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Holmium-Loaded PLLA Nanoparticles for Intratumoral Radiotherapy Via the TMT Technique: Preparation, Characterization, and Stability Evaluation after Neutron Irradiation

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This article describes the preparation of biocompatible radioactive holmium-loaded particles with appropriate nanoscale size for radionuclide intratumoral administration by the targeted multitherapy (TMT) technique. For this objective, holmium acetylacetonate has been encapsulated in poly-L-lactide (PLLA)-based nanoparticles (NP) by oil-in-water emulsion–solvent evaporation method. NP sizes ranged between 100 and 1,100 m being suitable for the TMT administration method. Elemental holmium loading was found to be around 18% wt/wt and the holmium acetylacetonate trihydrate (HoAcAc) encapsulation efficacy was about 90%. Different experiments demonstrated an amorphous state of HoAcAc after incorporation in NPs. The NPs were irradiated in a nuclear reactor with a neutron flux of 1.1×10^{13} n/cm^2/s for 1 h, which yielded a specific activity of about 27.4 GBq/g of NPs being sufficient for our desired application. Microscopic analysis of irradiated NPs showed some alteration after neutron irradiation as some NPs looked partially coagglomerated and a few pores appeared at their surface because of the locally released heat in the irradiation vials. Furthermore, differential scanning calorimetry (DSC) results indicated a clear decrease in PLLA melting point and melting enthalpy reflecting a decrease in polymer crystallinity. This was accompanied by a clear decrease in polymer molecular weights, which can be ascribed to a radiation-induced chain scission mechanism. However, interestingly, other experiments confirmed the chemical identity retention of both HoAcAc and PLLA in irradiated NPs despite this detected decrease in the polymer crystallinity and molecular weight. Although neutron irradiation has induced some NPs damage, these NPs kept out their overall chemical composition, and their size distribution remained suitable for the TMT administration technique. Coupled with the TMT technique, these NPs may represent a novel potential radiopharmaceutical agent for intratumoral radiotherapy.

Keywords holmium; nanoparticles; PLLA; radionuclide; tumor; irradiation; TMT technique

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INTRODUCTION

Despite the great progress achieved in the technologies used to investigate better treatment modalities, cancer will remain a major social and health concern for the next few years, with more than 11 million new cases diagnosed annually (Buono et al., 2007).

In oncology, nuclear medicine is playing an important role as a diagnostic and therapeutic tool. In the challenge against cancer, major advancement has been made toward its treatment with medical radioisotopes. Some researchers predict that over 80% of cancer types should be treatable with radioisotopes (Schenter, 2007). In current nuclear medicine, two general types of radiotherapy can be carried out to treat tumors: (a) external radiotherapy applying high-energy radiations and unfortunately inducing radionecrosis of normal adjacent tissues and resulting in several side effects and considerable complications (Kaylie et al., 2000) and (b) radionuclide-based therapies including arterial embolization (Kobeiter et al., 2007; Nijsen et al., 2002), metabolic radiotherapy (Alevizaki et al., 2006; Borbath et al., 2005), immunoradiotherapy (Andratschke et al., 2007; Garkavij et al.,

2005), and brachytherapy (Bladou et al., 2007; Rivard, 2007). Among these last radionuclide-based modalities, brachytherapy has the advantage to administer radioisotopes, such as yttrium-90 (^{90}Y), rhenium-186 (^{186}Re), rhenium-188 (^{188}Re), and holmium-166 (^{166}Ho), locally in the tumor mass to restrict the radiation delivery to a defined tumor area.

In this context, the recent development in pharmaceutical nanotechnology field has been efficiently exploited in the design of novel nano- and microscaled carriers to deliver various radioisotopes with the aim to improve the outcome of tumor radiotherapy and the quality of its diagnosis. Recently, different research groups worked on the incorporation of radioisotopes in microparticles for radionuclide local delivery. The elaboration of these microparticles is generally realized using the isotope in its radioactive form (Wunderlich et al., 2000) or nonradioactive one, needing a subsequent activation by neutron bombardment (Mumper & Jay, 1992). Various substances can be used to prepare these particles including albumin (Even & Green 1989), glass (Salem & Hunter, 2006), resin (Schubiger et al., 1991), and poly-L-lactide (PLLA) (Hafeli, Sweeney, Beresford, Sim, & Macklis, 1994).

Among these substances, PLLA is a biocompatible and biodegradable polymer. It belongs to the polyester family and degrades, depending on different parameters such as its molecular weight, crystallinity, and the release media components, in a timeframe of months to years.

On the contrary, among the most promising radioisotopes for radionuclide therapy application is holmium-166, which can be obtained from the neutron activation of holmium-165 having an abundance of 100% and a high neutron capture cross-section of 64 barn enabling to obtain high radioactivities is short activation times. From a therapeutic point of view, the interesting properties of holmium-166 include (a) a maximum beta energy of 1,853 keV being high and sufficient for a radionuclide-based therapy; (b) a physical half-life of 26.9 h, which is suitable for radiotherapy, medical staff safety, and transport feasibility perspectives; (c) a maximum radiation tissue diffusion range around 10 mm satisfying internal radiotherapy application; and (d) finally, ^{166}Ho emits gamma lines at energy levels of 81 keV, which is useful for imaging with gamma camera.

Recently, the group of Dr. Henri Mehier (Cerma France) invented a new promising technique for a multimodal intratumoral therapy that was called targeted multitherapy (TMT) (Hiltbrand et al., 2004). The first purpose of this technique is to treat solid tumors via thermoablation by applying hot vaporized water at 400°C under high pressure being reached with a hydropneumatic pump. This technology uses a perforated microtube to inject locally and mini-invasively the active agents under high kinetic energy. The invention consists precisely of a biocompatible microtube (Platinium–Irridium) that is made out of a flexible nonmagnetic and biocompatible alloy. It is resistant to both traction and high internal pressure up to 3,000 bars. These mechanical characteristics facilitate the surgical implantation by percutaneous insertion. The microtube is 20–50 cm long, its external diameter is 200 μm, and its internal diameter 100 μm. Its distal end is introduced in the tissue to be treated, and the pulses of hot water vapor are injected through the microtube being perforated with 4–5 narrow halls of a few microns diameter at its distal end administered in the tumor (Roux et al., 2006). The efficacy of TMT technique in inducing thermonecrosis has already been proven for treatment of cancers in animal models (Hiltbrand et al., 2003, 2004; Roux et al., 2006).

Therefore, the objective of this work is to describe the elaboration of radioactive ^{166}Ho-loaded PLLA nanoparticles (NPs) with suitable size range and holmium content for a subsequent intratumoral radionuclide administration via the TMT microtube into solid tumors. These NPs were prepared by a solvent evaporation of oil-in-water simple emulsion with an organic phase containing the PLLA polymer and holmium acetylacetonate, and an aqueous phase containing polyvinyl alcohol as a stabilizer. Different methods were used to characterize the obtained NPs including scanning electron microscope (SEM), Fourier transformed infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), X-ray diffraction (XRD), gel permeation chromatography (GPC), and differential scanning calorimetry (DSC). These NPs are irradiated in a subsequent step inside a nuclear reactor at high neutron flux, and the results of the neutron irradiation in terms of obtained radioactivities and influence on the NP characteristics are shown.

MATERIALS AND METHODS

Materials

The PLLA polymer was kindly supplied by Purac Biochem. BV (Gorinchem, The Netherlands). Holmium acetylacetonate trihydrate (HoAcAc) was purchased from SoltecVentures (USA). Polyvinyl alcohol (PVA, MW = 31 KDa, hydrolyzation degree = 88%), deuterated chloroform ($CDCl_3$), red nile, and potassium bromide were from Sigma Aldrich (Lyon, France). Dichloromethane (DCM) was from Laurylab (Lyon, France). Nitric acid (65%) and sulfuric acid (95%) were purchased from Carloerba (Lyon, France).

NPs Preparation

Ho-loaded NPs were prepared by an oil-in-water simple emulsion–solvent evaporation method as described by Hamoudeh et al. (2007a) with modification. The oil-in-water emulsion consisted of the following:

- Organic phase: HoAcAc (200 mg) was mixed with the polymer (200 mg) in 10 mL of DCM (Table 1).
- Aqueous phase: PVA was dissolved by gentle heating in continuously stirred water at 40°C. PVA was used at percentages ranging from 1 to 3% wt/vol. The organic phase was added into the aqueous one by mechanical stirring (Ultraturax T25, IKA, Germany) at a defined stirring speed for 1 min. The stirring speed ranged between 11,000 and 24,000 rpm.

TABLE 1
The Formulation Ingredients

	Organic Phase			Aqueous Phase	
Substance	HoAcAc	PLLA	Dichloromethane	PVA	Water
Amount or volume	200 mg	200 mg	10 mL	750–1,500–2,250 mg	75 mL

HoAcAC, holmium acetylacetonate trihydrate; PLLA, poly-L-lactide; PVA, polyvinyl alcohol.

DCM was thereafter evaporated by a rotative evaporator (R-144, Buchi, Switzerland) at 100 rpm for 15 min. The prepared NPs were separated by ultracentrifugation (four cycles at 50,000 rpm for 25 min at 20°C) (Beckman, Fullerton, USA) and washed with water in each cycle to eliminate the excess of PVA. Finally, volumes of 1 ml of NPs suspension were filled into 2-mL freeze-drying vials and freeze-dried.

Holmium Loading and Encapsulation Efficacy Determination

Two different methods were carried out to determine the holmium content in NPs.

Inductively Coupled Plasma Atomic Emission Spectroscopy

The determination of holmium content was performed using a spectrometer ARL 3580 (Thermo, Waltham, USA). A sample of 15 mg of prepared NPs was digested in a medium containing 1 vol of H_2SO_4 (95%) and 1 vol of fuming HNO_3 (68%). This was repeated in triplicate. The assay was linear between 0 and 50 μg/mL with a correlation coefficient of .999. To calculate the encapsulation efficacy (EE%), we applied the theoretical (in formulation) and experimental (experimentally determined) HoAcAc loadings in the following equation:

$$\text{HoAcAc } EE\,\% = 100 \times \text{experimental loading}/\text{theoretical loading}. \quad (1)$$

Thermal Neutron Activation Analysis

Thermal neutron activation analysis (NAA) analysis is based on the fact that each formed radioactive nuclide during irradiation decays with a specific half-life, emitting gamma rays of characteristic energy. After irradiation, the gamma ray-induced activity in the samples is measured with a high-resolution spectrometric system using high-purity germanium (HpGe) detector. The analysis has been carried out by bombarding the NPs with a neutron flux of 1×10^{11} n/cm^2/s for 30 min. About five determinations have been carried out for each sample.

Size Determination

The NPs size was determined by a photon correlation spectroscopy (PCS) using Zetasizer 3000 HSa (Malvern, England) at 25°C. Each measurement was performed in triplicate.

Scanning Electronic Microscopy

NP suspensions were deposited on a metallic probe then metallized with gold/palladium using a cathodic pulverizer techniques Hummer II (6 V, 10 mA). Imaging was realized on a FEG Hitachi S800 SEM at an accelerating voltage of 15 kV.

X-Ray Diffraction

X-ray powder diffractometry was carried out to investigate the microencapsulation process effect on the crystalline properties of both the polymer and the HoAcAc. The powder XRD patterns were recorded on a Siemens D500 operated with Cu Kα X-ray radiation, a voltage of 40 kV, and a current of 30 mA. The scans were conducted at a scanning rate of 1°min-1 in the 2θ range from 5 to 70°.

Fourier Transformed Infrared Spectroscopy

The polymer, HoAcAc, irradiated and nonirradiated NPs were characterized by infrared spectroscopy using a Unicam Mattson 5000 FTIR spectrometer at room temperature. The spectra were taken in KBr discs in the range of 3500–400 cm^{-1}.

NMR Characterization

^{1}H and ^{13}C NMR spectra were acquired on a Bruker DMX-300 SB spectrometer in chloroform $CDCl_3$ for the polymer before and after neutron irradiation. In each analysis, about 25 mg of samples were placed into 5-mm NMR tubes and then were dissolved in 2 mL of $CDCl_3$.

Differential Scanning Calorimetry

Thermal analysis was performed using a differential scanning calorimeter DSC TA 125 (TA Instrument, New Castle,

USA). About 10 mg of samples were introduced into aluminium pans and hermetically sealed. All samples were heated at a 2°C/min scanning rate between 25 and 200°C after a 5 min stabilization plate under nitrogen atmosphere. Pure PLLA and HoAcAc were used as controls. The instrument was calibrated with indium for melting point and enthalpy heat of melting.

Gel Permeation Chromatography

Polymer molecular weights were determined on a Waters GPC system equipped with an isocratic pump (Waters 515) operated at a flow rate of 1 mL/min with tetrahydrofuran (THF), an autosampler (Waters 717 plus), a column oven, and a refractive index (RI) detector Model (Waters 410) with integrated temperature controller maintained at 35°C. Data collection was performed with the software «Empower pro» of Waters Corporation. For molecular mass separation, a guard column (PLgel 5 μm), three Polymer Laboratories columns [2 × PLgel 5 μm Mixed C (300 × 7.5 mm)], and [1 × PLgel 5 μm 500 A (300 × 7.5 mm)] were used in-line at 35°C. Calibration was carried out using ND (narrow distributed)-polystyrene standards. The mobile phase was THF [high-performance liquid chromatography (HPLC) grade] stabilized with diether butyl-2,6 methyl-4 phenol. Polymer and NP samples were dissolved in THF, shortly sonicated in ultrasonic bath to better homogenize solutions. Chromatography was carried out after sample filtration through a 0.45-μm filter. Toluene (internal standard) was added to standards and samples as a flow rate corrector.

Neutron Irradiation

All irradiations were performed in the TRIGA reactor facilities in Pitesti, Institute for Nuclear Research (Romania). We used the irradiation channel with a thermal neutron flux of 1.1×10^{13} n/cm^2/s for 1 h. Irradiation was carried out in a sealed poly-propylene cylinder.

RESULTS AND DISCUSSION

Holmium Acetylacetonate Encapsulation Efficacy

In the field of nanotechnology, drug encapsulation efficacy is an important parameter to characterize drug carriers. In this investigation, concerning Ho-loaded NPs being designed for a subsequent neutron bombardment, higher holmium loadings in NPs enable obtaining higher radiotherapeutic doses at relatively shorter activation times (Hafeli et al., 1994; Nijsen et al., 1999).

The holmium loading, determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) method, was 18.4 ± 0.6% wt/wt corresponding to an encapsulation efficacy around 90%. Furthermore, the NAA results coincided with ICP-AES results but were somewhat higher (19.1 ± 0.95% wt/wt). The holmium loading in our study is relatively close to those reported for microscale-sized PLLA particles (17.0 ± 0.6% wt/wt) by Nijsen et al. (1999) and higher than the 9–10% loadings given by Mumper and Jay (1992). According to the above-mentioned neutron irradiation conditions, the activation of 1 g of ^{165}Ho-loaded NPs could yield a specific activity around 24.7 GBq being close to that reported by Mumper, Ryo, and Jay (1991), which was 25.9 GBq/g of microparticles (after converting masses and radioactivity units) with a quasi-similar neutron flux but with a longer irradiation time (3 h). In addition, assuming a necessary 1-day period to ship the activated NPs batch (1 g) to the hospital, we think that the obtained specific radioactivity from these NPs remains well sufficient for an intratumoral radiotherapy.

NPs Size and Morphology

In pharmaceutical technology field, the particle size is considered as an important parameter that influences the biopharmaceutical properties and the particles fate. Here, as it has been indicated above, the NPs are designed to be administered by the TMT microtube that is perforated at its distal end with holes of less than 50 μm (Hiltbrand et al., 2003). Figure 1A shows the NP size distribution results obtained by PCS. As it can be seen, the NPs size ranged between 100 and 110 nm with a mean diameter of 307 nm and a polydispersity index of 0.26. Furthermore, Figure 1B shows a SEM micrograph of prepared NPs. Considering our perspective of NPs injection by the TMT technique, we think that these NPs would have adequate size and size distribution for this purpose. To check that, an in vitro TMT injection of these NPs labeled with the fluorescent red nile substance, under a high pressure, was performed in a gel of agarose (at 2% agarose concentration). The NPs showed to get out rapidly and easily through the microtube holes and to diffuse in the gel in a circle of 5 mm radius around the microtube (Figure 2).

Several experiments were conducted to evaluate the influence of proceeding and formulation parameters on the NPs size. The results showed that the NPs size increases with the polymer amount in formulation and inversely decreases with the stabilizer amount and the applied stirring speed in accordance with our previous work on Re-loaded PLLA NPs (Hamoudeh, Salim, Barbos, Paunoiu, & Fessi, 2007b) and in agreement with published results elsewhere (Kwon, Lee, Choi, Jang, & Kim, 2001; Sahoo, Panyam, Prabha, & Labhasetwar, 2002).

Indeed, these variables' influences on the NPs size can be explained as follows:

- The polymer effect: the higher concentration in the organic phase results in an increase in its viscosity rendering it more resistant to shear forces and yielding larger droplets and subsequently larger NPs (Kyoung, Sang Bong, & Moo, 2005).

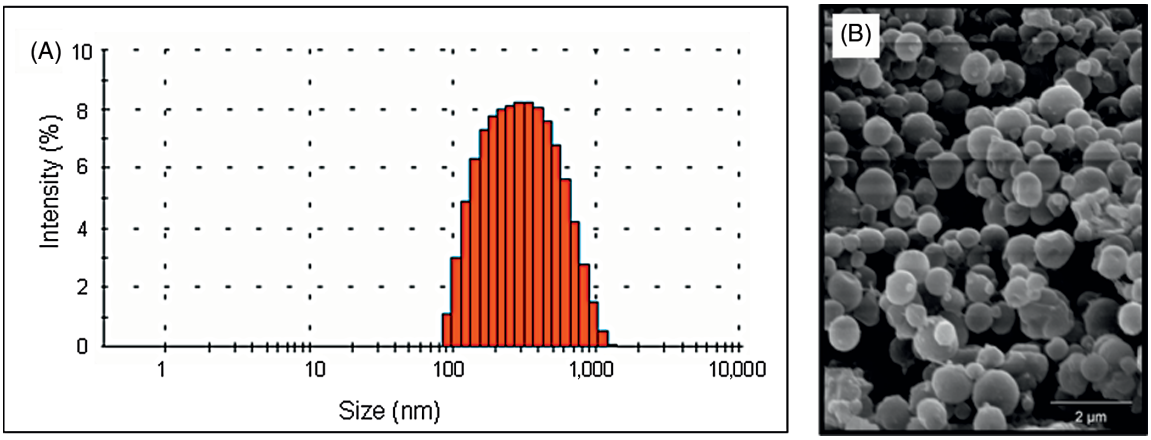

FIGURE 1. (A) Nanoparticle size distribution and (B) scanning electron microscope (SEM) micrograph of holmium acetylacetonate trihydrate (HoAcAc) -loaded NPs (bar 2 µm).

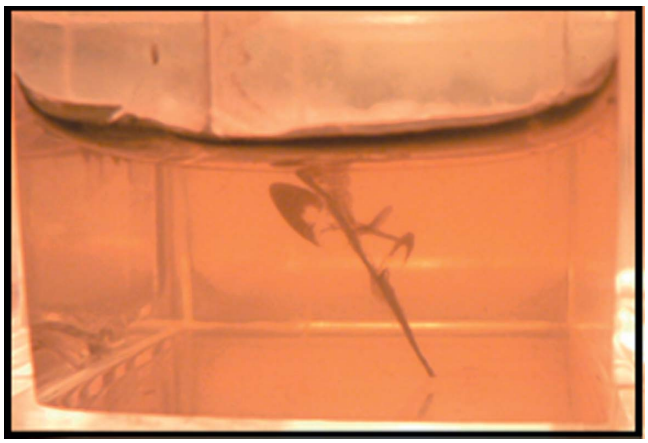

FIGURE 2. Fluorescent nanoparticle in vitro injection in agarose gel by the targeted multitherapy (TMT) technique.

- The stirring speed effect: the higher the applied stirring speed in emulsification, the more the available energy to disperse the organic phase in the aqueous one yielding smaller droplets and resulting in smaller NPs (Hamoudeh & Fessi, 2006; Kwon et al., 2001).
- The stabilizer effect: the increase in PVA concentration renders the external aqueous phase more viscous, which amplifies the shear forces' influence on the organic phase and allows obtaining smaller droplets (Sahoo et al., 2002).

Furthermore, we have investigated whether the NP size distribution and the morphology had been modified after neutron irradiation at the above-mentioned conditions. Generally, the most visible NP damage that can be induced by neutron irradiations is due to the local absorbed heat by NPs (Hafeli, Roberts, Pauer, Kraeft, & Macklis, 2001). Figure 3A–C shows HoAcAc-loaded NPs (18% wt/wt Ho loaded) before (A) and after neutron irradiation (B and C). As it can be noticed from the figure, the neutron irradiation induced some harmful effects on the NP morphology. These effects included partial agglomeration of some NPs, formation of a few pores at the surface of some NPs, and rarely, but, however, noticed in only a very small part of micrographs, some polymer fusion. The above-found morphological modifications have also been found in our previous work (Hamoudeh, Fessi, Mehier, Al Faraj, & Canet-Soulas, 2007c) and in that of Hafeli et al. (2001) concerning PLLA-based microparticles (22 µm) loaded with 30% wt/wt rhenium metal after very close neutron irradiation conditions (1.5×10^{13} n/cm^2/s during 1 h). According to the authors, the theoretical temperature increase without heat transfer from the activation vial can be obtained by applying the following equation supplied by the authors:

$$\frac{dT}{dt} = \frac{1}{c} \times \frac{dE}{dt}, \tag{2}$$

where dT/dt is the temperature increase per second (K/s), dE/dt is the energy deposited per kilogram per second (kJ/kg/s), and c is the PLLA-specific heat value, which is estimated to be 1.5 kJ/kg/K according to the authors. Considering now that the neutron irradiation-induced dose rate into our NPs in the reactor was 0.35 MGy/h (B. Barbos, Pitesti Nuclear Reactor, Institute for Nuclear Research Romania, personal communication, 2007) and that one Gray (Gy) equals 10^{-3} kJ/kg, the dose rate of 0.35 MGy/h equals 0.0972 kJ/kg/s. By entering these data into Equation 2, the temperature rise in our NPs was found to be 0.0648 K/s or 233.3 K/h. However, as mentioned above in this study, our previous study (Hamoudeh et al., 2007c), and that of Hafeli

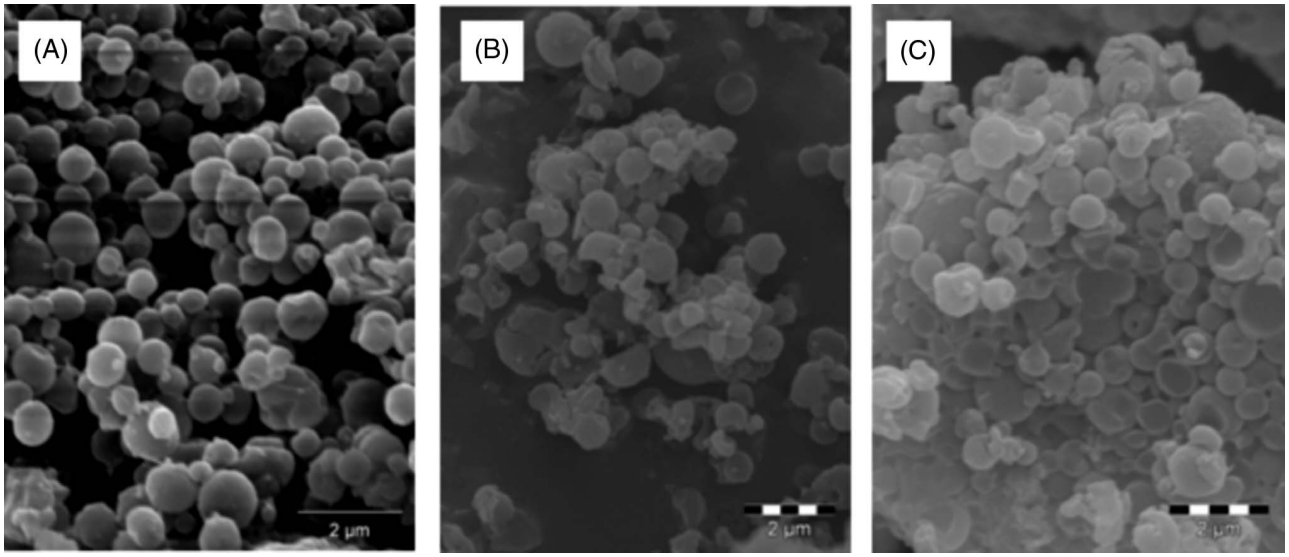

FIGURE 3. Scanning electron microscope (SEM) micrograph of holmium acetylacetonate trihydrate (HoAcAc)-loaded nanoparticles (NPs) before (A) and after (b and C) neuron at 1.1×10^{13} n/cm^2/s for 1 h (bar = 2 µm).

et al. (2001), we think that the polymer melting point (here, T_m = 177°C) has been reached as the polymer started to melt at the points where NPs touch each other but without a noticeable general polymer melting. Therefore, in our opinion, a heat transfer has certainly occurred from the NPs batch out to the activation vial.

Furthermore, pore formation may be ascribed to the lower specific heat value of holmium (0.16 kJ/kg/K) yielding by consequence a temperature increase of holmium atoms reaching up to 0.607 K/s, which is about 9.3 times higher than that of PLLA (see above). Therefore, in resemblance with previously reported results on Re-loaded PLLA NPs (Hafeli et al., 2001; Hamoudeh et al., 2007c), the holmium atoms in the irradiated NPs in this study may represent some local hot spots in the PLLA matrix and lead consequently to these surface pores.

X-Ray Diffraction

XRD is a useful tool to study crystal lattice arrangements and yields useful information on the degree of crystallinity of the sample. It has been frequently used to determine the crystalline/amorphous pattern of drugs after encapsulation in biomaterials (Redenti et al., 1996; Zhang & Shen, 2007). Figure 4 shows the XRD pattern of HoAcAc and Ho-loaded NPs. It was observed that the characteristic peaks of crystalline HoAcAc were almost absent in NPs. This indicated that HoAcAc was incorporated in amorphous state, which is in agreement with the results of Nijesn et al. (2001). The authors attributed the HoAcAc amorphization to a fast solvent removal during particle preparation that would lead to a more rapid solidification of the polymer matrix, impeding by consequence the crystallization of HoAcAc.

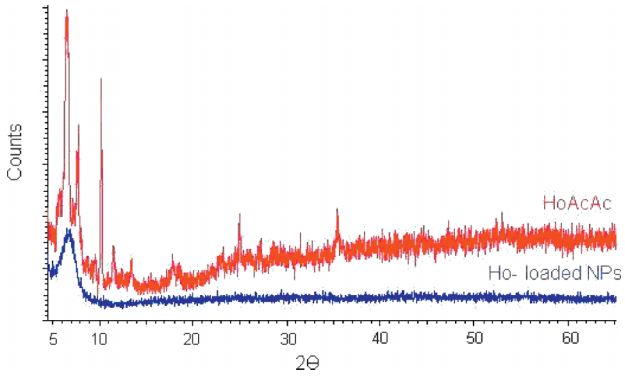

FIGURE 4. XRD patterns of holmium acetylacetonate trihydrate (HoAcAc) and HoAcAc-loaded NPs.

Fourier Transformed Infrared Spectroscopy

Figure 5A shows the characteristic absorption peaks of PLLA, which are evident at 1,750 cm^{-1} (carbonyl groups), 1,080 cm^{-1} (C–O–C stretching bands), and 1,450 cm^{-1} (C–H stretching in methyl groups) in agreement with published results elsewhere (Hamoudeh et al., 2007b; Lee, Isobe, & Senna, 1996; Paragkumar, Edith, & Six, 2006). Furthermore, the FTIR spectra of HoAcAc (Figure 5E) show peaks at 1 cm^{-1} (carbonyl groups) and 1,522 cm^{-1} (C=C stretching groups) in accordance with the works of Nijsen et al. (2001, 2002). All these characteristic peaks of both PLLA and HoAcAc were well found in the transmittance spectra of Ho-loaded NPs (Figure 5C) reflecting the success of HoAcAc encapsulation in the PLLA matrix without apparently significant interaction between HoAcAc and PLLA.

After irradiation, the intensity of the PLLA broad peak between 3,200 and 3,600 cm^{-1} increased in agreement with the

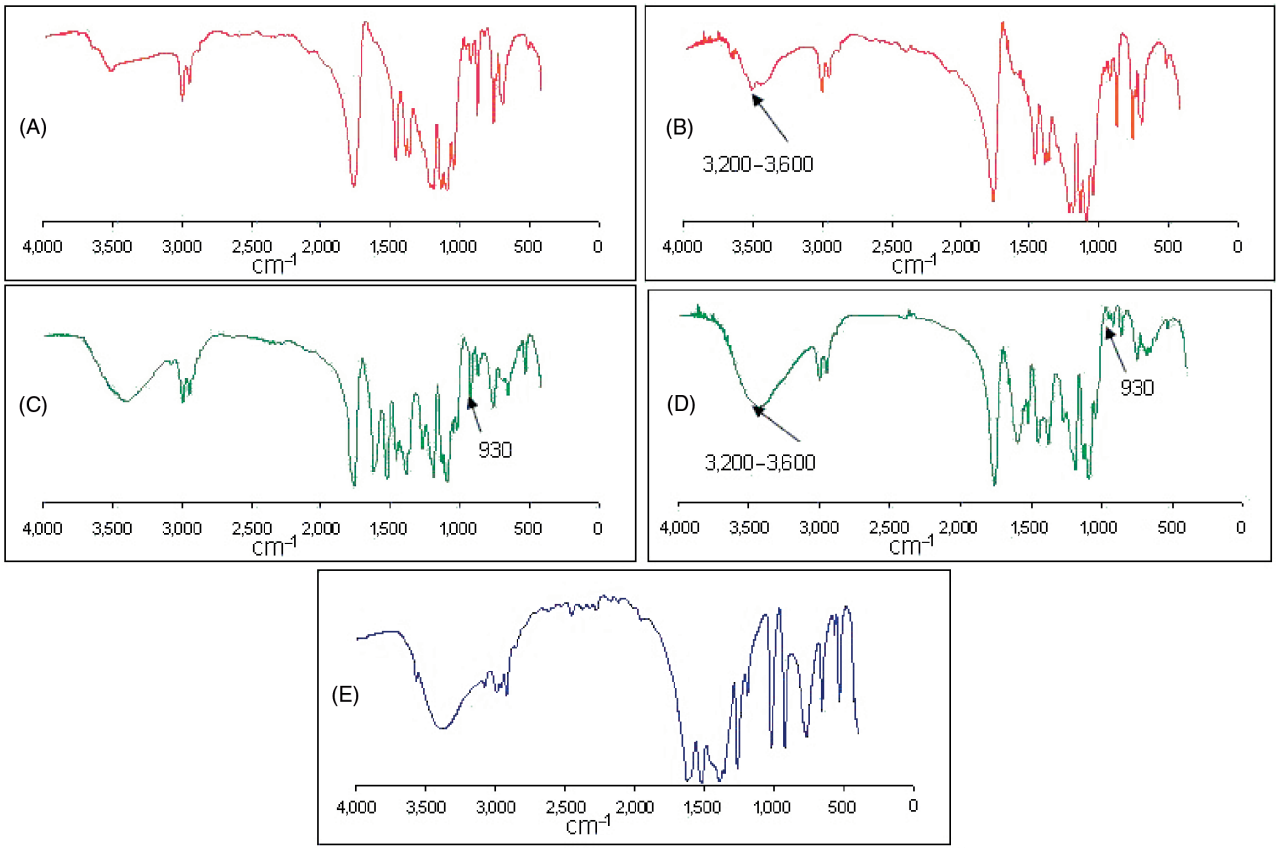

FIGURE 5. Fourier transformed infrared spectroscopy (FTIR) spectra of (A) poly-L-lactide (PLLA), (C) holmium acetylacetonate trihydrate (HoAcAc)-loaded NPs, and (E) HoAcAc before irradiation. FTIR spectra of (B) PLLA and (D) HoAcAc-loaded NPs after neutron irradiation.

results of Nijsen et al. (2002) and our previous work (Hamoudeh et al., 2007c) (Figure 5B and D). This broad peak corresponds to stretching vibrations of COOH end groups of PLLA chains, and its increase can be explained by a chain scission confirming the results of GPC (Section 3.7). Loo, Ooi, and Boey (2004), investigating the electron beam radiation effects on PLLA and poly(lactic-co-glycolic acid) (PLGA), attributed this intensity increase to the formation of alcohol groups upon irradiation. In addition, the peak that was present at 930 cm^{-1} in PLLA spectra became clearly shorter after irradiation, which has also been reported by Mumper and Jay (1992). However, again from Figure 5, the retention of the above-mentioned principal peaks in both PLLA and HoAcAc spectra after irradiation provided a good proof that irradiated NPs retained the overall chemical identity of the used HoAcAc and PLLA despite the noticed PLLA molecular weight and crystallinity decrease (Sections 3.6 and 3.7).

NMR Characterization

In ^{1}H NMR spectra of PLLA, the characteristic hydrogen shifts are evident at 1.58 (3H, d, J = 6.97 Hz) and 5.21 ppm (1H, q, J = 7.16 Hz) in accordance with the results of Mumper and Jay (1992) and Hamoudeh et al. (2007b). These peaks were well detected without difference in ^{1}H NMR spectra of PLLA after irradiation (data not shown). The ^{13}C NMR spectra of PLLA before and after neutron irradiation are shown in Figure 6. The characteristic peaks of PLLA were found at 16.99 (CH_3), 69.35 (CH), and 169.95 ppm (COO) in agreement with the results of Siedler et al. (2001) and Hamoudeh et al. (2007b). These peaks were again detected in PLLA after irradiation providing another proof that PLLA does maintain its chemical composition after neutron irradiation.

Polymer Therma Properties

The DSC technique has been widely used as a useful characterization method for drug delivery systems to get both qualitative and quantitative data about the physicochemical state of incorporated drugs in nano- and microparticles (Dubernet, 1995). Using the DSC allows determining the drug nature inside the polymer matrix. This nature can emerge from crystalline to amorphous state influencing by consequence the relevant in vitro release properties (Tayade & Kale, 2004).

Figure 7A shows the representative DSC scans for PLLA-, HoAcAc-, and Ho-loaded NPs. The PLLA thermal curve

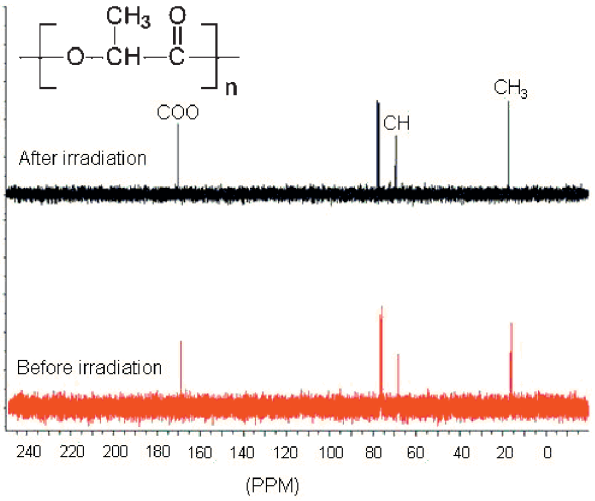

FIGURE 6. ^{13}C spectra of poly-L-lactide (PLLA) before and after neutron irradiation.

showed a melting point at 176–177°C with a melting enthalpy of 57 J/g indicating a semi-crystalline structure with a degree of crystallinity of around 60%, which was calculated as the percentage of the hereby used PLLA melting enthalpy devised by the melting enthalpy for a 100% crystalline PLLA ($\Delta H_f(100\%)$ = 95 J/g) (Sosnowski, 2002). The HoAcAc thermal curve showed, in turn, a large endothermic peak at 120–125°C in agreement with that found by Mumper and Jay (1992). Interestingly, the DSC curve of Ho-loaded NPs did not show this large peak ascribed to HoAcAc. This indicates that HoAcAc is in amorphous form in NPs, which is in accordance with the XRD results and other reported works elsewhere (Milicevic, Trifunovic, Galovic, & Suljovrujic, 2007; Mumper & Jay, 1992) for HoAcAc-loaded microparticles. Furthermore, this accords well with SEM micrographs of Ho-loaded NPs (Figure 1B) as no HoAcAc crystals could be visualized at the surface or out of NPs. Indeed, this is typical of what happens when a crystalline material becomes uniformly dispersed within a solid matrix at the molecular level. In such a case, the crystalline lattice is disrupted by intervening polymer molecules. As a result, the substance, here HoAcAc, is converted to the amorphous form and no longer exhibits melting behavior. Thus, the DSC results are highly indicative of the HoAcAc being uniformly dispersed throughout the polymer matrix rather than being outside the NP or else clustered within.

Furthermore, a sharp decrease in the PLLA melting enthalpy in Ho-loaded NPs was recorded reaching down to 15 J/g (Table 2). Such melting enthalpy decrease has also been found by Mumper and Jay (1992) and Nijsen et al. (2001). Indeed,

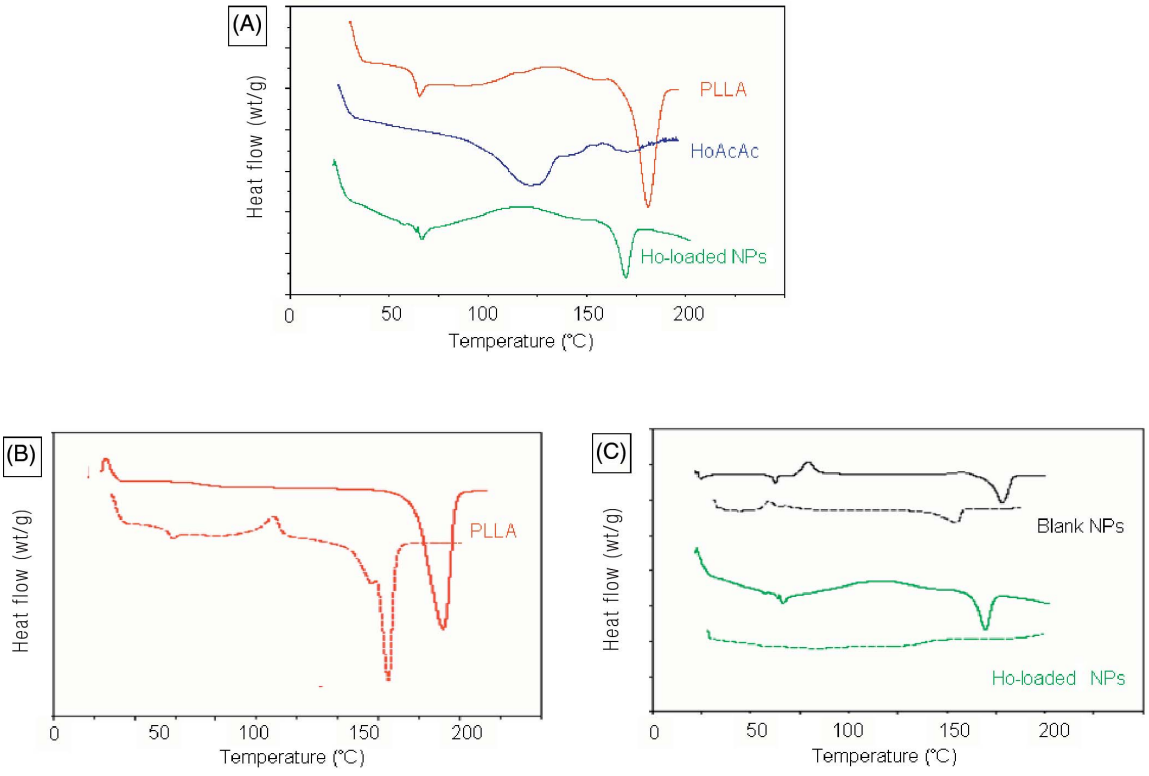

FIGURE 7. Differential scanning calorimetry (DSC) results of (A) poly-L-lactide (PLLA), holmium acetylacetonate trihydrate (HoAcAc), and HoAcAc-loaded NPs; (B) PLLA before and after neutron irradiation; and (C) HoAcAc-loaded NPs before and after neutron irradiation.

TABLE 2
DSC Results of PLLA, Blank, and Holmium-Loaded Nanoparticles before and after Neutron Irradiation

	Before Irradiation		After Irradiation	
Sample	T (°C)	Melting Enthalpy (J/g)	T (°C)	Melting Enthalpy (J/g)
PLLA	177	57	152	41
Blank NPs	173	53	141	29.3
Holmium-loaded NPs	172	15	ND	ND

DSC, differential scanning calorimetry; PLLA, poly-L-lactide; ND, not detected; NP, nanoparticle.

this decrease, which was not found in blank NPs, can be explained by the fact that HoAcAc may be molecularly dispersed in the PLLA matrix and acts as a plasticizer for PLLA (Mumper & Jay, 1992).

Insight to the stability of PLLA and NPs after irradiation was also obtained by DSC. Figure 7B shows along with Table 2 the neutron irradiation effect on PLLA thermal properties. After irradiation, free PLLA melting enthalpy decreased from 57 to 41 J/g and its melting point was shifted down to152°C. These findings indicate that a decrease in PLLA crystallinity after irradiation has occurred, which is in agreement with our previous work on dirhenium decacarbonyl-loaded PLLA NPs (Hamoudeh et al., 2007c) and other reported results by the groups of Nijsen et al. (2002), Mumper and Jay (1992), and Hafeli et al. (2001). The same decrease tendency was also recorded in blank NPs (Table 2), whereas the DSC curve of irradiated HoAcAc-loaded NPs showed a disappearance of the PLLA melting peak indicating probably a total loss of polymer crystallinity upon neutron irradiation (Figure 7C).

Polymer Molecular Weights

The neutron irradiation of PLLA and NPs resulted in a substantial decrease in the polymer molecular weight reaching about 30–35% of the initial number and weight average molecular weights (M_n and M_w) in all samples, which is in accordance with our previous work (Hamoudeh et al., 2007c). Furthermore, the polydispersity index (PI = M_w/M_n) increased from 1.9 to 2.1 in samples after neutron irradiation indicating that chain scission had occurred in a random way (Milicevic et al., 2007; Sintzel, Merkli, Tabatabay, & Gurny, 1997). Generally, two mechanisms can be responsible for the radiation-induced chain scissions of polymers such as PLLA: (a) unzipping during which terminal segments of polymer chains are preferentially cleaved inducing a decrease in the M_n although the M_w is not largely affected (Gilding & Reed, 1979) and by consequence the polymer polydispersity index increases. (b) A random cleavage of the polymer chains that leads to the decrease of both the M_n and M_w values and does not induce a profound increase in the PI value (Chu & Campbell, 1982; Volland, Wolff, & Kissel, 1994). Therefore, we think that the principal mechanism leading to the noticed clear decrease in PLLA molecular weights would be a random chain cleavage of PLLA chains, which accords again with other results published elsewhere in neutron irradiation-like conditions (Hafeli et al., 2001; Hamoudeh et al. 2007c; Nijsen et al., 2002).

CONCLUSION

The main objective of this article is to describe the elaboration, characterization, and neutron irradiation of HoAcAc-loaded PLLA NPs, a novel neutron-activatable radiopharmaceutical agent for intratumoral radiotherapy by the hereby described TMT technique. NPs were prepared with sizes ranging from 100 to 1,100 nm (mean diameter of 307 nm) being suitable for the TMT administration method. Elemental holmium loading was determined by ICP-AES and NAA methods and was around 18% wt/wt with an encapsulation efficacy reaching 90%. Different characterization methods showed that HoAcAc was incorporated in amorphous form. The NPs were irradiated in a nuclear reactor at a thermal neutron flux of 1.1×10^{13} n/cm^2/s for 1 h. SEM micrographs of irradiated NPs showed some signals of alteration. Some NPs were partially coagglomerated and some pores appeared at their surface giving evidence of a local temperature increase in the irradiation vials. Furthermore, DSC results showed a decrease in PLLA melting point and melting enthalpy in both blank and loaded NPs reflecting a decrease in the polymer crystallinity. Moreover, a clear irradiation-induced decrease in both PLLA molecular weights (M_n, M_w) was recorded but without largely increasing the polymer polydispersity index indicating that an irradiation-induced PLLA chain scission happened in a random way. However, interestingly, other conducted experiments confirmed that irradiated NPs retained the chemical identity of HoAcAc and PLLA in irradiated NPs. In conclusion, although neutron irradiation has induced some NP damage, holmium acetylacetonate-loaded PLLA NPs retained their overall chemical composition, and their size distribution remained suitable for the TMT administration technique. Coupled with the TMT technique, these NPs represent a novel promising radiopharmaceutical for intratumoral radiotherapy.

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