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COATINGS OF NANOPARTICLES APPLIED TO BRACHYTHERAPY TREATMENTS

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ABSTRACT

Brachytherapy is a treatment for cancer in which the radiation is placed close or in contact with the region to be treated saving the surrounding healthy tissues. Nanotechnology is the science that studies the properties of nanometric materials. Nanobrachytherapy in a new field that unites the advantages of brachytherapy with the small size in the nanoparticle, resulting in an even less invasive treatment. In view of the synthesis of the nanoparticles and their use, there is a fundamental role that is made by the coatings, which not only have the function of avoiding the aggregation of particles, but also stabilize and control their functional properties. Among the range of coatings, the most outstanding are polyethylene glycol (PEG) and gum arabica (GA). PEG improves the surface properties of nanoparticles and presents high stability under biomedical conditions. After the synthesis of gold nanoparticles was developed, PEG and gum arabica were successfully incorporated into the surface. In a vial of pyrex, 1 ml of coating agent and 1 ml of nanoparticles was left under gentle shaking for 2 hours. Incorporation was confirmed by DLS and HRTEM. GA requires further study.

1. INTRODUCTION

Cancer is characterized by anomalous growth of cells in organism impairing the functioning of the body.[1] When discovered in early stages, the disease can be treated using less invasive methods with better or equal results when compared with traditional procedures, such as chemotherapy, radiotherapy, and surgery.[2] The simpler approach is highly desirable especially because the main side effects are pain, abbreviation of life and death. Nowadays, cancer is responsible for 8,2 million deaths in worldwide. [2]

A type non-invasive treatment is brachytherapy. This treatment uses radiation in close contact or inside the tumor.[3] It can be classified as a permanent or temporary implant. In permanent implants, the dose is released during the whole radioactive decay. Temporary implants the dose is calculated in function of treatment time and number of sessions.[3] The main advantage of brachytherapy is the direct delivery of radiation into the target, sparing the healthy surround tissues and, consequently, reduces the amount of side effects.[3, 4]

For example, the size of the seed used in prostate brachytherapy is in millimetric scale. They are inserted in the prostate 3-5 at a time through a needle. Since around 100 seed are inserted,

the needle punctures the peritoneum approximatively 25 causing pain and discomfort to patient.

With the size issue in mind, the IPEN's brachytherapy sources production group is using its experience combined with nanotechnology. The nanometric scale have unique properties and promotes alteration in physical, chemical and biological aspects of the atoms/ molecules used.[5] The properties of materials, such as melting point, electrical conductivity, magnetic permeability, and chemical reactivity, are size-dependent (quantum effects rule).[6] By synthetizing a radioactive nanoparticle, a nanosource is created and can be used to treat cancer in a direct and easy way.

2. OBJECTIVES

For the correct synthesis of nanoparticles coatings are necessary to avoiding the aggregation of particles, stabilize, and control their functional properties. A advantage of coating on nanoparticle is the capacity to add molecules of interest, such as antibiotics and anti-inflammatories. Among the range of coatings, polyethylene glycol (PEG) and gum Arabic are largely used. PEG improves the surface properties, high stability under biomedical conditions and, have a positive affinity with blood plasma proteins. Nanoparticles coated with gum Arabic have their size controlled, which facilitates their penetration into the vasculature. This paper aims to developed and analyze coating reactions in an already developed gold nanoparticle synthesis.

3. BACKGROUND

Several papers report the use of PEG and GA as coating agents. But only two papers presented the methodology applied to a radioactive nanoparticle.

The first paper published in 2009 at Nanomedicine Journal entitled "Radioactive gold nanoparticles in cancer therapy: therapeutic efficacy studies of GA-^{198}AuNP nanoconstruct in prostate tumor–bearing mice" by Chanda et al [7]. The group, from the radiology department of Missouri University, presented a methodology to nanoparticle synthesis of Au198 coated by gum Arabic. The authors affirm that their new method is better than classical which consists in a reduction of Au^{3+} to $AuCl_4^-$ with NaBH4 or citrate, reagents with high toxicity.

The new methodology have a new reducing agent, THPA, a trimeric alanine with phosphines. The time of reaction is 5 minutes, but the THPAL synthesis is complicated and very expensive. $^{198}AuCl_4^-$ was activated in a nuclear reactor with a flow of $8x10^{13}$n /cm^2/s in HCl solution. After activation, 50-100 μL of $H^{198}AuCl_4$ is added with 6 mL of gum arabica along with 20 μL / 0.0337 THPAL for each mL of solution. Figure 1 shows the scheme and the final product.

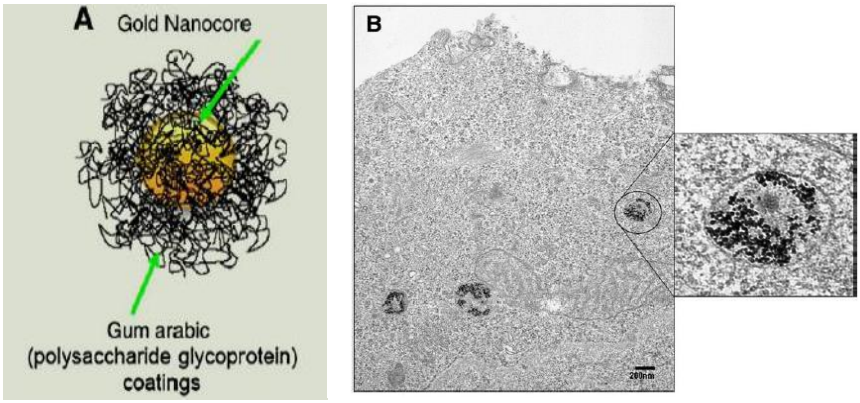

Figure 1: a) Scheme of nanoparticle by Chanda et al. b) Image of nanoparticle on tissue.

The analysis of the material, without the radioactive nucleus, was performed obtaining the following results:

- Mean diameter (without gum arabica coating): 7 ± 3 nm;
- UV-VIS Absorption: 540 nm;
- Average diameter (with gum arabica coating): 85 nm;
- Zeta potential: -24.5 ± 1.5 mV

In-vitro tests obtained the following results:

- Stable
- Without aggregation or decomposition

Hemocompatibility test:

- Without hemolysis

Platelet aggregation:

- Does not cause aggregation

In-vivo tests obtained the following results:

- Nanoparticles with 408 μCi were injected directly into the cancer (PC3 prostate cancer cells) and remained for three weeks. After analysis, the size of the cancer diminished up to 82%.
- After two weeks, 19.9% of the dose injected was found in the tumor, 0.91% in the liver, 0.13% in the kidney, 0.09% in the intestine, 18.5% in the carcass;
- The counts of red blood cells, platelets, lymphocytes and antibodies remained the same as the healthy animal.

The second paper presented 2015 at Journal of Materials Chemistry entitled "Paper Rapid, one-pot procedure to synthesize ^{103}Pd:Pd@Au nanoparticles en route for radiosensitisation and radiotherapeutic applications" [8] by Dr. Marc-André Fortin of Laval University.

The methodology starts from a radioactive precursor: 1mL of $^{103}PdCl_2$ (700mCi) was mixed with 500mL of H_2PdCl_4. In another tube, 200mL of DMSA was mixed with 100mL of Ascorbic Acid (AA). The solution changed color from yellow to brown (classic color change in nanoparticles) in 5 min. After this process, 24 mL of ^{103}Pd:Pd NP's and 12.5 mL of nanopure water with 0.8 mL of $HAuCl_4$ (0.1 M) were mixed. Two successive additions of AA (0.1M) 0.64 mL and 0.4 mL were performed.

After coating with gold, the nanoparticle was again coated with polyethylene glycol (PEG) 1,875 mL at 10 mg / mL for 2 hours. The summary of the synthesis can be seen in figure 2.

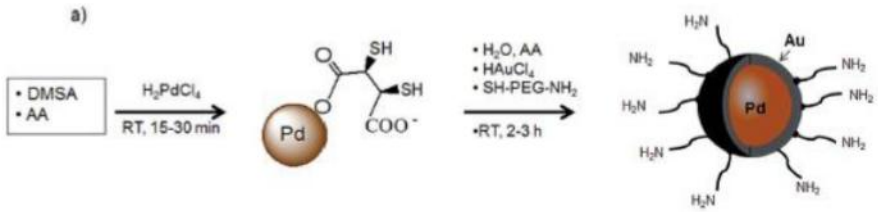

Figure 2: PEG -AuPd^{0}NP synthesis

The analysis of the material, without the radioactive nucleus, was performed obtaining the following results:

• UV results of the material before (a) and after (b) Pd^{0}NP of the synthesis reaction (figure 3);

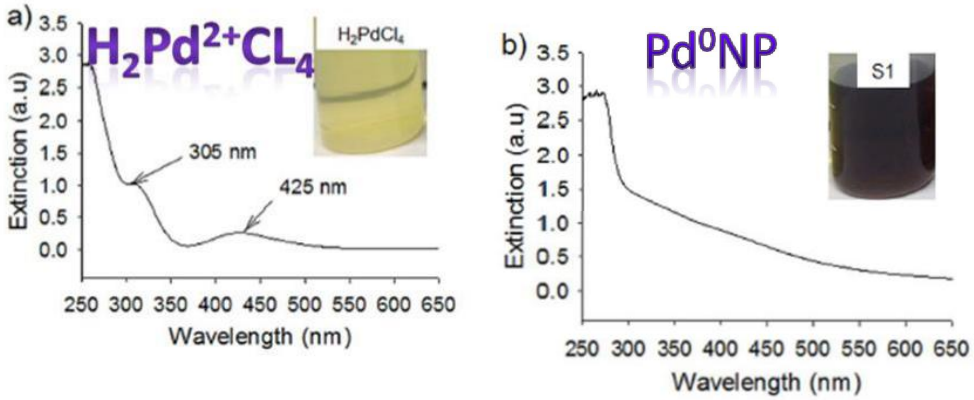

Figure 3: UV before and after of reaction

• TEM – Transmission Electronic Microscopy (Pd^{0}NP) and (PEG -AuPd^{0}NP) (Figure 4)

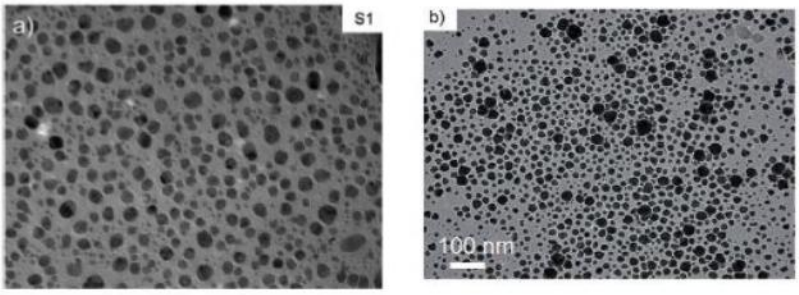

Figure 4: a) TEM of nanoparticles b) TEM of nanoparticles coated with PEG

• DLS - Dynamic Light Scattering: 9,3 nm to Pd^{0}NP and 20,2 nm to PEG -AuPd^{0}NP;

Cell proliferation analysis with the radioactive nucleus was performed. Nanoparticles were placed in 154 nm saline and NaCl solution for 7 days. The viability of PC3 cells was not affected by the presence of nano particles.

4. METHODOLOGY

Nanoparticles developed by Souza et. al. (will be published at Pannano2017) was prepared and used in all subsequent reactions. Then, 1 mL of NP solution was mixed with 1 mL of 1M coating agent solution in a 2 mL borosilicate glass vial. The vial was placed in an agitator at mild and mixed for two hours. PEG was obtained from Laysan Bio (5000 Mw). GA were purchased from Labsynth. All reactants were used without any further purification.

DLS and TEM were performed before and after the coating reaction.

DLS (Dynamic Light Scattering) is a method that uses a monochromatic and coherent laser that focuses on nanoparticles spreading light in all directions (Rayleigh scattering). Spread fluctuates due to Brownian motion (random motion of particles resulting from collision with the media particles) resulting in constructive or destructive interference. This information allows the quantification of the flotation resulting in the particle size distribution profile. [9] The particle size was measured by Nano S from Malvern at room temperature. A disposable plastic cuvette was used for each analysis.

TEM (Transmission Electron Microscopy) uses a beam of electrons with small wavelength to interact with the sample to then form an image. [9] Images were acquired in a FEI Tectai Spirit Biotwin operating at 120 kV. Drops of NP's were deposited in a copper grid and allowed to air dry.

5. RESULTS AND DISCUSSION

The coating reaction was uncomplicated and reproducible. Fine tuning of the different steps will be perfected in the future. The gold nanoparticle synthetized by the group developed presented a wine-red color indicating that all gold salts were consumed.

Figure 5-7 present DLS result for the uncoated AuNP's, PEG-AuNP's, and GA-AuNP's in number and intensity. A growth is observed indicating that the coating reactions were successful. Similar results were achieved for PEG and GA.

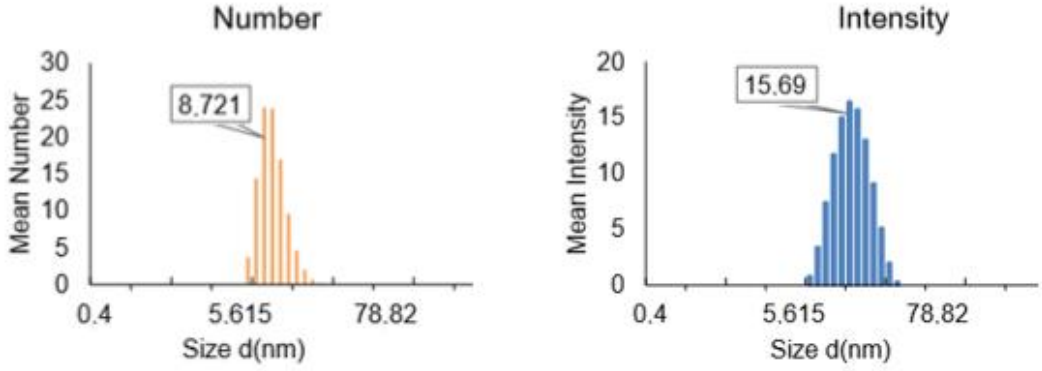

Figure 5: AuNP's DLS results in Number and Intensity. High peak and value on the graph.

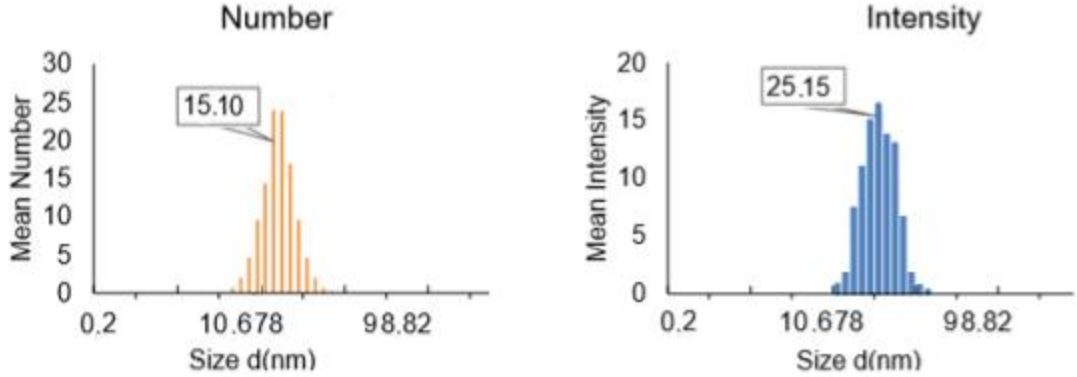

Figure 6: PEG-AuNP's DLS results in Number and Intensity. High peak and value on the graph.

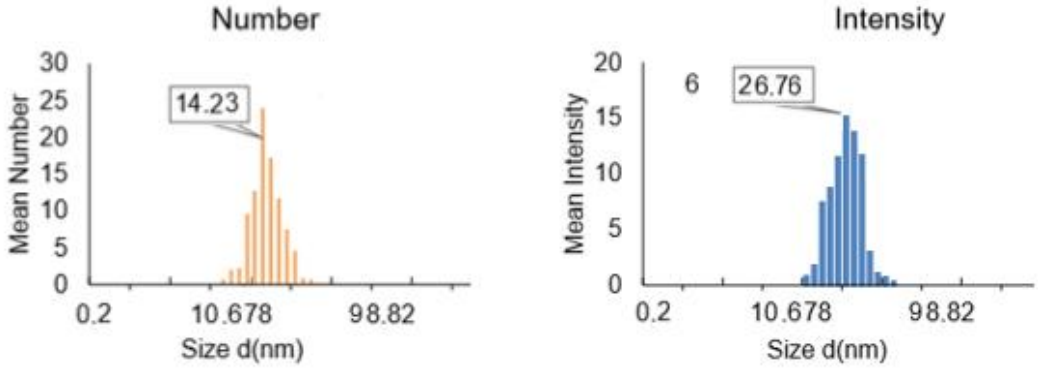

Figure 7: GA-AuNP's DLS results in Number and Intensity. High peak and value on the graph.

TEM images are presented in figures 8-10.

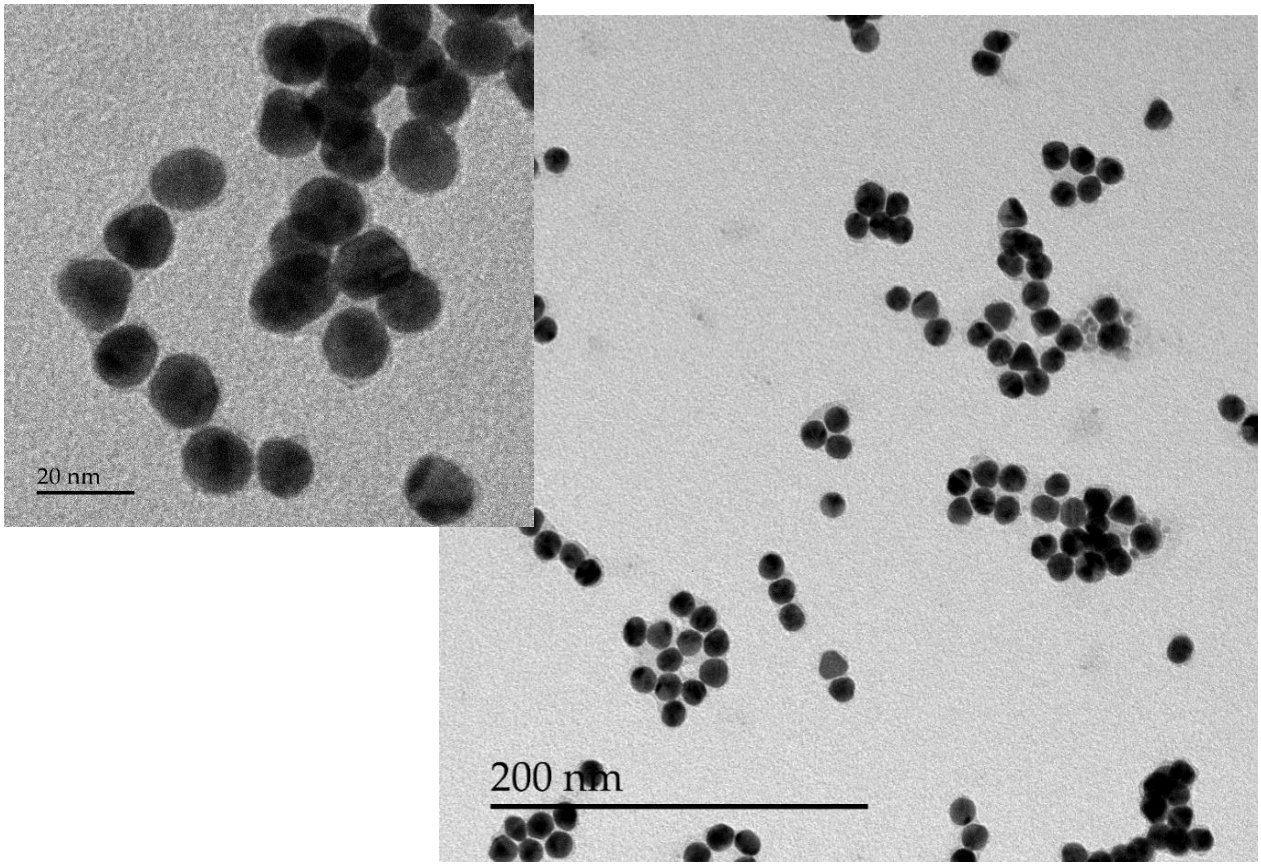

Figure 8: Uncoated AuNP TEM images.

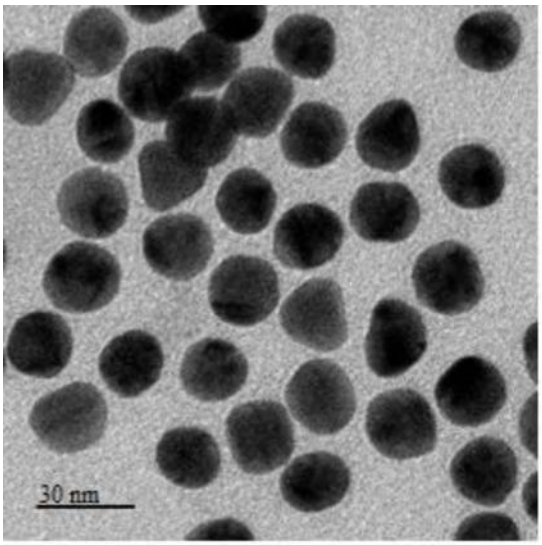

Figure 9: PEG-AuNP TEM images.

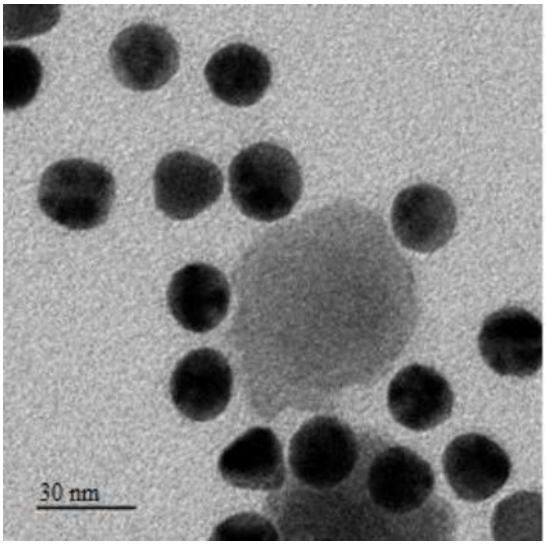

Figure 10: GA-AuNP TEM images.

The TEM images confirms the homogeneous results found in DLS, except the GA-AuNp's. It is possible to observe excess unreacted GA in figure 10. Studies are being performed with dialysis, centrifugation and even reaction optimization in order to access the possibility of cleaning the reaction.

6. CONCLUSION

Cancer is a devastating and prevalent disease that demands easy, cheap, and efficient treatments. With that in mind, we are combining our experience with brachytherapy sources production with nanotechnology to produce a less invasive nanosource. We already developed a gold radioactive nanoparticle. This work evaluated two coating agents, PEG and GA. DLS and TEM confirms the achievement of a nanoparticle and a good distribution. The GA-AuNp's presented an unreacted material. We are investigating purifying procedures. In future work, we will perform in-vivo and in-vitro validations.

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REFERENCES

1. Ministério da Saúde. Instituto Nacional de Câncer, *Incidência de Câncer no Brasil*, 2014.
2. Stewart, B. and C. Wild, *World Cancer Report 2014*, 2014, International Agency for Research on Cancer.
3. Russel, J., *A century of brachytherapy.* Nucl News, 2004. **v. 47**: p. p. 44-46.

4. Grimm, P. and J. Sylvester, *Advances in Brachytherapy.* Reviews in Urology, 2004. **6**(Suppl 4): p. S37-S48.
5. Alves, O.L., *Cartilha sobre nanotecnologia,* Agencia Brasileira de Desenvolvimento Industrial, Editor 2011: **Brasília**.
6. Adams, M., *Nanoparticles Technology Handbook*. 2015: NY RESEARCH PRESS. 346.
7. Chanda, N., et al., *Radioactive gold nanoparticles in cancer therapy: therapeutic efficacy studies of GA-198AuNP nanoconstruct in prostate tumor–bearing mice.* Nanomedicine: Nanotechnology, Biology and Medicine, 2010. **6**(2): p. 201-209.
8. Djoumessi, D., et al., *Rapid, one-pot procedure to synthesise 103Pd:Pd@Au nanoparticles en route for radiosensitisation and radiotherapeutic applications.* Journal of Materials Chemistry B, 2015. **3**(10): p. 2192-2205.
9. Róz, A.L.d., et al., *Técnicas de Nanocaraterização*. 2014: Campus - Grupo Elsevier. 256.