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ARTIGO ORIGINAL

Dating of skeletonized human remains: recent vs. archaeological

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Article received on the 21st of November of 2021 and accepted on the 15th of February of 2021

ABSTRACT

In this study, the FTIR-ATR technique was used to show possible structural mineral differences between recent and archaeological human bone, from spectral indices: CI (Crystallinity Index), C/P (Carbonate/Phosphate ratio), BPI (content of type B carbonate), API (content of type A carbonate) and C/C (relationship of carbonates (A+B) with type B carbonate). The archaeological sample had lower CI, C/P, BPI and API values than recent samples. Only C/C had similar values. The limitation of this study lies in its small sample, mainly the archaeological one. Even so, it was possible to show that with the FTIR-ATR technique it is possible to study the differences between the two types of human bone samples.

Keywords: Human Bone Dating; FTIR-ATR; Forensic Anthropology

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RESUMO

Neste estudo, a técnica FTIR-ATR foi utilizada para mostrar possíveis diferenças minerais estruturais entre osso humano recente e arqueológico, a partir dos índices espectrais: CI (Índice de Cristalinidade), C/P (relação Carbonato/Fosfato), BPI (teor de carbonato tipo B), API (teor de carbonato tipo A) e C / C (relação dos carbonatos (A+B) com carbonato do tipo B). A amostra arqueológica teve valores de CI, C/P, BPI e API mais baixos do que as amostras recentes. Apenas o C/C teve valores semelhantes. A limitação deste estudo reside na sua pequena amostra, principalmente a arqueológica. Mesmo assim, foi possível mostrar que com a técnica de FTIR-ATR é possível estudar as diferenças entre os dois tipos de amostras de osso humano.

Palavras-chave: faunas; mamíferos; zooarqueologia.

Dating human skeletal remains is one of the most important tasks in Forensic Anthropology (Ferreira and Cunha, 2012; Baptista, 2020; Baptista *et al.*, 2021). Its dating is extremely important to understand if we are facing a true forensic case. Depending on its result, it may give rise to a criminal investigation, depending on the legal framework of each country.

Human bone tissue is a heterogeneous structure and can be divided into two major components: inorganic component and organic component. The inorganic component provides mechanical rigidity and strength, while the organic one provides elasticity and flexibility (Datta *et al.*, 2008). The organic component comprises lipids and proteins (mainly collagen type I) and non-collagenous proteins, including osteocalcin, osteonectin, osteopontin, fibronectin and bone sialoprotein II, bone morphogenetic proteins (BMPs) and growth factors (Aszódi *et al.*, 2000). The inorganic component, bioapatite, is a carbonate substituted hydroxyapatite represented by the formula $Ca_{10}(PO_4)_6(OH)_2$. Carbonates (CO_3^{2}) can replace phosphate groups (PO_4^{3}) (type B substitution) or hydroxyl groups (OH) (type A substitution) in the bioapatite matrix (Rey *et al.*, 1989; Astala and Stott, 2005).

The organic and inorganic constituents of bone tissue provide a characteristic infrared spectrum therefore the FTIR-ATR method may be extremely suitable for providing detailed information on bone samples. It is an inexpensive technique, with fast and non-destructive analysis, has no saturation and optical dispersion problems, does not use any type of solvent for the sample and, thus, the probability of spectrum variation is minimal and the amount of sample required is very small (<1 mg) (Thompson *et al.*, 2009; Hollund *et al.,* 2013; Beasley *et al.,* 2014; Mamede *et al*., 2018; Marques *et al*., 2021).

This study aims to show possible mineral structural differences between archaeological human bone samples and recent human bone samples through spectroscopic indices: CI (Crystallinity Index) (Weiner and Bar-Yosef, 1990), BPI (amount of type-B carbonate) (Sponheimer and Lee-

Thorp, 1999; Snoeck *et al*., 2014), API (amount of type A carbonate) (Sponheimer and Lee-Thorp, 1999), C/P (Carbonate/Phosphate ratio) (Wright and Schwarcz, 1996; Thompson *et al*., 2009; Snoeck *et al*., 2014) and C/C (carbonate (A+B) to carbonate B type relationship) (Thompson *et al*., 2009; Snoeck *et al*., 2014) calculated from spectra obtained through FTIR-ATR analysis (Baptista, 2020).

Material and Methods

Sample

For this study 11 bone samples were chosen. The sample included 10 femurs, each from a different individual, from the 21st Century Identified Skeletal Collection (CEI/XXI) housed at the Laboratory of Forensic Anthropology, University of Coimbra (Ferreira *et al*., 2020), with a post-mortem interval (PMI) between 19 and 24 years, counting from 2020. The archaeological sample included a clavicle of one individual (PAVd'09_I.25 with a radiocarbon dating: 560 ± 30 BP, Cal AD 1310–1360, Cal BP 640–590, and Cal AD 1385–1425, Cal BP 565–525, Beta-438015) from the Collection of African Slaves of Valle da Gafaria (Ferreira *et al*., 2019). The PMI values were established from the year of death of each individual until 2020, when the samples were analysed. Cortical bone tissue was assessed (from the medial portion of the diaphysis), as it is less susceptible to diagenetic processes (Grupe, 1988). Prior to spectroscopic analysis the outermost layer of the compact bone was gently removed with a scalpel (Pedrosa *et al*., 2020), yielding a fine powder suitable for FTIR-ATR analysis (ca. to < 1mg).

FTIR-ATR Spectroscopy

FTIR-ATR data was recorded, for the powdered bone samples, in a Bruker Optics Vertex 70 FTIR spectrometer purged by CO_2-free dry air equipped with a Bruker Platinum ATR single reflection diamond accessory. A liquid nitrogen-cooled wide band mercury cadmium telluride (MCT) detector and a Ge on KBr substrate beamsplitter were used for the mid-IR interval (400- 4000 cm^{-1}). 128 scans were summed for each spectrum, at 2 cm^{-1} resolution, applying the 3-term Blackman–Harris apodization function, yielding wavenumber accuracy above 1 cm^{-1}.

Data analysis

Pre-processing of the FTIR data was performed with the OPUS Software (Bruker OPUS - Spectroscopy Software (2019)). The spectra were corrected regarding the wavelength dependence of the penetration depth of the electric field in ATR, for a mean refractive index of 1.25. After baseline correction, the spectra were normalized relative to the $\nu_3(PO4^{3-})$ phosphate band (at 1035 cm^{-1}).

Several relationships between specific infrared band intensities were calculated for a thorough analysis of the differences between the corresponding spectroscopic profiles (Fig. 1): Crystallinity Index (CI), carbonate/phosphate ratio (C/P), A-type carbonate content ratio (API), B-type carbonate content ratio (BPI), and carbonate (A+B) to carbonate B-type relationship (C/C). The values of these relationships were compared between the archaeological individual and the recent bone samples.

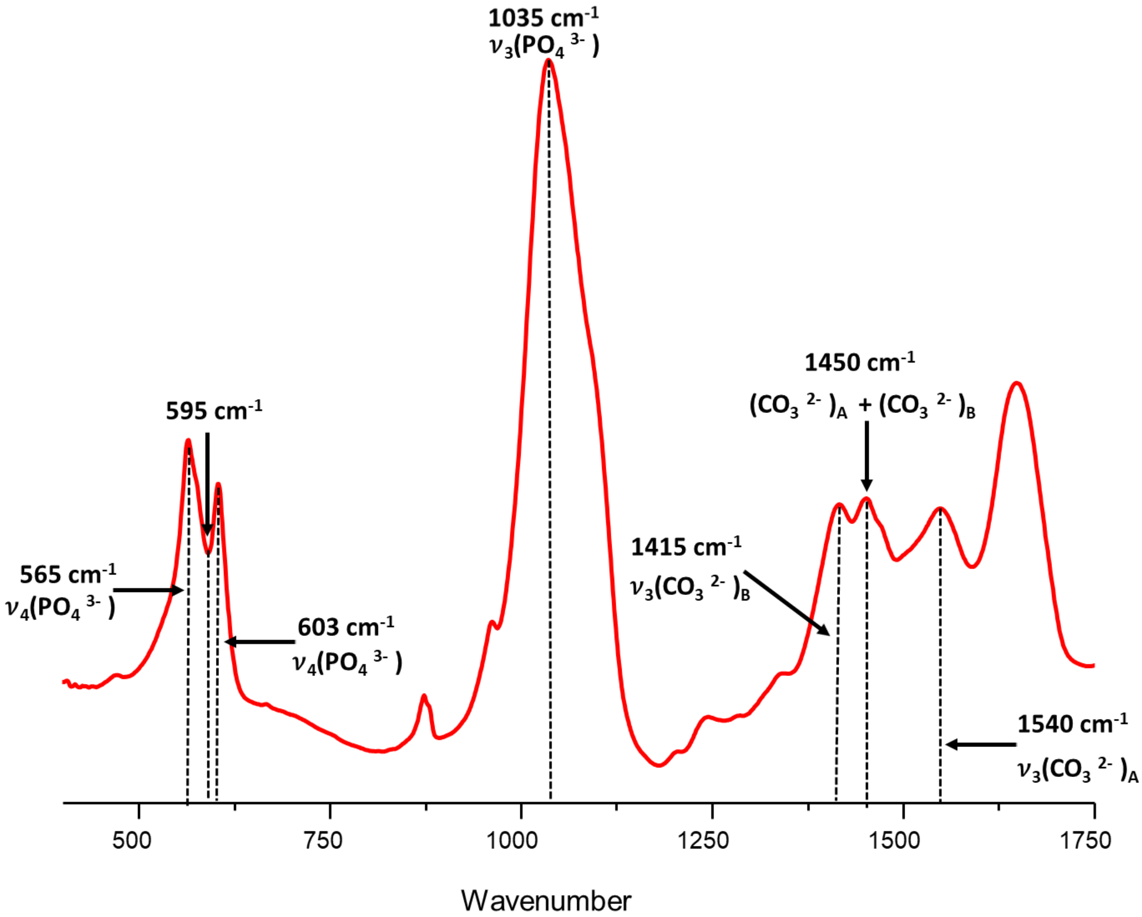

Figure 1 - FTIR-ATR spectrum showing the signals of interest for calculating the spectral indices of human bone samples used throughout this work to assess their chemical composition (Individual CEI/XXI 80 spectrum – recent bone sample).

Results

The results obtained from the indices calculated for the samples under study are summarized in Table 1. It can be observed in Figure 2 that the values calculated for the indices for recent and archaeological samples are comparable however there are differences between both spectra. Comparing the values obtained from the spectral indices for the archaeological bone sample (PAVd'09_I.25) with the values of the recent bone samples, it can be concluded that the API (0.3) and BPI (0.6) ratios are significantly lower, that CI (2.8) and C/P (0.27) also have slightly lower values than the rest. The C/C (0.99) has a similar value.

Table 1 - Spectroscopic indices calculated for the femur of each individual from the 21st Century Identified Skeletal Collection and for the clavicle of the archaeological sample from the Collection of African Slaves of Valle da Gafaria.

Individual	PMI (years)	CI	C/P	API	BPI	C/C
PAVd'09_I.25	560 ± 30 BP	2.80	0.27	0.30	0.60	0.99
CEI/XXI 69	24	3.07	0.42	1.02	1.33	0.96
CEI/XXI 58	21	2.89	0.37	0.89	1.16	0.98
CEI/XXI 82	21	3.17	0.41	1.08	1.31	0.99
CEI/XXI 89	20	2.96	0.40	0.98	1.23	0.97
CEI/XXI 51	20	3.08	0.40	1.01	1.30	0.98
CEI/XXI 87	20	3.08	0.39	0.99	1.26	0.98
CEI/XXI 63	20	3.14	0.41	1.05	1.34	0.98
CEI/XXI 80	19	3.21	0.30	0.82	0.97	0.99
CEI/XXI 14	19	2.99	0.37	0.83	1.20	0.94
CEI/XXI 6	19	3.05	0.40	0.98	1.28	0.96

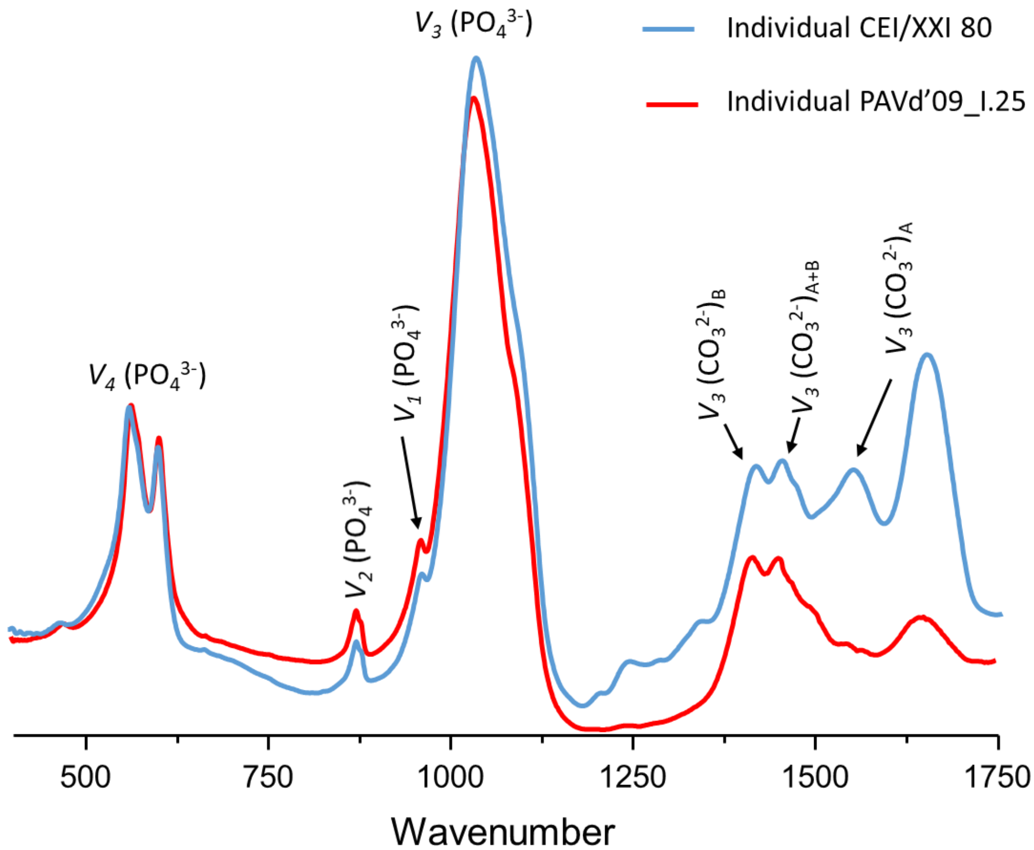

Figure 2 - Spectroscopic indices calculated for the femur of each individual from the 21st Century Identified Skeletal Collection and for the clavicle of the archaeological sample from the Collection of African Slaves of Valle da Gafaria.

Discussion

Dating human skeletal remains is one of the most important and yet problematic aspects in Forensic Anthropology (Ferreira and Cunha, 2012; Baptista, 2020; Baptista *et al.*, 2021). FTIR-ATR analysis allows you to gather important information about the chemical properties of bone tissue and better understand how they change. Also this method is extremely suitable to provide detailed information on bone samples through calculated indices. Furthermore, it is a non-destructive, inexpensive technique that requires small amounts of sample for analysis and does not require any type of sample preparation. Based on the results obtained in this study, we can say that, in general, CI, C/P, API and BPI indices tend to decrease over time, that is archaeological bones have lower CI, C/P, API and BPI values than recent ones. While C/C has a similar value between recent and archaeological samples. The limitation of this study is its small sample size, especially the archaeological sample.

However, it shows that with FTIR-ATR it is possible to observe human bone mineral structural differences between archaeological samples and recent samples and that those same differences might be used to distinguish between a forensic and a non-forensic case.

Acknowledgments

This work is carried out at the R&D Unit Centre for Functional Ecology – Science for People and the Planet (CFE), with reference UIDB/04004/2020, financed by FCT/MCTES through national funds (PIDDAC). The authors also acknowledge financial support from the Portuguese Foundation for Science and Technology (UIDB/00070/2020).

The authors state that they do not have any conflict of interest to declare.

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