

MICROSPHERES OF POLYESTHER LOADED WITH HOLMIUM-165: EFFECT OF GAMMA IRRADIATION ON THE POLYMERIC STRUTURE

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ABSTRACT

Biodegradable polymers containing radioactive isotopes have potential applications as delivery vehicles of beta radiation to the cancer tumors by brachytherapy. ¹⁶⁶Ho is an example of such radioisotope emitting high-energy beta particles, and also its gamma rays allow nuclear imaging in everywhere is applied. Among the biodegradable polymers, different types of poly(lactide) have been investigated in our laboratory, and poly(L-lactide) (PLLA) was used as substrate to prepare microparticles loaded with holmium acetylacetonate HoAcAc (PLLA-HoAcAc-MP). The aim of this study was to evaluate the stability of these microparticles to gamma radiation. The PLLA-HoAcAc-MP were irradiated in a nuclear reactor IEA-R1 at IPEN/CNEN-SP, and their stability studied out with gamma radiation of 25, 50 and 100 kGy doses. MP were characterized before and after irradiation by differential scanning calorimetry analysis (DSC), thermogravimetric analysis (TGA), scanning electron microscopy (SEM), X-ray diffraction (XRD) and confocal laser scanning microscopy (CLSM). Preliminary results showed that gamma radiation did not damage morphologically the prepared PLLA-HoAcAc-MP in the dose range studied, and this procedure may be an important tool for knowing the stabilities of the polymers studied as MP for possible application in brachytherapy.

1. INTRODUCTION

Cancer has been one of the major social and health concerns in the last decades, even with all the advances related to diagnostic modalities and treatment procedures. Between therapy options the use of nuclear medicine in oncology is of a particular importance as a rapidly developing therapeutic procedure, and also providing diagnostic multimodality [1]. The worldwide incidence of hepatic malignancies, both primary and secondary, exceeds 1000 000 new cases each year. Hence, the prognosis in patients suffering of these cancers has lead to the development of a directed therapy, which consists of intra-arterial administration of radioactive particles through a catheter into the liver. Biodegradable polymers actually have been the major focus of attempts to develop improved delivery systems for pharmaceutical research, and biodegradable polymers containing radioactive isotopes have potential applications in this scope as delivery vehicles of beta radiation to tumors by brachytherapy. Beta-emitters radionuclides are the most widely utilized in radiotherapy, possess suitable long path length and low linear energy transfer values (LET). Passing through tissues the ejected β -electrons interact with atoms, mainly of the abundant water molecules, leading to the

generation of excited and ionized atoms and free radicals which are responsible inducing single strand breaks in DNA [2]. ^{166}Ho , in addition to high-energy beta particles, is also a gamma rays emitter, so it allows acquiring a nuclear imaging of any tissue where present too. The commonly studied biodegradable polymers to controlled drug delivery are aliphatic polyesters: poly(lactide), poly(glycolide), polycaprolactone (PCL) and its copolymers [3].

Poly-L-lactide (PLLA) is a well-known biodegradable and biocompatible polymer, studied in a wide variety of applications like implantable medical devices, drug release matrices, and environmentally friendly packaging materials [4].

PLLA was selected to prepare holmium acetylacetonate microparticles because the crystalline domains of this polymer guarantee a good dimensional stability of the particles once irradiated in a nuclear reactor (and long time radionuclide retention, avoiding their escape of MP and damage health tissues), a procedure accompanied with temperatures of 50-60 °C. Other advantage considered is the lower susceptibility of PLLA crystalline state to a radiation-induced degradation compared with its amorphous state [5].

Polymer-based microparticles have many advantages over other materials specially their near-plasma density, biodegradability and biocompatibility. Polymer-based MP used for internal radionuclide therapy are mainly prepared by a solvent evaporation technique. In such process the polymer is dissolved in a suitable water immiscible volatile solvent, and the medicament is dispersed or dissolved in this polymeric solution; the resulting solution or dispersion is then emulsified by stirring in an aqueous continuous phase, thereby forming discrete droplets. In order that the MP should form, the organic solvent must first diffuse into the aqueous phase, and then evaporate at the water/air interface. As solvent evaporation occurs the MP harden, and free flowing ones can be obtained after suitable filtration and drying. The solvent evaporation method has been used for preparation of poly(L-lactic acid) microparticles (PLLA-MP) containing ^{166}Ho , ^{90}Y and $^{186}\text{Re}/^{188}\text{Re}$. Mumper et al. [6] and Nijssen group [7-9] prepared PLLA-MP loaded with holmium-165-acetylacetonate (HoAcAc). Briefly, HoAcAc complex and PLLA were dissolved in chloroform, and this solution was added in an aqueous polyvinyl alcohol (PVA) one, and stirred until the solvent had evaporated. MP were graded and collected according to their size, on stainless steel sieves of 20-50 μm .

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

Different types of poly(lactide) have been investigated in our laboratory [10]. For this purpose, the aim of this study was to investigate the stability of PLLA-HoAcAc-MP to gamma irradiation. PLLA-MP and PLLA-HoAcAc-MP were irradiated in a nuclear reactor, and their stability was evaluated with 25, 50 and 100 kGy gamma radiation doses. MP were characterized before and after its procedure by thermogravimetric (TGA) and differential scanning calorimetry (DSC) analysis, X-ray diffraction (XRD), 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(L-lactide) (PLLA inherent viscosity 0.90-1.20 dL g⁻¹), chloroform, acetylacetone, holmium (III) chloride hexahydrate, and poly(vinyl alcohol) (PVA) 98-99 % hydrolyzed (molecular weight 31,000-50,000), were obtained from Sigma Aldrich.

2.2 Methods

2.2.1 Preparation of the Ho-acetylacetonate complex

Acetylacetone (180 g) was dissolved in 1080 g water, and the pH brought to 8.52 with NH₄OH. Holmium chloride (10 g in 30 mL water) was added to this solution, and HoAcAc crystals were formed at room temperature after 24h. The crystals were collected by filtration, washed with water and dried in vacuum oven.

2.2.2. Preparation of HoAcAc-loaded and unloaded microparticles

PLLA-MP and PLLA-HoAcAc-MP were prepared by emulsion solvent extraction and evaporation technique. In the solvent evaporation method the polymer, or polymer and HoAcAc, were dissolved in an organic phase (e.g. chloroform) which was emulsified under stirring with PVA (2 % w/w) solution to form an oil/water emulsion. Stirring was continued for 3 h at 500-1000 rpm (depending on the used polymer), with air flow over the mixture to evaporate organic phase. MP were collected by centrifugation and washed, respectively, with water, 0.1 mol L⁻¹ HCl, and water again. The PLLA-MP and PLLA-HoAcAc-MP were suspended also in water and fractionated according to size using stainless steel sieves, pore size of 20 and 50 μm, and dried in freeze-dryer. Theoretical HoAcAc percentage in PLLA-HoAcAc-MP was 37,5% (w/w), assuming full incorporation of complex in the MP.

2.2.3. Irradiations

Irradiation was performed in the nuclear reactor IEA-R1 at IPEN/CNEN-SP. Gamma irradiation technique was performed in Gamma cell IPEN/CNEN-SP, using a ⁶⁰Co source with 25, 50 and 100 kGy doses, and a dose rate of 1.69 kGy h⁻¹.

2.3 Characterization

Thermogravimetric (TG) curves were obtained using a Mettler-Toledo TGA/SDTA 851 thermobalance, evaluated in the range of 25 to 600°C using a heating rate of 10°C min⁻¹, under nitrogen atmosphere (flow rate of 10 mL min⁻¹).

Differential Scanning Calorimeter (DSC) was carried out in an 822 Mettler-Toledo, exploring the range of 25-200 °C repeating the heating rate of 10°C min⁻¹ under nitrogen atmosphere

(flow rate of 50m min⁻¹). DSC apparatus was calibrated with Indium (m.p 156.61 °C; ΔH = 28.54 kJ kg⁻¹). MP crystallinity was calculated according to Eq. 1:

$$X_c(\%) = (\Delta H_f \times 100) / \Delta H_0 \quad (1)$$

ΔH_f = melting enthalpy of the sample, ΔH_0 = melting enthalpy of the 100% crystalline PLLA, which is assumed to be 93.7 (J g⁻¹) [11].

The SEM images were obtained using a Phillips XL 30 Microscope in magnitude of 5,000 X.

X-ray diffraction (XRD) analyses were performed in a Rigaku diffractometer model Miniflex II, using Cu K α radiation source ($\lambda = 0.15406$ nm). The diffractograms were recorded from $2\theta = 5^\circ$ to 50° with a step size of 0.05° and a scan time of 2 s per step.

Confocal laser scanning microscopy (CLSM) images were obtained using a LSM 500 - Carl Zeiss. The excitation wavelength of 488 nm is suitable to induce holmium electronic excitations involving its 4f orbitals in HoAcAc, resulting in an emission (fluorescence) most intense in the wavelength range between 505-530 nm.

3. RESULTS AND DISCUSSION

3.1 Thermal Analysis Techniques

The thermal stability of PLLA-MP were observed by TGA, Fig. 1, underlies that a sample irradiated with 25 kGy presented a higher thermal stability than a non irradiated one (around 30 °C). Both samples presented only one event of mass loss, ascribed to the decomposition of the polymer main chain occurring in the range of 230-390°C. Hence, the gamma irradiation with 25 kGy seemed to affect the chemical structure or molecular size in PLLA-MP chains.

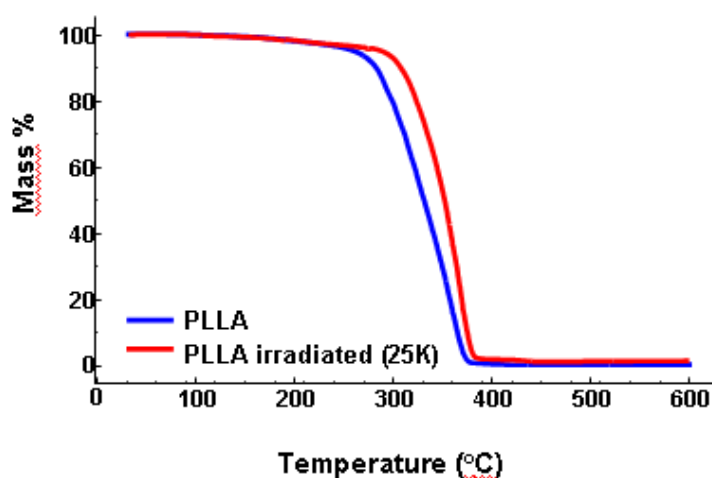


Figure 1. TG curves in N₂ atmosphere of PLLA-MP initial and after a 25 kGy irradiation gamma dose.

PLLA-MP DSC curves were shown in Fig. 2, and data were compiled in Table 1. The process associated to its glass transition temperature (about 50-65°C) is poorly defined to be discussed, but the exothermic event around 90°C is ascribed to a new kind of crystallite been formed in the PLLA matrix (in pure PLLA is ascribed to a cold crystallization); its displacement to 110°C and broadening as the irradiation dose is increased is be proposed to the cumulative radiolysis effects and changes in polymer chains alignment ruling the temperature range in which such event is observed [4,12]. An interesting feature to compare is that dirhenium decacarbonyl loaded PLLA nanoparticles displays an endothermic process exactly in the same temperature range however it is ascribed to a reversible transition of the rhenium complex [13], but in pure and cross-linked PLLA this temperature range contains one exothermic process [4,12]. Hence, it indicates the possibility of HoAcAc exhibit strong interactions with PLLA chains: the organized coordination sphere around holmium ion may induces some crystallization degree in the polymer amorphous structure very closer of the complex, similar to the rise of other kind of polymer phase sharing with the amorphous and initial PLLA crystallites the same polymer matrix.

Finally, melting temperature for all MP produced was observed about 150-160°C. Pure PLLA enantiomer melts as high as 207°C: the typical range commonly described between 170-180°C is ascribed to slightly racemization, impurities and/or presence of small and imperfect crystallites [14]. Crystallite effects are more likely due our experimental procedure. Once pure PLLA do not exhibit a so well-defined split in its melting temperature even if irradiated [4,12], it is proposed that in our PLLA-HoAcAc-MP should contains at least two kinds of PLLA crystallites: a pure one, far from molecules as the complex, and other one probably affected physically/chemically by the HoAcAc neighborhood. According to the Nijssen et al. [7], a PLLA non-irradiated good crystallinity film or microparticle exhibits its melting temperature (T_m) in 176 °C; therefore it was ascribed that the T_m anticipated in -16 °C to the prepared PLLA-MP reveals a structure with an amorphous character in some range. However, the MP degree of crystallinity (X_c) in Table 1 is consistent to the typical ones commonly related to this polymer, around 37°-47° [15-17], even at higher complex loads. It is well described that PLLA-HoAcAc-MP have fusion in colder temperatures related to pure PLLA-MPs ones, due the HoAcAc load disturbs in some extension the attractive forces and/or crystallinity intrinsically possible to the polymer net. Nijssen et al [7] investigating the relation between T_m in PLLA-HoAcAc films or microparticles and the amount of loaded holmium complex revealed a quantitative interdependence between them (Fig. 3 is exhibited data related to these PLLA films). Based in these experiments, if a PLLA-HoAcAc-MP with 17% of HoAcAc (weight of complex/weight of PLLA-HoAcAc-MP) exhibits fusion in (159±1)°C, therefore to our prepared PLLA-HoAcAc-MP with higher HoAcAc dosages (37,5% w/w) an even more anticipated melting process between 151-155°C is reasonable, even more if the MP considered is an irradiated one.

Besides of certain similarities the DSC curves have significantly different shapes, positions and peak areas, consequences of structural changes of amorphous/crystalline ratio induced by gamma irradiation to the prepared PLLA microparticles. From Fig. 2, PLLA-HoAcAc-MP irradiated with gamma-rays of 25 and 50 kGy show two endothermic peaks about 140-160°C, ascribed to fusion of the two kinds of PLLA crystallites (pure and the HoAcAc neighbor one) present in the MP. According to Nijssen, it is expected that PLLA matrix character is mainly an amorphous phase, but the HoAcAc domains can be also amorphous or even crystalline. XRD analyses in Fig. 4 and Fig. 5 offered useful informations to characterize the peaks present in DSC.

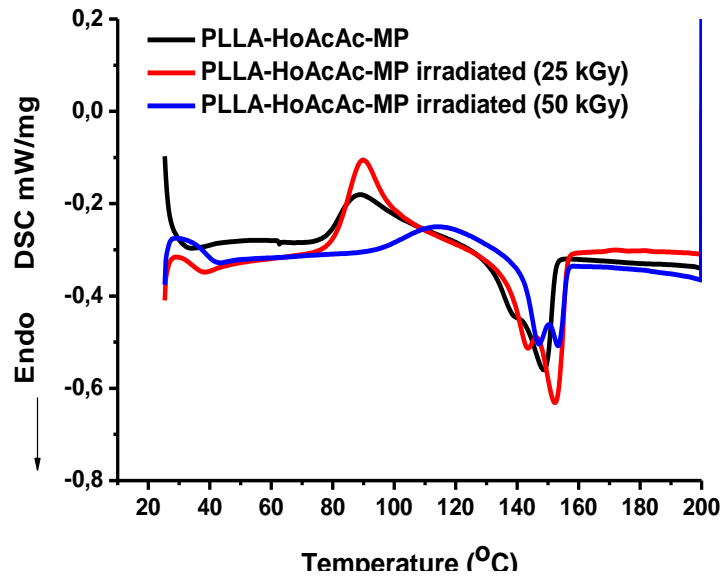


Figure 2. DSC curves to the PLLA-HoAcAc-MP initial and after irradiated with 25 and 50 kGy doses.

Table 1. DSC values for PLLA-HoAcAc microparticles initial and irradiated at 25 and 50 kGy doses.

Microparticles	T_m peak ($^{\circ}\text{C}$)	ΔH_m (J g^{-1})	X_c (%)
PLLA-HoAcAc	151	33	35
PLLA-HoAcAc irradiated (25 kGy)	165	35	38
PLLA-HoAcAc irradiated (50 kGy)	155	38	40

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

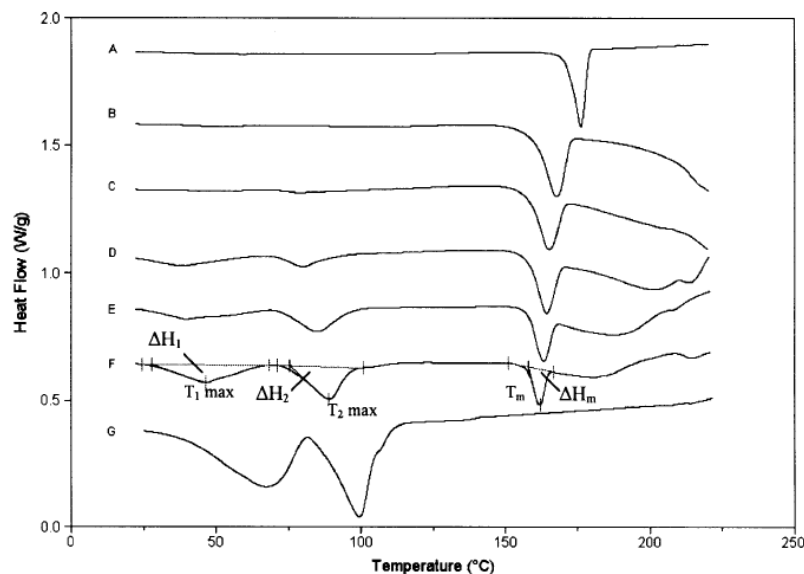


Figure 3. MDSC thermograms of films with a percentage of (HoAcAc weight/PLLA-HoAcAc-MP weight) in: 0% (A), 4% (B), 8% (C), 12% (D), 16% (E) e 20% (F). (G) is related to only HoAcAc (Adapted of Nijssen et al.) [7].

3.2 XRD Analysis

The initial PLLA-HoAcAc-MP exhibits in XRD spectra just one intense and broad band with peak at $6,9^\circ$, Fig. 4. Compared to Nijsen et al [7], Fig. 5, and Sarasua et al [18], this peak corresponds more likely to HoAcAc domains with relative crystalline character rather than a PLLA matrix. In Nijsen's PLLA-HoAcAc-MP with 16% Ho (w/w) load implied in a crystalline PLLA phase, even though HoAcAc domains could be crystalline or amorphous; in our initial PLLA-HoAcAc-MP there is a higher complex load, 37,5 % (w/w), but none band of crystalline PLLA in XRD spectrum. This reveals differences in the nature of interactions between PLLA and HoAcAc in the MP useful to analyze the obtained XRD spectrum.

For example, the Nijsen neutron irradiated PLLA-HoAcAc-MP [7] exhibit changes both in band broad and peak positions, underling HoAcAc domains rather than PLLA matrix. However, in our gamma irradiated PLLA-HoAcAc-MP the alterations are exclusively of peak intensity, revealing only a progressive loss of crystallinity in HoAcAc domains according to the radiation dosage, and the rise of a new crystalline phase not related to this complex. Generally PLLA exposed to gamma rays in doses below 250 kGy exhibits chain scission, decreasing the polymer molecular weight due: (1) backbone rupture of C-C bonds form alkyl radicals - which in presence of diffused oxygen form reactive peroxy ones - and carbonyl ones, and (2) C-O bond breaks provides also carboxyl and alcoxyl radicals to propagate more chain scission in polymer amorphous regions - and (3) hydrogen abstraction of methine groups around the most crystalline PLLA phases; above a 250 kGy dose predominates cross-linking effects in original and broken ones PLLA chains [4,19-21].

Despite of this, PLLA exposed until 50 kGy radiation have more to analyze than foreseeable defects and damage in morphology: broken tied chains defined new lamella, not uniformed distributed in the polymer surface, accounting for a local increase of crystallinity: needle-shape structures rise in the PLLA films with length and width of, respectively, 1-10 μm and 0,1-1 μm ; stronger radiations prevent their appearance [4]. Once their size is comparable to the PLLA-HoAcAc-MP ones, we propose that such crystal rearrangement comprising entire 50 kGy irradiated MP could be related to the new and well-defined peaks in XRD. Nijsen et al [7] characterizing irradiated PLLA-HoAcAc-MP obtained holmium dosages higher than expected based in the holmium complex added, proposing changes in its structure providing a superior percentage of holmium related to acetylacetonate ligands. In their assumption the acetylacetonate ligands in irradiated PLLA-HoAcAc-MP are labil, and able to coordinate with two holmium atoms simultaneously; hence this could explain the increase of holmium percentage related to acetylacetonate one - and leaving a positive charge in the holmium able to interact with PLLA carbonyl groups or any kind of anion or radical present or formed in the polymer matrix. Applying this assumption to our PLLA-HoAcAc-MP, non fully coordinated acetylacetonate should leave some carbonyl groups free, eventually able to physically interact with the PLLA carbonyl groups: their similarity in chemical nature is theoretically suitable to create some organization in an amorphous PLLA matrix. The remaining PLLA carboxyl groups, if closer, are likely to interact with holmium ion, also introducing higher organization in amorphous phases where HoAcAc is present. Acetylacetonate suffer radiolysis mainly by its enol form, which provides coordination to the holmium, losing methyl hydrogens [22]: this process should enhance the crystallinity decrease in HoAcAc domains, so its interaction with radicals generated by broken PLLA backbones should not be discarded. Finally, PLLA-HoAcAc-MP prepared by emulsion process using chloroform has residues of this solvent (ppm range) which suffer radiolysis too:

alkyl and chloride radicals probably have some influence in the PLLA crystallization and thermogravimetric features [23].

Therefore, we propose the appearing of the new bands in XRD spectrum ($2\theta = 16,6^\circ$, $18,9^\circ$ and $22,3^\circ$) are not related to HoAcAc already characterized in literature, assigned exclusively to the PLLA matrix with better crystallinity, consistent to Sarasua et al [18]. Our model to the prepared PLLA-HoAcAc-MP admits that higher gamma radiation doses inducing more loss of crystallinity in HoAcAc domains should provides gradually more acetylacetonate carbonyl groups released in contact with the PLLA matrix original and radiolysis damaged one, contributing in some extension to the reorganization in at least one kind of polymer crystallites dispersed in the matrix; holmium ions whose charge is not completely neutralized by anions should interact mainly with carboxyl free groups of PLLA chains, providing a new mechanism able to induce organization in the polymer net, and a new gain of crystallinity. This assumption is consistent to explain the inverse tendencies of HoAcAc and PLLA bands in XRD spectra and the split in the PLLA melting temperatures in DSC, in which a decrease of crystallinity in HoAcAc domains are concomitantly followed to gain in this property related to PLLA matrix; this interaction should also provides a higher stability (low release) found in HoAcAc-loaded microparticles.

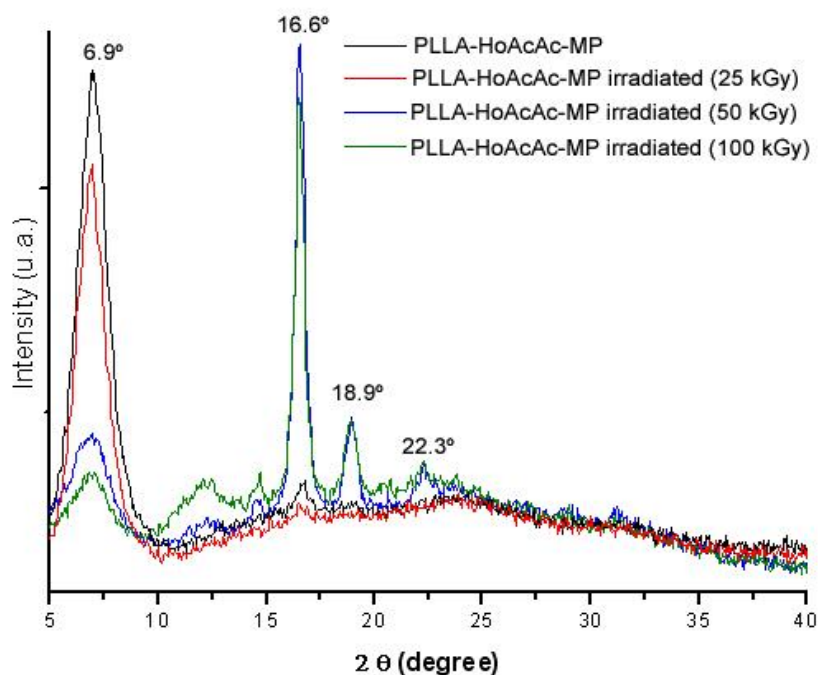


Figure 4. XRD spectra of PLLA-HoAcAc-MP initial and irradiated at 25, 50 and 100 kGy doses.

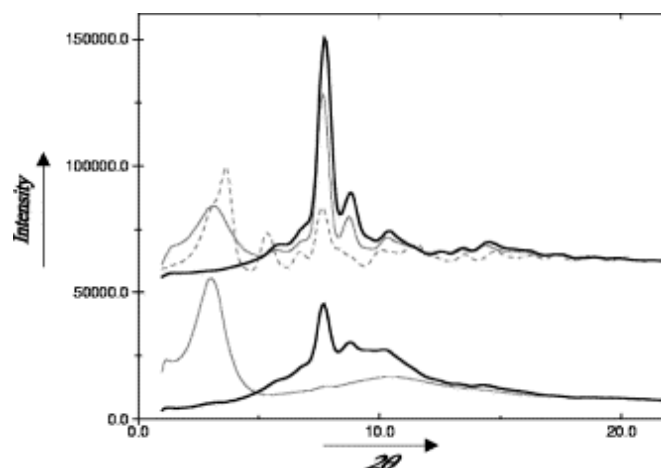
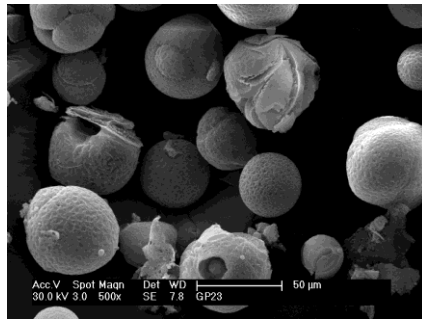


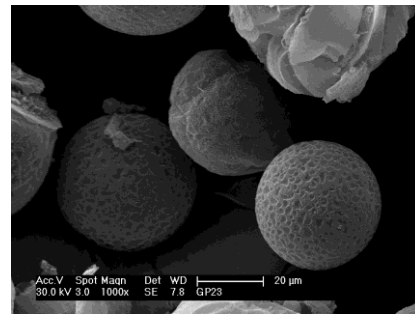
Figure 5. X-ray scattered intensity as function of diffraction angle 2θ . Arbitrary scaling applied to curves so as to match the intensities at 2θ of 22° . The upper three curves are shifted upwards by 55,000 units. Lower curves: PLLA-MP (thick line) and PLLA-HoAcAc-MP with 17% Ho (thin line). Upper curves: film of PLLA (thick line), film of PLLA loaded with 4% Ho (thin line) and film of PLLA loaded with 16% Ho (thin dashed line). In the latter curve, peaks either correspond to crystalline PLLA, amorphous Ho–Ho or crystalline HoAcAc (crystal form I). Extracted from Nijsen et al. [7].

3.3 Morphological Analysis

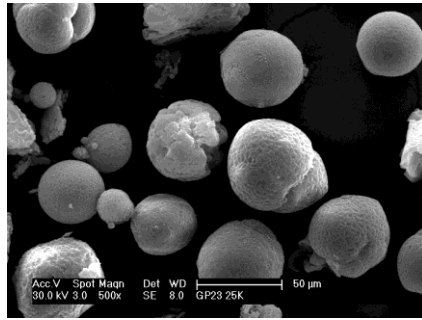
Fig. 6 is a representative SEM picture of PLLA-MP and PLLA-HoAcAc-MP with diameter of 20-50 μm . It reveals the major part of prepared MP spherical and slightly wrinkled. The PLLA-HoAcAc-MP surface irradiated was not affected after gamma irradiation of 25 kGy (Fig. 6b, 6e), however if irradiated in 50 kGy there are expressive effects especially in the low diameter MP, which did not resist and burst (Fig. 6c, 6f); once their size is compatible with the needle-shape structures of PLLA irradiated described by Milicevic et al [4], its reorganization to a more crystalline phase at some places of the amorphous phase of these MP probably is collapsed due the local heat and all the possible radiolysis effects. Despite of this, CLSM in Fig. 7a-c exhibits the characteristic emission of holmium III (fluorescence in 505-530 nm, resulting in a green color) related to the decay associated to a weak electronic excitation closer of 488 nm: like all lanthanide ions, holmium electronic properties are preserved even if coordinated with ligands, once its 4f electrons are shielded by outer 5s and 5p electrons [24,25]. The emission widespread in all the PLLA-HoAcAc-MP extension indicated a quite homogeneous distribution of the HoAcAc in the PLLA matrix of these MP.



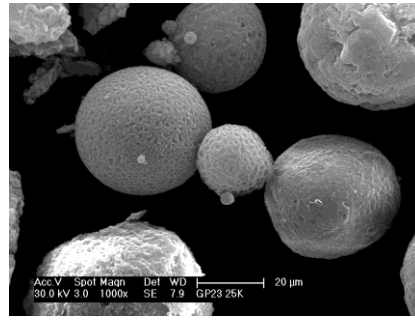
(a) PLLA-HoAcAc-MP



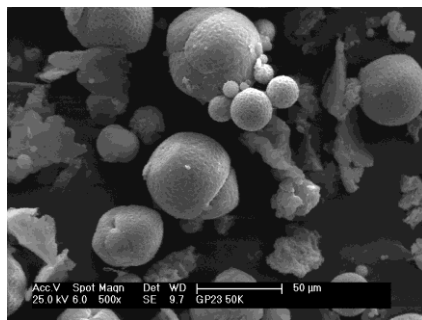
(d) PLLA-HoAcAc-MP



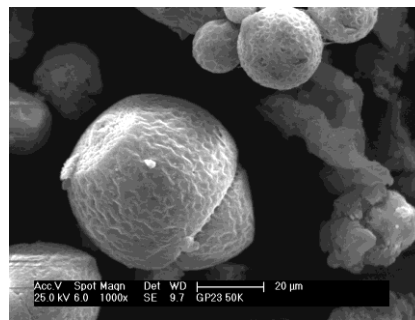
(b) PLLA-HoAcAc-MP 25 kGy



(e) PLLA-HoAcAc-MP 25 kGy

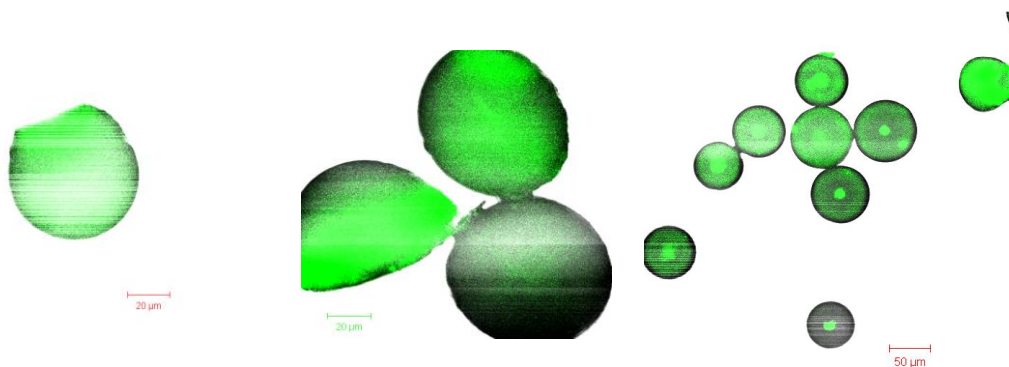


(c) PLLA-HoAcAc-MP 50 kGy



(f) PLLA-HoAcAc-MP 50 kGy

Figure 6. SEM images for initial PLLA-HoAcAc-MP (a, d) and irradiated with (b, e) 25 kGy dose and (c, f) 50 kGy dose. Magnification: (a, b, c) 500 x, (d,e,f) 1000 x.



(a) PLLA-HoAcAc-MP (b) PLLA-HoAcAc-MP (c) PLLA-HoAcAc-MP

Figure 7. CLSM images of initial PLLA-HoAcAc-MP exhibiting the holmium fluorescence. Magnification in (a,b): 1000 x; (c) 400 x.

4. CONCLUSIONS

This study showed preliminary results for the effect of gamma irradiation on the polymeric structure of Poly(L-lactide) microparticles loaded with holmium-165 (PLLA-HoAcAc-MP), characterized by their thermal behavior, XRD and morphology by SEM, and Confocal Laser Scanning Microscopy (CLSM).

XRD spectra showed an inverse tendency in crystallinity of microparticles after gamma irradiation: HoAcAc crystallinity decreased, and the PLLA one seems to increase once the applied radiation is crescent. In thermal analysis, it is ascribed that the 25 and 50 kGy irradiated samples show two peaks around 140-160°C, which may be attributed to the decreasing in HoAcAc crystallinity while the PLLA phase is reorganizing.

Gamma irradiation at 25 and 50 kGy doses had no significant effect on the PLLA-HoAcAc-MP of higher diameters in a morphological analysis, and in the 25 kGy the smaller MP ones are still preserved.

Therefore, the international established radiation sterilization dose (25 kGy) did not affect significantly the morphological properties of poly(L-lactide) microparticles loaded with holmium-165 studied here. So in a potential use of them to brachytherapy a choice for sterilization using gamma radiation is suitable, and even the use of a 50 kGy dose can be evaluated.

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REFERENCES

1. M. Hamoudeh, M. A. Kamleh, R. Diab, and H. Fessi, "Radionuclides delivery systems for nuclear imaging and radiotherapy of cancer", *Advanced Drug Delivery Reviews*, **60**, pp. 1329-1346 (2008).
2. G. Lliakis, "The role of DNA double strand breaks in ionizing radiation-induced killing of eukaryotic cells", *BioEssays*, **13**, pp. 641-648 (1991).
3. V. R. Sinha, K. Bansal, R. Kaushik, R. Kumria, and A. Trehan, "Poly-ε-caprolactone microspheres and nanospheres: an overview", *International Journal of Pharmaceutics*, **278**, pp. 1-23 (2004).
4. D. Milicevic, S. Trifunovic, S. Galovic, and E. Suljovrujic, "Thermal and crystallization behaviour of gamma irradiated PLLA", *Radiation Physics and Chemistry*, **76**, 1376-1380 (2007).
5. J. F. W. Nijssen, van M. J. Steenberg, H. Kooijman, H. Talsma, L. M. J. Kroon-Batenburg, M. van de Weert, P. P. van Rijk, A. de Witte, A. D. van het Schip, and W. E.

- Hennink, "Characterization of poly(L-lactic acid) microspheres loaded with holmium acetylacetonate", *Biomaterials*, **22**, pp. 3073–3081 (2001).
6. R.J. Mumper, and M. Jay, "Biodegradable radiotherapeutic polyester microspheres: Optimization and in-vitro/in-vivo evaluation", *Journal of Controlled Release*, **18**, pp. 193-204 (1992).
 7. J. F. W. Nijsen, van M. J. Steenbergen, H. Kooijman, H. Talsma, L. M. J. Kroon-Batenburg, M. van de Weert, P. P. van Rijk, A. de Witte, A. D. van het Schip, and W. E. Hennink, "Characterization of poly(L-lactic acid) microspheres loaded with holmium acetylacetonate", *Biomaterials*, **22**, pp. 3073–3081 (2001).
 8. M. A. D. Vente, B. A. Zonnenberg, and J. F. W. Nijsen, "Microspheres for radioembolization of liver malignancies", *Expert Review of Medical Devices*, **7**, pp. 581–583 (2010).
 9. W. Bult, R. Varkevisser, F. Soulimani, P. R. Seevinck, H. de Leeuw, C. J. G. Bakker, P. R. Luijten, A. D. van het Schip, W. E. Hennink, and J. F. W. Nijsen, "Holmium nanoparticles: preparation and in vitro characterization of a new device for radioablation of solid malignancies", *Pharmaceutical Research*, **27**, pp. 2205–2212 (2010).
 10. R. F. Costa, M. B. M. Azevedo, N. Nascimento, F. F. Sene, J. R. Martinelli, and J. A. Osso, "Production of Microspheres Labeled with Holmium-166 for Liver Cancer Therapy: The Preliminary Experience at IPEN-CNEN/SP", *International Nuclear Atlantic Conference - INAC 2009*; Rio de Janeiro, 27/9 a 02/10, ISBN: 978-85-99141-03-8 (2009).
 11. H. W. Kammer and C. Kummerlowe, "Poly (ϵ -caprolactone) Comprising Blends - Phase Behavior and Thermal Properties", *Advances in Polymer Blends and Alloys Technology*, **5**, ed. by Kier Finlayson, Technomic, USA, pp. 132-160 (1994).
 12. T. M. Quynh, H. Mitomo, N. Nagasawa, Y. Wada, F. Yoshii, and M. Tamada, "Properties of crosslinked poly(lactides) (PLLA & PDLA) by radiation and its biodegradability", *European Polymer Journal*, **43**, pp.1779-1785 (2007).
 13. M. Hamoudeh, H. Fessi, H. Mehier, A. A. Faraj, and E. Canet-Soulas, "Dirhenium decacarbonyl-loaded PLLA nanoparticles: Influence of neutron irradiation and preliminary in vivo administration by the TMT technique", *International Journal of Pharmaceutics*, **348**, pp.125-136 (2008) .
 14. D. Garlotta, "A Literature Review of Poly (Lactic Acid)", *Journal of Polymers and the Environment*, **9**, pp.63-84 (2001).
 15. E. W. Fischer, H. J. Sterzel and Wegner, G, "Investigation of the Structure of Solution Growth Crystals of Lactide Copolymers by Means of Chemical Reactions", *Kolloid-Zeitschrift and Zeitschrift fur Polymere*, **251**, pp.980-990 (1973).
 16. H. Tsuji and Y. Ikada, "Properties and morphologies of poly(L-lactide): 1. Annealing condition effects on properties and morphologies of poly(L-lactide)", *Polymer*, **36**, pp.2709-2716 (1996).
 17. H. Tsuji and Y. Ikada, "Blends of isotactic and atactic poly(lactide)s: 2. Molecular-weight effects of atactic component on crystallization and morphology of equimolar blends from the melt", *Polymer*, **37**, pp.595-601 (1996).
 18. J.- R. Sarasua, R. E. Prud'homme, M. Wisniewski, A. L. Borgne, and N. Spassky, "Crystallization and melting behavior of poly(lactides)", *Macromolecules*, **31**, pp.3895-3905 (1998).
 19. J. H. Collett, L. Y. Lim, and P. L, "Gamma-irradiation of biodegradable polyesters in controlled physical environments", *Polymer Preprints*, **31**, pp.468-469 (1989).
 20. M. C. Gupta and V. G. Deshmukh, "Radiation effects on poly-(lactic acid)", *Polymer*, **24**, pp.827-830 (1983).

21. P. Nugroho, H. Mitomo, F. Yoshii, and T. Kume, "Degradation of poly(L-lactic acid) by γ -irradiation", *Polymer Degradation and Stability*, **72**, pp.337-343 (2001).
22. W. H. Wolodarsky and J. K. S. Wan, "Electron spin resonance of γ -irradiated acetylacetone in adamantene matrix", *Canadian Journal of Chemistry*, **50**, pp.1096-1098 (1972).
23. S. W. Zielhuis, J. F. W. Nijsen, L. Dorland, G. C. Krijger, A. D. van het Schip, and W. E. Hennik, "Removal of chloroform from biodegradable therapeutic microspheres by radiolysis", *International Journal of Pharmaceutics*, **315**, pp.67-74 (2006).
24. J. -C. G. Bünzli and C. Piguet, "Taking advantage of luminescent lanthanide ions", *Chemical Society Reviews*, **34**, pp.1048–1077 (2005).
25. S. Dang, J. Yu , X. Wang, L. Sun, R. Deng, J. Feng, W. Fan, and H. Zhang, "NIR-luminescence from ternary lanthanide [Ho, Pr and Tm] complexes with 1-(2-naphthyl)-4,4,4-trifluoro-1,3-butanedionate", *Journal of Luminescence*, **131**, pp.1857-1863 (2011).