



## Mineralization changes substituted type B carbonate of $\text{PO}_4^{3-}$ ion in the bone minerals of an archaeological sample studied using fourier self deconvolution technique

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Received 26 January 2019; revised 21 December 2019

The aim of this study was to describe the compositional changes in the archaeological specimen using FTIR. Fourier deconvolution was applied in the carbonate ( $500\text{-}650\text{ cm}^{-1}$ ), phosphate ( $900\text{-}1200\text{ cm}^{-1}$ ), and in the amide region ( $1700\text{-}1600\text{ cm}^{-1}$ ). The deconvolution of the spectra in the phosphate region  $1200\text{-}900\text{ cm}^{-1}$  reveals six components  $960\text{ cm}^{-1}$ ,  $1010\text{ cm}^{-1}$ ,  $1020\text{ cm}^{-1}$ ,  $1030\text{ cm}^{-1}$ ,  $1110\text{ cm}^{-1}$  and  $1120\text{ cm}^{-1}$ . The bone mineral such as  $\text{CO}_3^{2-}$ ,  $\text{PO}_4^{3-}$  particle, collagen, and organic materials were studied in detail from the infrared spectra. The computed parameters such as collagen development, mineralization index, C/P proportion, crystalline index, and maturing of bone help us in characterizing the bone samples. The Low estimation of C/P proportion resulted in changes in Type B carbonate ( $\text{PO}_4^{3-}$ ) substitution. The absence of phosphate band  $\sim 1100\text{ cm}^{-1}$  and formation of  $\beta$  sheet structure were observed in the samples studied. Increased level of mineralization resulted in the increased in the crystalline nature of the bone samples. The study indicates the utilization of FTIR using self deconvolution technique which helps in understanding the compositional changes in the archaeological sample.

**Keywords:** Collagen matrix, FTIR, Bone, Mineralization index,  $\beta$  sheet structure

The characteristic bone tissues are calcium phosphates, water, and collagen alongside the other natural materials in small amounts. Collagen is the most abundant protein of the organic matrix in mineralizing tissues<sup>1</sup>. The bone crystals were found to contain significant and differing measures of carbonate and  $\text{HPO}_4^{2-}$  ions. The level of mineralization is steady with bone maturing/development and it is helpful for the portrayal of the archaeological specimens. The mineral part of the bone was poorly crystalline

quality for a better understanding of the mechanism of bone fragility<sup>3</sup>. IR spectroscopy has wide application in biomedical fields and has been utilized to assess the mineral substance, mineral crystallinity, phosphate, carbonate, and collagen substance in bones<sup>4-6</sup>. Various techniques, such as X-ray diffraction, FTIR, NMR, ESR are utilized to study bone tissue and other biological material to decide mineral structures<sup>7,8</sup>. FTIR is a nondestructive tool and it gives the conformation regarding the changes in absorption frequency and functional groups in the specimen. Hence it is appropriate to assess the variation in the chemical composition and mineralogy in the bone specimen. The most characteristic chemical groups such as  $\text{PO}_4^{3-}$ ,  $\text{CO}_3^{2-}$ ,  $\text{HPO}_4^{2-}$  present in the bone specimen was studied using FTIR spectrum<sup>9,10</sup>. It gives an efficient way of checking various characteristic groups and vibrations. Further, it gives data about the location of peak, intensity, width, and wavenumber range. FTIR allows to describe the nature of both mineral and collagen in bone tissue.

for either the phosphate or the hydroxyl group prompting to nonstoichiometric apatite mineral<sup>2</sup>. This substitution creates vacancies and distortion that may influence the crystal lattice and influence the mechanical quality of the bone. This progression is studied by Fourier self deconvolution in the selected region of  $500\text{-}650\text{ cm}^{-1}$ ,  $850\text{-}890\text{ cm}^{-1}$ ,  $900\text{-}1200\text{ cm}^{-1}$ . Delphine Farlay *et al.* showed that mineral maturity and crystallinity index are utilized to analyze the bone

The structure and the chemical composition of archaeological bone material may be modified by

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interaction with the burial environment. Archaeological bone constitutes an important in the understanding of ancient societies as they can give evidence of human or faunal occupation. As biomaterials, they register for their chemical and isotopic composition information on climate and environmental conditions<sup>11</sup>. Hence, the study aims to assess the crystallinity of HA minerals, collagen, and the changes in phosphate and carbonate content based on the Fourier self deconvolution method. Deconvolution of FTIR spectra offers a detailed qualitative and quantitative analysis of carbonate and orthophosphates ( $\text{HPO}_4^{2-}$  and  $\text{PO}_4^{3-}$ ). Keeping in view of the above discussion, the present study aims to look at the portrayal of the archaeological bone revealed from Kerala state of Kotapuram area, India utilizing Fourier transform infrared spectroscopy.

#### Site Details of Kottapuram Fort

Kottapuram Fort is an old stronghold territory close to Kodungalur Taluk, Thrissur area, Kerala state, India. Kottapuram is a piece of the Thrissur region and at the southernmost peripheral of the Kodungallur region. This region of Kerala is rich in the history and social convention of all the regulatory divisions of the state. Different sorts of ceramic, red ornamented pots, stoneware, globules, human and animal bones were found. This was exhumed by the archaeological study of India, Trivandrum, in the year April 2007. In 2007, the state branch of paleontology, Government of Kerala investigated this site deliberately and laid out a few trial trenches.

#### Materials and Methods

The two animal bones (Fig. 1A) KHB- Horse bone, KGB-Goat bone were gathered from the archaeological review in the location of Kotapuram region, Kerala, India. The bone specimen was cut with a microtome. A representative portion from the bone was ground into powder with steel mortar. For KBR pelleting sample 1-3 mg of ground, bone was blended with 100 mg of KBR using a hydraulic press tomake KBr pellets. FTIR spectra were acquired utilizing a Nicolet FTIR spectrometer controlled by OMNIC programming. The spectra were recorded in the region of  $4000\text{-}400\text{ cm}^{-1}$  with a spectral resolution of  $4\text{ cm}^{-1}$ . Each spectrum was the result of an average of 16 scans. The curve fitting calculation utilizes Gaussian bands to deliver the computed spectrum, which is compared with the experimental spectrum. The procedure is iterated until an acceptable fit is

acquired by a least square regression analysis. Initial peak positions were acquired from the second derivative spectra of the raw data information. In every situation, the goodness of fit (GOF) parameters was resolved to locate the best fit from the different algorithm. The measured % area of the computed band was utilized for the quantitative change in the compositions of the sample studied<sup>12</sup>.

#### Results and Discussion

Figure 1B demonstrates the normal FTIR spectra of the archaeological bone specimen collected at Kotapuram, Kerala state, India. Table 1 shows the tentative frequency assignment of the archaeological bone samples. A broad and weak band  $\sim 3444\text{ cm}^{-1}$  relates to the OH group. The Amide I band emerges because of C=O stretching from collagen. It relates to  $\sim 1633\text{ cm}^{-1}$  of the bone specimen. A strong band

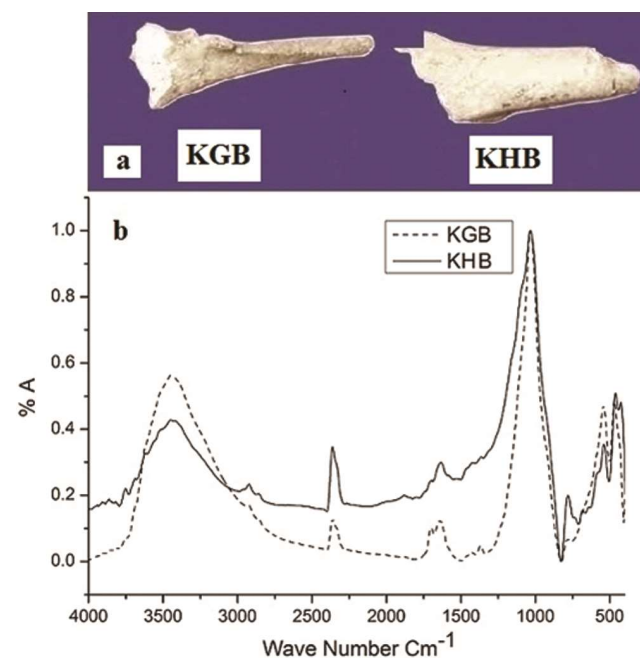


Fig. 1 — (A) A Photograph showing archaeological sample extracted from Kottapuram region, Kerala, India; and (B) Average FTIR spectra of bone sample

Table 1 — Tentative frequency assignment of the archaeological sample recently excavated from kottapuram place, India

KHB	KGB	Frequency Assignment
3435 (vw)	3444 (w)	OH group
1634 (w)	1634 (vw)	Amide I group
1030 (vs)	1029 (vs)	Phosphate group
542 (w)	544 (w)	$\text{PO}_4^{3-}$
476 (m)	479 (m)	

VS-Very Strong, m-Medium, W-Weak, VW-Very Weak

Table 2 — Computed values obtained from the FTIR spectra of the archaeological sample

Parameter	KHB	KGB
C/P	0.03	0.03
Collagen maturity	0.27	2.59
Crystalline Index	2.41	3.89
Degree of mineralization	18.61	23.28
Mineralization index	27.41	29.31

$\sim 1028\text{ cm}^{-1}$  associates to phosphate content. A weak band  $\sim 542\text{ cm}^{-1}$  and medium band  $\sim 476\text{ cm}^{-1}$  corresponds to the bending vibration of ( $\text{PO}_4^{3-}$ ) phosphate anion. For a quantitative study the ratio of the band region 900-1200/1600-1700 results in the degree of mineralization or maturing of bone<sup>13</sup>. The observed values show KGB has higher maturity and KHB has a lower maturity of the sample studied (Table 2).

The ratio of C/P demonstrates the level of carbonate substitution of the collagen framework in the bone specimens. The least value was observed for both the sample studied. The measured value of the C/P ratio by Melainc *et al.* falls within our results and it was in good agreement with the present findings distinguishing prehistoric bone samples<sup>14</sup>. This change in C/P proportion is credited by the reduction in substitution of Type B carbonate ( $\text{PO}_4^{3-}$  substitutions) and increment to Type A carbonate (OH substitutions) as shown in (Fig. 2A& 2B). The phosphate band was obtained from the results of curve fitting in the region 1200-900  $\text{cm}^{-1}$  (Fig. 2C). Consequently, phosphate substitution in mineral lattice demonstrates the increase in age of bone sample or development. The clearest change observed is an expansion in bone mineral crystallinity with maturing, demonstrating that mineral crystallinity increases with aging bones. This change is also correlated with an increase in the degree of mineralization of bone tissue.

It is observed in our case that the degree of mineralization increases in the KGB when compared to KHB. Crystallinity is contrarily identified with carbonate-to-phosphate proportion *i.e.*, lower carbonate-to-phosphate proportion should be connected with large crystals<sup>15</sup>. Carbonate-to-phosphate proportion was additionally connected with delicacy crack<sup>13</sup>. In bone, carbonate ions can substitute for both phosphate and hydroxide ions when the hydroxyapatite crystal lattice is formed<sup>16</sup>. The collagen maturity of the sample was calculated using the band area ratio of 1660/1690 which was obtained as results of the curve fitting

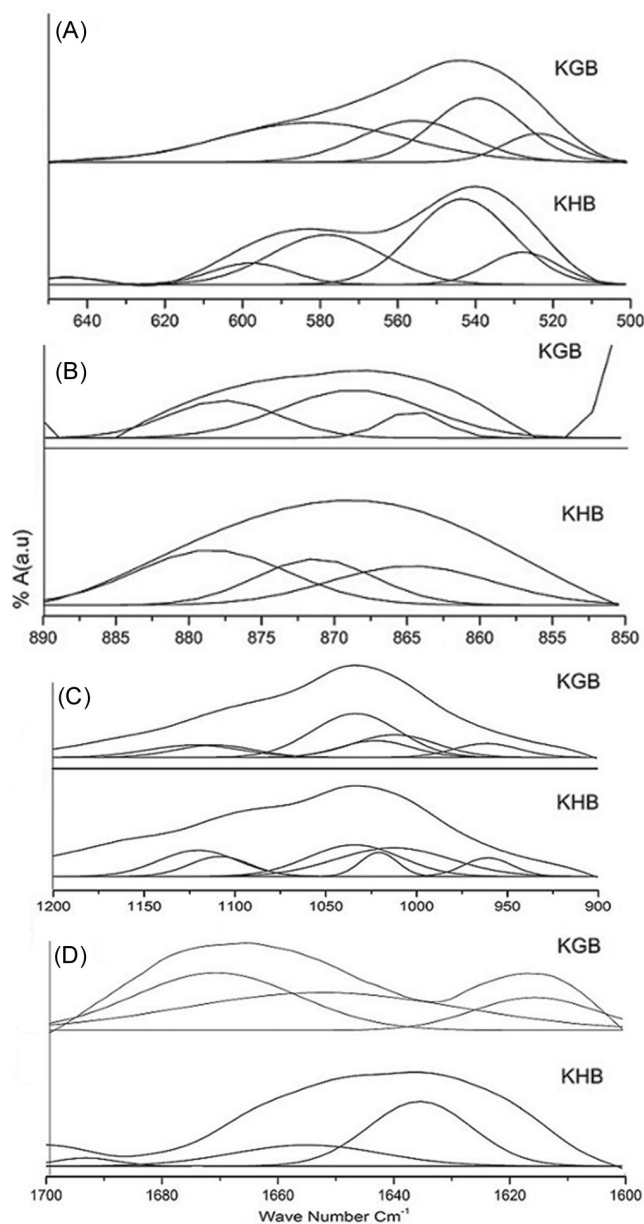


Fig. 2 — (A) Deduced curve fitting spectra of the bone sample in the region 640-500  $\text{cm}^{-1}$  of  $\text{HPO}_4$  band; (B) Deduced curve fitting spectra of the bone sample in the region 890-850  $\text{cm}^{-1}$  of carbonate band; (C) Deduced curve fitting spectra of the bone sample in the region 1200-900  $\text{cm}^{-1}$  of phosphate band; and (D) Deduced curve fitting spectra of the bone sample in the region 1700-1600  $\text{cm}^{-1}$  Amide region

analysis<sup>17</sup>. These studies demonstrate that its value washigh for KGB when compared to KHB samples (Table 2). FTIR information reflects the persistence of both existing mineral and further collagen maturity. Failure of reabsorbs of the collagen matrix of the bone would bring about increased mineral content clarifying the distinction between the mineral of the

Table 3 — The results of curve fitting analysis of the archaeological sample in 500-650  $\text{cm}^{-1}$  region

Sample Compositions	KHB		KGB	
	Frequency ( $\text{Cm}^{-1}$ )	% Area	Frequency ( $\text{Cm}^{-1}$ )	% Area
Labile $\text{HPO}_4$	530	12.53	524	10.02
Acid Phosphate	540	45.65	539	29.43
$\text{PO}_4^{3-}$	578	30.31	556	23.22
Labile $\text{PO}_4^{3-}$	597	9.21	582	37.33

bone specimen<sup>18</sup>. This increased collagen cross-link ratios (collagen maturity) could basically reflect the persistence of older bone.

Increasing mineralization density builds the quality of bone. Mineralization is a vital determinant of the micro hardness. Bone micro hardness is connected to Young's modulus of bone and is emphatically related to mineralization. The degree of mineralization of bone (DMB) not just impacts the mechanical resistance of bone additionally somewhat decides the bone mineral density. The crystallinity index was computed<sup>19</sup> using the ratio between the peak areas  $\sim 1030$  to  $1010 \text{ cm}^{-1}$ . The high value of the C.I index was observed for the KGB when compared to the KHB bone sample. This band  $\sim 1110 \text{ cm}^{-1}$  has great reproducibility in assessing the composition of the bone sample in an archaeological study<sup>20</sup>. The weakening of the crystallinity index (CI) may happen because the development of the  $\beta$  sheet arrangement as proved from curve fitting analysis which was discussed in the section Amide region.

#### Fourier self deconvoluted spectral analysis in the carbonate region

Figure 2A demonstrates the consequences of the curve fitting investigation performed in the region of  $650\text{-}500 \text{ cm}^{-1}$ . It indicates wide band focused  $\sim 524 \text{ cm}^{-1}$  and  $540 \text{ cm}^{-1}$  is assigned to acid phosphate contents whereas  $\text{HPO}_4^{2-}$  particle is promptly substituted *via* carbonate group. The decrease in the band area was seen at  $530 \text{ cm}^{-1}$  and  $540 \text{ cm}^{-1}$  for the KGB and when compared with the KHB sample (Table 3). Similar results were obtained from human and rabbit bone by Lisa *et al.* using infrared microspectroscopy<sup>1</sup>. As a result, in KHB sample the increase in  $\text{HPO}_4^{2-}$  ions creates an anionic vacancy which is compensated by the removal of  $\text{Ca}^{2+}$  from the lattice as it is proven from the C/P proportion. The principal  $\text{PO}_4^{3-}$   $\nu_4$  mode shows up at  $561 \text{ cm}^{-1}$  comparing to the crystalline apatite environment<sup>21</sup> with the band of  $580 \text{ cm}^{-1}$ .

Table 4 — The results of curve fitting analysis of the archaeological sample in  $850\text{-}890 \text{ cm}^{-1}$  region

Sample Compositions	KHB		KGB	
	Frequency ( $\text{Cm}^{-1}$ )	% Area	Frequency ( $\text{Cm}^{-1}$ )	% Area
Liabile $\text{CO}_3^{2-}$	865	32.63	866	26.87
B Type $\text{CO}_3^{2-}$	872	42.12	873	57.09
A Type $\text{CO}_3^{2-}$	879	20.25	879	16.04

Utilizing curve fitting examination, we assessed the relative site of carbonate substitutions for the bone sample as shown in (Fig.2B). The three sub-bands of the  $\text{CO}_3^{2-}$  are 879, 872, and  $865 \text{ cm}^{-1}$  are known as Type A (OH substitution) Type B ( $\text{PO}_3^{4-}$ ) and unstable  $\text{CO}_3^{2-}$  non-apatite carbonate band separately. The type B  $\sim 872 \text{ cm}^{-1}$  is suitable for the quantitative calculation of carbonate in the bone minerals. These changes in the molecular structure are recognized by FTIR. It is because the different ionic radii of the substitutions cause changes in the environment of the molecular group, which results in alterations to the characteristic vibration modes that are reflected in their infrared absorption spectra<sup>21</sup>. It is seen from the (Table 4) the band area of  $872 \text{ cm}^{-1}$  in KGB has a higher concentration of carbonate and smallest value as observed for KHB. The (Fig.2B) demonstrates Type A carbonate reductions and portion of Type B carbonate increments in the KGB sample. This shows the development of bone towards by substitution of OH group by  $\text{PO}_3^{4-}$  a group of hydroxyapatite of bone specimen. Additionally a weak band  $\sim 865 \text{ cm}^{-1}$  unstable  $\text{CO}_3^{2-}$  was observed in our study of the specimens.

#### Fourier self deconvoluted spectral analysis in the phosphate region

The deconvolution of the spectra in the region  $1200\text{-}900 \text{ cm}^{-1}$  reveals six components that were needed to get a satisfactory best fit for poorly crystalline apatite bone samples. (Fig. 2C). The position, intensity, and band area of each component were analysed. The results demonstrate critical distinction in the % area of the sub-bands of 960, 1010, 1020, 1030, 1110, and  $1120 \text{ cm}^{-1}$  (Table 5). The band  $\sim 960 \text{ cm}^{-1}$  corresponds to a symmetric stretching mode of phosphate ( $\text{PO}_4^{3-}$ ) consolidation. The intensive absorption band in the range of  $1040\text{-}1090 \text{ cm}^{-1}$  corresponds to the asymmetric stretching mode of  $\text{PO}_4^{3-}$ . The band  $\sim 1020 \text{ cm}^{-1}$  is connected with non-stoichiometric apatite (containing  $\text{HPO}_4^{2-}$ /or  $\text{CO}_3^{2-}$ ) and at  $\sim 1030 \text{ cm}^{-1}$

Table 5 — The results of curve fitting analysis of the archaeological sample in 900-1200  $\text{cm}^{-1}$  region

Sample Compositions	KHB		KGB	
	Frequency ( $\text{Cm}^{-1}$ )	% Area	Frequency ( $\text{Cm}^{-1}$ )	% Area
$\text{PO}_4^{3-} \nu_1$ symmetric stretch	961	8.02	961	9.13
$\text{PO}_4^{3-} \nu_3$ asymmetric stretch	1013	29.09	1012	19.06
$\text{PO}_4^{3-} \nu_3$ asymmetric stretch	1020	7.89	1022	12.11
$\text{PO}_4^{3-} \nu_3$ asymmetric stretch	1034	26.00	1033	38.29
$\text{PO}_4^{3-} \nu_3$ symmetric stretch	1108	10.73	1110	9.80
$\text{PO}_4^{3-} \nu_3$ symmetric stretch	1120	18.27	1122	11.59

to stoichiometric apatite<sup>22</sup>. Additionally the band  $\sim 1030 \text{ cm}^{-1}$  is connected with crystallinity.

The peak area ratio of 1030/1020 was measured to quantify the crystalline nature of the sample. The peak area ratio of 1030/1020 decreases with the KHB sample showing a decrease in crystalline nature. This information concurs with the finding in the KHB sample. Further the increase in the band area of the band  $\sim 1120 \text{ cm}^{-1}$  of the KHB and KGB sample prompting to development of maturation of bone, which, is supported by the increase in the mineralization index as seen from (Table 2). The increase in the band area of  $\sim 1020 \text{ cm}^{-1}$  emerges from  $\text{HPO}_4^{2-}$  substitute for the apatite lattice of a bone sample. This band decreases with KHB and increases in the KGB sample. As a result, the bone sample of KGB at higher age attains changes in mineral constitutions because of substitution of  $\text{CO}_3^{2-}$  by  $\text{HPO}_4^{2-}$  ions. This might be supported by an increment in Type B  $\text{CO}_3^{2-}$  in KGB when compared with KHB (Table 4).

#### Fourier self deconvoluted spectral analysis in the Amide region

The curve fitting was performed in the amide I band demonstrates the presence of a secondary structure of the collagen matrix. The adjustments in the collagen cross- linked peptides in the amide I region were resolved to examine the secondary structure of proteins. Amide I band (Peptide bond

Table 6 — The results of curve fitting analysis of the archaeological sample in Amide I region

Sample Compositions	KHB		KGB	
	Frequency ( $\text{Cm}^{-1}$ )	% Area	Frequency ( $\text{Cm}^{-1}$ )	% Area
$\beta$ sheet	1625	64.56	1624	39.32
$\alpha$ helices	1654	30.49	1656	43.84
$\beta$ turns	1693	4.95	1695	16.84

$\text{C}=\text{O}$  extends  $\sim 1650 \text{ cm}^{-1}$  is particularly sensitive to the secondary structure of proteins<sup>23</sup>. The (Fig. 2D) indicates FTIR spectra of the collagen framework of the archaeological specimen of the determined hidden groups in the amide I region as deduced from a curve fitting examination. The band centred  $\sim 1624 \text{ cm}^{-1}$  allocates to  $\beta$  sheet, 1660 to  $\alpha$  helix, and 1693 to  $\beta$  turns of the secondary structure of proteins. For lower mature bone (KHB) increase in  $\beta$  sheet and decrease in  $\alpha$  helix and  $\beta$  turns was observed when compared with the KGB. For  $\alpha$  helix structure, it increases as the bone matures. This is contradictory to our study that an increase in  $\beta$  sheet and  $\alpha$  helical results from diminishing in collagen development which was observed for the KHB sample (Table 6). This confirms the collagen development is because of  $\beta$  sheet arrangement of the protein structure. This might be because of the impact of the burial environment of the bone sample where structural alteration happens and this procedure is called a digenesis<sup>23</sup>. Comparable results were gotten for an archaeological specimen of animal bone where a reduction in the band area of  $\alpha$  helix and an expansion at random coils has been observed.

#### Conclusion

The change in the various functional groups of an archaeological bone sample was studied from FTIR spectra utilizing the Fourier self deconvolution technique. The computed parameters like crystalline index, collagen maturity and maturing of bone were obtained from the spectra. They were high for the sample KGB and low for the KHB sample. The phosphate band  $1022 \text{ cm}^{-1}$  accounted for a high crystalline index of the samples studied. It was in good agreement with the results of the deconvolution technique applied in the region  $900\text{-}1200 \text{ cm}^{-1}$ . The curve fitting in the Amide I region demonstrates the  $\beta$  sheet development of the secondary structure of the protein. Investigation in the  $850\text{-}890 \text{ cm}^{-1}$  demonstrates the substitution of phosphate ions by Type B carbonate indicating maturation of bone in

the studied samples. This Type B carbonate observed at  $872\text{ cm}^{-1}$  was suitable for the quantitative measurement in the bone minerals of the samples studied. It was clear from the IR spectra that both carbonate and phosphate ions have a significant impact on the pool of bone minerals. The results conclude that the various compositional changes in the archaeological bone studied might be altered by interaction with the burial environment. In this way, the utilization of FTIR spectra with self deconvolution strategies serves to describe the chemical compositions and in understanding the biochemical changes in the archaeological specimen.

### Conflict of interest

All authors declare no conflict of interest.

### Acknowledgment

The authors are grateful to the Archaeological Survey of India (ASI), Trivandrum, Kerala for providing the archaeological bone specimens. Similarly, the authors are grateful to the DST-FIST lab of the Department of Physics, Periyar University for taking the FTIR spectra.

### References

- 1 Lisa M, Miller, Vidyasagar V, Mark R, Richard M, Eleftherios P, Paschalis, Foster B & Adele L, *In situ* analysis of mineral content and crystallinity in bone using infrared microspectroscopy of the  $\nu_4\text{ PO}_4^{3-}$  vibration Boskey. *Biochimica et Biophysica Acta*, 1527 (2001) 11.
- 2 Bonel G, Contribution a l'etude de la carbonation des apatites. Part I. *Ann Chim*, 7 (1972a) 127.
- 3 Farlay D, Panczer G, Rey C, Delmas P & Boivin G, Mineral maturity and crystallinity index are distinct characteristics of bone mineral. *J Bone Miner Metab*, 28 (2010) 433.
- 4 Yamamoto T, Uchida K, Naruse K, Suto M, Urabe K, Uchiyama K, Suto K, Moriya M, Itoman M & Takaso M, Quality assessment for processed and serialized bone using raman spectroscopy. *Cell Tissue Bank*, 13 (2012) 409.
- 5 Salesse K, Dufour E, Lebon M, Wurster C, Castex D, Bruzek J & Zazzo A, Variability of bone preservation in a confined environment: the case of the catacomb of Sts peter and Marcellinus (Rome, Italy). *Palaeogeogr Palaeoclimatol Palaeoecol*, 416 (2014) 43.
- 6 Vincke D, Miller R, Stassart E, Otte M, Dardenne P, Collins M, Wilkinson K, Stewart J, Baeten V & Fernandez Pierna JA, Analysis of collagen preservation in bones recovered in archaeological contexts using NIR hyper spectral imaging. *Talanta*, 125 (2014) 181.
- 7 Boseky AL, Pleshko N, Doty SB & Mendelsohn R, Applications of Fourier Transform Infrared (FT-IR) Microscopy to the Study of Mineralization in Bone and Cartilage. *Cells Mater*, 2 (1992) 209.
- 8 Fernandes MC & Sellappan K, Elemental compositions and X ray diffraction studies of *Strobilanthes* species, *Indian J Biochem Biophys*, 56 (2019) 144.
- 9 Subashini S & Kumar KS, Physicochemical characteristics of calcium oxalate crystals in *Spinacia oleracea* L. *Indian J Biochem Biophys*, 54 (2017) 156.
- 10 Rai DV, Darbari R & Aggarwal LM, Age related changes in the elemental constituents and molecular behaviour of bone. *Indian J Biochem Biophys*, 42 (2005) 127.
- 11 Basu A, Sarkar A, Maulik U & Basak P, Three dimensional structure prediction and ligand-protein interaction study of expansion protein ATEXPA23 from *Arabidopsis thaliana* L. *Indian J Biochem Biophys*, 56 (2019) 20.
- 12 Karthikeyan S & Easwaran R, Analysis of a curve fitting model in the amide region applied to the muscle tissues of an edible fish: *Labeo rohita* fingerlings. *J Biol Phys Chem*, 13 (2013) 125.
- 13 Chadefaux C, Ho ASL, Gurlet LB & Reiche I, Cure fitting micro ATR FTIR studies of the amide I and amide II bands of Tyoe U collagen in archaeological bone materials. *e Pres Sci*, 6 (2009) 129.
- 14 Beasley MM, Bartelink EJ, Taylor L, Miller RM, Comparison of transmission FTIR, ATR and DRIFT spectra: implications for assessment of bone bio apatite diagnosis. *J Archaeol Sci*, 46 (2014) 16.
- 15 Soejoko DS, Sari YW, Dewi SU, Nurizati, Dahlan K & Atmadja DS, Composition of human bone mineral by FTIR and its relationship to the age. *J Med Phys Biophys*, 1 (2014) 1.
- 16 Gourion-Arsiquaud S, Lukashova L, Power J, Loveridge N, Reeve J & Boskey AL, Fourier transform infrared imaging of femoral neck bone: reduced heterogeneity of mineral-to-matrix and carbonate to phosphate and more variable crystallinity in treatment-naive fracture cases compared with fracture-free controls. *J Bone Miner Res*, 28 (2013) 150.
- 17 Fleet ME, The carbonate ion in hydroxyapatite: recent X-ray and infrared results. *Front Biosci (Elite Ed)*, 5 (2013) 643.
- 18 Paschalis EP, Verdelis K, Doty SB, Boskey AL, Mendelsohn R & Yamauchi M, Spectroscopic characterization of collagen cross links in bone. *J Bone Miner Res*, 16 (2001) 893.
- 19 Enzo S, Bazzoni M, Mazzarello V, Piga G, Bandieray P & Melis P, A study by thermal treatment and X-ray powder diffraction on burnt fragmented bones from tombs II, IV and IX belonging to the hypogeic necropolis of Sa Figu near Ittiri, Sassari (Sardinia, Italy). *J Archaeol Sci*, 34 (2007) 1731.
- 20 Legros R, Balmain N & Bonel G, Age related changes in mineral of rat and bovine Cortical bone. *Calcif Tissue Int*, 41 (1987) 137.
- 21 Rey CM, Shimuzu B, Collins B & Glimcher MJ, Resolution enhanced Fourier transform infrared spectroscopy study of the environment of phosphate ions in the early deposits of a solid phase of calcium phosphate in bone and enamel and their evolution with age I. Investigation in the  $\nu_3\text{ PO}_4$  domain. *Calcif Tissue Int*, 46 (1990) 384.
- 22 Rey C, Shimuzu M, Collins B & Glimcher MJ, Resolution enhanced fourier transform infrared spectroscopy study of the environment of phosphate ion in the early deposits of a solid phase of calcium phosphate in bone and enamel and their evolution with age: 2. Investigations in the  $\nu_3\text{ PO}_4$  domain. *Calcif Tissue Int*, 49 (1991) 383.
- 23 Legeros RZ, Apatites in biological systems. *Prog Cryst Growth Charact*, 4 (1981) 1.