

PHYSICOCHEMICAL CHARACTERIZATION OF SIX COMMERCIAL HYDROXYAPATITES FOR MEDICAL-DENTAL APPLICATIONS AS BONE GRAFT

CARACTERIZAÇÃO FÍSICO-QUÍMICA DE SEIS HIDROXIAPATITAS COMERCIAIS PARA APLICAÇÕES MÉDICO-ODONTOLÓGICAS COMO MATERIAL DE ENXERTO

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ABSTRACT

The aim of this work was to characterize six hydroxyapatites in granular form for applications in medicine and dentistry as bone graft and to compare with manufacturers' specification. These samples were produced by four different manufacturers, and all of them are easily available in the Brazilian market. Physicochemical characterization was carried out by using electron microscopy (SEM), X-ray diffraction (XRD), infrared spectroscopy (FTIR) and surface area (BET) measurements. The results show that just one hydroxyapatite sample exhibited characteristics in perfect agreement with the manufacturers' specifications. The studied parameters, namely crystallinity, surface area and composition may be used to estimate hydroxyapatite's biodegradability and as a criterion for quality control.

Uniterms: Hydroxyapatite; Calcium phosphate; Bone graft; Physicochemical characterization.

RESUMO

O objetivo desse trabalho foi caracterizar seis hidroxiapatitas granulares para aplicação em área médico-odontológica como material de enxerto. Estes materiais foram produzidos por quatro diferentes fabricantes e são todos facilmente disponíveis no mercado brasileiro. A caracterização físico-química foi realizada por meio de microscopia eletrônica de varredura, difração de raios-X, espectroscopia de infravermelho e área superficial específica (BET). Os resultados mostraram que apenas um dos produtos teve a caracterização perfeitamente de acordo com a especificação do fabricante. Os parâmetros de cristalinidade, área superficial e composição podem ser usados para estimar a biodegradabilidade da hidroxiapatita e como critério de controle de qualidade desses materiais.

Unitermos: Hidroxiapatita; Fosfato de cálcio; Enxerto ósseo; Caracterização físico-química.

INTRODUCTION

The use of biomaterials for hard tissue replacement has grown as a consequence of the increase in reconstructive surgeries that require, in most cases, adjuvant grafting⁸. Several applications of bone graft materials in medicine and dentistry, such as bone repair, augmentation and substitution can be highlighted¹¹. According to data from the Brazilian

Ministry of Health, the number of surgical procedures using hydroxyapatites as bone graft has more than doubled between 2002 and 2003. Bone graft may be obtained from different origins: autogenous (from the same individual, highly osteogenic), allogeneic graft (banked freeze-dried bones of human cadavers), xenogenous (usually bovine bone-derived) or synthetic calcium phosphate materials^{11,13}. Autografts are considered the "gold standard" for bone repair

and substitutions¹², but they could only be taken in limited amounts. On the other hand, allografts and xenografts are sometimes avoided due to the potential transmission of infectious diseases¹⁵.

Calcium phosphate (Ca-P) materials can be found in nature (coralline hydroxyapatite) or synthesized by precipitation methods using chemical reagents¹. Hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ - HA, is the most well known and studied calcium phosphate. In medical and dental fields, the term "hydroxyapatite" is sometimes used to describe any calcium phosphate material. Synthetic Ca-P grafts may be the chosen material, especially when large defects need to be filled. It is generally accepted that these bioceramics are only osteoconductive (have the ability to support tissue ingrowth and bone formation) and non-osteoinductive, which means ability to form bone when implanted in non-osseous sites³. In several cases, synthetic materials can be mixed with autogenous bone in order to increase the osteogenic behavior.

The bone graft material may be either resorbable or non-resorbable, with this classification being related to the extent of dissolution of Ca-P materials. The factors affecting the dissolution properties were similar to those affecting biodegradation or bioresorption^{9,11}. According to Ducheyne and Qiu⁸, the larger the solubility rate of the ceramic, the more pronounced the enhancement effect of bone tissue growth. Among several factors, chemical composition, particles size and crystallinity are likely to affect the ceramics solubility, which may be adjusted for the desired purpose. Different applications require materials with different resorption rates, which can be regulated by the mixture of several calcium phosphate phases.

The extent of calcium phosphate ceramics (CPC) dissolution in acidic buffer increases from crystalline hydroxyapatite to amorphous apatite, with tricalcium phosphate exhibiting medium solubility¹². Magnesium or carbonate substitutions were shown to cause reduction in crystal size and an increase of dissolution of synthetic apatites¹². For the same composition, the resorption increases with the decrease in crystallinity and with the increase in surface area⁵. Crystallinity is highly dependent on sintering temperature: the higher the sintering temperature, the more perfect the crystal and thus the lower the degradation rate. Resorbable calcium phosphate materials are usually unsintered Ca-P materials.

Changes in composition and crystallinity of calcium phosphate-based materials from one manufacturer to another or even from different batches of the same manufacturer were observed. These variations can be a consequence of lack of control in the manufacturing process and directly affect the clinical result, becoming a limiting factor for the use of this material¹¹.

The purpose of this study was to determine the physicochemical properties of six commercial hydroxyapatite granules available in the Brazilian market, comparing the results obtained with information from manufacturers' specifications.

MATERIALS AND METHODS

A total of six hydroxyapatite samples in granular form were used in this study: two were imported (HA-4 and HA-6), although they are easily available in the Brazilian market, and four of them were made in Brazil. Concerning the degradation rate, four samples were indicated by the producer as resorbable materials (from HA-3 to HA-6) and the other two – HA-1 and HA-2 – as non-resorbable. According to the products' specifications, the hydroxyapatite granules were either of bovine origin (named "natural") – HA-5 and HA-6) or by using synthetic routes (HA-1, HA-2, HA-3 and HA-4). Information following packing was quite variable. The manufacturers' names were not disclosed to protect their identities.

The granules were bought from or, alternatively, donated by manufacturers and characterized without further treatment. The following characteristics were determined: granules morphology, size range and surface area. The presence of other phases besides hydroxyapatite, crystallinity and chemical groups were also investigated. Size range was determined by vibratory sieving using 75, 125, 250, 350, 420 and 600mm aperture screen size (ABNT). Scanning electron microscopy (SEM - ZEISS, DSM 940A model) was used to investigate granules morphology and granules porosity. The specific surface area of each hydroxyapatite was determined by BET analysis which estimates of surface area by nitrogen adsorption at 77K⁷.

X-ray diffraction (XRD) allows the identification of other crystalline phases besides hydroxyapatite and the unit cell parameters. Crystallinity was determined by the methodology used by Landi, et al.¹⁰, which results in the following formula:

$$Xc = 1 - \left(\frac{V_{112/300}}{I_{300}} \right) \times 100,$$

where Xc = the sample crystallinity in %, I_{300} is the intensity of (300) reflection and $V_{112/300}$ is the intensity of the hollow between (112) and (300) reflections. XRD analysis was carried out in a Rigaku MINIFLEX XRD operating at 30 kV, 15mA and CuK α radiation. Data was obtained in the range of 5-100 $^\circ$ 2 θ .

Fourier-transform infrared spectroscopy (FTIR, ABB Bomem Inc., MB series, Quebec, Canada) equipped with reflectance attachment was also used in order to confirm XRD results and to detect some groups, such as CO_3^{2-} . The spectra were collected at room temperature at a nominal resolution of 4.00 and number of sample scans equal to 1000. The FTIR spectra were recorded in the 400 – 4000 cm^{-1} range using specular reflection.

One gram of each granule was randomly separated and crushed into a fine powder for XRD and FTIR analysis. All procedures were conducted in duplicates and run in separated experiments.

RESULTS

Table 1 shows the particle size obtained and the range size indicated in the manufacturer's specifications. Despite some error associated with the methodology used, the only sample that clearly is in accordance with these specifications

was sample HA-6. Figure 1 shows SEM image (50x magnification) and the BET specific surface area for each condition. It is possible to observe that sample HA-3 exhibits the smallest particle size, which corresponds to a high surface area (49.6 m²/g) while sample HA-6 exhibits large particles (250 - > 600 nm) but also large pores. Thus, the highest specific surface area of HA-6 (84.5 m²/g) is probably a consequence of the porosity observed on these granules, which is compatible with the bovine bone origin. Samples HA-3 and HA-4 exhibited intermediate values of surface area, and, for samples HA-1, HA-2 and HA-5, the BET specific surface areas were relatively low.

Figure 2 shows the XRD pattern (displayed range in 20-60°) of all samples. Only hydroxyapatite was identified (9-432

TABLE 1- Comparison between particle size determined by sieving and the manufacturers' specifications. HA-samples in granular form

SAMPLE	Particle size (µm)	
	Sieved	Manufacturer data
HA-1	< 75 - 600	250-450
HA-2	250 - > 600	500-1000
HA-3	<75 - 125	250-1000
HA-4	< 75 - 420	300-400
HA-5	< 75 - 600	250-450
HA-6	250 - > 600	250-1000

JCPDS card) with the three most intense peaks corresponding to (211), (300) and (002) planes, respectively. The crystallinity percentage was also indicated in Figure 2 and shows that HA-1, HA-2 and HA-5 have highest, HA-3 intermediate and HA-4 and HA-6 the lowest crystallinity.

Figure 3 shows the FTIR spectra of the samples examined. The bands at 640 and 3575 cm⁻¹ are characteristic OH-absorption bands of HA. The band at 1650 cm⁻¹ was assigned to adsorbed H₂O. The absorption bands at 1092, 1044, 1036, 960, 602, 573, and 475 cm⁻¹ were assigned to the vibration in the PO₄³⁻ group of the hydroxyapatite (HA). The C-O vibration in the CO₃²⁻ group was located in the 1414-1545 cm⁻¹ range and at 875 cm⁻¹, corresponding to A-type and B-type of carbonated apatite, respectively, and were largely variable from one sample to another. The triplet around 2000 cm⁻¹ was not identified. All granules examined show a composition compatible with that of hydroxyapatite containing variable CO₃²⁻ substitution. The samples were assumed to have high, medium or low CO₃²⁻ substitution, based on the comparison of the relative intensity of CO₃²⁻ bands to the phosphate band. For example, HA-1, HA-5 and HA-6 were classified as high CO₃²⁻ content, which may improve "in vivo" degradation. HA-3 and HA-4 were considered as containing intermediate CO₃²⁻ content, while carbonate substitution was almost absent on sample HA-2.

All granules that have exhibited non-expected results were re-characterized in order to confirm previous results. In the case of samples HA-1 and HA-5, new granules were bought and all methodology was again applied. No discrepancies were observed between samples from different batches.

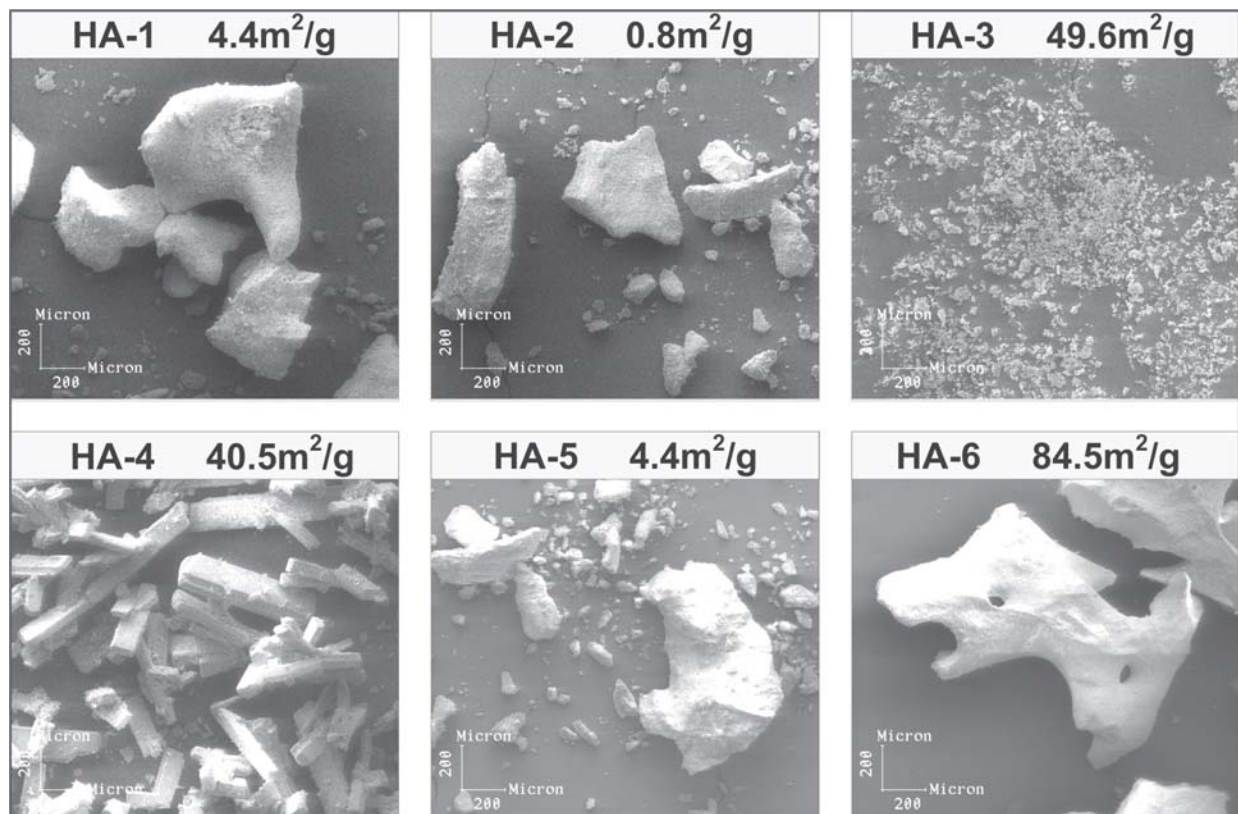


FIGURE 1- Scanning electron micrography (50x magnification) and the BET specific surface area for each hydroxyapatite examined

DISCUSSION

Synthetic and natural calcium phosphate-based materials may be a suitable alternative for the use of autogenous graft. However, the use of these materials is sometimes avoided due to lack of information or to contradiction between manufacturers' specifications (clinical indication) and clinical results. The results showed that the hydroxyapatite examined had variable physicochemical properties, which is in disagreement with the manufacturers' specifications. This discrepancy may affect the materials' performance.

Properties such as solubility and surface reactivity are highly dependent on the calcium phosphate composition and surface texture. Such characteristics will strongly affect the nature of the biologically equivalent (carbonated) apatite formed when calcium phosphates enter in contact with bone tissue⁸. In fact, the low efficiency of calcined hydroxyapatite granules in producing bone-like apatite was attributed to the slow dissolution capacity of this sample, probably related to the small quantity of tricalcium phosphate (TCP) produced during heat treatment¹⁴. With the aim of increasing degradability in biological fluids, some authors use biphasic

(HA + TCP) instead of pure hydroxyapatite¹².

Particle size and size range are very important, as they directly affect the surface area available to react with cells and biological fluid. Ducheyne and Qiu⁸ pointed out the relevance of a straight distribution in order to allow an adequate vascularization. If the distribution is large, the smaller particles tend to obstruct the spaces among the larger particles, thus reducing vascularization. Conversely, if the distribution is kept narrow, HA resorbability will increase with reducing particle size, i.e., with the increase in specific surface area. This is the case of HA-3 when compared with HA-1. Both are synthetic in origin, and the resorbability attributed by the manufacturer of HA-3 may be a consequence of the higher surface area value. Conz⁷ (2003) analyzed the particle size distribution determined by laser light scattering (LLS), showing that HA-1 and HA-5 exhibited the larger distribution, when compared with the other examined hydroxyapatites. HA-1 granules were in the range of 16-450nm and HA-5 in the range of 0.5-204nm.

Another mean to increase resorbability by the increase in the surface area is to produce porous granules. According to several authors^{2,6,13,16,17}, pore diameter should be higher than 100µm for the continued bone ingrowth. Furthermore, the quantity of new bone formed is directly proportional to pore size⁴. Porous granules may be artificially made by convenient processing of synthetic HA¹⁴ or may be consequence of the use of cancellous medullar bovine bone.

Considering that all samples examined were composed only of hydroxyapatite and the tendency of parameters surface area, crystallinity, and carbonate substitution in affecting biodegradability, these three parameters can be used to estimate the degradation behavior to make a comparison with manufacturers' indications. Table 2 summarizes data obtained for all hydroxyapatites studied. Samples HA-1 and HA-5 exhibited similar results, although they were produced by the same company and described as non-resorbable and resorbable, respectively. As they have low surface area (4.4 m²/g) coupled with high crystallinity (> 90%) they can be assumed to be non-resorbable. However, the relatively high carbonate content may attenuate this tendency. For all samples – except HA-5 - the manufacturers' specifications were not contradicted by the qualitative estimation of biodegradability. However, only sample HA-6 granule exactly matches these specifications. In order to quantify the

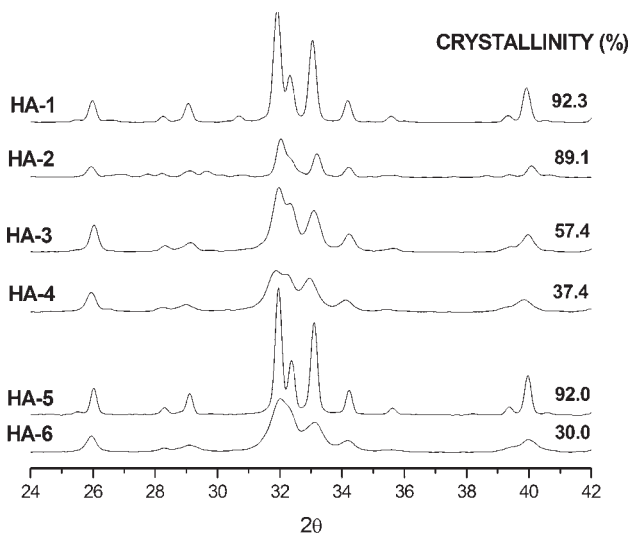


FIGURE 2- XRD pattern (displayed range in 20-60°) of all hydroxyapatite samples. No other phase than hydroxyapatite was identified. Crystallinity varied from 30 to 92 %

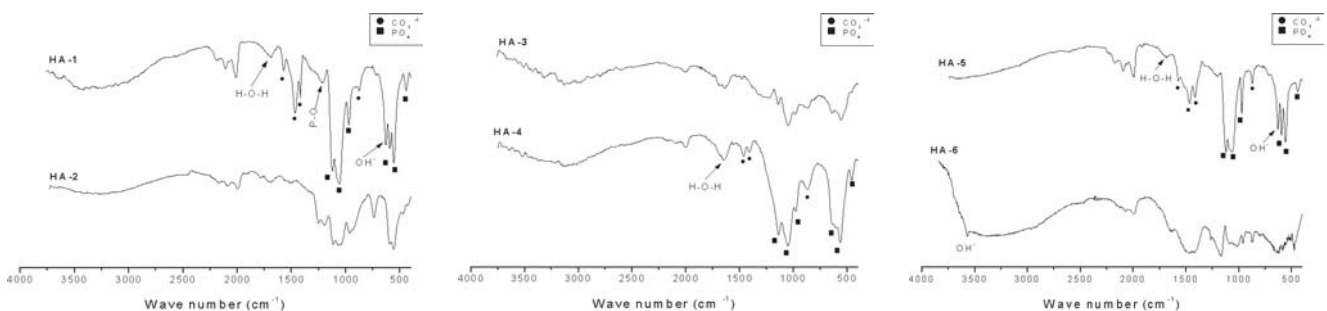


FIGURE 3- FTIR spectra of samples examined.
 a) HA-1 and HA-2
 b) HA-3 and HA-4
 c) HA-5 and HA-6

TABLE 2- Correlation between expected biodegradability and the manufacturers' specifications

Manufacturers' specification	Surface Area	Cristallinity	Carbonate %	Expected Biodegradability
HA-1(synthetic, non-resorbable)	low	high	high	moderate-low
HA-2(synthetic, non-resorbable)	low	high	low	low
HA-3 (synthetic, resorbable)	moderate	moderate	moderate	moderate
HA-4 (synthetic, resorbable)	moderate	low	moderate	moderate
HA-5 (natural HA, resorbable)	low	high	high	moderate-low
HA-6 (inorganic bovine bone, resorbable)	high	low	high	fast

biodegradability of these materials, the ISO/FDIS 10993-14:2001 degradation test should be used.

Although only six commercial hydroxyapatite granules were analyzed in this paper, it is interesting to point out the urgent need for the development of a Brazilian standard for this kind of products. The reliability of medical and dental professionals may be increased if these standards were approved and quality control was implemented.

CONCLUSIONS

The six granules examined were composed by basically pure hydroxyapatite. Strong differences were observed in terms of crystallinity, surface area and carbonate content and, therefore, different "in vivo" behavior can be expected. For all samples – except for HA-5 - the manufacturers' specifications were not contradicted by the qualitative estimation of biodegradability. However, only sample HA-6 exhibited characteristics in perfect agreement with these specifications.

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