POLY(3-HYDROXI-BUTYRATE-CO-3-HYDROXY-VALERATE) (PHB-HV) MICROPARTICLES LOADED WITH HOLMIUM ACETYLACETONATE AS POTENTIAL CONTRAST AGENTS FOR MAGNETIC RESONANCE IMAGES

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Mariangela de Burgos M de Azevedo1
Vitor HS Melo1
Carlos RJ Soares1
Lionel F Gamarra2
Caio HN Barros3,4
Ljubica Tasic

1Centro de Biotecnologia, IPEN/CNEN-SP, São Paulo, Brazil; 2Hospital Israelita Albert Einstein, São Paulo, SP, Brazil; 3School of Chemical and Bioprocess Engineering, University College Dublin, Dublin, Ireland; 4Instituto de Química, UNICAMP, SP, Campinas, Brazil

Introduction: Biodegradable polymers that contain radioactive isotopes such as Holmium 166 have potential applications as beta particle emitters in tumor tissues. Also, Ho(III) is paramagnetic, which makes it suitable as a contrast agent for magnetic resonance (MR) images.

Methods: Holmium acetylacetonate (Ho(acac)3) loaded poly(3-hydroxy-butyrate-co-3-hydroxy-valerate) microspheres, with 5% or 8% of 3-hydroxy-valerate (HV), were prepared by emulsification/evaporation process within 20–53 μm size. Microspheres characterization was done using scanning electron microscopy, energy-dispersive X-ray, and infrared spectroscopies. The release of holmium(III) in sodium phosphate buffer (pH 7.4) was followed for 9 days with inductively coupled plasma. Finally, T2 and T2* magnetic resonance images (MRI) were acquired and compared with the MRI of the inclusion complex of holmium acetylacetonate in some β-cyclodextrins.

Results: Holmium acetylacetonate loading, evaluated by thermogravimetry, was up to 20 times higher for copolymer with 5% of HV. It was shown that microspheres loaded with Ho(acac)3 exhibited an accumulation of Ho(III) on their surfaces but were stable over time, as no expressive release of holmium(III) was detected in 9-day exposition to sodium phosphate buffer. Holmium acetylacetonate in both microspheres or inclusion complexes was very efficient in obtaining T2 and T2* weighted images in magnetic resonance, thus, might be used as contrast agents.

Conclusion: This is the first description of the use of inclusion complexes of holmium acetylacetonate in biodegradable polymers as contrast agents. New investigations are underway to evaluate the resistance of PHB-HV polymer microparticles to nuclear activation to assess their potential for use as radiopharmaceuticals for the treatment of liver cancer.

Keywords: holmium acetylacetonate, PHB-HV microparticles, microspheres, MRI

Correspondence: Mariangela de Burgos M de Azevedo
Centro de Biotecnologia, IPEN/CNEN-SP, São Paulo, Brazil, 05508-000
Email mariangela.burgos@cnpq.pq.br

Introduction
The concept of nano- or microtechnological materials involves ones with sizes in the range of 1–100 nm or up to tenths of millimeters, respectively, and their application in human health is growing rapidly.1 Cancer is a critical public health problem worldwide that has brought great burden to society. In 2016, around 1,685,210 new cases and 595,690 cancer deaths occurred just in the United States.2 Many of the known cancer therapy modalities, such as chemo-, radio-, photodynamic therapy among others, apply substances or materials that can be classified in one of the above cited categories.3,4 A great number of substances able
to inhibit key enzymes, cytoskeleton synthesis, or with antimitotic, apoptotic and necrotic activities are used in cancer treatments, such as etoposide, teniposide, taxol, methotrexate, cisplatin, and derivatives.5–10 All of these compounds belong to nano- or micro-world. Tumors’ treatments and choices of specific therapeutic modality depend on tumor sensitivity. For example, to minimize maleficent effects in radiotherapeutic techniques, radionuclides, and radioisotopes can be complexed into micro-technological materials made from pure mixtures or composites.

Radioactive decays are hardly supported by cancer tissues. By stopping an essential metabolic process or inducing apoptosis, tumor tissues are quickly destroyed by direct or indirect effects of radioactive particles, gamma rays, auger electrons release, and generation of free radicals.11 Alpha and beta particles, as well as gamma rays, propagate in a great range, and thus have more potential to destroy cancer tissues. One of the most suitable targets to beta particles is the water molecule and hydroxyl radical is the first of many new high-energy species and other radicals that destroy cell structures.12 The high-energy alpha particles propagate less in tissues but cause more irreparable damages in the genetic material, while high-energy gamma radiation breaks chemical bonds.13

However, all kind of therapeutic agents usually explored to eliminate cancer are also absorbed by healthy cells: patients may suffer side effects as physical weakening, necrosis or burns depending on the method used. Thus, micro- and nanotechnology concepts have been employed to address many medical issues, prepare better delivery systems, and to overcome drug cancer resistance mechanisms.14–20 Semiconductor nanoparticles (“quantum dots”) are proposed as new modalities for fluorescence-based diagnostics and photodynamic therapy.21 Antibodies improve absorption of gold nanoparticles in cancer cells and were proposed as new image-based diagnostics and photo-thermal therapies.22,23 All cells have specific proteins on their membranes, which enable recognition and specific-based delivery of agents into the cells with great selectivity. Nutrients, covertures, vesicles, and matrixes also enhance delivery and cytotoxicity of the known therapeutic agents.14,24,25

Inorganic oxides with radioisotopes have been used since 1960s to combine the advantages of both radiotherapy and embolization procedures: they are denser and sediment closer to the spot where applied.26,27 Resins as matrix for radiopharmaceuticals are lighter, and so distribute better in tissues.28,29 Yttrium-90 is the most used beta particles source to prepare these internal radiotherapy materials, which are injected in main arteries that supply blood to cancer tissues.30–32 Among advantages of using it in such manner are controlled and higher radiation dose which is locally administrated, which causes less damage to surrounding healthy tissues. It can be preferred to chemotherapeutic agents with similar efficiency for procedures of monitoring and acquiring images because of the less collateral effects, and even to avoid a risky surgery. Nevertheless, there is a need to develop and design better delivery systems for all radiotherapy agents. Radiation delivery systems and embolization might be either pure materials or compound-loaded solid matrixes with particle sizes not greater than 20 µm as not to block hepatic microcirculation.33 Also, it is important to say that X-ray computed tomography allows visualization of catheters in real time to deliver particles during procedures of internal radiotherapy.34,35

Many recent researches use holmium-166 as beta particles source. This rare earth forms stable complexes with many ligands.36–41 Beta emissions of 166Ho and 90Y in tissues have ranges of 2.1 and 3.6 mm, respectively, and the treatments using the first one are less aggressive with minor damage on healthy surrounding tissues. Gamma-ray emission of 166Ho has less energy than the one coming from the 90Y, but it is more abundant, so a scintigraphy diagnostic of tissues retaining holmium-coordinated compounds has become possible.42–49 Ho(III) is paramagnetic and replaces its inner sphere waters quickly; thus, this rare earth element can act as contrast agent to acquire magnetic resonance images (MRI) as a substitute for the gadolinium compounds.50–53 Finally, luminescent emission of this trivalent cation in visible spectra (500–530 nm) allows obtaining images of compounds in samples and in vivo by fluorescence-based techniques.34,54

Many inconveniences in the use of oxides and resins as matrixes to produce particles for medical therapies come from their long-time stability in the human body what brings a risk of embolization in healthy organs. Recently, the biodegradable matrixes are being preferred to host radionuclides.56 Their lower time of stability gives them properties of controlled delivery systems: (1) release time of radionuclides is longer than in free formulations, (2) they maintain plasma concentration for a long time, (3) they also locomote in vivo through physical and/or chemical principles.57 Radioisotope loaded polymeric
matrixes change their properties with the radioactive decays dose, such as degradation time in vivo. For example, gamma rays may induce radiolysis in polymers during their industrial processing or sterilization or even promote polymerization or cross-linking of matrixes, which depend on polymer nature and radiation dose.\textsuperscript{58–67}

An ideal procedure to obtain radiopharmaceuticals at lower costs and in greater amounts involves the development of microdevices loaded with the chosen radionuclide and posterior activation in a nuclear reactor. Emulsification process is commonly used in chemistry, food, and (radio) pharmaceutics due to the nature of the materials that can dissolve or disperse in drops, micelles, or vesicles.\textsuperscript{68–71}

Although some disadvantages in obtaining radiopharmaceutical particles involve damage or aggregation during a nuclear activation of radioisotopes in biodegradable polymers matrixes, these materials are extensively used to carry and deliver radiation as they enable an easier absorption by tumors. Many of studied materials are cellular nutrients or substrates for tissue engineering and when used to deliver radiation, cancer cells suffer radiation damages by absorbing those particles.\textsuperscript{72,73} One model for the designed microparticle radiopharmaceuticals to the hepatic cancer intern radiotherapy procedure was produced by emulsification/evaporation of solvent, where holmium acetylacetonate was loaded into the poly(L-lactic acid) microspheres.\textsuperscript{32,74–82} Acetylacetonate complexes are commonly used to extract and recover metal transition ions due to their stability that provide a suitable retention of the ions.\textsuperscript{83–86}

The high cost of poly(L-lactic acid) limits its applications despite its better crystallinity and slower degradation under biological conditions.\textsuperscript{87,88} A substitute polyester to prepare microparticle-based biodegradable radiopharmaceuticals is poly(3-hydroxy-butyrate-co-3-hydroxy-valerate), PHB-HV. Poly(hydroxyl-alkanoates) are easily extracted from microorganisms and very much applicable for biotechnological purposes.\textsuperscript{89,90} For instance, cultures of the bacteria \textit{Alcaligenis eutrophus} supplied by sugar as a carbon source and in scarcity of nitrogen nutrients produce massively these polymers like a nutritional stock and if sugar is replaced by the propionic acid, \textit{Alcaligenis eutrophus} produces copolymers of 3-hydroxy-butyrates and 3-hydroxy-valerates instead.\textsuperscript{91}

Poly(3-hydroxy-butyrate), PHB is the most studied poly(hydroxyl-alkanoate), although it is a brittle material and inadequate for many industrial and clinical applications; its copolymers or blends with other polyesters enhance mechanical properties, such as elasticity.\textsuperscript{92} Therefore, we prepared holmium acetylacetonate loaded PHB-HV microdevices using raw poly(3-hydroxy-butyrate) with 5% and 8% of 3-hydroxy-valerate by emulsification/evaporation of solvent. These microparticles were evaluated as potential radiopharmaceutical and contrast agent by T\textsubscript{2} and T\textsubscript{2}* weighted MRI. It is worth saying that holmium acetylacetonate microspheres are known for the latter property.\textsuperscript{93}

The transverse relaxation time (T\textsubscript{2}) is the decay constant of recovery of xy component or the vector of magnetization of nuclear spin initial or its equilibrium value. Physically, it depends on changes in nuclear spin magnetization: magnetic fields applied in real samples have random and transient changes of the local alignment, and/or own sample may have an instrinsical inhomogeneity; the diffused nuclear spin precession frequency defines a band of radiofrequency absorption. This is an intramolecular interaction caused by spin–spin nuclear and/or electronic events. Large molecules may have an intrinsic magnetic field, but their internal components can affect it easily and reduce T\textsubscript{2} relaxation time; small molecules and free flow water are less disturbed. Lipid rich tissues or muscles have water as a structure material, and T\textsubscript{2} lower than blood water. Convention of T\textsubscript{2} weighted images on magnetic resonance (MR) is that white color represents locals rich in free water.\textsuperscript{94} White color indicates sites of greatest T\textsubscript{2} like kidneys, gonads, inflammation, fluid, crystals, protein-rich tissues, and blood derivatives. Tissues which are rich in fats or the spinal lipid portion appear white or gray according to the free water content in the range of T\textsubscript{2} values typical for tissues with more fix water molecules as liver, pancreas, muscle, cartilage, or tissues rich in collagen. Finally, poor water-free tissues such as bone, tissues without water (lungs) and fast-moving fluids exhibit lower T\textsubscript{2}, and also water bounded to paramagnetic elements – electronic spin facilitates the nuclear spin relaxation process and, thus, appear black in these images.

Finally, all real systems exchange energy by intermolecular interactions, so the energy is distributed in states denoting rotational, vibrational, electronical, or other energy levels in an atom or a molecule. Different chemical compositions in the environment, such as the presence of coagulum and microparticles and inhomogeneity of applied magnetic field affect the distribution of states. To the nuclear spin precession frequencies, it causes dephasing in T\textsubscript{2} relaxation times and frequencies, and it has a relaxation time constant denoted as T\textsubscript{2}*.\textsuperscript{95,96} T\textsubscript{2} and T\textsubscript{2}* weighted images of the holmium acetylacetonate loaded
PHB-HV microspheres were compared to inclusion complexes between holmium acetylacetonate and β-cyclodextrin or hydroxyl-propyl-β-cyclodextrin, because these host-guest systems dissolve in water. At this stage, the obtained materials were not capped by hydrophilic materials, but once other hydrophobic materials easily suffer anchoring by the opsonist, proteins that induce immunological reaction of macrophages, were considered as materials with great possibility to be applied in cancer treatments as they are. We focused on 20–53 μm sized particles because of their potential application in hepatic cancer brachytherapy procedures.

**Materials and methods**

**Materials**

Holmium chloride hexahydrate, poly(vinyl alcohol) (MM 13,000–23,000 g mol⁻¹, 98% hydrolyzed), poly(3-hydroxy-butyrate-co-3-hydroxy-valerate) (PHB-HV) with 5% or 8% amounts of 3-hydroxy-valerate in copolymer were obtained from the Sigma-Aldrich. Acetylacetone and chloroform were from Vetec (Brazil). Ammonium hydroxide and nitric acid were obtained from commercial suppliers and used as obtained.

**Preparation of holmium acetylacetonate**

Holmium acetylacetone was prepared by a modified procedure related by Nijsen et al. Acetylacetone, 225 mL, was dissolved in 1350 mL of water, and pH was adjusted to 8.5 by dropwise addition of concentrated ammonium hydroxide. This solution was stirred for 30 mins, and its pH corrected when changed. Holmium chloride hexahydrate (379.38 g mol⁻¹), 9.45 g, was dissolved in 200 mL of water containing 5 drops of concentrated nitric acid and stirred for 2 hrs. HoCl₃.6H₂O solution was added to the acetylacetone one, and pH was adjusted to 8.5 adding drops of ammonium hydroxide. Reaction continued for 24 hrs under stirring. The orange powder was separated by filtration, washed with water and dried. Mass of 7.0 g (54.4% yield) was obtained and assigned to [Ho(acac)₃(H₂O)]×2H₂O as number of inner sphere water molecules was confirmed by thermogravimetric studies.

**Preparation of the β-cyclodextrin-holmium acetylacetonate and the hydroxyl-propyl-β-cyclodextrin inclusion complexes (Scheme 1A)**

The inclusion complex between β-cyclodextrin and Ho(acac)₃, β-CD-Ho(acac)₃, was prepared by dissolving 236.85 mg of β-cyclodextrin in 50.0 mL of water and suspending 52.0 mg of Ho(acac)₃ in it, after stirring for 1 hr. Then, the suspension was left in the refrigerator for 3 days. Other comparative system was prepared from 277.46 mg of hydroxyl-propyl-β-cyclodextrin (HP-β-CD) and 50.1 mg of Ho(acac)₃ using the aforementioned procedure to obtain a new inclusion complex, HP-β-CD-Ho(acac)₃.

**Emulsification process to prepare PHB-HV microspheres (Scheme 1B)**

PHB-HV 5%-MS

Polymer – 3.0 g of poly(3-hydroxy-butyrate) (PHB) was dissolved in 80 mL of chloroform under sonication and
heating at 50°C. This solution was added dropwise into 480 mL of a 1% (w/w) aqueous solution of poly(vinyl alcohol), under the stirring at 1120 rpm for 15 mins. Residual chloroform was removed decreasing the stirring speed to 700 rpm and for additional 3 hrs. The emulsion was centrifuged with 1 L of water, 250 mL of 0.1 mol L⁻¹ hydrochloric acid and again with 1 L of water; and filtered using stainless steel sieves. PHB-HV 8% microspheres (20–53 μm) were obtained in 3.5% yield (107.4 g). To prepare Ho(acac)₃ loaded PHB-HV 5% microspheres (PHB-HV 5%/Ho(acac)₃-MS), 1.8 g of Ho(III) complex was added in the organic phase with polyester, and the procedure was repeated. PHB-HV 5% Ho(III)-loaded microspheres (20–53 μm) were obtained in a yield of 30% (900.5 g) relative to added polyester mass.

PHB-HV 8%-MS

Using the same procedure, by just changing the ratio of HV applied (as illustrated in Scheme 1) microspheres of 20–53 μm in a yield of 22.0% (330.4 g) were obtained. To prepare the Ho(acac)₃ loaded PHB-HV 8% microspheres, PHB-HV 8%-Ho(acac)₃-MS, 0.9 g of the Ho(III) complex in chloroform with polyester was added, and the procedure was repeated. PHB-HV 8%-Ho(acac)₃-MS with diameters of 20–53 μm in a yield of 8.9% (584.0 g) relative to the initial polymer mass used were obtained.

Stability tests in sodium phosphate buffer, pH 7.4

Samples of PHB-HV/Ho(acac)₃ microspheres were studied in sodium phosphate buffer at pH 7.4, which corresponds to physiological pH of human blood. Ten glass flasks were separated. One was filled with 50.00 mL of sodium phosphate buffer and used as a blank probe to evaluate sodium contaminants. The microspheres were put into other flasks as indicated in Table 1, and also 50.00 mL of sodium phosphate buffer was added. Each flask was labeled according to the days of experiment (1–9 days) at the room temperature (25°C). Solution was filtered and Ho(III) concentration by inductively coupled plasma (ICP) atomic emission spectroscopy assay was measured. In this study, a theoretical full holmium acetylacetonate radioactive sample would exhibit 0.39% of the initial radioactivity considering the half-life time of holmium-166.

Characterizations

Scanning electron microscopy (SEM) images were acquired in the Philips XL30 Microscope, with JEOL.
multicontrast turbo-spin of each SEM images of PHB-HV 5%-MS in: Philips XL30 Microscope (TE weighted MR images. For this purpose, a disperses in 2% of the Carl Zeiss, excitation value vs the concentration of the line of 1.541 e \( \frac{1}{C_0} \). The transverse relaxivity value, \( r_2 \), was determined by the linear least square’s adjustment of the slope of the graph 1/T vs the concentration of the microparticles. In a similar way, the calculation of \( r_1 \) value has performed to determine the efficiency of the contrast agent.

Results and discussion
SEM images and EDS spectra

Figure 1A–F shows PHB-HV 5%-MS SEM images. The microparticles are spherical but slightly wrinkled and show sizes ranging from 20 to 50 µm. The observed morphology of the MS probably indicates the effects of evaporation of residual organic solvent after particle formation and/or inherent crystallization of the polyester. Once the PHB-HV 5% dissolution in chloroform required sonication and heat obtained copolymer showed to be sensible to the amount of chloroform present, for example, higher stirring may speed the solvent evaporation and the polyester may form an irregular surface.

PHB-HV 8%-MS SEM images (Figure 2A–F) display a more wrinkled morphology than the PHB-HV 5%-MS microspheres. Their appearance resembles an exfoliated deformed system with sizes ranging from 20 to 30 µm. Again, this may be associated with a high stirring speed.

![Image of SEM images](https://example.com/image1)

![Image of SEM images](https://example.com/image2)

![Image of SEM images](https://example.com/image3)

![Image of SEM images](https://example.com/image4)

![Image of SEM images](https://example.com/image5)

![Image of SEM images](https://example.com/image6)

**Figure 1** SEM images of PHB-HV 5%-MS in: Philips XL30 Microscope (A–C) and Tabletop Microscope TM3000 (D–F). Magnifications: (A) 100×, (B) 500×, (C) 1000×, (D) 200×, (E) 500×, and (F) 2000×.

**Abbreviations:** SEM, scanning electron microscopy; PHB-HV, poly(3-hydroxy-nutyrate-co-3-hydroxy-valerate); MS, microspheres.
and fast chloroform removal. Also, this material is somewhat more elastic due to the higher amounts of 3-hydroxyvalerate as copolymer.

PHB-HV 5%-Ho(acac)₃-MS SEM images in Figure 3A–F show more spherical microspheres with a smoother surface and with smaller pore sizes than the observed for HB-HV 5%-MS (Figure 1A–F). PHB-HV 5%-Ho(acac)₃-MS showed sizes ranging from 20 to 40 μm. Therefore, it is proposed that holmium acetylacetonate added up some plasticizer property to this copolymer.

In PHB-HV 8%-Ho(acac)₃-MS images (Figure 4A–F) similar morphology changes were noted: microspheres had less defects and were more compact with sizes ranging from 40 to 50 μm. These might indicate that the Ho(III) complex was accumulated on the external parts of the microspheres, once the Ho(III) complex aspect seen by SEM images (Figure 5A and B) was in form of the elongated plates.

In both microspheres the EDS spectra were acquired, Figure 6A and B, which also confirmed the presence of Ho (acac)₃ loading, judging by the appearance of intense and typical peaks.

**CLSM images**

The typical green fluorescence of Ho(III) is homogeneously dispersed in PHB-HV 5%/Ho(acac)₃-MS microspheres (Figure 7A and B), so it is deduced that the complex is perfectly mixed inside the polyester matrix. Darker microparticles in the lower portion of the images are out of focus materials in the scanning process. However, in PHB-HV 8%/Ho(acac)₃-MS (Figure 7C and D), the typical Ho(III) green fluorescence is not continuous in greater microspheres and this contradicts their EDS spectral data (Figure 6B). These microparticles displayed the most wrinkled surfaces; thus, it can be assumed that Ho(III) inhomogeneous loading occurred in some parts, or that the irregular layers blocked some of the fluorescence emitted by external parts of these microspheres.

**Infrared spectroscopy**

The comparative infrared spectra of raw Ho(acac)₃ and polyesters and the complex loaded polyester microspheres are shown in Figure 8. The assignment of FT-IR data is shown in Table 2. Raw polyesters and the
corresponding microparticles FT-IR spectra are quite similar. However, microspheres loaded with Ho(III) showed some characteristic modes of holmium acetylacetonate.

Comparing normal modes of raw PHB-HV 5% and 8%, the only distinction observed was in ν(C=O) wave-number, which showed higher energies for the polyester with 8% of 3-hydroxy-valerate (Table 2, Figure 8). This might have occurred because of packing effects in accordance with the dimensions of the unit cells (obtained by XRD studies, item 4.4). For the PHB-HV 5%/Ho(acac)₃-MS, it was seen that one ν(C–H) band had higher energy that might be associated to steric influences of loaded material on the aliphatic portions of copolymer. Thus, an expressive amount of complex inside the copolymer matrix might have provoked the observed shift. Both microspheres infrared spectra indicate coordination of acetylacetonate and Ho(III) by enol tautomer (1600 cm⁻¹, ν(C=O)) and 1520 cm⁻¹ (ν(C=C)). Another indicative for a great loading of complex in the PHB-HV 5%/Ho(acac)₃-MS could be associated with weaker bands of Ho(acac)₃ assigned as π(C–H) near 760 cm⁻¹, and ν(H-O) around 490 cm⁻¹ and 648 cm⁻¹.

**X-ray diffraction (XRD) data**

XRD of the two raw PHB-HV (Figure 9A) materials revealed crystallinity of these materials in short and medium distances, observed through intense peaks in 20 =13.3° and 16.7°. Some long-range ordering by peaks at 20=20–22° could be attributed to preserved tendency in their respective Ho(acac)₃ loaded microspheres. Typical peaks and crystalline planes of poly(3-hydroxy-butyrate) PHB are: 13.5° (020), 16.9° (110), 20.0° (021), 21.5° (101), 22.4° (111), 25.6° (121), and 27.2° (040). Once they were noted in the copolymers, the amount of 3-hydroxy-valerate did not affect crystallization of main polyester – PHB. However, in PHB-HV 8% microspheres (MS), these peaks were more shifted to lower 20 values than in PHB-HV 5% MS, so 3-hydroxy-valerate gave more elasticity to polyester and defined crystallinity in a more compact manner, as already proposed in assignments of ν(C=O) of this material.

The PHB-HV 8%/Ho(acac)₃-MS XRD was quite similar to PHB-HV 8% (Figure 9B). Small loss of intensity could be attributed to: (1) holmium acetylacetonate loading that interacted with polymer as plasticizer, and therefore part of polyester became amorphous, or (2) possible effect caused by emulsification. Polyester and Ho(III) loaded microspheres showed very similar peaks at 21.44° and 22.28°. Nevertheless, the peak at 21.76° lost definition and resolution in microspheres with Ho(III). This might suggest a decrease of crystallinity and structural change. Peaks above 30° were displaced to lower 20, what might be indicative for better packing in presence of Ho(acac)₃. Despite all, none of the characteristic peaks of holmium acetylacetonate were noted in XRD illustrated in Figure 9B, neither as a crystalline system (Figure 9C) or a more amorphous one (Figure 9D) which could reveal quite a
small loading in terms of weight of Ho(III) in the microspheres.

In PHB-HV 5%/Ho(acac)3-MS XRD (Figure 9B), an expressive loss of XRD intensity of polyester peaks in relation to the raw material (Figure 9A) was observed, indicating that the complex loading was high enough to disturb crystallization of organic matrix. Mostly, this could not be attributed to a simple effect of emulsification, because a new and broad peak at 6.48° (Figure 9B) is seen, similar to the most intense peak of Ho(acac)3 in an amorphous condition (Figure 9D). It could be related also to some loss of luminescence in the PHB-HV 5%/Ho (acac)3-MS bigger microspheres previously shown in Figure 7A and B. Probably, acetylacetonate was not linked to Ho(III) in an ideal geometry and could not induce strong antenna effect nor enhance Ho(III) fluorescence.

Using Bragg’s Law equation, 20 diffraction angles were converted into interplanar distances, which could be related to the Miller indices (hkl) and, finally, unit cell parameters (abc): in orthorhombic cell, the diffraction planes were related to hkl indices by Equation 1. It was also possible to calculate the polyesters’ crystallinities by Sherrer formula in Equation 2.

\[
d = \left[ \frac{(h^2/a^2) + (k^2/b^2) + (l^2/c^2)}{4}\right]^{1/2} \quad (1)
\]

\[
T = \frac{0.9 \lambda}{(B \cos \theta_B)} \quad (2)
\]

We have used the planes 020 (13.5°), 110 (16.9°), and 021 (20.0°) as intense peaks, unaffected by Ho(III) load. Poly(3-hydroxy-butyrate) unit cell parameters were: a =5.69 Å, b =13.04 Å, and c =5.90 Å. The peak related to the plane 110 at 16.72° was used as reference to determine average sizes of crystallites using the Sherrer formula. The results are summarized in Table 3.

The PHB-HV unit cell was revealed to be slightly larger than that of PHB because of steric hindrance. In

**Figure 6** The EDS spectra of (A) PHB-HV 5%/Ho(acac)3-MS; and (B) PHB-HV8%/Ho(acac)3-MS, both acquired in Tabletop Microscope TM3000.

**Abbreviations:** EDS, energy-dispersive x-ray; PHB-HV, poly(3-hydroxi-butyrate-co-3-hydroxy-valerate); MS, microspheres.
PHB-HV 5%/Ho(acac)$_3$-MS parameters (Table 3) it was noted a slight increase in 00l and 0k0 directions related to PHB-HV 5%, which did not occur in PHB-HV 8%/Ho(acac)$_3$-MS, unit cells’ parameters exhibited a slight shortening in h direction and large increase in l one: despite a low Ho(III) load, its plasticizing effect was noted, a fact that might justify the particles’ smoother surfaces seen in Figure 4A–F.

Finally, comparing unit cells’ parameters of PHB-HV 5% or 8%, it was noted that the last polymer cell was shorter in 001 direction. This might be explained by a low complex loading, for example, if the 001 direction is important – when copolymer crystallites are smaller, they are less affected by any distortion or poor crystallization and leave enough room for the insertion of holmium acetylacetonate. On the other hand, PHB-HV 5%/Ho(acac)$_3$-MS showed larger cell parameter in y plane (direction 0k0), therefore, this direction is proposed as preferential one for Ho(III) insertion.

**Thermogravimetry**

This method was used to quantify holmium acetylacetonate loading in PHB-HV/Ho(acac)$_3$-MS. To identify the sequence of Ho(acac)$_3$ thermal decomposition events, an analogy to lanthanum acetylacetonate study was explored.$^{106,107}$

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**Figure 7** CLSM images of: (A and B) PHB-HV 5%/Ho(acac)$_3$-MS; and (C and D) PHB-HV 8%/Ho(acac)$_3$-MS.

**Abbreviations:** CLSM, confocal laser scanning microscopy; PHB-HV, poly(3-hydroxy-butyrate-co-3-hydroxy-valerate); MS, microspheres.

**Figure 8** Infrared spectra of raw materials, polyesters, and micromaterials.
Table 2 Assignments of normal modes to raw Ho(acac)₃, polyesters, and to microspheres (MS)

| Ho(acac)₃ cm⁻¹ | PHB-HV 5% cm⁻¹ | PHB-HV 8% cm⁻¹ | PHB-HV 5%/Ho(acac)₃ cm⁻¹ | PHB-HV 8%/Ho(acac)₃ cm⁻¹ | Assignments |
|----------------|----------------|----------------|--------------------------|--------------------------|--------------|
| 3421           | 3437           | 3437           | 3437                     | 3437                     | ν(O–H)       |
| 2997           | 2977           | 2977           | 2977                     | 2977                     | ν(C–H)asym   |
| 2666           | 2935           | 2935           | 2935                     | 2935                     | ν(C–H) asym   |
| 2924           | 2877           | 2877           | 2877                     | 2877                     | ν(C–H) sym    |
| 1605           | 1728           | 1736           | 1724                     | 1724                     | ν(C=O) carboxylic |
| 1524           | 1524           | 1524           | 1524                     | 1524                     | ν(C=O) enol   |
| 1450           | 1454           | 1454           | 1458                     | 1458                     | ν(C=O) + ν(C=CH₃) |
| 1396           | 1381           | 1381           | 1385                     | 1385                     | ν(C=O)+ ν(C=C) |
| 1358           | 1358           | 1362           | 1358                     | 1358                     | ν(C=H) sym    |
| 1281           | 1281           | 1281           | 1281                     | 1281                     | ν(C=C–O) asym |
| 1265           | 1227           | 1227           | 1227                     | 1227                     | ν(C=O)+ ν(C–CH₃) |
| 1192           | 1184           | 1184           | 1180                     | 1184                     | ν(CH₂)       |
| 1134           | 1134           | 1130           | 1130                     | 1130                     | ν(CH₂)       |
| 1099           | 1099           | 1099           | 1099                     | 1099                     | ν(CH₂)       |
| 1057           | 1057           | 1057           | 1057                     | 1057                     | ν(O–CH₂–C) asym |
| 1018           | 980            | 980            | 980                      | 980                      | ν(CH₂) + ν(C=O) |
| 933            | 933            | 933            | 930                      | 930                      | ν(CHOH–CH₂) + (O) |
| 922            | 910            | 910            | 910                      | 910                      | ν(C–CH₃)     |
| 895            | 895            | 895            | 895                      | 895                      | ν(C–C–O) asym |
| 825            | 825            | 825            | 825                      | 825                      | ν(CH₂)       |
| 764            | 760            |                |                          |                          | m(C=H)       |
| 698            | 675            | 679            | 675                      | 679                      | ν(O–H) asym   |
| 656            | 621            | 621            | 621                      | 621                      | ν(CH₂) + ν(Ho–O) |
| 602            | 513            | 517            | 513                      | 513                      | ν(C=O)       |
| 536            | 513            |                |                          |                          | ν(C=O) enol   |
| 494            | 459            |                | 459                      | 459                      | ν(HO–O)       |
| 474            | 459            |                | 459                      | 459                      | ν(HO–O)       |
| 424            | 420            |                | 420                      | 428                      | ν(C=C–CH₃)    |

Abbreviations: op, out of phase mode; ip, in phase mode; inp, in plane mode; outp, out plane mode; ν, stretching; δ, in plane bending; ρ, "rocking" deformation; ω, "wagging" deformation; τ, "twist" deformation; “sym”, symmetric mode; “asym”, asymmetric.

Propyne (C₃H₄) is released from acetylacetonates in a thermal event. Theoretical sequence for Ho(acac)₃ decomposition is proposed in Table 4. It was possible to identify inorganic residue after full decomposition of polyester of microspheres; in PHB-HV this occurred around 330°C.₁⁰₈

The thermogravimetric curve and the first derivate for Ho(acac)₃ (Figure 10A) were split into events separated by the blue lines, and the determined percentages of residual matter were: (1) 93.08%, (2) 89.47%, (3) 74.21%, (4) 66.50%, (5) 60.75%, and 49.82%.

It was proposed that after first two mass loss events – Ho (acac)₃ was fully dehydrated, because of the similarity between theoretical percentage of mass residue calculated in Table 4 (89.53% to event II) and experimental one (89.47%). However, the water molecules release order, as could be modeled by the experimental curve, was of two water
molecules in the event I (Figure 10A – under average temperature of 92.46°C) rather than one molecule based in predictions the structure resolved by Kooijman et al. Ho (III) inner sphere is therefore composed of two water

Table 3 Unit cell parameters of PHB-HV and PHB-HV loaded with Ho(acac)₃ microspheres (MS)

| Material           | 2θ (hkl)                  | Unit cell parameters a (Å) | b (Å) | c (Å) | Crystallite size (Å) |
|--------------------|---------------------------|---------------------------|-------|-------|----------------------|
| PHB-HV 5%          | 13.36°, 16.72°, 19.6°      | 5.79                      | 13.28 | 6.01  | 158.95               |
|                    | (020), (110), (021)        |                           |       |       |                      |
| PHB-HV 5%/Ho(acac)₃ | 13.26°, 16.72°, 19.82°     | 5.79                      | 13.40 | 6.03  | 152.84               |
|                    | (020), (110), (021)        |                           |       |       |                      |
| PHB-HV 8%          | 13.32°, 16.76°, 20.02°     | 5.79                      | 13.28 | 5.94  | 155.84               |
|                    | (020), (110), (021)        |                           |       |       |                      |
| PHB-HV 8%/Ho(acac)₃ | 13.36°, 16.78°, 19.86°     | 5.75                      | 13.28 | 6.07  | 155.84               |
|                    | (020), (110), (021)        |                           |       |       |                      |
molecules. This assignment explored the following calculation for complex with molar mass when 1 and 3 waters were present, respectively, 480.27 and 516.30 g mol\(^{-1}\). If we compare the theoretical value for residual mass of (480.27 g mol\(^{-1}\)/516.30 g mol\(^{-1}\)) × 100% = 93.01%, with the one observed in event I (93.08%), two water molecules must be a part of the structure. The temperature of event I was lower than the boiling point of pure water, and could indicate that Ho(acac)\(_3\) has two outer sphere (hydration) water molecules, and one water in Ho(III) inner sphere, which was lost under 137.29°C (event II in Figure 10A). Finally, the disposition of ligands around Ho(III) is attributed as asymmetric, considering the uncommon seventh point of coordination and the energy split of ν(Ho–O) indicated in Table 2.

Following the sequence of thermal events proposed in Table 4, Figure 10A did not exhibit – even in the first derivative of the thermogravimetric curve – a percentage of residual mass indicative for the distinct releases of first and second propynes (events III and IV), but only the sum of both (theoretical residual mass of 74.01%, and experimental one of 74.21%). Thus, a mixed salt was formed with two acetylacetonates coordinated similarly to Ho(III). Their thermal events occurred at an average temperature, and this is consistent to the assignment of infrared spectral data, which revealed more than one energy band to the ν(Ho–O), because of more than one type of chemical bond strength. Third propyne release and conversion of this intermediate salt to holmium acetate (event V in Table 4, theoretical residual mass of 66.25%) – proposed in an average temperature of 282°C (event 4 in Figure 10A, mass residue of 66.50%) – was observed due to the changes in the first derivative of the thermogravimetric curve, although it was partially hidden by the former thermal event.

The event 5 in Figure 10A is unexpected following the analogies to the Ln(acac)\(_3\) in Table 4, since no chemical transformation could provide an experimental mass residue of 60.75%. Compound related is assigned as a mixed salt intermediate Ho(CH\(_3\)COO)\(_3\)×2Ho(OH)\(_2\) (event V in Figure 10A, mass residue of 66.50%) – was observed due to the changes in the first derivative of the thermogravimetric curve, although it was partially hidden by the former thermal event.  

### Table 4 Thermal events proposed and residual mass percentage to Ho(acac)\(_3\)×3H\(_2\)O thermogravimetry

| Thermal event                                                                 | Theoretical percentage of residual mass |
|------------------------------------------------------------------------------|-----------------------------------------|
| Ho(acac)\(_3\)×3H\(_2\)O → Ho(acac)\(_3\)×2H\(_2\)O                           | [498.29 g mol\(^{-1}\)/516.30 g mol\(^{-1}\)] × 100% = 96.51% |
| (I – elimination of hydration water)                                         |                                         |
| Ho(acac)\(_3\)×2H\(_2\)O → Ho(acac)\(_3\)                                   | [462.26 g mol\(^{-1}\)/516.30 g mol\(^{-1}\)] × 100% = 89.53% |
| (II – elimination of inner sphere water molecules)                          |                                         |
| Ho(acac)\(_3\) → Ho(CH\(_3\)COO)\(_3\) + C\(_3\)H\(_4\)                     | [422.19 g mol\(^{-1}\)/516.30 g mol\(^{-1}\)] × 100% = 81.77% |
| (III – first release of propyne)                                             |                                         |
| Ho(CH\(_3\)COO)\(_3\)×(acac) → Ho(CH\(_3\)COO)\(_3\) + C\(_3\)H\(_4\)       | [382.13 g mol\(^{-1}\)/516.30 g mol\(^{-1}\)] × 100% = 74.01% |
| (IV – second release of propyne)                                             |                                         |
| Ho(CH\(_3\)COO)\(_3\)×(acac) → Ho(CH\(_3\)COO)\(_3\)+C\(_3\)H\(_4\) + C\(_3\)H\(_4\) | [342.06 g mol\(^{-1}\)/516.30 g mol\(^{-1}\)] × 100% = 66.25% |
| (V – holmium acetate formation)                                              |                                         |
| 2Ho(CH\(_3\)COO)\(_3\) → Ho\(_2\)(CO\(_3\))\(_3\)+3CH\(_3\)COCH\(_3\)        | [509.89 g mol\(^{-1}\)/(2,516.30 g mol\(^{-1}\))] × 100% = 49.38% |
| (VI – holmium carbonate formation)                                           |                                         |
| Ho\(_2\)(CO\(_3\))\(_3\) → Ho\(_2\)O\(_2\)CO\(_2\)                         | [421.87 g mol\(^{-1}\)/(2,516.30 g mol\(^{-1}\))] × 100% = 40.86% |
| (VII – holmium oxycarbonate formation)                                       |                                         |
| Ho\(_2\)O\(_2\)CO\(_3\) → Ho\(_2\)O\(_2\) + CO\(_3\)                        | [377.86 g mol\(^{-1}\)/(2,516.30 g mol\(^{-1}\))] × 100% = 36.59% |
| (VIII – holmium oxide formation)                                             |                                         |
100°C, excluding the possibility of adsorbed or hydration water molecules in particles. The first mass loss in these microspheres occurred at an average temperature of 200°C, which did not correspond to poly(vinyl alcohol) degradation: the main event was at 300°C, and an additional one at 400°C could be noted. However, as indicated in Table 3, the infrared spectra of these materials did not reveal an expressive band denoting a considerable amount of this polymer. Hence, the mass losses are assigned to PHB-HV degradation, independent of the 3-hydroxy-valerate amounts, at an average temperature of 277°C (Figure 10B and C). And the thermal events at 201°C and 415–425°C were assigned to holmium acetylacetonate degradation, although the process at 200°C certainly included some matrix degradation. Absence of mass loss of hydration (< 100°C) and crystallization waters (commonly between 100°C and 200°C) accounted for a replacement of these molecules in the Ho(III) inner sphere by carbonyl and/or carboxyl residues of copolymer, especially in PHB-HV 5%. Hence, the physical or chemical interaction did not induce strong steric consequences to the system.

For PHB-HV 8%/Ho(acac), the percentage of mass residue was so small that the complex loading could not be analyzed according to thermal events of raw complex analogies. Peaks in the first derivative were under temperatures of 239°C and 400°C. However, the complex must be quantified as dehydrated material: there were no hydration or crystallization water molecules. Above 400°C, the salt Ho₂(CH₃COO)₄(CO₃) was formed and maintained as a stable material because the first derivative was constant. Based on this, complex loading was calculated as 0.76% (2 x 462.26 g mol⁻¹)/626.05 g mol⁻¹) = 1.12% in mass. It is interesting that the infrared spectrum
of PHB-HV 8%Ho(acac)₃-MS (Figure 8) exhibited enol normal modes (Table 2) so as the data shown in Figure 6B, which displayed characteristic absorption of holmium. Most of the data obtained by these techniques are of surface components. This is also consistent with the smoother surface of PHB-HV 8%Ho(acac)₃, where the plasticizing effect of HV was noted.

For the PHB-HV 5%Ho(acac)₃-MS, XRD spectrum in Figure 9D revealed that the complex is amorphous. Hence, many properties including thermal degradation are similar to the raw material. Hence, the same quantification process determined that 16.25% × (2 × 462.26 gmol⁻¹)/626.05 gmol⁻¹ = 22.15% residual mass. Therefore, PHB-HV 5%Ho(acac)₃-MS are the most promising to the internal radiotherapy aims.

Holmium release essays
Table 5 summarizes the data obtained in the calibration curve for Ho(III) concentration in water observed by ICP. The signal related to sodium phosphate buffer (0 day), and the directed and corrected ICP intensities after discounting the blank read to determine real Ho(III) concentration released by microspheres in ppm. Figure 11A and B illustrate the calibration curve and quantification of Ho(III) according to the number of days that microspheres were exposed to sodium phosphate buffer (pH =7.4).

For all microspheres, the results for stability in the buffer (Table 5) were quite positive: there were no tendencies of a cumulative increase in the concentration of holmium over days under exposure to sodium phosphate buffer (pH 7.4), and Ho(III) concentrations were in the range of 10⁻³ ppm. This could be indicative that under experimental conditions, the release and reuptake of Ho (III) are in a dynamic equilibrium; or simply that the amount of Ho(III) released by microspheres is so low that it is below the detection limit of the equipment. This result opens the possibility of using both kinds of microspheres for medical procedures.

MRI assays
The MR images of the samples dispersed or dissolved in water with agar (Figure 12-I) were acquired according to sample distribution shown in Figure 12-II(A), to define a gelatin more solid system in which water is forming chemical bonds with the polysaccharide matrix, becoming somewhat more “structured” due to interactions within itself and with the agar net. The PHB-HV 5%/Ho(acac)₃-MS dispersed in a 2% agar revealed a dark phantom as

Table 5 ICP calibration curve and holmium(III) quantification in the sodium phosphate buffer solutions after removing the microparticles, according to days of exposition to sodium phosphate buffer.

| Days in buffer | PHB-HV8%Ho(acac)₃-MS | ICP (c/s) | [Ho³⁺]⁻buffer signal (ppm) | [Ho³⁺]⁺buffer signal (ppm) |
|---------------|---------------------|-----------|---------------------------|---------------------------|
| 0             | 1947                | 0.13391   | -1.35 × 10⁻³               | -1.12 × 10⁻³               |
| 2.00          | 23,293              | 0.13348   | -1.32 × 10⁻³               | -1.14 × 10⁻³               |
| 4.00          | 38,069              | 0.13355   | -1.34 × 10⁻³               | -1.15 × 10⁻³               |
| 6.00          | 57,028              | 0.13352   | -1.56 × 10⁻³               | -1.37 × 10⁻³               |
| 8.00          | 77,014              | 0.13353   | -1.57 × 10⁻³               | -1.38 × 10⁻³               |
| 10.00         | 96,903              | 0.13354   | -1.58 × 10⁻³               | -1.39 × 10⁻³               |
seen under $T_2$ weighted scan images of protons, Figure 12-I(A1–A5), confirming contrast agent property in this image modality relative to a 2% agar standard. Hence, the paramagnetic nature of the holmium(III) in the wrinkled microspheres affected transverse relaxation time of water, and the darkening is proportional to microsphere (and holmium acetylacetonate) mass present in each sample. Usually, it would be unexpected a $T_2$ relaxation to influence in the water protons caused by lanthanide ions since they usually replace inner sphere waters too fast to affect the nuclear spin relaxation by intermolecular events. But in microspheres, the Ho(III) concentration is much higher, so an expressive part of water molecules is affected by short distance effects to provide better images as shown in Figure 12-I(B) A1–A4. Once the paramagnetism of Ho(III) is additive, the whole material had a full magnetic effect comparable to the superparamagnetic nanoparticles with high Fe(III) concentration and provided an intense magnetic field at surface that interacted with water molecules. This result was analogous to the holmium(III) acetylacetonate microspheres reported by Bult et al.; but this is the first description of this effect to a complex-loaded PHB-HV matrixes. The proof of this effect is related to the particle systems and their $T_2^*$ weighted images illustrated in Figure 12-I(C)A1–A4. It is known that dephasing of spin protons precession frequencies is quicker under the effects of local inhomogeneities of the magnetic field. Once the PHB-HV 5%/Ho(acac)$_3$-MS are wrinkled on the surface, the magnetic fields around them are more complex and multidirectional than in a perfect spherical micromaterial.

Interestingly, the contrast agent effect is also present in the $T_2$ (Figure 12-I(B)B1–B2) and $T_2^*$ (12-I(C)B1–B2) weighted scan of the PHB HV 8%/Ho(acac)$_3$-MS, but more mass of microsphere had to be used as to have a similar amount of holmium acetylacetonate in surface interacting with water molecules. This is consistent with the discussion of EDS signal and infrared spectrum signals, so as the contrast agent properties, come from surface elements. On the other hand, it also reveals that in order to produce contrast agent microspheres, only a small load of the holmium acetylacetonate is enough. According to the complex loading, it is possible to prepare an analogous material suitable for MRIs and/or for internal radiotherapeutic purposes, once all the retained PHB-HV/Ho(acac)$_3$-MS in tissues theoretically can be seen by this image technique. Therefore, the elimination of the tumor tissues can be evaluated time by time-lapse observations using the MR images without any injection of other contrast agent, thus, avoiding risks of cumulative toxicity in the period prior to biodegradation of the microparticles.

A final feature is obtained comparing the contrast agent properties of Ho(acac)$_3$ loaded polyester matrixes to Ho(III) inclusion complexes with β-cyclodextrin (Figure 12-I(C)C1) or hydroxyl-propyl-β-cyclodextrin, Figure 12-I(C)C2. These host-guest systems exhibited the Ho(III) expected contrast in $T_2$ weighted images as they showed white phantom images; these inclusion complexes broke spatial ordering of internal water hydrogen bonding
and with polysaccharide net as the lanthanide ions quickly replace inner sphere water molecules. However, they are not able to cause a dephasing in protons’ spin precession frequencies such as the PHB-HV/Ho(acac)3 microspheres, once their T2* weighted images (Figure 12-I(C)–C2) had the same color of a 2% agar standard.

To determine the T2, the curves of MRI signal intensity of the samples indicated in (a) in the function of TEs as shown in Figure 12-II were obtained. It was shown that higher microparticle concentrations exhibited lower values of T2 (Figure 12-III). Values for r2 were determined from the linear correlation between the inverse of the T2 values as follows (shown in Figure 12-IV): T2(A1) = 31.5 ± 0.6 ms, T2 (A2) = 34.6 ± 0.7 ms, T2 (A3) = 44.4 ± 0.9 ms, T2 (A4) = 49.9 ± 0.9 ms, T2 (A5) = 64.2 ± 1.6 ms, and T2 (control) = 78.7 ± 1.9 ms, and the concentrations of the corresponding microparticles, the r2=(25 ± 3)×10^-4 mg·ms·mL and r1=(3.1 ± 0.6)×10^-4 mg·ms·mL. Taking into account that an MRI contrast agent for T2-weighted images is effective for high values of the r2/r1 ratio, the ratio value of 8.06 obtained, indicate to be an adequate contrast for images T2-weighted for a magnetic field of 3 T magnetic field.112–114

**Figure 12** Relaxometry characterization of the PHB-HV microparticles loaded with holmium acetylacetonate. (I) Schematic drawing of samples (A) microsphere added (A1) 7.7 mg; (A2) 5.0 mg; (A3) 3.0 mg; (A4) 2.0 mg; and (A5) 1.0 mg. PHB-HV 8%/Ho(acac)3-MS, mass of microsphere added (B1) 20 mg; (B2) 10 mg. Inclusion complex of β-cyclodextrin and Ho(acac)3, initial concentration of 9.12 × 10^-3 mol L^-1 (C1), and hydroxy-propyl-β-cyclodextrin and Ho(acac)3, initial concentration of 1.94 × 10^-3 mol L^-1 (C1). (B) T2 and (C) T2* weighted MRI of the corresponding samples indicated in (A). (II) Curves of MRI signal intensity of the samples indicated in (A) in the function of TEs. (III) T2 values determined from the curves showed in item (II). (IV) Calculation of the value of r2 from the linear correlation between the inverse of the T2 values of the samples (A1–A5) and the concentrations of the corresponding microparticles.

**Abbreviation:** PHB-HV, poly(3-hydroxy-nutyrate-co-3-hydroxy-valerate)
Conclusions

The PHB-HV microspheres loaded with holmium(III) acetylacetonate, in which the Ho(III) loading was inversely proportional to the amount of 3-hydroxy-valerate in the copolymer used during the emulsification/evaporation of solvent were successfully produced. The microspheres produced were mostly wrinkled and were loaded with 22% or 12% of Ho(III). Microspheres with higher Ho(III) loading in a PHB copolymer with more poly(3-hydroxy-valerate) 8% were indicated as more suitable to internal radiotherapy procedures. An accumulation of Ho(III) was detected on the surface of these microspheres, and the XRD revealed the preferential direction toward which the Ho(III) complex interacted or simply precipitated in the polyester matrix. No expressive release of Ho(III) was detected in a 9-day exposition period to sodium phosphate buffer (pH 7.4), suggesting the microspheres are highly stable in this simple simulation of the human fluids. Despite the different amounts of holmium (III) acetylacetonate loading, both microspheres proved to be very efficient to obtain T2 and T2* weighted images in the MR. The Ho(III) loaded PHB-HV microspheres showed different properties when compared to the Ho (III) inclusion complexes with β-cyclodextrins. When discussing the cancer tissues diagnostics, either Ho(III) microspheres or host-guest inclusion systems can be used to acquire MR images of patients. Tests with the culture cells and biological fluids, as well as in vivo tests are still being conducted as to reveal the Ho(III) microspheres clinical potential for application and risks.

Nevertheless, this was the first report on the use of inclusion complexes of holmium acetylacetonate in biodegradable polymers as contrast agents, evaluating the endurance of the microparticles under nuclear activation and Ho(III) microsphere use as safer radiopharmaceuticals, although it still needs further testing, we hopefully might have great potential for clinical use.

Abbreviations

CLSM, confocal laser scanning microscopy; EDS, energy-dispersive X-ray; HP-β-CD, hydroxyl-propyl-β-cyclodextrin; Ho(acac)3, holmium(III) acetylacetonate; Ho(acac)3-MS, holmium(III) acetylacetonate microspheres; ICP, inductively coupled plasma; PHB-HV, Poly(3-hydroxi-butyrato-co-3-hydroxy-valerate); MRI, magnetic resonance images; MS, microspheres; PHB, poly(3-hydroxy-butrate); SEM, scanning electron microscopy; XRD, X-ray diffraction.

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Disclosure

The authors report no conflicts of interest in this work.

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