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CHARACTERIZATION OF PVDF/HAP COMPOSITES FOR USE IN MEDICINE AREA

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Abstract. *In mid-17th century (1600) the man already was worried about restoring or replacing damaged parts of the human bony tissue by implants. Engineers and researchers of different areas are working to investigate solutions for the problems that persist until present. In this aspect the biomaterials (composites and blends) represent an important and significant role in the health of the modern society, contributing for the development of new materials with wide application in the medical area. Thus, this work shows preparation and characterization of composites with poly(vinylidene fluoride) (PVDF) and hydroxyapatite (HAP), in order to analyse the incorporation of HAP in PVDF, as also to investigate their mechanical properties and cytotoxicity (biocompatibility), aiming the use of this kind of composite in bony restoration and bony filling. The films were prepared by casting method. The PVDF pellet shape was dissolved in dimethylacetamide (DMA) and a HAP/DMA emulsion was also prepared. Soon afterwards they were mixed in several proportions 100/00, 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70 in weight and left to dry in greenhouse. Homogeneous and flexible films were obtained and characterized by scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), X-ray diffractometry (XRD), contact angle measurement, mechanical and cytotoxicity assays.*

Keywords: *Poly(vinylidene fluoride), Polymer composites, Biomaterials, Hydroxyapatite.*

1. INTRODUCTION

Studies in the area of the health disclose the growing search for substances that replace lost bony parts in traumatic or nontraumatic events. The metals have been widely used for major implants. There are, however, various problems related to metallic materials in the human body due to corrosion, wear, and/or negative tissue reaction [Park]. In the search for bioactive prothesis, improvements of materials that which contain hydroxyapatite (HAP) have aroused innumerable researches [Suchanek]. Composite materials that cause ostogenesis in the place of the injury have been wide studied. Biodegradable polymers, artificial bony cells and morphogenetic proteins with HAP loads have been searched in the attainment of natural bones [Inoue, Ikada, Laurencin, Yaszemki and Payne, Yamazaki and Oida].

In this sense, poly(vinylidene fluoride) (PVDF) membranes have been studied for diverse applications [Buonomenna, Ghosh, Pham, Chang], however for applications in bony tissue regeneration nothing is observed in the literature. For the anti-bacterial property of PVDF membrane [Hu], the aim of this work is to verify the maximum incorporation of HAP in this type of membrane and to analyse the results of the assays and tests that are considered basics for the application of PVDF/HAP composites as barrier on bony defects.

Beyond the studies of the physical-chemical properties of these types of membranes the biocompatibility of the same ones must be verified, since it is intended to use them as devices in processes of tissue regeneration. The first *in vitro* test of biocompatibility is the cytotoxicity assay. After verify that the material showed no toxicity the studies must be continued and other assays must be carried out to each stage to confirm its biocompatibility, along the attainment process of the device as finished product.

2. MATERIALS AND METHODS

2.1 Preparation of Composites

The composites were prepared in a container dissolving PVDF *pellet* shape in dimethylacetamide (DMA) with continuous agitation and under controlled temperature at 100°C. In another container an emulsion of HAP/DMA was formed without heating, only with agitation. After complete dissolution of PVDF the HAP emulsion was added, the temperature and agitation were kept until reaching the necessary viscosity to pour the mixture on a glass plate. After that the mixture was left to dry in a greenhouse at 110°C for 4 hours to eliminate the DMA solvent. The obtained PVDF/HAP films were homogeneous and flexible in the proportions of 100/00, 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70 in weight.

2.2 Characterization of Composites

The properties of PVDF/HAP composites were examined using scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), X-ray diffractometry (XRD), contact angle measurement, mechanical and cytotoxicity assays. It is necessary to emphasize that samples for mechanical and cytotoxicity assays were irradiated in a Co-60 source with 25 kGy dose to verify possible interference of structural modifications in the mechanical properties and to sterilize samples for cytotoxicity assay of biocompatibility.

Scanning Electron Microscopy (SEM). Samples of PVDF/HAP composite in proportions of 100/00 and 60/40 (in weight) were used to SEM and EDS observations. These samples were covered with thin film of carbon in their surfaces which topographies were obtained by the manufacturing process. The analysis was conducted in such way to observe possible heterogeneity in the surface texture in a Phillips XL-30 unit.

Energy Dispersive Spectroscopy (EDS). According to SEM, the samples were exposed to X-ray EDS with the possible biggest surface area to get the most representative spectrum for each sample.

X-ray Diffractometry (XRD). Powder X-ray data were collected in the 2θ interval from 23 to 100° with a 0.01° step-size and 5s step-time by using a SIEMENS D5000 diffractometer with copper radiation monocromatized by a graphite crystal. The diffractometer conditions were set at 40 kV and 30 mA.

Contact Angle Measures. The hanging drop method had used to measure the contact angle (θ) on surface of PVDF/HAP samples in study, using a measurer of contact angle (goniometer: TATEC, Model Cam-micron). The contact angle measures had been carried out in the following conditions: room temperature with 75% relative humidity of air, since θ varies very little with the temperature, according to Adam, which did not find detectable variations for the water in some solid hydrocarbonates in an interval between 20°C and 35°C. The reading of contact angle was carried out 20 seconds after deposition of the drop on the surface of the samples for establishment of the balance of the involved forces. Each θ value was a average of 4 measures, with maximum deviation of ± 2. The measures had been carried out in the samples that had presented better regularity surface, because this kind of analysis demands plain and uniform surfaces (little roughness), that is, the results of the angle contact measures become more reliable. The surface free energy data, polar and dispersion components of distilled water used for the measures of contact angle are: (γ_{LV} = 72,8 mN/m; γ_L^p = 51,0 mN/m e γ_L^d = 21,8 mN/m) [Comyn, Wu].

Mechanical Testing. The mechanical behavior of PVDF/HAP films was obtained from tensile testing. Three proof bodies were tested in each of the following proportions: 100/00, 70/30 and 60/40 (in weight). In the proportions mentioned earlier, the films testing was carried out without irradiation and with irradiation. The tensile testing was carried out in a universal mechanical testing machine, Instron 4400. The load cell used corresponded to 50 kgf, and the crosshead movement speed during the testing was 5 mm/minute. After the completion of each tensile testing and consequent result of the stress-strain curve, it was then determined the ultimate strength and the total strain of the film tested.

Cytotoxicity Assay. Cytotoxicity assay is a procedure adopted as an *in vitro* method for screening the toxicity of materials. The assay was carried out following International Standardization Organization - ISO 10993 and methodology presented in previous papers [Rogero and Malmonge, Rogero and Lugão]. Monolayer cell line of NCTC clone 929 obtained from American Type Culture Collection (ATCC) was used in 96 microplate wells which received serially diluted extracts of PVDF and PVDF/HAP membranes samples. The extract was obtained by membrane immersion in a flask with cell culture medium MEM (minimum Eagle´s medium) with 5% calf fetal serum and non essential aminoacids and incubated at 37°C during 24h. PVC *pellets* were used as negative control and the extract was obtained by the same way as membranes. 0.02% phenol solution was used as positive control and received the same treatment of extract dilution. The aim of controls is to verify the assay performance, positive control has to be a material which causes toxicity and negative has to be no toxic. The cytotoxic effect of each material was measured by the percentage of viability of cells when in contact with extract of testing material and was quantitatively assessed by measuring the uptake of neutral red by the viable cells. The optical densities (DO) were measured in a microplate ELISA reader spectrophotometer Sunrise – Tecan in 540nm filter and calculated cell viability % in relation to cell control. Plotting the cell viability % against extract concentration it could obtain the cytotoxicity index represented by $IC_{50\%}$ in the viability curves. The cytotoxicity index represents the concentration of the extract which kills or injury 50% of cell population in the assay.

3. RESULTS AND DISCUSSION

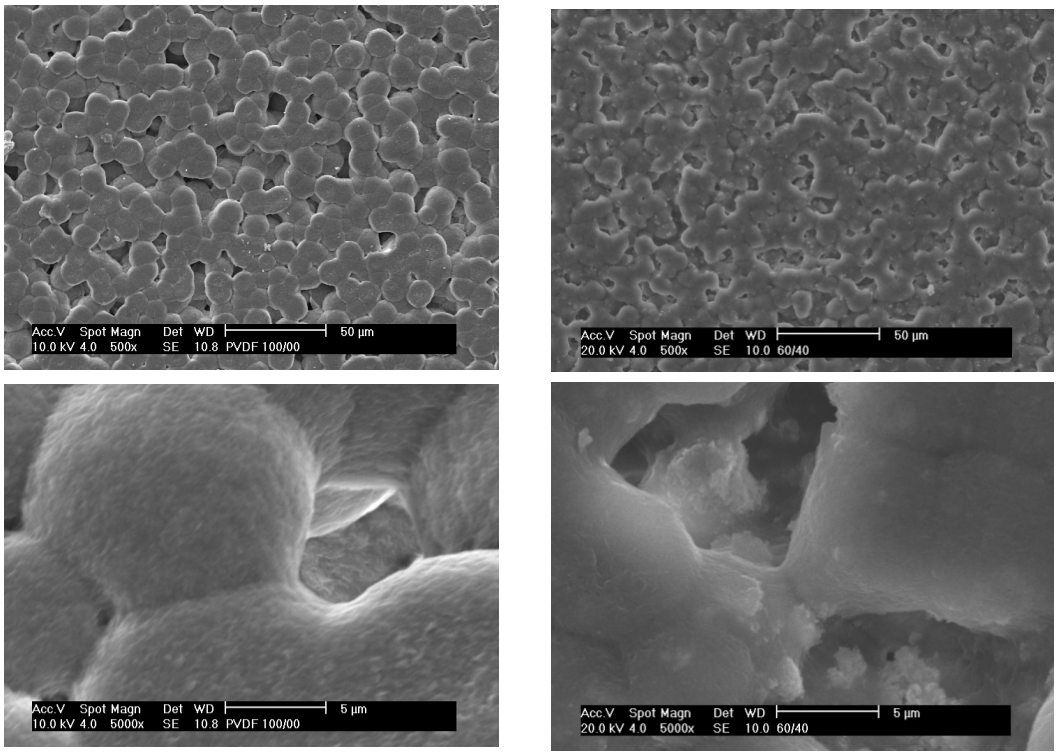
Amongst the studied compositions, the maximum limit of hydroxyapatite inconporated in PVDF polymer was 40%, this amount does not cause saturation of the polymeric matrix, that

is, appearance of HAP as powder shape on the membrane surface. In the composition of PVDF/HAP 30/70 the polymeric matrix did not support the high amount of load, did not occur the formation of film-composite and almost all HAP was deposited on the deep of the glass plate.

3.1 Scanning Electron Microscopy (SEM)

Figure 1 shows the SEM images with 500 and 5000 times magnification. It is possible identify PVDF polymer having a globular structure with intense porousity among the globes. The PVDF/HAP composites show the HAP fase filling part of the original porous spaces observed in pure PVDF.

According to Sheldon, the filler phase of a polymeric composite can acts as a crystal nucleant agent (or in rare cases, as anti-nucleant agent), affecting the size or the perfection of the polymer crystal. The addiction of HAP in the PVDF matrix, suggest modify the formation process of the polymer grains, inducing alteration in size and morphology of the pores.



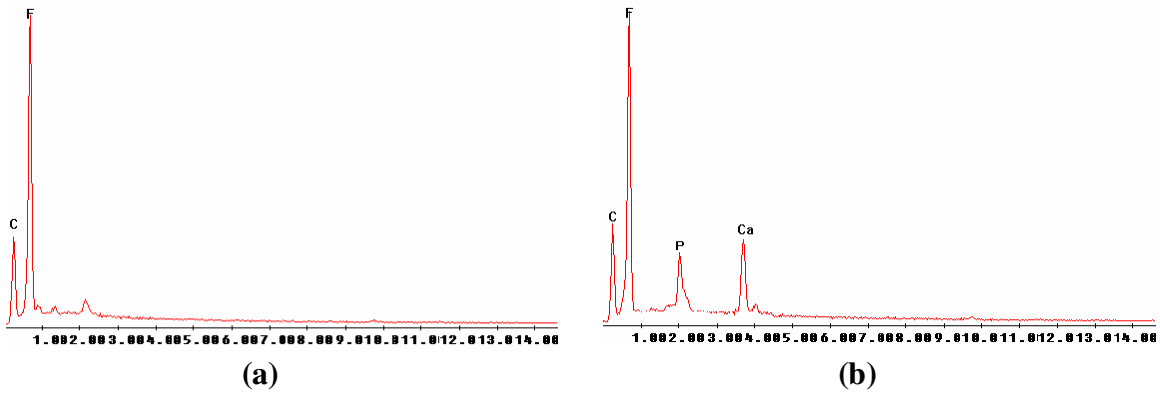
(a) **(b)**

Figure 1. SEM images with 500 and 5000 times magnification: (a) pure PVDF membrane and (b) PVDF membrane with 40% in weight of HAP.

3.2 Energy Dispersive Spectroscopy (EDS)

The spectrum of the Fig. 2, obtained by EDS, shows the most important chemical elements of the PVDF membranes composition 100/00 and 60/40. These elements were identified as Carbon and Fluorine, due to the PVDF structure, and Calcium (Ca) and Phosphorus (P) due to the incorporated HAP.

A semi-quantitative relationship of the chemical elements can be calculated by means the height proportion of their peaks in the spectrum. The Ca/P relationship of the HAP usually is 1.65 and the observed was 1.15. This difference can indicate a chemical reaction among HAP and PVDF during the synthesis process. This question can be solved by X-ray Diffraction and/or Fourier Transformed Infra-Red techniques.



(a) **(b)**

Figure 2. X-ray Spectra by EDS: (a) pure PVDF membrane and (b) PVDF membrane with 40% in weight of HAP.

3.3 X-ray Diffractometry (XRD)

The incorporation of HAP in the PVDF membranes was confirmed by XRD. Figure 3 shows the X-ray diffraction patterns of composites PVDF/HAP in the ratio 100/00 (slice graphic) and 60/40 (thick graphic) and its HAP XRD patterns (triangle up graphic - PDF n. 86-740). According to Lovinger, PVDF present Bragg peak positions at 2θ equal to 20°, 27° and 40°. As it can be observed in Fig. 3, XRD of pure PVDF membrane shows its characteristic diffraction patterns indicating that the polymer was successful formed. By comparison between the HAP XRD patterns (triangle up graphic) and XRD of PVDF/HAP 60/40 (thick graphic) one can conclude that the HAP was incorporate in the PVDF membrane. The PVDF diffraction patterns remain the same after the membrane being impregnated by HAP, indicating that the PVDF polymer structure was not modified during composites synthesis.

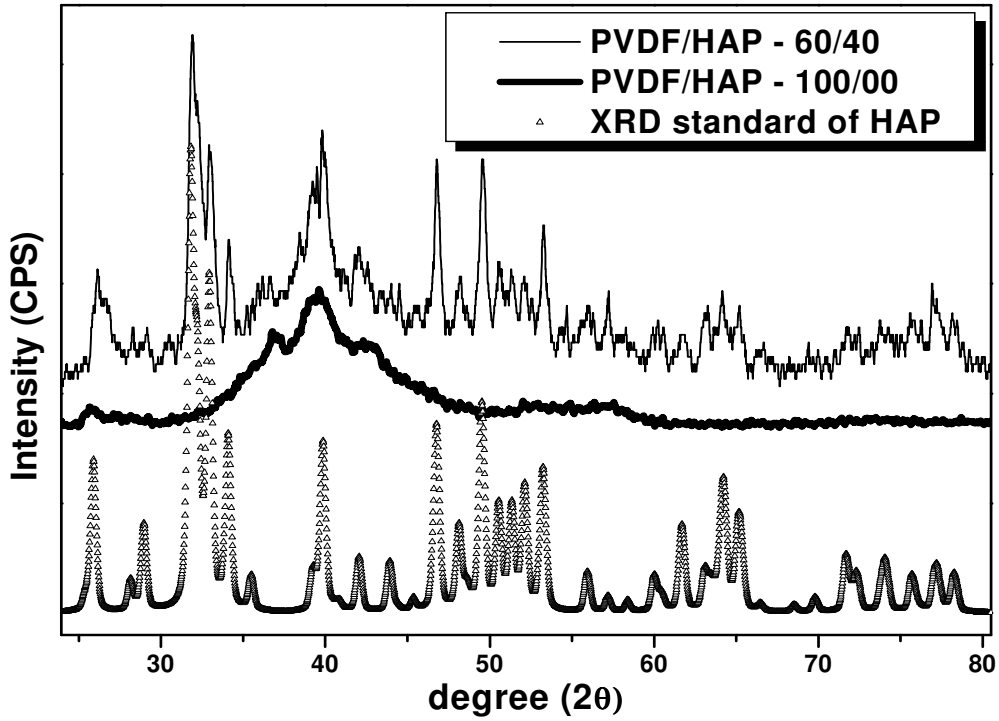


Figure 3. XRD patterns of PVDF/HAP: 100/00 (slice graphic), PVDF/HAP - 60/40 (thick graphic) and HAP phase (triangle up graphic - PDF n. 86-740).

3.4 Contact Angle Measures

Table 1 presents the contact angle values (θ) with the respective standard deviations of the PVDF/HAP films-composites surface, using distilled water as liquid of measure.

Table 1: Contact angle values (θ) of the PVDF/HAP composite-films surface with the respective standard deviations.

PVDF/HAP Composites						
100/00	**90/10**	**80/20**	**70/30**	**60/40**	**50/50**	**40/60**
83±2.0°	94±0°	98±0°	110±0°	115±0.5°	41±1.5°	19±3.8°

It is observed in the Tab. 1 that pure PVDF presents a contact angle of 83° and θ tends to increase with the HAP addition, as load concentration increases in the polymeric matrix. This behavior can be occurred, according to micrographs presented in the Fig. 1, due to mineral load modify the process of formation of the grains of polymer. Possibly the size, the shape of the pores and the surface characteristics were modified, causing a reduction in the superficial wet ability of the composites. However, for the compositions above 60/40 proportions the contact angle undergoes a high reduction, due to saturation of the polymeric matrix by the mineral load, which causes the appearance of HAP as powder shape on the surface of PVDF/HAP films, causing an increase in the wet ability of the material.

3.5 Mechanical Testing

Table 2 shows the obtained results of the ultimate strength and the total strain of proof bodies of PVDF/HAP films in the various presented proportions. It is clearly noticed that the addition of HAP reduces both the strength and strain of PVDF, making the film more brittle and less resistant mechanically, in spite of presenting certain flexibility. This can be observed in the handling of the film when the proof bodies are put in the testing machine. If necessary caution was not taken at the moment of fixing the proof bodies to the testing machine grips, they would fall apart right at handling. It is also possible to observe that the flexibility of the films is reduced with the increase of HAP load in the PVDF polymeric matrix. In the Tab. 2 it is observed that, in general, there is no evidence of any significant alteration in the mechanical properties in tensile testing relating to the irradiated and non-irradiated proof bodies. The only alteration likely for consideration is the limit of resistance of pure PVDF (without addition of HAP), whose values after the irradiation decreased from 45.2 MPa to 33.7 MPa. For further illustration of the addition effect of HAP in the mechanical behavior of PVDF films, Fig. 5 shows the stress-strain curves obtained from the tensile testing of proof bodies in the proportions of PVDF/HAP equivalent to 100/00, 70/30 and 60/40, all of them without irradiation.

Table 2. Values of ultimate strength and total strain obtained from the tensile testing of PVDF and PVDF/HAP.

Proportion PVDF/HAP	Ultimate Strength [MPa]	Total Strain [%]
100/00 without irradiation	45.2 ± 1.2	11.3 ± 2.3
100/00 irradiated	33.7 ± 0.3	10.9 ± 0.3
70/30 without irradiation	19.1 ± 1.1	3.9 ± 0.4
70/30 irradiated	19.0 ± 2.8	3.6 ± 0.2
60/40 without irradiation	12.8 ± 1.0	3.5 ± 0.4
60/40 irradiated	10.4 ± 1.6	3.0 ± 0.2

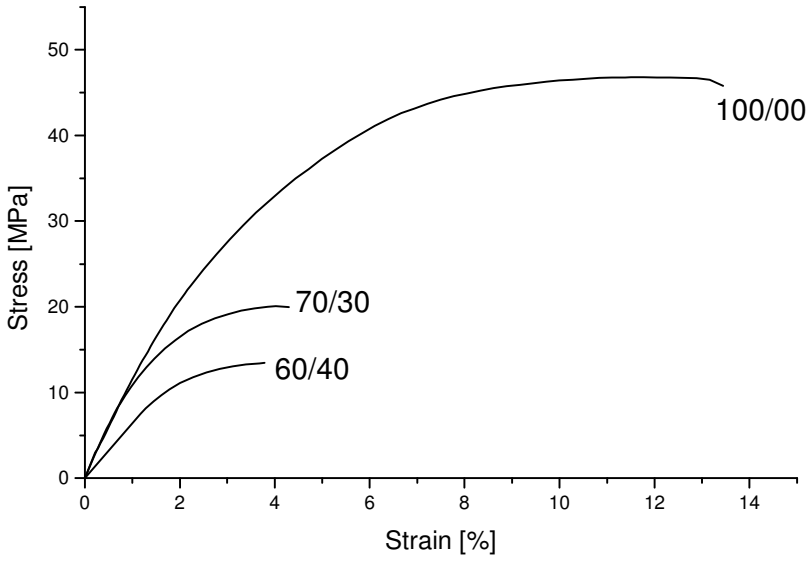


Figure 5. Stress-strain curves obtained from the tensile testing of PVDF/HAP in the following proportions: 100/00, 70/30 and 60/40.

3.6 Cytotoxicity Assay

The cell viability percentage of PVDF and PVDF/HAP membrane samples in the serially diluted extracts obtained in the cytotoxicity assay are shown in the Tab. 3 as well as the negative and positive controls.

Table 3. Results of cell viability percentage of PVDF and PVDF/HAP membranes in the cytotoxicity assay by the neutral red uptake method.

Extract Conentration (%)	Cell Viability % ± dp			
	Negative Control	Positive Control	PVDF Membrane	PVDF/HAP Membrane
100	99 ± 11	0 ± 0	105 ± 6	90 ± 9
50	107 ± 6	29 ± 1	108 ± 4	101 ± 8
25	100 ± 4	68 ± 12	105 ± 1	125 ± 13
12.5	98 ± 3	89 ± 14	108 ± 4	110 ± 9
6.25	97 ± 3	89 ± 6	108 ± 4	117 ± 9

The data of cell viability percentage projected in the graphic against extract concentration (Fig. 6) showed cell viability curves of cytotoxicity assay. All the tested samples presented the same behavior of negative control, the viability curves are above the cytotoxicity index ($IC_{50\%}$) line indicating no toxic effect. In the other hand the positive control presented cytotoxic behavior with $IC_{50\%}$ = 37 which means that the extract of positive control, in a dilution of 37% caused damage in 50% of the cell population in this assay.

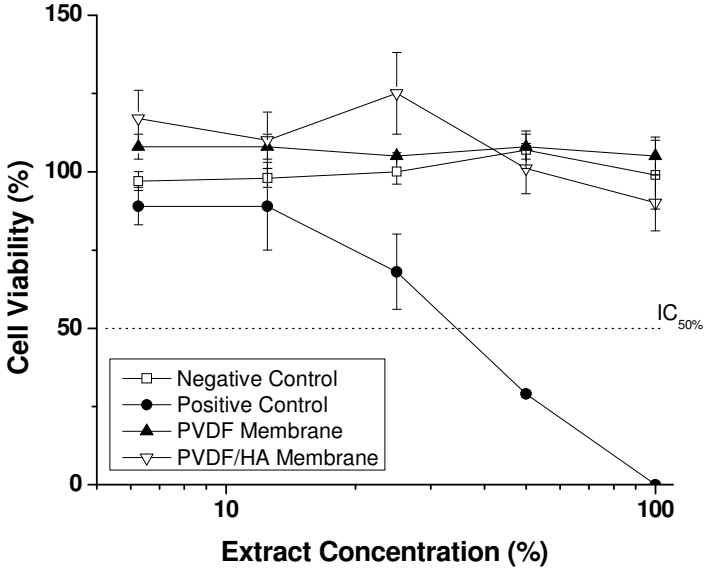


Figure 6. Cell viability curves of PVDF and PVDF/HA membranes in the cytotoxicity assay by neutral red uptake methodology.

4. CONCLUSIONS

The maximum limit of HAP incorporated in PVDF polymeric matrix was 40%, forming sufficiently homogeneous PVDF/HAP composites by naked eye and SEM.

The morphology and size of pores in the PVDF matrix had been modified by addition of HAP, although the polymer crystalline structure remains the same. This suggests that HAP incorporated did not react with the PVDF matrix during the synthesis process, that is, there was not structural modification in the obtained composite films. The addition of HAP also makes PVDF more brittle and less resistant mechanically, in spite of presenting certain flexibility at handling, which is reduced with the increase of load in the polymeric matrix.

The superficial wet ability of PVDF/HAP composites tends to decrease as the percentage in weight of HAP increases in the polymeric matrix. This characteristic can bring some difficulty to the material application in the implants area, because it is known how much bigger the wet ability of material, better its interaction with the tissues and liquids of the body. However, only by *in vivo* tests it is possible to get a definitive conclusion with regard to this property, these will be carried out in future works.

The PVDF and PVDF/HAP membranes showed no toxicity in the first *in vitro* assay of biocompatibility study, therefore having possibility to be used as biomaterial in medical/dentistry devices. But it could not forget that depending on the use of these devices other tests of biocompatibility must be carried out according to the international standardization as ISO 10993.

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