

CRYSTALLINE PHASES IDENTIFICATION IN BIOMATERIALS

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Abstract. Brazilian granulated bone grafting products derive from bovine bone structure, were analyzed by WDXRF, LOI, DRX and FTIR techniques. The major crystalline phases appears like perfect hydroxyapatite and that one with less calcium. Some others calcium and phosphorous compounds appear in significant percentages that vary from product to product, what can makes the grafting functional differences of each other.

Introduction.

Focusing to avoid taking grafting material from donor areas of the patient, biomaterials have been used in the most of surgical cases where tissue reposition or replacing it is necessary. These materials were defined in 1986 by European Society as “any substance, drug or their combinations, synthetic or natural, to be used as tissue or function reposition in the body by a period or definitely (Gomes, 2002). Regarding the origin, the biomaterials can be classified as:

HOMOGENOUS – obtained from a donor of similar kind to the receptor. Theses materials are those ones more similar to the autogenous grafting materials (Lynch et al., 1999; Okamoto et al., 1994).

HETEROGENOUS OR XENOGENOUS – obtained from a donor of different kind to the receptor (Fonseca et al., 1997).

ALOPLASTIC – synthetic materials identified as metal, ceramic or plastic and usually named “implant”. Their most important function is to fill tissue defects without physiological incorporation (Okamoto et al., 1994).

The most used heterogeneous grafting material, product commercially available in the market in granulated configuration, by the surgeons in bone reconstruction procedures is the non organic or mineral bovine bone matrix. This biomaterial has been exhaustively investigated during the last two decades with good evidences of bio-compatibility and bio-efficiency as an osteo-conductor material (Jensen et al., 2006).

The mineral portion of the bone tissue is basically constituted of HYDROXYAPATITE $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, but minor crystalline phases as CaCO_3 , $\text{Ca}_8 \text{H}_2 (\text{P O}_4)_6$, $\text{Ca}_3 (\text{P O}_4)_2$, etc... appears in the bone structure. These compounds are important to the nutrient diffusion, cellular migration, repairing and remodeling processes (LeGeros, 2002).

Some authors warn about the contamination possibility of bovine contagious diseases to the human been by the bone grafting material (Sogal & Tofe, 1999). But, Wenz et al.



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(2001) in a review, discussed the blocking up mechanism to the prion, responsible agent of the bovine spongyform encephalopathy already knew as Creutzfeldt-Jakob disease, and concluded that the physical-chemistry processing of the biomaterial eliminates the risk of this disease transmission. Indeed, the biomaterial manufacturing process can modify the original bone structure and consequently affecting its bio-functionality.

The present study selected three mineral bovine bone matrix products commercially offered in the Brazilian domestic market, and by wavelength dispersive x-ray fluorescence (WDXRF), loss of ignition (LOI), X-ray powder diffraction (XRD) with Rietveld refinement and Fourier transform spectroscopy (FTIR) techniques shows the resulting structure derived from the production procedure of each one. In this way, taking as references, bovine bone tissues in natural state and ash condition were analyzed in the same protocol that one applied to the commercial products.

Materials and Methods.

Five samples were analyzed by WDXRF and XRD/Rietveld as follows:

- Bovine bone as natural - TOM;
- Bovine bone as thermally treated at 600 °C - TOC;
- Commercial bovine bone Graft - A;
- Commercial bovine bone Graft - B;
- Commercial bovine bone Graft - C.

The used equipments and parameters to analyze the samples are listed in the Table I.

Table I. Data of the used analytical techniques.

METHOD	EQUIPMENT	PARAMETERS
WDXRF	WD RIGAKU-RIX3000	Ca/P by Fundamental parameters (Bone ash standard NIST-1400)
LOI	MUFFLED FURNACE	950 °C
XRD	RIGAKU DENKI MULTIFLEX	Cu K α radiation, graphite monocromator, step 0.02 ^o and 6 sec/step.
FTIR	NICOLET NEXUS 670	KBr Pellets 3:1

The XRD data refinement by Rietveld technique (Rietveld, 1969) was done by the software GSAS (General Structure Analysis System) that is a comprehensive system for the refinement of structural models to both x-ray and neutron diffraction data created by Allen C. Larson and Robert B. Von Dreele of Los Alamos National Laboratory.

Results and Discussion.

The chemical analysis of the samples by WDXRF technique is presented in the Table II.



Table II. Chemical composition of the samples.

Elem./Comp.(%)	A*	B*	C*	TOM**	TOC***
Ca	41,8±0,5	55,3±0,5	60,4±0,5	55,3±0,5	55,6±0,5
P	16,5±0,5	20,0±0,5	14,1±0,5	20,9±0,5	20,0±0,5
O	39,9±0,5	23,0±0,5	23,5±0,5	22,9±0,5	22,3±0,5
Mg	0,65±0,05	0,67±0,05	0,91±0,05	-----	0,74±0,05
Na	0,52±0,05	0,49±0,05	0,80±0,05	0,68±0,05	0,64±0,05
Si	-----	0,25±0,05	-----	-----	-----
S	0,42±0,05	0,13±0,05	0,07±0,05	0,11±0,05	0,10±0,05
Fe	0,09±0,02	0,12±0,05	0,13±0,03	0,08±0,02	0,12±0,05
Sr	0,03±0,01	0,03±0,01	0,03±0,01	0,03±0,01	0,04±0,02
Cl	-----	-----	-----	-----	0,10±0,05
K	-----	-----	-----	-----	0,04±0,02
Zn	-----	-----	-----	-----	0,03±0,01

* Commercial product

** Mineralized bone tissue

*** Bone ash

The values of the samples obtained by WDXRF in its former condition, do not show carbon and hydrogen contents. So, to verify the correct Ca/P rate, it is necessary to burn all samples in LOI procedure firstly.

The values obtained by LOI, and the final Ca/P ratio are showed in the Table III.

Table III. LOI and Ca/P ratio of the samples.

SAMPLE	Ca (%)	P (%)	Ca/P RATIO	LOI (%)
A	25,4	11,8	2,2	35,2
B	28,4	13,1	2,2	28,5
C	39,3	18,1	2,2	1,2
TOM	26,3	14,3	1,8	29,6
TOC	41,6	16,8	2,5	1,0
BONE ASH NIST 1400 (Determined)				
NIST Standard	38,1	18,6	2,0	1,4
BONE ASH NIST 1400 (Certified)				
NIST Standard	38,2	17,9	2,1	1,2

All the commercial products show the same Ca/P ratio, but the obtained low mass loss percentage to the product C, comparing to bone ash, it suggest be thermally treated in its process production.

The XRD refinements, by Rietveld technique (Rietveld, 1969) using the software GSAS (General Structure Analysis System), was conducted utilizing $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ 'P 63/m' symmetry, number ICSD 99358. The quality of the refinement χ^2 and the mean crystallite size obtained for the samples is presented in Table IV. The x-ray diffraction refinements pattern presented in Figure 1, shows different degree of crystallization, where the similarity between product C and TOC patterns and mean crystallite size suggest both were treated in the same way, i. e., thermally treated.



Table IV- The mean crystallite size and the refinement χ^2 obtained for the samples.

Sample	Mean crystallite size	χ^2
A	7,4 nm	1.4
B	10,2 nm	3.0
C	111,8 nm	2.7
TOM	10,9 nm	2.6
TOC	131,6 nm	3.0

The product C has a mean crystallite size up to 10 times of the mean crystallite size of products A and B. If the inverse relationship crystallite size: absorption rate in the body is valid, the product C is the less resorbable product comparing with A and B.

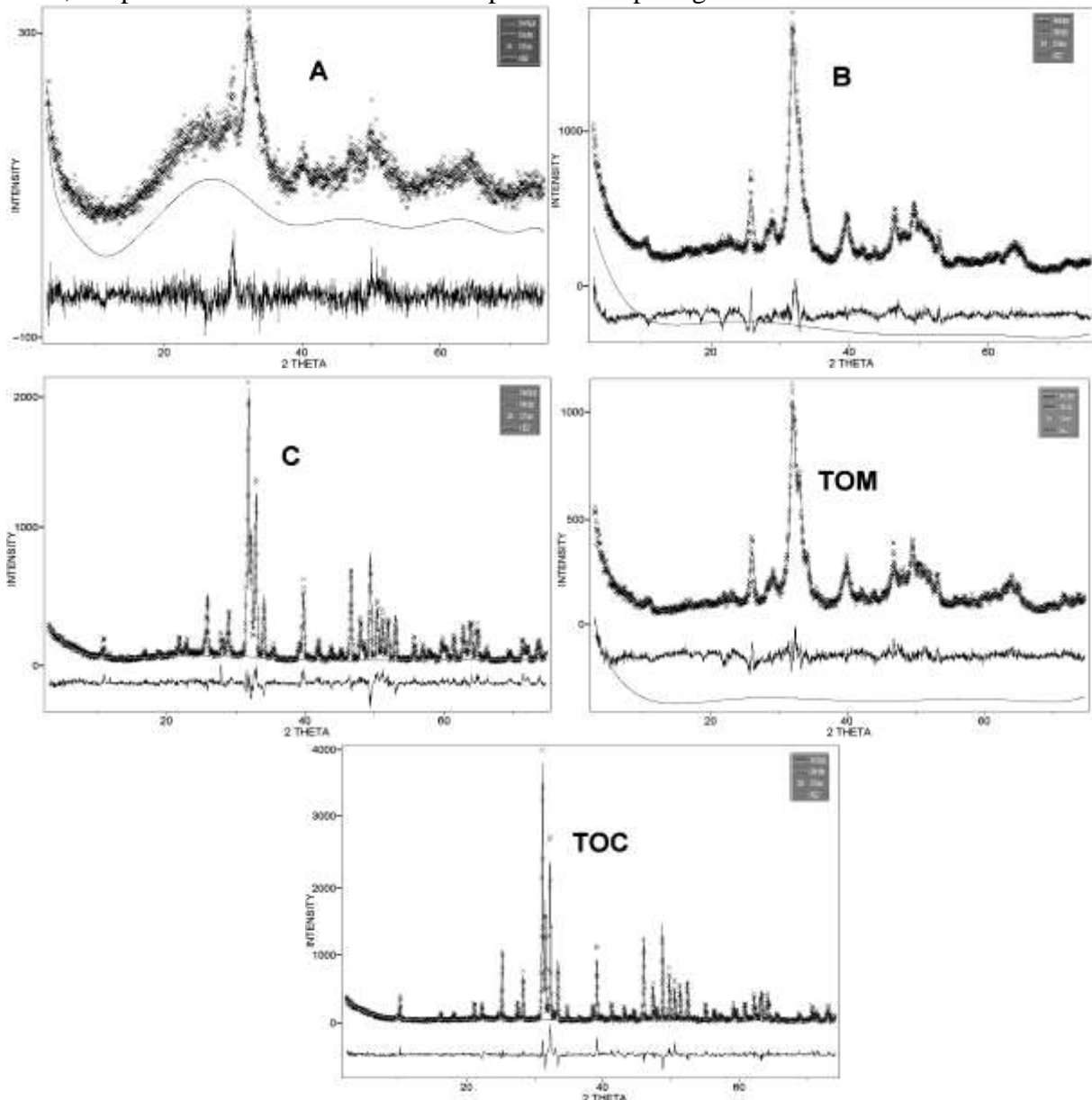


Fig. 1. Rietveld refinements of the samples.



The FTIR spectra show the analyzed samples have different profiles and vary in their composition, except to the hydroxyapatite that is a constant and majority phase for all samples. All the hydroxyapatite peaks are according to the literature (Santos et al., 2005; el Feki et al., 1991; Liu et al., 2007; Li et al., 2002; Kong et al., 2004; Silva et al., 2003; Wang et al., 2007). Some peaks in all samples were identified as referred to calcium defective apatite (Siddharthan et al., 2005; Nair et al., 2006). Specific papers about FTIR in bones (Paschalis et al., 2003; Boskey et al., 2003; Magne et al., 2001) also confirm the massive presence of Hydroxyapatite but presences like carbonated apatite and collagen occurs as well. The samples A, B and TOM showed these secondary compounds in their spectra. The sample C suggests be burned to ash due to lack of carbon peaks in its FTIR spectrum. Figure 2 shows the FTIR spectrum for each analyzed sample.

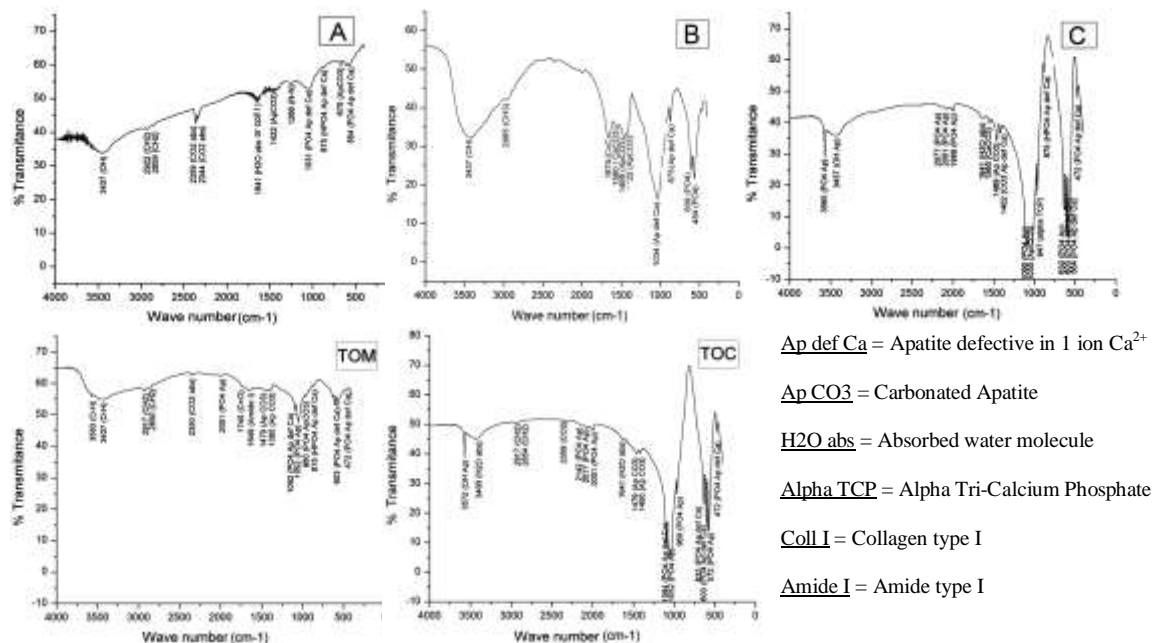


Fig. 2. FTIR spectra of the analyzed samples.

Conclusions.

By the analytical information obtained in this study, it is possible conclude the following considerations:

1. All products have the chemical and crystalline characters defining similarity in its basic composition;
2. The presence of thermal processing is strongly suggested occurs in the product C, and obviously in the TOC sample, when analyzed by LOI, XRD and FTIR methods;
3. All commercial products have the same Ca/P ration suggesting the major phase be hydroxyapatite, but not the only, in its composition;



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4. The product C has the major mean crystallite size and suggest be the less resorbable of all.

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