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COMPARATIVE STUDY OF HYDROXYAPATITE PREPARED BY THE AUTHORS WITH SELECTED COMMERCIALLY AVAILABLE CERAMICS

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The paper presents study of hydroxyapatites from different sources - obtained by the authors: a) Nat.HA, derived from animal bones, b) synthetic made by a wet method, - in comparison with commercial powders; Habiocer®, and Merck®). All hydroxyapatites were characterized using X-ray diffraction (XRD); Fourier transformed infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM-EDS) methods. Calcium was determined by titration with EDTA (ethylenediaminetetraacetic acid) in the presence of thymolphthalein and calcein mixture whereas phosphorus was determined with the spectrophotometric method. Content of microelements was determined by the AAS and ICP method. The specific surface of the materials was measured by BET method. Volume of micro and mesopores was also determined. Synthetic hydroxyapatite, prepared by the authors by wet method was of low crystallinity, high surface area and porosity, while XRD and FT-IR characteristics were similar to commercial Merck®. In contrast, the highest crystallinity, while the lowest surface area and porosity caused possibly by sintering of the material characterised Nat.HA (animal bone calcined at 800^{o}C). XRD and FT-IR characteristics of commercial Habiocer® indicated high, (similar to Nat.HA) crystallinity, however, surface area and pores were much higher than those of Nat.HA.

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1. Introduction

Bone is a living connective tissue that provides structural support to body organs and is an emergency reservoir of calcium in case that the concentration of that element in blood needs to be corrected because of disease, or some pathological processes throughout life [1].

The main components of bone are: organic (mostly collagen fibres) and inorganic mineral phase, known as biological hydroxyapatite which represents 65-70 wt% of natural bone [2-5]. Collagen fibres are responsible for bone resilience, while mineral component for bone stiffness. The most compatible with bone mineral phase is non-stoichiometric calcium phosphate of "apatitic structure" [2]. It differs from stoichiometric hydroxyapatite of formula $Ca_{10}(PO_4)_6(OH)_2$ with molar ratio of Ca/P = 1.67. Biological apatites - constituents of bone, enamel, dentine and pathologically calcified tissues are nonstoichiometric, mostly Ca-deficient with respect to phosphate (Ca/P < 1.67) unless carbonate ion is incorporated, then Ca/P is higher than 1.67. The difference between stoichiometric hydroxyapatite and bone mineral lies also in the impurity content, which is associated mostly with ions substitution for calcium sites in the bone hydroxyapatite structure.

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For the last few decades much attention has been given to develop new biologically relevant materials useful for skeletal tissue reconstruction on surgically operated patients. Most of them are based on synthetic hydroxyapatite (HAp) [2-7]. Such materials are biocompatible, bioactive, osteoconductive, non-toxic, noninflammatory and have an ability to form strong bonds with the living hard tissue. The chemical and crystallographic similarity to biological apatites makes synthetic materials intimately integrating with the surrounding host bone by a strong interface [8].

Synthetic bioceramics for skeletal tissue reconstruction are already commercially available in a large assortment. The aim of the present paper was to demonstrate similarities and differences in physicochemical characteristics of selected commercial materials under the name of hydroxyapatite (Habiocer®, Merck®) in comparison to prepared by the authors: synthetic HA (precipitated from a water solution) and Nat.HA (calcined animal bone).

2. Experimental

2.1 Materials

2.1.1 Prepared by the authors

a) Preparation method of hydroxyapatite from natural animal bone has been developed in the Institute of Inorganic Chemistry and Technology, Cracow University of Technology [9]. As a raw material deproteinised and defatted bone pulp - (bone sludge delivered from a slaughterhouse) was applied. Two stage calcining process in chamber oven with electric heating in air atmosphere at temperatures 600^{o}C and 850^{o}C, respectively for each stage for 3 hours produced hydroxyapatite of natural origin (Nat.HA). Sieve fraction below 0.063 mm was used in all tests.

b) Preparation of synthetic hydroxyapatite was realized by wet method [10]. Starting solutions were prepared from reagent grade H_3PO_4 (Solution A) and $Ca(OH)_2$ (Solution B). pH at a level of 12 was adjusted with the help of $NH_3 \cdot H_2O$. Concentration in solution (A) was 0.300 mol/dm^3. Concentration in solution (B) was 0.500 mol/dm^3. Solution (A) = 100 cm^3, was added dropwise into 100 cm^3 of solution (B). Molar ratio of Ca/P was ~1.67. The slurry obtained was stirred for 60 minutes at room temperature, then left to maturate for the next 24 hours, after that the precipitate was filtered off. The filter cake was washed with distilled water, covered with filter paper and left to dry at room temperature.

Commercial materials

Two commercially available synthetic hydroxyapatites: Habiocer® (Chema-Elektromet, Poland) and Merck® were used for comparative study.

2.2 Methods

Phosphorus content was determined by spectrophotometric method according to [11] with Marcel Media UV-VIS spectrophotometer after former mineralization of the sample in mixture of concentrated hydrochloric and nitric acids. Calcium was determined by titration method according to [12] in the presence of calcein and thymolphthalein as indicators.

Phase composition was analysed using X-Ray diffraction (XRD) method with the use of Philips X'Pert diffractometer with graphite monochromator, Cu K_α, (λ = 0.1518nm), and Ni filter. Contents of microelements: Cu, Co, Ni, Cr and Cd were determined by AAS method with Analyst 300 Perkin Elmer Spectrometer. Contents of heavy metals such as Pb, Hg and As were measured by the ICP (inductively coupled plasma).

Infrared investigations FT-IR were realized with spectrophotometer Scimitar Series FTS 2000 Digilab in range of middle infrared 400-4000 cm^{-1}. 0.0007 g sample was pressed in 0.2000 g of KBr. Number of scans 16 and resolution 4 cm^{-1} characterized these measurements.

The microstructure of samples was examined using scanning electron microscope S-4700 Hitachi supported by chemical analysis carried out using energy dispersive X-ray spectroscope (EDS) at 20.0 kV and 15.0 mA.

Specific surface of powders was evaluated by BET method using apparatus ASAP 2405 Micromeritics Inc. USA. Surface and volume of pores were also tested.

3. Results and discussion

Results of chemical analyses of the hydroxyapatites is summarised in Table 1.

Table 1. Chemical composition of hydroxyapatites

Element	Unit	Synthetic hydroxyapatite Habiocer®	Nat.HA (from animal bone)	Synthetic hydroxyapatite by wet method	Synthetic hydroxyapatite Merck®	ISO 13779
Calcium	%	41.3	38.8	37.2	38.7	-
Phosphorus	%	19.3	17.8	16.9	17.7	-
Molar ratio Ca/P		1.66	1.68	1.70	1.69	-
Cobalt	ppm	17.68	7.88	9.66	9.87	-
Copper	ppm	6.42	6.09	4.36	4.21	-
Nickel	ppm	12.10	10.72	7.99	7.57	-
Chromium	ppm	2.79	9.50	5.66	5.26	-
Cadmium	ppm	11.54	1.98	5.53	5.73	10.0
Lead	ppm	0.39	0.59	0.29	0.066	10.0
Mercury	ppm	0.0093	0.0043	0.0066	0.0062	1.50
Arsenic	ppm	0.46	0.19	0.21	0.125	1.00

The highest content of calcium and phosphorus was found in Habiocer®. Calcium and phosphorus content in Nat.HA was similar to those in commercial hydroxyapatite Merck®, however it was significantly lower than in commercial Habiocer®. The molar ratio Ca/P in the analysed samples was ranging within 1.66 – 1.70. Content of two main components in Nat.HA derived from animal bone could be affected by an occurrence of microelements such as magnessium, natrium and also carbonate group, usually present in natural bone tissue. Cadmium, lead, mercury and arsenic – heavy metals are on the level accepted by ISO standard 13779 [13]. Content of cobalt was the highest in Habiocer® sample. In contrast, the level of Co content in all the other HA powders was lower and similar to each other. The lowest quantity of chromium was recorded in Habiocer®, whereas the highest was determined for hydroxyapatite of natural origin.

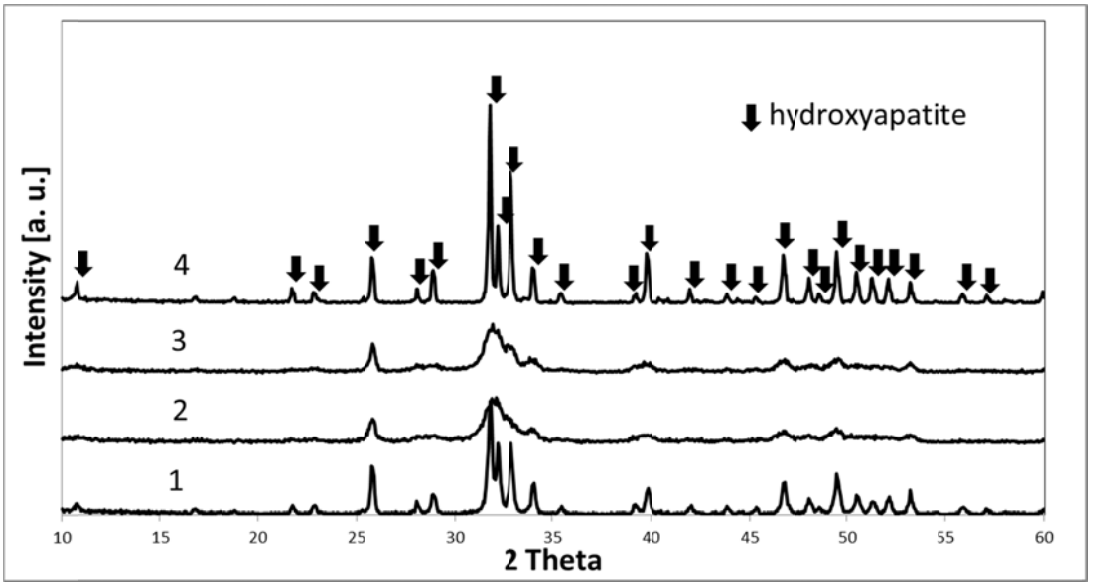

Fig. 1. XRD pattern of hydroxypaptite: 1 – Habiocer®, 2 – Merck®, 3 – synthetic, 4 – Nat.HA

Fig. 1. presents X-ray diffraction patterns of four samples of hydroxyapatite. X-ray analyses showed that hydroxyapatite was the only crystalline phase indentified in all the materials. However, the crystallization degree (crystallinity) was different. The hydroxyapatite of natural origin was characterised by the highest relative intensity of most reflexes typical for high crystallinity. High crystallinity was also recorded in case of Habiocer®, although the relative intensity was lower to some extent than in natural HA in Fig. 1.4. The XRD patterns of commercial hydroxyapatite - Merck® (Fig.1.2) and synthetic HA, obtained by the authors by wet method (Fig.1.3) indicated poor crystallinity of these powders. The broad peaks suggested that their particles could be of a nanometric size. It has to be noted, that mineral component of natural bone has been known as poorly crystallised hydroxyapatite [14] with XRD pattern usually similar to that of synthetic (Fig. 1.3), obtained by the authors. High-temperature treatment of bone causes organic component burnt out and the mineral component re-crystallised. As a result much higher, than the initial, crystallinity, such as illustrated in Fig. 1.4. has been observed.

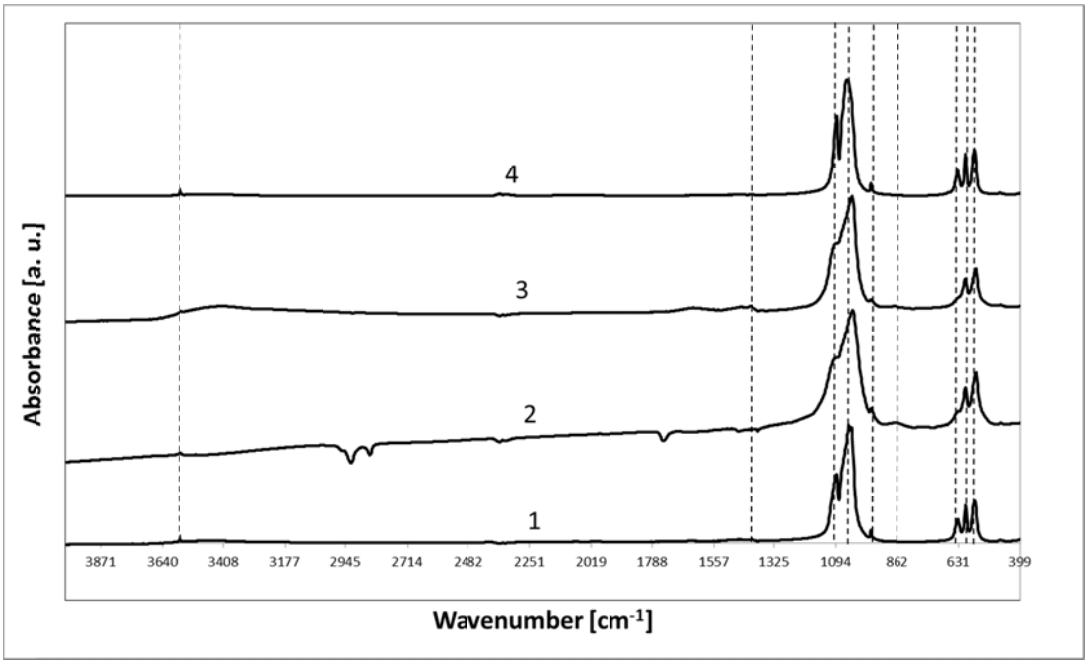

Fig. 2. FT-IR spectra of hydroxypaptites: 1 – Habiocer®, 2 – Merck®, 3 – synthetic, 4 – Nat.HA

All FT-IR spectra shown in Figure 2 are characteristic of hydroxyapatite according to [15-17]. The band (Fig.2.1- Fig.2.4) within high wave number range (3600 – 3400 cm^{-1}) and the band ~ 630 cm^{-1} (Fig. 2.1 and Fig.2.4) are typical of O-H group in hydroxyapatite. The absence of bands characteristic of C-H and C-C vibrations in Fig.2.4 confirms that the whole organic material was removed from animal bone during calcining process yielding natural crystalline Nat.HA. Absorption band at wave number ~ 960 cm^{-1} has been assigned to ν_1 - symmetric stretching mode of P - O. Absorption bands recorded within wave number range of 570-636 cm^{-1} were assigned to ν_4 asymmetric deformation of O – P – O vibrational mode in PO_4. The bands of a very low intensity at 1427and 885cm^{-1} observed for synthetic powder by wet method (Fig.2.3) might possibly correspond to stretching vibrations of CO_3^{2-}. Lower resolution of the P – O (ν_3 asymmetric stretching mode of PO_4) band in the region of 1000- 1100 cm^{-1} and the absence of the shoulder for the OH librational mode, typical for hydroxyapatite, at about 630 cm^{-1} reflect low crystallinity of Merck® sample (Fig.2.2) and synthetic apatite (Fig.2.3). The observed decrease in FT-IR resolution bands is in agreement with lower crystallinity phenomena described by LeGeros [2], and also in accordance with the XRD patterns in Fig.1.2, and Fig.1.3.

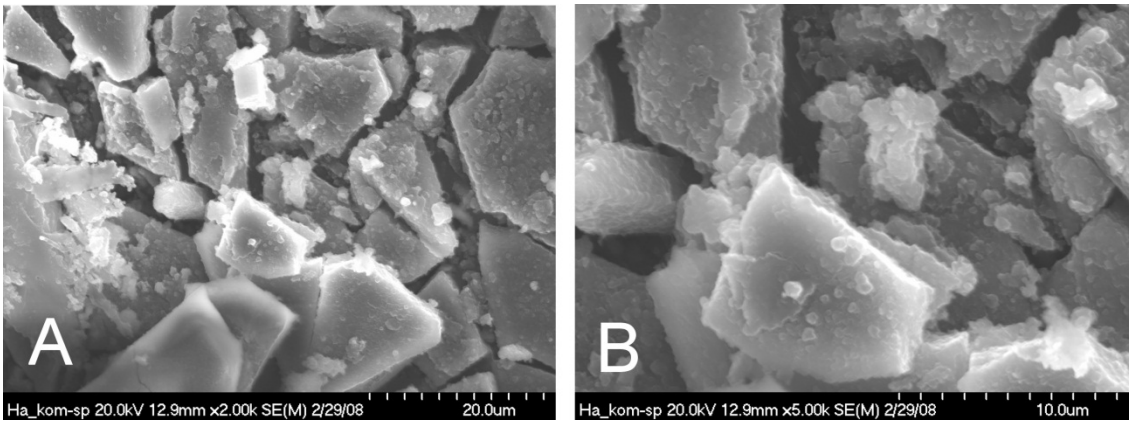

Fig. 3. SEM pictures of hydroxyapatite purchased from Merck®

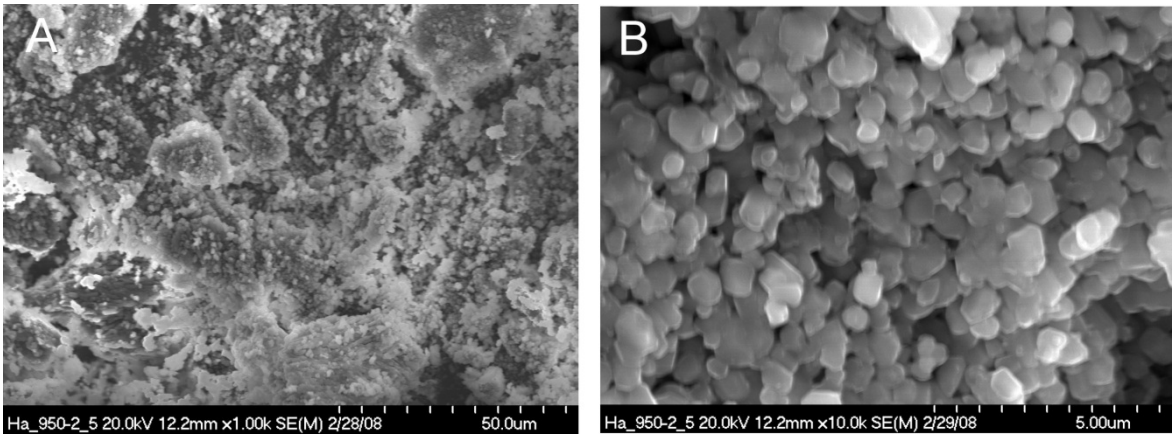

Fig. 4. SEM pictures of hydroxyapatite natural origin (Nat.HA)

Figures 3 and 4 are showing representative SEM pictures of commercial product (Merck®) and hydroxyapatite obtained through animal bone calcining (Nat.HA), respectively. The hydroxyapatite powders demonstrated diversified propensity to agglomeration. The highest propensity to agglomeration showed HA from Merck®. Hydroxyapatite from Merck® is in the form of large clusters of different size with sharp edges. Nat.HA exhibited sintered grains of regular sphere-like shape with diameter approximately 1μm.

The specific surfaces of obtained powders were characterized by BET method. Surface and volume of pores were also investigated. Results are presented in Table 2.

Table 2. Characteristics of surface area and pores of the analysed hydroxyapatites.

Parametr	Unit	Synthetic hydroxyapatite Habiocer®	Nat.HA	Synthetic by wet method	Synthetic hydroxyapatite Merck®
BET surface area	[m^2/g]	26.74	4.10	77.62	56.47
Langmuir surface area	[m^2/g]	34.19	5.26	98.61	71.90
Cumulative adsorption surface area of mezopores	[m^2/g]	28.91	4.69	80.67	62.02
Micropore area	[m^2/g]	0.88	0.30	11.58	8.17
Cumulative adsorption pore volume of mezopores	[cm^3/g]	0.21	0.00755	0.61	0.26
Micropore volume	[cm^3/g]	0.00039	0.000135	0.00504	0.00359
Average pore diameter	[nm]	12.93	73.67	251.63	153.30

Hydroxyapatite synthesized by the wet method is characterised by the highest, while Nat.HA by the lowest values of parameters indicated in Table 2 such as respectively: BET surface (77.62 and 4.10), surface of mezopores (80.67 and 4.69), micropore area (11.58 and 0.30). It is worth to emphasise that there is some similarity between relatively high listed above characteristics of commercial Merck® and synthetic obtained by the authors by wet method hydroxyapatite. Micropore area for natural and synthetic Habiocer® was below 1 m^2/g. Micropore volume was below 0.005 cm_3/g for all tested samples. The average pore diameter was high for synthetic HA by wet method (251.63nm) and for Merck® (153.3nm) whereas for Habiocer® and Nat.HA the respective pore diameters were much smaller (12.93 and 73.67) in nm.

3. Conclusions

Comparative study of four different types of hydroxyapatite revealed that physicochemical properties and morphology of the powders depended on the origin/preparation method.

Synthetic hydroxyapatite, prepared by the authors by wet method had XRD and FT-IR characteristics similar to commercial Merck®. They were of low crystallinity, however, surface area and porosity were high.

In contrast XRD and FT-IR of Nat.HA (animal bone calcined at 800^{o}C) was characterised by the highest crystallinity. However, sintering of that material was possibly the cause of the lowest BET surface area and porosity.

XRD and FT-IR characteristics of commercial Habiocer® indicate high, (similar to Nat.HA) crystallinity. That may suggest possible re-crystallisation in which thermal treatment of the material might have been involved, however, surface area and pores were much higher than those of Nat.HA.

Acknowledgements

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