possibly part of the same structure, hence the phosphorous atom in the structure may result in local distortation of the carbonate resulting in changes in the local environment of the oxygen associated with the carbonate resulting in the shift,

3.4.4.3 Oxygen K-edge TEY of bone samples calcined at 400°C

The spectra for all bone samples calcined at 400°C shown in Figure 3.17 appear to match with those of phosphates rather than carbonates, whereas, the dried bone is made up of a mixture of carbonate and phosphate. The TEY spectra provide information from about the top 100 nm of the powdered sample. Hence, it is clear enough to say that the carbonate is found on the surface. The primary peak position at 537.37 eV for the sample corresponds to the beta-TCP peak at 537.2 eV. The second peak at 539.94 eV is close to beta TCP peak at 539.86 eV rather than the alpha TCP peak at 540.14 eV. Hence it is likely that the spectra matched beta TCP rather than alpha TCP, because of two reasons. Firstly, the peak position is much closer to beta TCP and secondly, the relative peaks heights of the spectra matches better with that of beta TCP. Hence, it can be concluded that the bone samples calcined at 400°C samples are largely made up of beta TCP. A small amount of carbonate is also present in the sample; the corresponding peak position is at 534.54 eV. The samples calcined at 400°C are powdered samples the surfaces of the powder do not appear to have extra carbonate and the powder is coming from all parts of the bone and not just the surface of bone.

3.4.4.4 Oxygen K-edge of TEY Bone calcined at 700°C

There is no much change takes place in terms of oxygen when the samples are further heated up to 700°C, see Figure 3.18. This is evident from the spectrum of 700°C, which looks almost identical to the 400°C. Only exception being, Bovine 700°C sample which seems to have little more carbonate content than other samples at 534.54 eV. Hence, the chemical composition with respect to oxygen remains beta TCP even after heating up to 700°C.

3.4.5 Oxygen K-edge FY

3.4.5.1 Oxygen K-edge FY of Model Compounds

Figure 3.19 shows oxygen K-edge FY XANES spectra of all the model compounds. Distinctive features of each spectrum are the differentiators of their respective compounds. The beta TCP and alpha TCP are very similar, but the relative heights of the peak at 537.20 eV and 539.94 eV are different. The pre-edge peaks at 531.89 eV and 529.81 eV are common for the two tri-calcium phosphate standards. The pure HA standard with its relative height of the peaks at 537.71 eV and 540.85 eV, and its shape are similar to beta TCP, though, they are shifted towards the higher energy. The presence of a pre-edge peak at 534.79 eV and its separation from the white line are the clear differentiators of HA from tri-calcium phosphates.

The carbonate spectrum is significantly different from other model compounds, with its features and its primary absorption peaks at 534.45 eV, 537.91 eV, 541.14 eV and 545.87 eV. Also, a small pre-edge peak is present at 534.45 eV which is also present in alpha TCP and beta TCP. The CaO spectrum has a pre-edge peak at 529.81 eV, and its first peak is at 534.79 eV is same as the pre-edge peak of pure HA standard. The second and third peaks are present at 536.79 eV and 540.00eV, respectively, with a small shoulder at 542.00eV. The characteristic features of all these standards are very unique. This Oxygen K-edge FY spectra are very important in differentiating pure HA and beta TCP, because of its distinctive pre-edge peaks.

The oxygen K-edge FY spectra of model compounds are very similar to its respective oxygen K-edge TEY spectra, except for few peaks. In pure HA, the pre-edge peak at 530.81 eV for TEY is absent in FY. The pre-edge peaks for carbonate and CaO are clearer for FY spectra, than for TEY. The CaO peak at 534.46 eV is shifted towards the higher energy at 536.79 eV for FY and it is less dominant than its TEY counterpart. Even though, the peak positions for carbonate for TEY and FY are same, their relative height intensities between the peaks at 534.45 eV and 541.14 eV are different.

3.4.5.2 Oxygen K-edge FY of Dried Bone samples

The oxygen K-edge FY spectra of dried bones of deer and bovine, shown in Figure 3.20, are similar to beta TCP standard. The oxygen K-edge FY spectra of all the dried bone samples have their white lines at 537.33 eV are a match to beta TCP at 537.20 eV. The peak at 532.85 eV corresponds to carbonate and between the two samples, it is very dominant in dried deer bone sample than dried bovine bone. The same pre-edge peak for the dried chicken bone sample is very dominant than any other sample. This indicates the presence of more carbonate in dried chicken bone surface.

The relative height of peaks at 537.33 eV and 540.03 eV for deer and bovine dried bone samples are corresponds to beta TCP standard, but the chicken dried bone sample looks more like alpha TCP standard. The dominant carbonate peak at 532.85 eV shows the presence of carbonate in the sample, and the other carbonates peaks at 534.45 eV, 537.91 eV, 541.14 eV and 545.87 eV should also be present in the dried chicken bone spectrum. Hence, it can be hypothesized that the overlap of carbonate peak at 541.14 eV and the beta TCP peak at 539.94 eV results in the formation of peak of dried chicken bone sample at 540.03 eV. That is, the relative peak heights between the peaks at 537.33 eV and 540.03 are coming from the combination of beta TCP and carbonate, but not from alpha TCP.

Hence, it is possible to conclude that deer and bovine dried bone samples are primarily composed of beta TCP, on the other hand, the chicken is composed of beta TCP and carbonate. The FY spectra provide the information of the bulk, which is less of carbonate. This supports that the result of Oxygen K-edge TEY, that dried bone samples are covered with carbonate on the top surface, not in the bulk.

3.4.5.3 Oxygen K-edge FY of bone samples calcined at 400^{o}C

The oxygen K-edge FY XANES spectra for all the bone samples calcined at 400^{o}C are shown in the Figure 3.21, and they match with the beta TCP standard. The amount of carbonate present in the bone samples calcined at 400^{o}C is less than that of the surface of the dried bone

samples. The bone samples calcined at 400^{o}C are powdered samples, which comes from the surface and bulk of the bone samples. On the other hand, the dried samples are chunk of bones and the surface can also be studied. The white line at 537.32 eV, the pre-edge peak at 531.89 eV and the post-edge peak at 540.02 eV of the spectra of all the bone samples calcined at 400^{o}C are corresponds to the beta TCP standard. Even though, the spectrum of beta and alpha TCP are similar, the relative peaks heights at 537.32 eV and 540.02 eV are different. And the relative peak height of all the bone samples calcined at 400^{o}C match with the beta TCP standard. The pre-edge peak at 534.28 eV is coming from the carbonate standard. Hence it is clear from the FY spectra that bones calcined at 400^{o}C are primarily composed of beta TCP and with little amount carbonate in it.

3.4.5.4 Oxygen K-edge FY of bone samples calcined at 700^{o}C

The pre-edge peak at 531.89 eV, the white line at 537.31 eV and the post-edge peak at 540.0 eV in the oxygen K-edge spectra of all the bones calcined at 700^{o}C, shown in Figure 3.22, corresponds to the beta TCP standard. And, the pre-edge peak at 543.28 eV matches with the carbonate standard. This shows that the bones calcined at 400^{o}C and 700^{o}C have a very common chemistry; with the primary component being beta TCP and along with some carbonate.

3.4.6 Phosphorous K-edge TEY

3.4.6.1 Phosphorous K-edge TEY of Model compounds

The phosphorous K-edge TEY XANES spectra of all the model compounds are shown in the Figure 3.23 and are studied widely [18] [19]. All the model compounds have their white line at 2152.04 eV and two post-edge peaks at 2162.78 eV and 2169.30 eV, respectively. There is no pre-edge peak present in any of the model compounds. The spectra of $CaHPO_4$ and CaH_2PO_4 H_2O are identical, with the same white line peak and two post-edge peak positions. Most of the spectra look similar, with only difference being a peak or shoulder at 2155 eV. The $Ca_2P_2O_7$ has a post-edge shoulder at 2155 eV, which is absent in all other standards. The

presence of a post-edge peak at 2154.81 eV is unique for pure HA and beta TCP standards. And, there is no distinctive features between pure HA and beta TCP standards.

3.4.6.2 Phosphorous K-edge TEY of Dried Bone samples

All the phosphorous K-edge TEY spectra of dried bone samples have their white line at 2152.14 eV, and two post-edge peaks at 2162.6 eV and 2169 eV; with no pre-edge peaks, see Figure 3.24. These features are common for all the model compounds. The peak at 2154.9 eV corresponds to the post-edge shoulder for $Ca_2P_2O_7$standard or pre-edge peaks for the pure HA and beta TCP standards. The 2154.9 eV is clearly stronger than a shoulder, and it should be coming from the beta TCP or pure HA standard. Hence, it is possible to conclude that all the dried bone samples are made of pure HA or beta TCP with some other trace compounds as contamination.

3.4.6.3 Phosphorous K-edge TEY of bones calcined at 400^oC

The phosphorous K-edge TEY spectra of all the dried bone samples and that of bone samples calcined at 400^{o}C are similar in its structure and are shown in the Figure 3.25. The only difference being, the peak at 2154.7 eV for the bone samples calcined at 400^{o}C is more prominent than the peak at 2154.9 eV for dried bone samples. So, it is possible to conclude that chemistry of bone calcined at 400^{o}C are pure HA or beta TCP and the heating of dried bone samples at 400^{o}C make the bone samples purer, with less of contamination.

3.4.6.4 Phosphorous K-edge TEY of bones calcined at 700^oC:

The phosphorous K-edge TEY spectra of bones calcined at 700^{o}C look very similar to the beta TCP and pure HA spectra, see Figure 3.26. The post-edge peak feature at 2154.85 eV is a perfect match to that of the pure HA and beta TCP standards. It is possible to say, that the phosphorus contamination in the bone samples are mostly eliminated by heating at 700^{o}C and the bone samples looks to have a very cleaner HA or beta TCP in it.

3.4.7 Calcium K-edge TEY

3.4.7.1 Calcium K-edge TEY of Model Compounds

The calcium K-edge TEY XANES spectra of the model compounds used are shown in the Figure 3.27. The The calcium K-edge TEY XANES spectra are used in the past to study osteoporosis and other calcium phosphate compounds [18] [17]. The calcium K-edge TEY of pure HA and beta TCP are different from other model compounds with the presence of a pre-edge peak at 4040.40 eV. The carbonate spectrum has a dominant absorption peak at 4060.50 eV, which is almost absent in other standards. The pre-edge peaks at 4045.7 eV for pure HA and at 4044.73 eV for CaO are well defined, when compared to the other standards, which is a shoulder at 4045 eV. The post edge shoulder is used to differentiate otherwise similar pure HA and beta TCP; the post-edge shoulder at 4060.50 eV for beta TCP is not present in the pure HA standard. The pure HA has a split white line, which is absent in all other standards.

In summary, the pure HA and beta TCP standards can be differentiated by three structural differences in their respective spectrum. Firstly, the presence of a post-edge peak at 4060.50 eV in the beta TCP spectrum, secondly, the absence of split white line in the beta TCP spectrum and thirdly, the pronounced pre-edge peak at 4045.70 eV in the pure HA spectrum. The $Ca(OH)_2$ spectrum has no pre-edge peak at 4040.50 eV and its white line is shifted towards the higher energy at 4051.73 eV. The same is true for CaO as well with one exception, that is, it has a strong defined pre-edge peak at 4044.73 eV.

3.4.7.2 Calcium K-edge TEY of Dried Bone samples

The calcium K-edge XANES spectra of all the dried bone samples have no obvious difference in its structure, see Figure 3.28. The white line for all the dried bones are at 4049.91eV is a match for the pure HA and beta TCP and they have their white lines at 4050.40 eV. Also, the presence of pre-edge peak at 4040.54 eV corresponds to beta TCP and pure HA. The dominant post-edge peak at 4060.50 eV is the characteristic feature of the carbonate spectrum, which is present in all the dried bone samples. But, they are not as strong as the

carbonate peak. It looks as if the peak is coming from the beta TCP and also the post-edge shoulder is very similar to the beta TCP spectrum. The pre-edge shoulder at 4046.01 for all the dried bone samples corresponds to the carbonate, $Ca(OH)_2$, and beta TCP standards. Due to the absence of the pre-edge peak at 4040.50 eV for the carbonate and $Ca(OH)_2$ standards, it is less likely to have them in the dried bone chemistry. Hence, it is clear that the primary content of all the dried bone samples are made up of beta TCP.

3.4.7.3 Calcium K-edge TEY of bone samples calcined at 400^{o}C

The white lines for all the calcium K-edge TEY spectra of bone samples calcined is at 400^{o}C are at 4050.24 eV and they matches with the pure HA and beta TCP standards. Since, the white lines of other model compounds are only shifted 0.3 eV, it is not possible to conclude that the bones are made up of pure HA or beta TCP. The calcium K-edge TEY spectra of bone samples calcined at 400^{o}C are shown in Figure 3.29. The pre-edge peak at 4045.9 eV and all other post-edge peaks at 4071.6 eV, 4080.1 eV and 4096.4 eV are present in all the standards. To determine the chemistry of the bone samples calcined at 400^{o} C, the post-edge shoulder at 4060.5 eV and the pre-edge shoulder at 4045.9 eV are considered. The presence of a pre-edge peak at 4040.54 eV confirms that the bone samples calcined at 400^{o}C are made up of beta TCP or pure HA. Even though, the pre-edge peak at 4045.9 eV matches with the 4045.7 eV of both the standards, the shape is quite differentiable between pure HA and beta TCP standards. This along with the presence of a post-edge shoulder at 4060.5 eV, it is possible to say that the bone samples calcined at 400^{o}C are primarily made up of beta TCP.

3.4.7.4 Calcium K-edge TEY of bone samples calcined at 700^{o}C

The absorption peaks of most calcium K-edge TEY XANES spectra of the bone samples calcined at 700^{o}C are common for all the standards, see Figure 3.30. The white line at 4050.3 eV and pre-edge peak at 4040.7 eV corresponds to the pure HA and beta TCP standards. The pre-edge shoulder at 4045.3 eV looks very similar to pre-edge shoulder of beta

TCP at 4045.7 eV than pure HA. The shape and peak position at 4060.50 eV of the post-edge shoulder of all the bone samples calcined at 700°C are identical to the beta TCP standards.

3.5 XRD spectra

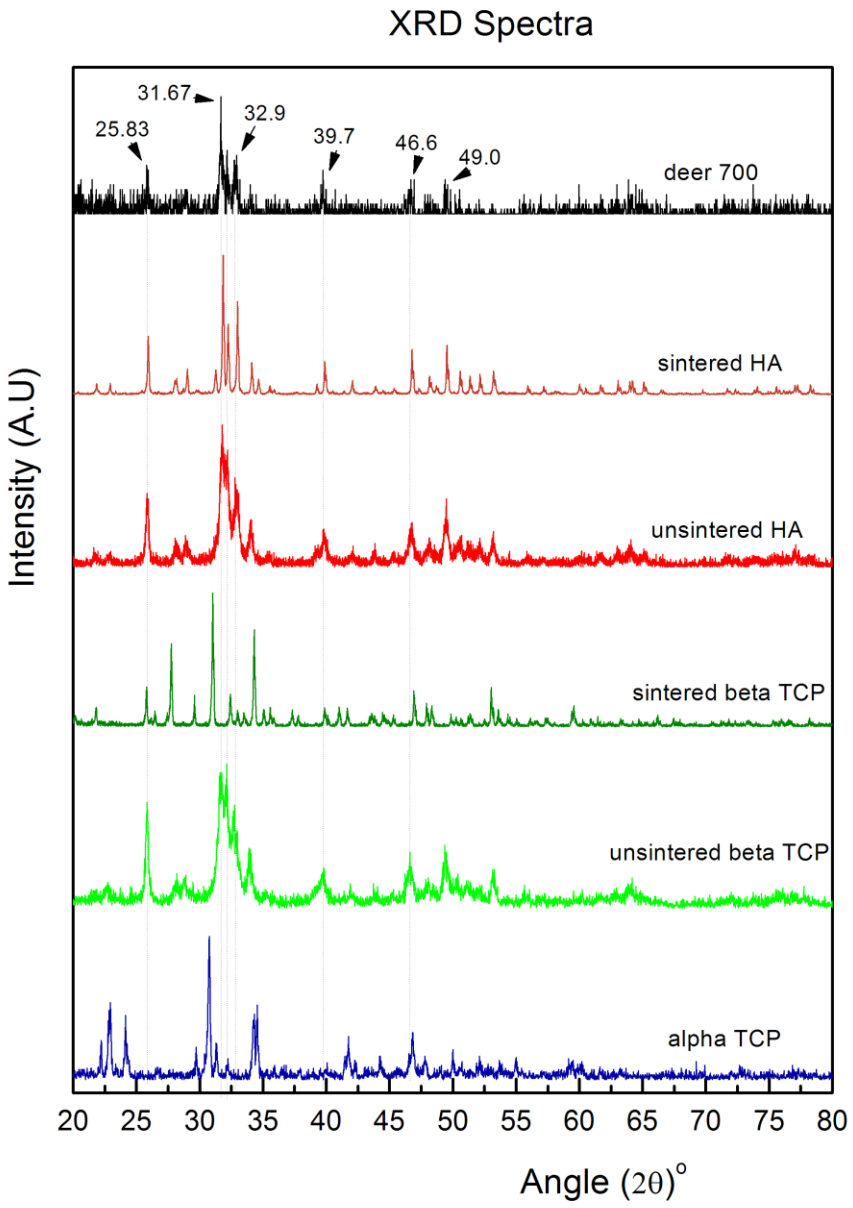


Figure 3.1 XRD spectra - Comparison of deer bone sample calcined at 700°C and standards

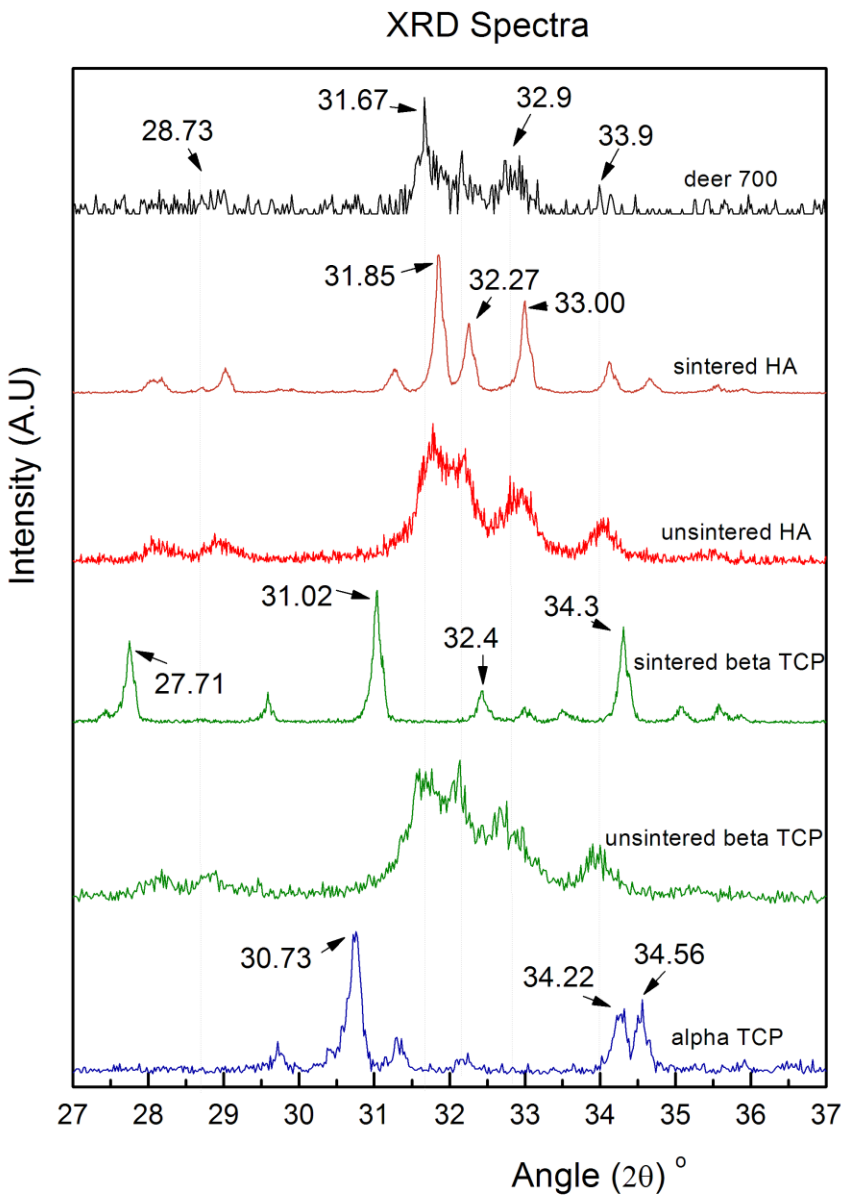


Figure 3.2 XRD spectra - Comparison of deer bone sample calcined at 700°C and standards - region of primary peaks

3.6 XANES spectra

3.6.1 Phosphorous L edge FY

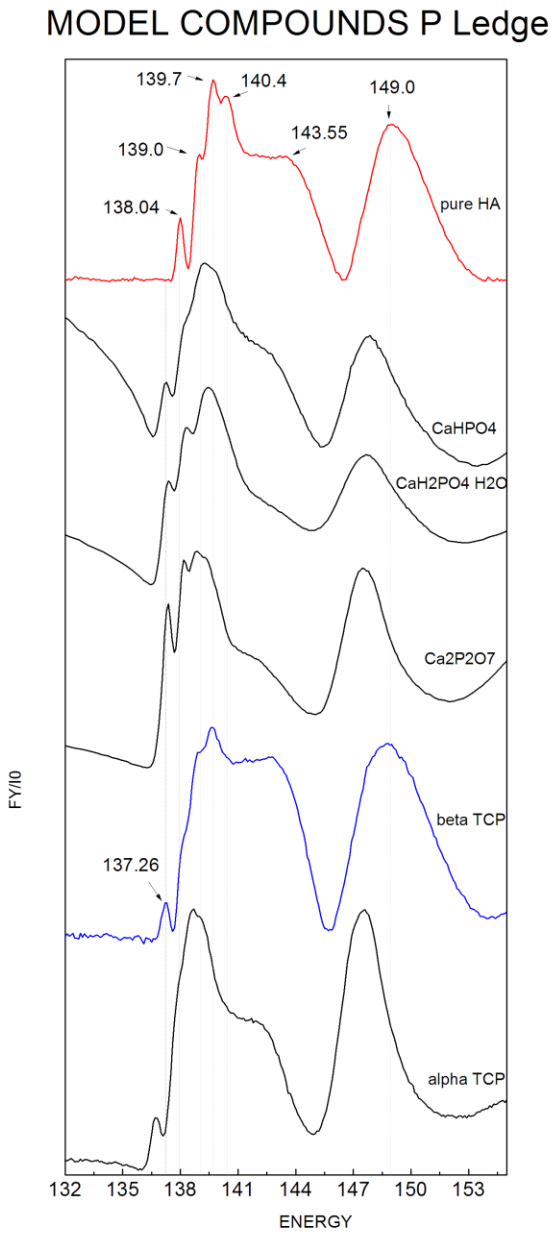


Figure 3.3 Phosphorous L-edge FY XANES spectra of Model Compounds

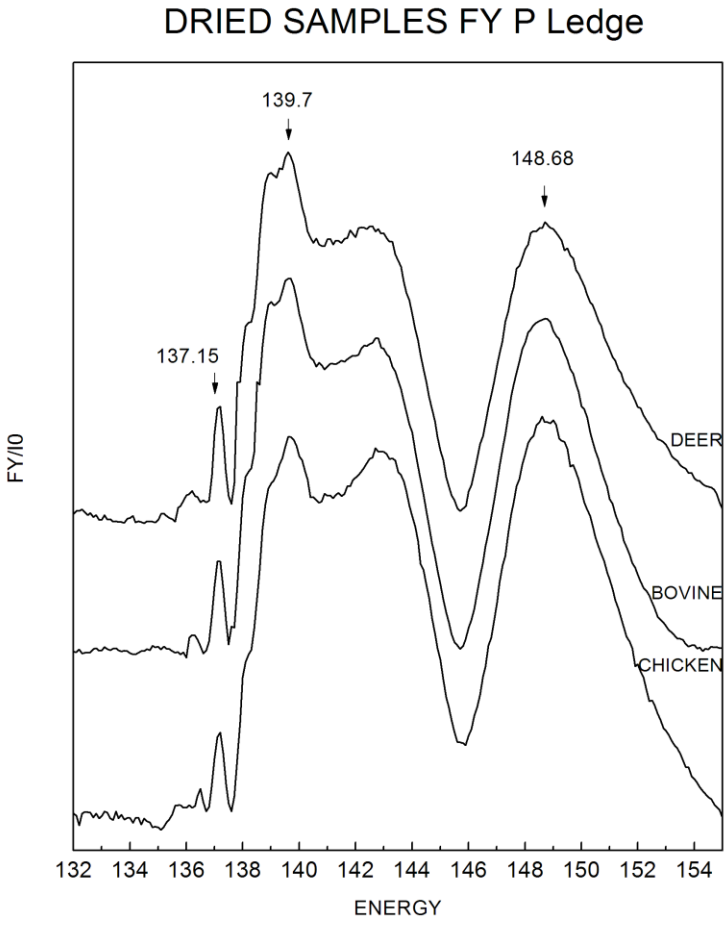


Figure 3.4 Phosphorous L-edge FY XANES spectra of dried bone samples

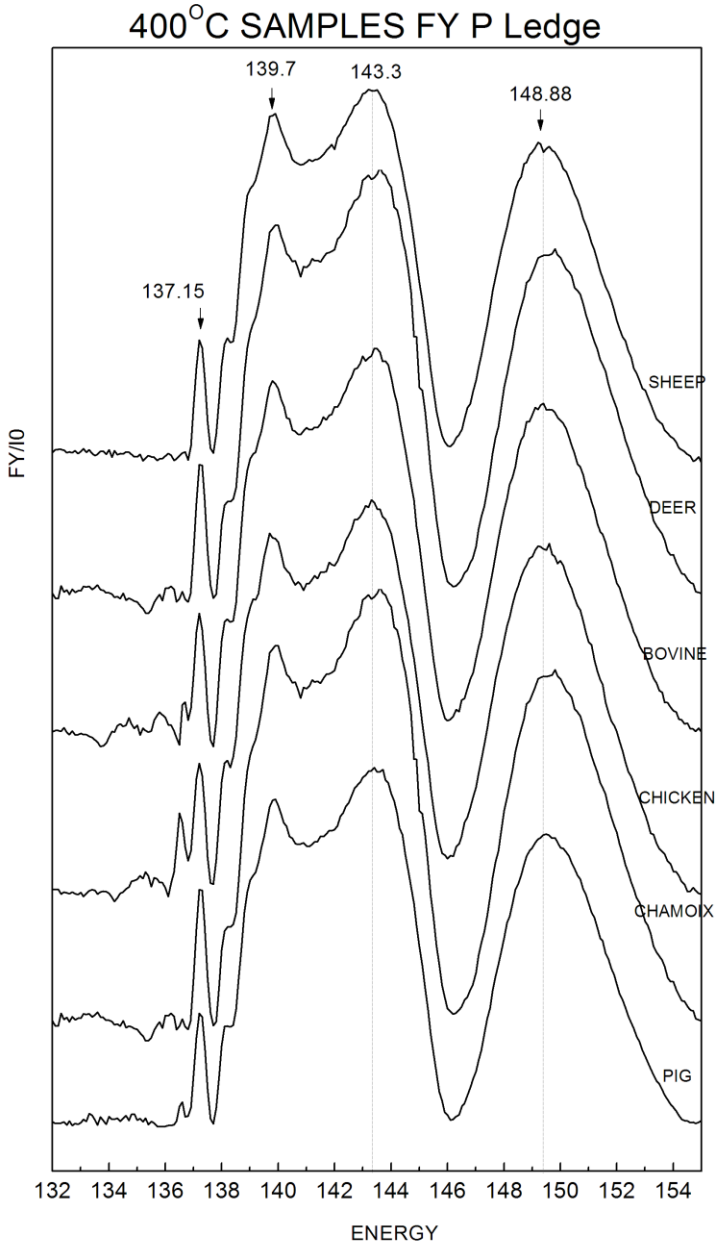


Figure 3.5 Phosphorous L-edge FY XANES spectra of bone samples calcined at 400°C

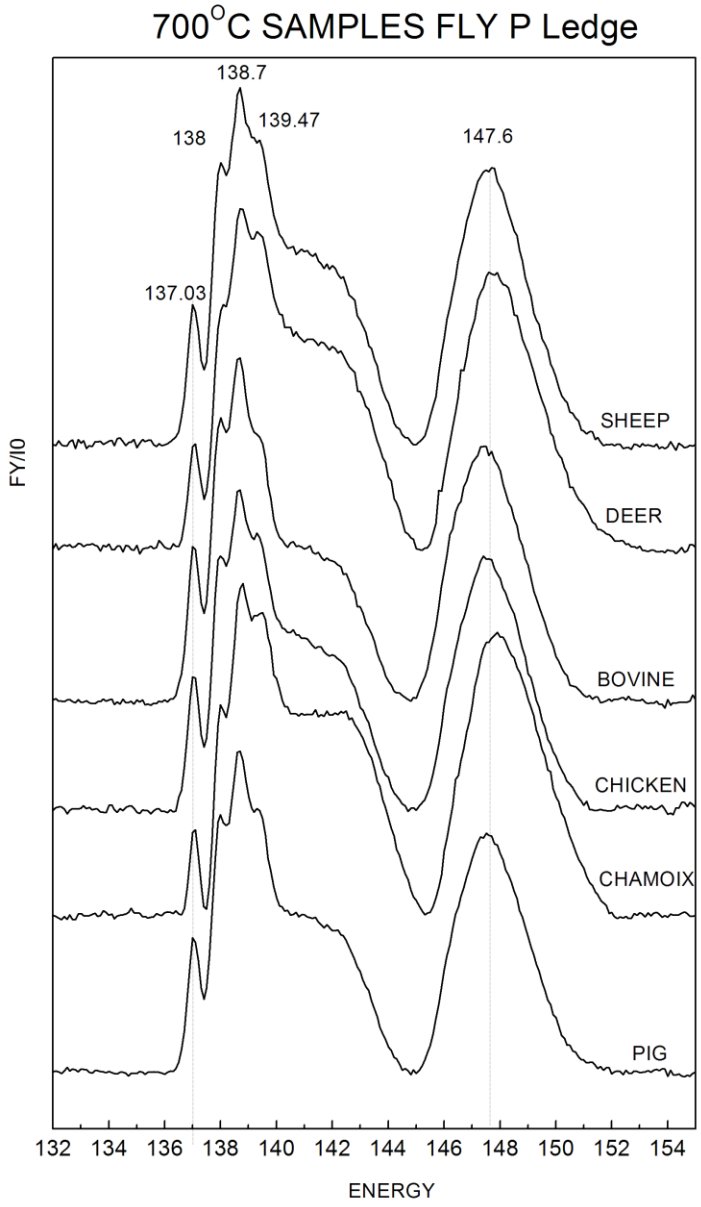


Figure 3.6 Phosphorous L-edge FY XANES spectra of bone samples calcined at 700°C

3.6.2 Calcium L-edge TEY

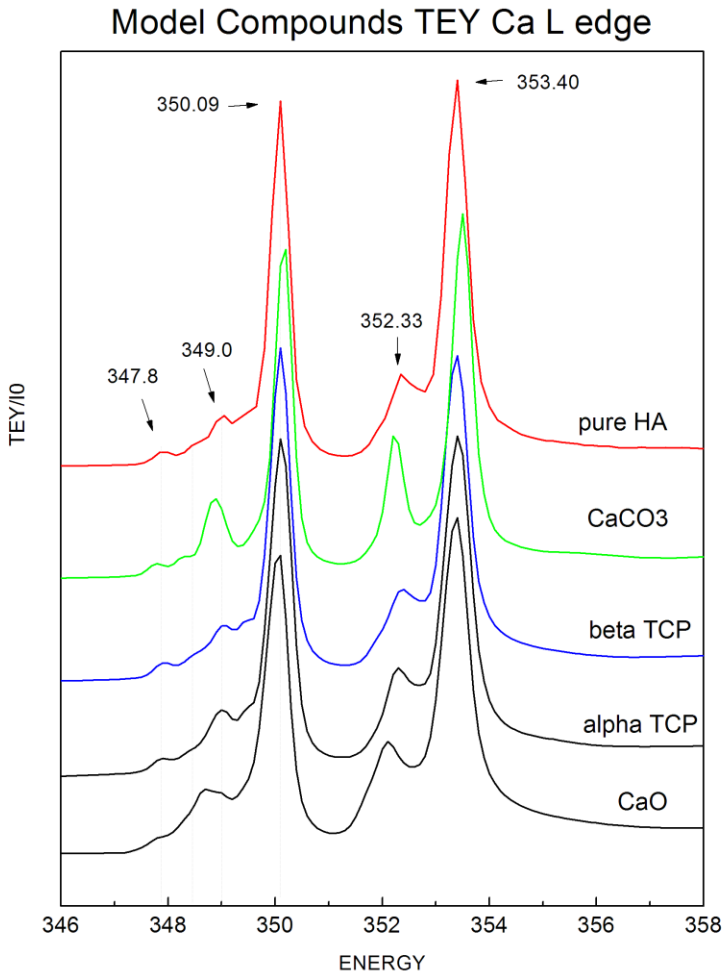


Figure 3.7 Calcium L-edge TEY XANES spectra of Model Compounds

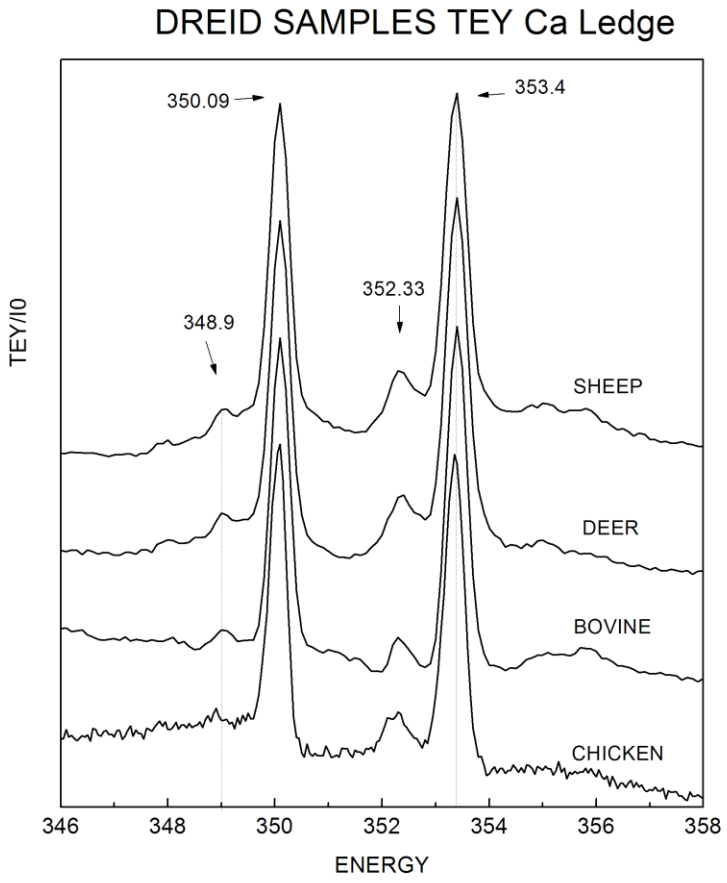


Figure 3.8 Calcium L-edge TEY XANES spectra of Dried Bone samples

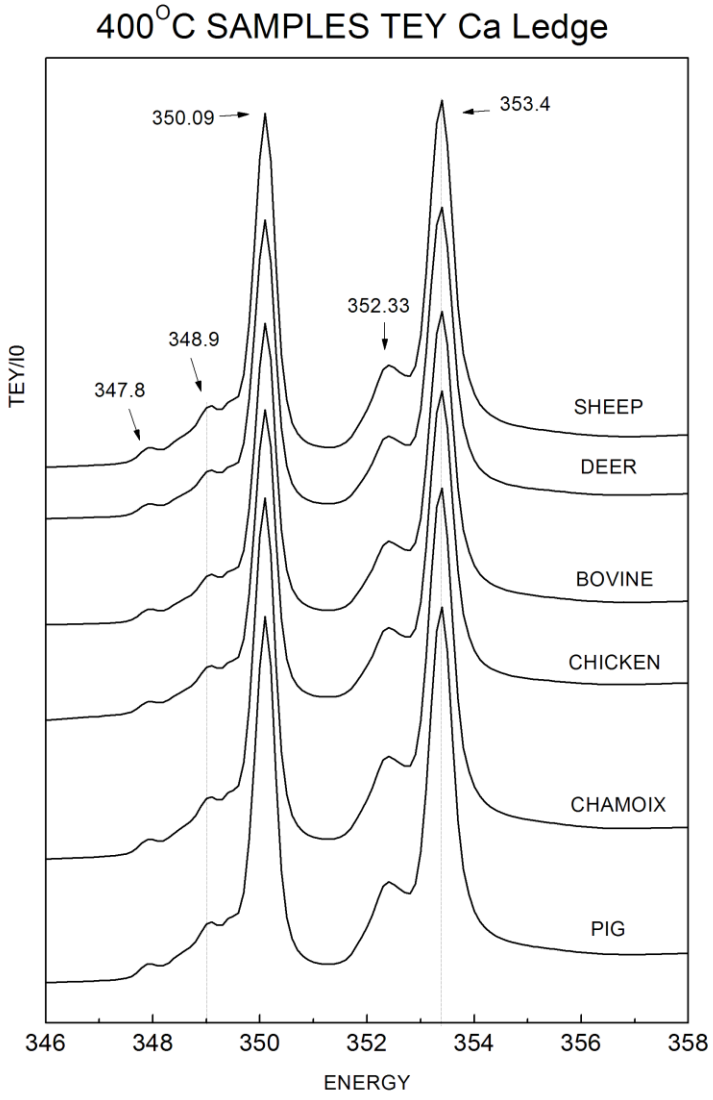


Figure 3.9 Calcium L-edge TEY XANES spectra of bones calcined at 400°C

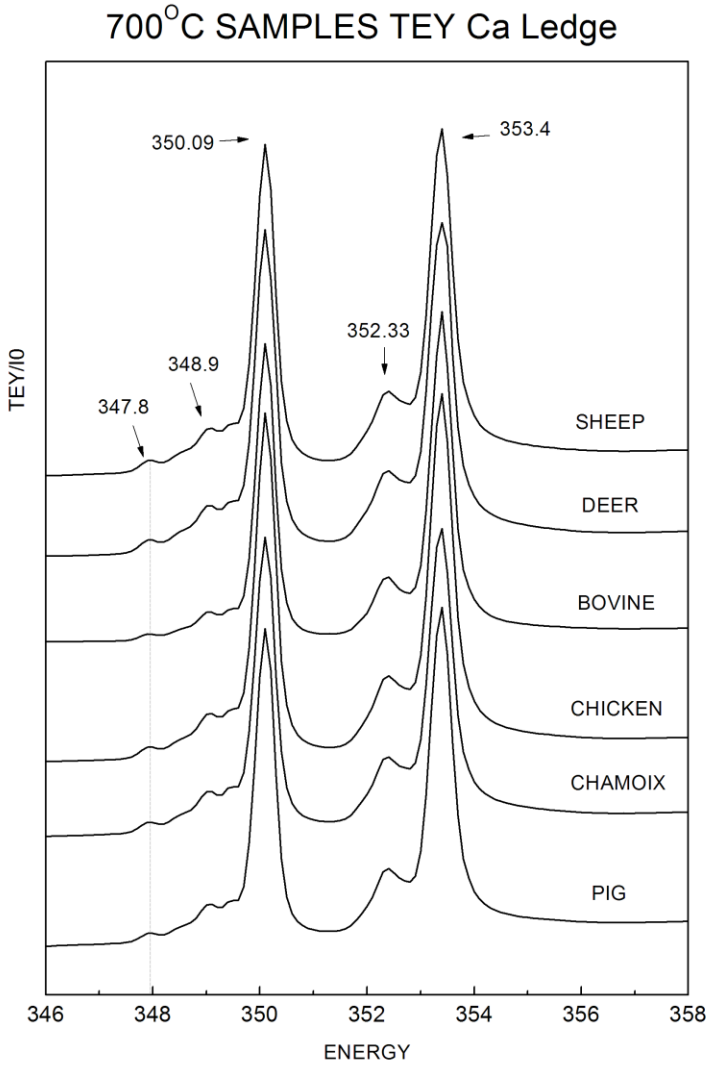


Figure 3.10 Calcium L-edge TEY XANES spectra of bones calcined at 700°C

3.6.3 Calcium L-edge FY

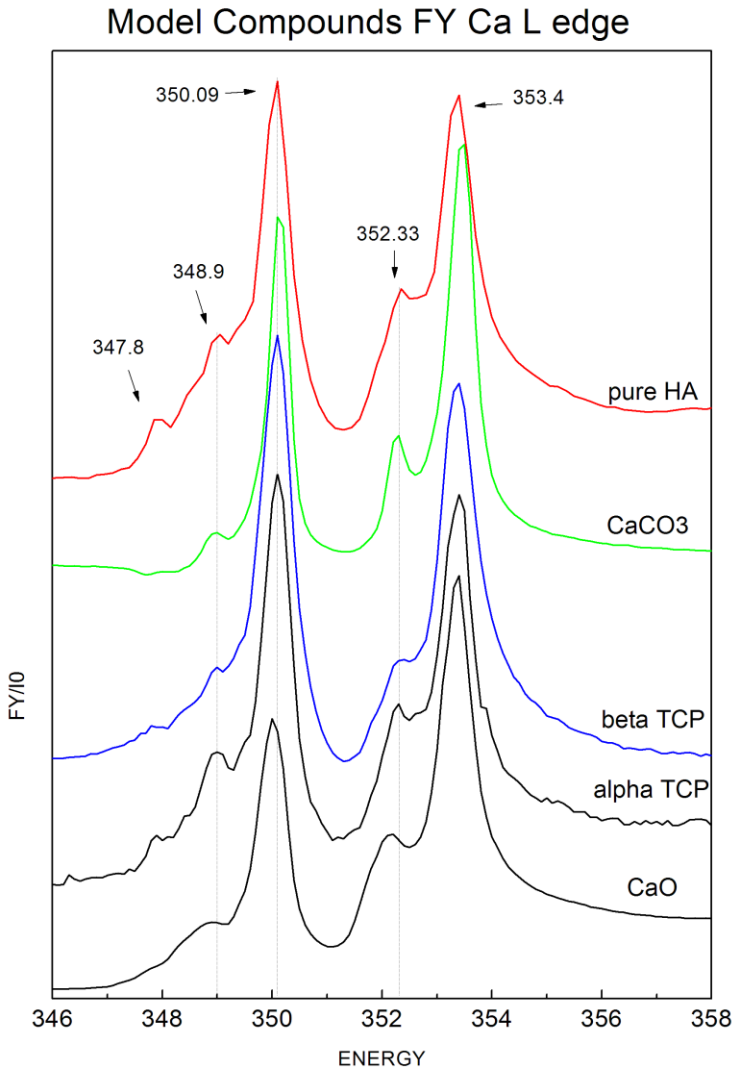


Figure 3.11 Calcium L-edge FY XANES spectra of Model Compounds

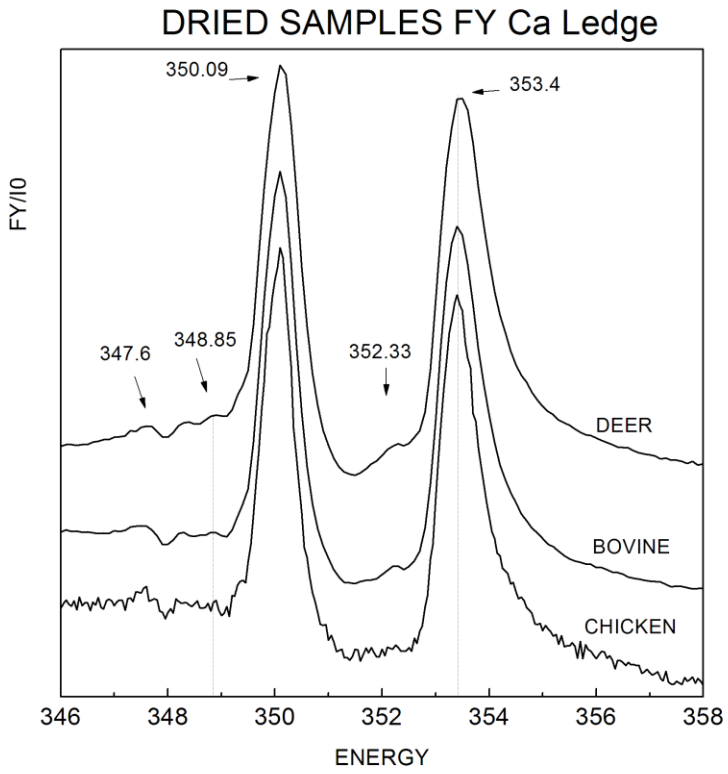


Figure 3.12 Calcium L-edge FY XANES spectra of Dried Bone samples

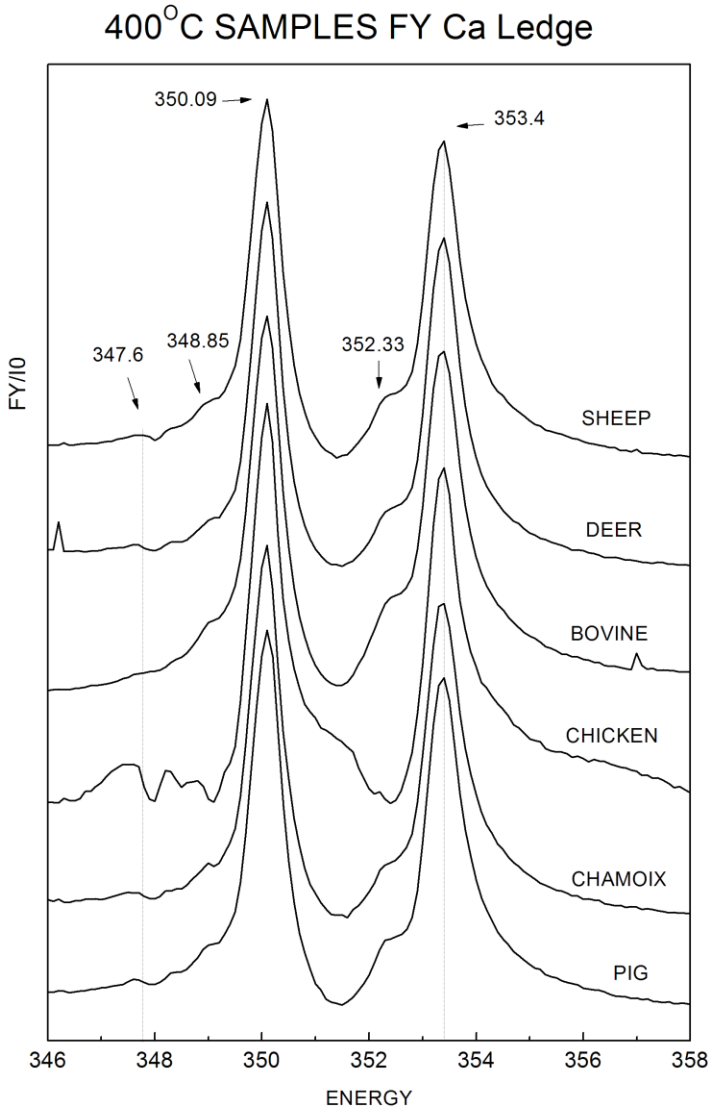


Figure 3.13 Calcium L-edge FY XANES spectra of bone samples calcined at 400°C

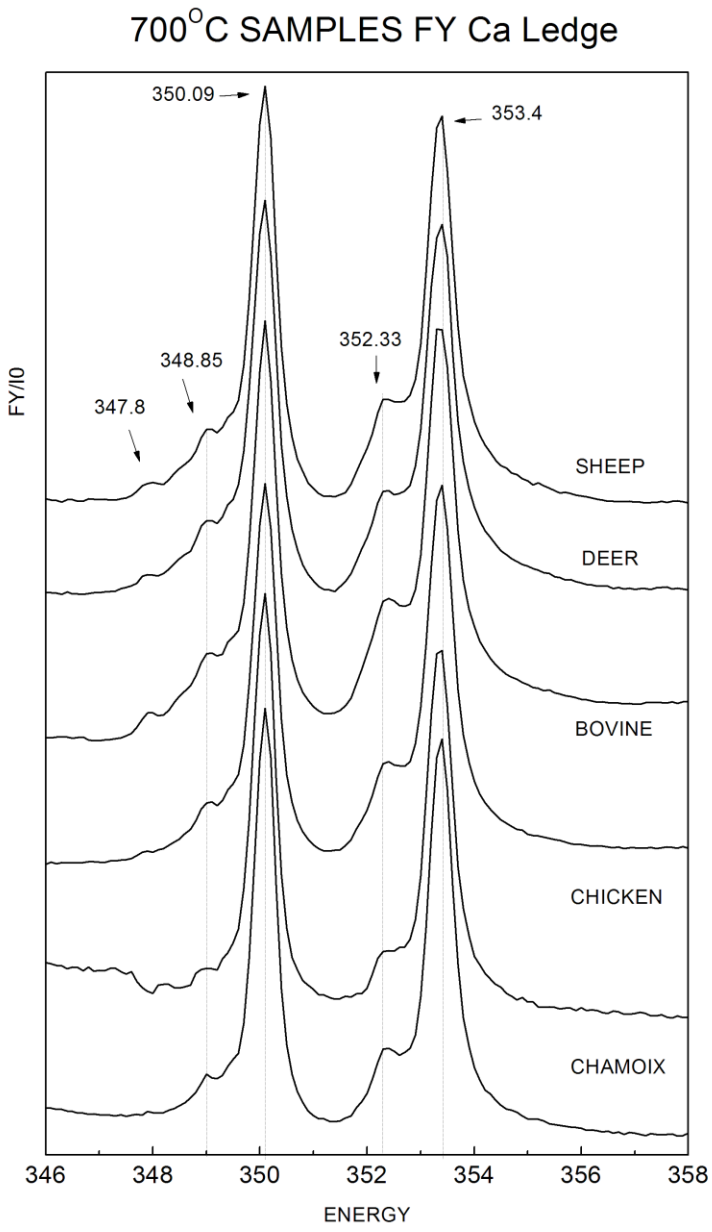


Figure 3.14 Calcium L-edge FY XANES spectra of bone samples calcined at 700°C

3.6.4 Oxygen K-edge TEY

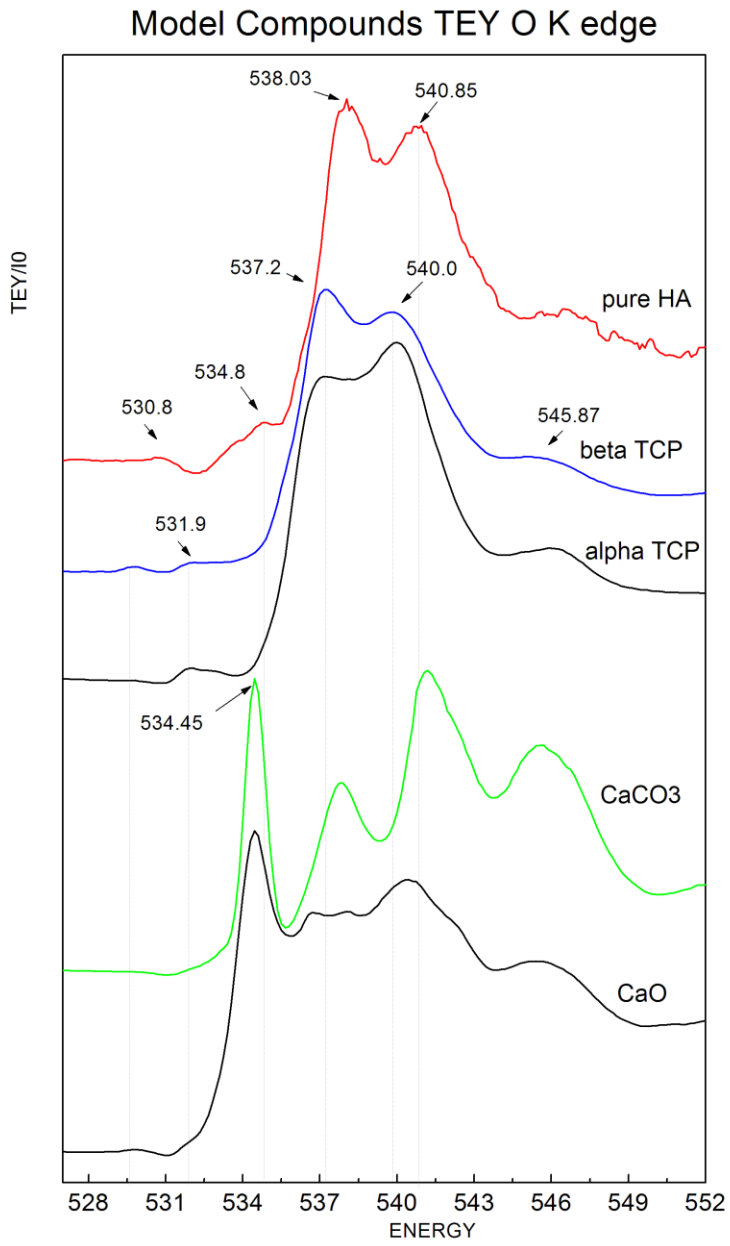


Figure 3.15 Oxygen K-edge TEY XANES spectra of Model Compounds

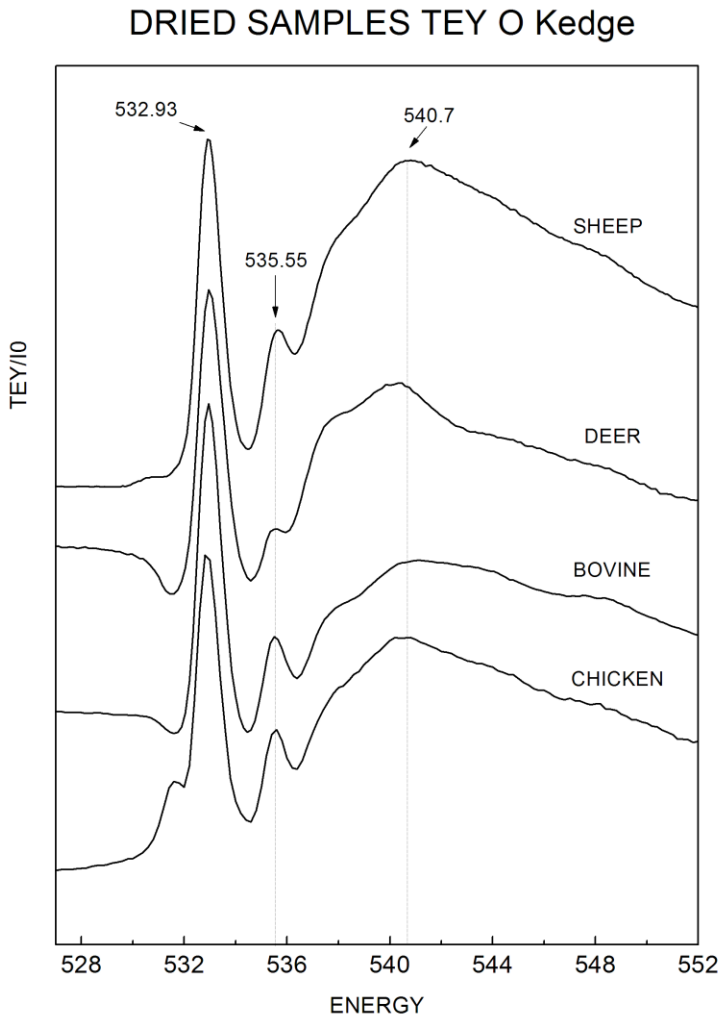


Figure 3.16 Oxygen K-edge TEY XANES spectra of Dried bone samples

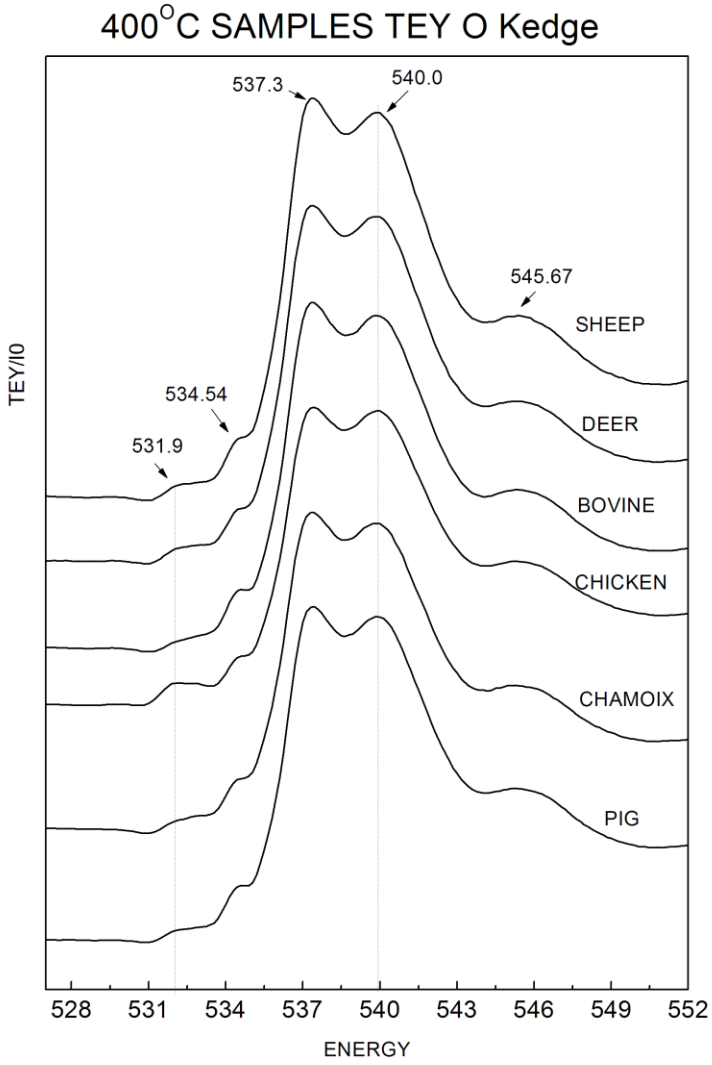


Figure 3.17 Oxygen K-edge TEY XANES spectra of bone samples calcined at 400°C

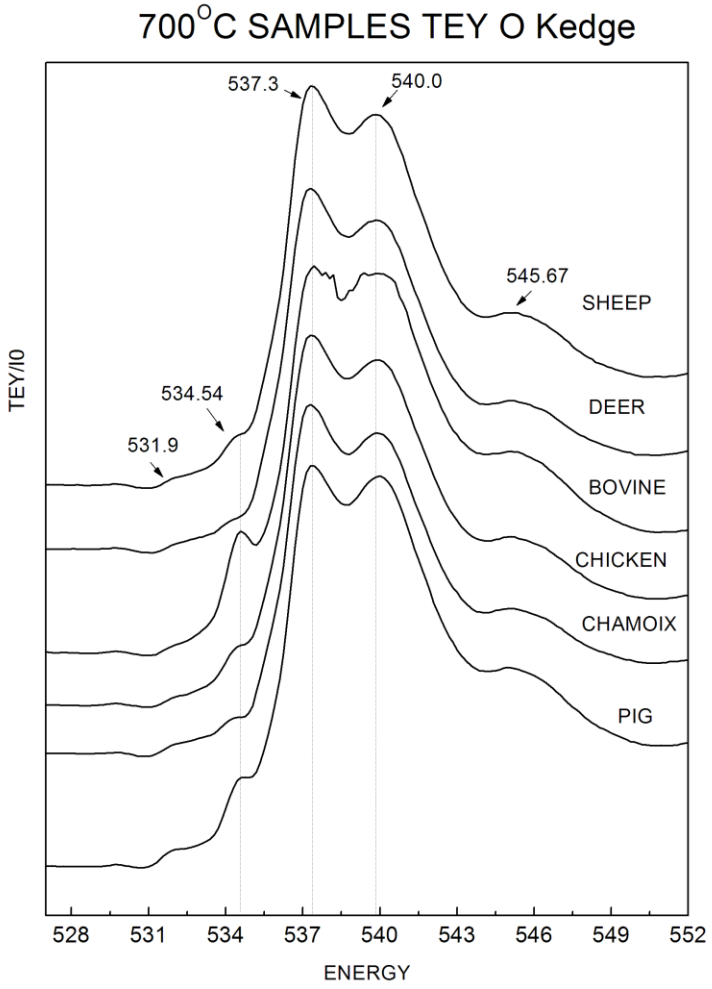
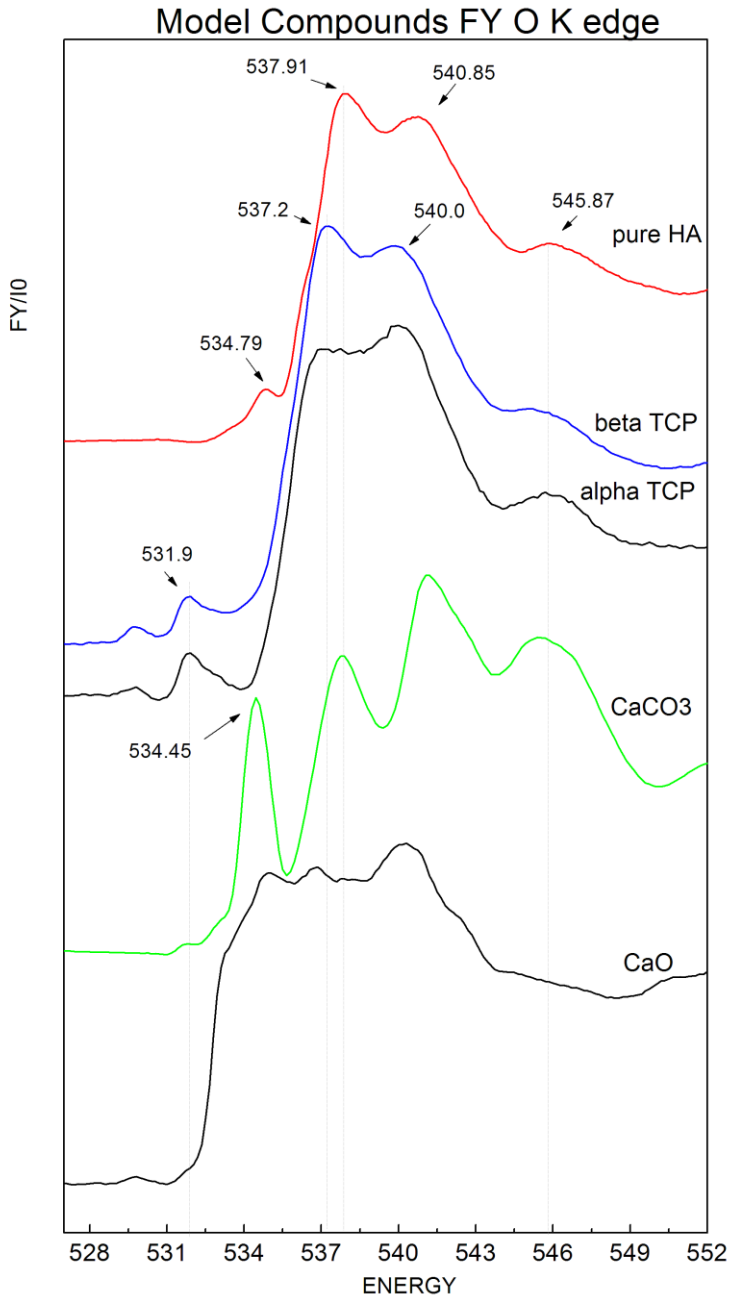


Figure 3.18 Oxygen K-edge TEY XANES spectra of bone samples calcined at 700°C

3.6.5 Oxygen K-edge FY



3.7

Figure 3.19 Oxygen K-edge FY XANES spectra of Model Compounds

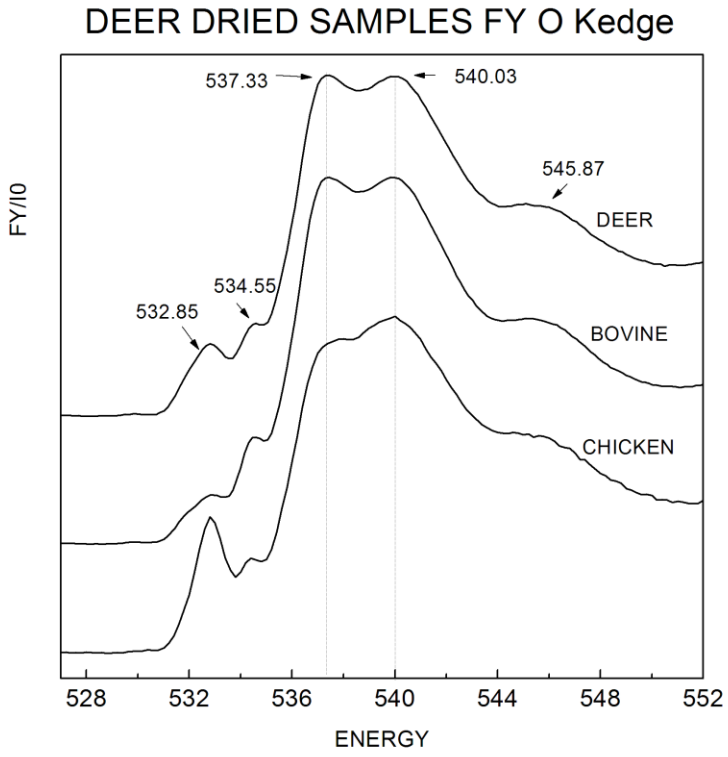


Figure 3.20 Oxygen K-edge FY XANES spectra of Dried Bone samples

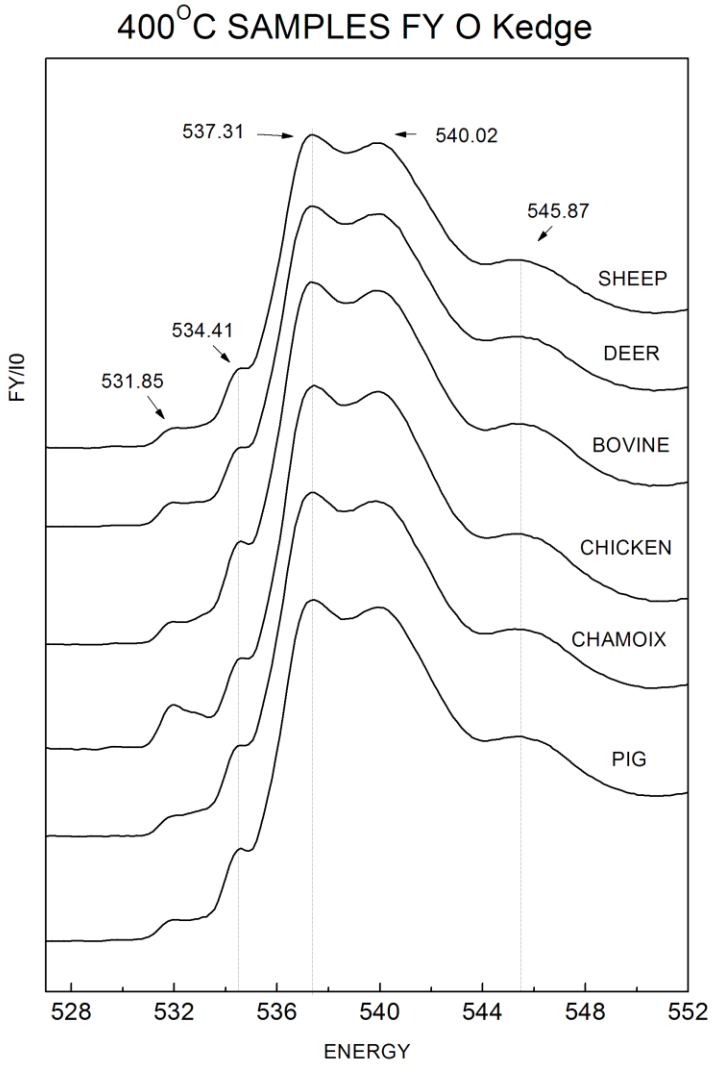


Figure 3.21 Oxygen K-edge FY XANES spectra of bone samples calcined at 400°C

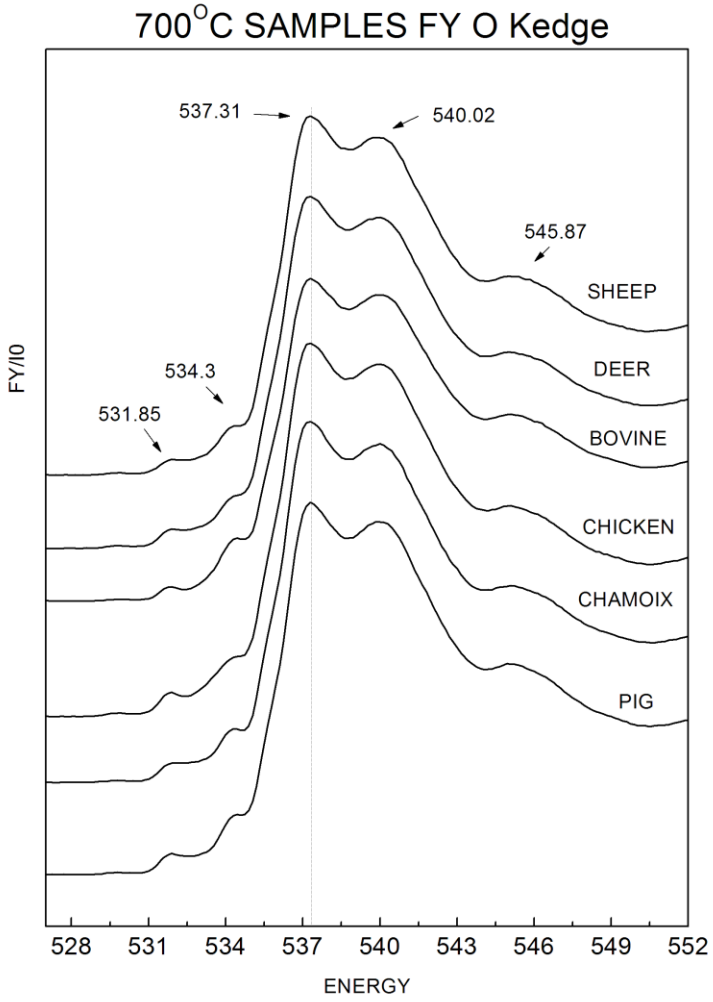


Figure 3.22 Oxygen K-edge FY XANES spectra of bone samples calcined at 700°C

3.7.1 Phosphorous K-edge TEY

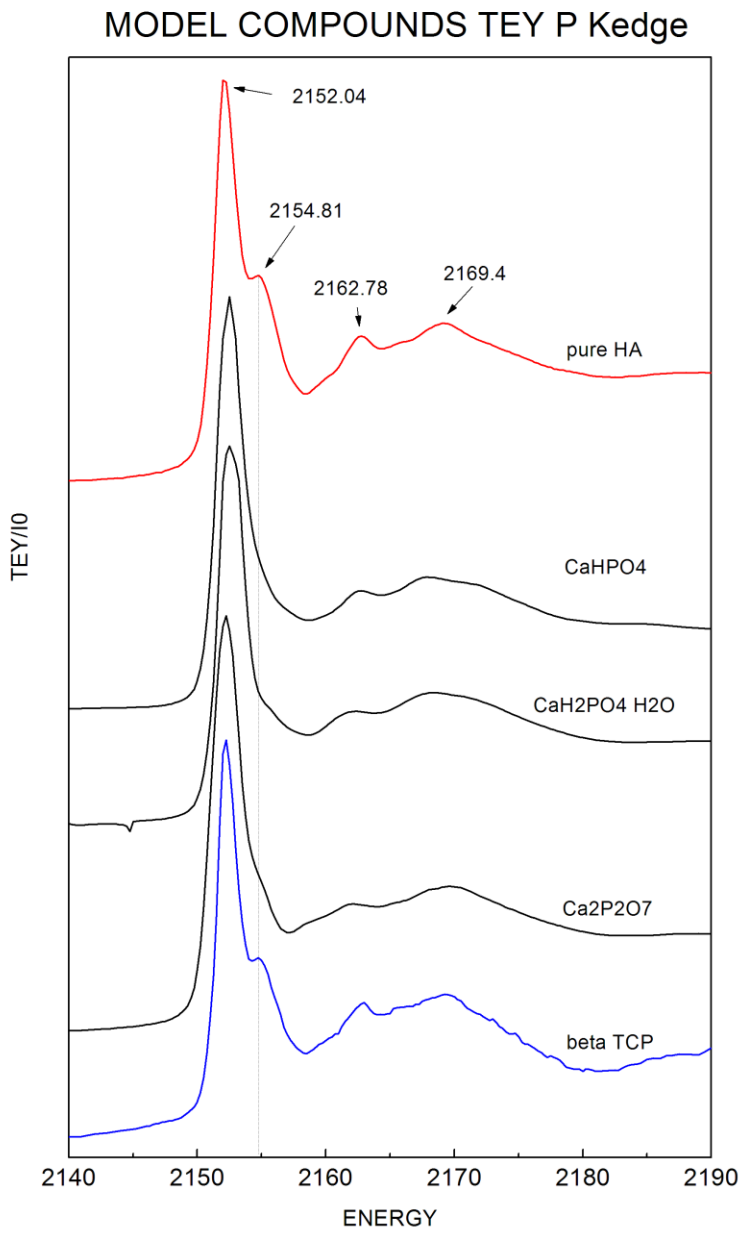


Figure 3.23 Phosphorous K-edge TEY of XANES spectra Model Compounds

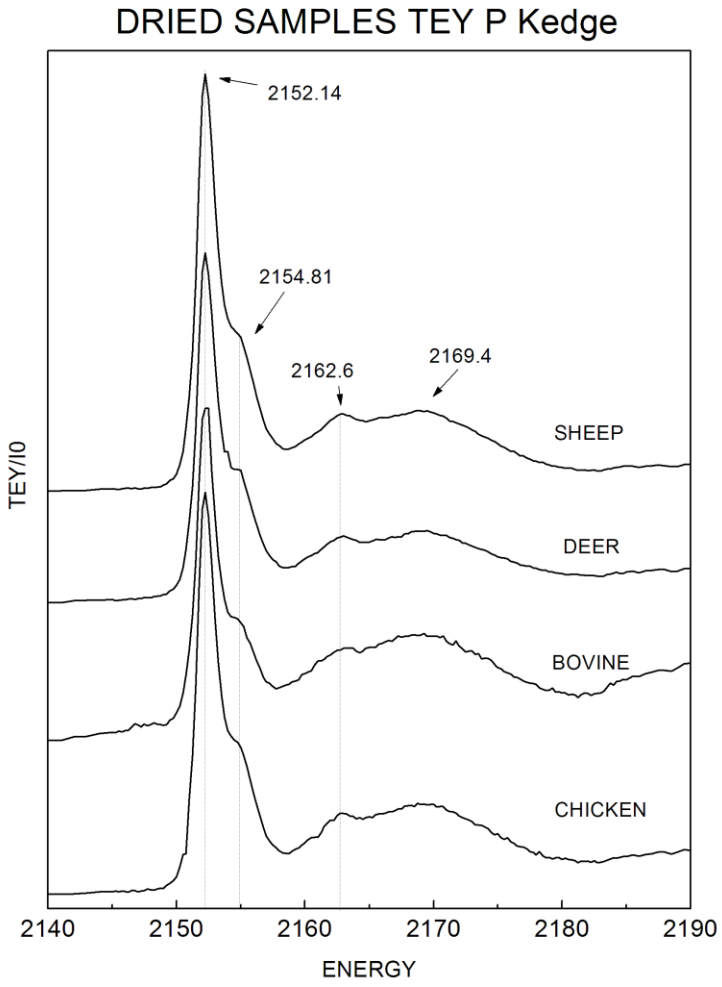


Figure 3.24 Phosphorous K-edge TEY XANES spectra of dried bone samples

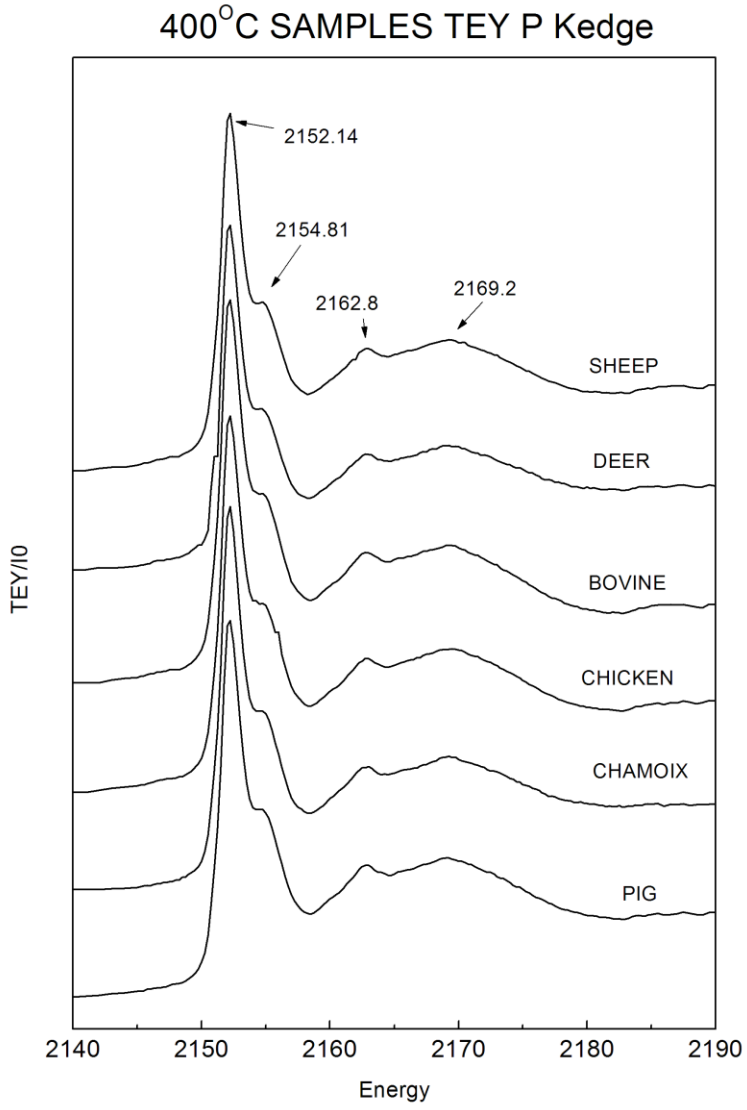


Figure 3.25 Phosphorous K-edge TEY XANES spectra of bone samples calcined at 400°C

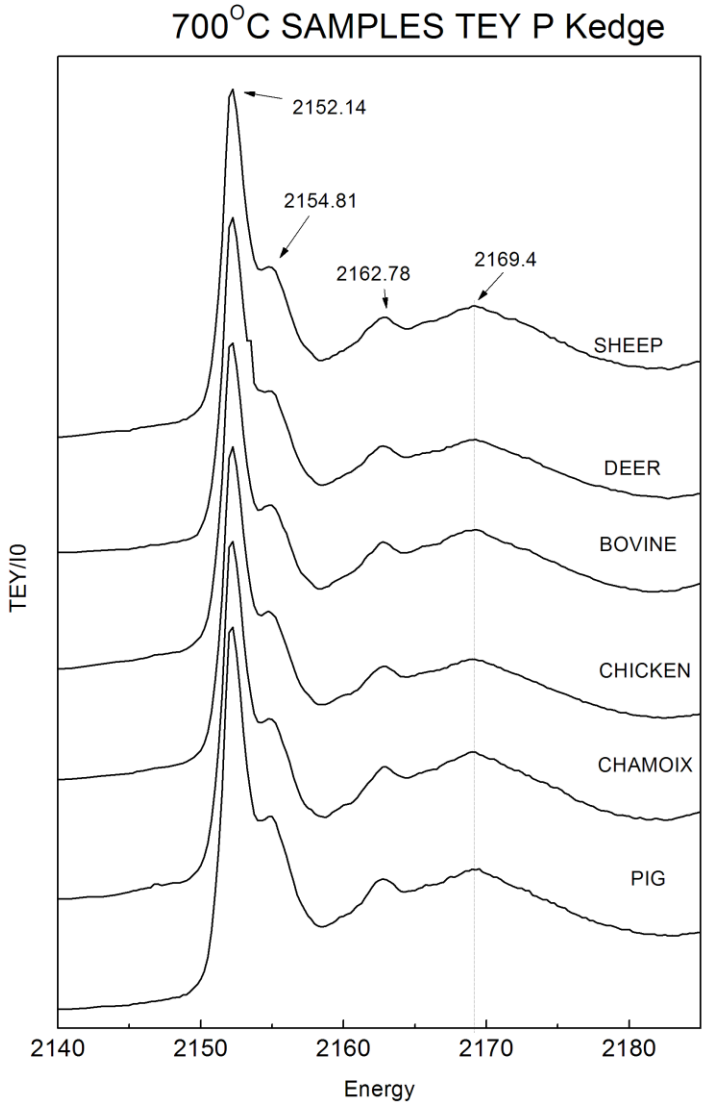


Figure 3.26 Phosphorous K-edge TEY XANES spectra of bone samples calcined at 700°C

3.7.2 Calcium K-edge TEY

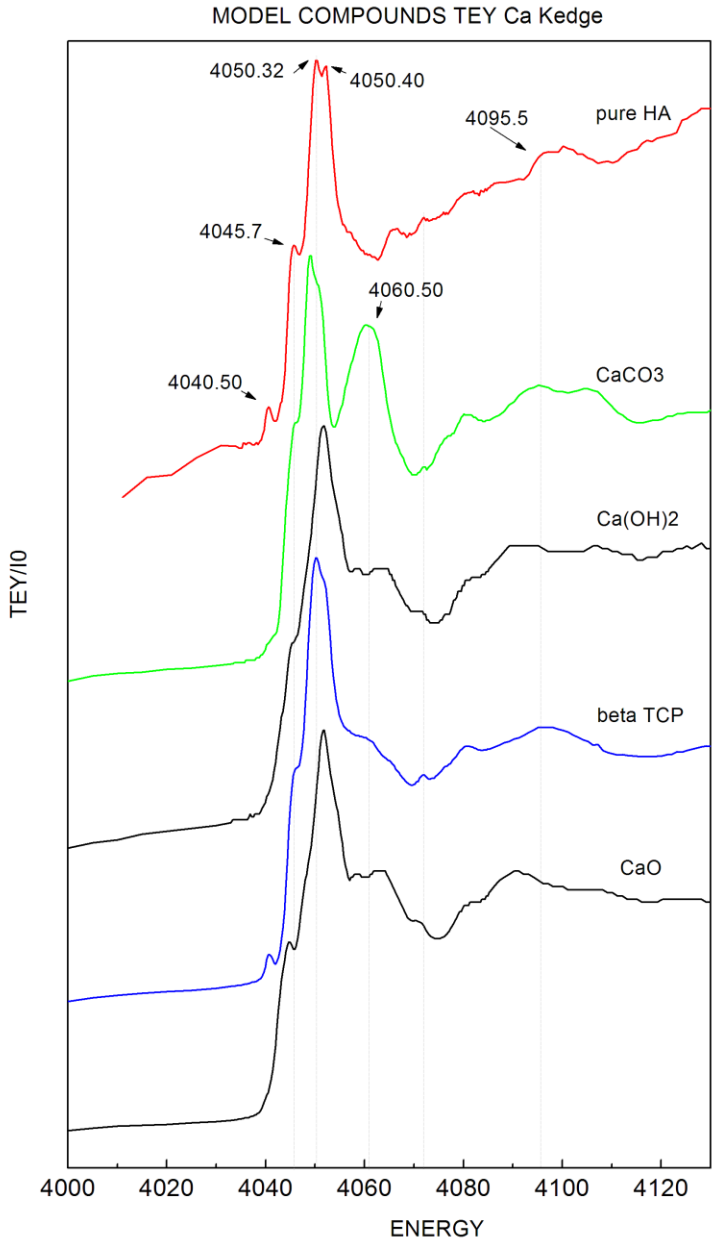


Figure 3.27 Calcium K-edge TEY XANES spectra of Model Compounds

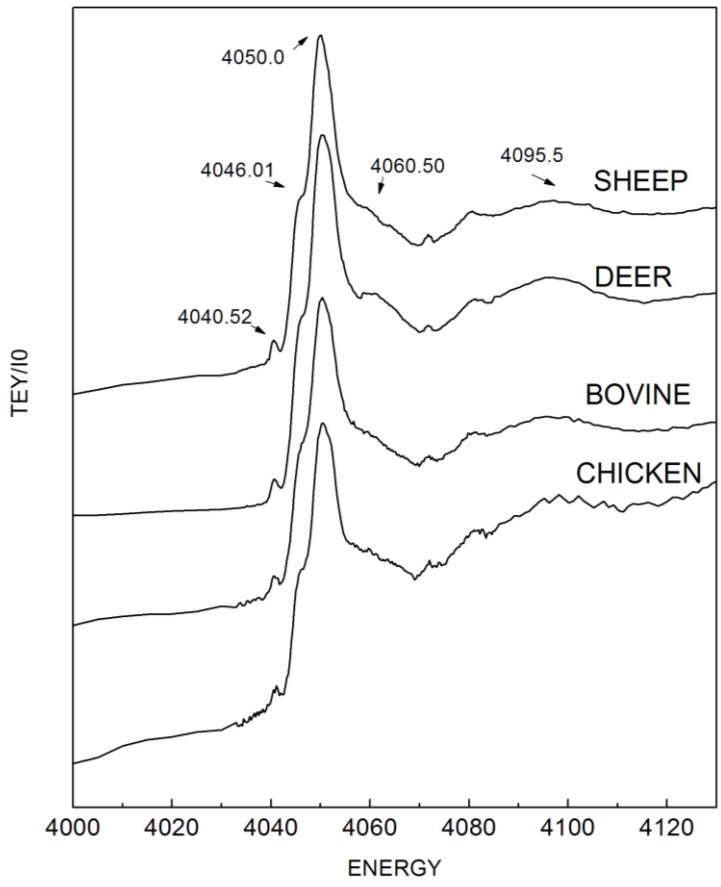


Figure 3.28 Calcium K-edge TEY XANES spectra of Dried Bone Samples

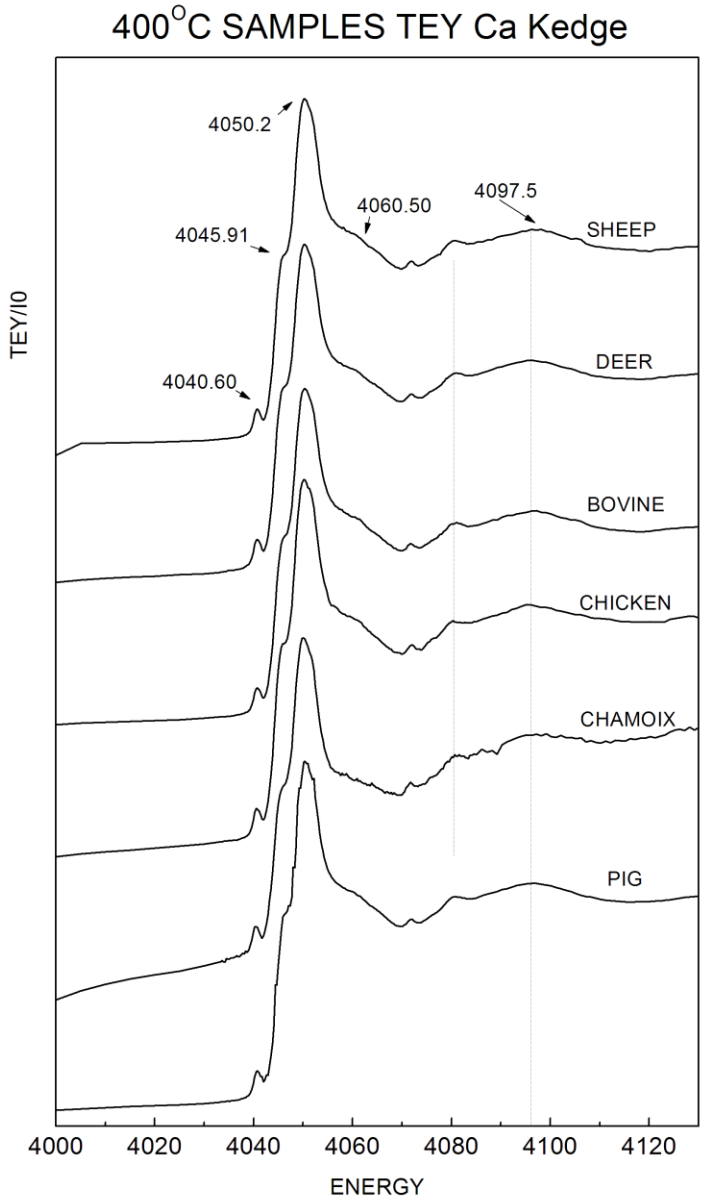


Figure 3.29 Calcium K-edge TEY XANES spectra of bone samples calcined at 400°C

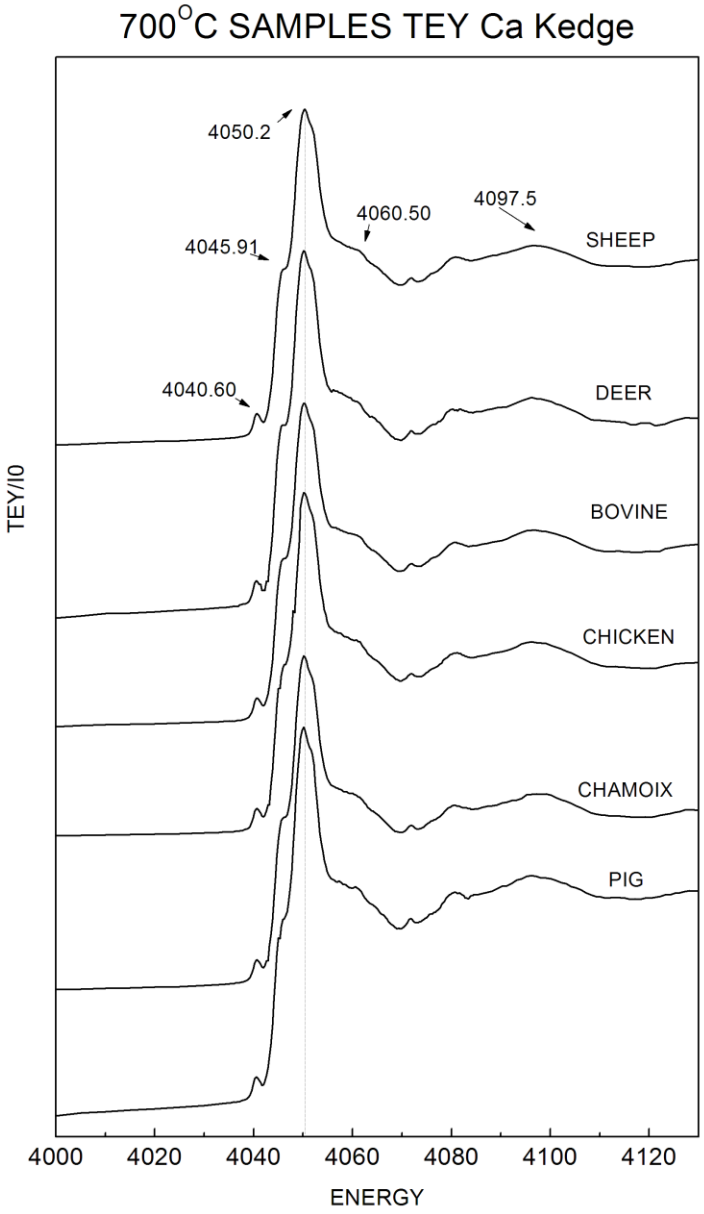


Figure 3.30 Calcium K-edge TEY XANES spectra of bone samples calcined at 700°C

3.8 Conclusion

The XANES study is very helpful in understanding the surface and the bulk property of the bones. The XANES TEY spectra of oxygen K edge for all dried bones show the presence of carbonate in the sample. On the other hand, the XANES FY spectra of oxygen K edge for all dried bone samples, the intensity of the carbonate peak is very low. The fluorescence yield gives the bulk information of the sample, since its penetration depth is about few 1000 Angstrom. Whereas, the penetration depth of total electron yield is about few 10s of Angstrom, this is very close to the surface of the sample. And, hence the surface of the dried bone samples has more carbonate than in the bulk. Since, all the calcined bones are powdered samples and they come from all part of the bone. Hence, they show similar characteristic features that of the bulk of the dried bone.

The XRD results shows, that the bone samples have beta TCP or HA, and since the spectrum of both the standards looks similar, it is hard to discriminate their individual presence.

The XANES study is much more clear in differentiating the beta TCP from the HA. The following are the observed differences in the spectra of the two standards:

Calcium K edge

- The post-edge peak at 4060.50 eV is present in the beta TCP spectrum, but not in HA spectrum.
- The pronounced pre-edge peak at 4045.70 eV is present only in the pure HA spectrum.

Oxygen K edge FY

- Presence of pre-edge peak at 534.79 eV in HA standard spectrum
- The separation of pre-edge peak at 534.79 eV from the white line is the clear differentiator of HA from beta TCP standards

Oxygen K edge TEY

- The peak at 534.84 eV is the differentiator peak, which is present only in the HA spectrum
- The beta TCP spectrum also has a pre-edge peak at 529.5 eV and 532 eV which are absent in HA standard spectrum.
- The separation between the pre-edge peaks and the white line is large in the spectrum for beta TCP standard than that of HA standard.

These clear differentiators help to identify the presence of beta TCP in the bone sample. Even though, the presence of beta TCP is evident from the XANES study, the bone mineral is a mixture of HA and beta TCP. The presence of HA in the bone mineral is well documented and this study will add the possibility of presence of beta TCP.

CHAPTER 4

CHARACTERIZATION OF DRIED AND CALCINED BONES BY FTIR

4.1 Introduction

The FTIR is sensitive to the different molecular bond vibrations, like, stretching, bending, etc. The absorption of the incident IR rays depends on the configuration of the atom and the presence of the functional groups in the sample [24]. The change in bond angle and length can be easily determined by FTIR spectroscopy. FTIR is sensitive to all the functional group in the organic and inorganic content of the bone..

4.2 Experimental procedure

The FTIR spectra are carried in the Thermo-Fisher FTIR instrument at Characterization Center for Materials and Biology (CCMB), University of Texas at Arlington. All the model compounds and the dried and calcined bone samples are acquired in the range from 500 to 4000 cm^{-1}. The aperture value is fixed at 50.00 and the sample gain at 8.0, the resolution is 4.0 cm^{-1} with the step size 3.85 cm^{-1}. The value for the number of scans for background and sample are set at 32. These settings are kept constant for all the standards, dried bones and calcined bones. The spectra are background subtracted with Originpro software and are normalized between 0 – 1. This helps in comparing the organic and inorganic content in the dried and calcined samples within the same species.

4.3 Sample preparation

The recovered fresh bones are cut into small fragments and are preserved in the freezer. After removing the attached soft parts from the bone samples, they are further break into small fragments of about few centimeters in dimension. They are then put into the test tubes and are mixed with water to remove the still attached soft tissues. The jet of compressed air is blown in to dry the fresh samples. The plastic dryers are employed to remove the liquids

present in the bone sample. Then the samples are placed in the vacuum chamber, and the air is removed to create vacuum inside the chamber. The temperature is maintained at 20^{o}C -25^{o}C and the sample is placed inside the vacuum for about 5 days. Thus the dried bone samples are prepared, except for the deer dried bones. The deer dried bone samples are dried naturally for about 6 months. The deer bone sample is the only exception, which are dried under natural conditions, which are similar to the formation of archaeology dried bone specimens.

The calcined bone samples are prepared by heating the dried bone samples in an oven, which has a maximum capability of 1800^{o}C. The bone samples are placed in the oven and the temperature of the oven is increased slowly to reach 400^{o}C. It temperature is increased slowly from room temperature to 400^{o}C in about 40 minutes and the temperature is maintained for one hour, in air. The temperature is brought to room temperature after decreasing the temperature from 400^{o}C to 22^{o}C for about an hour. Thus the first set of calcined bones are prepared, which are calcined at 400^{o}C. The same process is repeated for preparing the other set of calcined bones, by changing the temperature to 700^{o}C and maintained if for one hour in air. Thus the bone samples calcined at 700^{o}C are prepared.

4.4 Results and discussion

4.4.1 FTIR spectra of bone samples

The bone is made up of organic and inorganic components. The hydroxyapatite and collagen are the main components of inorganic and organic phase of bone, respectively. FTIR can be used to identify different functional groups in both organic and inorganic constituents of the bone. In order to examine the effect of calcination on the structure and chemistry of bone, the dried and calcined samples of bones of the same species are compared. The comparison of FTIR spectra of sheep dried bone samples and calcined bone samples at 400^{o}C and 700^{o}C are shown in the Figure 4.1 on page 72. Similar FTIR spectra of deer, bovine and chicken are shown in Figure 4.2, Figure 4.3 and Figure 4.4, respectively. The comparison of FTIR spectra of chamoix and pig bone samples calcined bone at 400^{o}C and 700^{o}C are shown in Figure 4.5 and

Figure 4.6, respectively. The spectra have phosphate bands in the region of 530 cm^{-1} - 650 cm^{-1} and 900 cm^{-1} - 1100 cm^{-1}, and the relative intensity of the phosphate peaks grow stronger when the bone samples calcined. The peaks in the region of 2600 cm^{-1} to 3600 cm^{-1} and 1500 to 1800 cm^{-1} corresponds to the organic phase arising from collagen in the bone samples. These peaks become weaker on calcining the bone.

The FTIR spectra from 500 cm^{-1} to 4000 cm^{-1} are divided into three spectral regions for detailed analysis.

4.4.2 Spectral region 530 cm^{-1} to 1200 cm^{-1}

The FTIR spectrum of deer dried bone sample in the region 530 cm^{-1} to 1200 cm^{-1} is shown in the Figure 4.7 along with peak assignments. This spectral region is called the "fingerprint" region, because of its prominent peaks and easy identification [9]. The spectral region from 530 cm^{-1} to 650 cm^{-1} is the v_4 phosphate, and it has peaks at 560 cm^{-1} and 600 cm^{-1} [26]. The peak at 560 cm^{-1} corresponds to the peak of hydroxyapatite (HA), calcium hydroxide and beta tri-calcium phosphate (TCP) standards shown in Figure 4.11. The spectrum of beta TCP and HA standards have a peak at 600 cm^{-1}, which matches with the v_4 phosphate peak of the spectra of bone samples. The peak at 876 cm^{-1} is assigned to the carbonate, which is coming from the inorganic phase of the bone sample [28]. The peak at 876 cm^{-1} of the bone samples corresponds to calcium carbonate, calcium hydroxide and calcium oxide standards, and their spectra are shown in Figure 4.11.

The spectral region between 900 cm^{-1} and 1100 cm^{-1} is the location of the intense peaks in the entire spectra which spans the 500 cm^{-1} to 4000 cm^{-1} range. The peak at 960 cm^{-1} is assigned to the v_1 phosphate band [28] [27] [Ilaria] and its mode of vibration is symmetric stretching [29] [30]. The peaks at 1020 cm^{-1} and 1090 cm^{-1} corresponds to the v_3 phosphate [ilaria][27], and are due to the asymmetric stretching vibration of the phosphate ions [29] [30].

The v_3 phosphate peaks at 1092 cm^{-1} is coming from the stoichiometric apatite of the bone samples and the peak at 1020 cm^{-1} arises from the non-stoichiometric apatite which may contain CO_3^{2-} or HPO_4^{2-} or both in the bone apatite [31].

These v_1 and v_3 phosphate peaks at 960 cm^{-1}, 1020 cm^{-1}, and 1090 cm^{-1} matches with the peaks of beta TCP and pure HA standards, see Figure 4.11.

4.4.2.1 Dried bone samples

The FTIR spectra of dried bone samples of different species are compared in the Figure 4.8. All the spectra look similar in their shape and peak positions which imply that the bones of different species are of similar chemical composition. The spectrum of deer bone dried sample is little different with its fine resolved peaks at 960 cm^{-1} and 1090 cm^{-1}, compared to other spectra. And, the peaks at 560 cm^{-1} and 600 cm^{-1} are very prominent. This difference could be caused by the difference in the drying process among the samples. All the dried samples are artificially dried, except for the deer sample. The deer dried samples are prepared by natural dehydration of the fresh deer bone samples for months. On the other hand, dryers are employed to dry the bone samples of other species and, they are placed in vacuum for 5 days to liberate the liquids through evaporation. This time frame could possibly cause an increase in crystallization of the apatite minerals in the deer dried samples and hence, results in a well resolved phosphate peaks at 560 cm^{-1} and 600 cm^{-1} [10]. The carbonate peak at 876 cm^{-1} comes from the inorganic phase of the bone and is very similar in all the dried bone samples. The v_1 and v_3 phosphate peaks in the spectral region of 950 cm^{-1} to 1100 cm^{-1} are less pronounced. The broad peaks in the v_1, v_3 phosphate region are due to the poor crystallinity of the bone mineral [30].

4.4.2.2 Bone samples calcined at 400^oC

The FTIR spectra of bone samples calcined at 400^oC are shown in the Figure 4.9 on page 80. The spectrum of bones calcined at 400^oC of different species look identical to each other. Their v_4 phosphate peaks at 560 cm^{-1} and 600 cm^{-1}, are predominant than that of dried

bone samples, especially the peak at 560 cm^{-1}. The peak 560 cm^{-1} is very pronounced in all the spectra of bone samples calcined at 400^{o}C and it is intense only in the deer dried spectrum. This shows that the chemistry of bone samples heated at 400^{o}C are same for all the species, and are independent of their drying process. The v_1 and v_3 phosphates peaks at 960 cm^{-1}, 1020 cm^{-1} and 1090 cm^{-1} are little more pronounced than the peaks in their respective dried sample spectra indicating that the phosphates are becoming more pronounced after calcination.

4.4.2.3 Bone samples calcined at 700^{o}C

The comparison of the spectra of bone samples calcined at 700^{o}C of different species is shown in the Figure 4.10 on page81. They all very similar, but, when compared to the spectra of dried bone samples, the bone samples calcined at 700^{o}C show presence of a new peak at 630cm^{-1}. This new peak is present in all the bone samples calcined at 700^{o}C, irrespective of its species. The peak at 630cm^{-1} is also absent in the spectra of bone samples calcined at 400^{o}C. So, it is clear that the heating of bone sample at 700^{o}C is the only cause for the appearance of this peak in the spectra. The peak originates from the decomposition of the phosphate by the liberation of OH [28] [10], and this shows that the crystallinity of the bone mineral is increased when heated above 700^{o}C.

The intensity of carbonate peak at 876 cm^{-1} declines, when the bone sample is heated and it is due to the decomposition of the calcite [10]. The v_1 and v_3 phosphate peaks at 960 cm^{-1}, 1020 cm^{-1} and 1090 cm^{-1} are more resolved in the spectra of bone samples calcined at 700^{o}C. It is possible to say that the size of the apatite crystal increases due to heating, and causes the v_1 and v_3 phosphate peaks to be more resolved [10].

4.4.3 Spectral region 1200cm^{-1} to 2000 cm^{-1}

The peak assignments in the spectral region of 1200cm^{-1} to 2000 cm^{-1} are shown in the spectrum of the dried deer bone sample in the Figure 4.12 on the page83. This spectral region is mostly composed of peaks from the organic phase of the bone sample. The peaks at 1236 cm^{-1}, 1534 cm^{-1} and 1635 cm^{-1} correspond to the amide group and they originate from the

cross-linked collagen of the bone [10] [9]. The peak at 1534 cm^{-1} corresponds to the amide II, which arises from the combined effect of C-N stretch and N-H in-plane bending. And, the amide I peak at 1635 cm^{-1} is due to the C=O stretch [30] [29]. The peak at 1412 cm^{-1} corresponds to v_3 carbonate vibration, which comes from the mineral phase of the bone samples [32]. And it matches with the peaks of calcium carbonate, calcium hydroxide and calcium oxide shown in the spectra of model compounds in the Figure 4.16 on page 87. As it is visible from the spectrum of carbonate standard, the primary peak at 1390 cm^{-1} is buried under the protein peaks and is barely visible as a shoulder in the spectra of bone samples. The spectra of model compounds shows weak peak at 1745 cm^{-1} belongs to the calcium carbonate and calcium hydroxide standards.

4.4.3.1 Dried bone samples

The FTIR spectra of different species of dried bone samples are shown in the Figure 4.13. The amide groups are most dominant in this spectral region from 1200 cm^{-1} to 2000 cm^{-1}. The amide I peak at 1635 cm^{-1} and amide II at 1534 cm^{-1} arises from the protein content of the bone material, and this shows that the dried bone samples have plenty of it. Also, the presence of amide III peak at 1236 cm^{-1} attribute to the above statement. This is a complex spectral region with overlapping vibration bands of amide and carbonate bands generated by the organic and inorganic phase of the bone samples, respectively. The spectra of bovine dried bone samples has an additional carbonate peak at 1740 cm^{-1}, and is absent in the spectra of other dried bone samples. Other than this difference, the spectra of dried bone samples of different species appear very similar and from this, it is possible to say that, the difference in the drying process does not cause any change to the organic phase of the bone.

4.4.3.2 Bone samples calcined at 400^{o}C

The comparison of the FTIR spectra of different species of bone samples calcined at 400^{o}C are shown in the Figure 4.14 and they all look similar. When compared to the spectra of dried bone samples, it is clear that the intensity of the amide peaks is drastically declined. The

amide III peak at 1236 cm^{-1} is absent in the spectra of bone samples calcined at 400^{o}C. The predominant amide peaks of spectra of the dried bones at 1635 cm^{-1} and 1534 cm^{-1} are less pronounced in the spectra of bone samples calcined at 400^{o}C. The gradual decline of the intensity of carbonate peaks is also observed. This is hard to visualize from the spectra of bone samples calcined at 400^{o}C, since, the plots show a normalized value. But, this decline in intensity can be clearly seen in the species specific plots, see Figure 4.2 on page 73. It is possible to conclude that the calcined bone samples are more likely to lose its organic content.

4.4.3.3 Bone samples calcined at 700^{o}C

The disappearance of the organic phase in the bone is clearly evident from the FTIR spectra of bone samples calcined at 700^{o}C, shown in Figure 4.15. The intensity of the entire spectral region from 1200 cm^{-1} to 2000 $cm^{-1}$is significantly declined, with no trace of amide peaks. It could be possible to say that the required temperature to drive-off all the collagen content in the bone sample is 700^{o}C. The carbonate peak at 1412 cm^{-1} also experienced a decline in intensity and is due to the decomposition of the calcite when heated [10]. Thus escaping carbonate and the carbonate from the heating chamber can possibly get into the apatite structure. The carbonate ions replacing the OH- sites are called type A carbonate apatite and have their peaks at 879 cm^{-1}, 1535 cm^{-1} and 1463 cm^{-1}. If the carbonate ion replaces the phosphate sites, they are called type B carbonate apatite and have their peaks at 866 cm^{-1}, 873 cm^{-1}, and 1422 cm^{-1}, 1456 cm^{-1}. The carbonate ion can replace both of these monovalent and trivalent anionic sites and they are called type AB carbonate apatite. The type AB carbonate apatite has peaks at 1416 cm^{-1}, 1452 cm^{-1}, 1470 cm^{-1}, 1500 cm^{-1}, and 1568 cm^{-1} [33]. The presence of carbonate peak at 876 cm^{-1} in all the samples could possibly from the type A carbonate apatite. The presence of a small shoulder at 1505, and 1546 cm^{-1} in most of the samples matches with the type AB carbonate apatite. The sharp peak at 1484 cm^{-1}in the spectrum of pig bone sample calcined at 700^{o}C could be coming from the type AB carbonate. It is possible to conclude two points from this spectral region. Firstly, a small amount of carbonate

apatite is present along with the bone apatite in the samples, and secondly, the organic content of the bone samples are driven-off by heating it above 700^{o}C.

4.4.4 Spectral region 2000cm^{-1} to 4000 cm^{-1}

The FTIR spectra of deer dried bone samples from 2000 cm^{-1} to 4000cm^{-1} with its peak alignments are shown in the Figure 4.17. The intensity of peaks in this spectral region is relatively low when compared to the phosphate peaks, and is much weaker for the calcined bone samples. The peak at 2852 cm^{-1} is asymmetric CH_2 stretch and it arises from the organic component of the bone sample. The peak at 2924 cm^{-1} corresponds to the symmetric CH_2 and asymmetric CH_3 stretch of the organic phase of the bone sample [9]. The broad peak at 3400 cm^{-1} is due to the overlap of contribution from water and amide in the bone sample.

4.4.4.1 Dried bone samples

The FTIR spectra of different species of dried bone samples in the spectral region from 2000 cm^{-1} to 4000 cm^{-1} are shown in the Figure 4.18 on page 89. The symmetric and asymmetric stretches of CH are pronounced along with the peak for the water. The CH_2 symmetric stretch at 2852 cm^{-1} and the CH_2 asymmetric stretch and CH_3 symmetric stretch at 2924 cm^{-1} are predominant in the spectra of bovine dried bone samples. The CH_2 asymmetric stretch is barely visible in the chicken and deer dried bone samples. This shows some variation among the spectra of different animal species. Since, the difference is seen in the organic part of the spectrum, the results may not be reproducible.

4.4.4.2 Bone samples calcined at 400^{o}C

The FTIR spectra of different species of bone samples calcined at 400^{o}C in the region from 2000 to 4000 cm^{-1} are shown in the Figure 4.19. All these spectra look identical to each other, and have less pronounced peaks corresponding to the CH stretch. The intensity of the entire spectral region is decreased, which can be observed from the spectra of dried and burnt bone samples of same species, see Figure 4.2. The presence of water is evident from the

broad peak at 3400 cm^{-1}. This supports the previous argument that the heating of the bone samples drives off the organic content in the bone sample.

4.4.4.3 Bone samples calcined at 700^{o}C

The FTIR spectra of different species of bone samples calcined at 700^{o}C in the spectral region from 2000 $cm^{-1}$to 4000 cm^{-1} have just a single peak, and are shown in the Figure 4.20 on page 91. The peak is at 3570 cm^{-1} and it corresponds to the free OH group and it arises from the mineral phase of the bone sample [34]. Hence, it still remains in the spectra of the bone samples. Total disappearance of the organic phase of the bone samples is observed. This also affirms the previous statement that 700^{o}C is possibly the temperature required to remove all the collagen content of the bone.

4.5 FTIR Spectra

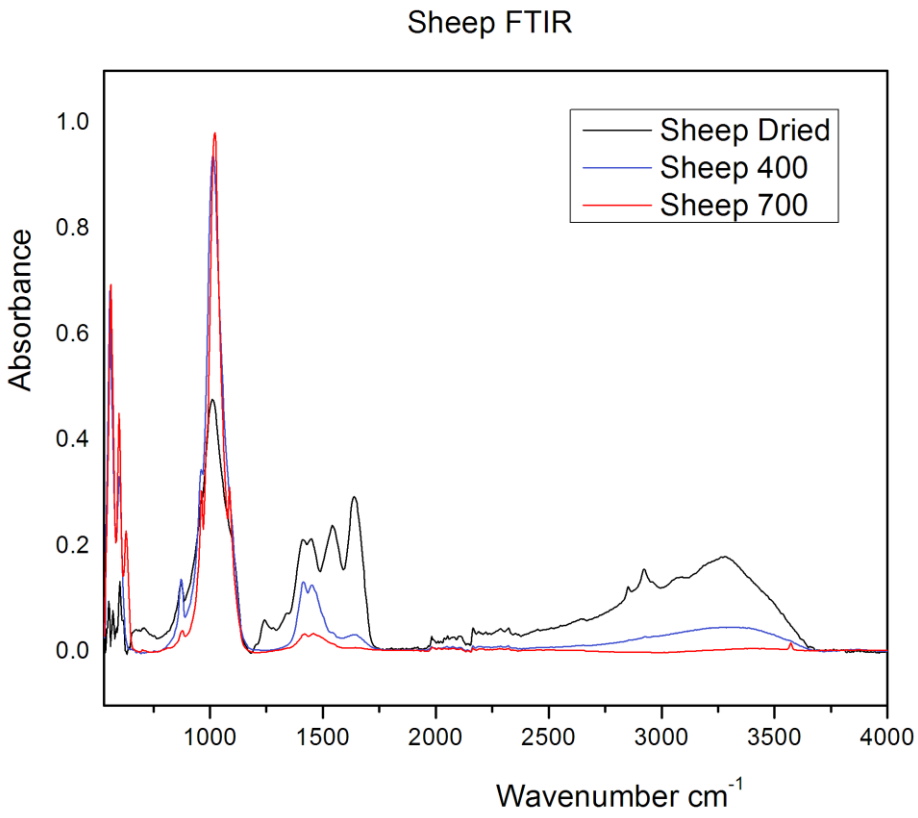


Figure 4.1 Comparison of FTIR spectra of sheep dried bone, calcined bone at 400°C and 700°C

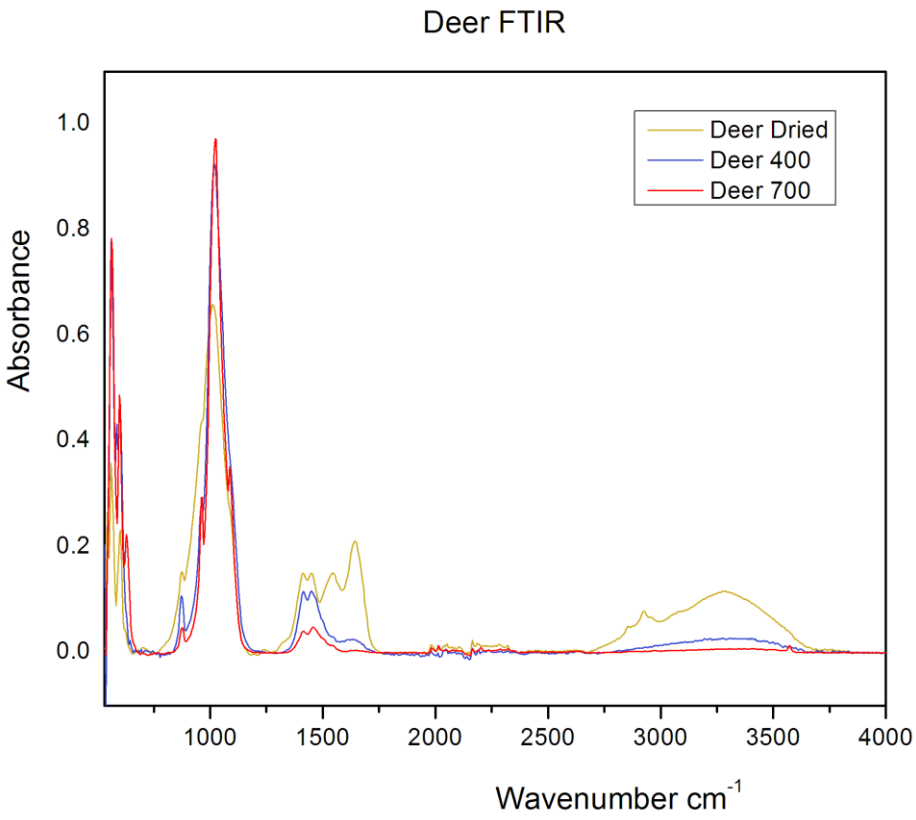


Figure 4.2 Comparison of FTIR spectra of deer dried bone, calcined bone at 400°C and 700°C

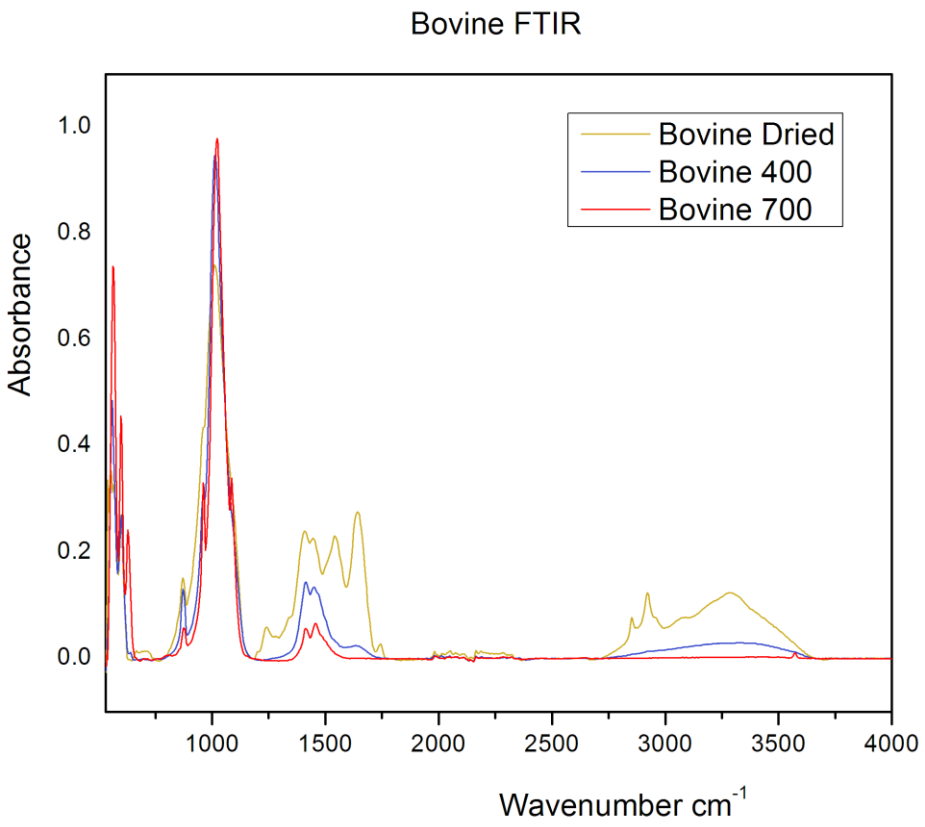


Figure 4.3 Comparison of FTIR spectra of bovine dried bone, calcined bone at 400^{o}C and 700^{o}C

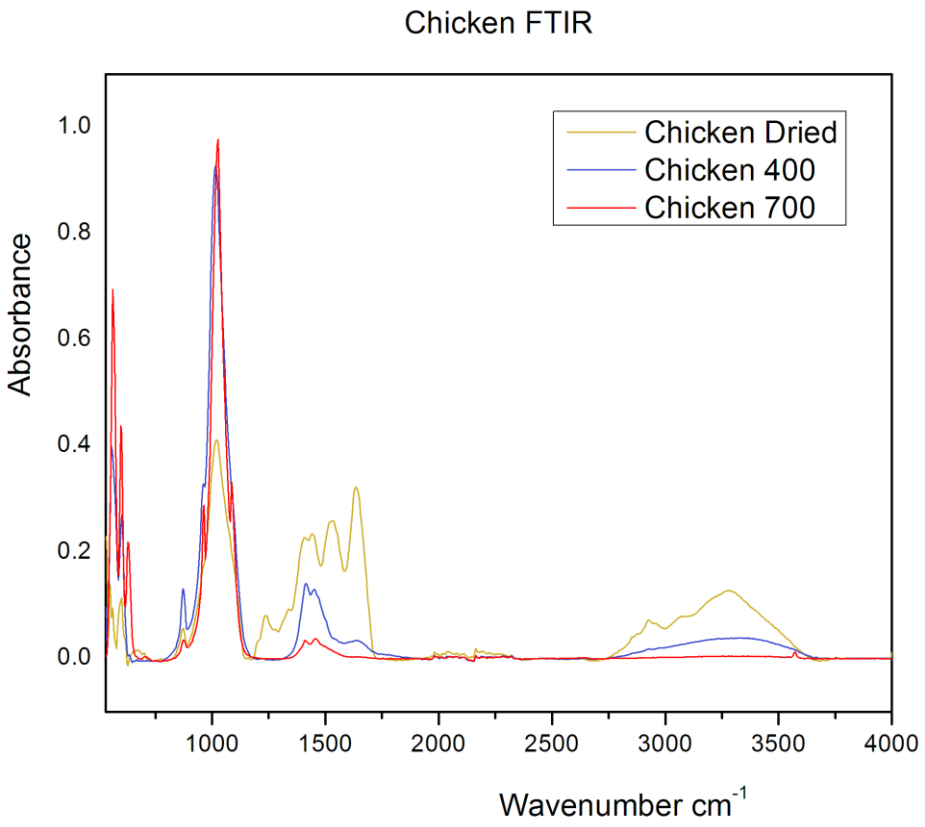


Figure 4.4 Comparison of FTIR spectra of chicken bone, calcined bone at 400°C and 700°C

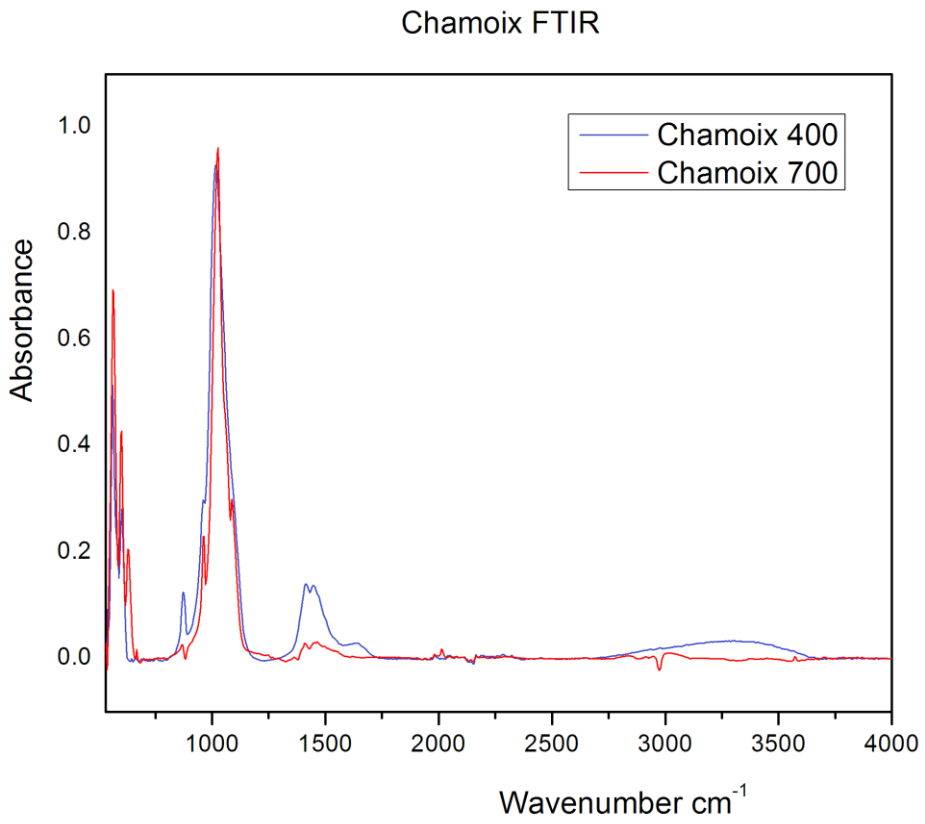


Figure 4.5 Comparison of FTIR spectra of chamoix bone samples calcined bone at 400°C and 700°C

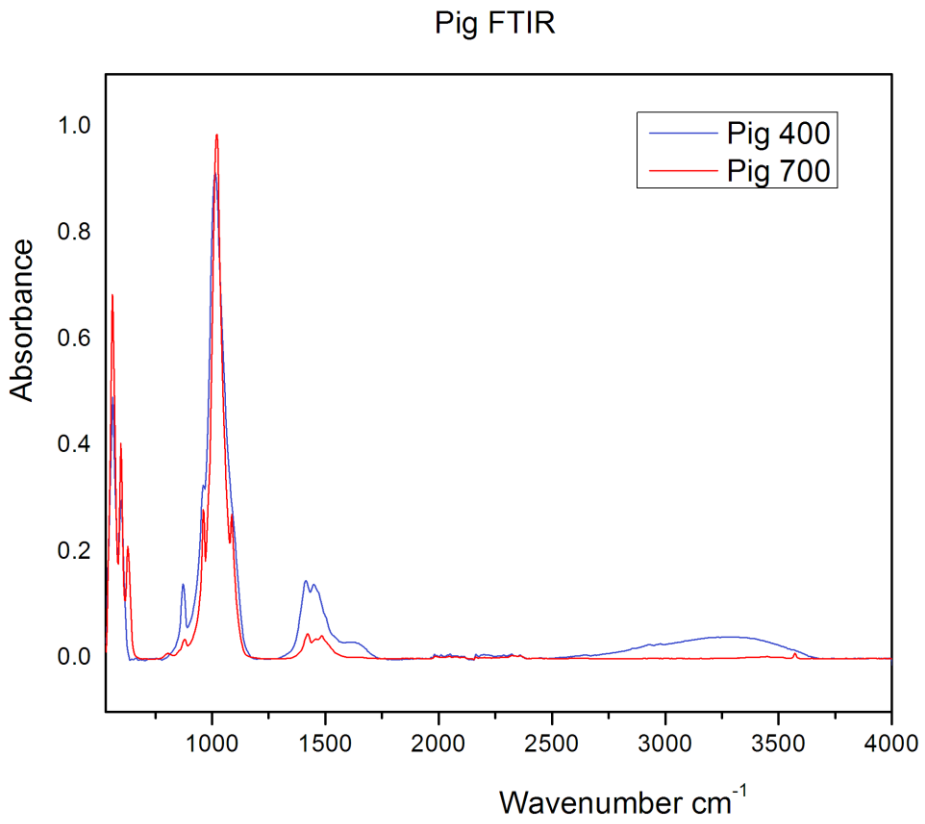


Figure 4.6 Comparison of FTIR spectra of pig bone samples calcined bone at 400°C and 700°C

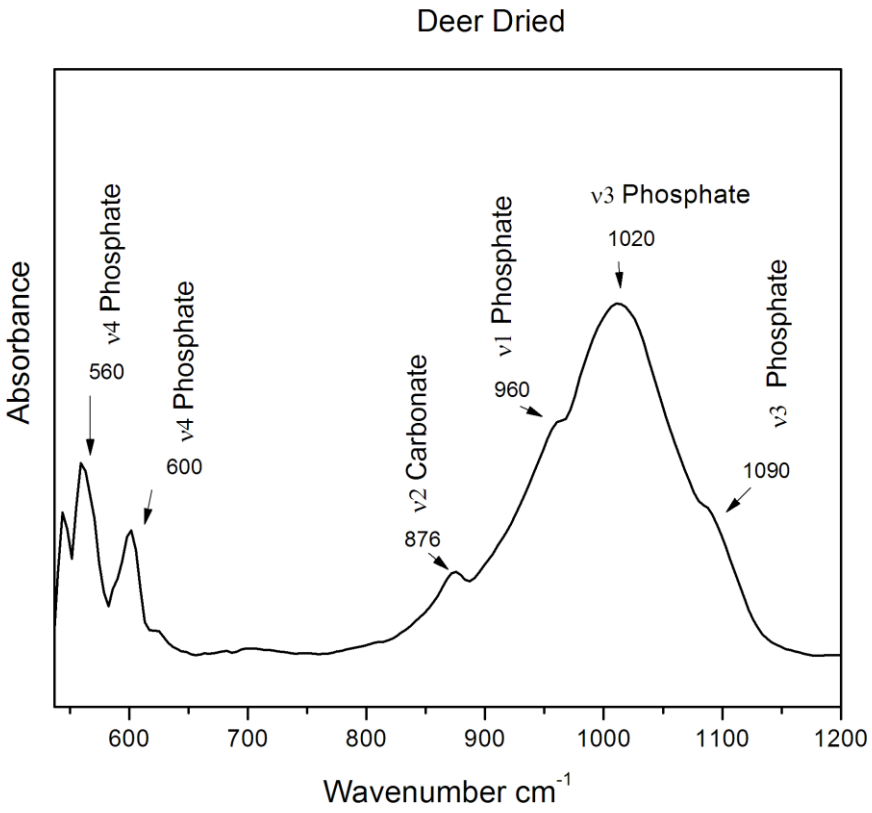


Figure 4.7 FTIR Spectrum of deer dried bone samples with its peak assignments in 500 cm^{-1} to 1200 cm^{-1} region

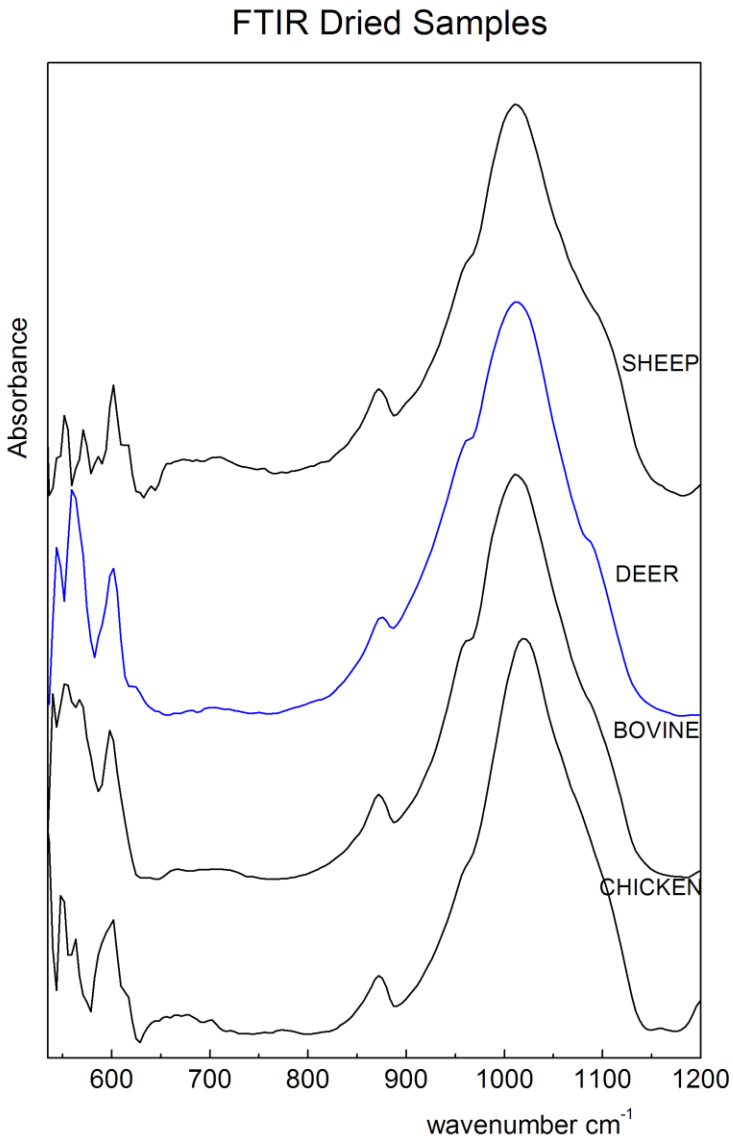


Figure 4.8 FTIR Spectra of dried bone samples in 500 cm^{-1} to 1200 cm^{-1} region

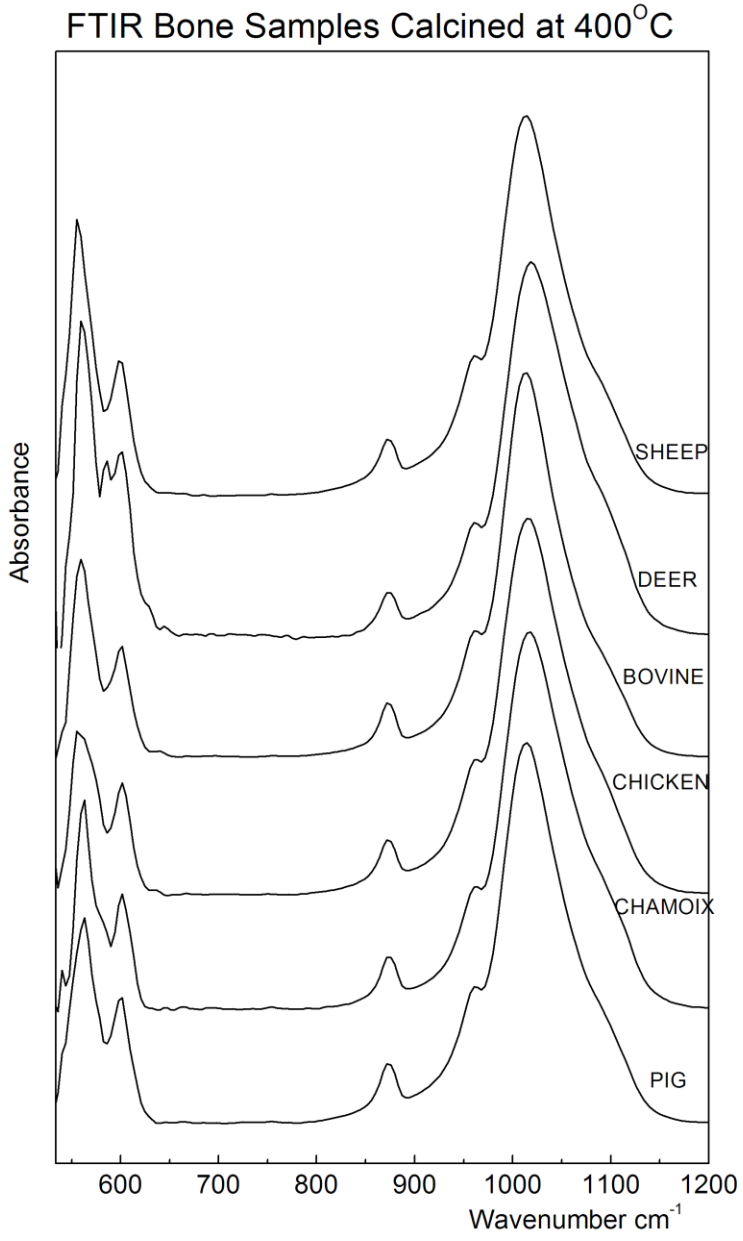


Figure 4.9 FTIR Spectra of bone samples calcined at 400°C in 500 cm^{-1} to 1200 cm^{-1} region

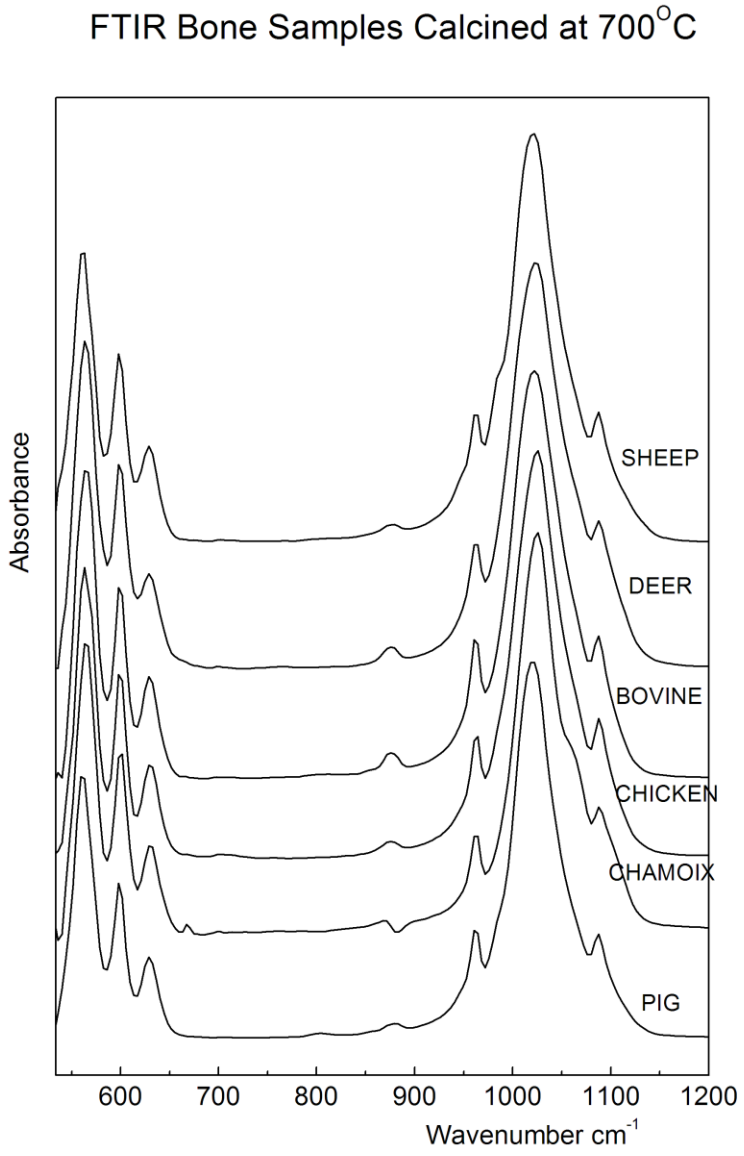


Figure 4.10 FTIR Spectra of bone samples calcined at 700°C in 500 cm^{-1} to 1200 cm^{-1} region

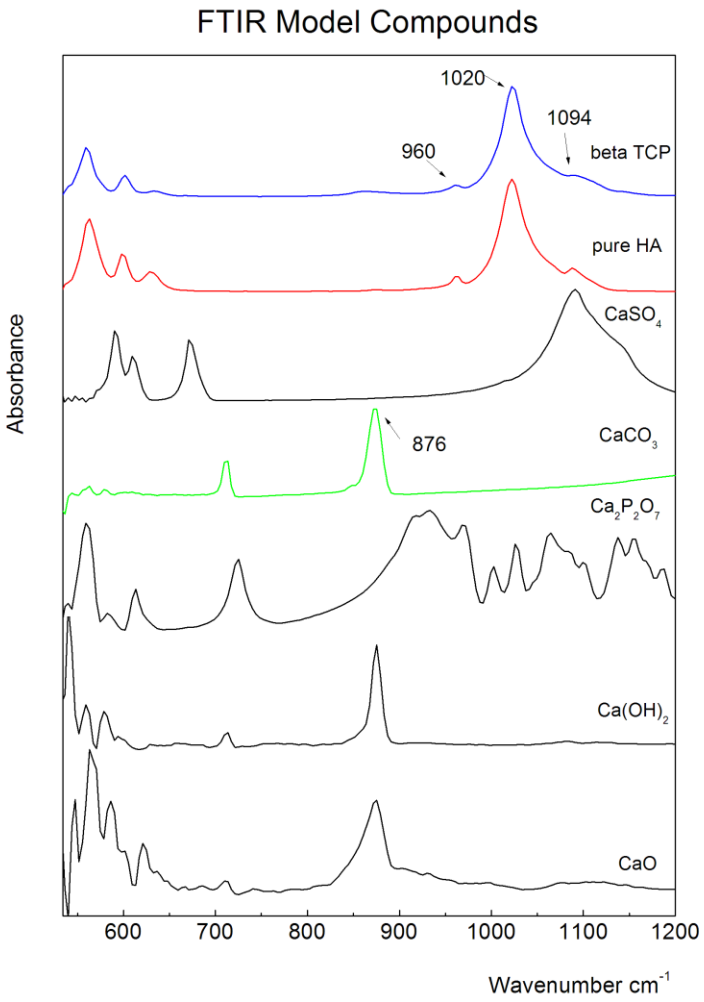


Figure 4.11 FTIR Spectra of Model Compounds in 500 cm^{-1} to 1200 cm^{-1} region

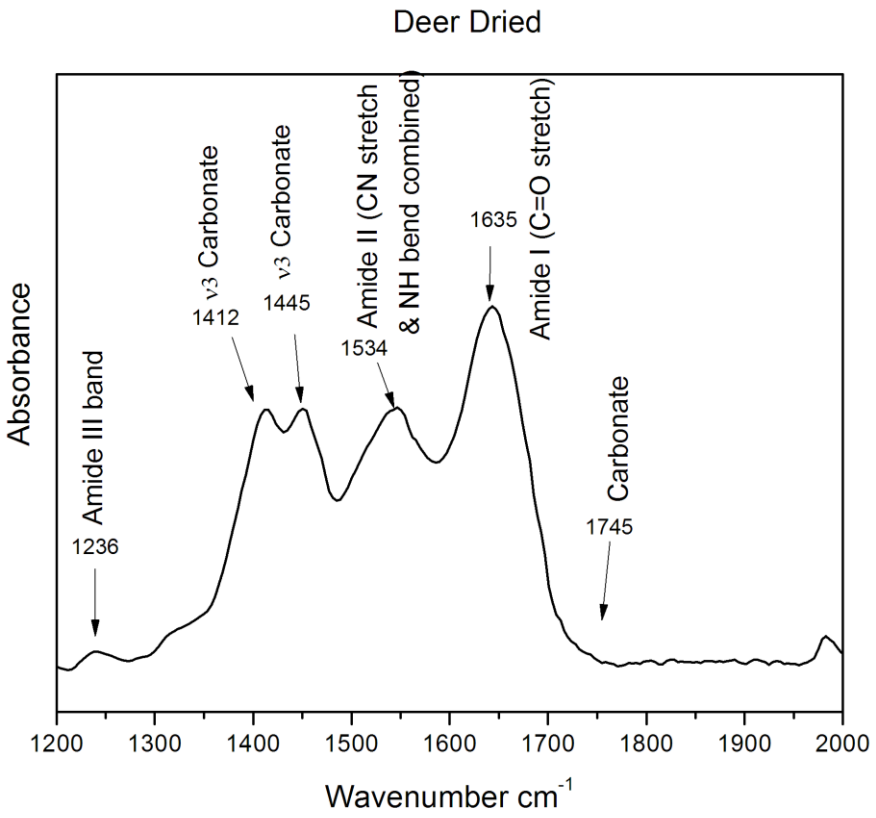


Figure 4.12 FTIR Spectrum of deer dried bone sample with its peak assignments in 1200 cm^{-1} to 2000 cm^{-1} region

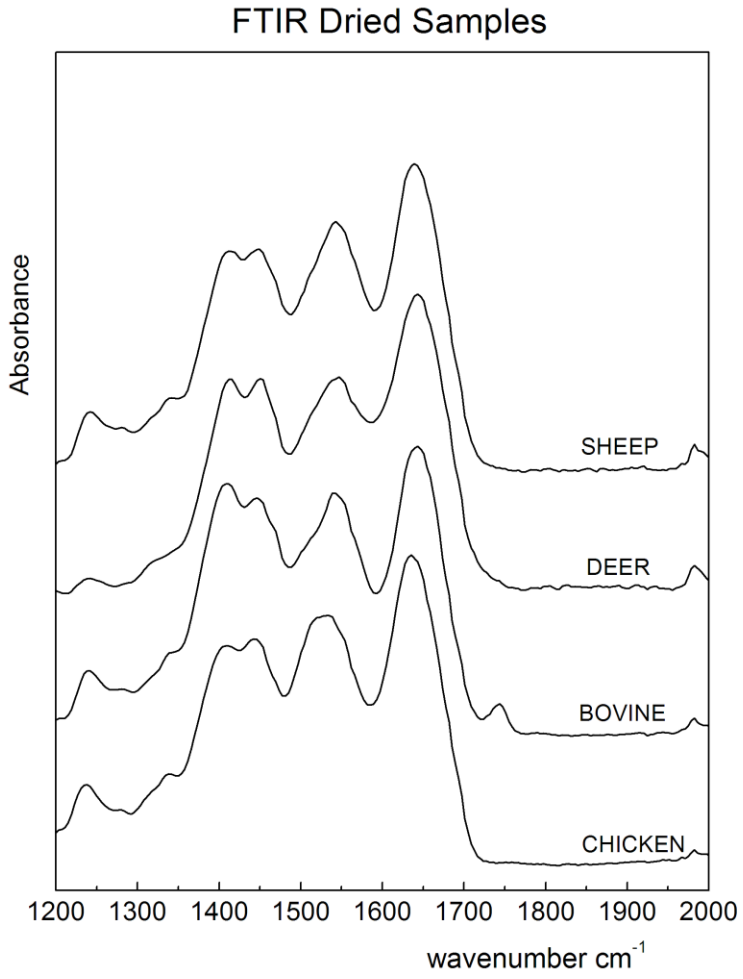


Figure 4.13 FTIR Spectra of dried bone samples in 1200 cm^{-1} to 2000 cm^{-1} region

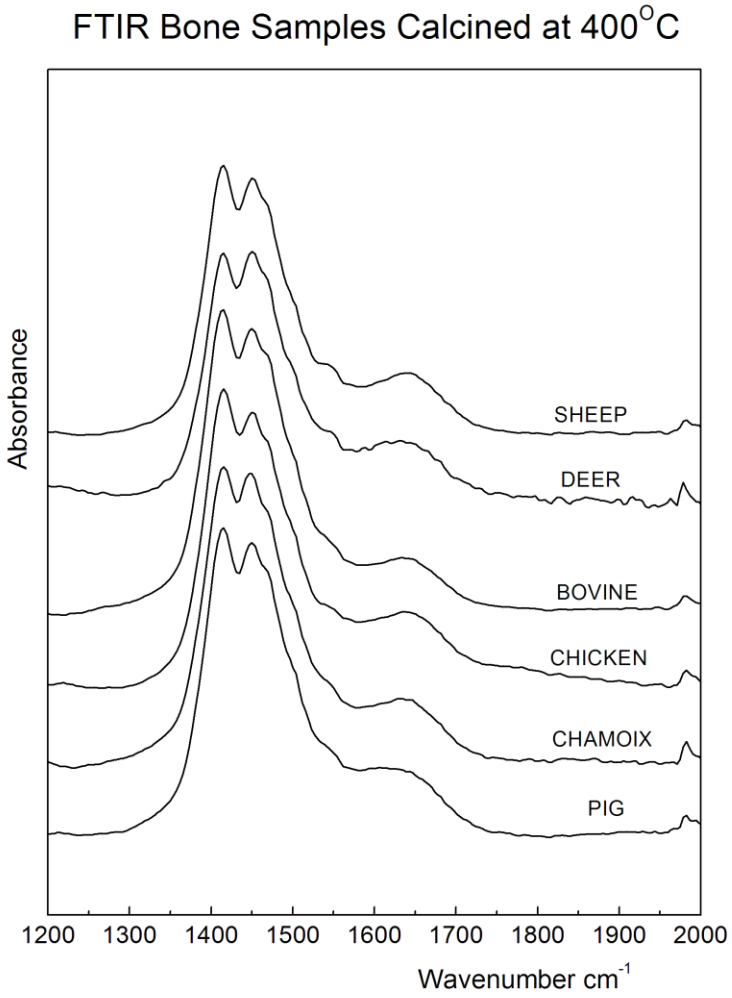


Figure 4.14 FTIR Spectra of bone samples calcined at 400°C in 1200 cm^{-1} to 2000 cm^{-1} region

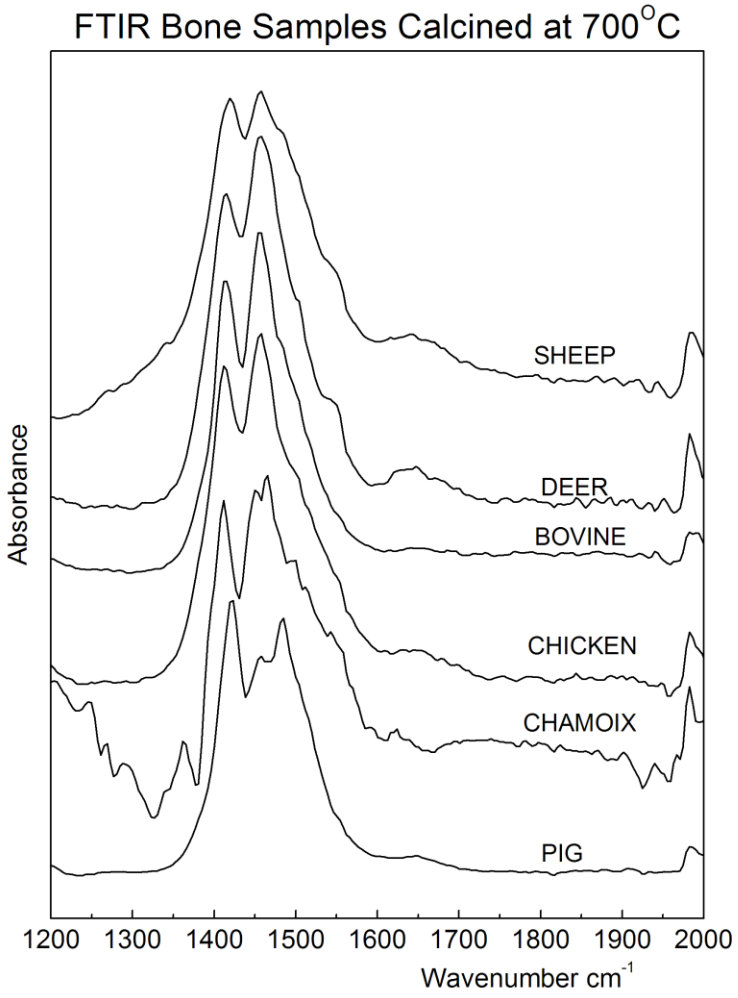


Figure 4.15 FTIR Spectra of bone samples calcined at 700°C in 1200 cm^{-1} to 2000 cm^{-1} region

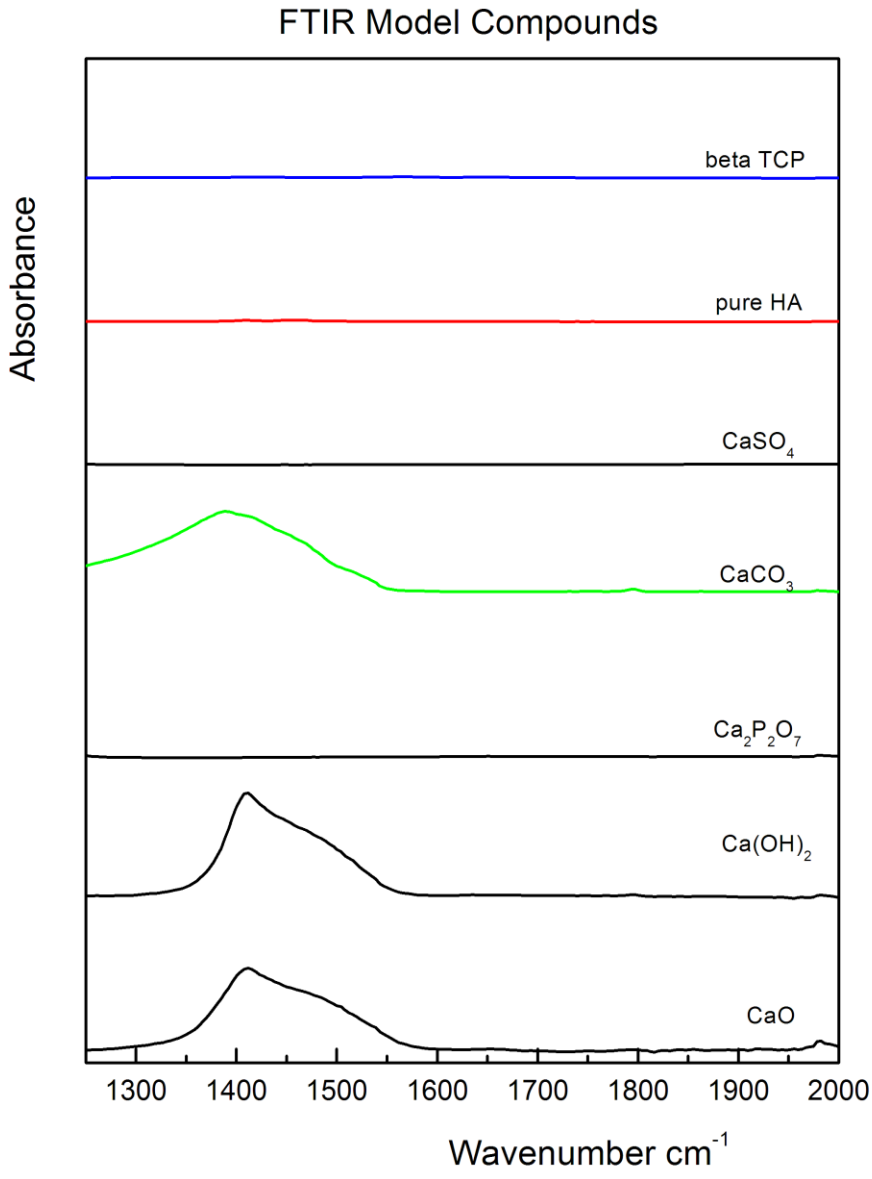


Figure 4.16 FTIR Spectra of Model Compounds in 1200 cm-1 to 2000 cm-1 region

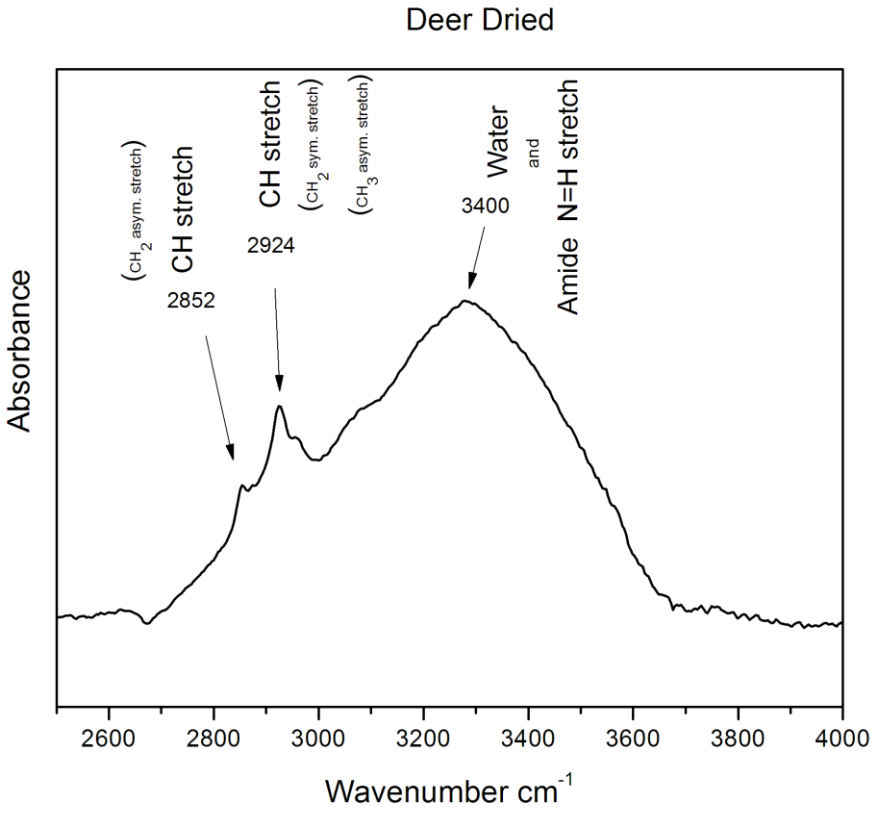


Figure 4.17 FTIR Spectrum of deer dried bone samples with its peak assignment in 2000 cm^{-1} to 4000 cm^{-1} region

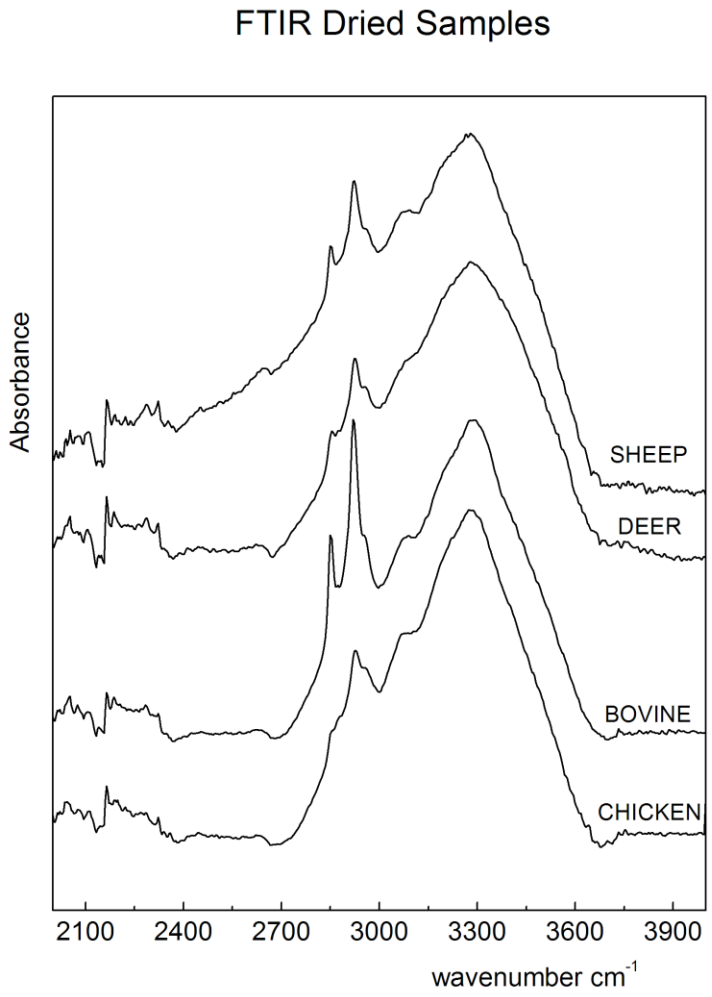


Figure 4.18 FTIR Spectra of dried bone samples in 2000 cm^{-1} to 4000 cm^{-1} region

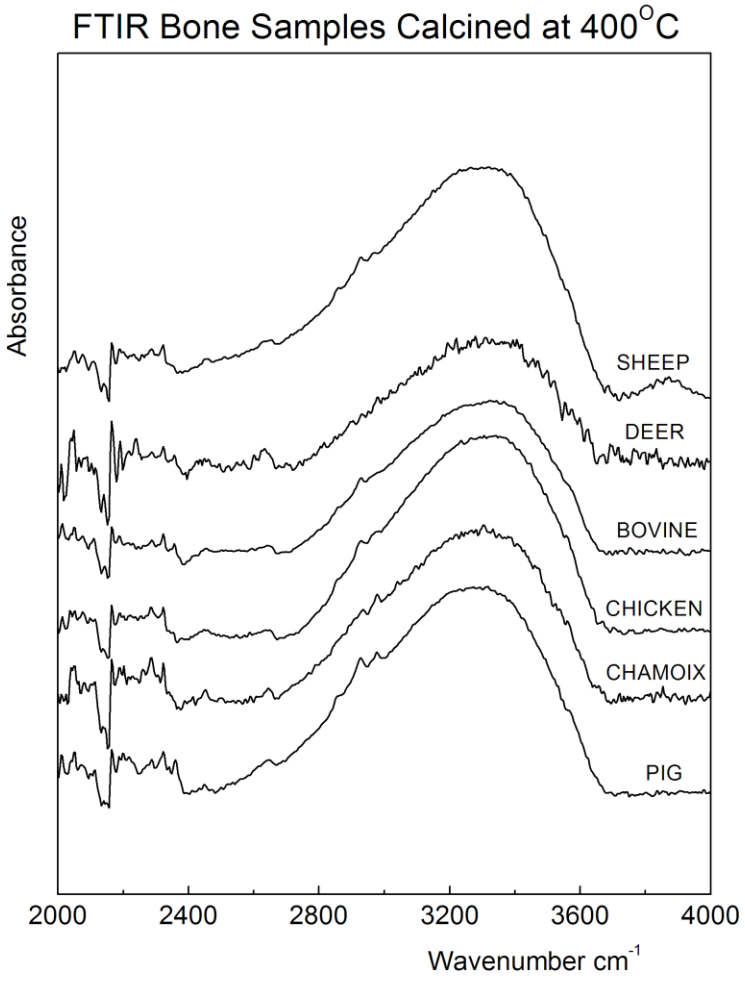


Figure 4.19 FTIR Spectra of bone samples calcined at 400°C in 2000 cm^{-1} to 4000 cm^{-1} region

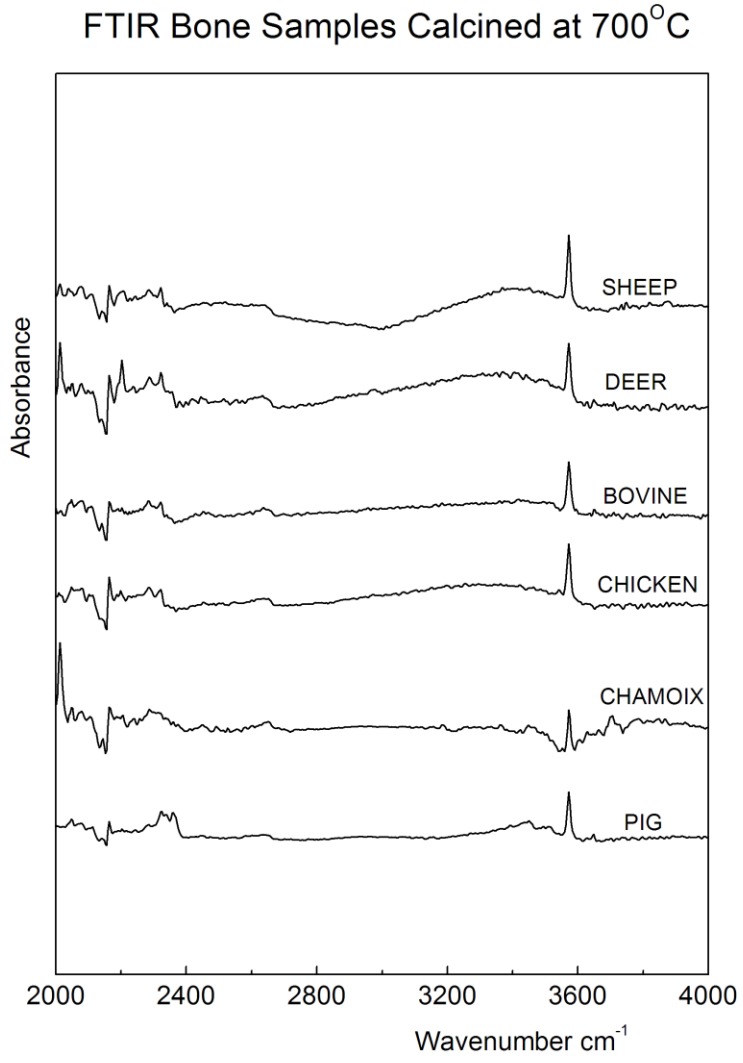


Figure 4.20 FTIR Spectra of bone samples calcined at 700°C in 2000 cm^{-1} to 4000 cm^{-1} region

4.6 Conclusion

FTIR study is sensitive to different functional groups in both organic and inorganic constituents of the bone. The presence of the collagen in all the dried bone samples is clearly observed in their FTIR spectra. The difference in the drying process is clearly identified in the FTIR studies. The spectra of the deer dried bone, which undergone a different drying process, has pronounced phosphate peaks. All the other dried bone samples which are artificially dried have similar spectra. This indicates that the FTIR is sensitive to the drying process of the bones and can be utilized to identify the possible drying process.

The FTIR spectra of calcined bones are very identical and this indicates that the process of drying the bone samples does not have any influence in the calcined bones. Since, there is no specific change in the bone chemistry among the different species of the available bone samples. All the bone samples are composed of same organic and inorganic content, irrespective of its species.

The decline in the organic content in the bone is clearly seen in the FTIR spectra. This is due the fact that the heating drives off the organic content of the bone. The decline in the intensity of the organic peaks is observed in the bone samples are calcined at 400^0C. These organic peaks are completely absent in the bone samples calcined at 700^0C. This shows that the organic content of the bone samples are driven off from the bones, when the bones are calcined at 700^0C. This also indicates that the required temperature to completely deproteinize the bone samples is 700^0C.

CHAPTER 5

CONCLUSION

The presence of the calcium phosphate is seen in the XRD, but it is hard to differentiate between the beta TCP and pure HA standard. The XANES study helps in discriminating the presence of beta TCP and pure HA. The presence of beta TCP is clearly observed in the oxygen Kedge TEY, oxygen Kedge FY and calcium K edge spectra. But the presence of HA is not completely ruled out, since, the structure of both beta TCP and HA are very similar. hence, the beta TCP and HA are present in the bone mineral, but in above mentioned beamlines, the content of beta TCP is more than the HA. The presence of the carbonate on the surface of the dried bone samples are observed in the oxygen K edge TEY spectra. And, in the oxygen K edge FY spectra, which correspond to the bulk of the bone, the carbonate content is comparatively less. On the surface of dried bone samples has carbonate and phosphates, but the carbonate content is more.

The FTIR spectrum is sensitive to the functional groups of organic and inorganic part of the bone. The FTIR spectra show the clear difference between the dried and calcined bone samples. The collagen content in the bone samples calcined at 400^{o}C are very less when compared to the dried bone samples. At 700^{o}C, all the organic content of the bones are disappeared and are seen from the FTIR spectra of bone samples calcined at 700^{o}C which has just the inorganic peaks.

The bone chemistry does not change with the species. The drying process will certainly be observed by the FTIR spectra. The change in organic content is visible from the FTIR spectra and hence, the approximate calcined temperature can be determined and it can be clearly discriminated from the dried bone samples.

CHAPTER 6

FUTURE WORKS

There are some possible future works that can be carried out based on the results from this study. The change in drying process of the fresh bone causes some noticeable changes in the FTIR spectra. More information could be obtained by making a detailed study by using different possible drying processes. For instance, the dehydration of fresh bones in different pH conditions, humidity, etc, can be studied with FTIR.

It is clear from the XANES and FTIR studies, that the chemistry of the calcined and dried bones have hydroxyapatite and beta TCP. But, at this point the proportion of these calcium phosphates is unknown. By preparing few standards with different proportion of HA and beta TCP, could possibly help in identifying their quantity in the bone mineral. The XANES spectra of these standards can be compared with the XANES spectra of dried and calcined bones. Physical mixing of the samples will give peaks of HA and beta TCP and hence, the standards should be sintered or prepared by other process like, precipitation.

In this study, XANES is used for the identification of the species. By curve fitting the absorption peaks, it is possible to do a semi-quantitative study of the species. The ratio of mineral to organic component of the bone can be identified by curve fitting the FTIR spectra. Since, the collagen is clearly picked up in the FTIR study; it will be worth studying the proportion of organic and inorganic content.

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BIOGRAPHICAL INFORMATION

The author of the paper Jayapradhi Rajendran was born in Tiruppur, Tamil Nadu, India. He completed his Bachelor of Engineering in Electrical and Electronics from Anna University, Tamil Nadu, India. For masters he chose Materials Science and Engineering from University of Texas at Arlington to understand the contribution of different materials in electronics and other engineering applications. On the process of understanding the various analysis methods of materials, he started working under the guidance of Dr. Pranesh Aswath and involved himself in the characterization of the archeological bones. The challenge in characterizing the calcined bones and his interest towards the field of archaeology are the driving force for this research. He is currently working on couple of journal papers in the area of characterizing calcined bones and whishes to be a significant contributor for an industry and science in general.