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PRODUCTION OF MICROSPHERES LABELED WITH HOLMIUM-166 FOR LIVER CANCER THERAPY: THE PRELIMINARY EXPERIENCE AT IPEN-CNEN/SP

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ABSTRACT

Microspheres labeled with therapeutic radionuclides for malignances of liver are widely used in many countries. The internal radionuclide therapy uses a permanently implanted device, such as Therasphere® or SIR-Spheres®, or a biodegradable device that provides structural support for the radionuclide of choice and causes the tumor reduction. Three different types of material supports have been investigated, i.e., biodegradable polymer-based, glass-based and resin-based microspheres. Nowadays there is a project concerning the labeling of these 3 materials with ^{166}Ho being developed at IPEN-CNEN/SP and coordinated by the Radiopharmacy Directory. ^{166}Ho($t_{1/2}$=26.8h) is a beta minus emitter (E_{max}=1.84 MeV), with right properties for radiotherapy and can be produced with the low power Brazilian Nuclear Reactor IEA-R1m. The aim of this work is to describe the stage of development of this project. The initial experience used resin-based microspheres, a cation exchange resin labeled with ^{166}Ho, it showed the essential characteristics for liver therapy. Preliminary results of the preparation of glass-based microspheres labeled with ^{165}Ho showed that 5% of Ho_2O_3 was incorporated in an aluminosilicate glass, through the process of spheronization by flame, which produced spherical microspheres with 20-40μm particle size. The preparation of biodegradable material, polymer-based microspheres, is in its initial stage and the objective is to prepare and label with ^{165}Ho different polymer-based microspheres. These combined efforts have been done to offer a national radiotherapeutic product for the the Brazilian nuclear medicine community at fair value and also to offer a viable possibility of treatment for patients affected by liver malignances.

1. INTRODUCTION

The global incidence of hepatocellular carcinoma is high (more than 600.000 cases per year) and still increasing, even though liver metastases have a greater incidence, originating from other tumors in other organs and drained by the portal vein. Commonly, the metastases are derived from colon rectal cancer, with approximately one million new cases per year [1]. In both cases, the only curative treatment is the surgical resection, depending on the location of the tumor [2]. Unfortunately, most patients are not candidates for this type of treatment. Novel options for this type of patients are required.

In recent years, micro and nano spheres have been extensively used for therapeutic purposes in several varieties of diseases. These new materials are designed to maximize the bioavailability of conventional drugs with reduced side effects. The particulate material used

as vehicle may be based on synthetic or natural biodegradable polymers, glass, ceramics, etc [3].

Local radionuclide therapy using radioactive microspheres is a promising therapy for non-operable group of patients suffering from liver malignances [4]. Two ^{90}Y microspheres products are currently commerciallly available and in clinical use: glass-based Therasphere® (MDS Nordion, Canada) and resin-based microsphres SIR-Sphere® (SIRteX, Medical Ltd., Australia).

The internal radionuclide therapy consists in placing the radioactive material near the tumor, which can deliver high doses directly to malignant cells, sparing healthy tissues from toxic effects. The local radiotherapy can be applied to any tumor that is accessible by a vascular catheter [5]. This technique is particularly applicable in highly vascularized tumors or those tumors, which have a dominant vascular artery to fill it, such as the liver. The liver, due to its anatomical position, receives all the blood drained from the digestive tract, becoming a propitious organ to the establishment of cancer [6].

For internal radionuclide therapy in the liver, the size of the microspheres is considered ideal between 20-50 μm to reach the arterioles of the liver completely. A catheter in the hepatic artery introduces the spheres after the administration of vasoactive drugs. The microspheres can be made of biodegradable polymer, ion exchange resins and ceramic (glass) material [4].

This work chosen radionuclide is ^{166}Ho that is produced by the nuclear reaction ^{165}Ho (n,γ) ^{166}Ho. Holmium-165 has a natural abundance of 100% and neutron capture cross-section of 64 barns. ^{166}Ho is a β^- emitter (E_{max}=1.84 MeV) with maximum soft-tissue range of 8.4mm and a half-life of 26.8 hours. In addition, ^{166}Ho also emits gamma photons (0.081 MeV) that can be imaged with a gamma camera, but are of low enough photon yield (5.4%) to result in limited absorbed radiation dose to surrounding tissue.

The production of ^{166}Ho is feasible in the IEA-R1 Reactor at IPEN-CNEN/SP, because it does not need high power and high neutron fluxes. In addition, holmium is a highly paramagnetic element and suitable for (quantitative) MRI [7].

Nowadays there is a project concerning the labeling of 3 materials (glass, resin and biodegradabel polymer) with ^{166}Ho being developed at IPEN-CNEN/SP and coordinated by the Radiopharmacy Directory. The aim of this work is to describe the stage of development of this project.

2. MATERIALS AND METHODS

2.1. Glass microspheres

Holmium doped- aluminum silicate glasses were prepared by mixing Al_2O_3, SiO_2 , MgO, and Ho_2O_3 using a silica mortar and pestle during 20 min. Other oxides were also present in the composition (Lu_2O_3, K_2O and NbO_5). The material was then melted in an alumina crucible at 1600^oC in a crucible type electric furnace. The liquid was held at this temperature for 2 hours and stirred each 30 min by using a silica rod to assure the liquid homogenization and gas release. The liquid was then cast in a stainless steel mold at room temperature, and ground by

using an alumina ball mill. Stainless steel sieves were used to obtain particles in the size range of 20 -50 μm.

The glass composition was selected based on the phase diagram of Fig. 1. Holmium oxide was added up to 5 ^{wt}/o to the selected glass composition.

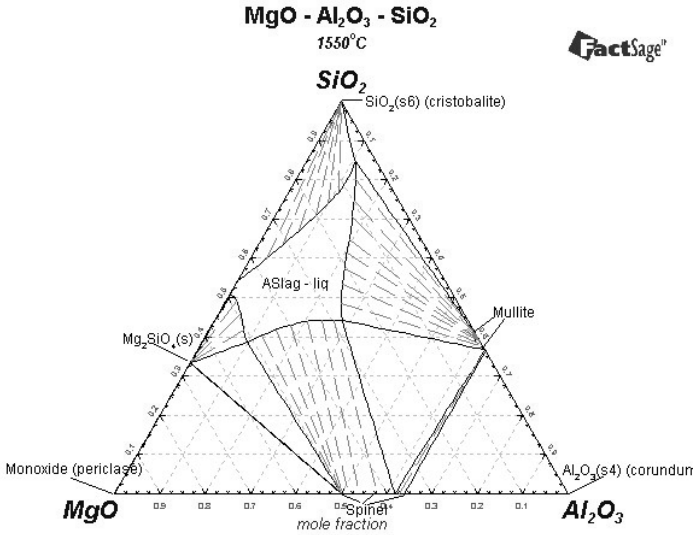

Figure 1 –MgO – Al_2O_3- SiO_2 phase at 1550^{o}C- Courtesy of FactSage

Glass particles with irregular shapes were transformed to microspheres by using a process known as 'spheronization by flame'. The glass particles were transformed in microspheres by exposing them to a hot flame. A torch burning a mixture of oxygen and petrol liquefied gas was used for this purpose. The viscosity of the glass particles gets lower and their shape becomes spherical. The microspheres were collected in a metal cylinder. More details can be found elsewhere [8,9].

The glass microspheres were characterized by optical microscopy and by the energy dispersive spectrometry of X-rays fluorescence (EDX Shimadzu model 720). The glass microspheres were also irradiated in a Eppendorf® vial (1mL) inside sealed aluminum containers (7 cm height, 2.1 cm diameter) in selected positions of the nuclear reactor IEA-R1 at IPEN/CNEN-SP. The medium neutron flux was 1.0 x10^{13}n.s^{-1}.cm^{-2} for 1 hour.

2.2. Resin microspheres

The cation exchange resin of choice was the SP Sepharose High Performance. Samples of high purity Ho_2O_3 were irradiated inside sealed aluminum containers (7 cm height, 2.1 cm diameter) in selected positions of the nuclear reactor IEA-R1 at IPEN/CNEN-SP. The neutron flux was 1.0x10^{13} n.s^{-1}.cm^{-2} for 1 hour. The dissolution of Ho_2O_3 occurred in acid medium

(1N HCl) and the resin maintains its properties in pH 4 to 10, therefore the pH of the target solution was adjusted to neutral. A small column containing the resin was assembled and the ^{166}Ho was percolated though the resin (labeling process). The stability tests were performed with 0.9% saline.

The particle size distribution of Sepharose used in this work was determined using a laser optical equipment (CILAS, 1064).

2.3. Polymer microspheres

Nijsen *et al*. [10] loaded poly (L-lactic) acid microspheres with holmium, a cold method, which involves two steps. The first step was the preparation of Ho-acetylacetonate. Acetylacetonate (18.5mL) was dissolved in water (108mL). The pH range was 3.5-4.5. Ammonium hydroxide was added to the stirring acetylacetonate solution until pH 8.5. Holmium chloride hexahydrated (10g in 30mL water) was stirred into this solution and Ho-AcAc crystals were allowed to form at room temperature for 24 hours.

The second step was the synthesis of holmium loaded microspheres. Ho-AcAc complex (1g) and acid lactic (0.5mL) were added to continuously stirred chloroform (12.6mL). Polyvinyl alcohol (2g) was dissolved in water (100mL) at 40ºC. Stirred (500rpm) the solution was stirred (500rpm) until the chloroform was evaporated. The formed microsperes were washed sequentially with water, 0.1N HCl and water.

3. RESULTS AND DISCUSSION

3.1. Glass Microspheres

Fig.2 shows the micrograph of microspheres observed by an optical microscope. The original glass particles with irregular shapes become now spheres with crack free surfaces and no apparent porosity.

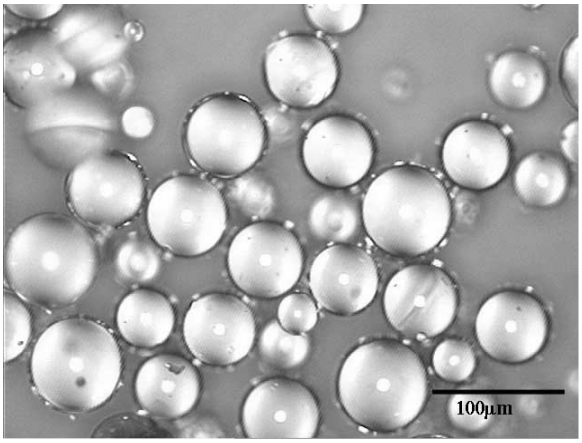

Figure 2 – View of microspheres observed by an optical microscope.

The X-rays diffraction patterns have shown halos which are characteristic of amorphous phases and no indication of diffraction peaks that could be related to crystalline phases.
The final composition of the glass microspheres were determined by the energy dispersive spectrometry of X-rays fluorescence (EDX Shimadzu model 720), which indicated that the amount of Ho_2O_3 was in the range of 4.9 to 5.4 $^{wt}/_{o}$, which is very close to the nominal composition. Besides Mg, Si, and Al, other elements were also detected such as Fe, and Ti, in minor amounts.

The activity of ^{166}Ho obtained at the end of the irradiation of Ho-glass (53mg) with the neutron flux of $1.1x10^{13}n.s^{-1}.cm^{-2}$ during 1 hour was 891 MBq (24 mCi).

The main impurities of the Ho-glass were identified by gamma spectroscopy (HPGe), performed three days after irradiation, as shown the Table 3.

Table 3. Impurities of glass microspheres

Radionuclide	Initial Activity (mCi/g)	Impurities (%)	Half life	Eγ (keV)
^{177}Lu	0.74 ($2.73x10^{7}$Bq)	21.63	6.7 days	136.7
^{177m}Lu	$9.20x10^{-3}$($3.40x10^{5}$Bq)	0.26	155 days	129.3
^{24}Na	$9.48x10^{-4}$($3.51x10^{4}$Bq)	0.02	15 hours	1368.0

The impurity in higher concentration was ^{177}Lu, produced by neutron activation of Lu present in the glass microspheres. The other impurity, ^{24}Na, came from the neutron activation of Al, also present in the glass preparation. The level of impurities is high, but it can be reduced by using the microspheres just after the irradiation.

3.2. Resin Microspheres

The activity of ^{166}Ho obtained at the end of the irradiation of Ho_2O_3 (100 mg) with the neutron flux of $1.1x10^{13}n.s^{-1}.cm^{-2}$ during 1 hour was 60300 MBq (1630mCi). The results of the incorporation of ^{166}Ho in the resin and its stability can be seen in Table 4.

Table 4. Labelling of Sepharose with ^{166}Ho

Resin	Sepharose
Volume	10mL
Mass of $^{166}Ho_2O_3$ (mg)	100
% ^{166}Ho resin	97.2
% ^{166}Ho loading waste	1.0
% ^{166}Ho Saline 0.9%elutions	1.8

The result of the labeling was excellent. It is possible to label 10 mL of resin with 100mg of Ho_2O_3 or 87mg of Ho.

The appropriate particle size of the resin microspheres (20-50μm) is an important aspect, because bigger particles can sediment on the capillary and smaller can migrate to another organ [4]. Figure 3 shows the results of the particle size determination of the Sepharose.

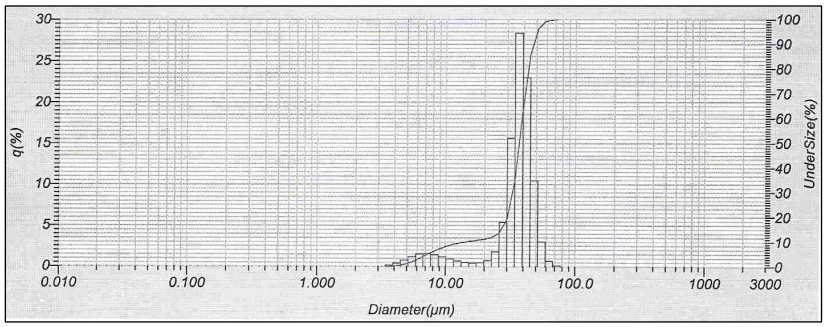

Figure 3. Nominal size distribution of Sepharose

The medium particle size is 37μm, considered ideal for selective delivery of radioisotopes to liver tumor

3.3. Polymer Microspheres

The preparation of crystals of Ho-AcAc was very effective. The separation of the crystals was carried out by the centrifuge and / or Millipore filter 5μm and 0.22 micrometers. The drying was done in the furnace (30º-60º), unlike Nijsen et al. [10] where the Ho-AcAc was dried under nitrogen gas. The crystals dried at 60 ° C and did not lost their integrity during the 48 hours that remained in the furnace. All these changes resulted in an average yield of (39.0 ± 11.8)%. The results of yield of the reaction obtained experimentally were higher than those obtained by Mumper et al. [11] (27.7% ± 4.9)%.)%.

The preparation of acid lactic-based microspheres was not successful, but the first step of the preparation was very effective. The preliminary stage of this project is to prepare different polymer-based microspheres, such as lactic acid, glycolic acid and poly(3-hydroxybutyrate) polymers and check which of these materials has the best performance when labeled with ^{166}Ho. Polymer- based microspheres have many advantages, in particular their near-plasma density, biodegradability and biocompatibility [4].

3.4. Irradiation of Holmium Oxide and Glass Microsphere

Table 5 shows the results of simulations performed with different conditions of irradiation in the reactor.

Table 5. Activity of ^{166}Ho Produced at IEA-R1 Nuclear Reactor

Irradiation Time (h)	Neutron Flux ($n.s^{-1}.cm^{-2}$)	Specific Activity Ho_2O_3(Ci/g)	Specific Activity Glass microsphres (Ci/g)	Note
1	$1.1x10^{13}$	0.41 (15.17GBq/g)	0.02 (0.89GBq/g)	Experimental Value
1	$4.0x10^{13}$	1.49 (55.13GBq/g)	0.08 (3.24 GBq/g)	Best neutron flux in the present
1	7.0×10^{13}	2.60 (96.20GBq/g)	0,15 (5.70GBq/g)	Highest neutron flux in the future*
60	4.0×10^{13}	48.18 (178GBq/g)	2.84 ($1.05 x10^2$GBq/g)	Best conditions in the present
120	4.0×10^{13}	58.99 (218GBq/g)	3.47 ($1.28 x10^2$GBq/g)	Increasing the operation time
120	7.0×10^{13}	102.93 (380GBq/g)	6.08 ($2.24x10^2$ GBq/g)	increasing the neutron flux and the operation time*

* If the nuclear reactor operates at 5MW

Although the IEA-R1 nuclear reactor do not operate in the maximal power (5MW), it is possible to produce sufficient activity of ^{166}Ho for radiotherapeutic dose (5.8GBq) [12] for patients in both ^{166}Ho samples, using the best conditions of the reactor in the current days (60 hours, neutron flux of $4.0x10^{13}n.s^{-1}cm^{-2}$).

According to studies by Turner *et al*. [12], the standard dose of microspheres labeled with ^{166}Ho for use in humans is 5.8 GBq (157mCi). According to this work, about 0.8 grams of Ho (^{166}Ho) is retained per gram of resin. Assuming the highest specific activity obtained (Table 5) in the current conditions of the Nuclear reactor IEA-R1, could label 10mL of resin with 152Ci (~5600GBq) of ^{166}Ho, more than enough for therapeutical applications.

3. CONCLUSION

This work showed the results so far of the project aiming the preparation of microspheres labeled with ^{166}Ho at IPEN-CNEN/SP. The glass and resin based microspheres have already good and promising results and the polymer based is in its initial stage of development.

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