Radioactive holmium phosphate microspheres for cancer treatment

Arranja, A. G.; Hennink, W. E.; Denkova, A. G.; Hendrikx, R. W.A.; Nijsen, J. F.W.

DOI
10.1016/j.ijpharm.2018.06.036

Publication date
2018

Document Version
Final published version

Published in
International Journal of Pharmaceutics

International Journal of Pharmaceutics 548 (2018) 73–81

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Radioactive holmium phosphate microspheres for cancer treatment

A.G. Arranja[a,b,c,d], W.E. Hennink[b], A.G. Denkova[c], R.W.A. Hendrikx[e], J.F.W. Nijsen[a,d,f,*]

[a] Department of Radiology and Nuclear Medicine, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands
[b] Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Science for Life, Faculty of Science, Utrecht University, 3508 TB Utrecht, The Netherlands
[c] Radiation Science and Technology, Delft University of Technology, Mekelweg 15, 2629 JB Delft, The Netherlands
[d] Radboudumc, Department of Radiology and Nuclear Medicine, Geert Grooteplein Zuid 10, 6525 GA Nijmegen, The Netherlands
[e] X-Ray Facilities, Department of Materials Science and Engineering, Delft University of Technology, Faculty of 3mE, Mekelweg 2, 2628 CD Delft, The Netherlands
[f] Quirem Medical B.V., Zutphenseweg 55, 7418 AH Deventer, The Netherlands

ARTICLE INFO

Keywords:
Microspheres
Emulsification
Holmium
Selective internal radiation therapy
Neutron irradiation
Holmium phosphate

ABSTRACT

The aim of this study was the development of radioactive holmium phosphate microspheres ($HoPO_4$-MS) with a high holmium content and that are stable in human serum for selective internal radiation therapy (SIRT) of liver cancer. To this end, holmium acetylacetonate microspheres (HoAcAc-MS) were prepared (34.2 ± 1.0 μm in diameter, holmium content of 46.2 ± 0.8 and density of 1.7 g/cm^3) via an emulsification and solvent evaporation method. The concentration of HoAcAc in the organic solvent, the temperature of emulsification and the stirring speed were varied for the preparation of the HoAcAc-MS to obtain microspheres with different diameters ranging from 11 to 35 μm. Subsequently, the AcAc ligands of the HoAcAc-MS were replaced by phosphate ions by simply incubating neutron irradiated HoAcAc-MS in a phosphate buffer solution (0.116 M, pH 4.2) to yield radioactive $HoPO_4$-MS. The obtained microspheres were analyzed using different techniques such as SEM-EDS, ICP-OES and HPLC. The prepared $HoPO_4$-MS (29.5 ± 1.2 μm in diameter and a density of 3.1 g/cm^3) present an even higher holmium content (52 wt%) than the HoAcAc-MS precursor (46 wt%). Finally, the stability of the $HoPO_4$-MS was tested by incubation in human serum at 37 °C which showed no visible changes of the microspheres morphology and only 0.1% of holmium release was observed during the 2 weeks period of incubation. In conclusion, this study shows that stable radioactive $HoPO_4$-MS can be prepared with suitable properties to be used for cancer therapy.

1. Introduction

Liver cancer is the fifth most diagnosed cancer type in men and the second most common cause of death from cancer in the world (Ferlay et al., 2015). Actually, more than 700.000 people are diagnosed with primary liver tumors every year worldwide (American Cancer Society) and almost 50% of the patients with colorectal carcinoma will develop metastatic liver cancer (over 1 million patients) (Kelly and Kemeny, 2017). Particularly in these patients, spreading of the tumor to the liver is the major cause of death due to the low response to chemotherapy and external radiotherapy (Valderrama-Treviño et al., 2017; Zarour et al., 2017). Moreover, very few treatment alternatives are available for these group of patients. Hepatic resection is used in up to 15% of the cases with reported 5-year survival rates ranging from 25% to 51%. Unfortunately, most of these patients are not eligible for hepatic resection due to the characteristics of the metastatic lesions (size, number and location) and the presence of extra hepatic disease (Misiakos et al., 2011). Due to the extremely aggressive nature and poor prognosis of primary and metastatic liver cancer, it remains an important public health issue (Soerjomataram et al., 2012; Zarour et al., 2017). Thus, different alternative therapies have been proposed over the last years.

Selective internal radiation therapy (SIRT) of the liver is a type of radionuclide therapy consisting in placing radioactive microspheres with a diameter between 20 and 50 μm in the direct vicinity of the liver tumors in order to deliver high radiation doses directly to malignant cells leaving healthy tissue unaffected (Anderson et al., 1991; Dendy et al., 2017; Meade et al., 1987; Rognoni et al., 2016). This local radionuclide therapy using microspheres loaded with beta-emitting isotopes is a very promising therapeutic modality for inoperable patients suffering from liver malignances. Therefore, several generations of microspheres for SIRT have been developed in recent years comprising different radionuclides, such as yttrium-90 (^{90}Y) and holmium-166 (^{166}Ho), embedded in matrix materials such as biodegradable polymers, ion exchange resins and glass (Meade et al., 1987; Nijsen

* Corresponding author at: Radboudumc, Department of Radiology and Nuclear Medicine, Geert Grooteplein Zuid 10, 6525 GA Nijmegen, The Netherlands.
E-mail address: Frank.Nijsen@radboudumc.nl (J.F.W. Nijsen).

https://doi.org/10.1016/j.ijpharm.2018.06.036
Received 1 May 2018; Received in revised form 13 June 2018; Accepted 14 June 2018
Available online 15 June 2018

et al., 2002a; Pasciak, 2017). Currently, two ^{90}Y and one ^{166}Ho-based microspheres formulations are commercially available and in clinical use for liver radioembolization: ceramic-based TheraSphere® (MDS Nordion, Canada) and resin-based SIR-Spheres® (SIRteX, Medical Ltd., Australia) containing ^{90}Y, and polymer-based microspheres QuiremSpheres® (Quirem Medical B.V., The Netherlands) loaded with ^{166}Ho (Nijsen et al., 2002a; Pasciak, 2017; Prince et al., 2017).

The holmium loaded microspheres have shown similar results to the other products for SIRT in unresectable liver metastases (Prince et al., 2017; Smits et al., 2012, 2010) and their efficacy in neuroendocrine and head and neck tumors is currently being investigated (van Nimwegen et al., 2017). These microspheres loaded with the radioactive ^{166}Ho are produced upon neutron activation of ^{165}Ho-based microspheres (^{165}Ho + n → ^{166}Ho, cross section 66 barn) (Nijsen et al., 2001; Zielhuis et al., 2006). ^{166}Ho emits high-energy beta-minus particles (β^-) (E_{max} = 1.74 (48.7%) and 1.85 (50%) MeV) with a relatively short half-life of 26.8 h (^{166}Ho → ^{166}Er + β^-) and a maximum soft-tissue range of 8.4 mm, making this isotope very suitable for use as a therapeutic radionuclide. Importantly, ^{166}Ho also emits low-energy gamma photons (6.2% 81 keV, 0.93% 1.38 keV), that enables the visualization of its distribution and quantification by single-photon emission computed tomography (SPECT) imaging after administration of the microspheres. Moreover, ^{165}Ho is paramagnetic which allows its visualization using magnetic resonance imaging (MRI) for personalized treatment and monitoring of treatment progression and efficacy (Smits et al., 2013). Therefore, a medical device for SIRT based on ^{166}Ho has considerable advantages of using common imaging modalities such as SPECT and high resolution MRI when compared to the ^{90}Y isotope based products.

The commercially available formulation of ^{166}Ho loaded microspheres (QuiremSpheres®) has a mean of 19 wt% (range 16.6–20.4%) of ^{166}Ho content and this radioactive element is stably incorporated in a polymeric matrix of the biodegradable poly-L-lactic acid (PLLA). In the present work, ^{166}Ho-loaded microspheres were prepared with more than three-fold higher holmium content which are very attractive because they have a higher specific activity per microsphere. This enables the administration of lower volumes of radioactive sample which (1) may provide more personalized treatments adjusted in activity and number of spheres, and (2) allows the use of these microspheres for intratumoral injections as very small volumes of microspheres containing a high specific activity are usually required for these applications (Bakker et al., 2017; van Nimwegen et al., 2017). Moreover, the higher specific activity can offer several other advantages such as (3) maximize the dose delivered to the tumor, (4) promote higher visibility in imaging modalities (SPECT, MRI and CT) (Prince et al., 2017; Seevinck et al., 2007), and (5) allows an increase of the time for transportation and logistics of the product from the reactor to the hospital.

Many particles loaded with ^{165}Ho have been developed in a wide range of sizes and holmium loading contents. For example, silica-based nanoparticles with 80–100 nm in diameter and 18 wt% ^{165}Ho-loading (Di Pasqua et al., 2013; Kim et al., 2017) as well as particles with 400 nm in diameter and 28 wt% of ^{165}Ho-loading (Marcon et al., 2017) have been prepared for brachytherapy of ovarian cancer and glioblastoma respectively. Even smaller nanoparticles (20–50 nm) loaded with ^{165}Ho were fabricated by nanotemplate engineering for the treatment of ovarian cancer metastases (Di Pasqua et al., 2012). Other polymer-based particles have also been produced composed of alginate with mean diameters of 570 (Oerlemans et al., 2015) and 159 μm (Zielhuis et al., 2007), poly(lactic acid) with 7.2 μm (Mumper et al., 1992) and 100–1000 nm (Hamoudeh et al., 2008) and chitosan (Kim et al., 2006) for a wide variety of applications in cancer therapy. Nevertheless, all these particles have a ^{165}Ho-loading content lower than 28 wt% which greatly limits their application.

In previous work of our group, Bult et al. showed that microspheres with a high holmium content of 45 wt% can be obtained by using solely the holmium acetylacetonate complex (HoAcAc) (Bult et al., 2012; Bult et al., 2007, 2009). In the present work, the effect of the main process parameters on the preparation of the holmium acetylacetonate microspheres (HoAcAc-MS) and the influence of neutron activation on the HoAcAc-MS by which process non-radioactive ^{165}HoAcAc-MS are converted into the beta and gamma-emitting ^{166}HoAcAc-MS were investigated. It is however shown that these radioactive microspheres were not stable in water for injection. Therefore, the AcAc ligands were replaced by phosphate ions to yield radioactive holmium phosphate microspheres with a high holmium content ($HoPO_4$-MS). The stability of these $^{166}HoPO_4$-MS was finally examined in human serum.

2. Materials and methods

2.1. Materials

Holmium chloride ($HoCl_3{\cdot}6H_2O$; MW 379.38; 99.9%) was obtained from Metall Rare Earth Limited. Acetylacetone (AcAc; ReagentPlus®; MW 100.12; greater than99%), polyvinyl alcohol (PVA; MW 30.000–70.000; 87–90% hydrolyzed) and sodium phosphate monobasic (NaH_2PO_4, MW 119.98; greater than99.0%) were obtained from Sigma-Aldrich. Absolute methanol was supplied by Biosolve and perchloric acid (MW 100.46; 70%) by ACROS Organics. Ammonium hydroxide (NH_4OH; EMSURE®; MW 35.05; 28–30%), hydrochloric acid (HCl; EMPROVE®; MW 36.46; 37%), chloroform (EMPROVE®) and nitric acid (SUPRAPUR®; 65%) were supplied by Millipore.

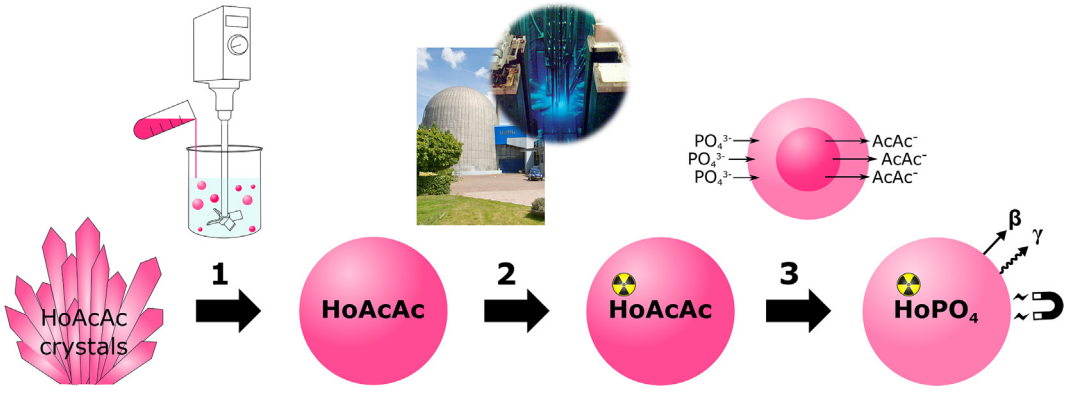

Fig. 1. Schematic overview of the preparation of radioactive holmium phosphate microspheres: (1) holmium acetylacetonate microspheres (HoAcAc-MS) were prepared by dissolution of holmium acetylacetonate crystals in chloroform followed by solvent evaporation. The obtained microspheres were (2) neutron activated in a nuclear research reactor where the ^{165}HoAcAc-MS were converted into radioactive ^{166}HoAcAc-MS. Finally, (3) the AcAc ligands were exchanged by phosphate ions by simply incubation of the ^{166}HoAcAc-MS in a phosphate buffer solution to yield radioactive holmium phosphate microspheres ($HoPO_4$-MS).

2.1. Methods

2.1.1. Preparation of HoAcAc microspheres

A solvent evaporation method was used to prepare HoAcAc microspheres (HoAcAc-MS) (Fig. 1, step 1). For this purpose, crystals of holmium acetylacetonate (HoAcAc) were prepared as previously reported by Nijsen et al. (Nijsen et al., 1999). Briefly, an aqueous solution of holmium chloride was mixed with a diluted solution of acetyl acetone and the pH of this solution was adjusted to 8.5 with ammonium hydroxide solution. Complexes of holmium and acetylacetonate were formed overnight, which were subsequently collected and washed with water three times and dried under vacuum at 48 °C for at least 48 h. For the preparation of the HoAcAc-MS, 10 g of HoAcAc crystals were dissolved in chloroform (186 g) and the obtained homogeneous solution was then added to an aqueous solution of PVA (1000 g water with 2% w/w PVA). Overhead four blades propeller stirrers (IKA Eurostar power digi-visc) were used to vigorously stir the mixture in two liters baffled beakers to obtain an oil-in-water (o/w) emulsion which was stirred at a constant speed and temperature. The temperature of the emulsion was controlled using jacketed beakers and a constant flow of nitrogen (12 L/min) was applied for 72 h. Different parameters in this procedure were varied to assess their influence on the final properties of the microspheres: (1) concentration of HoAcAc in the organic solvent (5.4, 6.3 and 7.5 ww%), (2) temperature of emulsification (15, 25 and 35 °C) and (3) stirring speed (300, 400, 500 and 600 rpm).

After the emulsification/solvent evaporation procedure, the HoAcAc microspheres were formed (Fig. 1, step 1). The microspheres were collected by centrifugation, washed three times with water and sieved according to the desired size (typically 20–50 μm) using an electronic sieve vibrator (TOPAS EMS 755) and an ultrasonic processor (Hielscher UP200S). Finally, the sieved microspheres were dried at room temperature for 5 h under ambient pressure followed by vacuum drying at room temperature for 72 h.

2.1.2. Neutron activation of HoAcAc microspheres

Dry ^{165}HoAcAc-MS were neutron activated in the pneumatic rabbit system (PRS) of the research nuclear reactor facility operational at the Department of Radiation Science and Technology of the Delft University of Technology, The Netherlands. Irradiation was performed in polyethylene vials (Vente et al., 2009; Vente et al., 2010) and irradiated with a thermal flux of 4.97×10^{16} n m^{-2} s^{-1}, epithermal flux of 8.13×10^{14} n m^{-2} s^{-1} and fast neutrons flux of 3.48×10^{15} n m^{-2} s^{-1}. Different amounts of ^{165}HoAcAc-MS (50, 100 and 600 mg) were irradiated for 2, 4, 6 or 8 h to yield radioactive ^{166}HoAcAc-MS (Fig. 1, step 2). The maximum temperature during irradiation was monitored with temperature indicator strips that were attached to the vials immediately prior to irradiation (Digi-Sense, Cole-Parmer). The radioactive ^{166}HoAcAc-MS were allowed to decay for at least one month before handling the samples to reduce exposure of the operator to radiation. The decayed neutron irradiated HoAcAc-MS were analyzed by Scanning Electron Microscope-Energy Dispersive X-ray Spectroscopy (SEM-EDS), x-ray powder diffraction (XRD), Inductively Coupled Plasma-Optical Emission spectroscopy (ICP-OES), High Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC–MS) to determine the effects of neutron irradiation on the surface properties and chemical composition of the HoAcAc-MS (methods described below).

2.1.3. Stability of HoAcAc microspheres in water after neutron activation

After neutron activation, the stability of the irradiated HoAcAc-MS was evaluated by incubating the microspheres in water for injection (100 mg in 10 mL). The morphological properties of the microspheres were analyzed by optical microscopy and the possible holmium leakage was determined by gamma-scintillation counting of the metastable isotope ^{166m}Ho (2480 Wizard2 Automatic Gamma Counter, Perkin Elmer) over a period of 48 h. The metastable isotope ^{166m}Ho has a very long half-life (~1200 y) and is formed in a fixed ratio of 7 ppm relative to ^{166}Ho during irradiation of ^{165}Ho, enabling an accurate measurement of the ^{166}Ho release from the microspheres even after the ^{166}Ho non-metastable isotope has been decayed (Seppenwoolde et al., 2005).

2.1.4. Formation of $HoPO_4$ microspheres after neutron activation of HoAcAc microspheres

To form $HoPO_4$-MS (Fig. 1, step 3), the irradiated HoAcAc-MS were incubated (approximately 100 mg per 10 mL) in a phosphate buffer solution (0.116 M NaH_2PO_4, pH 4.2) with gentle shaking (tube roller shaker) at room temperature. The formation kinetics of the $HoPO_4$-MS was determined by HPLC analysis by measuring the acetylacetonate released from the microspheres in the supernatant at different time points. Light microscopy was used to observe the microspheres during exchange of the AcAc ligands by the phosphate ions. The final $HoPO_4$-MS were characterized by size distribution analysis, light and scanning electron microscopy and the holmium and phosphorus contents were determined by ICP-OES. Finally, the chemical composition of the $HoPO_4$-MS was determined by XRD.

2.1.5. Holmium release during formation of $HoPO_4$ microspheres and stability in human serum

After forming of the $HoPO_4$-MS (Fig. 1, step 3), the phosphate buffer was removed by centrifugation and replaced by human serum (approximately 100 mg in 10 mL). Human serum was collected from blood donors of the University Medical Center of Utrecht after incubation of whole blood in Clot Activator Tubes and centrifugation to collect serum. The stability of the $HoPO_4$-MS was tested at 37 °C under constant shaking in a C24 incubator shaker (New Brunswick Scientific, Edison, USA). The microspheres were centrifuged and samples of human serum were collected at different time points to quantify the amount of ^{166m}Ho in the supernatant (8 mL). After incubation, the microspheres were washed with water three times, dried under vacuum and the holmium and phosphorous contents were determined by ICP-OES.

2.1.6. Characterization of HoAcAc crystals, (radioactive) HoAcAc microspheres and radioactive $HoPO_4$ microspheres

The HoAcAc crystals, HoAcAc-MS (before and after neutron irradiation) and radioactive $HoPO_4$-MS were characterized for their holmium (Ho) and phosphorous (P) contents using ICP-OES. Samples of 20 to 50 mg were dissolved in 50 mL of 2% nitric acid and the holmium concentration of the solutions was measured at three different wavelengths (339.9, 345.6 and 347.4 nm) and the phosphorous concentrations at two wavelength (213.6 and 214.9 nm) using an Optima 4300 CV (PerkinElmer, Norwalk, USA).

The HoAcAc-MS (5 to 15 mg) were dissolved in methanol (10 mL) and the acetylacetonate (AcAc) content was determined by HPLC analysis using an Alliance HPLC system using a C18 column (XSelect CSH C18 3.5 μm 4.6 × 150 mm, Waters) at 40 °C and a 70:30 mixture of methanol and water with 0.1% perchloric acid as the mobile phase (0.5 mL/min). Detection was done at 280 nm.

The water content of vacuum dried HoAcAc crystals and HoAcAc-MS was determined using a Karl Fisher Coulometer (831, Metrohm) by dissolving samples of approximately 20 to 50 mg in 1 mL of methanol. The Karl Fisher method could not be applied to the $HoPO_4$-MS because these microspheres are not soluble in methanol. Therefore, the water content in the $HoPO_4$-MS after vacuum drying the microspheres at room temperature was determining by thermogravimetric analysis (TGA, TA Instruments Q-50) using a samples of approximately 25 mg.

The size distribution of HoAcAc-MS (before and after sieving) and one month decayed radioactive $HoPO_4$-MS was determined using a Coulter counter equipped with an orifice of 100 μm (Multisizer 3, Beckman Coulter, Mijdrecht, The Netherlands).

The density of the HoAcAc crystals and the different microspheres with a sample amount of approximately 250 mg was determined in

water using a 25 cm^3 specific gravity bottle (Blaubrand NS10/19, DIN ISO 3507, Wertheim, Germany).

For identification of the chemical compounds present in non-irradiated and neutron irradiated HoAcAc-MS, 5 mg of samples were dissolved in 1 mL of methanol (n = 4 for each condition tested) and analyzed by GC–MS in a Shimadzu Gas Chromatograph-Mass Spectrometer GCMS-QP2010 system equipped with a VF-5 ms column (35 m × 0.25 mm × 0.25 μm film thickness). The injection temperature was 265 °C and the column oven was set at 50 °C (8 min hold) and increased to 290 °C at 100°/min (6 min hold). The carrier gas was He at a constant flow rate of 1.0 mL/min. The total ion chromatograms (TIC) were obtained and analyzed using the GC–MS Solution v272 software. The identification of the compounds of the different peaks was performed by MS using the mass spectral library NIST 2011. The MS was operated in full scan mode over 35–500 *m/z*. The MS transfer line temperature was held at 300 °C and the ion source temperature at 200 °C. After identification, the area under the peak from the TIC was used for quantification of the acetylacetonate present in the samples.

XRD patterns of the HoAcAc crystals, HoAcAc-MS before irradiation, decayed neutron activated HoAcAc-MS and decayed $HoPO_4$-MS were obtained by depositing a small amount (around 5 mg) of the different sample on a Si-510 wafer and analyzed using a Bruker D8 Advance diffractometer in Bragg-Brentano geometry with a Lynxeye position sensitive detector.

Optical microscopy (AE2000 Motic) was used to investigate the morphological properties of the microspheres (sphericity and surface damages) obtained under the various preparation conditions. The surface composition of the HoAcAc crystals, HoAcAc-MS, decayed radioactive HoAcAc-MS and $HoPO_4$-MS was studied using a SEM-EDS (JEOL JSM-IT100, InTouchScope™, Tokyo, Japan).

3. Results and discussion

3.1. Preparation of holmium acetylacetonate microspheres (HoAcAc-MS)

Microspheres with a holmium content of 45 wt% were previously prepared using a conventional emulsification and solvent evaporation process (Bult et al., 2012, 2007, 2009). These holmium acetylacetonate microspheres (HoAcAc-MS, Fig. 2B) were prepared by dissolution of holmium acetylacetonate crystals (Fig. 2A) in chloroform followed by solvent evaporation (Fig. 1, step 1).

Several parameters of the preparation process can influence the properties of the obtained microspheres. To this end, the influence of the (1) concentration of HoAcAc in the organic solvent (5.4, 6.3 or 7.5 ww%), the (2) temperature of emulsification (15, 25 and 35 °C) and the (3) stirring speed (300, 400, 500 and 600 rpm) on the size of the HoAcAc-MS were studied. It was observed that the concentration of HoAcAc in the organic phase is critical to obtain microspheres in the final dispersion without contamination with HoAcAc crystals. When relatively high concentrations of HoAcAc in the organic phase were used (6.3 or 7.5 ww%), the HoAcAc-MS were contaminated with HoAcAc crystals (Fig. S1). Using 5.4 ww% of HoAcAc in chloroform resulted in the formation of only HoAcAc-MS (Fig. 2B). The mean size, chemical composition and density of the obtained HoAcAc-MS are reported in Table 2. The density of the HoAcAc crystals precursor (1.6 g/cm^3) is very similar to that the HoAcAc-MS (1.7 g/cm^3), demonstrating that the microspheres are non-porous.

HoAcAc-MS were also prepared at different temperatures (15, 25 and 35 °C; Table 1). An increasing diameter of the microspheres was observed with higher temperature of the mixture: the average mean diameter for microspheres prepared at 15 °C was 27 μm whereas at 35 °C the formed microspheres had a diameter of 33 μm (Table 1). Similar findings were reported in previous studies where the temperature of emulsification was also varied to prepare polymeric microspheres (Mateovic-Rojnik et al., 2005; Sahoo et al., 2007; Yang et al., 2000a; Yang et al., 2000b). The vapor pressure of chloroform is 16.8, 26.2 and 39.5 kPa at at 15, 25 and 35 °C (http://www.ddbst.com/ddb.html). Thus, higher temperatures provide a greater driving force for evaporation leading to a faster hardening of the droplets to finally yield into microspheres. This rapid solvent removal at higher temperatures will shorten the time to further decrease the size of the droplets by the applied shear forces during stirring (Scheme S1) (Li et al., 1995; Maia et al., 2004; Mateovic-Rojnik et al., 2005).

The effect of the stirring speed on the size of the microspheres was also studied and it was observed that the average diameter of the microspheres decreased from 31.2 ± 1.2 μm to 11.1 ± 1.6 μm with increasing stirring speed from 300 to 600 rpm respectively (Fig. 3). Likely, a higher stirring speed yielded due to the higher shear forces an emulsion with smaller chloroform droplets finally resulting after evaporation of the solvent in smaller HoAcAc microspheres.

The research on the examined parameters resulted in a standard and reproducible method for the preparation of HoAcAc-MS under the following conditions: 5.4 wt% of HoAcAc in chloroform as the dispersed phase, 25 °C and 300 rpm at a fixed concentration of emulsifier (2%)

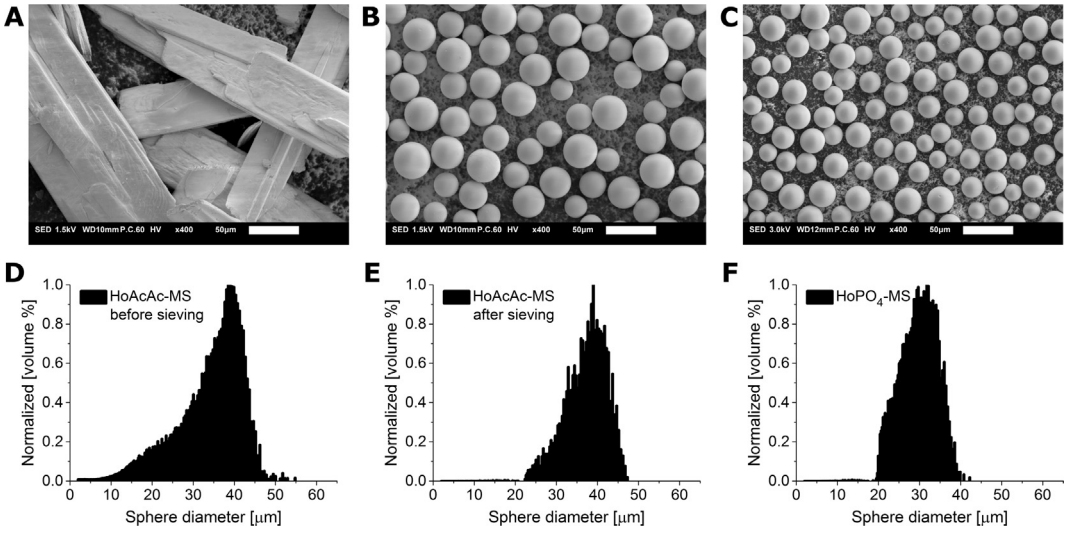

Fig. 2. Scanning electron microphotographs of (A) holmium acetylacetonate crystals used for the preparation of (B) holmium acetylacetonate microspheres (HoAcAc-MS), and (C) holmium phosphate microspheres ($HoPO_4$-MS). Microphotographs were acquired at a magnification of 400× and scale bars correspond to 50 μm. Size distributions of the HoAcAc-MS (D) before and (E) after sieving, and of the (F) $HoPO_4$-MS.

Table 1
Influence of temperature of emulsification on the average diameter, density and yield of holmium acetylacetonate microspheres (HoAcAc-MS, n = 6 for each temperature). HoAcAc-MS were prepared at different temperatures using an o/w emulsification and solvent evaporation followed by sieving through 50 and 20 μm sieves.

Temperature [°C]	Size before sieving [μm]	Size after sieving [μm]	Density [g/cm^3]	Yield 20–50 μm [g]	Yield 20–50 μm in terms of Ho recovery [%]
15	26.7 ± 0.8	28.6 ± 1.1	1.76 ± 0.06	2.52 ± 0.25	33.8 ± 3.4
25	31.2 ± 1.2	34.2 ± 1.0	1.71 ± 0.05	3.39 ± 0.33	45.5 ± 4.4
35	33.2 ± 1.8	34.7 ± 0.9	1.72 ± 0.07	4.26 ± 0.27	57.2 ± 3.6

Table 2
Characteristics of HoAcAc crystals (n = 6), the standard HoAcAc-MS (n = 12) and radioactive $HoPO_4$-MS (n = 6).

Sample	Calculated structure	Ho [wt%]	P [wt%]	AcAc [wt%]	H_2O [wt%]	Density [g/cm^3]	Mean diameter [μm]
HoAcAc crystals	$Ho(AcAc)_3{\cdot}H_2O$	35.2 ± 1.5	n.a.	60.1 ± 1.6	3.7 ± 0.4	1.61 ± 0.12	n.a.
HoAcAc-MS	$Ho_2(AcAc)_3{\cdot}2H_2O$	46.2 ± 0.8	n.a.	46.8 ± 1.2	5.2 ± 0.7	1.71 ± 0.05	34.2 ± 1.0
$HoPO_4$-MS	$HoPO_4{\cdot}3H_2O$	52.0 ± 2.3	9.7 ± 0.5	less than 0.01	20 ± 2.1	3.12 ± 0.08	29.5 ± 1.2

n.a. not applicable.

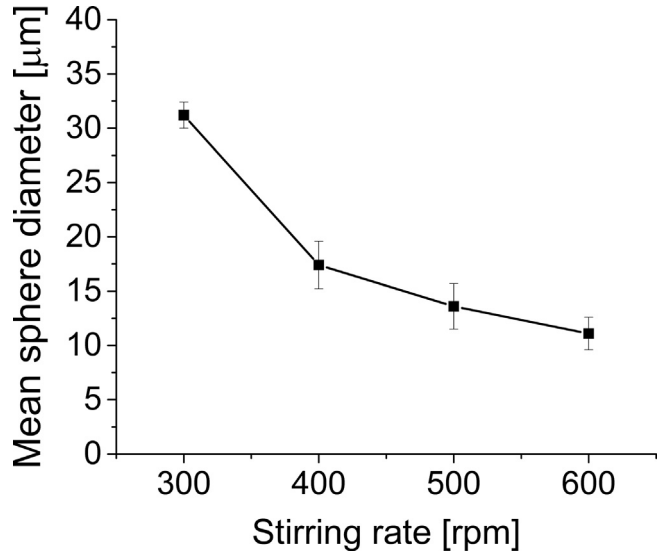

Fig. 3. Effect of emulsion stirring speed on the mean diameter of the obtained holmium acetylacetonate microspheres (HoAcAc-MS, n = 3 for each stirring speed). The temperature of emulsification (25 °C), concentration of HoAcAc in chloroform (5.4 ww%), concentration of emulsifier (2%) and duration of emulsification (72 h) were fixed.

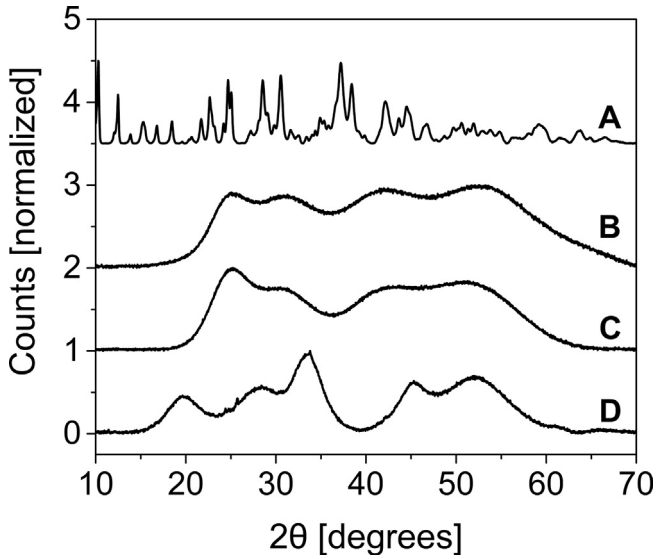

Fig. 4. X-Ray powder diffraction patterns of (A) HoAcAc crystals, (B) HoAcAc-MS, (C) radioactive HoAcAc-MS (600 mg irradiated for 4 h measured after 2 months) and (D) radioactive $HoPO_4$-MS.

and 72 h of emulsification/solvent evaporation. The so obtained microspheres (n = 12) had a mean size of 31.2 ± 1.2 μm and, after sieving through 50 and 20 μm sieves, a mean size of 34.2 ± 1.0 μm. The used parameters yields 3.4 ± 0.3*g* of microspheres after sieving. The recovery of holmium was ca. 45% from 10*g* HoAcAc crystals (density 1.6 g/cm^3 and holmium content 35.2 ± 1.5% (w/w)) to HoAcAc-MS (density of 1.7 g/cm^3 and a holmium content of 46.2 ± 0.8 (w/w)).

The HoAcAc crystals and the HoAcAc-MS were characterized using different techniques. The results show that HoAcAc crystals are formed through the complexation of one holmium atom with three acetylacetonate molecules and one molecule of water (Table 2), resulting in a highly crystalline complex (Fig. 4A). These results are in line with previous findings (Kooijman et al., 2000; Nijsen et al., 2001). After dissolving this complex in chloroform and emulsification of obtained solution in an aqueous solution, the obtained HoAcAc-MS have a composition of one and a half AcAc molecules and one water molecule per holmium atom and per water molecule. The new complex of the holmium and AcAc is not crystalline but amorphous as observed by XRD (Fig. 4B). As suggested by Bult et al., a network is formed in which one and a half acetylacetonate molecule is complexed with one holmium ion in an amorphous state (Bult et al., 2009).

3.1. Neutron activation of HoAcAc-MS

HoAcAc-MS prepared under standard conditions were neutron activated to convert the non-radioactive ^{165}Ho into the beta and gamma-emitting ^{166}Ho (Fig. 1, step 2). In previous studies, several irradiation parameters were varied to investigate their influence on the obtained radioactive HoAcAc loaded poly(l-lactic acid) microspheres (Nijsen et al., 2002b; Nijsen et al., 1999; Vente et al., 2009). It was observed that the irradiation facility, duration of irradiation as well the size of the samples influenced the final properties of the microspheres. In the present study, the tested parameters were the irradiation time (2, 4, 6 and 8 h) and the amount of microspheres per vial (50, 100 or 600 mg). The different irradiation times allow to evaluate the effect of increasing doses of neutron irradiation on the microspheres, whereas the amount of sample per vial is important to optimize the amount of microspheres that will be administered to the patients (Smits et al., 2012).

In comparison with the current clinically used ^{166}Ho loaded microspheres (QuiremSpheres®), the substantially higher holmium content of the HoAcAc-MS (46 wt%) resulted in a higher radioactivity per

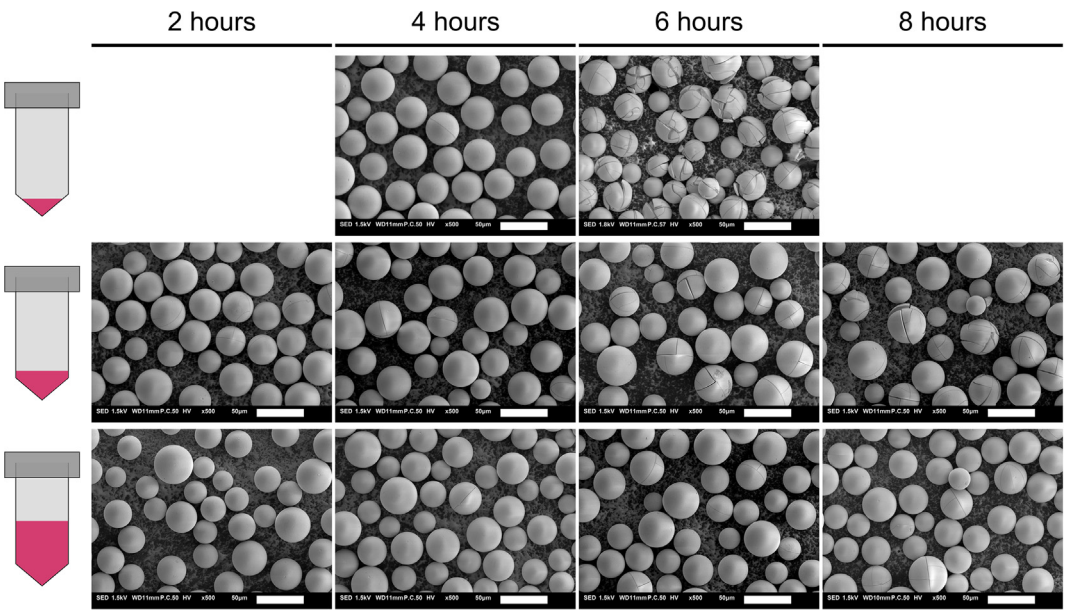

Fig. 5. Scanning electron photographs of HoAcAc-MS irradiated for different irradiation times (2, 4, 6 and 8 h) in different amounts: (top) 50 mg, (middle) 100 mg and (bottom) 600 mg. Microphotographs were acquired at a magnification of 500× and scale bars correspond to 50 μm.

mg of sphere by a factor of 2.5. Within 6 h of neutron activation of 600 mg of HoAcAc-MS, an activity of 47 GBq can be produced at the end of bombardment (thermal flux 4.97×10^{16} n m^{-2} s^{-1}) compared to 19 GBq for the QuiremSpheres®.

It was observed that both the irradiation time and volume of sample in the irradiation vial had an effect on the final appearance of the HoAcAc-MS. After 2 or 4 h irradiation, SEM analysis of the microspheres showed a smooth spherical appearance of the microspheres identical to non-irradiated microspheres regardless the amount of microspheres used per vial (Fig. 5). However, when longer irradiation periods were used (8 h), the microspheres displayed cracks on their surface. Unexpectedly, the amount of microspheres per vial had a substantial effect on the damages of the microspheres: when relatively low amounts of microspheres were irradiated (50 or 100 mg), the microspheres showed surface damages with irradiation times of 6 and 8 h. On the other hand, when a larger amount of microspheres was irradiated (600 mg), no significant damages were observed up to 8 h irradiation.

Due to the fact that some of the neutron activated and thereafter decayed HoAcAc-MS presented cracks on their surface after irradiation, HoAcAc-MS before and after neutron activation were analyzed by GC–MS to detect whether degradation compounds are formed. Fig. 6 shows that indeed several compounds were formed during irradiation of the HoAcAc-MS. Some of the compounds detected were methyl acetate and acetone (mass spectrums are displayed in Fig. S2) suggesting that the radiation induced degradation of the AcAc molecule. The radiation responsible for the degradation of the acetylacetonate molecule are the gamma rays which are present in the reactor during neutron irradiation of the microspheres. This is in line with the literature (Barker, 1963; Hummel, 1995) reporting that the dominating processes in the degradation of ketones upon exposure to ionizing radiation is the break of the C–C bond adjacent to the C=O bond and chemical reactions with radiolytic products of water (e.g. hydrated electrons, HO_2, H_3O^+, OH^-, H_2O_2 and H_2) (Čech, 1974; Rama Rao et al., 1970).

The degradation products formed during irradiation have boiling points around 60 °C (boiling points of acetone and methyl acetate are 56 °C and 57 °C respectively) and the average temperature reached during irradiation of the samples was 54–60 °C. It can be therefore expected that the evaporation of these volatile degradation products during irradiation will result in surface cracking of the microspheres. Moreover, the amount of microspheres per vial plays a particular role in the heat transfer and therefore influences the heating degree of the samples. When smaller samples are used, there is less heat conductivity resulting in a faster heating up and thus a higher temperature of the microspheres which in turn will result in a higher driving force for

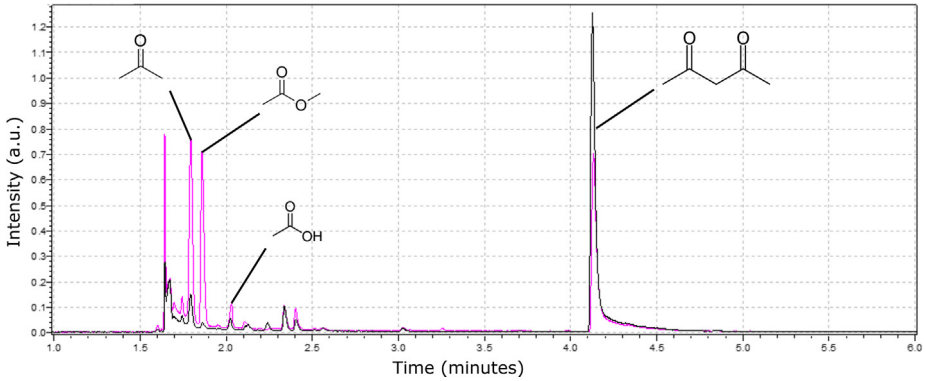

Fig. 6. Total ion chromatogram (TIC) obtained from the GC–MS analysis of non-irradiated HoAcAc-MS (black line) and irradiated HoAcAc-MS (pink line) (600 mg of HoAcAc-MS irradiated for 4 h). The peaks detected on the TIC were used for the identification of the different compounds using mass spectrometry.

evaporation of volatile degradation products and subsequently in the formation of cracks. The AcAc content of the HoAcAc-MS after irradiation under different conditions was quantitatively determined by GC–MS and HPLC and it was observed that this content decreased with increasing irradiation time (Fig. S3). The XRD pattern of the irradiated HoAcAc-MS remains similar to the non-irradiated HoAcAc-MS (Fig. 4C) showing that, after neutron activation, the microspheres remained amorphous.

3.2. Stability of HoAcAc-MS in water after neutron activation

The stability of the irradiated HoAcAc-MS was investigated by incubating the particles in water for injection. Non-irradiated HoAcAc-MS are stable in water. On the other hand, it was observed that the HoAcAc-MS irradiated for 4 h in a vial of 600 mg were not stable in water for injection and released more than 90% of their radioactive ^{166m}Ho within 2 days (Fig. S4a). Optical microscopy observations revealed a progressive degradation of the microspheres with complete disintegration of the microspheres after 48 h incubation in water for injection (Fig. S4b). The instability of the irradiated microspheres points to irradiation damage.

Interestingly, in previous work from our group, it was observed that when the irradiated HoAcAc-MS were incubated in a phosphate buffer, the microspheres remained spherical and holmium release was not observed (Bult et al., 2012). The reasons for this stability in a phosphate buffer were further investigated in the present study.

3.3. Formation of $HoPO_4$-MS by incubation of irradiated HoAcAc-MS in a phosphate buffer

As pointed out above, irradiated HoAcAc-MS incubated in phosphate buffer were stable (Bult et al., 2012). In this work, after incubation of the irradiated HoAcAc-MS in a phosphate buffer (0.116 M, pH 4.2) for 1 h at room temperature (Fig. 1, step 4), the resulting microspheres were characterized by ICP-OES. It was found that they contain 9.7 wt% of phosphorus, 52 wt% of holmium and no traces of AcAc were detected (less than 0.01%) (Table 2). This revealed that during incubation of the irradiated HoAcAc-MS in the phosphate buffer, the AcAc ligands were replaced by phosphate ions forming new complexes of phosphate and ^{166}Ho.

The formation kinetics of the $^{166}HoPO_4$ complexes was also monitored by HPLC through the release of AcAc over time (Fig. 7A). It was observed that 100% of the AcAc present in the HoAcAc-MS was releases within 1 h of incubation in phosphate buffer. Interestingly, imaging of the microspheres by optical microscopy showed the formation of a ring-like structure that increased over time and correlated with the amount of AcAc released (Fig. 7B). Likely, the AcAc ligands are exchanged by phosphate ions resulting in $HoPO_4$, which has different refractive index than the HoAcAc.

The exchange reaction was performed at pH 4.2 because acetylacetone is essentially in its non-charged state (pKa = 9.0) decreasing the stability of the HoAcAc complexes (Smith, 2010; Stary and Liljenzin, 1982). This in turn favors the release of the AcAc and subsequent binding of the phosphate ions. During the exchange reaction, it was observed that a maximum of 0.28 ± 0.02% (n = 8) of ^{166m}Ho was released during 1 h incubation. Moreover, the geometry of the microspheres was retained (Fig. 7B) while acetylacetone and its degradation products were completely exchanged with phosphate ion to yield radioactive $^{166}HoPO_4$-MS. Thermogravimetric analysis of the $HoPO_4$-MS revealed a total weight loss of 21.5 wt% which corresponds to water loss (Fig. S5). Altogether, these results show that the final $HoPO_4$-MS have a chemical composition of one holmium atom per phosphate group complexed with three water molecules (Table 2).

After exchange of the AcAc by the PO_4 ions, it was observed by optical (Fig. 7B) and scanning electron microscopy (Fig. 2C) that the microspheres retained their spherical shape without alterations of their surface morphology. It was further observed that the mean diameter of the microspheres decreased from 34.2 ± 1.0 µm to 29.5 ± 1.2 µm (Fig. 2B and 2C, Table 2), which corresponds to a reduction of the microspheres volume of approximately 30% and consequently resulted in an increase of the microsphere's density from 1.7 to 3.1 g/cm^3 (Table 2). XRD analysis shows that $HoPO_4$-MS are fully amorphous (Fig. 4D).

3.4. Stability of radioactive $HoPO_4$-MS in human serum

Anticipating the use of the $HoPO_4$-MS for internal radionuclide therapy, the stability of the microspheres was tested in human serum. The microspheres had a very good stability and less than 0.1% of ^{166m}Ho was released during their incubation in serum for two weeks (Fig. 8A). This is in line with the literature where it is described that rare earth metal phosphates are practically insoluble in aqueous media (Kijkowska and LeGeros, 2005) and explains the high stability of these microspheres in serum. Furthermore, no changes of the microspheres were observed by optical microscopy (Fig. 8B) and the size distribution remained constant during the 2 weeks period of incubation.

4. Conclusions

The study shows that stable radioactive $HoPO_4$ microspheres with a

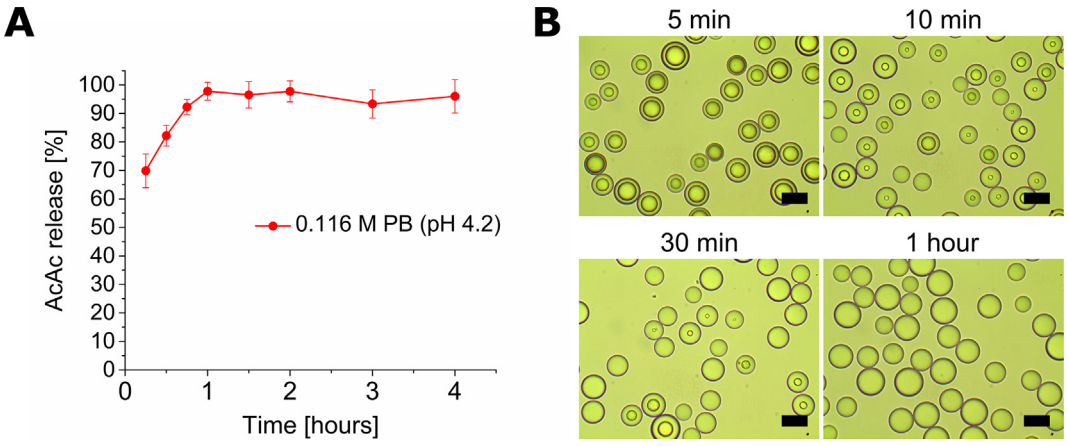

Fig. 7. (A) Release of the AcAc from the irradiated HoAcAc-MS incubated in 0.116 M phosphate buffer (pH 4.2) resulting in formation of radioactive $HoPO_4$-MS. (B) Optical microphotographs of radioactive holmium microspheres incubated for different times (5 min to 1 h) in the 0.116 M phosphate buffer at pH 4.2. The progressive binding of phosphate ions to the radioactive HoAcAc-MS can be observed with optical microscopy through the formation of a ring-like structure in the microspheres as early as 5 min until the disappearance and thus complete exchange at 1 h. Scale bars correspond to 25 µm.

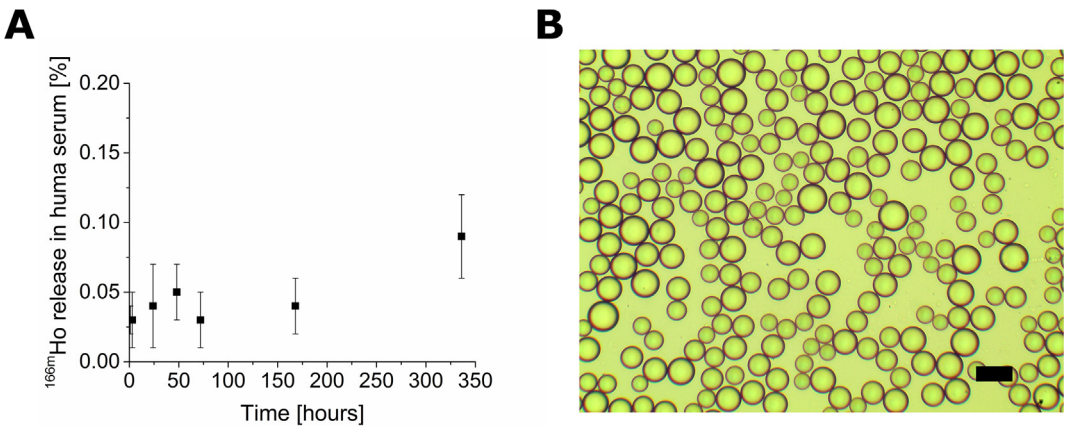

Fig. 8. HoAcAc-MS were irradiated for 4 h followed by incubation in phosphate buffer solution to produce radioactive $HoPO_4$-MS, which were subsequently incubated for 2 weeks at 37 °C under constant shaking in human serum: (A) quantification of ^{166m}Ho release in the human serum and (B) optical micrograph of radioactive $HoPO_4$-MS incubated in human serum after two weeks. Scale bar corresponds to 50 μm.

very high holmium content for internal radionuclide therapy can be produced. The previously described HoAcAc-MS with a 46 wt% holmium content were prepared and the influence of different processing parameters was investigated to define the optimal preparation conditions. The most critical parameter was the stirring speed and the mean diameter of the HoAcAc-MS could be easily tailored from 11 to 31 μm. It was also observed that after irradiation the microspheres are unstable in water for injection. Therefore, holmium phosphate microspheres ($HoPO_4$-MS) were prepared from the neutron irradiated HoAcAc-MS by incubating the microspheres in a phosphate buffer. The radioactive $HoPO_4$-MS that have a high holmium content (52 wt%) are very stable in human serum (holmium release less than 0.1%). It is concluded that these $HoPO_4$-MS are attractive microspheres for application in the SIRT technology for liver malignancies and for intratumoral treatment of solid tumors. Future studies will focus on the pre-clinical testing of these microspheres.

Acknowledgments

This work was supported by the NWO Innovation Fund for Chemistry (IFC) and the Launchpad for Innovative Future Technology (LIFT) [Project number 731.015.411]. The authors would like to acknowledge the help of many people. Mies van Steenbergen of the Department of Pharmaceutic of the Utrecht University for helping with the HPLC method and for all the help in the lab. Pascal Wijten of the section of Inorganic Chemistry and Catalysis of Utrecht University for assistance in acquiring the GC-MS profiles. Adrie Laan and Baukje Terpstra of the Reactor Institute Delft for helping with the SEM-EDS and the ICP-OES measurements. Mehmet Sarilar, Baukje Terpstra and Delia van Rij for performing the neutron irradiation of the samples. Carla de Wals from the company Quirem Medical is also acknowledged for preparing the HoAcAc crystals used in this work. Finally, we would like to thank Angelique Barten for participating in discussions related to this work.

5. Declaration of interest

JFW Nijsen is inventor on the patents related to the HoAcAc-MS and $HoPO_4$-MS which are assigned to University Medical Center Utrecht Holding BV and/or Quirem Medical (patent families: USA Patent No. 6,373,068 B1, PCT/NL03/00485, EP07112807.8, 10190254.2, P114198PC00, P112614NL00). He is co-founder and chief scientific officer of Quirem Medical, and has a minority share in the company Quirem Medical. The activities of J.F.W. Nijsen within Quirem Medical are approved and supported by Dirkjan Masman (Director Technology Transfer Office Radboudumc) and Mathias Prokop (Head of Radiology and Nuclear Medicine at Radboudumc). All authors have revised and have approved the final manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijpharm.2018.06.036.

References

Anderson, J.H., Angerson, W.J., Willmott, N., Kerr, D.J., McArdle, C.S., Cooke, T.G., 1991. Regional delivery of microspheres to liver metastases: the effects of particle size and concentration on intrahepatic distribution. Br. J. Cancer 64, 1031–1034.
Bakker, R.C., Lam, M.G.E.H., van Nimwegen, S.A., Rosenberg, A.J.W.P., van Es, R.J.J., Nijsen, J.F.W., 2017. Intratumoral treatment with radioactive beta-emitting microparticles: a systematic review. J. Radiation Oncol.
Barker, R., 1963. Gamma-radiolysis of liquid acetone. Trans. Faraday Soc. 59, 375–385.
Bult, W., de Leeuw, H., Steinebach, O.M., van der Bom, M.J., Wolterbeek, H.T., Heeren, R.M.A., Bakker, C.J.G., van het Schip, A.D., Hennink, W.E., Nijsen, J.F.W., 2012. Radioactive Holmium Acetylacetonate Microspheres for Interstitial Microbrachytherapy: An In Vitro and In Vivo Stability Study. Pharm. Res. 29, 827–836.
Bult, W., Nijsen, J.F., van het Schip, A.D., 2007. A particle comprising an organic lanthanide metal complex. EP07112807.8 / P81585EP00.
Bult, W., Seevinck, P.R., Krijger, G.C., Visser, T., Kroon-Batenburg, L.M.J., Bakker, C.J.G., Hennink, W.E., van het Schip, A.D., Nijsen, J.F.W., 2009. Microspheres with Ultrahigh Holmium Content for Radioablation of Malignancies. Pharm. Res. 26, 1371–1378.
Čech, R., 1974. Postirradiation changes in the solutions of acetylacetone. Chem. Pap. 28 (1), 47–50.
Dendy, M.S., Ludwig, J.M., Kim, H.S., 2017. Predictors and prognosticators for survival with Yttrium-90 radioembolization therapy for unresectable colorectal cancer liver metastasis. Oncotarget 8, 37912–37922.
Di Pasqua, A.J., Huckle, J.E., Kim, J.K., Chung, Y., Wang, A.Z., Jay, M., Lu, X., 2012. Preparation of neutron-activatable holmium nanoparticles for the treatment of ovarian cancer metastases. Small 8, 997–1000.
Di Pasqua, A.J., Yuan, H., Chung, Y., Kim, J.K., Huckle, J.E., Li, C., Sadgrove, M., Tran, T.H., Jay, M., Lu, X., 2013. Neutron-activatable holmium-containing mesoporous silica nanoparticles as a potential radionuclide therapeutic agent for ovarian cancer. J. Nucl. Med. 54, 111–116.
Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D., Bray, F., 2015. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int. J. Cancer 136, 9.
Hamoudeh, M., Fessi, H., Salim, H., Barbos, D., 2008. Holmium-Loaded PLLA Nanoparticles for Intratumoral Radiotherapy Via the TMT Technique: Preparation, Characterization, and Stability Evaluation after Neutron Irradiation. Drug Dev. Ind. Pharm. 34, 796–806.
Hummel, A., 1995. Radiation Chemistry: The Chemical Effects of Ionizing Radiation and Their Applications. IRI-TUD, Delft.
Valderrama-Treviño, A.I., Barrera-Mera, B., Ceballos-Villalva, J.C., Montalvo-Javé, E.E., 2017. Hepatic Metastasis from Colorectal Cancer. Euroasian J. Hepato-Gastroenterol. 7, 166–175.
Kelly, C.M., Kemeny, N.E., 2017. Liver-directed therapy in metastatic colorectal cancer. Expert Rev. Anticancer Ther. 17, 745–758.
Kijkowska, R., LeGeros, R.Z., 2005. Preparation and Properties of Lanthanide Phosphates. Key Eng. Mater. 284–286, 79–82.
Kim, J., Luo, Z.-X., Wu, Y., Lu, X., Jay, M., 2017. In-situ formation of holmium oxide in

pores of Mesoporous Carbon Nanoparticles as substrates for neutron-activatable radiotherapeutics. Carbon 117, 92–99.
Kim, J.K., Han, K.-H., Lee, J.T., Paik, Y.H., Ahn, S.H., Lee, J.D., Lee, K.S., Chon, C.Y., Moon, Y.M., 2006. Long-term Clinical Outcome of Phase IIb Clinical Trial of Percutaneous Injection with Holmium-166/Chitosan Complex (Milican) for the Treatment of Small Hepatocellular Carcinoma. Clin. Cancer Res. 12, 543–548.
Kooijman, H., Nijsen, F., Spek, A.L., van het Schip, F., 2000. Diaquatris(pentane-2,4-dionato-O, O')holmium(III) monohydrate and diaquatris(pentane-2,4-dionato-O, O') holmium(III) 4-hydroxypentan-2-one solvate dihydrate. Acta Crystallogr. C 56, 156–158.
Li, W.-I., Anderson, K.W., Deluca, P.P., 1995. Kinetic and thermodynamic modeling of the formation of polymeric microspheres using solvent extraction/evaporation method. J. Control. Release 37, 187–198.
Maia, J.L., Santana, M.H.A., Ré, M.I., 2004. The effect of some processing conditions on the characteristics of biodegradable microspheres obtained by an emulsion solvent evaporation process. Braz. J. Chem. Eng. 21, 01–12.
Marcon, L., Gehan, H., Khoshnevis, M., Marmuse, L., Carozzo, C., Louis, C., Fonce, F., Tillement, O., 2017. Synthesis of Highly-loaded Holmium-165 Siloxane Particles for Brachytherapy of Brain Cancer and Injectability Evaluation in Healthy Pig. J. Nanomed. Nanotechnol. 8.
Mateovic-Rojnik, T., Frlan, R., Bogataj, M., Bukovec, P., Mrhar, A., 2005. Effect of preparation temperature in solvent evaporation process on Eudragit RS microsphere properties. Chem. Pharm. Bull. 53, 143–146.
Meade, V.M., Burton, M.A., Gray, B.N., Self, G.W., 1987. Distribution of different sized microspheres in experimental hepatic tumours. Eur. J. Cancer Clin. Oncol. 23, 37–41.
Misiakos, E.P., Karidis, N.P., Kouraklis, G., 2011. Current treatment for colorectal liver metastases. World J. Gastroenterol. 17, 4067–4075.
Mumper, R.J., Mills, B.J., Ryo, U.Y., Jay, M., 1992. Polymeric microspheres for radionuclide synovectomy containing neutron-activated holmium-166. J. Nucl. Med. 33, 398–402.
Nijsen, J.F., van het Schip, A.D., Hennink, W.E., Rook, D.W., van Rijk, P.P., de Klerk, J.M., 2002a. Advances in nuclear oncology: microspheres for internal radionuclide therapy of liver tumours. Curr. Med. Chem. 9, 73–82.
Nijsen, J.F., van Het Schip, A.D., van Steenbergen, M.J., Zielhuis, S.W., Kroon-Batenburg, L.M., van de Weert, M., van Rijk, P.P., Hennink, W.E., 2002b. Influence of neutron irradiation on holmium acetylacetonate loaded poly(L-lactic acid) microspheres. Biomaterials 23, 1831–1839.
Nijsen, J.F.W., van Steenbergen, M.J., Kooijman, H., Talsma, H., Kroon-Batenburg, L.M.J., van de Weert, M., van Rijk, P.P., de Witte, A., van het Schip, A.D., Hennink, W.E., 2001. Characterization of poly(l-lactic acid) microspheres loaded with holmium acetylacetonate. Biomaterials 22, 3073–3081.
Nijsen, J.F.W., Zonnenberg, B.A., Woittiez, J.R.W., Rook, D.W., Swildens-van Woudenberg, I.A., van Rijk, P.P., van het Schip, A.D., 1999. Holmium-166 poly lactic acid microspheres applicable for intra-arterial radionuclide therapy of hepatic malignancies: effects of preparation and neutron activation techniques. Eur. J. Nucl. Med. 26, 699–704.
Oerlemans, C., Seevinck, P.R., Smits, M.L., Hennink, W.E., Bakker, C.J.G., van den Bosch, M.A.A.J., Nijsen, J.F.W., 2015. Holmium–lipiodol–alginate microspheres for fluoroscopy-guided embolotherapy and multimodality imaging. Int. J. Pharm. 482, 47–53.
Pasciak, A., 2017. Handbook of Radioembolization. CRC Press, Taylor & Francis Group, LLC.
Prince, J.F., van den Bosch, M., Nijsen, J.F.W., Smits, M.L.J., van den Hoven, A.F., Nikolakopoulos, S., Wessels, F.J., Bruijnen, R.C.G., Braat, M., Zonnenberg, B.A., Lam, M., 2017. Efficacy of radioembolization with holmium-166 microspheres in salvage patients with liver metastases: a phase 2 study. J. Nucl. Med. 15, 197194.
Rama Rao, K.V.S., Shastri, L.V., Shankar, J., 1970. Radiation chemistry of tris(acetylacetonato) cobalt(iii) in aqueous solutions. Radiation Effects 2, 193–200.
Rognoni, C., Ciani, O., Sommariva, S., Facciorusso, A., Tarricone, R., Bhoori, S., Mazzaferro, V., 2016. Trans-arterial radioembolization in intermediate-advanced hepatocellular carcinoma: systematic review and meta-analyses. Oncotarget 7, 72343–72355.
Sahoo, S.K., Dhal, S., Mohapatro, P., Behera, B.C., Barik, B.B., 2007. Effect of processing temperature on Eudragit RS PO microsphere characteristics in the solvent evaporation process. Pharmazie 62, 638–639.
Seevinck, P.R., Seppenwoolde, J.H., de Wit, T.C., Nijsen, J.F., Beekman, F.J., van Het Schip, A.D., Bakker, C.J., 2007. Factors affecting the sensitivity and detection limits of MRI, CT, and SPECT for multimodal diagnostic and therapeutic agents. Anticancer Agents Med. Chem. 7, 317–334.
Seppenwoolde, J.H., Nijsen, J.F., Bartels, L.W., Zielhuis, S.W., van Het Schip, A.D., Bakker, C.J., 2005. Internal radiation therapy of liver tumors: qualitative and quantitative magnetic resonance imaging of the biodistribution of holmium-loaded microspheres in animal models. Magn. Reson. Med. 53, 76–84.
Smith, M.B., 2010. Chapter 8 - Cd Disconnect Products: Nucleophilic Species that Form Carbon-Carbon Bonds, Organic Synthesis (Third Edition). Academic Press, Oxford, pp. 623–779.
Smits, M.L., Elschot, M., van den Bosch, M.A., van de Maat, G.H., van het Schip, A.D., Zonnenberg, B.A., Seevinck, P.R., Verkooijen, H.M., Bakker, C.J., de Jong, H.W., Lam, M.G., Nijsen, J.F., 2013. In vivo dosimetry based on SPECT and MR imaging of 166Ho-microspheres for treatment of liver malignancies. J. Nucl. Med. 54, 2093–2100.
Smits, M.L., Nijsen, J.F., van den Bosch, M.A., Lam, M.G., Vente, M.A., Mali, W.P., van Het Schip, A.D., Zonnenberg, B.A., 2012. Holmium-166 radioembolisation in patients with unresectable, chemorefractory liver metastases (HEPAR trial): a phase 1, dose-escalation study. Lancet Oncol. 13, 1025–1034.
Smits, M.L.J., Nijsen, J.F.W., van den Bosch, M.A.A.J., Lam, M.G.E.H., Vente, M.A.D., Huijbregts, J.E., van het Schip, A.D., Elschot, M., Bult, W., de Jong, H.W.A.M., Meulenhoff, P.C.W., Zonnenberg, B.A., 2010. Holmium-166 radioembolization for the treatment of patients with liver metastases: design of the phase I HEPAR trial. J. Exp. Clin. Cancer Res. CR 29 70 70.
Soerjomataram, I., Lortet-Tieulent, J., Parkin, D.M., Ferlay, J., Mathers, C., Forman, D., Bray, F., 2012. Global burden of cancer in 2008: a systematic analysis of disability-adjusted life-years in 12 world regions. Lancet 380, 1840–1850.
Stary, J., Liljenzin, J.O., 1982. Critical evaluation of equilibrium constants involving acetylacetone and its metal chelates. Pure Appl. Chem., p. 2557.
van Nimwegen, S.A., Bakker, R.C., Kirpensteijn, J., van Es, R.J.J., Koole, R., Lam, M., Hesselink, J.W., Nijsen, J.F.W., 2017. Intratumoral injection of radioactive holmium ((166) Ho) microspheres for treatment of oral squamous cell carcinoma in cats. Vet. Comp. Oncol. 8, 12319.
Vente, M.A., Nijsen, J.F., de Roos, R., van Steenbergen, M.J., Kaaijk, C.N., Koster-Ammerlaan, M.J., de Leege, P.F., Hennink, W.E., van Het Schip, A.D., Krijger, G.C., 2009. Neutron activation of holmium poly(L-lactic acid) microspheres for hepatic arterial radio-embolization: a validation study. Biomed. Microdevices 11, 763–772.
Vente, M.A.D., de Wit, T.C., van den Bosch, M.A.A.J., Bult, W., Seevinck, P.R., Zonnenberg, B.A., de Jong, H.W.A.M., Krijger, G.C., Bakker, C.J.G., van het Schip, A.D., Nijsen, J.F.W., 2010. Holmium-166 poly(L-lactic acid) microsphere radioembolisation of the liver: technical aspects studied in a large animal model. Eur. Radiol. 20, 862–869.
Yang, Y.-Y., Chia, H.-H., Chung, T.-S., 2000a. Effect of preparation temperature on the characteristics and release profiles of PLGA microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. J. Control. Release 69, 81–96.
Yang, Y.-Y., Chung, T.-S., Bai, X.-L., Chan, W.-K., 2000b. Effect of preparation conditions on morphology and release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion method. Chem. Eng. Sci. 55, 2223–2236.
Zarour, L.R., Anand, S., Billingsley, K.G., Bisson, W.H., Cercek, A., Clarke, M.F., Coussens, L.M., Gast, C.E., Geltzeiler, C.B., Hansen, L., Kelley, K.A., Lopez, C.D., Rana, S.R., Ruhl, R., Tsikitis, V.L., Vaccaro, G.M., Wong, M.H., Mayo, S.C., 2017. Colorectal Cancer Liver Metastasis: Evolving Paradigms and Future Directions. Cellular Mol. Gastroenterol. Hepatol. 3, 163–173.
Zielhuis, S.W., Nijsen, J.F., de Roos, R., Krijger, G.C., van Rijk, P.P., Hennink, W.E., van het Schip, A.D., 2006. Production of GMP-grade radioactive holmium loaded poly(L-lactic acid) microspheres for clinical application. Int. J. Pharm. 311, 69–74.
Zielhuis, S.W., Seppenwoolde, J.H., Bakker, C.J., Jahnz, U., Zonnenberg, B.A., van het Schip, A.D., Hennink, W.E., Nijsen, J.F., 2007. Characterization of holmium loaded alginate microspheres for multimodality imaging and therapeutic applications. J. Biomed. Mater. Res. A 82, 892–898.