

## Importance of FTIR Spectra Deconvolution for the Analysis of Amorphous Calcium Phosphates

This content has been downloaded from IOPscience. Please scroll down to see the full text.

2015 IOP Conf. Ser.: Mater. Sci. Eng. 77 012027

(<http://iopscience.iop.org/1757-899X/77/1/012027>)

View [the table of contents for this issue](#), or go to the [journal homepage](#) for more

Download details:

IP Address: 78.84.175.214

This content was downloaded on 10/03/2015 at 17:50

Please note that [terms and conditions apply](#).

# Importance of FTIR Spectra Deconvolution for the Analysis of Amorphous Calcium Phosphates

Agnese Brangule<sup>1,2</sup>, Karlis Agris Gross<sup>1</sup>

<sup>1</sup>Institute of Biomaterials and Biomechanics, Riga Technical University, P.Valdena 3, LV-1048,

<sup>2</sup>Riga, Latvia Riga Stradiņš University, Dzirciema 16, LV-1007, Riga, Latvia.

**Keywords:** amorphous calcium phosphate, bone, spectroscopy, FTIR-DRIFT, deconvolution

## Abstract.

This work will consider Fourier transform infrared spectroscopy – diffuse reflectance infrared reflection (FTIR-DRIFT) for collecting the spectra and deconvolution to identify changes in bonding as a means of more powerful detection. Spectra were recorded from amorphous calcium phosphate synthesized by wet precipitation, and from bone. FTIR-DRIFT was used to study the chemical environments of  $\text{PO}_4$ ,  $\text{CO}_3$  and amide. Deconvolution of spectra separated overlapping bands in the  $\nu_4\text{PO}_4$ ,  $\nu_2\text{CO}_3$ ,  $\nu_3\text{CO}_3$  and amide region allowing a more detailed analysis of changes at the atomic level. Amorphous calcium phosphate dried at 80 °C, despite showing an X-ray diffraction amorphous structure, displayed carbonate in positions resembling a carbonated hydroxyapatite. Additional peaks were designated as A1 type, A2 type or B type. Deconvolution allowed the separation of  $\text{CO}_3$  positions in bone from amide peaks. FTIR-DRIFT spectrometry in combination with deconvolution offers an advanced tool for qualitative and quantitative determination of  $\text{CO}_3$ ,  $\text{PO}_4$  and  $\text{HPO}_4$  and shows promise to measure the degree of order.

## Introduction

Amorphous calcium phosphate (ACP) plays an important role in the formation of biomaterials [1]. The diversity of possible arrangements within the apatite structure requires sensitive tools to detect changes from different synthesis conditions, especially for amorphous structures, that are even more difficult to characterize. Fourier transform infrared (FTIR) spectroscopy offers a method for monitoring changes in the chemical groups. This work will show how FTIR-DRIFT spectroscopy provides a simple, non-destructive technique to obtain yet unexplored information about amorphous calcium phosphates.

The aim of this study was to apply curve fitting deconvolution for investigating overlapping bands in the  $\nu_4\text{PO}_4$ ,  $\nu_2\text{CO}_3$ ,  $\nu_3\text{CO}_3$  band region in synthesized amorphous calcium phosphates and  $\nu_3\text{CO}_3$  and amide band in natural human bone. Deconvolution is based on J.K. Kaupinens et. al. methodology introduced in the 1980's [2] – a mathematical procedure for resolving overlapped peaks in a complex FTIR spectrum, also referred to as “resolution enhancement” without changes to the experimental spectral resolution [3].

The vibration band  $\nu_4\text{PO}_4$  was chosen in this study due to the  $\nu_4\text{PO}_4$  562  $\text{cm}^{-1}$ , 575  $\text{cm}^{-1}$  and 603  $\text{cm}^{-1}$  peak overlap with the  $\text{HPO}_4$  apatitic peak at 550  $\text{cm}^{-1}$  [4]. Chemical determination of  $\text{HPO}_4^{2-}$  ions by colorimetry cannot be distinguished from  $\text{PO}_4^{3-}$ . Another method by Gee and Dietz [5] that condenses  $\text{HPO}_4^{2-}$  ions into pyrophosphates  $\text{P}_2\text{O}_4^{4-}$  cannot be used for powders containing carbonate  $\text{CO}_3^{2-}$  ions. Carbonate ions interfere with  $\text{P}_2\text{O}_4^{4-}$  ions and partially prevent  $\text{P}_2\text{O}_4^{4-}$  ion formation [6]. Consequently, this spectral method is thus the only viable technique for determining the  $\text{HPO}_4^{2-}$  content in carbonated ACP.



Carbonate ion concentration can be determined by heating ACP to 1200 °C to liberate CO<sub>2</sub> for collection in an absorption cell. Carbonates can then be determined by calorimetric titration [7]. This analysis method only gives information on the quantity of carbonates. Deconvolution of  $\nu_2\text{CO}_3$  and  $\nu_3\text{CO}_3$  bands in FTIR spectra gives information about the apatite structure. Le Geros observed and interpreted IR peak splitting for the CO<sub>3</sub> to represent A and B type carbonate substitution models – representing OH and PO<sub>4</sub> replacement respectively [8]. Some researchers also reported A1 type, A2 type and B type substitution doublets [9-11] or A-B type apatites, and “nonapatitic” or “labile” CO<sub>3</sub><sup>2-</sup>, PO<sub>4</sub><sup>3-</sup> and HPO<sub>4</sub><sup>2-</sup> [12,13].

Human bone consists mainly of collagen fibers and inorganic compounds, which can be approximated as carbonate containing hydroxylapatite [14,15]. Deconvolution of FTIR spectra will be shown to give information about overlapped amide I, amide II and amide III peaks in collagen, and inorganic  $\nu_3\text{CO}_3$  bands in the 900 – 1900 cm<sup>-1</sup> region [15].

## Materials and Methods

### Materials

Three different carbonate containing ACPs were synthesized at room temperature and dried for FTIR analysis. The first two powders were prepared by mixing a solution containing Ca(NO<sub>3</sub>)<sub>2</sub> and 30 % ammonia with another solution consisting of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> and (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>. Drying of these first two wet powders was conducted in air at 20 °C for 72h, or in a convection oven at 80 °C for 24h. The third ACP was a Zn enriched ACP was prepared by mixing the solution (Ca(NO<sub>3</sub>)<sub>2</sub>, Zn(NO<sub>3</sub>)<sub>2</sub> and 30 % ammonia) with (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> and (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> solution followed by freeze-drying at -50 °C for 48 h.

Bone was sourced from human vertebra - stored at room temperature in air for 40 years.

### Methods

**Fourier transform infrared spectroscopy – diffuse reflectance infrared reflection.** The functional group changes were determined by FTIR–DRIFT (PerkinElmer Spectrum One,) at 450–4000 cm<sup>-1</sup>, at a resolution of 4 cm<sup>-1</sup>, with an average of 8 scans, sampled by diamond sampling sticks. A special preparation method was not required for synthesized powders. Powder for bone analysis was obtained from the inside of bone vertebra.

Table 1. Domains, assignments and literature for nanocrystalline apatite

Domain, Assignments	IR (cm <sup>-1</sup> )	Reference
HPO <sub>4</sub> apatitic	551	12
$\nu_4\text{PO}_4$	562, 575 and 603	12
PO <sub>4</sub> non-apatitic	617	12
non-apatitic	866	12
$\nu_2\text{CO}_3$ type B	871	12
$\nu_2\text{CO}_3$ type A	880	12
$\nu_2\text{CO}_3$ type B	1460-1470	12
$\nu_2\text{CO}_3$ type B doublet	~1455 and ~1410	11
non-apatitic	1500	12
type A	1540	12
$\nu_2\text{CO}_3$ type A1 doublet	~1540 and ~1455	11
$\nu_2\text{CO}_3$ type A2 doublet	~1565 and ~1505	11
B + non-apatitic	1420	12
Amide I	~1660 – 1690	15
Amide II	~1500 – 1600	15
Amide III	~1242	15

X-ray powder diffraction (XRD). Powders were characterized by X-ray diffraction (Bruker D8 ADVANCE diffractometer). Diffraction patterns were recorded from  $5^\circ$  to  $60^\circ$  using Cu K radiation ( $\lambda = 1.54 \text{ \AA}$  generated at 40 mA and 40 kV) at a step size of  $0.2^\circ$ . Analysis. The FTIR spectra were viewed and smoothed with freeware software Specwin32. Baseline correction and curve-fitting analysis was performed using MagicPlotStudent software with wavenumbers from literature as a guide for  $\nu_4\text{PO}_4$ ,  $\nu_2\text{CO}_3$ ,  $\nu_3\text{CO}_3$ , amide I, amide II, amide III groups (Table 1). Deconvolution involved both Lorentzian and Gaussian curve fitting.

## Results and Discussions

All synthesized ACPs displayed an X-ray amorphous structure - a broad low intensity peak centred at  $30^\circ$ , Fig 1. All three patterns appear similar.

FTIR-DRIFT spectra of three ACPs are shown in Fig 2. Phosphate bands ( $\nu_1$ ,  $\nu_2$  and  $\nu_4\text{PO}_4$ ) and carbonate bands ( $\nu_2$ ,  $\nu_3\text{CO}_3$ ) were detected. By using information from the literature (Table 1) we can infer that  $\text{PO}_4$  bands overlap  $\text{HPO}_4$  bands. Only small shifts in the IR spectra suggest a change in the chemical group placement.

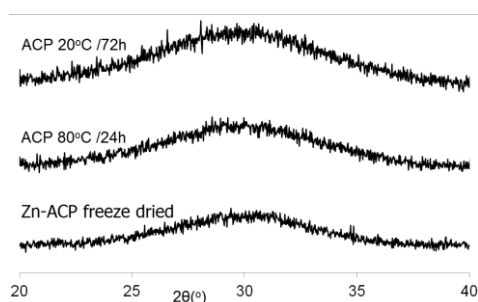


Fig 1. XRD patterns of air dried ACP,  $80^\circ\text{C}$  dried ACP, freeze-dried Zn-ACP.

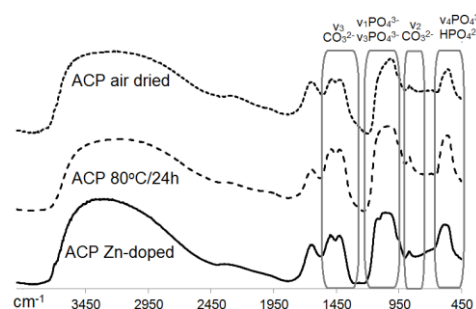


Fig 2. FTIR-DRIFT spectra of air dried ACP,  $80^\circ\text{C}$  dried ACP, and freeze-dried Zn-ACP

Deconvolution of the  $500\text{--}700 \text{ cm}^{-1}$  region of all three FTIR spectra revealed the presence of  $\text{HPO}_4^{2-}$  ( $550 \text{ cm}^{-1}$ ) only in Zn-doped ACP (Fig 3).

Spectra obtained in the  $\nu_2\text{CO}_3$  band region ( $800\text{--}920 \text{ cm}^{-1}$ ) show a considerable change in peak shape and position. A shoulder at  $\sim 880 \text{ cm}^{-1}$  was observed for the  $80^\circ\text{C}$  dried powder, that was not present for the air dried powder. Using peak assignments from the literature, we can infer the presence of an A type substituted carbonate group. More detailed information on the structure of apatites can be obtained after deconvolution of the spectra. Deconvolution of the  $800\text{--}920 \text{ cm}^{-1}$  region displays only non-apatitic carbonate in air-

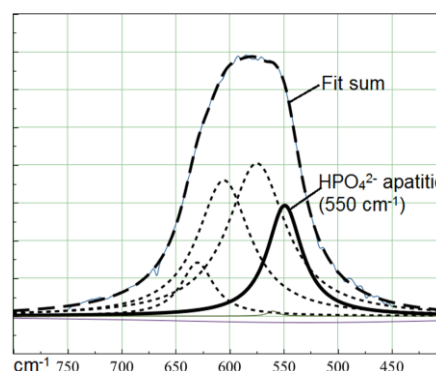


Fig 3. Deconvolution of Zn doped ACP showing  $\nu_4\text{PO}_4^{3-}$  and  $\text{HPO}_4^{2-}$  band ( $450\text{--}700 \text{ cm}^{-1}$ )

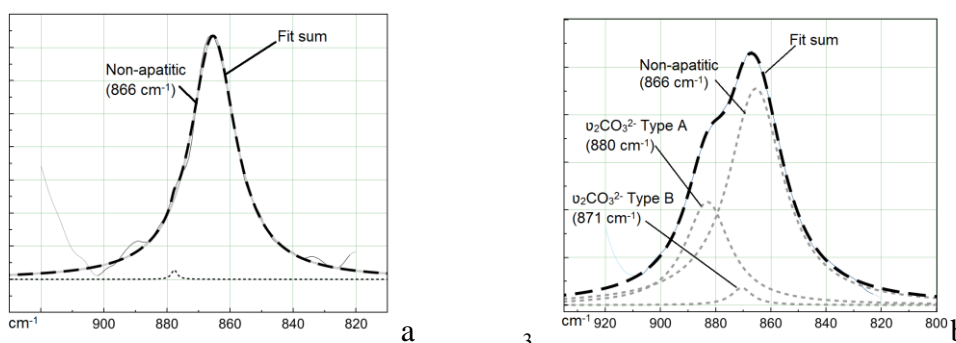


Fig 4. Deconvolution of band ( $800\text{--}920 \text{ cm}^{-1}$ ) showing the  $\nu_2\text{CO}_3^{2-}$  in a) air dried ACP and b)  $80^\circ\text{C}$  dried ACP

dried ACP but additional A-type and B-type carbonate for 80 °C dried powders (Fig 4a and b). The carbonate type can be changed by drying at different conditions [16]. Air-dried powder mainly contained non-apatitic  $\text{CO}_3^{2-}$ , but drying at 80 °C produced more A-type and B-type carbonates as shown by peaks at 880  $\text{cm}^{-1}$  and 871  $\text{cm}^{-1}$ , respectively.

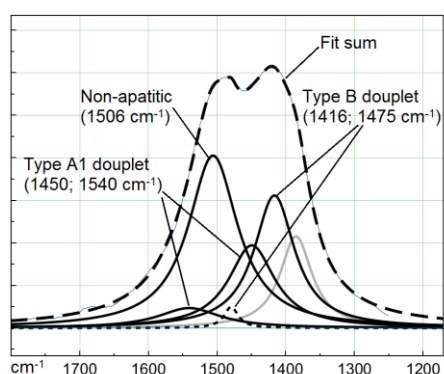


Fig 5. Deconvolution of  $\nu_3\text{CO}_3^{2-}$  band (1200—1800  $\text{cm}^{-1}$ ) for 80 °C dried ACP

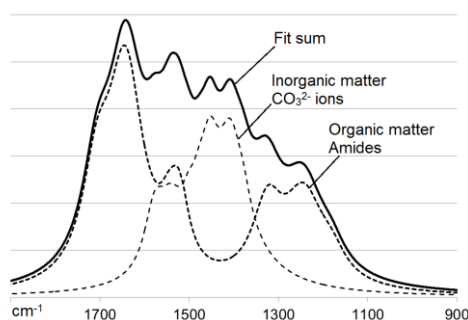


Fig 6. Deconvolution of human bone showing convoluted organic amide and inorganic carbonate bands (900 —1900  $\text{cm}^{-1}$ )

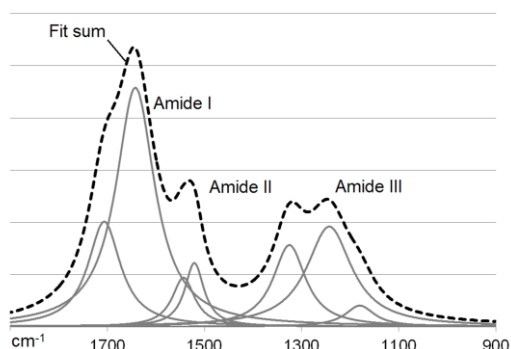


Fig 7. Deconvolution of human bone showing deconvoluted and convoluted amide band

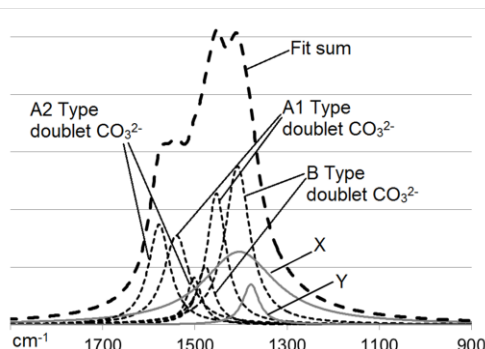


Fig 8. Deconvolution of human bone showing deconvoluted and convoluted carbonate band

## Conclusions

Deconvolution of FTIR spectra offers a more detailed qualitative and quantitative analysis of carbonate ("non-apatitic" vs "apatitic") and orthophosphates ( $\text{HPO}_4^{2-}$  and  $\text{PO}_4^{3-}$ ). The carbonate band ( $1400 - 1600 \text{ cm}^{-1}$ ) for the air dried powder shows non-apatitic positions, but drying at  $80^\circ\text{C}$  showed peaks that coincided with crystalline carbonated apatite.

FTIR-DRIFT provides a sensitive, non-destructive tool for the analysis of bone, and with deconvolution can distinguish the inorganic carbonate bands from the organic amide bands.

Deconvolution of ACP spectra provides a new ability that may be used to track the transition of the amorphous phase to a crystalline apatite in bones as well as providing a tool to establish the influence of processing and drying conditions on the state of the amorphous calcium phosphate. The change in the bonding marks the first change from an amorphous to a crystalline state and so this tool will be able to bridge the gap in understanding between the amorphous state and nanocrystalline states.

**Acknowledgements.** A special thanks to M. Pilmane from the Riga Stradins University of Latvia, for providing the vertebra bone for FTIR analysis.

## References

- [1] LeGeros R Z In Brown P W 1994 Constantz B (Eds.) Hydroxyapatite and Related Materials 3-28
- [2] Kauppinen J K *et al* 1981 *Appl. Spectrosc.* **35** 271–76
- [3] Barth A Harris P 2009 Infrared spectroscopy – Past and Present. In: Biological and Biomedical Infrared Spectroscopy 1–52
- [4] Rey C 1990 *et al Calcif. Tissue Int.* **46** 384-94
- [5] Gee A Dietz V R 1953 *Ann. Chem.* **25** 1320-1324
- [6] Elliott J C 1994 Structure and Chemistry of the Apatites and Other Calcium Phosphates
- [7] Markovic M *et al* 2004 *J. Res. Natl. Inst. Stand. Technol.* **109** 553-568
- [8] LeGeros R Z *et al* 1969 **25** 5-7
- [9] Elliott J C *et al* 2002 *Adv. X-Ray Anal.* **45** 172-181
- [10] Wopenka B Pasteris J D 2005 *Materials Science and Engineering: C* **25** 131–143
- [11] Fleet M E *et al* 2004 *Am. Mineral.* **89** 1422-1432
- [12] Eichert D *et al* 2007 J.B. Kendall (Eds.), Nova Science Publishers 93-145
- [13] Rey C *et al* 1989 *Calcif. Tissue Int.* **21** 267-273
- [14] Farre B *et al* 2014 *J. Afr. Earth Sci.* **92** 1-13
- [15] Figueiredo M M *et al* 2013 *J. Struct Biol* **181** 207–222 doi:10.1016/j.jsb.2012.12.005.
- [16] Brangule A Gross K A 2015 *Key Eng. Mat.* **631** 99-103, doi:10.4028/www.scientific.net/KEM.631.99