J. Mater. Environ. Sci. 7 (3) (2016) 761-769
ISSN : 2028-2508
CODEN: JMESC

Novel Tricomponenets composites Films From Polylactic Acid/ Hydroxyapatite/ Poly- Caprolactone Suitable For Biomedical Applications

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Received 12 Nov 2015, Revised 14 Jan 2016, Accepted 25 Jan 2016
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Abstract
This study focuses on the development of a composite based on polylactic acid (PLA) and treated hydroxyapatite (HAp_t). The use of poly-caprolactone (PCL) as an adjuvant has significantly improved the resistance and the rigidity of the composite, which could extend the physico-chemical and biological applications of this material. The amount of PCL needed to optimize the traction and physico- chemical properties of the membranes were evaluated. Several methods for the preparation of different types of composites were developed. The transmission electron microscopy (SEM) analysis results showed a textural uniformity of the membranes. The physico-chemical studies have allowed providing mechanisms of interactions between composites phases.

Keywords: Composite, Hydroxyapatite, Poly-l-lactic acid, Polycaprolactone, Biodegradable, Biomaterials.

1. Introduction
Hydroxyapatite is the major inorganic component of human hard tissues. It is often used in biomedical applications by reason of excellent cell adhesion due to the favorable adsorption capacity of the HAp surface for bioactive substances such as cell-adhesive proteins [1]. The use of hydroxyapatite combined with a suitable polymer may further improve osteo-generating properties of this material. Therefore, HAp and its composites with polymers have been widely used in orthopedic and dental applications. Other important applications of HAp are cell culture substrates to study cell behavior, supports for drug delivery [2], and cell scaffolds for tissue engineering [3].
The Polylactic acid (PLA) has been used successfully as a polymeric biomaterial for fastening devices in orthopedic surgery, because of its biocompatibility and bioresorbability. Several attempt to improve the PLA mechanical properties by blending it with other polymers and composites have been presented in several studies [4- 6]. In one study it was shown that, the fragility of PLA [7] could be enhanced by blending it with Poly-caprolactone (PCL) which is a bioresorbable polymer and flexible with a low glass transition temperature (60 °C) [8, 9]. However, PLA is better than PCL in terms of cell adhesion and proliferation, due to its hydrophilic properties [7]. The polycaprolactone (PCL) is hydrophobic, it has no physiological active site, making it unfavorable for cell growth when comes into contact with the living body. The addition of PCL was proposed to improve the physicochemical properties of PLA. In this paper, we describe the synthesis and characterization of a tricomponents composite of HAp_t/ PLA/ PCL.
Currently, there is a great difficulty to develop an ideal material for the substitution of damaged bone. As we know, perfect substitution materials for bone repair should have good biocompatibility and a suitable biodegradation rate as well as higher mechanical property to support the growth of new bone tissue.

Hydroxyapatite (HAp), which is the main inorganic constituent of bone, has been extensively investigated due to its excellent biocompatibility and bioactivity with human tissue [10, 11]. Especially, a nano-scaled HAp with extraordinary properties such as high surface area to volume ratio and ultra fine structure similar to that of biological apatite, which is of a great effect on cell-biomaterial interaction [12], has been reported to be used for the treatment of bone defects, and it could bond to living bone in implanted areas [13 ,14]. However, the fragility and low mechanical strength of n-HAp made it unsuitable to be applied in load-bearing sites [15, 16]. Thus, many scientists turn to the researches on n-HAp/organic polymer composites [17, 18].

2. Experimental

2.1. Materials

Hydroxyapatite (HAp_t) was synthesized at our laboratory. The polylactic acid (PLA) (99 %), poly-caprolactone (PCL) (99 %), Calcium Nitrate $Ca(NO_3)_2.4H_2O$ (99%), Ammonium Hydrogen Phosphate $(NH_4)_2HPO_4$(99%), and Dimethylformamide (DMF) were purchased from Aldrich. High purity distilled water was used throughout the whole experiment.

2.2. Synthesis of membrane HApt/PLA /PCL

Hydroxyapatite was prepared by wet process using diammonium phosphate $[(NH_4)_2HPO_4]$, calcium nitrate$[Ca(NO_3)_2.4H_2O]$ and polyethylene glycol 1000 (PEG-1000). An aqueous solution of (PEG-1000) was added in variable proportions, to n aqueous solution of of $Ca(NO_3)_2.4H_2O$. After the solution of the polymer and calcium were well mixed, the phosphate solution was added drop by drop. The reaction mixture was stirred for 30 minutes. The resulting precipitate was filtered, washed and dried at 50 °C [19- 21]. In the second step, the composites were prepared using different proportions of HApt /PLA/PCL (50/50/00, 60/40/00, 70/30/00, 50/10/40, 50/20/30, 50/30/20 and 50/40/10). Polycaprolactone was first dissolved in ethyl acetate, then a solution of polylactic acid was prepared in dimethylformamide and added to the PCL solution. The mixture was heated to a temperature of 40 ° C for 1 hr. At the end of the heating period a dispersion of hydroxyapatite / (PEG 1000) in was DMF added to the PLA/PCL solution. The produced mixture was stirred for 5 hours at 40 °C then poured into a petri dish and dried at room temperature overnight to produce a very thin membrane. A schematic diagram of the process is depicted in Fig. 1.

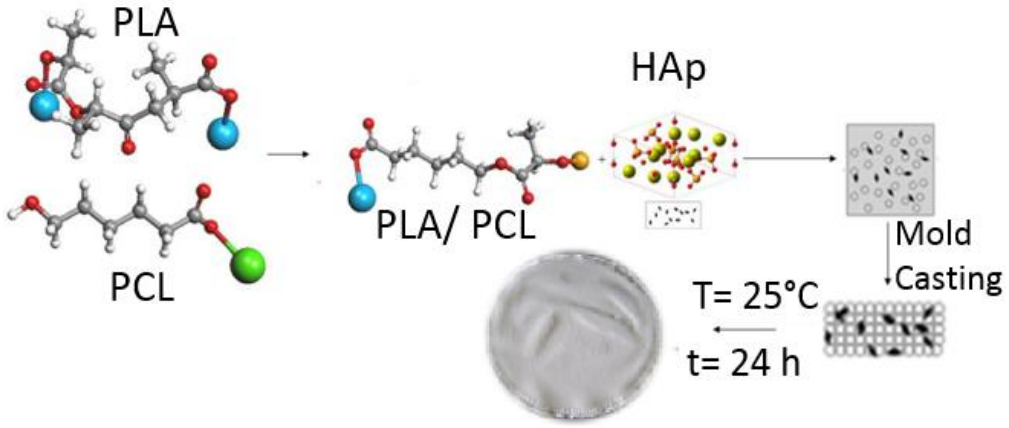

Figure 1: Schematic representation of synthesis route of composite.

2.3. Measurements

The film analysis was performed using several spectroscopic and microscopic methods, ATR-FTIR was performed using a Schimadzu FT-IR 300 series instrument (Shimadzu Scientific Instruments). Emission-scanning electron microscopy (SEM) was performed using a SU 8020, 3.0 KV SE(U). Standard thermogravimetric analysis was performed on the composite using a TA Instrument (TGA Q500 and Q50), with a temperature range of 20–900 °C at a heating rate of 10 °C/min. Reaction products mostly dissolved in the aqueous phase and this phase was analyzed by the total organic carbon (TOC) analysis (TOC/ TN Analyzer multi N/C 2100/2100). Instead of total organic carbon (TOC), total carbon (TC) was used to represent the carbon content into the aqueous phase, because this value covers all carbon (organic and inorganic) existing in the aqueous effluent, which is needed to close the carbon balance.

2.4. Studies in vitro swelling and biodegradable composites

Swelling and biodegradability of composites were studied by immersing the membranes in water at 37°C and in a biological medium PBS at a pH of 7.4). The samples were dried and weighed (W1). The water absorption (expressed in percentage) was calculated by comparing the initial weight (W0) with the wet weight after swelling (W1) as shown in equation (1).

$$\text{Water absorption}(\%)=[(W_1 - W_0)/ W_0]100 \qquad (1)$$

The mass loss was calculated by comparing the initial weight(W_0) with the weight after immersion in the biological medium, and dryingat40 °Cfor30 min (W_2) as shown in equation(2).

$$\text{Weight loss}(\%)= [(W_2 - W_0) /W_0]100 \qquad (2)$$

3. Results and Discussion

3.1. FTIR analysis

Infrared absorption spectra Fourier transforms of the different compositions are presented in Fig. 2. Figure 2a also shows the IR spectrum of the PLA, the absorption band of the carbonyl group (CO) is located to1757 cm^{-1}. The characteristic absorption bands of C-H bonds of methyl group of PLA are located at 2994cm^{-1} and 2946 cm^{-1}. The band at 3503 cm^{-1} could be attributed to the hydroxyl (OH) groups of the polymers.

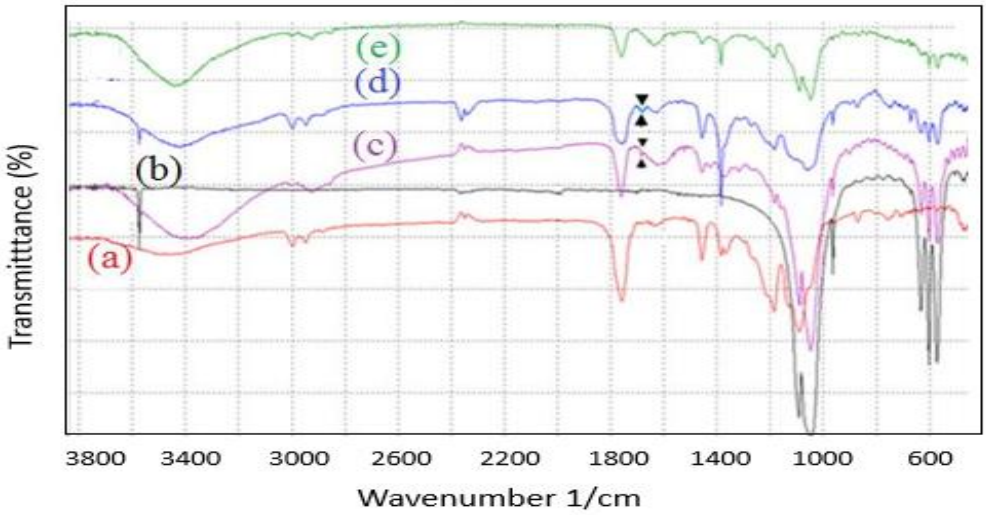

Figure 2: FTIR spectra of the HAp/PLA composite, with molar ratios: a=PLA, b= HAp, c = 70/30, d =60/40 and e=50/50.

Figure.2.b also shows the IR spectrum of hydroxyapatite HAp_t. It is consensus on the sharp bands at 3572 cm^{-1} and 630 cm^{-1} due the OH stretching and bending modes in crystal lattice of HAp, respectively. However, the bands corresponding to PO_4^{3-} groups of apatite network are shown at frequencies of 1090 to 1047,962,602 and 572 cm^{-1}.Figure.2.c,d and e shows the IR spectra of composites based hydroxyapatite at weight ratios of , c = 70/30, d = 60/40 and e= 50/50.As seen in the Figure, the absorption band of the carbonyl group (CO)is located to 1757 cm^{-1}. The characteristic absorption bands of C-H bonds of methyl group of PLA are located at 2994 cm-1 and 2946 cm-1. The band at 3503 cm^{-1}could be attributed to the hydroxyl (OH) groups of the polymers. Whoever, the bands of the PO_4^{3-} at frequencies of 1090 to 1047 cm^{-1},962 cm^{-1},602 $cm^{-1}$and 572 cm^{-1}.FT-IR analysis HAp_t /PLA membranes show the appearance of a new band near 1683 cm-1.This band could be a attributed to the carboxyl group of PLA complexed with Ca^{2+} of HAp_t. In addition, the characteristic absorption band of hydroxide ions at 3550 $cm^{-1}$was not changed [22].

From the infrared absorption the interactions between the composite components are as shown in the diagram depicted in Fig.3. Craboxyl groups form an octahedral complex with the calcium of HAp_t.

Indeed the calcium ions are aligned along a hexagonal shaft (columns for mingdense of calcium atoms Ca1).Other Ca2ionssurroundedbythephosphategroupsto form less dense columns, in which are located the hydroxyl anions.

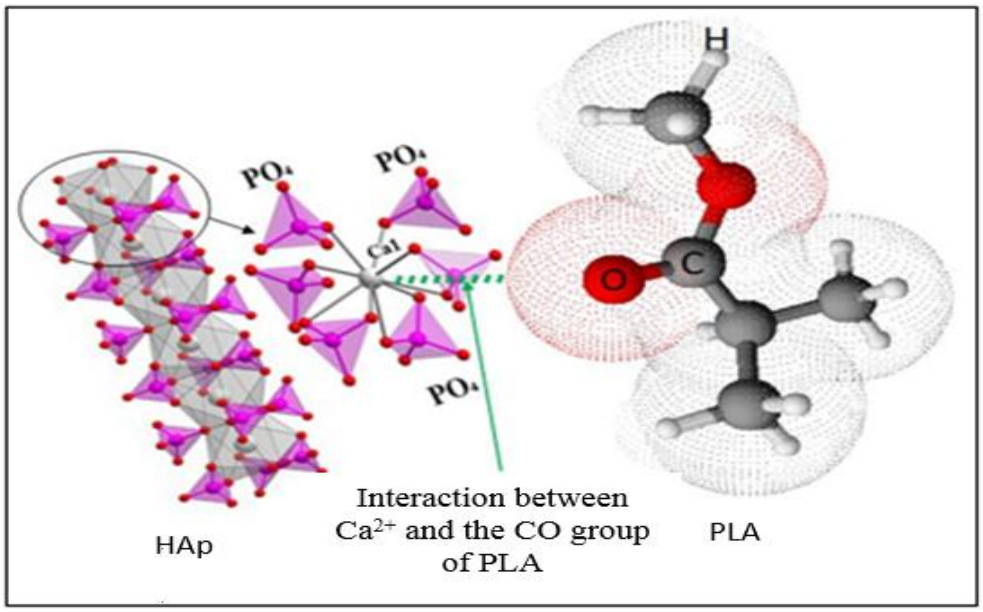

Figure 3: **A s**chematic model of the complexation between the carboxyl groups of PLA and $Ca^{2+}$of HAp_t.

The mechanism of shape memory effect in polymers may be that thermoplastic shape memory polymers have at least two separated phases, namely, stationary phase and reversible phase, where the domains with the highest thermal transition stabilize the permanent shape and a second phase having another thermal transition serves as switch. The shape fixity of amorphous PLA polymers depends on a random winding of molecular chains. However, the shape memory effect of composite based hydroxyapatite is improved obviously. Therefore, it is further indicated that there was physical crosslinking by connection of Ca bonding hydroxyapatite and PLA. The amorphous PLA polymer in the composite was selected as Ca groups in HAp_t particle was designed as shown in Figure 3. The molecular chains of PLA polymer are random winding and prevent reversible phase from bringing irreversible strain that depends on the physical winding during inter- transition from glassy state to rubbery state. However, in the composite the interpenetrating networks were formed due to a physical crosslinking by the Ca bonding, which served as a stationary phase during shape memory recovery, as shown in Figure 3. Interaction between ions Ca1and the COOH of the polymers lightly changes the environment of the carbonyl group, which could be responsible for the appearance of the new absorption band of the C=O group as shown above: the strong carbonyl band with normal frequency is unaffected by the HAp_t/ PLA interactions, however the new low intensity band is corresponding to those influenced by Ca ions and COOH interaction.

3.2. Improvement the physical properties of the composite membranes

The SEM micrographs of HAp modified with polyethylene glycol (HAp_t) is shown in Fig. 4. The small particles are seen agglomerated with an average diameter of 50–60 nm. The SEM image shows the presence of pores, these pores are beneficial for the circulation of the physiological fluid throughout the coatings when it is used as a biomaterial in bone implantation. Pure HAp_t sintered at 900 °C for 2 h in stagnant air exhibits the morphology of clusters of grains structure as shown in Fig. 4.

The use of PLA with single crystals of treated hydroxyapatite provides thin membranes with low mechanical resistance, having varying random shapes, ranging from 1 to 4 cm2 of surface. This disadvantage was solved by using a mixture of two the polymers PLA and PLC in different proportions of.

The results were very satisfactory and the developed composite membranes acquired very good mechanical and physical properties. The mechanical properties of HAp_t/ PLA composites were improved by adding PCL, a bioresorbable and flexible polymer. The PCL was used as a plasticizer to reduce brittleness of PLA. It may play a role of armoring thereon. The mixture of PLA/PCL polymer with the HAp_t was thus envisaged to improve the physicochemical properties of the processed composites [8].

The imaging scanning electron microscopy confirmed the miscibility of the mixture of two organic-inorganic matrices HAp_t/ PLA/ PCL with molar ratios: a= 50/ 50/ 00 and b= 50/ 20/ 30.

A shown in Fig. 4 the fine particles of hydroxyapatite (about 20 microns) are dispersed homogeneously.

The morphological and chemical properties of synthetic hydroxyapatite and composite based hydroxyapatite could be modulated by varying the method and conditions of synthesis. Several different methods that could be used to synthesize HAp are reported in the literature including chemical precipitation [19], hydrothermal techniques [23], sol–gel, solid state and mechano- chemical methods [24, 25].

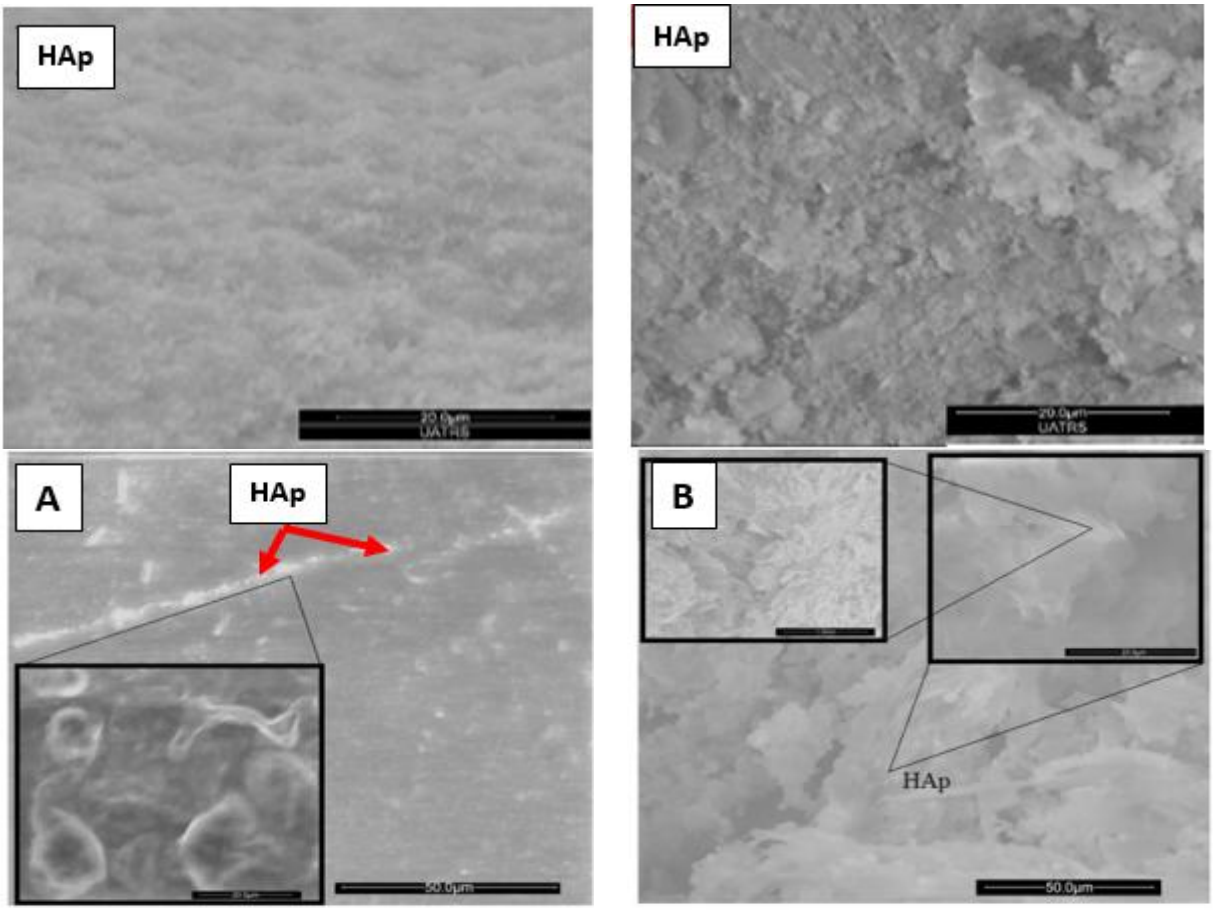

Figure 4: SEM micrographs of two membranes surfaces of HAp_t/PLA/PCL with a molar ratio of a = 50/50/00 and b =50/20/30.

According to the results of SEM, we investigated the influence of the rate of PLA on the particle size, we found that the particle size of HAp increases when the rate of PLA decreases. Dependence is linear in the range studied. This is probably due to the fact that a higher concentration leads to a dispersion of HAp particles inside the polymer and prevents agglomeration of HAp particles (Fig 5).

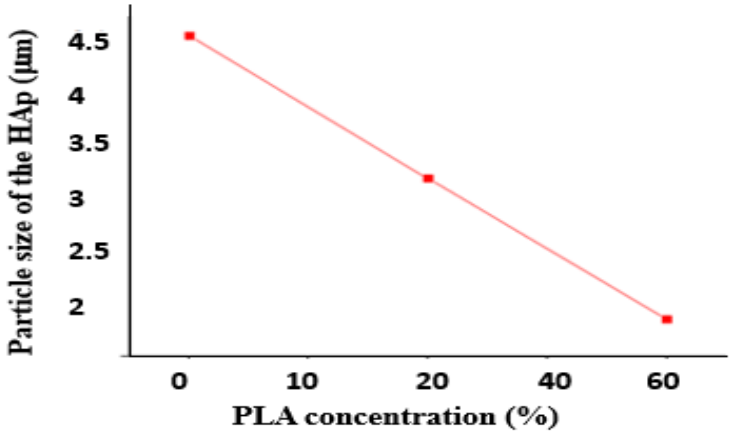

Figure 5: particle size of HAp.

3.4. Thermal study of composite membranes HAp$_t$/ PLA/ PCL

The thermal stability of composites HAp$_t$/ PLA and HAp$_t$ / PLA/ PCL, between 20 °C and 1000 ° C was studied using thermogravimetric analysis under inert gas. The TG/ DTA analytical curves of PLA and PLA/ PCL, HAp$_t$ and composite based of HAp$_t$are shown in Fig.6.

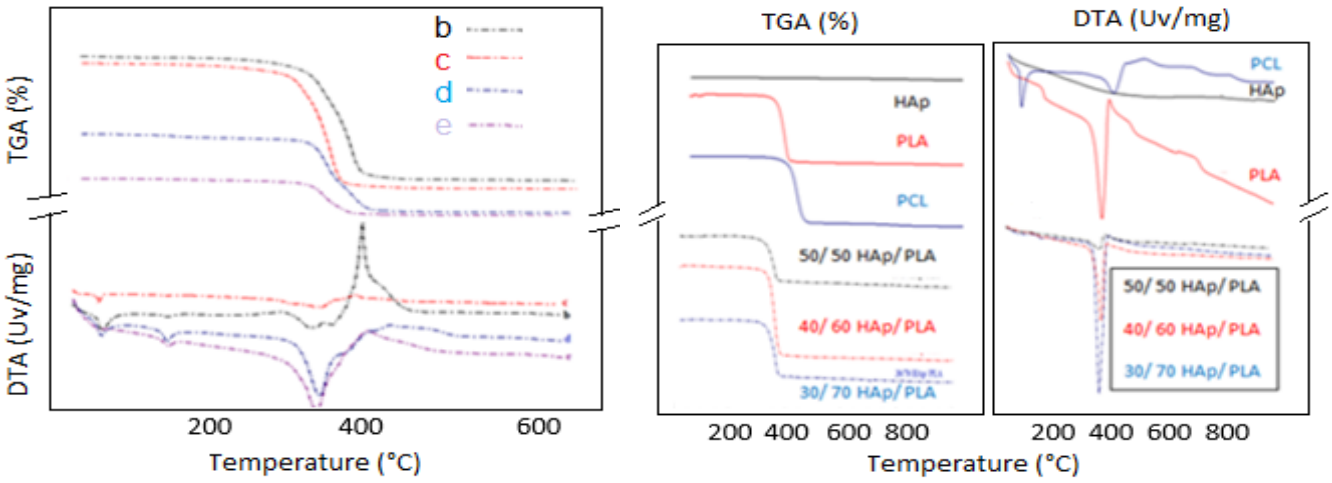

Figure 6: TGA (1) and DTA (2) curves of the composites HApt/ PLA and *HAp$_t$*/ PLA / PCL, with molar ratios of b = 50/30/20, c =50/10/40 d = 50/20/30 and e =50/40/10 (Heating rate: 10 ° C / min).

Table 1. TGA/ DTA results for HAp/ PLA/PCL composite.

HAp$_t$/PLA	*β(°C/min)*	*Td(°C)*
00/100	10	364.55
50/50	10	366.81
40/60	10	372.65
30/70	10	356.83
HAp$_t$/PLA/PCL	*β(°C/min)*	*Td(°C)*
0/0/100	10	407.06
50/40/10	10	361.97
50/20/30	10	364.01
50/10/40	10	367.40
50/30/20	10	385.41

The TG/DTA curves of PLA, PLA/PCL has two distinct regions. The first region, between 25 and 100°C, is due to the loss of water, and then a second loss begins around 350 and 400°C, is due to the decomposition of organic matters and the loss of (CO_2) fom the polymer.The curves of thermal degradation show that the composite HAp$_t$/PLA/PCL: 50/30/20 has the highest thermal stability [26]. There was a slight increase in the thermal degradation onset temperature for the proportion HAp$_t$/ PLA which is equal to 40/60. For composites HAp$_t$/PLA/PCL, the onset of degradation is improved to respectively about 361.97 ° C, 364.01 ° C, 367.40 ° C and 385.41 °C for molar ratios (50/40/10), (50/20/30) (50/10/40) and (50/30/20), the results are summarized in table. 1. The biodegradability of membranes HAp$_t$/PLA /PCL in PBS solution at pH 7.4 and 37 °C is shown in Fig. 7. All samples displayed similar degradation kinetics.

The initial mass loss of the membrane increases with increasing molar ratios of HAp particles degradation in PBS. This means that, the membrane surface is not only become coarse, but also porous and permits access of water during degradation. As shown in Fig. 8 the swelling kinetics duringthe24 hour period, and the swelling of the composite HAp_t/PLA/PCL increases with soaking time in water and PLA /PCL molar ratio [26].

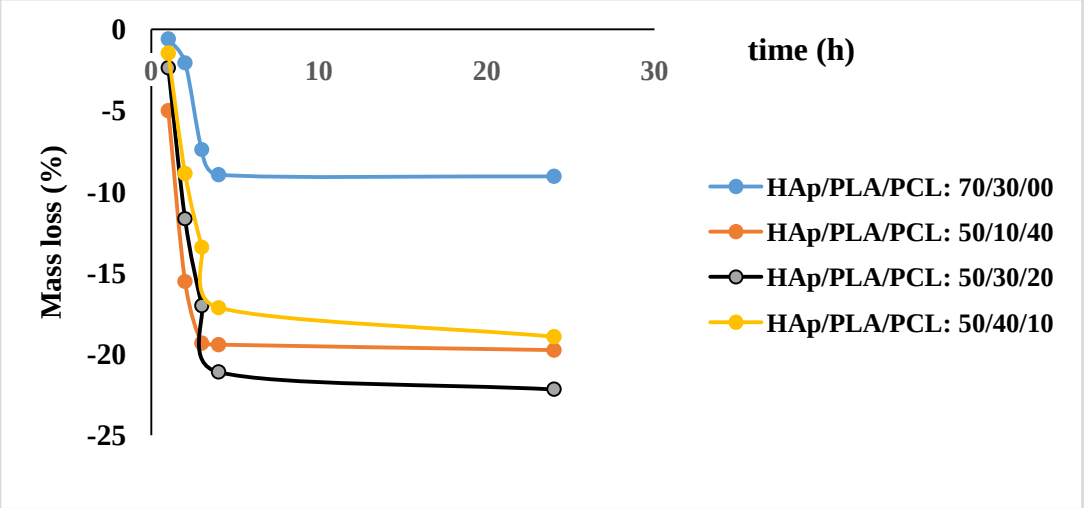

Figure 7: Biodegradability at 37 ° C of membranes HAp_t/PLA/PCL in PBS

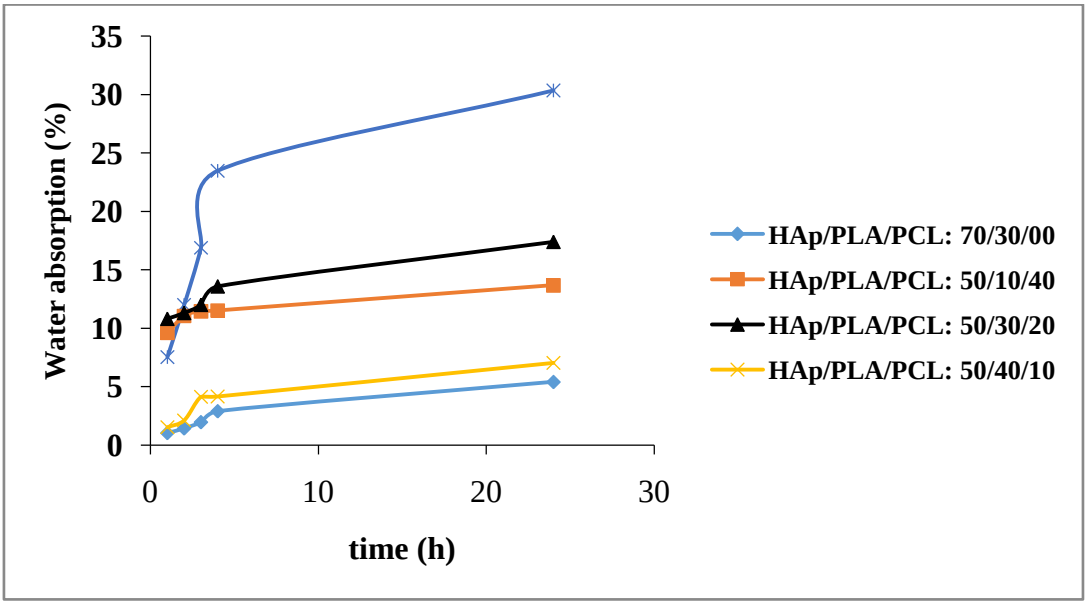

Figure 8: Swelling ratio of composite HAp_t/PLA/PCL.

- The composite with a molar ratio of 70/30/00 showed a swelling ratio of 5.4%.
- The composite 50/40/10 present inflation rate of 13.68%.
- The composite of the molar ratio of 50/ 30/ 20 showed a swelling ratio of 17.39%.
- Thiscould be due to the presence of the hydrophilic polymer.

3.5. Total organic carbon production

Measurements indicated that carbon production for PLA is higher than that of composites. As we can see from the graph Fig. 9, the TOC results indicated also that the introduction of HAp up to 50% decrease the CO_2 formation. The TOC level of composites has been related to % of HAp changing.

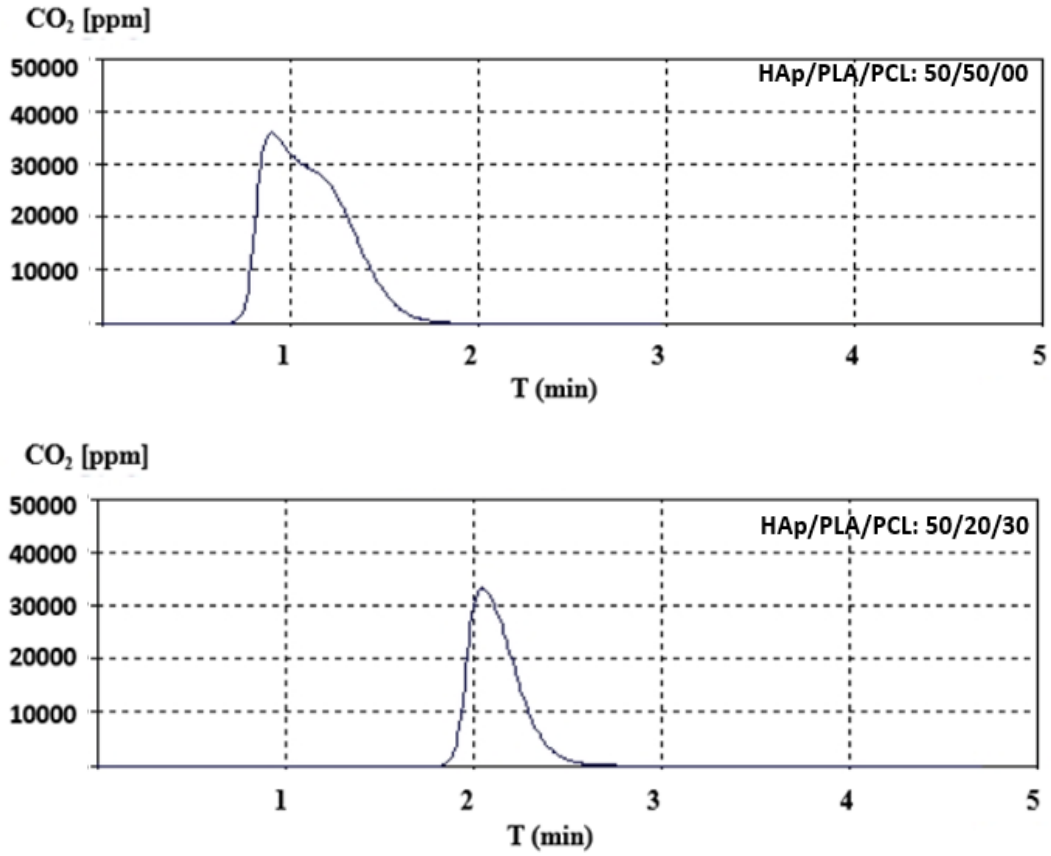

Figure 9: Plot of TOC vs. time for HAp_t/ PLA/PCL composite scaffold with a molar ratio of a = 50/50/00 and b =50/20/30.

Conclusion

Synthesis of HAp_t/PLA/PCL membranes was successfully achieved through dispersion of HAp_t particles homogeneously in the composite. The composites membrane was characterized by FT-IR and TGA/DTA. Swelling and weight loss results showed an interaction between the organic matrix and the HAP matrix by the mean of hydrogen bonding and carboxyl-metal complexation. Morphology analysis showed that the composite has good compatibility between the organic matrix and inorganic matrix (HAp_t and PLA), which allow the production of very fine particles. Therefore, membranes with well-controlled parameters could be produced.
The presence of the mineral phase in the polymer matrix provides the "bioactive" character desired for bone regrowth. Finally, the use of a polymer matrix can benefit from the technical shaping of plastics and a resorbable nature.

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