

# Composites between hydroxyapatite and poly( $\epsilon$ -caprolactone) synthesized in open system at room temperature

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The hydroxyls present on the surface of hydroxyapatite (HA) granules, annealed at 700 °, 900 ° and 1100 °C, are able to initiate the polymerization of  $\epsilon$ -caprolactone (CL), not only at 185 °C under vacuum, but also at room temperature in open system. A polymer layer ionically linked to the substrate is formed on HA surface, enhancing the compatibility between the organic phase and the inorganic one in composite biomaterials. We studied the characteristics of the polymer, produced by the reaction carried out at room temperature in open system, as well as the percentages of the poly( $\epsilon$ -caprolactone) (PCL) ionically bonded to the HA structure and of the "free" one. Both percentages appear very dependent on the annealing temperature; in particular, HA annealed for 1 h at 1100 °C is the most efficient initiator of the reaction leading to ionically bonded PCL. The percentages of "free" polymer are much higher than at 185 °C under vacuum. Its formation is attributed to the role of water in opening the CL rings, and to the presence of  $\text{CO}_3^{2-}$  and  $\text{HPO}_4^{2-}$  ions in the HA annealed at lower temperatures. The presence of water appears to be the limiting factor for the production of PCL not bonded to the HA structure.

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## 1. Introduction

Hydroxyapatite (HA), the main inorganic component of the bone structure, is widely used as inorganic filler in composites with polymeric materials [1]. Many papers were published in past years, regarding composites containing HA as a biological mimetic material within polymer matrixes. These studies seldom investigated the possibility of bonding HA chemically to the matrix macromolecules, either directly or through coupling agents [2]. In most cases, after having considered as obvious a physical interaction between them, the research regarded only the usefulness of HA as an emulsifier and possibly as a bio-stimulator of adhesion

processes either to the bone or to the tooth. HA was considered mostly a biocompatible strengthening agent for the composites, when they are used as dental cements. Such filler was thought useful mainly to modify the physical properties of the composite produced, like density, consistency, and rheological behaviour during application and setting; in the matrix alone, in fact, these properties were often not suitable for tooth repair. Among the matrixes used were also poly(alkenoic acid)s and other carboxyl-containing compounds [3–5], the nature of which could suggest the hypothesis of acid-base interactions between the carboxyl groups and HA. Interactions between the carboxylate groups of

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the polymers and the hydroxyl or phosphate groups of HA, strong enough to explain the observed permanent adsorption, were proposed [5]. In other cases, adsorption of polyacids on HA surface was used to make it compatible with bioactive poly(ester-ether)s [6, 7].

It is very interesting the reactivity of HA towards cyclic monomers. The hydroxyl groups,  $[\text{OH}]^1$ , present on the surface of HA granules, are able to initiate the ring-opening polymerization of  $\epsilon$ -caprolactone (CL). The polymerization was carried out at 185 °C under vacuum without catalysts by us [8], and later at 130 °C under argon in the presence of stannous octoate by German researchers [9]. It must, however, be pointed out that the latter technique is not an improvement of that developed by us, since it makes use of a potentially toxic tin compound and from a chemical viewpoint introduces an interfering factor as catalytic agent.

We found that such a polymerization can give rise to a poly( $\epsilon$ -caprolactone) (PCL) layer, ionically bonded to the surface of the granules, which is able to enhance the compatibility between HA and the polymer in composite biomaterials [8]. The ionic bonds between the HA granules and the PCL layer were ascertained detaching the polymer by the neutralization of  $-\text{COO}^-$  with dilute HCl [8]. The bonds were confirmed also by the presence of a band at 1552–1558  $\text{cm}^{-1}$  in the FT-IR spectra of the composites, attributed to the antisymmetric stretching of the carboxylate anion [10]. An initiation mechanism for the synthesis of PCL, both “free” and ionically bonded to the HA surface, was proposed in previous papers [10, 11].

More recently the authors have ascertained that the same reaction occurs also in open system at room temperature [10–12]. It is known that HA is a typical osteoconductive material, able to orient tissue reconstruction towards the growth of osteoblasts, rather than that of fibroblasts [13]. A HA/poly( $\epsilon$ -caprolactone)-*block*-poly(oxyethylene)-*block*-poly( $\epsilon$ -caprolactone) copolymer composite stimulates new bone formation in the rabbit [14]. The macromolecular phases of such composites are totally bioresorbable [8, 14, 15], and the extra-cellular matrix of natural bone may be seen as a composite of HA with collagen and non-collagenous proteins [16]. Therefore, HA/PCL formation, in conditions near to the physiological ones, might be useful for the fabrication of *in situ* temporary scaffolds for bone repair. Moreover, the presence of bioresorbable PCL chains, ionically bonded to the HA surface through  $-\text{COO}^-$  end groups, may favour the adhesion to HA of the newly formed collagen, a macromolecule having  $-\text{COOH}$  groups in its structure. These groups can form ionic bonds with the cationic sites, which remain free on the HA surface after the bioresorption of the PCL chains.

<sup>1</sup>The notation of a chemical group within square brackets indicates the “concept” of the group itself, whichever may be the form, in which it is present in a structure.

## 2. Materials and methods

### 2.1. Materials

CL (Fluka “purum”) was purified by distillation over  $\text{CaH}_2$ , at a pressure of 5 mmHg. The purity of the fraction collected at 96–98 °C was about 99.8%, as checked by gas chromatography. Its specific weight at 20 °C was 1.076 g/ml.

HA was prepared, following the standard procedure for a “biomedical grade” one, by precipitation method [8, 17]. The obtained powders, accurately washed and dried, were annealed for 1 h at 700 °, 900 ° and 1100 °C, respectively, then milled into almost spherical granules.

High purity calcium pyrophosphate (CPP, Aldrich 99.9+%) was used, without further purification, for spectral comparison alone, to demonstrate absence at higher temperatures of its formation, due to excess of  $[\text{HPO}_4]$  groups in the HA lattice for the well known reaction:  $2\text{HPO}_4^{2-} \rightarrow \text{P}_2\text{O}_7^{4-} + \text{H}_2\text{O}$  [18].

### 2.2. Polymerization procedure

The HA was mixed with the monomer in open system, in the weight ratios of 1:2, 1:1 and 2:1, respectively. In particular, 1 g of each HA sample was mixed with 1.86 ml of CL for the first ratio, 1.5 g with 1.39 ml for the second, and 2 g with 0.93 ml for the third one, in order to have a total weight of 3 g in each experiment. The mixture was then allowed to stay some days at room temperature, until a solid powder or mass was formed. To recover both soluble and insoluble polymer fractions, the product was repeatedly extracted with  $\text{CHCl}_3$ , treated twice with HCl and repeatedly extracted again with  $\text{CHCl}_3$ , according to the procedure already used [8].

### 2.3. Analytical techniques

Transmission Fourier-transform infrared (FT-IR) spectra of the three HA samples and of the composites were carried out in sintered KBr pellets, using a Perkin-Elmer 1600 apparatus. Those of both “free” and bonded PCL fractions were carried out casting on a KBr surface the  $\text{CHCl}_3$  solution of each polymer sample. Total reflection FT-IR measurements were carried out on HA and CPP by means of a Perkin Elmer Spectrum One FT-IR Spectrometer; the apparatus was equipped with a Perkin-Elmer Universal ATR Sampling Accessory and a Perkin Elmer Spectrum Spotlight FT-IR Imaging System.

Thermogravimetric analysis (TGA) was performed by a Perkin Elmer TGA6 Thermogravimetric Analyzer, equipped with a Perkin Elmer Thermal Analysis Gas Station (TAGS). The measurements were carried out at the heating rate of 10.00 °C/min under  $\text{N}_2$  flow, in the temperature range between 30 and 1000 °C.

The amount of carbonate group,  $[\text{CO}_3]$ , was evaluated by means of both FT-IR and elemental analysis. In the first case, the ratio of the absorbance of the band between 1390 and 1530  $\text{cm}^{-1}$  to that of the phosphate one at about 575  $\text{cm}^{-1}$  is linearly related to the  $[\text{CO}_3]$  content

within HA. Its computation as weight percentage (wt%) was made through the equation reported in the literature [19].

The elemental analysis of the carbon, used to calculate the amount of  $[\text{CO}_3]$  group present in the HA composition, was performed by an elemental automatic analyser (Finnigan FlashEA 1112, with TCD detector), equipped with a mass spectrometer. The oxidation tube was kept at  $1000^\circ\text{C}$ , the reduction one at  $680^\circ\text{C}$ .

The specific surface area (SSA) values of the HA granules were measured by the Brunauer-Emmet-Teller (BET) method [20] with a Sorptly 1750 “Carlo Erba Strumentazione” analyser. With the term “specific surface area” we indicate the whole surface ( $\text{m}^2$ ) developed per unit of weight (g).

The analysis of porosity was carried out by mercury intrusion with a Pascal 140–240 Thermoquest analyser.

### 3. Results

The FT-IR spectra of the nine composites, obtained by reacting the three HA powders with CL, in the three weight ratios of 1:2, 1:1 and 2:1, show the absorption bands characteristic both of HA and PCL. A typical spectrum is shown in Fig. 1.

The spectrum contains the absorption bands characteristic of HA. The sharp peak at  $3572\text{ cm}^{-1}$  is due to the stretching of the  $\text{O}-\text{H}^-$  ions. The bands of the phosphate groups are the wide band at  $1044\text{ cm}^{-1}$ , with the  $960\text{ cm}^{-1}$  peak of  $\text{P}-\text{O}$  stretching on its shoulder, and the triplet at  $572$ ,  $602$  and  $632\text{ cm}^{-1}$ . The spectrum shows also the typical PCL absorption bands. Most significant are the aliphatic polyester band at  $1734\text{ cm}^{-1}$ , due to  $-\text{C}=\text{O}$  stretching, the doublet at  $2940$  and  $2866\text{ cm}^{-1}$ , due to  $-\text{CH}_2$   $\nu_{\text{as}}$  and  $\nu_{\text{s}}$ , and various less sharp peaks around  $1500\text{ cm}^{-1}$ . It is noteworthy the weak absorption at  $1558\text{ cm}^{-1}$ , attributable to the antisymmetric stretching of the carboxylate anion. These results confirm those obtained both under vacuum at  $185^\circ\text{C}$  [8] and in the preliminary studies in open system at room temperature [10]. The extraction with  $\text{CHCl}_3$  leaves the spectra quite unchanged, only diminishing the intensity of the PCL absorption bands.

The extraction of the reaction products with  $\text{CHCl}_3$ , before and after the treatment with  $\text{HCl}$ , allows the separation of two different fractions of PCL, “free” (Fraction a) and ionically bonded to the HA granules (Fraction b). The weight percentages of the two fractions are reported in Table I, together with the values of SSA, those of the

TABLE I Weight percentages of “free” PCL (Fraction a) and of PCL ionically bonded to the HA granules (Fraction b), in the polymerization with HA annealed at different temperatures ( $T$ ), with different HA to CL weight ratios

Exp.	$T$ ( $^\circ\text{C}$ )	SSA ( $\text{m}^2\text{ g}^{-1}$ )	HA:CL (wt/wt)	TS ( $\text{m}^2$ )	Fraction a (%)	Fraction b (%)	BPUS ( $\% \text{ m}^{-2}$ )
1	1100	$1.11 \pm 0.06$	1:2	$1.11 \pm 0.01$	$87.4 \pm 0.5$	$12.6 \pm 0.5$	$11.351 \pm 0.352$
2	1100	$1.11 \pm 0.06$	1:1	$1.67 \pm 0.01$	$85.2 \pm 0.5$	$14.8 \pm 0.5$	$8.862 \pm 0.248$
3	1100	$1.11 \pm 0.06$	2:1	$2.22 \pm 0.01$	$82.5 \pm 0.5$	$17.5 \pm 0.5$	$7.883 \pm 0.189$
4	900	$1.26 \pm 0.06$	1:2	$1.26 \pm 0.01$	$92.8 \pm 0.5$	$7.2 \pm 0.5$	$5.714 \pm 0.348$
5	900	$1.26 \pm 0.06$	1:1	$1.89 \pm 0.01$	$96.3 \pm 0.5$	$3.7 \pm 0.5$	$1.958 \pm 0.255$
6	900	$1.26 \pm 0.06$	2:1	$2.52 \pm 0.01$	$95.4 \pm 0.5$	$4.6 \pm 0.5$	$1.825 \pm 0.191$
7	700	$17.99 \pm 0.90$	1:2	$17.99 \pm 0.11$	$91.4 \pm 0.5$	$8.6 \pm 0.5$	$0.478 \pm 0.027$
8	700	$17.99 \pm 0.90$	1:1	$26.99 \pm 0.11$	$97.0 \pm 0.5$	$3.0 \pm 0.5$	$0.111 \pm 0.018$
9	700	$17.99 \pm 0.90$	2:1	$35.98 \pm 0.11$	$94.7 \pm 0.5$	$5.3 \pm 0.5$	$0.147 \pm 0.013$

SSA: Specific surface area values of the HA granules. TS: Total surfaces of the granules. BPUS: Percentages of ionically bonded PCL per unit surface.

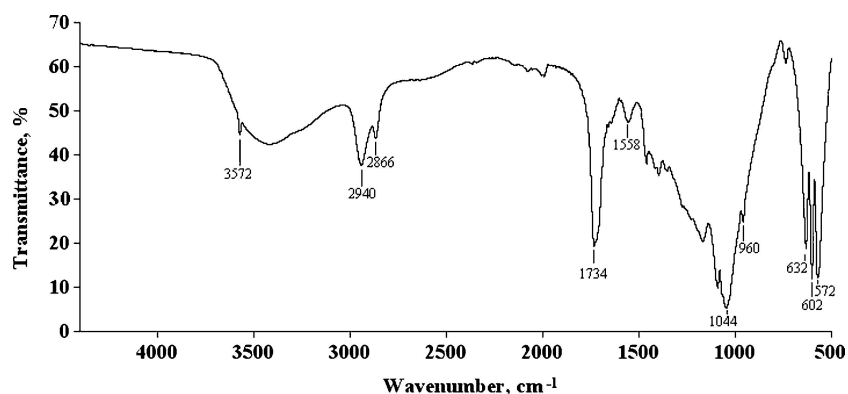


Figure 1 FT-IR spectrum of a composite obtained by reacting HA, annealed for 1 h at  $1100^\circ\text{C}$ , with CL in 1:1 weight ratio (sintered KBr pellet).

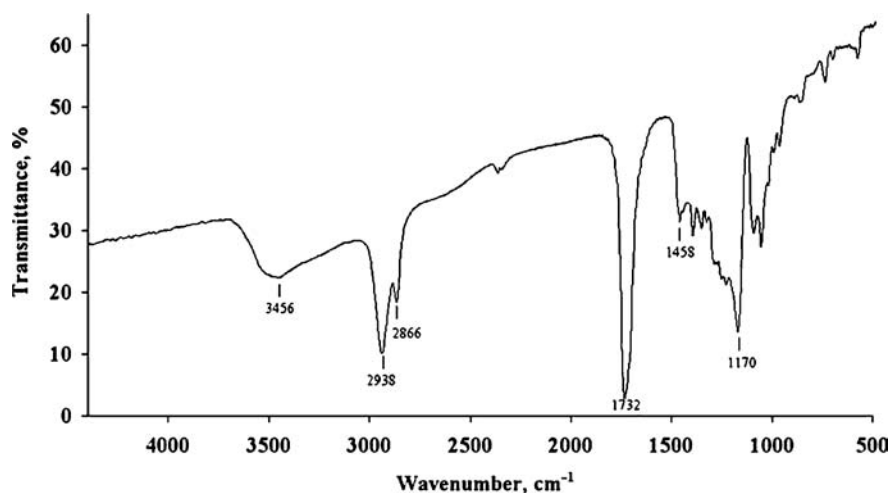


Figure 2 Typical FT-IR spectrum of one of the eighteen fractions listed in Table I (casting on KBr).

total surface of all the HA granules used in each experiment (TS) and the percentages of bonded polymer per unit surface (BPUS).

The FT-IR spectra of fraction b), after detaching with HCl, are not distinguishable from those of fraction a); all of them are typical PCL spectra (Fig. 2). They show, in addition to the wide band of the organic hydroxyls at  $3456\text{ cm}^{-1}$ , the doublet at  $2938$  and  $2866\text{ cm}^{-1}$ , due to methylene  $\nu_{\text{as}}$  and  $\nu_{\text{s}}$ , as well as the absorption at  $1458\text{ cm}^{-1}$ , due to the scissoring of the same group. The spectra show also the typical polyester absorption at  $1732\text{ cm}^{-1}$ , as well as the dense group of peaks under  $1400\text{ cm}^{-1}$ , mostly attributable to the ester groups too, like the  $\nu(\text{C}-\text{O})$  band at  $1170\text{ cm}^{-1}$ . A comparison with Fig. 1 shows the disappearance of both the HA bands and the absorption at  $1558\text{ cm}^{-1}$ .

All the macromolecular materials recovered from both fractions are mixtures of oligomers, having molecular weights too low, to be determined by the viscometric method.

The HA used in our study was a para-stoichiometric one. The prefix para- is used to indicate that the starting composition of the HA powders contains  $[\text{CO}_3]$  and  $[\text{HPO}_4]$  groups that are regular substitutes of  $[\text{PO}_4]$  groups in the crystalline lattice, but have the ratio  $[\text{HPO}_4]/[\text{CO}_3] = 3/2$  [case (3) of the Appendix with  $y/z = 3/2$ ]. Then, after proper annealing of them, it is possible to reach a final composition with the stoichiometric formula  $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ . The HA powders, green and annealed at all temperatures, have shown the absence, in their FT-IR spectra made in KBr pellets, of the absorption peaks at  $915$  and  $745\text{ cm}^{-1}$ , characteristic of a P—O—P vibration present in CPP [21]. A comparison of the total reflection FT-IR spectra of the three HA powders (everyone annealed at one of the three prefixed temperatures and used to start CL polymerization) with that of CPP is given in Fig. 3. In the range of  $1400$  to  $700\text{ cm}^{-1}$ , where the main pyrophosphate absorption bands are present, the spectrum of CPP is totally different from those of the three HA samples. It is therefore

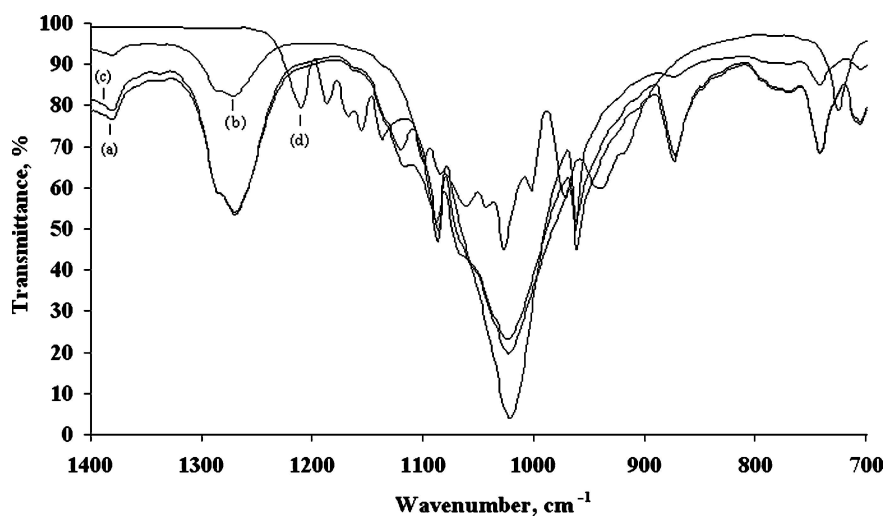


Figure 3 Total reflection FT-IR spectra of HA annealed for 1 h at  $700^\circ\text{C}$  (a), HA annealed for 1 h at  $900^\circ\text{C}$  (b), HA annealed for 1 h at  $1100^\circ\text{C}$  (c) and CPP (d).

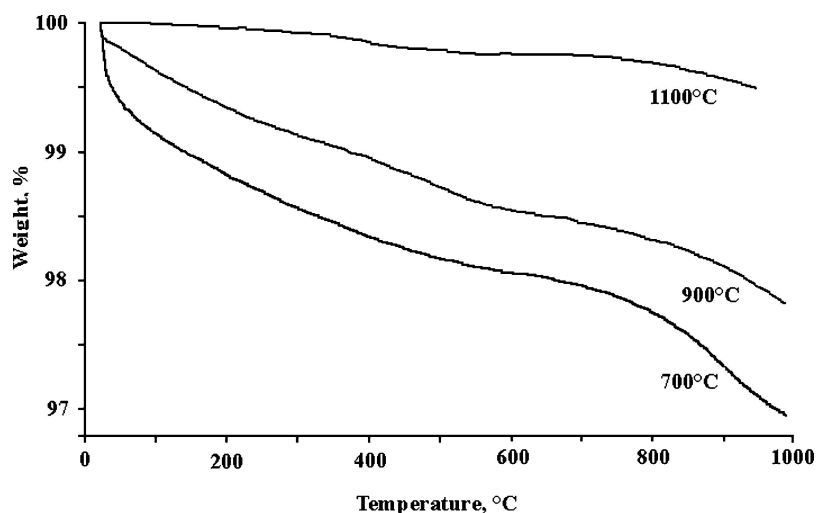


Figure 4 TGA traces of HA annealed for 1 h at 700, 900 and 1100 °C. Heating rate: 10.00 °C/min under N<sub>2</sub> flow.

apparent that the reaction (8) of the Appendix does not occur at any annealing temperature, and then no excess of [HPO<sub>4</sub>] groups is present into the HA lattice.

Moreover, X-ray diffraction on the powders annealed at high temperature (1100 °C) displayed a spectrum with only diffraction peaks belonging to HA. Conversely, no other weak spurious peak of crystalline phases different from HA or humps of the background, indicating presence of amorphous phase of unknown chemical composition, was present. The occurring of the reaction (3) in the HA annealed at both 700 and 900 °C, but not in that annealed at 1100 °C, is confirmed by TGA (see Fig. 4).

The trace of the HA annealed at 1100 °C shows, as expected, no significant weight loss in the range between 30 and 1000 °C, which is totally below the annealing temperature. A greater weight loss is given by the HA annealed at 900 °C, and that annealed at only 700 °C show the sharp signal of a decomposition between 800 and 1000 °C.

To ascertain how much of the two groups, [HPO<sub>4</sub>] and [CO<sub>3</sub>], remained after heating the powders at a certain temperature, resort to chemical and spectrometric investigations became necessary. Because a quantitative investigation on HPO<sub>4</sub><sup>2-</sup> is not easy, analytical evaluation of the amount of carbonate present was attempted. It was carried out by elemental analysis of carbon, as well as by infrared spectroscopy, made by both transmission and total reflection FT-IR. As the starting HA powders were ascertained to be para-stoichiometric, the amount of [HPO<sub>4</sub>] present was simply assumed as the value coming from the equation: [HPO<sub>4</sub>] =  $\frac{3}{2}$ [CO<sub>3</sub>]. The values, both as [CO<sub>3</sub>] content (wt%), coming from the two different investigative techniques (FT-IR and elemental analysis of carbon) applied on the powders utilised for the CL polymerization, are reported in Table II. One can see that the values coming from FT-IR analysis agree sufficiently with those coming from elemental

analysis of carbon, almost as regarding their degrees of magnitude.

Table III shows the absolute differences,  $\Delta$ , between the values, listed in Table I, of any PCL fraction (a or b), obtained with HA annealed at 1100 °C, and of the corresponding one, obtained with HA annealed at the lower temperatures (700 ° and 900 °C). Each  $\Delta$  refers to the experiments performed with the same HA to CL weight ratio. With HA annealed either at 700 °C or at 900 °C, it is possible to observe that the  $\Delta$  differences

TABLE II Values of the [CO<sub>3</sub>] content (wt%), from both FT-IR absorbance spectra and C elemental analyses, for the HA powders annealed at three different temperatures (*T*)

<i>T</i> (°C)	From FT-IR	From carbon elemental analysis
700	0.349(±0.050)	0.231(±0.003)
900	0.153(±0.002)	0.172(±0.005)
1100	0.028(±0.001)	Not detectable

TABLE III Absolute values of the differences between the whole percentages of bonded PCL ( $\Delta$ ) with HA annealed at the temperatures *T*<sub>1</sub> and *T*<sub>2</sub>, and of the differences between the corresponding percentages per unit surface ( $\Delta_{sp}$ ), at different HA to CL weight ratios. The listed values have associated an error of ±0.5

<i>T</i> <sub>1</sub> - <i>T</i> <sub>2</sub> (°C)	HA:CL	$\Delta$	$\Delta_{sp}$
1100-900	1:2	5.4	5.6
1100-700	1:2	4.0	10.9
1100-900	1:1	11.1	6.9
1100-700	1:1	11.8	8.8
1100-900	2:1	12.9	6.1
1100-700	2:1	12.2	7.7

TABLE IV Porosity parameters of the HA powders annealed at the indicated temperatures.

Annealing temperature	700 °C	900 °C	1100 °C
Total cumulative volume (mm <sup>3</sup> /g)	1240.057	1063.990	818.370
Total porosity (% v/v) <sup>a</sup>	58.63	63.461	60.729
Apparent density (g/cm <sup>3</sup> ) (bulk density = 3.12)	1.97	1.91	1.94

<sup>a</sup> Value referred to the volume of mercury penetrated into the whole porosity in comparison with the total volume occupied by the powder with its apparent density.

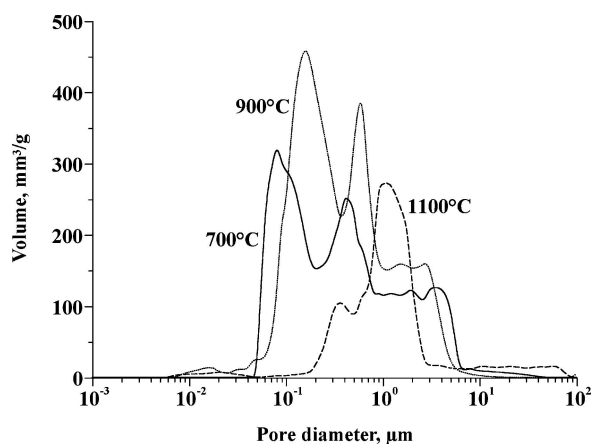


Figure 5 Porosity profiles of the hydroxyapatite powders annealed for 1 h at the indicated temperatures.

are practically the same. On the contrary, the differences between the percentages of bonded PCL per unit surface (BPUS), reported as  $\Delta_{sp}$ , have quite similar values only at 1:1 and 2:1 HA to CL weight ratios. At 1:2 ratio, the  $\Delta_{sp}$  value with HA annealed at 900 °C is a little more than half the  $\Delta_{sp}$  value with HA annealed at 700 °C (see Table III). The comparison between the values of  $\Delta$  and of  $\Delta_{sp}$  points out the role, which the surface area of the granules has in the formation of either “free” or ionically bonded PCL.

Another factor, which can affect the surface reactivity of HA, is the porosity of the granules. It might influence also the percentages of the two PCL fractions, since a PCL chain grown within a pore of sufficiently small diameter might be held adherent, only by capillarity forces, to the inner surface of the pore, even without an ionic bond. The porosity parameters, evaluated by Hg intrusion for the three HA kinds, are listed in Table IV. The corresponding curves of the variation, with the pore diameter, of the total volume able to be filled by Hg, are shown in Fig. 5.

#### 4. Discussion

In the CL polymerization, initiated by the surface hydroxyls of HA under vacuum at 185 °C, the quantity of

“free” PCL, evaluated as about the 0.50% of the total polymer, was negligible with respect to that ionically bonded to the HA surface [22]. Carrying out the reaction in open system at room temperature (Fig. 6), the ring-opening action of water in the reaction (a) enhances its product very much, decreasing that of reaction (b). Moreover, another factor operates to enhance the production of “free” PCL. The presence of the [HPO<sub>4</sub>] and [CO<sub>3</sub>] groups, on the surface of the HA annealed at lower temperatures, was ascertained by both FT-IR and elemental analysis. It must be pointed out that the elemental analysis technique is an analytical method more trustworthy than FT-IR for quantitative determinations. However, as already observed, the values coming from FT-IR agree sufficiently with those coming from elemental analysis of carbon, almost as regarding their degrees of magnitude. These groups behave negatively on the production of bonded polymer. This fact can reflect, in a more general way, on a lower capability of carbonate-apatites to bond with acidic groups (e.g. with carboxylic groups of collagen molecules) than stoichiometric HA. The [HPO<sub>4</sub>] group can initiate the polymerization of CL by the mechanism (c) in Fig. 6, with the formation of further “free” PCL. The [CO<sub>3</sub>] anion can link a water molecule through hydrogen bonds, and then transfer it to a CL ring, starting a “free” propagating chain, according to the mechanism (a). As shown in Table I, the percentages of the “free” PCL are always higher than those of the PCL ionically bonded to HA. From a comparison between the results obtained with the three different kinds of HA, that annealed at 1100 ° seems the most effective to produce PCL ionically bonded to the substrate. Such effectiveness appears more evident from the percentages of BPUS.

Anyway, the influence of the [CO<sub>3</sub>] groups content on the different polymerization mechanisms activated appears to depend more on its variation on a broad scale with varying HA annealing temperature, rather than on its absolute values. The results indicate that only between 900 ° and 1100 ° the surface characteristics of the HA grains undergo a substantial change. The TGA measurements (Fig. 4) show that in this temperature interval there is a sensible weight loss, attributable to the reaction (3) in Appendix. Both at 700 ° and 900 °C, [CO<sub>3</sub>] and [HPO<sub>4</sub>] groups are able to activate the (a) and (c) reaction mechanisms of Fig. 6 in a similar extent; only the drastic decrease of them, reached at 1100 °C, grants for a significant decrease of this activation.

As expected, Table II shows that the higher is the annealing temperature, the lower is the [CO<sub>3</sub>] group content in HA. It was shown above (see Fig. 6) how the presence of [CO<sub>3</sub>], as well as [HPO<sub>4</sub>], groups on the surface of the HA granules can induce the formation of further “free” PCL. Then, the practical absence of [CO<sub>3</sub>] group in the HA annealed at 1100 °C, compared with the significant quantities found in those annealed at lower temperatures (see Table II), can explain the much

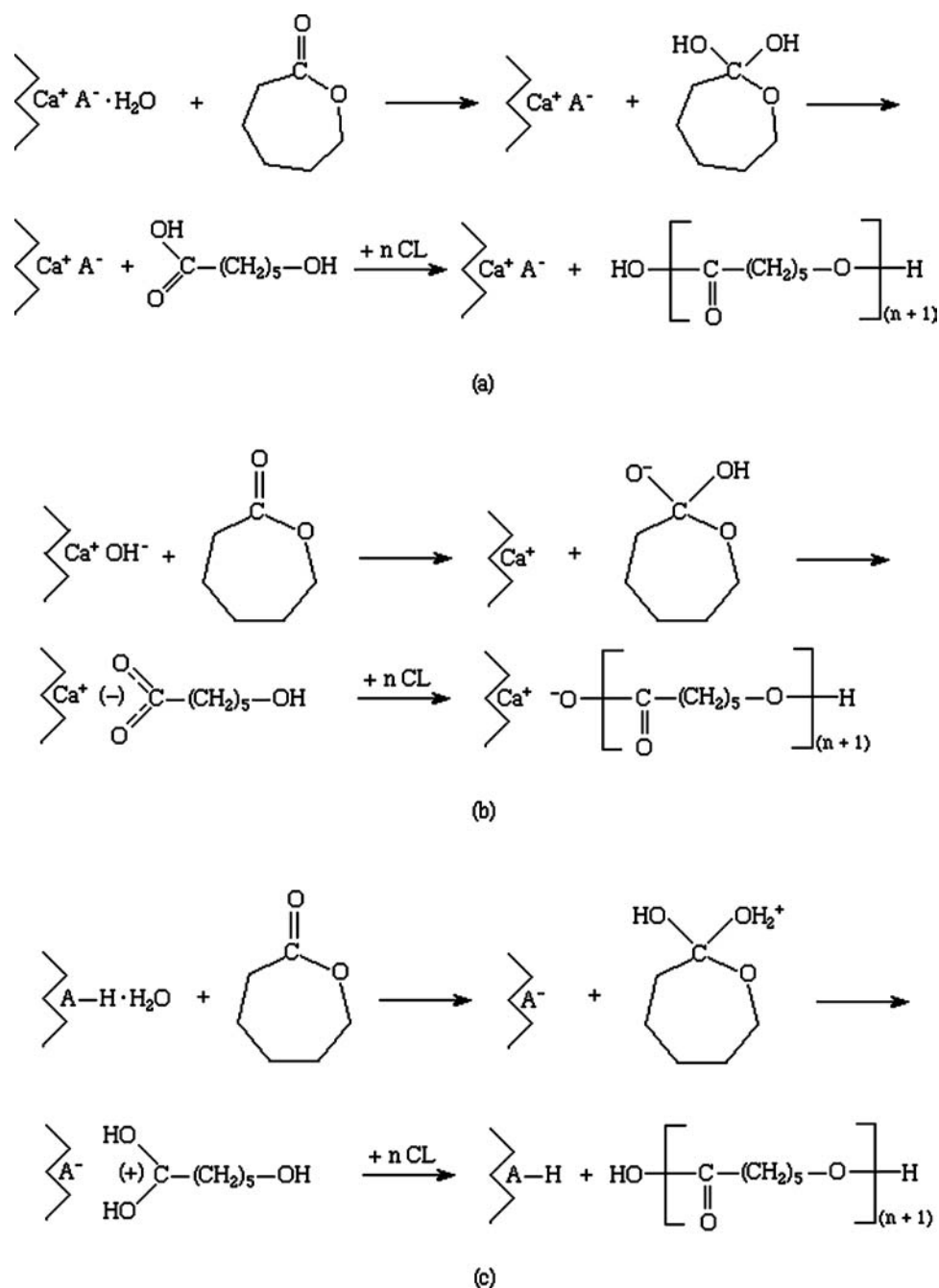


Figure 6 Possible initiation mechanisms for the polymerization of CL by HA at room temperature in open system: (a) "free" PCL from initiation with the participation of water, [A] being either [OH] or [CO<sub>3</sub>]; (b) PCL ionically bonded to the granule surface from basic initiation; (c) "free" PCL from acid initiation.

higher percentages of PCL ionically bonded to the HA granules in the experiments 1–3 of Table I.

Nevertheless, Table II shows a whole [CO<sub>3</sub>] content at 900°C lower than at 700°C, although in Table I the whole percentages of bonded and "free" PCL produced by the two HA kinds are approximately the same. This apparent discrepancy can be explained by the fact that the initiation of not bonded PCL chains, involving [CO<sub>3</sub>] and [HPO<sub>4</sub>] groups, occurs only on the surface of the HA granules. Between 700 and 900°C, the annealing

can induce rearrangements in the HA lattice. It is likely that, as the total amount of these groups diminishes, they migrate towards the surface of the grains, since the internal lattice tends to increase its thermodynamic stability, by minimising the number of defects. Although the condensation reaction (3) of the Appendix can occur easier on the surface, these groups, that substitute the regular [PO<sub>4</sub>] ones, are pushed outwards in substitution of their corresponding groups: [CO<sub>3</sub>] for the [CO<sub>3</sub>] disappeared as gaseous CO<sub>2</sub>, [HPO<sub>4</sub>] for the [HPO<sub>4</sub>]

transformed into regular  $[\text{PO}_4]$ . Therefore, the amount of groups, able to activate the reaction mechanisms (a) and (c) of Fig. 6, remains substantially the same at both temperatures. Another possible explanation is the value of specific surface area (SSA) of the HA annealed at  $700^\circ\text{C}$ , much higher than those of the other HA kinds (see Table I, experiments 7–9). So, although the concentration, on the surface of the granules, of the groups able to start bonded PCL chains through the mechanism (b) is lower than in the HA annealed at  $900^\circ\text{C}$ , the greater total surfaces (TS) make their absolute quantities quite similar. This explanation is supported also by the values of the percentages of bonded PCL per unit surface (BPUS), which are less than  $10^{-1}$  times for the HA annealed at  $700^\circ\text{C}$ , than for that annealed at  $900^\circ\text{C}$  (Table I). Obviously, both factors can act at the same time to obtain the same effect.

As regarding the values of BPUS obtained with each kind of HA at different ceramics to monomer ratios, one can see from Table I that they decrease much more from the 1:2 to the 1:1 ratio, than from the 1:1 to the 2:1 ratio. It means that HA quantities less than CL ones favour the formation of bonded PCL, whereas HA quantities equal or more than CL ones favour the formation of non-bonded PCL. Such behaviour can be explained by taking in account the kinetics of the different reactions that occur simultaneously. Reaction (b) of Fig. 6, which is the only one leading to PCL ionically bonded to HA, has the lowest kinetic constant, as it is deducible from the values of fraction b) in Table I, always smaller than those of fraction a) are. Nevertheless, its reaction rate is strongly dependent on the concentration of the CL present on the surface of the granules. On the contrary, the true initiator of reaction (a), and, somehow, also of reaction (c), is the water present in the reaction mixture, which depends only on the environmental humidity, which was equal for all the experiments, carried out together at the same time. The fact that the water can initiate the polymerization also in a layer, relatively remote from the HA surface, makes the formation of “free” PCL quite independent of the HA to CL ratio. Another experimental datum points out the importance of the role of water as a limiting factor in both mechanisms (a) and (c). Indeed, when the polymerization is carried out under vacuum at  $185^\circ\text{C}$ , the amount of PCL not bonded ionically to the HA structure is always negligible, independently of the temperature, at which HA was annealed [8, 22]. This result can be easily explained as follows: the only water present in such conditions is the not removable moisture, and its complete consumption makes no more effective the two mechanisms producing “free” PCL (see Fig. 6). Since the biochemical reactions, leading to the formation of organic tissues around the natural bone, occur in an aqueous environment, such an active role of water in the CL polymerisation induced by HA must be taken in account. The biochemical addition of another amino acid to a growing polypeptide

is not a ring-opening transesterification, like the propagation of the PCL chain; anyway, one may see it as the displacement of an ester bond by an amino group.

There is, however, a number of contributory causes in producing the probability of successful contact among molecules of CL and activating sites on HA grains. They are, e.g., the increase of the specific gravity of the HA grains with annealing temperature (which brings to a reduction of the developed surface), the increase of the grain size for sintering action with annealing temperature, *etc.* These factors can explain the small fluctuations found for the values of  $\Delta$  difference of Table III. It must be pointed out that the reduction of both specific and total surface is much greater between  $700^\circ$  and  $900^\circ\text{C}$  than between  $900^\circ$  and  $1100^\circ\text{C}$  (see Table I), so causing a mutual compensation of the differences. The  $\Delta_{\text{sp}}$  differences, shown in Table III, are more representative of the reactivity of the different HA kinds, being referred to the unit surface. The differences observed between the HA annealed at  $1100^\circ$  and at  $700^\circ\text{C}$ , always greater than those between  $1100^\circ$  and  $900^\circ\text{C}$ , confirm that the surface reactivity of HA towards CL, through mechanism (b) of Fig. 6, increases with decreasing  $[\text{CO}_3]$  and  $[\text{HPO}_4]$  groups on the surface.

The highest values of  $\Delta$  difference are present when the HA to CL weight ratios are 1:1 and 2:1. This fact is due mainly to the increasing values of total HA surface with decreasing the quantity of monomer in the reacting mixture (see Table I). Indeed, the corresponding values of  $\Delta_{\text{sp}}$  show a different behaviour: between  $1100^\circ$  and  $900^\circ\text{C}$  they have about the same order of magnitude at the different HA to CL ratios, whereas between  $1100^\circ$  and  $700^\circ\text{C}$  they decrease with increasing HA to CL ratio. Such behaviour may be due to the fact that the HA annealed at  $700^\circ\text{C}$  is the most representative one of a not fired HA. As one can see in Fig. 4, it shows, in addition to the greatest weight loss between  $800^\circ$  and  $1000^\circ\text{C}$ , due to the  $[\text{CO}_3]$  and  $[\text{HPO}_4]$  decomposition, also a significant weight loss between  $30^\circ$  and  $200^\circ\text{C}$ , attributable to the water originally present on the HA surface. In the TGA trace of the HA annealed at  $900^\circ\text{C}$  the first weight loss is also present, although in a minor extent, while the second one is much less pronounced. So, for this kind of HA, the water, involved in the (a) and (c) mechanisms of Fig. 6, originates near exclusively from the environmental humidity, which is independent of the surface characteristics. On the contrary, for the HA annealed at  $700^\circ\text{C}$ , the quantity of water present is one of the characteristics of the surface. It must be pointed out, however, that this water is adsorbed very weakly on the HA surface, and can be removed nearly completely simply by pumping HA under vacuum. Indeed, the CL polymerization by HA under vacuum at  $185^\circ\text{C}$  gives only traces of “free” PCL [8, 22].

As concerns the variation of the values of  $\Delta$  difference with the same HA to CL ratio, it has to be considered



that, of the total number  $N_0$  of CL molecules present in the same volume  $V$ , those able to reach an activating site are only  $N_S < N_0$ . Then, the number of activated chains is proportional to  $N_S = 4\pi\alpha D_S(T_{HA})N_G r_G^2$ . In the above equation,  $N_G$  is the number of the grains;  $r_G$  is their mean radius;  $D_S(T_{HA})$  is the surface density of the activating sites, i.e. their number per unity of surface, strongly dependent on the annealing temperature  $T_{HA}$ , as deducible from the BPUS values (see Table I). As concerns the factor  $\alpha$ , it accounts the CL molecules displaced inside a multilayer shell all around each grain; these molecules can contribute in different ways to the reaction mechanism (b) in Fig. 6.

All other molecules ( $N_0 - N_S$ ), the most of them, follow the other mechanisms, because they are in close contact each other. Obviously, new chains of PCL can be initiated in the bulk only by dispersed water molecules; however, additional “free” PCL can arise also from chain transfer reactions. Such reactions were proposed to be present also in the polymerisation carried out at 185 °C under vacuum [8], where the mechanisms (a) and (c) can be due only to residual moisture. The value of  $N_S$  increases with the increase of the amount  $N_G$  of grains of HA introduced, however in a scanty extent, as demonstrated by the values of  $\Delta$  difference coming from HA to CL weight ratios 1:1 and 2:1.

The attribution of a greater influence to this trend to the single  $[\text{CO}_3]$  group or to  $[\text{HPO}_4]$  group or to quantify how much one is more active than the other is not possible at the moment with the data at disposal. However, it has to be pointed out that in this powder the amount presumably present of  $[\text{HPO}_4]$  group is greater than that of  $[\text{CO}_3]$  group.

The curves in Fig. 5 indicate that the whole porosity trends towards a decrease of smaller pores and towards the formation of larger pores with increasing the annealing temperature. This was expected as a direct consequence of sintering processes. Some interesting parameters are reported in Table IV. It must be pointed out, however, that the whole porosity values are not totally significant. Indeed, a portion of the measured porosity (that at highest sizes) is not really present within each single grain, but rather it comes from the interstices formed by the random arrangement of packing assumed by the granules of the powder that fills the measurement cell of the porosimeter. These interstices have a higher dimensional limit that corresponds to the same dimensional order of magnitude as that of the grains. Their lower dimensional limit is instead very undefined. During the measurements, the mercury of the porosimeter penetrates into these interstices and the instrumental measurement is not able to distinguish this mercury from that penetrated into real pores of the grains.

It is likely that the porosity with greater seeming pore diameter be due mainly to these interstices, whose volume increases with increasing the HA grains dimensions. It is known that HA crystallite size increases with

firing temperature [8]. This fact contributes on one side to decrease the total porosity; on the other side, to increase the volume of the seeming pores with greater diameter in the three curves of Fig. 5, volume that is greater for the HA annealed at higher temperatures. As concerns the grains in themselves, it is known that they increase their volume and form clusters with increasing their annealing temperature [8]; consequently, the overall specific surface of the powders decreases.

From the above considerations, it follows that the whole porosity measured by mercury intrusion includes also a spurious contribute, which is not quantifiable from the data available in this work, and which depends strongly on both the shapes of the grains and the roughness of their surfaces. Nevertheless, Table IV shows that the total porosity values remain substantially constant with varying the annealing temperature. As concerns the pores with finest size, it must be remarked that there is a threshold steric factor that imposes a dimensional limit of the pores, under which the CL monomer molecules cannot enter the pore. This limit corresponds to the diameter of the greater cross-section of the CL molecule (in its ring configuration), approximately calculated to be 0.15  $\mu\text{m}$ . This also is a portion of porosity, whose developed surface has to be excluded as possible reacting area. It is possible to say that there is no clear distinction between the actual exterior surface of the grains and that one existing within the pores. Taking into account all the above considerations, an evaluation of the amount of the surface developed by all the pores that theoretically are able to adsorb monomers by capillarity has established to be no more than about 30% of the total surface. This accent could have validity provided that the porosity would introduce some different influence (if any) on the behaviour of the ionically bonded PCL molecules, event however not demonstrated. The data arising from the porosity measurements do not allow to state whether there are pores able to link firmly PCL chains only by capillarity. However, the fact that all PCL bonded to HA can be detached only by treating with dilute HCl indicates undoubtedly that there are not.

## 5. Conclusions

The results obtained give interesting information about the ability of HA granules to form a “compatibilizing layer” of ionically bonded PCL on their surface [22] also in open system at room temperature. In particular, we have found that a “stoichiometric” HA, annealed for 1 h at 1100 °C, is the more suitable one to attain this purpose, since it is composed by nearly pure hydroxyl pentacalcium triphosphate. One must remark the importance of the formation of bioresorbable PCL chains, ionically bonded to the HA surface through  $-\text{COO}^-$  end groups. Such chains, although very short,

may favour the adhesion between the HA grains and the newly formed collagen. Indeed, when the PCL chains are completely reabsorbed by the surrounding tissues, the cationic sites, left free on the HA surface, can form ionic bonds with the  $-\text{COOH}$  groups, present along the collagen polypeptide chains.

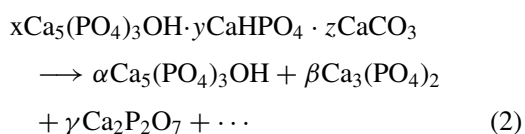
## Appendix

An actual “biomedical grade” HA can normally undergo substitution of  $\text{PO}_4^{3-}$  ions with  $\text{CO}_3^{2-}$  and  $\text{HPO}_4^{2-}$  ones. Then, its stoichiometric formula can be written as:



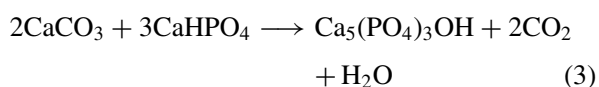
The presence of such substituting groups comes from synthesis, but, if their stoichiometric ratio was respected during the synthesis time, they disappear when annealed at temperatures higher than  $1000^\circ\text{C}$ , as shown by TGA and by the tests for the presence of carbon.

Heating a modified HA will cause reactions, strongly dependent on  $x$ ,  $y$  and  $z$  values, as well as on temperature, which can lead to a series of products:

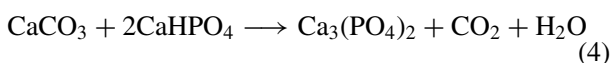


There are special conditions for preferential compound formation, as shown by the following reactions [18].

For  $y/z = 3/2$  the  $y$  and  $z$  substituting groups can react, giving rise to pure HA:



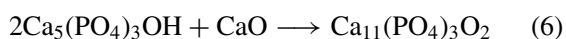
For  $y/z = 2$  we have:



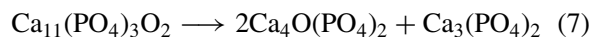
If  $y = 0$ , the carbonate present gives rise to:



This is a calcium rich hydroxyapatite; the CaO formed can in its turn produce, at a temperature lying between  $1200^\circ$  and  $1300^\circ\text{C}$ , the following reaction inside the apatite lattice:



This intermediate compound decomposes very easily with temperature, according to the following reaction:

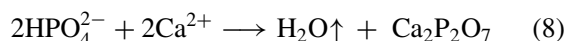


The compounds, formed as shown in Equation 7, leave the apatite lattice producing separate phases.

In Equation 3 the starting HA is chemically stoichiometric, so that the heating simply restores its original composition, leading to pure HA.

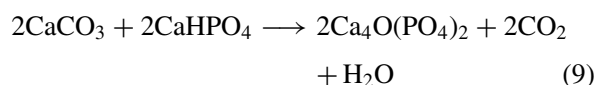
Equation 4 is another special example of substituted HA, chemically non-stoichiometric, the heating of which leads to formation of tricalcium phosphate (TCP).

If  $z = 0$ , the hydroxyapatite powder will result polluted with calcium pyrophosphate, the formation of which occurs from pyrolysis of the  $y$  fractional part:



Where the reacting ions are present into the HA lattice, while the calcium pyrophosphate (CPP) is formed as a separate phase.

If  $y/z = 1$ , the hydroxyapatite powder will result polluted with tetracalcium oxy-phosphate (TeCP):



However, the TeCP formation shown in Equation 9 occurs only at temperatures above  $1200^\circ\text{C}$ ; at lower temperatures, as the three ones used in the present work, separate structures of TCP and CaO are formed. It must be noted that the latter is a substance quite dangerous in biomedical applications. Indeed, it causes an excess of  $\text{Ca}^{2+}$  in the neighbouring tissues, so altering their electrolytic equilibrium. In addition, the strong pH enhancement induced by that alkaline-earth oxide, or by the corresponding hydroxide  $\text{Ca}(\text{OH})_2$ , can seriously damage the cells. It is obvious that, for intermediate values of  $x$ ,  $y$  and  $z$ , a mixture of non-apatitic substances will be present, in many cases formed by involving partially also  $x$  fraction.

## Acknowledgements

The authors thank Dr. Anna Mevoli of ENEA Centro Ricerche, Brindisi (I) and Dr. Pierluigi Buldini of ISTE-CNR, Faenza (I), for having carried out the elemental analysis of carbon, as well as Ms. Andreana Piancastelli of ISTE-CNR, Faenza (I), for having carried out the porosity and specific surface instrumental measurements.

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Received 20 May 2004  
and accepted 6 June 2005