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VIBRATIONAL SPECTROSCOPY STUDY OF ANCIENT BONES FROM ARCHAEOLOGICAL SITES IN JORDAN

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Abstract

This paper will study the molecular structure of human bone fragments in the field of conservation science. The fragments were excavated from the sites of Tell al-Husn and Al Yasileh in Jordan. The applying of Raman spectroscopy and Fourier transformation infrared for comparison and Analysis of bone as a new method for hard tissue primary bone dating in the field of archaeometry. A collection of bone fragments were prepared for measurements using this new technique in dating from different ancient sites in Jordan. It is shown that the bone objects are dated back around to 2,000 years (Roman period), determined by a typological study of pottery by the excavators of these sites. This preliminary dating by vibrational spectroscopy as a preliminary method was used to classify the bone fragments if they are archaeological artifacts.

Keywords*: FTIR; FT-Raman; SEM; Hydroxyapatite; Bone; Dating.*

Introduction

Bone is a composite material that consists of inorganic mineral phase (calcium phosphates approx. 60%-70%) and organic components (30%). The major organic part of the bone matrix types I collagen (90%) and non-collagenic proteins such as osteocalcin and osteonectin (10%), which causes the elasticity of the bone, while the mineral part the hydroxyapatite (OHAp) in charge providing the characteristic hardness ossified tissue [1-5]. The bone is composed of two sections. The external thin layer (cortical bone or compact bone, amounting to 80% of the bone mass) encloses a porous, spongy inner space (cancellous bone, 20% of the bone mass), which is filled with bone marrow. Porosity plays a role in mass transport and accessibility of the cells an important role. The bone is surrounded by the inner layer of the so-called periosteum, two distinct layers: a thick, outer fibrous layer and a thin, inner cambium layer that is adjacent to the bone matrix [6]. Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ is composed of calcium, phosphorous, oxygen, and hydrogen atoms. It is a triangular structure consists of a central $Ca(OH)_2$ and three surrounded neighboring $Ca_3(PO_4)_2$ molecules, having a hexagonal unit cell and P63/m space group. Its study and characterization are of great importance in the field of biomaterials because OHAp is the main constituent of bone [7, 8]. There is a process that affects the ancient bone called the substitution of ions and groups within the apatitic lattice. Apatites are thermodynamically the most stable phases among the calcium phosphates and, therefore, can be considered as the probable end product in many reactions. Over time, the F^{-1} replaces the OH^{-1}. This substitution of F^{-1} will give information about the dating of bone. It is an accumulated process over time.

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Raman spectroscopy has been increasingly applied in archaeology and most of the art objects. The spectroscopy applied to study the composite of the artifacts. It is a powerful technique to characterize the molecular structure of ancient materials. The molecular structure is fundamental to determine which wavelengths will be absorbed or transmitted or which will be scattered. The transmittance of light across a range of infrared wavelengths makes it possible to detect the changes in bone apatite structure at the molecular level, which can be compared to semi-quantitative analysis using the relative concentrations of infrared functional groups based on peak properties, height, shift, or peak area ratios [9, 10].

This present work will concentrate on the role of Raman spectroscopy and FTIR in the field of ancient bones in archaeological science. It introduces FTIR and Raman spectroscopies with emphasis on archaeological applications is specified, and the merits of scattering experiments in the archaeometric field also go to be discussed. A brief outline of the development of Raman spectroscopy in archaeology is presented and, finally, some of the occasional applications of the method to problems of archaeological nature are described through a case study of Tell al-Husn archaeological site in Jordan.

The vibrational spectroscopies FT Raman and FT-Infrared methods with emphasis on archaeological applications, particularly bone study and FTIR spectroscopy, have become an important tool for ancient bone research, particularly for the analysis of bones [10-17]. This study endeavors to show the integration of both spectroscopies specify and merit both scattering and absorption experiments in the archaeometric field. A brief outline of the development of Raman spectroscopy in archaeology is presented and, finally, some of the occasional applications of the method to problems of archaeological nature described through the case study of Tell al-Husn archaeological site in Jordan.

This research explores firstly, the study of human bone in the past from the Iron, Persian, Hellenistic, Roman, and Islamic periods. Secondly, it concentrated on hard tissues human objects in which field archaeologists set about making and using artifacts classifications to meet a variety of practical needs to use in the dating phenomena. The consultation of scientific methods of materials classification, in combination with archaeology by typological classification of the archaeological material, will ultimately enable us to be more specific in materials exploration. The study in these ways will converse the role of scientific techniques in the cultural heritage and archaeological materials. These previous techniques, which come mainly from physics and chemistry, and biology, enable conservators and archaeologists to conserve and preserve the tangible objects in cultural heritage, such as bone samples in this study.

The present work will concentrate on vibrational spectroscopies, both Fourier Transformation Infrared and Fourier Transformation-Raman of various archaeological bone objects. Raman spectroscopy is a very important and not an expensive method to reveal the historical and archaeological aspects of these important sites in Jordan. It is an excellent method for studying several types of artifacts like pigments, glass, and bone. Mainly it is a new trend in the bone dating field. A collection of bone fragments is prepared for measurements from the stores of the Faculty of Archaeology and Anthropology at Yarmouk University. It is shown that bone objects from Tell al-Husn dated back to 3,500 years. However, bone samples from Al *Yasileh* are about 1,200 years old, of late Roman-early Byzantine. This age is consistent with that estimated by previous archaeological (typology) studies [18-22].

Tell al-Husn is an ancient and continuously settled village; it is located in al-Husn region about 8 km south of Irbid and 73 km north of Amman in Jordan. The site has a settlement area since the Chalcolithic period (4,000 BC), throughout the Bronze, Iron, Persian, Hellenistic, Roman, Islamic periods, and even up to the present [23]. The first season of excavations at Tell al-Husn conducted by the Faculty of Archaeology and Anthropology at Yarmouk University, with the support of the Jordanian Department of Antiquities, during this work, a significant collection of bone, teeth, pottery, glass, metal objects excavated. The study of these artifacts objects is significant to reveal the historical and archaeological aspects of this significant site in the northern part of Jordan [23-28].

The Al *Yasileh* archaeological site is located about nine kilometers east of the city of Irbid, with ruins spread over an area of more than 1,000m^2. Al *Yasileh* has a strategic geographical position, located on the King's Highway that transverses Wadi al-Warran, and close to the trade routes connecting Syria and Palestine. In antiquity, the people of Al *Yasileh* developed a system of cisterns, channels, and wells to conserve rainwater. In addition, one of the most important water sources in the area is al-Mu'alaqah spring, in Wadi Warran, which is about one km north of the site. Several unpaved roads are leading to the site from ar-Ramtha and Huwwarah. The sessions for excavations were undertaken by the Faculty of Archaeology and Anthropology at Yarmouk University collaborated with the Jordanian Department of Antiquities [20-22, 29-31].

Experimental

A collection of bone fragments has been compiled for measurements with FTIR, Raman, SEM, and XRD techniques for archaeological bone fragments from sites in Jordan. It is confirmed that the bone objects are dated about 2,000 years ago, which was determined by the typical pottery study done from the excavators of these sites. This dating by vibrational spectroscopy use as a preliminary method for bone age classification in the field of archaeological dating. Sixteen different human's fragments were collected from the store of Faculty of Archaeology at Yarmouk University, the bone fragments sample were excavated from Tell al-Husn [3,4,8,11,14,16], and Al Yasileh [5-7,9,10,12,13,15] in northern Jordan. An FTIR spectrometer (model 670, Agilent, USA) coupled to a microscope (model 620, Agilent) and ATR accessory with a germanium prism are used for data acquisition in reflection mode, the IR spectra in the range from 750 to 4,000cm^{-1} at a spectral resolution of 4 cm^{-1} using the single-channel MCT detector, 64 scans were averaged for each spectrum.

Raman spectrometer with a single-mode diode laser (model Xtra, Toptica, Germany) with 785nm emission was coupled to a microscope, and the microscope was coupled to the Raman spectrometer (RXN1 microprobe, Kaiser Optical System, USA). Laser light with an intensity of 100mW focused on the sample with a 100×/NA 0.9 objective (Nikon, Japan). The Raman signal was detected on a Peltier-cooled (-60°C), back-illuminated, deep-depletion CCD chip (Andor, Ireland). Spectra re-obtained over the spectral region of 300 to 3,450 cm^{-1} at a spectral resolution of 4 cm^{-1} with a step size of 1μm and 6 seconds exposure time plus 3 seconds dwell time per Raman spectrum. In addition, this study will cover several scientific methods and techniques, which are used at the University of Friedrich Schiller and Institute of Photonic Technology in Jena (Germany) on the characterization of several artifacts samples, dated from prehistoric to roman times. In particular, vibrational spectroscopy as Raman spectroscopy and Fourier Transformation Infra-Red spectroscopy are very important for archaeological objects.

Results and discussion

Raman and FTIR can measure bone crystallization based on various factors and simultaneously measure the organic part degradation of the bone, the degradation of the collagen/amide depend on the amount of free water present in the bone; it is inversely proportional to the signal amplitude in Raman and FTIR spectra as shown in Figure 1. This work investigated many ancient bone samples from a Tell al-Husn site and Al Yasileh in the northern part of Jordan, as we showed in previous sections. FT-Raman and FTIR used to analyze archaeological bone (1,400 to 3,500 years old). Spectral data analysis of vibrational spectroscopy was performed using OMNIC 7.3 Software (ThermoNicolet). This study has focused on the range between 700–2,200 cm^{-1} and 2750-3125 cm^{-1} spectral motivated area, where the group of apatites as inorganic materials and their interaction with the organic matter are clear. Data analysis was achieved using OMNIC 7.3 version (ThermoNicolet) Software. The phosphate band (600-850) represents the mineral part of the bone. The absorption band centered at 1745 cm^{-1} corresponds to the carbonyl stretching vibration band of the organic part of the ancient bone. The spectrum contributes to the PO$_4$ region (900–1,150 cm^{-1}) stretching vibration bands. The carbonate stretching vibration band (1,600-1,900 cm^{-1}) can, therefore, be used as a fingerprint of the ancient collagen distribution in the samples, the spectral regions are shown in Figure 1.

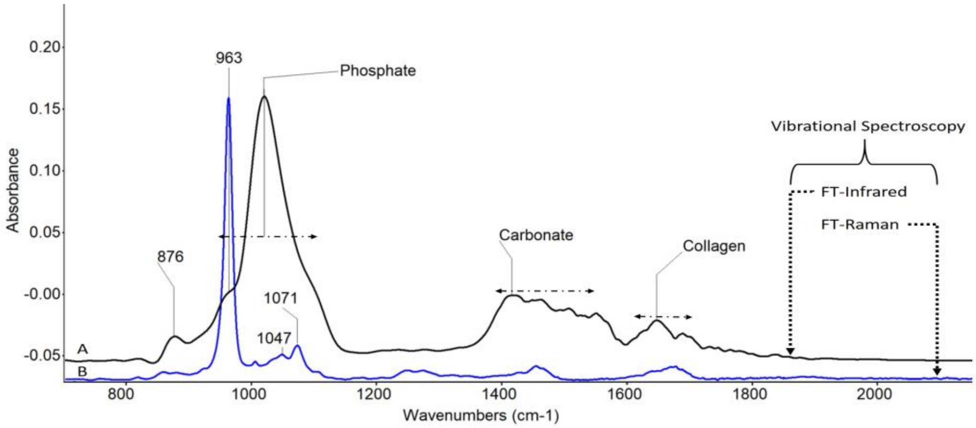

Fig. 1. The FT-Infrared spectra of the ancient human bone (A) and (B) FT-Raman Spectrum of the same sample.

The results of radiocarbon isotope are used to determining the absolute dating of those bone samples to compare with the preliminary method by vibrational spectroscopies, the age results measured by comparing the intensity ratio of CH Raman peak at 2,941 cm^{-1} to the intense peak of phosphate band at 963 cm^{-1}. The intensities investigation was used to estimate the age of the archaeological samples. It is a simple way to date the ancient bone to the intensity ratio of the organic Raman spectral band at 2,942 cm^{-1} of the organic fraction to the inorganic Raman spectral band of phosphate at 963 cm^{-1} [37], this ratio decreases with the bones ages or the degradation of the organic part of the protein are directly proportional to the age, this gives good results by using the archaeological bones from Tell al-Husn. This method will provide us a preliminary dating to know if the bone is archaeological or not; afterward, the sample is sent the sample to radiocarbon dating. This strategy used to conserve the time and financial resources - the cost of ^{14}C dating is expensive.

Figure 2, shows the ratio of the organic fraction at 2,950 cm^{-1} to the inorganic fraction at 963 cm^{-1} and indicates the degree of crystallization as a function of time.

During the loss of the organic portion as a function of age, the crystallization of the mineral part of the bone changes, e.g. at 0.11, which means the time lapse of 1,100 years after burial, which corresponds to the period of 3,000 years after burial. The samples 1, 9, and 15 are measured by Radiocarbon dating peak on the vertical y-axis before calibration (1,100-1,450 years), after calibration the old of the samples is about 1385±53 BP before present, where BP dates obtained by subtracting 1950 years from conventional age B.P. the calibrated age also presents an irregular distribution, resulting from projecting the probability of each point of the conventional age on the calibration curve. Therefore, the presence of "saw teeth" (wiggles) on the calibration curve, at a point on the conventional age distribution may correspond to more than one point of the age distribution calibrated, as is the case shown. After calibration, the actual date is found, with a 95.4% probability, in the interval 675 - 900 cal AD. The rest of the samples are measured by ^{14}C [28]; the results of ^{14}C agreed with the FTIR and Raman results, it represents the age of the samples.

It is argued that an inversely proportional to age and quantity of the organic part in the sample or decrease in the relative intensity of the organic component of the enamel or bone with increasing burial period of the hard tissue bone [37]. This trend is better represented in Figure 3, where the intensity of the peak on 2,941cm^{-1} to the peak on 963 cm^{-1}, these results suggest that for the bone examined, the organic component is released in proportion to the burial period [37].

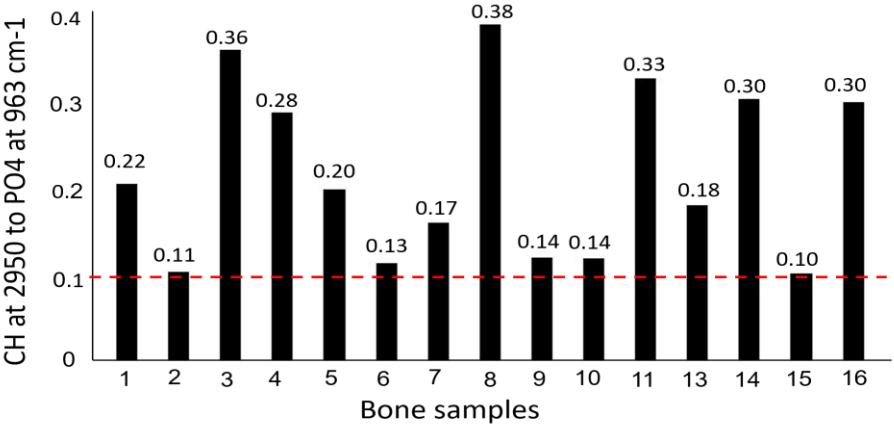

Fig. 2. An organic fraction from Tell al-Husn Samples, and samples from Al Yasileh.

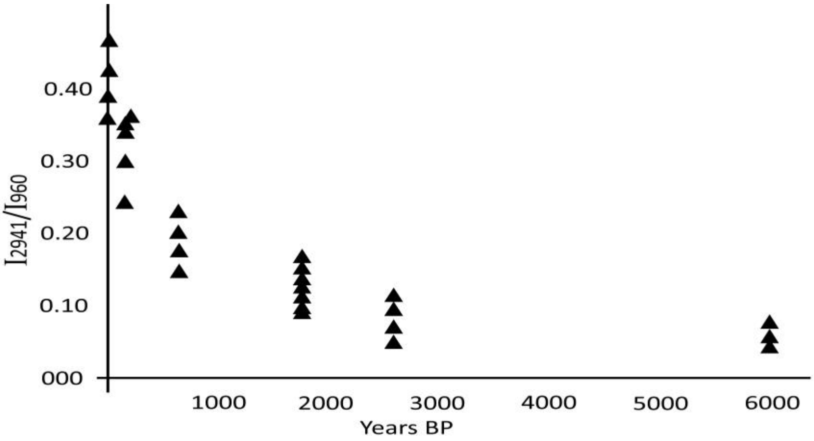

Fig. 3. I 2941/I 960 relative intensities as a function of the tooth burial period [37].

The results of ^{14}C Somewhat compatible with Raman results. Radiocarbon dating of human bone is yielded a conventional ^{14}C age of 1385 ± 53 yr BP. Following calibration using the IntCal13 atmospheric curve [38, 39]. The raw data are represented by the standard distribution Gaussian or normal date of the conventional carbon-14 date (green curve) on the horizontal axis, now, to be calibrated with the help of the calibration curve-the wiggly red dashed line-in order to gain calendar years. The straightforward interception method at one or two standard deviations visually examines the overlap between the measurement made and the reference curve. In contrast, the standard method is used to calculate a probability density function for the age of the sample with the help of a calibration program. Figure 4 shows the ^{14}C concentrating curve for the seventh century, the late Byzantine period in Al Yasileh site in Jordan.

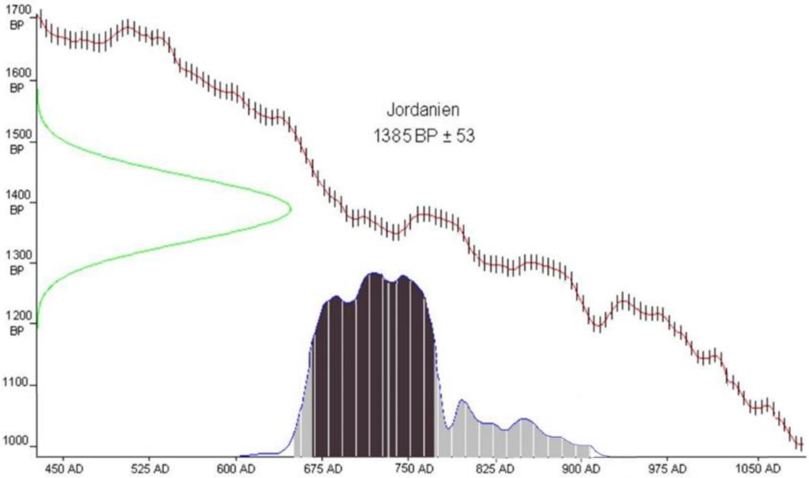

Fig. 4. Radiocarbon ^{14}C dating of bone from Al Yasileh.

SEM micrograph shows the crystalline nature of ancient bone with hydroxyapatite crystal size distributions. A representative SEM image for a sample shows the average crystal dimensions of approximately 100 to 1,000 nm (Fig. 5). The observed SEM crystal dimension has higher diversity in size and shape. This discrepancy may be due to the decay factors affected the crystal properties as age and buried environment. Bone mineral crystals are found within the collagen fibrils, and the fibril structure and organization control the size of the crystals and their orientation [40].

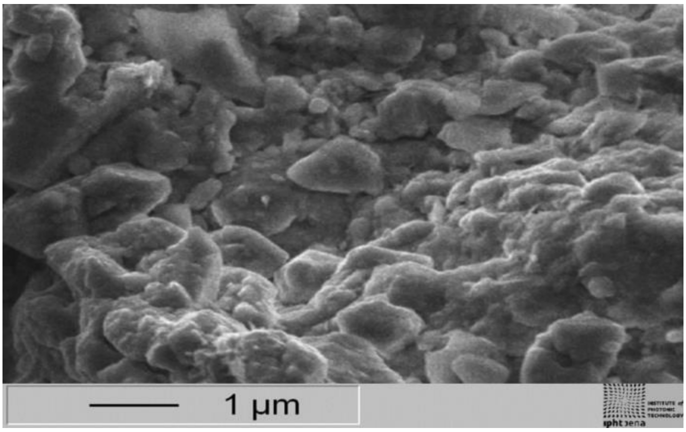

Fig. 5. SEM micrograph of archaeological bone from Tell al-Husn.

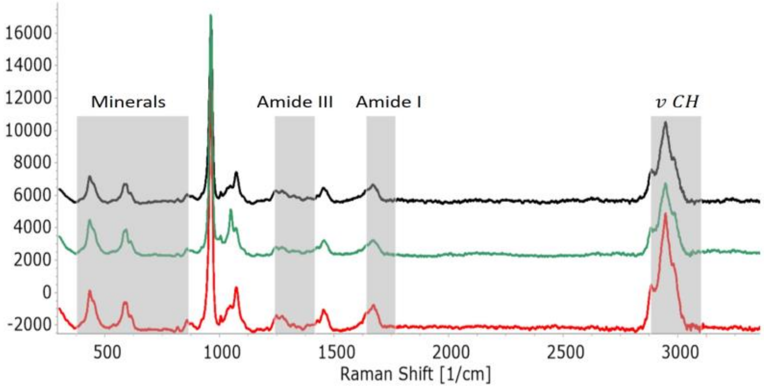

Fig. 6. Typical Raman spectrum of ancient human bone showing the most spectral regions of mineral and organic parts.

Bone consists of the three main components mineral, organic, and water on several hierarchical levels. The mineral fraction of bone is a highly impure, carbonated apatite matrix doped between collagen fibril crosslinks and fibril ends. Figure 5 shows the Raman spectra of ancient bone, the phosphate ν1 band (963 cm^{-1}), and the bands connected with collagen (amide I at 1,598-1,642.4 cm^{-1} and amide III at 1,298 cm^{-1}) are the most important components for bone examination. Widening one component to 963 gives information about the crystal formation of apatite, which in turn provides information about the age of the bone. The other important part is the organic bone resorption, which gives information about the age of the bone. In this study, the same Tell al-Husn specimen, whose culture was dated by radiocarbon AMS, is examined by dating 16 human bone fragments taken from a grave complex with a vertical shaft with radiocarbon.

Conclusions

The Fourier Transform Raman and FTIR spectroscopy were used to analyze archaeological bones (1,000 to about 3,800 years ago). ^{14}C was used to determine the age of these samples and then examined the age results by comparing the intensity ratio of the CH^{2-} Raman peak at 2,950 cm^{-1} with the intensity peak of the phosphate band at 963 cm^{-1}. We used this study to classify the age of the archaeological artifacts as preliminary dating. It is a simple method of dating of old bone to determine the intensity ratio of the organic Raman spectral band at 2,950 cm^{-1} to the inorganic Raman spectral band at 963 cm^{-1}. Figure 3 shows the relative intensities I 2950/I 963 of Jordan's old bone. The 16 samples with an intensity ratio of less than or equal to 0.1 were measured over a period of (1,000 to 24,000 years ago). Moreover, rest samples were in the range of 1,000 years ago. ^{14}C dating of some samples of bones found at the same excavation sites. The results of ^{14}C supported this study support the results of the vibrational spectroscopy; Raman and FTIR, are consistent chronologically with the age determination by the conventional and expensive of radiocarbon method ^{14}C.

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