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MICROSPHERES OF POLY(ε-CAPROLACTONE) LOADED HOLMIUM-165: MORPHOLOGY AND THERMAL DEGRADATION BEHAVIOR

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ABSTRACT

Polycaprolactone (PCL), being one of the most important biocompatible and biodegradable aliphatic polyester, provides many potential biomedical. The preparation of biodegradable materials, polymer-based microspheres, is being developed by our group and the goal is to prepare and label with Ho-165 different polymer-based microspheres. The use of radionuclide-loaded microspheres is a promising treatment of liver malignances. PCL microspheres can be loaded with holmium acetylacetonate (HoAcAc). PCL and PCL/HoAcAc microspheres were prepared by an emulsion solvent extraction/evaporation technique. The PCL/ HoAcAc microspheres were irradiated in a nuclear reactor IEA-R1 at IPEN/CNEN-SP to radionuclide activation. Gamma irradiation was performed at 25 and 50 kGy doses. The microspheres were evaluated by differential scanning calorimetry analysis (DSC), thermogravimetric analysis (TG), Fourier transformed infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). In the CLSM images were observed emission in 488 nm characteristic of holmium. The SEM surface image of PCL/HoAcAc microspheres showed more roughness than PCL microspheres. TG of PCL/HoAcAc microspheres showed a substantial weight loss above 200°C, indicating decomposition of HoAcAc. The residual weight indicates the presence of Ho_2O_3. Gamma irradiation at 25 and 50 kGy doses had no effect on the PCL/HoAcAc microspheres, which indicates that the chemical composition of the microspheres had not change.

1. INTRODUCTION

Biodegradable polymers have been the major focus of attempts to develop improved delivery systems for pharmaceutical research. The commonly studied biodegradable polymers for controlled drug delivery are the aliphatic polyesters; poly(lactide), poly(glycolide), polycaprolactone (PCL) and their copolymers [1]. Polycaprolactone, being one of the most important biocompatible and biodegradable aliphatic polyester, provides many potential biomedical such as the matrix material for bone substitutes, and drug carriers for controlled release [2]. PCL is semi-crystalline aliphatic polyester, belongs to the group of biodegradable polymers due to the susceptibility to hydrolytic cleavage of the ester bond. This property, along with good biocompatibility and easy processing (melting point at 60 °C), makes PCL an interesting substrate for biomaterials and tissue engineering. Moreover, PCL is suitable for controlled drug delivery due to its high permeability to many drugs and non-toxicity [1]. Microspheres can be prepared either by PCL alone, or by using copolymers with PCL or PCL blends in order to obtain the desired release characteristics.

On the other hand, biodegradable and non-biodegradable materials containing radioisotopes have been widely investigated as a carrier material for the radiation inside the cancer tumor, in order to provide a high and localized dose of beta radiation [3-6]. Internal radiotherapy

using microspheres containing beta radioisotopes represents an alternative for cancer treatment, especially for the cancers where the response to chemotherapy and external radiotherapy is poor [7-9].

The preparation of biodegradable materials, polymer-based microspheres, is being developed by our group and the goal is to prepare and label with Ho-165 different polymer-based microspheres [10]. $^{166}Ho(t_{1/2}=26.8h)$ is a beta minus emitter (Emax=1.84 MeV), with high properties for radiotherapy and can be produced with the low power Brazilian Nuclear Reactor IEA-R1. Generally microspheres of 20-50 μm range are administered into the hepatic artery of patients suffering from liver malignancies; they will lodge in and around the tumor and irradiate the surrounding tissue [11,12].

Ideal properties of microspheres for intra-arterial therapy include high mechanical stability to resist at radionuclide activation and sterilization procedures usually performed via gamma irradiation, high chemical stability to resist elution of radioactive label, macrophage removal, or radiolysis and uniform size.

For this purpose, PCL microspheres can be loaded with holmium acetylacetonate (HoAcAc). The PCL and PCL/HoAcAc microspheres were irradiated in a nuclear reactor IEA-R1 at IPEN/CNEN-SP to radionuclide activation and subjected to gamma irradiation at 25 and 50 kGy dose. The microspheres were evaluated by differential scanning calorimetry analysis (DSC), thermogravimetric measurements (TG), Fourier transformed infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM).

2. EXPERIMENTAL

2.1. Materials

All chemicals were commercially available and used as obtained. Poly(ε-caprolactone (molecular weight 65,000 g/mol), chloroform, acetylacetone,. holmium(III) chloride hexahydrate, polyvinyl alcohol were obtained from Sigma Aldrich.

2.2. Methods

2.2.1 Preparation of the Ho-acetylacetonate complex

The 180 g of acetylacetone was dissolved in 1080 g water. The pH of this solution was brought to 8.52 with NH_4OH. Holmium chloride (10 g in 30 ml water) was added to this solution, and HoAcAc crystals were formed at room temperature in 24h. The crystals were collected by filtration, washed with water and dried in vacuum oven.

2.2.2. Preparation of HoAcAc-loaded and unloaded microspheres

PCL and PCL/ HoAcAc microspheres were prepared by an emulsion solvent extraction/evaporation technique. In the solvent evaporation method, the required amount of polymer and polymer and HoAcAc were dissolved in an organic phase (e.g. chloroform) which was emulsified under stirring with polyvinyl alcohol (PVA) (2 % w/w) solution to

form an oil/water emulsion. Stirring was continued for 2 h at about 500 rpm, to evaporate the organic phase. The microspheres formed were collected by centrifugation and washed with water, 0.1 mol.L^{-1} HCl and water, respectively. The microspheres were suspended in water and fractionated according to size using stainless steel sieves of 20 and 50 μm.

2.2.3. Irradiations

Neutron irradiation was performed in nuclear reactor IEA-R1 at IPEN/CNEN-SP. Gamma irradiation technique was performed in Gamma cell IPEN/CNEN-SP using a ^{60}Co source at 25 and 50 kGy doses.

2.3. Characterization

Thermogravimetric measurement (TG) was recorded with a Mettler-Toledo TGA / SDTA 851 thermobalance in nitrogen atmosphere, from 25 up to 600 °C at a heating rate of 10 °C min^{-1}.

Differential Scanning Calorimeter (DSC) was carried out in an 822 Mettler-Toledo under nitrogen atmosphere at a heating rate of 10 °C min^{-1}, in the temperature range of 0 to 100 °C. DSC apparatus was calibrated with Indium (m.p 156.61 °C; ΔH = 28.54 kJ kg^{-1}). Crystallinity was calculated according to Eq. 1:

$$Xc(\%)=(\Delta Hf \times 100) / \Delta H_0 \quad (1)$$

ΔHf = melting enthalpy of the sample, ΔH_0 = melting enthalpy of the 100% crystalline PCL which is assumed to be 136.4 (J g^{-1}) [12].

The SEM images were obtained in a Phillips XL 30 Microscope in magnitude of 5,000 X using samples covered with gold in a Sputter Coater BAL-TEC SCD 050.

Infrared spectroscopy was performed at Nicolet 6700 FT-IR spectrometer equipped with ATR. The spectra were measured in transmittance mode in a wave number range of 4000-400 cm^{-1}.

Confocal laser scanning microscopy (CLSM) was obtained in a LSM 500 – Carl Zeiss. The excitation wavelength was 488 nm and wavelength fluorescence were 505-530 nm.

3. RESULTS AND DISCUSSION

The thermal stability of the PCL and PCL/HoAcAc microspheres were determined by TG, Fig. 1. The TG curves of PCL/HoAcAc irradiated at 25 and 50 kGy are overlapping. The PCL microspheres have only one event of mass loss that was ascribed to the decomposition of polymer main chain, at 350 °C. For the PCL/HoAcAc microspheres there are two decomposition steps. TG of PCL/HoAcAc microspheres showed a substantial weight loss above 200°C, indicating decomposition of HoAcAc. The steps of decomposition are in agreement to Vogelsager et al. [13]. The residual weight indicates the presence of Ho_2O_3 [4].

The radiation induced reactions occur preferentially in the amorphous regions, causing scission and cross linking of chains [14]. Gamma irradiation at 25 and 50 kGy doses had no effect on the PCL/HoAcAc microspheres, which indicates that the chemical composition of the microspheres had not change.

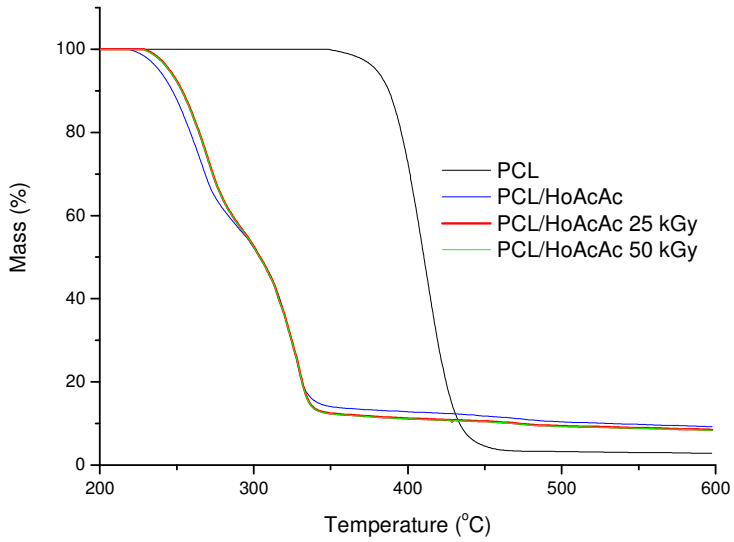


Figure. 1: TG curves, in N_2 atmosphere, at a heating rate of 10 ºC min^{-1} for PCL, PCL/HoAcAc microspheres original and irradiated at 25 and 50 kGy doses.

DSC curves were showed in Fig. 2. All the data of microspheres were compiled in Table 1. The melting temperature for all microspheres produced was observed about 55 °C. The degree of crystallinity (X_c) of microspheres was calculated according to Eq. 1. It was observed a slight decrease in the X_c for the microspheres irradiated at 25 and 50 kGy doses. This observation suggests the destruction of the crystalline phase of polymer.

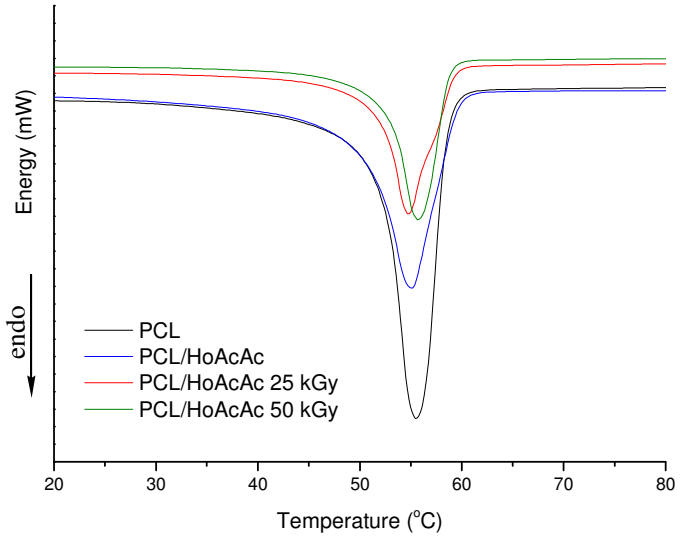


Figure. 2: DSC curves for PCL, PCL/HoAcAc microspheres original and irradiated at 25 and 50 kGy doses.

Table 1. DSC values for PCL, PCL/HoAcAc microspheres original and irradiated at 25 and 50 kGy doses.

Microspheres	T_m (°C)	ΔH_m (J g^{-1})	X_c (%)
PCL	55.5	53.3	39.1
PCL/HoAcAc	54.8	52.9	38.8
PCL/HoAcAc 25 kGy	55.0	49.4	36.2
PCL/HoAcAc 50 kGy	55.5	47.9	35.1

T_m: melting point; ΔH_m: melting enthalpy; X_c: degree of crystallinity.

The ATR-FTIR spectra (Fig. 3) show the characteristics absorption peaks of PCL and PCL/HoAcAc microspheres. The strongest band and their assignments are gathered in Table 2 in agreement to Elzein *et al.* and Roa *et al* [15, 16]. The HoAcAc complex band is in 1500 cm^{-1} [17].

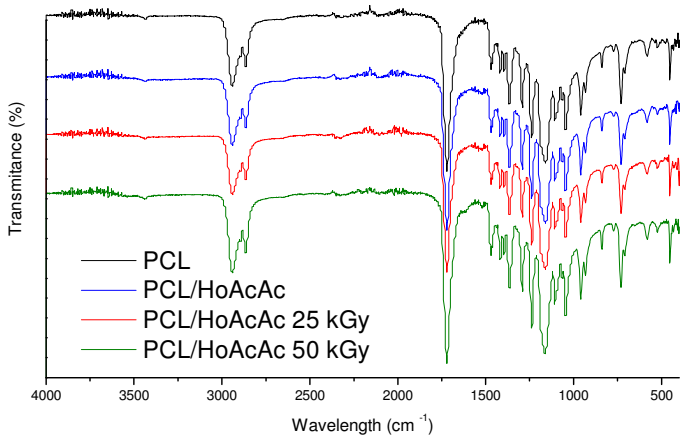
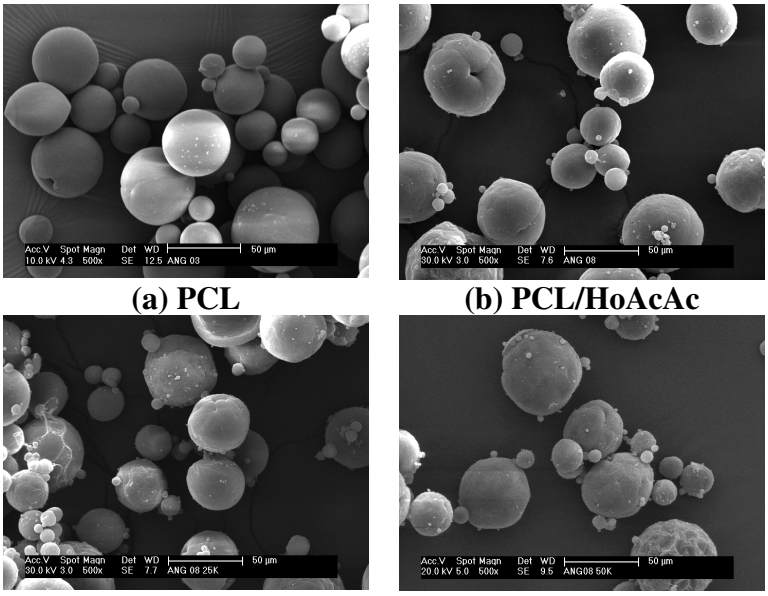


Figure 3: Infrared spectra of PCL and PCL/HoAcAc microspheres original and irradiated at 25 and 50 kGy doses.

Table 2: Characteristics infrared bands of PCL and PCL/HoAcAc microspheres.

Position (cm^{-1})	Assignment
2943	Asymmetric CH_2 stretching
2865	Symmetric CH_2 stretching
1720	Carbonyl stretching
1293	C–O and C–C stretching in the crystalline phase
1237	Asymmetric COC stretching
1160	OC–O stretching
1107	Angular CH_2 deformation
733	Rocking CH_2 deformation

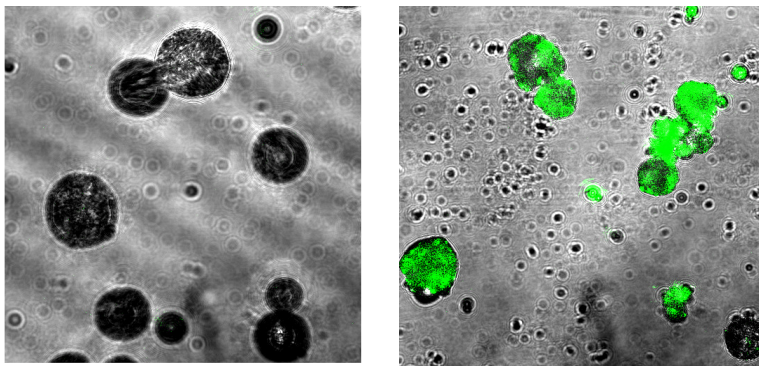
The surface morphology of the microspheres was analyzed by SEM. In Fig. 4a representative picture of microspheres with a diameter of 20-50 μm are observed. It demonstrates that the particles are spherical and smooth. PCL/HoAcAc microspheres (Fig 4b,c,d) showed more roughness than PCL microspheres (fig 4a). However, the microspheres retained their spherical character. The SEM analysis demonstrates that surface of PCL/HoAcAc irradiated at 25 and 50 kGy was not affected after gamma irradiation (Fig 4c,d). Probably there were HoAcAc crystals present on the surface of the PCL/HoAcAc microspheres. The CLSM images were observed in Fig. 5. The emission in 488 nm (green color in images, Fig. 5b) is characteristic of holmium.



(a) PCL **(b) PCL/HoAcAc**

(c) PCL/HoAcAc (25 kGy) **(d) PCL/HoAcAc (50 kGy)**

Figure 4: SEM images for PCL (a), PCL/HoAcAc microspheres original (b) and irradiated at 25 (c) and 50 (d) kGy doses.



(a) **(b)**

Figure 5: CLSM images for PCL (a), PCL/HoAcAc (b) microspheres.

The main impurities of the PCL/HoAcAc microspheres were identified by gamma spectroscopy (HPGe) in DIRF-GPD laboratories at IPEN, CNEN-SP. The radionuclides impurities found were: ^{166m}Ho, ^{169}Yb, ^{175}Yb, ^{177}Lu e ^{24}Na. The radionuclide contaminant ^{166m}Ho is intrinsic to the process of activating the ^{165}Ho.

4. CONCLUSIONS

This study showed that PCL microspheres were formed with and without holmium. The microspheres were characterized in a morphological and thermal behavior. Gamma irradiation at 25 and 50 kGy doses had no effect on the PCL/HoAcAc microspheres, which indicates that the chemical composition of the microspheres had not change. Preliminary studies for the preparation and activation of microparticles using the PCL / HoAcAc showed a promising result for possible application in the treatment of liver tumors by brachytherapy.

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