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## Low temperature method for the production of calcium phosphate fillers

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### Abstract

**Background:** Calcium phosphate manufactured samples, prepared with hydroxyapatite, are used as either spacers or fillers in orthopedic surgery, but these implants have never been used under conditions of mechanical stress. Similar conditions also apply with cements. Many authors have postulated that cements are a useful substitute material when implanted in vivo. The aim of this research is to develop a low crystalline material similar to bone in porosity and crystallinity.

**Methods:** Commercial hydroxyapatite (HAp) and monetite (M) powders are mixed with water and compacted to produce cylindrical samples. The material is processed at a temperature of 37–120 degrees C in saturated steam to obtain samples that are osteoconductive. The samples are studied by X-ray powder diffraction (XRD), Vickers hardness test (HV), scanning electron microscopy (SEM), and porosity evaluation.

**Results:** The X-ray diffractions of powders from the samples show patterns typical of HAp and M powders. After thermal treatment, no new crystal phase is formed and no increase of the relative intensity of the peaks is obtained. Vicker hardness data do not show any relationship with treatment temperature. The total porosity decreases by 50–60% according to the specific thermal treatment. Scanning electron microscopy of the surfaces of the samples with either HAp 80%-M 20% (c) or Hap 50%-M 50% (f), show cohesion of the powder grains.

**Conclusions:** The dissolution-precipitation process is more intensive in manufactured samples (c) and (f), according to Vickers hardness data. The process occurs in a steam saturated environment between 37 degrees and 120 degrees C. (c) (f) manufactured samples show pore dimension distributions useful to cellular repopulation in living tissues.

### Background

From about 1990, calcium phosphate manufactured samples, typically produced with hydroxyapatite, are used as spacers or fillers in orthopedic reconstruction and as fillers in maxillofacial applications, [1-10], however, these

implants have never been used under mechanical stress. Also, cements have been increasingly employed in craniofacial trauma surgery and dentistry [11-14], and after time these implants are reabsorbed and replaced by newly formed mineralized matrix.

At present many researchers prefer to produce macroporous ceramics, which are biologically compatible and show osteoinductive properties [15,16]. That is when they are implanted *in vivo* in ectopic areas, new mineral bone matrix is detectable after only six months.

If porous ceramics are prepared with organic matrices, such as collagen type I [17,18], and bone marrow cells [19,20], they show osteogenic activity even if implanted in bone tissue and ectopic areas of experimental animals and *in vitro* cultures.

The cements are obtained from powders mixed in water and hardened for a short time at 37 °C [21,22]. The preferred phosphates are monetite ( $\text{CaHPO}_4$ ) and brushite ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) [22-25]. These products give hydroxyapatite precipitates, so the cements harden and often are suggested as repair materials in those regions that are not under mechanical stress.

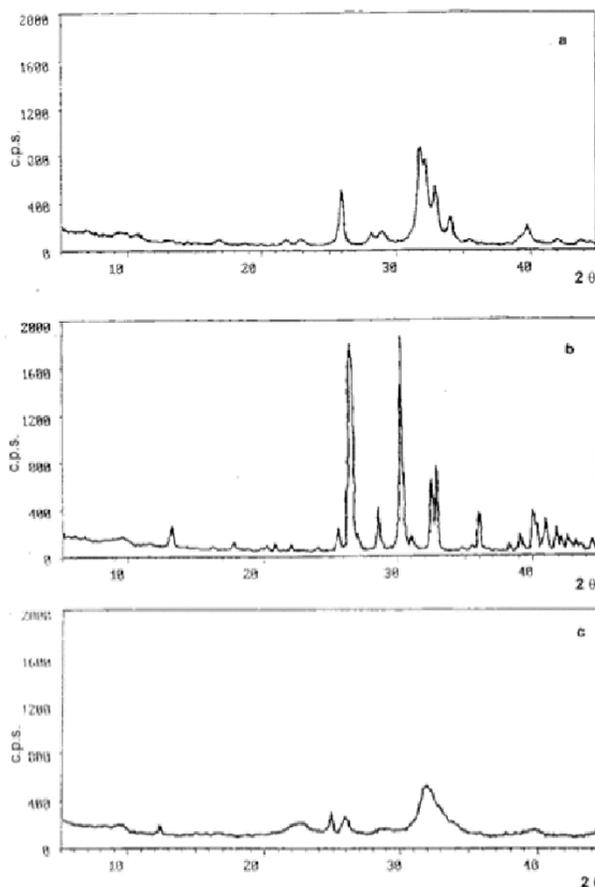
Many authors postulate that such compounds are useful as substitute materials when implanted *in vivo*. The replacement of these materials by *ex novo* formed bone occurs by a combination of osteoconduction and implant resorption [12-14].

The aim of the present research is to obtain low crystalline materials similar to bone in porosity and crystallinity. Our manufactured samples are characterized by X-ray powder diffraction (XRD), scanning electron microscopy (SEM), Vickers hardness test (HV) determination, porosity evaluation and calcium solubility of the powders used to produce the fillers.

The data obtained are extremely useful in evaluating the chemico-physical properties of manufactured samples and should be extremely useful in interpreting the morphological and X-ray diffraction data. The relationships among these data could help facilitate production of the fillers that we have studied.

## Methods

Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ), (HAp) and monetite ( $\text{CaHPO}_4$ ), (M) from Aldrich (Sheboygan, WI, USA) are used to prepare manufactured samples. Both powders are mechanically mixed for one minute, then the powders are wetted with distilled water and milled by a hand operated pestle for one minute more. The cylindrical specimens (1.27 cm D, 6 mm H) are enclosed in a steam saturated atmosphere at one of the following temperatures 37, 60, 80, 100, and 120 °C, and with a maximum vapour tension of 1 atm. These conditions were used so as not to increase the powder crystalline status. Thirty samples were prepared at each temperature and at each composition.



**Figure 1**

X-ray diffraction patterns of powders; X-ray powder diffraction patterns of: a) commercial hydroxyapatite powder, b) commercial monetite powder, c) compact bovine bone powder.

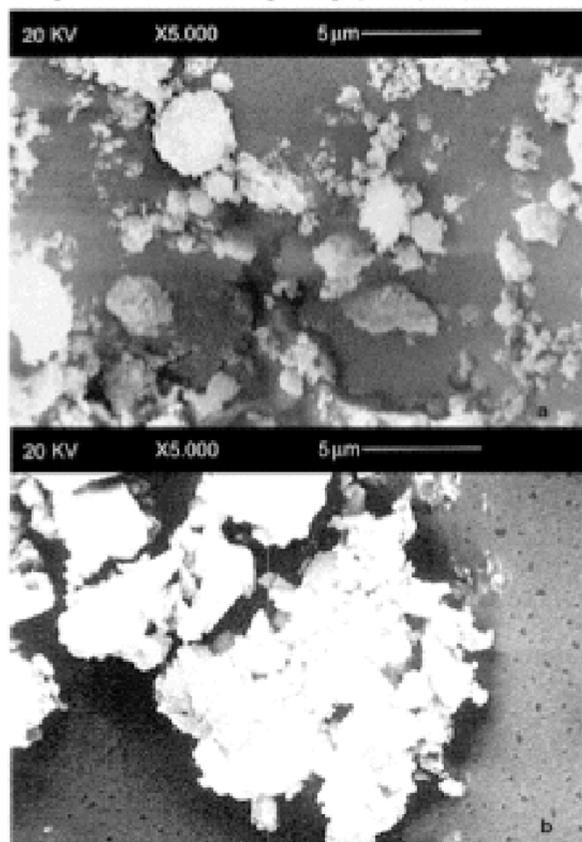
Bovine bone samples, used as reference, were obtained from the local slaughter-house. The bone was washed with acetone (analytical grade), then manually crashed by means of an iron hammer and homogenized in a stainless steel blender running at full speed (21000 rpm).

## Results

### Characterization of calcium phosphate powders

The powders are characterized by X-ray powder diffraction (Philips model 1730/10) and by scanning electron microscopy (Jeol model JSM-T330) and morphometry. Powder solubility in water at 25 °C, (72 hours) has been evaluated by calcium complexometric quantitative analysis [26].

Two suspensions are prepared by mixing either 30 g of HAp or 30 g of M in 1 l of distilled water. Six more



**Figure 2**  
Electron scanning micrographs of pure powders; Electron scanning micrographs of: a) commercial hydroxyapatite powder, b) commercial monetite powder.

mixtures are prepared suspending HAp and M at known weight ratios, HAp/M (table 1). Water is added to each mixture (1 l to 30 g), incubated at 25°C, and the calcium concentrations are determined at 3, 6, 12, 24, 48 and 72 hours, by the complexometric method [26].

The XRD traces for the hydroxyapatite and monetite powders are presented in figure (1a,1b). Each peak is compared with those of pure hydroxyapatite and monetite by ASTM standards [27]. Also shown, in figure 1c, is the X-ray diffraction pattern of bovine bone, showing its low crystallinity.

Morphometric and statistical analysis, of scanning electron microscopy studies (figure 2a) shows that the hydroxyapatite powder is constituted of grains between 1 and 7 micron diameter. The grains (figure 3a) that are 1–2 microns diameter, represent 65.8% of the total, those

**Table 1: Composition of HAp/M mixtures.**

Mixtures names	Composititon (% in weight of hydroxyapatite and moneite)	Hydroxyapatite/Monetite (moles ratios)
a	Hap 100%	/
b	Hap 90%-M 10%	2.4:1
c	Hap 80%-M20%	1:1
d	Hap 70%-M30%	2:3
e	Hap 60%-M40%	2:5
f	Hap 50%-M50%	1:3
g	Hap 30%-M70%	1:8
h	M 100%	/

Composition of the studied HAp/M mixtures and their molar ratios.

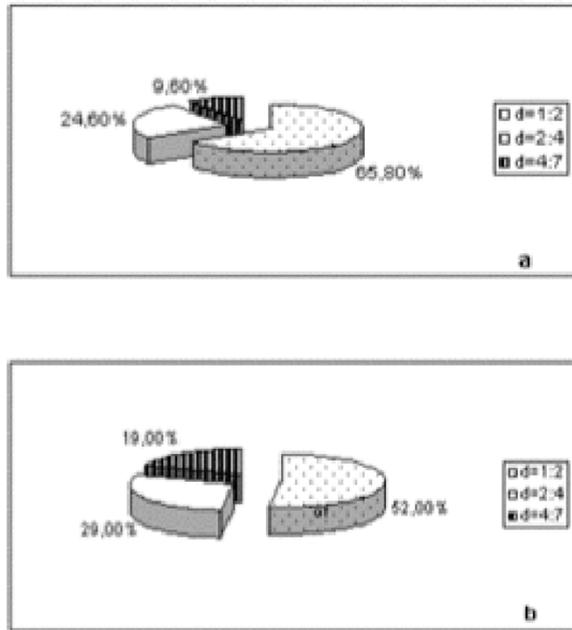
that are 2–4 microns are 24.6% and those between 4 and 7 microns are 9.6%. Aggregates less than 2 microns are composed of smaller grains that are about 100 nm in diameter.

Observing monetite powder by scanning electron microscopy (figure 2b), we see that it is composed of three groups of aggregates: the first consists of grains 1–2 microns diameter and constitute 52% of the total, the second is grains 2–4 microns diameter (29%), and the third, grains 4–6 microns diameter (19%) (figure 3b).

The hydroxyapatite and monetite solubilization time courses of the several mixtures at 25°C for 72 h is reported in figure 4a. The calcium concentration from HAp/M suspensions is higher than in both pure HAp and M. The Ca<sup>2+</sup> concentration at 72 hours in hydroxyapatite suspension is 0.23 mmoles/l and in monetite suspension is 0.78 mmoles/l. In mixture (c) (table 1) at 72 h, the Ca<sup>2+</sup> concentration is 1.51 mmoles/l, and in mixture (g) is 1.61 mmoles/l. The curves in figure 4a show that the solubility gradually increases over 72 hours reaching a plateau. The pH values of the several suspensions over the 72 hours are presented in figure 4b. The values of both the HAp and M solutions are within the 6.7–7.0 pH range, and those of their mixtures are within 6.0–6.6 pH range. Clearly, the HAp and M powder mixtures with water excess induce chemical reactions such as monetite hydrolysis. Most probably this happens because monetite is the most soluble and acidic compound.

**Characterization of manufactured samples**

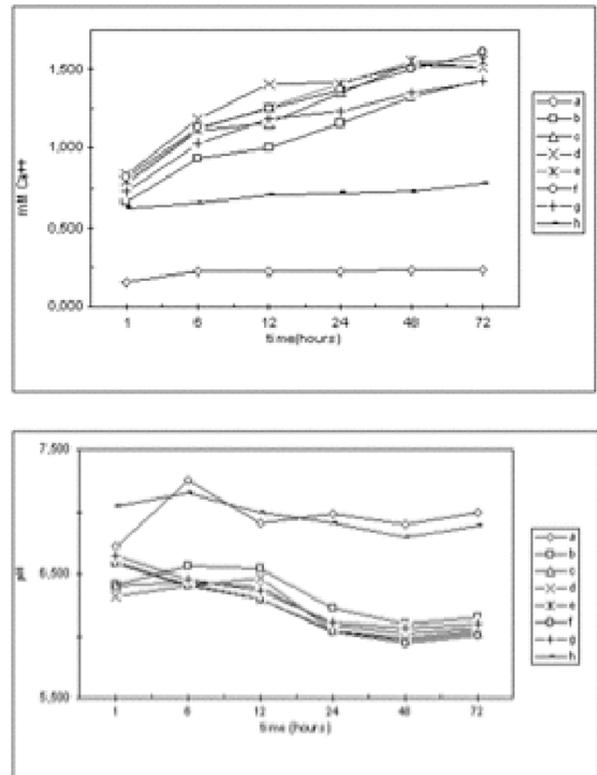
All of the calcium phosphate samples that we produced were characterized by X-ray powder diffraction (XRD), Vickers hardness test (HV), scanning electron microscopy (SEM), and porosity evaluation.



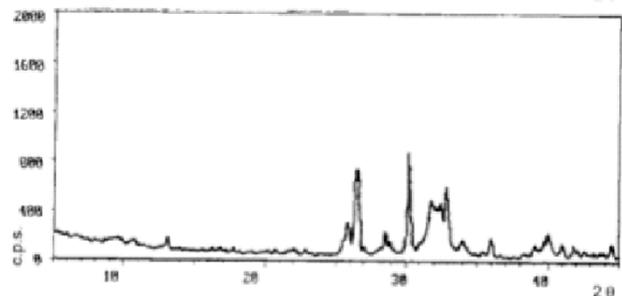
**Figure 3**  
Frequency distribution of particle dimension in powders; Frequency distribution of particle dimensions of: a) commercial hydroxyapatite powder, b) commercial monetite powder.

Vickers hardness tests (HV) was carried out by Microhardness tester (Type M) Shimadzu (Japan) and the porosity was evaluated by a mercury porosimeter (Porosimeter 2000) Carlo Erba (Italy).

The X-ray diffraction patterns of powders from our samples (e.g. mixtures (a), (h), (f)) show patterns typical of HAp and M powders (figure 1a and 1b, figure 5, respectively). After thermal treatment at temperatures between 37°C and 120°C, neither any new crystal phase formed nor was there an increase of the relative intensity of the peaks (that is a crystallinity increase), for example see in figure 6 the mixtures (h) and (f) at 120°C, but not the mixture (a) alone.



**Figure 4**  
Calcium concentration and pH values of the several mixtures; a) Final calcium concentration in water of HAp, M and HAp/M mixtures measured within 72 hours from mixing, b) pH values of HAp, M and HAp/M mixtures measured within 72 hours from mixing.

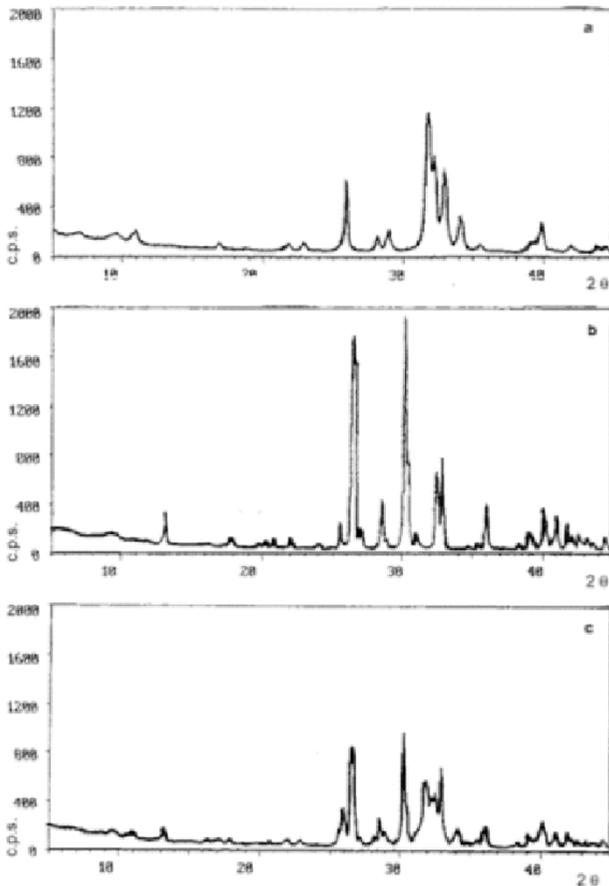


**Figure 5**  
X-ray diffraction pattern of specimen; X-ray powder diffraction patterns of untreated manufactured sample f.

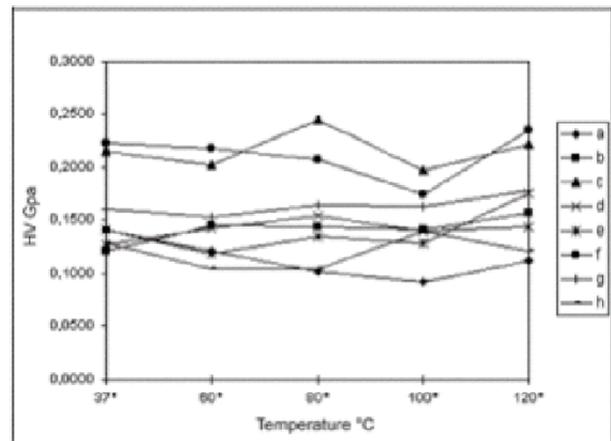
**Table 2: HV data (GPa) and standard deviation (SD) of manufacts made by eight mixtures HAp/M treated in a steam saturated environment at 37-60-80-100-120°C.**

MISCEL A	37°		60°		80°		100°		120°	
	HV	SD								
a	0.1420	0.0158	0.1210	0.0144	0.1020	0.0150	0.0930	0.0186	0.1130	0.0203
b	0.1210	0.0236	0.1450	0.0264	0.1440	0.0178	0.1420	0.0285	0.1570	0.0322
c	0.2150	0.0160	0.2020	0.0308	0.2460	0.0264	0.1970	0.0295	0.2220	0.0362
d	0.1280	0.0107	0.1430	0.0105	0.1550	0.0211	0.1400	0.0080	0.1440	0.0160
e	0.1420	0.0052	0.1190	0.0050	0.1360	0.0184	0.1290	0.0153	0.1760	0.0240
f	0.2230	0.0254	0.2180	0.0180	0.2080	0.0479	0.1750	0.0250	0.2360	0.0291
g	0.1610	0.0222	0.1530	0.0179	0.1650	0.0135	0.1630	0.0196	0.1780	0.0192
h	0.1290	0.0152	0.1050	0.0195	0.1050	0.0223	0.1400	0.0151	0.1210	0.0291

HV data (GPa) and standard deviations (SD) of manufactured samples made with the mixtures described in table 1.



**Figure 6**  
X-ray powder diffraction patterns of specimens; X-ray powder diffraction patterns of samples treated at 120°C and produced with: a) hydroxyapatite (sample a), b) monetite (sample b), c) hydroxyapatite/monetite (sample f).



**Figure 7**  
HV data of specimens; HV data of manufactured samples made with the mixtures described in table 1 at the several treatment temperatures.

Vickers hardness data are reported in table 2 and in figure 7 as the mean of 30 independent determinations for each mixture and each treatment temperature in saturated steam environment, and are expressed as GPa, according to the designation E 92-82 from ASTM standards [28] and SI units. An unpaired Student-t test was performed on the data for the same mixture at different temperatures, and also for the mixtures at each temperature, as shown in table 3.

**Table 3: Results of unpaired t Student-t test performed on the HV data**

	M100%	HAp30%+M70%	HAp50%+M50%	HAp60%+M40%	HAp70%+M30%	HAp80%+M20%	HAp90%+M10%
HAp 100%	p < 0.01	p < 0.005	p < 0.0005	n.s.	p < 0.005	p < 0.0005	p < 0.005
HAp90%+M10%	n.s.	p < 0.0005	p < 0.0005	p < 0.005	n.s.	p < 0.0005	
HAp80%+M20%	p < 0.0005	p < 0.0005	p < 0.1	p < 0.0005	p < 0.0005		
HAp70%+M30%	n.s.	p < 0.0005	p < 0.0005	p < 0.0005			
HAp60%+M40%	p < 0.005	p < 0.0005	p < 0.0005				
HAp50%+M50%	p < 0.0005	p < 0.0005					
HAp30%+M70%	p < 0.0005						
	M100%	HAp30%+M70%	HAp50%+M50%	HAp60%+M40%	HAp70%+M30%	HAp80%+M20%	HAp90%+M10%
HAp 100%	p < 0.005	p < 0.0005	p < 0.0005	n.s.	p < 0.0005	p < 0.0005	p < 0.0005
HAp90%+M10%	p < 0.0005	n.s.	p < 0.0005	p < 0.0005	n.s.	p < 0.0005	
HAp80%+M20%	p < 0.0005	p < 0.0005	p < 0.025	p < 0.0005	p < 0.0005		
HAp70%+M30%	p < 0.0005	p < 0.025	p < 0.0005	p < 0.0005			
HAp60%+M40%	p < 0.005	p < 0.0005	p < 0.0005				
HAp50%+M50%	p < 0.0005	p < 0.0005					
HAp30%+M70%	p < 0.0005						
	M100%	HAp30%+M70%	HAp50%+M50%	HAp60%+M40%	HAp70%+M30%	HAp80%+M20%	HAp90%+M10%
HAp 100%	n.s.	p < 0.0005					
HAp90%+M10%	p < 0.0005	p < 0.0005	p < 0.0005	p < 0.1	P < 0.05	p < 0.0005	
HAp80%+M20%	p < 0.0005	p < 0.0005	p < 0.005	p < 0.0005	p < 0.0005		
HAp70%+M30%	p < 0.0005	p < 0.005	p < 0.0005	p < 0.005			
HAp60%+M40%	p < 0.0005	p < 0.0005	p < 0.0005				
HAp50%+M50%	p < 0.0005	p < 0.0005					
HAp30%+M70%	p < 0.0005						
	M100%	HAp30%+M70%	HAp50%+M50%	HAp60%+M40%	HAp70%+M30%	HAp80%+M20%	HAp90%+M10%
HAp 100%	p < 0.0005	p < 0.0005	p < 0.0005	p < 0.0005	p < 0.0005	p < 0.0005	p < 0.0005
HAp90%+M10%	n.s.	p < 0.1	p < 0.0005	p < 0.025	n.s.	p < 0.0005	
HAp80%+M20%	p < 0.0005	p < 0.0005	p < 0.01	p < 0.0005	p < 0.0005		
HAp70%+M30%	n.s.	p < 0.0005	p < 0.0005	p < 0.005			
HAp60%+M40%	p < 0.025	p < 0.0005	p < 0.0005				
HAp50%+M50%	p < 0.0005	p < 0.05					
HAp30%+M70%	p < 0.0005						
	M100%	HAp30%+M70%	HAp50%+M50%	HAp60%+M40%	HAp70%+M30%	HAp80%+M20%	HAp90%+M10%
HAp 100%	n.s.	p < 0.0005					
HAp90%+M10%	p < 0.0005	p < 0.01	p < 0.0005	p < 0.025	p < 0.1	p < 0.0005	
HAp80%+M20%	p < 0.0005	p < 0.0005	p < 0.1	p < 0.0005	p < 0.0005		
HAp70%+M30%	p < 0.0005	p < 0.005	p < 0.0005	p < 0.0005			
HAp60%+M40%	p < 0.0005	n.s.	p < 0.0005				
HAp50%+M50%	p < 0.0005	p < 0.0005					
HAp30%+M70%	p < 0.0005						

Results of unpaired t Student-t test performed on the HV data obtained from manufactured samples prepared with different mixtures and treated at each temperature.

There was no relationship found between HV and treatment temperature of the mixtures. Much more significant is the relationship of HV with mixture composition, and only in two of the samples is HV higher than in the other mixtures. That is the specimens with HAp/M mixtures (c) and (f), that have HV values within the 0.20–0.25 GPa range. HV values of specimens obtained with HAp have a mean value of 0.11 GPa, those from M have a mean value of 0.10 GPa.

The total porosity, as determined by mercury porosimeter, in samples (c) and (f) decreases in the range of 50–60%,

**Table 4: Porosity percentage of specimens**

Samples	untreated	37°C	60°C	120°C
a	67.45	71.78	59.36	50.14
c	59.35	50.63	49.02	52.55
f	61.44	41.36	37.21	48.67

Porosity percentage of a, c, f manufactured samples (see Table I) at the described temperatures.

Pore distribution analysis of the (c) and (f) samples after thermal treatment shows three groups by pore sizes. They are characterized by pores 0.004–0.07 microns in diameter, 0.07–0.4 microns and 4–7 microns (about 1%). Figure 8 shows the pore distribution analysis of sample (f) thermally untreated (figure 8a), and treated at 60°C (figure 8b). By comparison, a sample of compact bovine bone shows both 0.01–0.1 micron and 0.6–6 micron pores (figure 8c).

Scanning electron microscopy of the surfaces of specimens (c) and (f) (figures 9a,9b) show a cohesion of the powders grains. Here (c) is more compact in comparison to (f), despite the presence of small pores. Observing the fracture surfaces of samples (c) and (f) we see long crystals of monetite with small grains of the hydroxyapatite above them (figures 10a,10b). In the (c) sample the monetite crystals are spaced further apart, in the (f) sample the monetite crystals overlap each other.

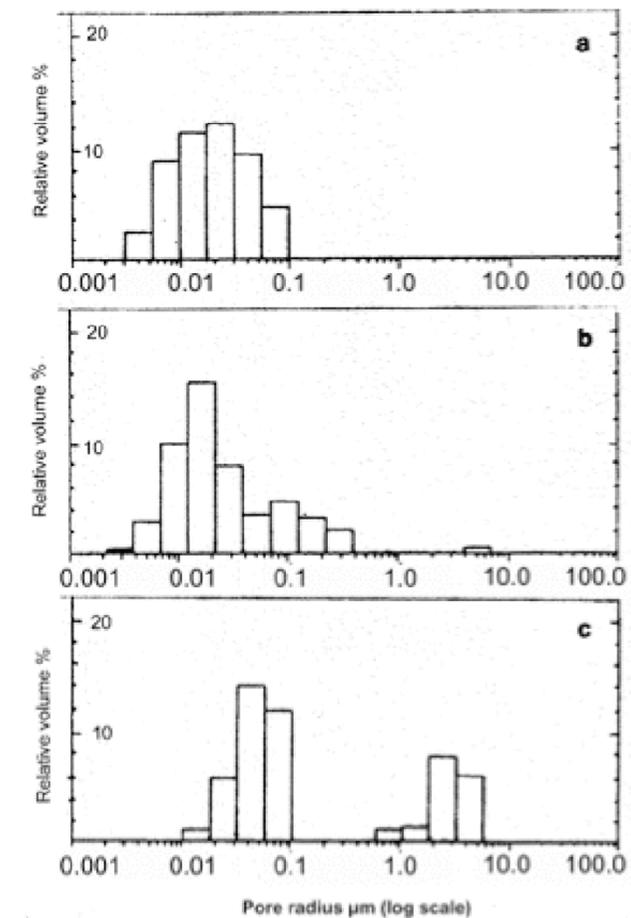
## Discussion

As described in the section above, all tested HAp/M powders mixtures were more soluble in water than those of pure compounds. According to these data, we assumed that in samples made from HAp/M mixtures, a partial solubilization of the chemicals occurs. This process should be useful to enhance the mixing of the reactants and the production of fillers composed of newly formed HAp crystals in the microspaces among the grains, and to obtain an higher coherence in manufactured samples.

The results described demonstrate the complexity of the systems, even those with heterogeneous compositions and prepared with constant parameters such as temperature. To interpret the phenomena that occur in the processes that compact these specimens, it is useful to consider that these processes occur within micropores [29]. In such microspaces, many processes occur at grain and micropore interfaces, which probably contain an oversaturated environment.

The manufactured samples are treated at temperatures between 37° and 120°C in a steam saturated environment to allow powders to solubilize without alteration of their crystallinity. Calcium phosphates are then precipitated into the grain interfaces without increasing HAp and M crystallinity, as demonstrated by X-ray diffraction studies of pure compounds. The thermal process always determines an higher coherence in the products [30,31]. This hypothesis is supported by the porosity % results, which decrease with thermal treatment, even if slightly, in samples (c) and (f), (table 4).

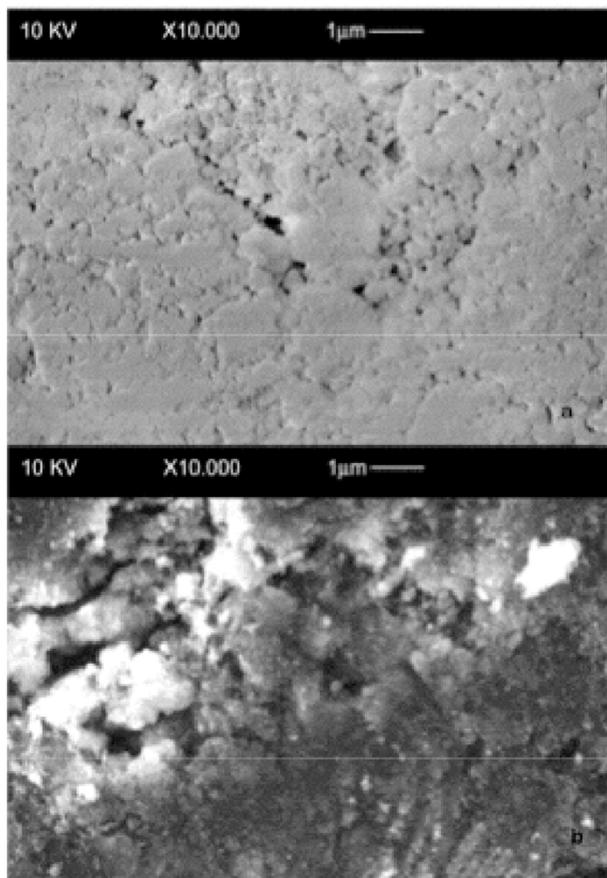
In HAp/M samples, the solubilization and reprecipitation processes are accelerated,  $\text{Ca}^{2+}$  and  $\text{HPO}_4^{2-}$  ion concentra-



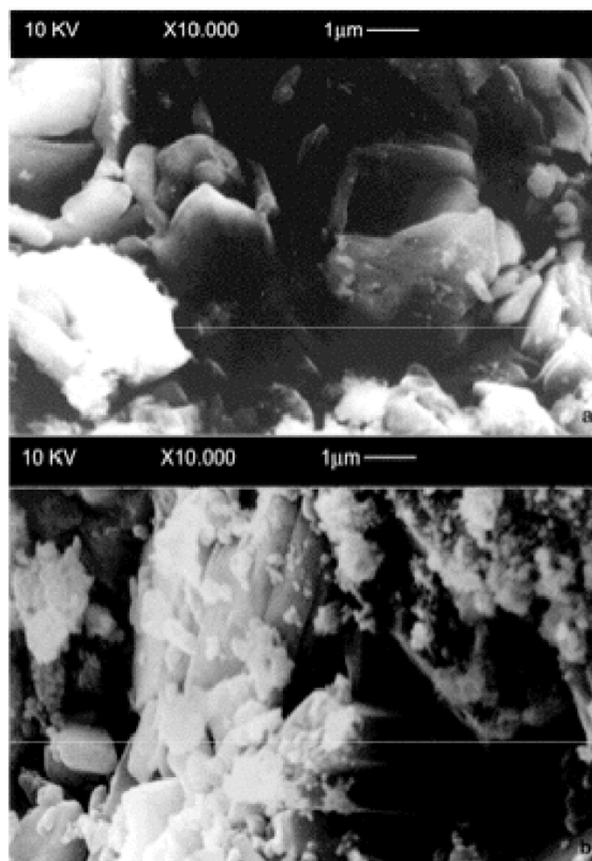
**Figure 8**

Distribution of pore volume in percentage; Relative volume percentage distribution of pores in function of pore radiuses: a) sample f untreated, b) sample f treated at 60°C, c) sample of compact bovine bone.

tions increase and then the ions are precipitated as HAp. Moreover, we should consider solubility of the initial inorganic components, and the formation of hydrolysis products (also at supersaturation, near the interfaces). They could precipitate by nucleation on grain surfaces (figures 10a,10b), producing intermediates with several solubility products. It is also useful to consider mechanical tests that show that temperature and vapour tension gradients can occur throughout the specimen thickness (table 2, figure 7). It is useful to note that standard treatment procedures do not necessarily give the same hardening with cement-like reaction within the several shapes and volumes of the samples. Scanning electron microscopy images of both external and fracture surfaces (that is external and internal surfaces) (figures 9a,9b and



**Figure 9**  
 Electron scanning micrographs of external surface of specimens; a) External surface by scanning electron micrographs of type c (table 1) manufactured sample. b) External surface by scanning electron micrographs of type f (table 1) manufactured sample.



**Figure 10**  
 Electron scanning micrographs of fracture surfaces of specimens; a) Fracture surface by scanning electron micrographs of type c (table 1) manufactured sample. b) Fracture surface by scanning electron micrographs of type f (table 1) manufactured sample.

10a,10b) of the samples show different coherence of the grains, some times with several crystals distributions between the external surfaces and the interior regions of the specimens. Hydroxyapatite is fragile, based on its low reactivity in freshly distilled water, (i.e., pH = 7), so that its grains do not sufficiently set to give mechanical consistency to the specimen.

Samples of (c) and (f) mixtures show the highest HV values (0.20–0.25 GPa). These results demonstrate that the HAP to M stoichiometric ratios (1:1 and 1: 3) optimize the solubilization-precipitation process at grain interfaces.

To compare with our results from the fillers and cements tested, we used a specimen of bovine compact bone with the same instrument and the same experimental conditions, and determined the mean HV value of 0.42 GPa. The morphology of sample (c) by scanning electron microscopy of the fractured surface is shown in figures 9a and 10a. Samples (c) and (f) show three groups by pore size, whereas a specimen of compact bovine bone show 10–100 nm pore size and 0.6–5 micron macropores. On the basis of the previous discussion of hydroxyapatite and monetite specimens, we obtained samples with mechanical properties useful to apply as either cements or fillers at mild temperature and pressure conditions. Such products can be further enhanced by the use of other inorganic

components the bone, and can easily be reabsorbed and repopulated by the surrounding bone tissue.

## Conclusions

In summary, based on our experimental data, we conclude that a solubilization-precipitation process occur, which is more intensive in specimens of 80% hydroxyapatite (HAp) with 20% monetite (M) (c), and of 50% HAp with 50% M (f), as demonstrated by Vickers hardness data. The manufacturing process is carried out at low temperature and in a steam saturated environment to obtain more consistent materials, without altering powders crystallinity. These HAp/M samples show pore dimension distribution, which is useful to cellular repopulation if implanted in living tissues.

## Authors' contributions

Calafiori Anna Rita: Manufactured samples preparations, calcium solubility tests, HV and porosity determinations of manufactured samples;

Marotta M.: Scanning electron microscopy micrographs and their evaluation;

Nastro A.: Acquisition and evaluation of X-ray diffractions and their evaluation;

Martino G.: Director of research.

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