2011 International Nuclear Atlantic Conference - INAC 2011
Belo Horizonte,MG, Brazil, October 24-28, 2011
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: 978-85-99141-04-5

MICROSPHERES WITH AN ULTRA HIGH HOLMIUM CONTENT FOR BRACHYTHERAPY OF MALIGNANCIES

Raphael A. Lira[1]*, Douglas M. Myamoto[1],Jaime R. Souza[1], Nanci Nascimento[1], João A. Osso Junior[2], José R. Martinelli[3] and Mariângela de Burgos M. de Azevedo[1]

[1] Centro de Biotecnologia
[2] Centro de Radiofarmácia
[3]Centro de Ciências e Tecnologia de Materiais
Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
*r.arivar@gmail.com

ABSTRACT

The overall objective of this work is to develop biodegradable microspheres intended for internal radiation therapy which provides an improved treatment for hepatic carcinomas[1]. The most studied brachytherapy system employing microspheres made of holmium-biopolymer system is composed by poly(L-lactic acid) (PLLA) and holmium acetylacetonate (HoAcAc)[2]. The importance of the holmium high content in the microspheres can be interpreted as follow from a therapeutic standpoint, to achieve an effective use of microspheres loaded with HoAcAc , a high content of holmium is required to yield enough radioactivity with a relatively low amount of microspheres.The usual amounts of holmium that are incorporated in the microspheres composed by poly(L-lactic acid) and HoAcAc are 17.0 ± 0.5% (w/w) of holmium, which corresponds to a loading of about 50% of HoAcAc[3]. Different approaches have been investigated to increase that value. One updated approach towards this direction is the production of microspheres with ultrahigh holmium as matrix using HoAcAc crystals as the sole starting material without the use of biopolymer [4,5]. Likewise, in the search of microspheres with increased holmium content , it has been demonstrated that by changing the HoAcAc crystal structure by its recrystallization from crystal phase to the amorphous there is lost of acetylacetonate and water molecules causing the increasing of the homium content. Microspheres were prepared by solvent evaporation, using holmium acetylacetonate (HoAcAc) crystals as the sole ingredient. Microspheres were characterized by using light and scanning electron microscopy, infrared and Raman spectroscopy, differential scanning calorimetry, X-rays difraction, and confocal laser scanning microscopy.

1. INTRODUCTION

The liver cancer affects every year 600,000 patients, the surgical resection is only eligible for a limited number of patients, limiting the 5 years survival rate to only 3-5% of the patients [6,7]. Also, a very common type of metastasis cancer to the liver is the colorectal carcinoma, which affects every year over a million and presents a mortality of half-million of patients yearly. The surgical resection is only eligible for 10% to 20%, and the protocols of the latest chemotherapeutic treatments are associated to a survival of patients with unresectable hepatic metastasis for almost two years.

The radioembolization treatment has shown to have very high response rates, and it is a very good option for cancer liver treatment. Currently microspheres with the beta emitter 90-Y are used to patients with unresectable hepatic malignancies [8,9]. The development of glass

microspheres containing 90-Y has been well described [10] and showed a path toward such brachytherapy. More recently studies with polymers (mainly PLLA) have been reported using 166-Ho as radionuclide[2, 11, 12]. The lack of quantitative imaging for 90Y [10] and its high relatively half-life has lead to the development of microspheres loaded with 166-Ho.

The importance of the holmium high content in the microspheres can relatively be interpreted as follows: a) from a therapeutic standpoint, to achieve an effective use of microspheres loaded with HoAcAc , a high content of holmium is required to yield enough radioactivity with a relatively low amount of microspheres; b) from the viewpoint of the microspheres irradiation, namely, small content of 165-Ho would require longer irradiation times , which would be subjected to undesirable radiation doses in the reactor core [2, 3]. The usual amounts of holmium that are incorporated in the microspheres composed by poly(L-lactic acid) and HoAcAc are 17.0 ± 0.5% (w/w) of holmium, which corresponds to a loading of about 50% of HoAcAc [3]. Different approaches have been investigated to increase this number. One updated approach towards this direction is the microspheres with ultrahigh holmium as matrix using HoAcAc crystals as the sole starting material without the use of biopolymer [4,5]. Likewise, in the search of materials with higher amounts holmium, it has been demonstrated that changes of the HoAcAc crystal structure by its recrystallization from crystal phase to the amorphous promotes the lost of acetylacetonate and water molecules causing the increasing of holmium content. The 166Ho(t1/2=26.8h) is a beta emitter (Emax=1.84 MeV) which can be used to several radiotherapy techniques , and it can be produced in the Research Nuclear Reactor IEA-R1 (located in Sao Paulo, Brazil),. 166-Ho is also a gamma emitter, which can be used to produce images linked to the location of this radioisotope in the human body [2,11,12].

The aim of the present work is to study the polyester, PHB-HV poly(hydroxybutyrate-hydroxyvalerate) for preparation of microparticles with 166-Ho. The PHB is a member of the family of polyhydroxyalkanoates (PHAs) which has attracted much attention as biocompatible and biodegradable thermoplastic with potential application in medical fields. These biopolymers are polyesters of various hydrocarboxylic acids, which are produced by numerous micro-organisms under unfavorable growth conditions in the presence of excess carbon source [13].

The microspheres are produced from the polymer and the Holmium Acetyl Acetonate (HoAcAc), which is a complex with high thermal and mechanic stability, and have a high weight percentage of holmium [14].

2. EXPERIMENTAL

2.1. Materials

All chemical compounds were commercially available and used as received. Chloroform, acetylacetone, holmium(III) chloride hexahydrate, polyvinyl alcohol were provided by Sigma Aldrich.

2.2. Methods

2.2.1 Preparation of the Holmium-acetylacetonate complex (HoAcAc)

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

2.2.2. Recrystallization of Ho-acetylacetonate complex

The HoAcAc were recrystallized by dissolving the required amount of HoAcAc in chloroform, and after evaporation of the solvent.

2.2.3. Preparation of HoAcAc-loaded and unloaded microspheres with PHB-HV

PHB-HV (5% and 8%) and PHB-HV/HoAcAc microspheres were prepared by an emulsion solvent extraction/evaporation technique. In the solvent evaporation method, the required amount of polymer and HoAcAc were dissolved in an organic phase (e.g. chloroform) which was emulsified under stirring with polyvinyl alcohol (PVA) (2 % w/w) solution to form an oil/water emulsion. Stirring was continued for 2h at about 500 rpm, to evaporate the organic phase. The formed microspheres were collected by centrifugation and washed with water, 0.1 mol.L-1 HCl and water, respectively. The microspheres were suspended in water and fractionated according to size using stainless steel sieves of 20 and 50 μm.

2.2.4. Preparation of microspheres with high content Holmium

Microspheres were prepared by an emulsion solvent extraction/evaporation technique. In the solvent evaporation method, the required amount of HoAcAc were dissolved in an organic phase (e.g. chloroform) which was emulsified under stirring with polyvinyl alcohol (PVA) (2% w/w) solution to form an oil/water emulsion. Stirring was continued for 24 h at about 600 rpm to evaporate the organic phase. The formed microspheres were collected by centrifugation and washed with water, 0.1 mol.L-1 HCl and water, respectively. The microspheres were suspended in water and fractioned according to size using stainless steel sieves of 20 and 50 μm.

2.2.5. Irradiations

The samples were irradiated with a neutron flux in a range of (0.9 - 1.1)x1013 n.cm-2s-1 for 1 hour at the IEA-R1 Research Nuclear Reactor located at IPEN/CNEN-SP. After the irradiation the samples were analyzed by Gamma Spectrometry using an HPGe detector from Canberra in order to measure the 166Ho activity and also the level of radionuclidic impurities. The highest specific activities of 166Ho were: 21.3 MBq.g-1 for the ultrahigh holmium sample, 11.5 MBq.g-1 for PHB-HV polymer, and 5.8 MBq.g-1 for PLLA polymer (the same for both viscosities employed).

When a neutron has lost most of its kinetic energy due to thermal motion, it is often called a thermal neutron or a slow neutron. Such neutrons do not have sufficient energy to eject particles from atomic nuclei but can be captured by a nucleus to give an isotope, like the 165-Ho studied here. The isotope is often formed in an excited state and returns to the ground state, emitting the excess energy in the form of one or more quanta of □ radiation. This □radiation can then cause ionization and excitation in the medium and will initiate typical radiation-chemical changes. Hence the effects that come along with the nuclear reactions, as

those brought about by slow neutrons or by radioactive decay are closely related to radiation chemistry. [15,16]. For that reason the effects of ionizing radiation on the polymeric microspheres were also evaluated.
Gamma irradiation technique was performed in a Gamma cell at IPEN/CNEN-SP using a 60Co source which provides 25 and 50 kGy doses.

2.3. Characterization

The thermogravimetric gravimetry curve (TG) was recorded with a Mettler-Toledo TGA / SDTA 851 thermobalance in nitrogen atmosphere, in the range of 25 - 600 °C at 10 °C min^{-1}.

Differential Scanning Calorimeter (DSC) was carried out in an 822 Mettler-Toledo under nitrogen atmosphere at a heating rate of 10°C min^{-1}, in the temperature range of 20 to 100°C. DSC apparatus was calibrated with Indium (m.p 156.61°C; ΔH = 28.54 kJ kg^{-1}). Crystallinity was calculated according to Eq. 1:

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

Where Xc = the crystallization percentage, ΔHf = melting enthalpy of the sample, ΔH_0 = melting enthalpy of the 100% crystalline HoAcAc which is assumed to be 115 (J g^{-1}) [17] and PHB-HV 109 (J g^{-1}) [18].

The SEM images were obtained in a Phillips XL 30 Microscope in magnitude of 5,000 times using samples covered with gold in a Sputter Coater BAL-TEC SCD 050 and a JEOL model JSM-7401 apparatus with an operating voltage of 1.0 kV.

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

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

X-ray diffraction (XRD) analyses were performed using a Rigaku diffractometer model Miniflex II using Cu Kα radiation source (λ = 0.15406 nm). The diffractograms were recorded from2θ = 05° to 50° with a step size of 0.05° and a scan time of 2 s per step.

3. RESULTS AND DISCUSSION

The size distribution obtained by sieving is on the **Table 1.** and we can see that the percentage of microspheres with 20-53μm are relatively high. This size of the microspheres was described has the ideal size to the treatment to liver tumor. [19]

Table 1. Microspheres size distribution.

Microspheres	75μm	53μm	20μm
PHB-HV 5%	7,5%	16,5%	76%
PHB-HV 8%	8%	24%	68%
HoAcAc	13%	27%	60%

The morphology determined by scanning electron micrograph showed spherical particles with smooth surface in the ultra high holmium, and slightly wrinkled in the microspheres of PHB-HV.

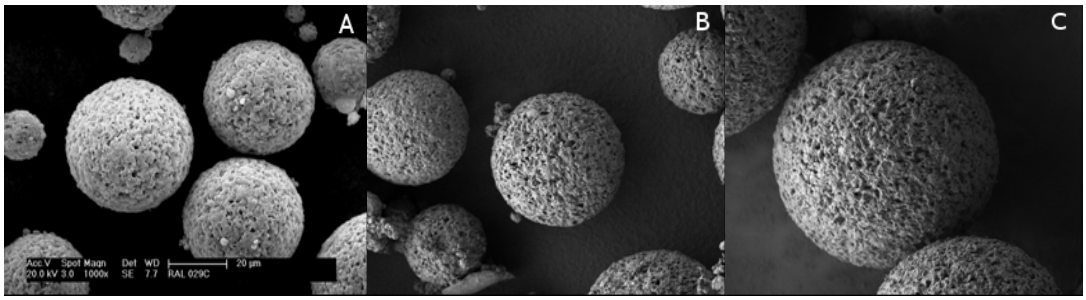

Fig. 1. A scanning electron micrograph of non gamma irradiated microspheres of PHB-HV 5% of HV, B scanning electron micrograph of microspheres gamma irradiated 25kGy and C scanning electron micrograph of irradiated microspheres with 50kGy.

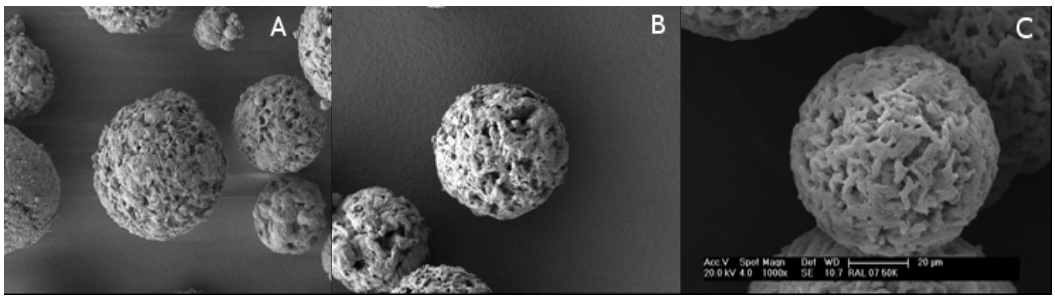

Fig. 2. A scanning electron micrograph of non gamma irradiated microspheres of PHB-HV 8% of HV, B scanning electron micrograph of microspheres gamma irradiated 25kGy and C scanning electron micrograph of irradiated microspheres with 50kGy.

The **Fig. 1.** and **Fig. 2.** showed that the surfaces of the microspheres formed from PHB-HV 5% and 8% of valeric acid are uniformly slightly wrinkled and resist very well to the gamma irradiation.

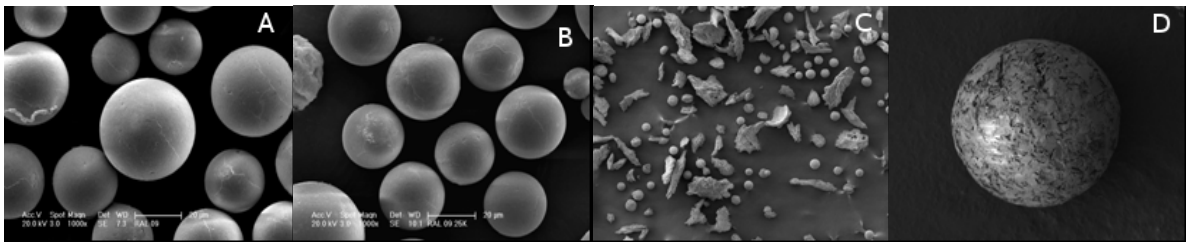

Fig. 3. A scanning electron micrograph of non gamma irradiated microspheres of High Holmium Content, B scanning electron micrograph of gamma irradiated at 25kGy microspheres, C and D scanning electron micrograph of microspheres after gamma irradiation at 50kGy (magnitude 500x and 1000x respectively).

The **Fig. 3.** showed that the microspheres formed only with the HoAcAc are a very smooth surface but not resist to the gamma irradiation of 50kGy.

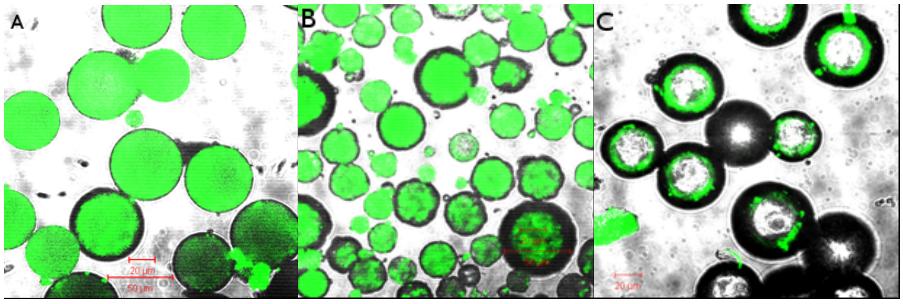

Fig. 4. CLSM images of A PHB-HV 5%, B PHB-HV 8% and C High Holmium Content microspheres.

The emission in 488 nm (green color in the images, Fig. 4b) is characteristic of holmium. We can observe that the microspheres containing only the HoAcAc are hollow and in the microspheres of PHB-HV the holmium are distributed in throughout the microspheres.

In the HoAcAc crystals, holmium is surrounded by three acetylacetonate ligands, and three water molecules [2,15], after recrystallized with chloroform. We can see in the **Fig. 5.** that the crystals are more unstructured. This was explained by a loss of acetylacetonate and water ligands caused by a structural rearrangement of the acetylacetonate ligands around holmium (4).

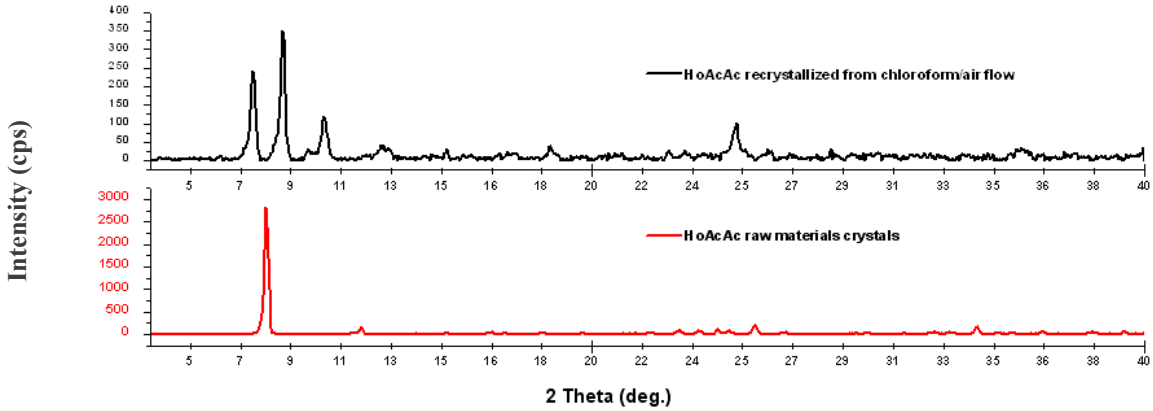

Fig. 5. X-ray scattered intensity as a function of the diffraction angle 2□ X-ray Diffraction of HoAcAc and HoAcAc recrystallized from $HCCl_4$.

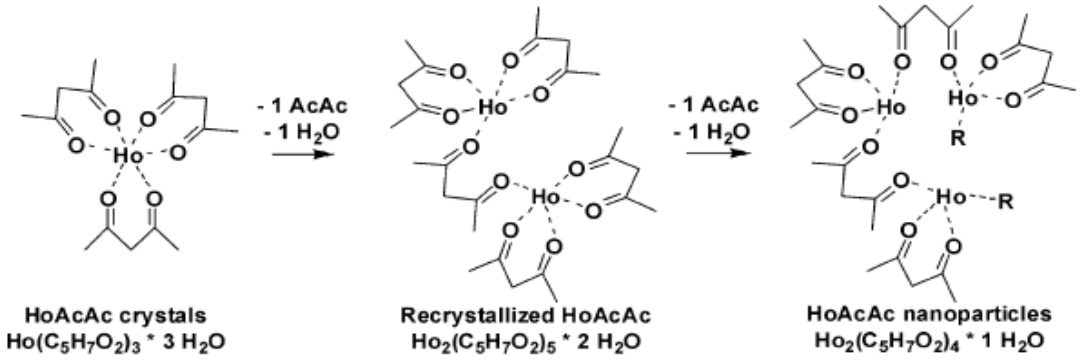

Fig. 6. Schematic representation of recrystallization of HoAcAc and formation of the microspheres. Adapted from Bult e col. 2010 (4)

Fig. 5 and **6** showed that the recrystallization of HoAcAc can increase the amount of holmium in the microspheres. This is very important result for the therapy of radioembolization, since higher concentrations of Ho in the microspheres increase the radiotherapeutic action and reduce the amount of particles used.

The thermal stability of the microspheres was determined by TGA and DSC. For the PHB-HV microspheres there are two decomposition steps showed on **Fig. 7** and **8**. The TGA curve for PHB-HV (5% and 8%) and HoAcAc microspheres showed a substantial weight loss above 200°C, indicating decomposition of HoAcAc. The residual weight indicates the presence of Ho2O3. Doses of 25kGy had no effects on PHB-HV 5% and 8%. In **Fig. 9.** we can see from the TG and DSC curves that the melting temperature of HoAcAc microspheres are 106°C and that they degraded after 175ºC. All the DSC data of microspheres were compiled in Table 1.

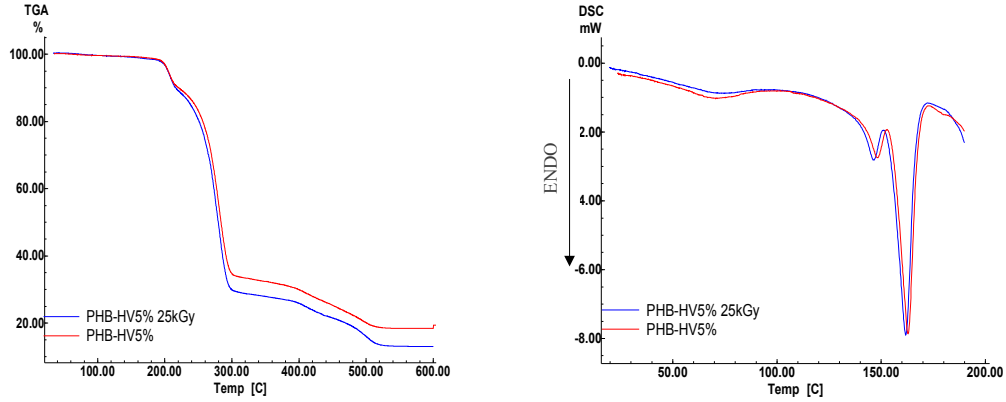

Fig. 7. TGA (A) and DSC (B) curves of non-irradiated microspheres of PHB-HV 5% and microspheres irradiated with gamma 25kGy.

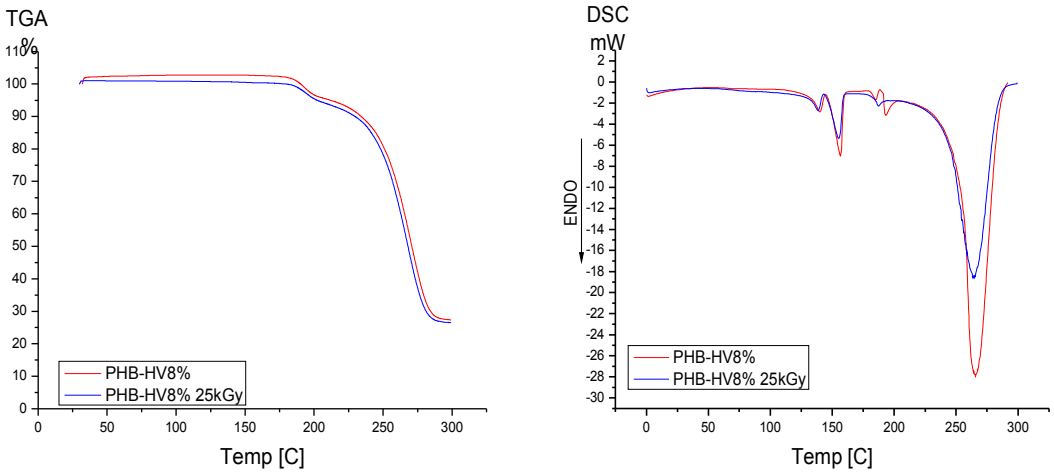

Fig. 8. TGA (A) and DSC (B) curves of non-irradated microspheres of PHB-HV 8% and gamma irradiated with 25kGy.

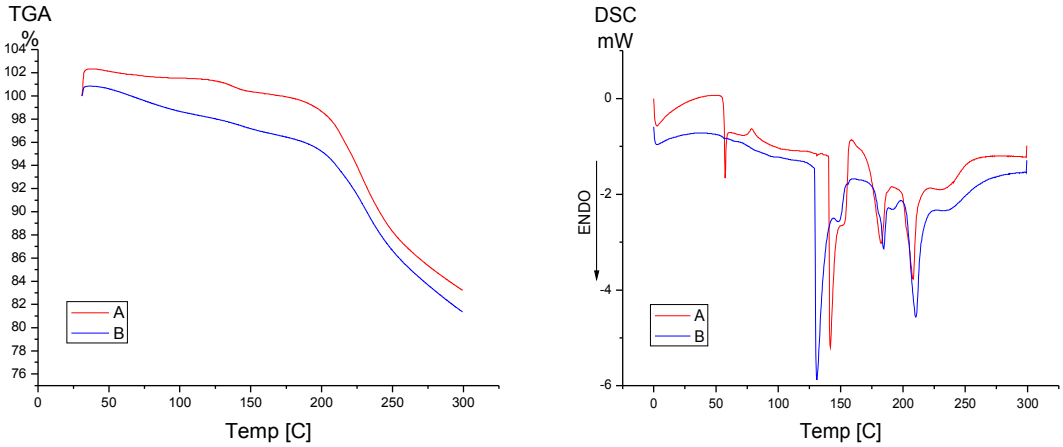

Fig. 9. TGA and DSC of HoAcAc microspheres (A) non-irradiated and (B) gamma irradiated with 25kGy.

Table 2. DSC values for PHB-HV and HoAcAc microspheres non-irradiated and irradiated at 25kGy dose.

Microspheres	**Tm (ºC)**	**ΔHm (J g-1)**	**Xc (%)**
PHB-HV 5%	162.87	38.26	35.10
PHB-HV 5% 25kGy	161.74	38.47	35.29
PHB-HV 8%	156.67	36.92	33.87
PHB-HV 8% 25kGy	155.25	39.16	35.93
HoAcAc	141.6	36.25	31.52
HoAcAc 25kGy	130.91	41.31	35,92

Tm: melting point; ΔHm: melting enthalpy; Xc: degree of crystallinity.

The results of the **Table 2.** showed that the degree of crystallinity increased slightly with the radiation.

No substantial changes in the DRX pattern are noticed in **Fig. 10.** due to the crystallinity of the irradiated microspheres which is in agreement with the SEM that shows that the irradiation had no effect on the microspheres.

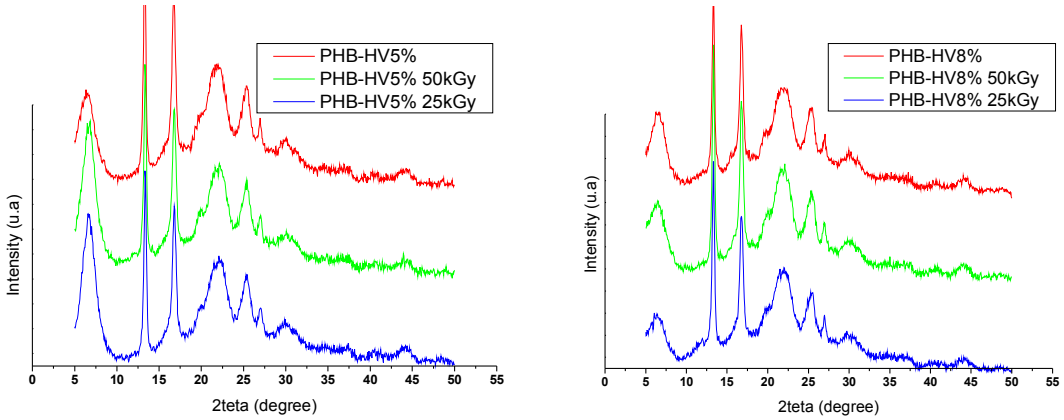

Fig. 10 DRX spectra of PHB-HV 5% and 8% microspheres.

The FTIR spectra in the **Fig. 11.** show the characteristics absorption peaks of PHB-HV5% and HoAcAc microspheres. The bands had not changed with the irradiation, which indicates that the chemical composition had not changed.

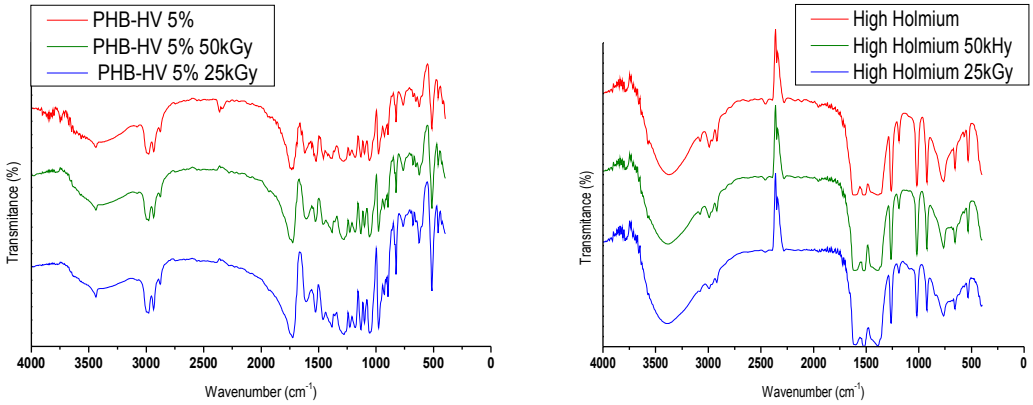

Fig. 11 Infrared spectra of PHB-HV 5% and HoAcAc microspheres non-irradiated and irradiated at 25 and 50 kGy doses.

4. CONCLUSIONS

This paper presents the preliminary data concerning the use of HV5% PHB-HV and HV8% PHB-HV 8% copolymers as possible candidates to replace PLLA as a matrix to produce microspheres for the therapy that uses HoAcAc in internal selective radiotherapy. In addition, a method was adapted to obtain microcapsules with a high content of Holmium, justifying the intrinsic capacity to contain a higher concentration of Holmium per gram in a sphere; they

were the ones that, to date, provided the best income of radioactivity per gram of sample. This result opens up excellent opportunity to use these materials in internal selective radiotherapy.

The morphology and thermal behavior of the microspheres were evaluated. Gamma irradiation at 25 and 50 kGy doses had no effect on the PHB-HV microspheres, which indicates that the chemical composition of the microspheres had not been changed.

Samples were irradiated with a neutron flux and some undesirable contaminants were detected, such as Na-24. New studies must be performed to assess the possible change in structural and chemical properties of these compounds due to the irradiation for potential future clinical use.

ACKNOWLEDGMENTS

We would like to thank Eleosmar Gasparin CQMA/IPEN-CNEN and Yasko Kodama CTR/ IPEN-CNEN for thermal analysis, Celso Vieira CCTM/IPEN-CNEN for SEM images, Elisabeth Somessari, Carlos G. da Silveira CTR/IPEN-CNEN for the samples irradiation, DIRF-GPD/IPEN-CNEN for the neutrons irradiation and Adriana Napoleão Geraldes CQMA/IPEN-CNEN for general analysis.

REFERENCES

1. Renata F. Costa, Mariangela B. M. Azevedo, Nanci Nascimento, Frank F. Sene, José R. Martinelli, João A. Osso **International Nuclear Atlantic Conference - INAC 2009**, ABEN ISBN: 978-85-99141-03-8;
2. J. F.W. Nijsen, B. A. Zonnenberg, J. R.Woittiez, D.W. Rook, I. A. Swildens-vanWoudenberg, P. P. van Rijk, andA.D. van het Schip. Holmium-166 poly lactic acid microspheres applicable for intraarterial radionuclide therapy of hepatic malignancies: effects of preparation and neutron activation techniques. *Eur. J. Nucl. Med*. **26**:699–704 (1999) doi:10.1007/s002590050440.
3. Zielhuis, S. W.; Nijsen, J. F. W.; de Roos, R.; Krijger, G. C.; van Rijk, P. P.; Hennink, W. E.; van het Schip, A. D., "Production of GMP-grade radioactive holmium loaded poly(L-lactic acid) microspheres for clinical application", *International Journal of Pharmaceutics*, **331**: 69-74, 2006.
4. Bult, W.; Varkevisser, R.; Soulimani, F.; Seevinck, P.R.; Leeuw, H de.; Bakker, C.J.G.; Luijten, P.R.; van het Schip, A.D.; Hennink, W.E.; Nijsen, J.F.W.; "Holmium Nanoparticles: Preparation and In Vitro Characterization of a New Device for Radioablation of Solid Malignancies", *Pharmaceutical Research*, **27**: 2205-2212, 2010.
5. 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., "Microspheres with Ultrahigh Holmium Content for Radioablation of Malignancies", *Pharmaceutical Research*, **26**: 1371-1378, 2009.
6. F. Kamangar, G. M. Dores, and W. F. Anderson. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. *J. Clin. Oncol*. **24**:2137–2150 (2006) doi:10.1200/JCO.2005.05.2308.

7. D. M. Parkin, F. Bray, J. Ferlay, and P. Pisani. Global cancer statistics, 2002. *Ca Cancer J. Clin*. **55**:74–108 (2005) doi:10.3322/canjclin.55.2.74.
8. M. A. D. Vente, M. Wondergem, I. van der Tweel, M. A. A. J. van den Bosch, B. A. Zonnenberg, M. G. E. H. Lam, A. D. van het Schip, and J. F. W. Nijsen. Yttrium-90 microsphere radioembolization for the treatment of liver malignancies: a structured meta-analysis. *Eur. Radiol*. 2008 doi:10.1007/s00330-008-1211-7.
9. G. van Hazel, A. Blackwell, J. Anderson, D. Price, P. Moroz, G. Bower, G. Cardaci, and B. Gray. Randomised phase 2 trial of SIR-Spheres plus fluorouracil/leucovorin chemotherapy versus fluorouracil/leucovorin chemotherapy alone in advanced colorectal cancer. *J. Surg. Oncol*. **88**:78–85 (2004) doi:10.1002/jso.20141.
10. T. Gupta, S. Virmani, T. M. Neidt, B. Szolc-Kowalska, K. T. Sato, R. K. Ryu, R. J. Lewandowski, V. L. Gates, G. E. Woloschak, R. Salem, R. A. Omary, and A. C. Larson. MR Tracking of Ironlabeled Glass Radioembolization Microspheres during Transcatheter Delivery to Rabbit VX2 Liver Tumors: Feasibility Study. *Radiology*. **249(3):**845–854 (2008).
11. S.W. Zielhuis, J. F.W. Nijsen, J. H. Seppenwoolde, C. J. G. Bakker, G. C.Krijger, H. F. Dullens, B.A. Zonnenberg, P. P. van Rijk,W. E. Hennink, and A. D. van het Schip. Long-term toxicity of holmiumloaded poly(L-lactic acid) microspheres in rats. *Biomaterials*. 28:4591–4599 (2007) doi:10.1016/j.biomaterials.2007.07.012.
12. M. A. Vente, J. F. Nijsen, T. C. de Wit, J. H. Seppenwoolde, G. C. Krijger, P. R. Seevinck, A. Huisman, B. A. Zonnenberg, T. S. G. A. M. Van den Ingh, and A. D. van het Schip. Clinical effects of transcatheter hepatic arterial embolization with holmium-166 poly(L: -lactic acid) microspheres in healthy pigs. *Eur. J. Nucl. Med. Mol. Imaging*. **35**:1259–1271 (2008) doi:10.1007/s00259-008-0747-8.
13. Lai, S.-M., Don, T.-M., Huang, Y.-C., 2005. Preparation and properties of biodegradable thermoplastic starch/poly (hydroxybutyrate) blends. *Journal of Applied Polymer Science* **100**, 2371–2379.
14. Mumper, J. M.; Jay, M., "Formation and Stability of Lanthanide Complexes and Their Encapsulation into Polymeric Microspheres", *The Journal of Physical Chemistry*, **96**: 8626-8631, 1992B.
15. A.R. Denaro, G.G. Jayson, *Fundamentals of Radiation Chemistry*, Butterworth, London, England (1972).
16. J.W.T. Spinks, R.J. Woods, *An Introduction to Radiation Chemistry*, Wiley, New York, USA (1990).
17. H. Kooijman, F. Nijsen, A. L. Spek, and A. D. van het Schip. 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 dehydrate. *Acta Crystallogr C*. **56**:156–158 (2000) doi:10.1107/S0108270199013566.
18. Scandola M, Focarete ML, Adamus G, Sikorska W, Baranowska I, Swierczek S, Gnatowski M, Kowalozuk M, Jedlinski Z. Polymer blends of natural poly(3-hydroxybutyrate-co-3-hydroxyvalerate and a synthetic atactic poly(3-hydroxybutyrate) – Characterisation and biodegradation studies. *Macromolecules* 1997;30:2568–2574.
19. S. W. Zielhuis, J. F. W. Nijsen, R. Figueiredo, B. Feddes, A. M. Vredenberg, A. D. Schip, W. E. Hennink, "Surface characteristics of holmium-loaded poly(L-lactic acid) micro-spheres", *Biomaterials*, **26**, pp. 925-932 (2005).