NEW GOLD-198 NANOPARTICLE SYNTHESIS TO BE USED IN CANCER TREATMENT

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ABSTRACT

Gold nanoparticles (NPs) have been intriguing scientists for over 100 years. Recently, they have been studied for new applications such as cancer treatment. Although the synthesis of gold nanoparticles is extensively reported, in the majority of cases the methodology is confused and/or not clear. We describe a new synthesis methodology for radioactive gold-198 NPs. Gold-198 was activated in IPEN IEA-01 nuclear reactor to a neutron flux with 10^{13} order. After that, chloroauric acid ($HAuCl_4$) was formed by dissolving the radioactive gold with aqua regia and performing repeated heating cycles. 0.1 mM $HAuCl_4$ containing 100 µL of 1 M NaOH was prepared in a flask equipped with a reflux condenser. The solution was brought to boil and stirred with a PTFE-coated magnetic stir-bar. Then 5 mL of sodium citrate was rapidly added. The reaction turns from light yellow to clear, black, dark purple until the solution attained a wine-red color (2–3 min). Dynamic light scattering (DLS) confirmed 8 nm particles. The presence of gold-198 (197.968 g/mol; half-life: 2.69517; decay mode: β-; average energy: 1.3723 MeV) was confirmed by an ORTEC HPGe detector. DLS was performed after complete decay confirming the 8 nm diameter maintenance. We were able to achieve radioactive gold-198 NPs and are performing further studies such as: coating reactions, in-vitro and in-vivo studies.

1. INTRODUCTION

1.1 Nanoscience

Nanoscience is the ramification that studies, produces and applies particles that are in the nanoscale (nm). In nanomaterials, the energy levels of the electrons are not continuous as when compared to the bulk form. They are discrete due to the confinement of the electronic wave function in up to three physical dimensions. That results in a change in surface area and electron confinment, making the change in materials properties such as melting point, fluorescence, electrical conductivity, magnetic permeability, and chemical reactivity.[1-3]
Maybe the most important result of the nanoscale quantum effects is the concept of "tunability" of properties. It means that a researcher can change the proprieties behavior to

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ISBN 978-85-99141-08-3
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serve a determined propose. For example, changing the fluorescence color of a particle can be used to identify diseases. [1]

Adding to their importance, the size of nanomaterials is similar to most of molecules and structures in the human biological systems such as DNA (2nm). A majority of biological processes occur at the nanoscale giving scientists models and templates to imagine and construct new processes. This fact can enhance their work in medicine, imaging, computing, printing, chemical catalysis, materials synthesis, and many other fields. [1]

Gold nanoparticles are a special type among others. About 87,000 papers were published since 1996. The use of these particles dates back to the 9th century in Mesopotamia when artisans unknowingly used gold nanoparticle to generate a glittering effect on the surface of pots. [1]

Michael Faraday provided the first synthesis description, in scientific terms, of the optical properties of nanometer-scale metals in his 1857 paper describing the production of colloidal gold by the reduction of chloroauric acid by phosphorous. [4]

Gold nanoparticles (AuNPs) interaction with light is environment, size and physical dimensions dependent. Consistent with the Fermi liquid model, plasmons are explained as a negatively charged electron cloud coherently displaced from its equilibrium position around a lattice made of positively charged ions.[5] When a small spherical AuNP is exposed to light, the oscillating electric field causes the conduction electrons to oscillate coherently. Because of the displacement of the electron cloud, a restoring force arises from Coulomb attraction between electrons and nuclei resulting in oscillation of the electron cloud relative to the nuclear framework. [6] The reflection in NPs with size much smaller than photon wavelength are non-propagating excitations, called localized surface plasmons (LSPs), due to the resulting plasmon oscillation being distributed over the entire particle volume. Such a coherent displacement of electrons from the positively charged lattice generates the restoring force that pulls the polarized electrons back to the lattice. When the wavelength is greater than the size of the NPs, a uniform and oscillating electric field is formed resulting in an in-phase electrons oscillation. Nevertheless, this collective oscillation is constrained by the reduced dimensions of the NP in which the electrons are confined, leading to a significant absorption of the wavelengths around green. That is why AuNPs appear with the complementary color, which is red. [2, 7-10] A few examples of gold nanoparticles being studied are:

- Therapeutic Agent Transport: The large ratio of surface area-volume of gold nanoparticles enables their surface to be coated with hundreds of molecules (including ligands, antibodies, and therapeutic, diagnostic and targeting agents). [11, 12]
- Photodynamic Therapy: Heat can be generated by gold nanoparticles when excited by light with 700 to 800 nm wavelengths. When positioned inside a tumor, the particles rapidly heat up, killing tumor cells. [13, 14]

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- Sensors: Gold nanoparticles can be used in Raman spectroscopy as substrates to enable/improve measurements of vibrational energies of chemical bonds. This strategy can be used for proteins detection. [15]
- Probes: In dark-field microscopy, gold nanoparticles can produce an array of colors that can be used for biological imaging applications. [16]
- Diagnostics: Gold nanoparticles are also used to detect biomarkers in the diagnosis of heart diseases, cancers, and infectious agents. [17]
- Treatment: gold nanoparticle can be used to improve the radiation therapy dose delivery or can be used for treatment when activated to gold-198. [18]

1.2 Cancer

Cancer is a term used generically to represent a group of more than 100 illnesses, including malignant tumors from different locations. According to World Health Organization (WHO), it is a leading cause of death worldwide. Only in 2012, 14 million new cases of cancer were diagnosed and a total of 8.2 million deaths were accounted, about 13% of worldwide deaths. Recent studies carried out by the International Agency for Research on Cancer (IARC) estimates that in 2030 will be 26.4 million new cases diagnosed, with 17 million deaths accounted worldwide [19, 20].

According with National Cancer Institute (INCA) in Brazil, 600,000 new cases of cancer will be diagnosed in 2018-2019. The most incidents, excluding non-melanoma skin cancer are prostate and lung cancer in males and breast and cervical cancer in females [21].

It is imperative that new and feasible forms of treatment be developed. Most people in countryside don't have access to linear accelerator radiation therapy and cancer surgery. Brachytherapy, the type of radiation therapy that uses a radiation source in direct contact with the site of interest, might be interesting as a full or adjuvant treatment.

1.3 Nanobrachytherapy

The idea to combine nanotechnology and brachytherapy is extremilly new. It might be a good cancer treatment cancer due to its effectiveness and simplicity. Radioactive AuNPs can be injected directly onto the treatment site by a seringe. [22]

Gold-198 decays with a half- life of ca. 2.7 days to stable mercury-198 by emission of ß particles with 0.960 Mev maximum energy and γ rays of energies 0.412 (most abundant), 0.68 and 1.09 Mev. The energy is suited for internal irradiation of human organs [20, 21]. It can be easily activated by IPEN's IEA 01 research reactor (4.5 MeV).

Chanda et al. [17] published a gold-198 nanoparticle coated with arabic gum. The authors state that their new method is better than the classic ones that consisted in the reduction of Au^{3+} in $AuCl_4^-$ with $NaBH_4$ or citrate, compounds that the author claims are highly toxic. Instead of the traditional route, the researchers synthesized a novel reducing agent that was

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named THPAL-alanine trimeric with phosphines. $^{198}AuCl_4^-$ was activated in a nuclear reactor with a flow of $8x10^{13}n$ /cm^2/s in acid solution of HCl. After activation, 50-100 µL of $H^{198}AuCl_4$ is added with 6 mL of arabic gum along with 20 µL / 0.0337 THPAL for each mL of solution. This reaction occurs in 5 minutes, although the synthesis of THPAL is complicated and expensive.

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

- Mean diameter (without the gum arabic coating): 7 ± 3 nm;
- Average diameter (with gum arabic coating): 85 nm;
- Zeta potential: -24.5 ± 1.5 mV

The second paper published by Fortin et. al. [18] used a mixture of 1mL $^{103}PdCl_2$ (700 mCi) with 500 mL H_2PdCl_4. In another tube, 200mL of DMSA (2,3- dimercaptosuccinic acid) 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 NPs and 12.5 mL of nanopure water with 0.8 mL of $HAuCl_4$ (0.1 M) were mixed. Two successive additions of AA (0.1 M) mL and 0.4 mL were performed. After coating with gold, the underwent again through a coating reaction, but this time with polyethylene glycol (PEG) 1.875 mL with 10 mg / mL for 2 hours. The analysis of the material by DLS - Dynamic Light Scattering obtained was: 9.3 nm for PdNp and 20.2 nm for PEG- AuPdNP.

Common oxidation states of gold include Au^{+1} (Au[I] or aurous compounds), Au^{+3} (Au[III] or auric compounds), and its non-oxidized state Au^0 (Au [0]). The Au^0 is the final desirable state for nanoparticles. So, the major step involving the synthesis of AuNps is reducing Au^{+1} or Au^{+3} to Au^0 by adding and electron donor (reduction agent) in the reaction. The precursor of choice for the majority of researches is chloroauric acid, $HAuCl_4$ with gold in its Au^{+3} oxidation state. [2, 4]

Although the synthesis of gold nanoparticles is extensively reported, in the majority of cases the methodology is confuse and/or not clear. Besides that, the toxicity of the reactants must be taken into account when developing products for medical applications.

The classical synthesis method was presented by Turkevitch in 1951 [5] using sodium citrate (cited as Na_3citrate, Nacit or simply citrate) as a reducing agent. The publication resulted in several others with some differences to the basic method presented. Mainly, the variations involve $HAuCl_4$/Na_3citrate ratio, pH control and temperature influence in nanoparticle size and stabilization. Turkevitch method for sodium citrate are as follows: 95 mL of chloroauric acid solution (containing 5 mg Au) were heated to the boiling point and 5 mL of 1% sodium citrate solution was added to the boiling solution with good mechanical stirring. The reaction mixture was colorless for 12 s after the addition of the citrate, and then it turned purpleish-

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blue within a fraction of a second. After 5 minutes the final color was deep wine red. The best results were obtained with 5-50 mg citrate addition.[5]

The current paper will present a new methodology for producing gold-198 nanoparticles for future evaluation as a nanobrachytherapy source.

2. METHODOLOGY

All reactants were used without any further purification. All glassware and magnetic stir bars were first cleaned with aqua regia (HCl and HNO_3 in a 3:1 volumetric ratio) and thoroughly rinsed with nanopure water (Barnstead, 18.2 MΩ, Dubuque, USA) before use. The same nanopure water was used to prepare aqueous solutions. $HAuCl_4$ was only handled with plastic spatulas to avoid corrosion.

2.1 Nuclear Activation

Nuclear activation was performed in gold foils (Goodfellow AU004925. Disc thickness: 0,25 μm; specific density: 483 μg.cm^{-2}; purity: 99.99+ %) at IPEN's IEA-01 reactor. Final activity for 8 hours irradiation cycle was calculated by using equation 1 [23]. Confirmation of gold-198 was obtained by an ORTEC HPGe detector.

$$A = \frac{M \,.N \,.\theta \,.\sigma \,.\phi}{P} \,(1 - e^{-\lambda t}) \qquad (1)$$

Table 1: Initials meaning for equation 1

Initial	Meaning	Unity
A	activity	bequerel
P	Atomic weight	g/mol
M	mass	g
N	avogrado	6.02×1023
θ	Isotopic abundance	%
λ	decay	time^{-1}
ϕ	reactor flow	n.cm^2/s^{-1}
σ	cross section	cm^2 ****
t	Irradiation time	tempo

**** 1 barn = 10^{-24} cm^2

2.2 Synthesis

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After the activation process, gold foils were transformed in $HAuCl_4$ by dissolution using aqua regia (HCl and HNO_3 – Sigma) following:

1. About 0.7 g of gold metal in disks are placed in a 250 ml beaker without being cut. Seventy ml of royal water (HCl and HNO_3 in a volume ratio of 3: 1) are added slowly;
2. The mixture is stirred and gradually heated to 50 °C. When gold dissolution is finished (about one minute), the temperature is gradually raised to 70-80 °C;
3. The solution is continuously heated until it is concentrated to 30 ml.
4. HCl is slowly added to the hot solution until the brown nitric vapors are completely eliminated. The final volume reached is about 40 ml;
5. The solution is kept warm until it is concentrated to 30 ml;
6. The procedure described in items 4 and 5 is repeated 3 times until no brown nitric vapor is formed. The presence of acid vapors is verified by a litmus paper (pH indicator);
7. The solution is concentrated (by heating) to 15 ml;
8. Chloroauric acid in liquid form is ready for use.

Fifty milliliters of freshly prepared 0.1 mM $HAuCl_4$ containing 100 µL of 1 M NaOH (Casa Americana, 99,99%) was prepared in a 100 mL three neck flask equipped with a reflux condenser (figure 1). The solution was brought to boil (80-90 °C) while being stirred with a PTFE-coated magnetic stir-bar. Then 5 mL of 34 mM Na_3Ctr was rapidly added. The reaction turns from light yellow to clear, black, dark purple until the solution attained a wine-red color (2-3 min). The reaction was allowed to proceed for 7 more minutes (completing 10 in total). The vial was removed from the heat plaque and allowed to cool down for 10 minutes still under reflux. After this period, the reflux system was shut down and dynamic light scattering (figure 2) analysis were performed.

Confirmation of gold-198 production was obtained by an ORTEC HPGe detector (GEM-65970-B). Dynamic light scattering (Anton Paar – Litesizer 500) analysis were performed after decay confirming size maintenance. Transmission electron microscopy (JEM-2100F) results were also performed.

3. RESULTS AND DISCUSSION

The final radioactive activity obtained was 1.7×10^{13} Bq (0.45 mCi) for a neutron flux of 8×10^{13} $n.cm^2/s^{-1}$.

Aqua Regia dissolves gold, though neither HCl and HNO_3 have the power to dissolve it alone, because in combination, each acid performs a different task. Nitric acid is a powerful oxidizer, which dissolves virtually undetectable amount of gold, forming ions of gold (Au^{3+}). The hydrochloric acid provides chloride ions (Cl^-), which react with the ions gold to produce tetrachlorourate (III) anions, also in solution. The reaction with hydrochloric acid is an equilibrium reaction that favors the formation of chlorourate anions ($AuCl_4^-$). This results in

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the removal of gold ions from the solution allowing further oxidation of the remaining gold. The gold foil them became chlorouronic acid.

The reaction them was performed by using sodium citrate as an electron donor. Glassware scheme is shown in figure 1. As expected, the reaction color turned to light yellow, black-purple wine-red confirming all the consumption of gold salts. Gold-198 presence was confirmed by HPGe detector (figure 2).

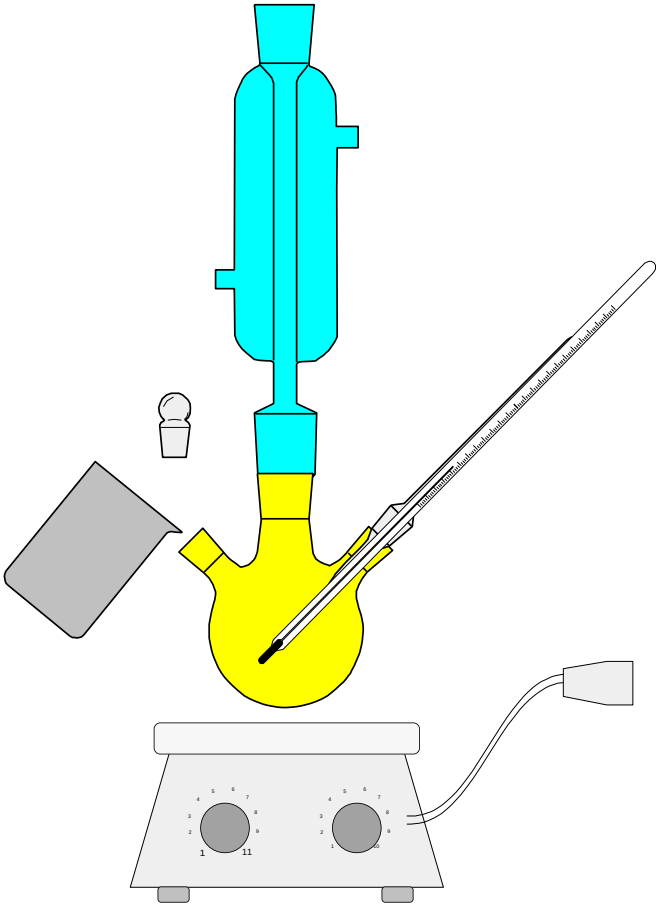

Figure 1: Glassware scheme.

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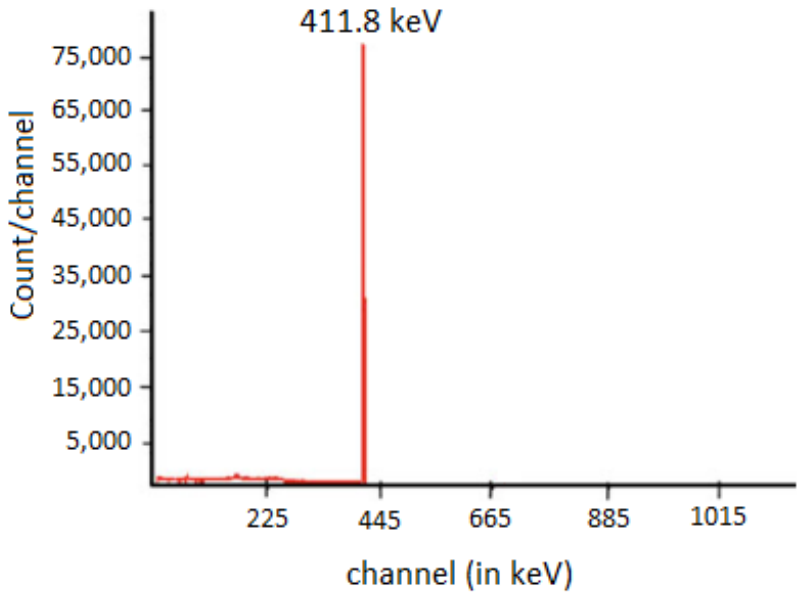

Figure 2: HPGe detector confirming gold-198 presence.

DLS analysis was then performed before and after decay (3 months after). Size was maintained (figure 3 and 4).

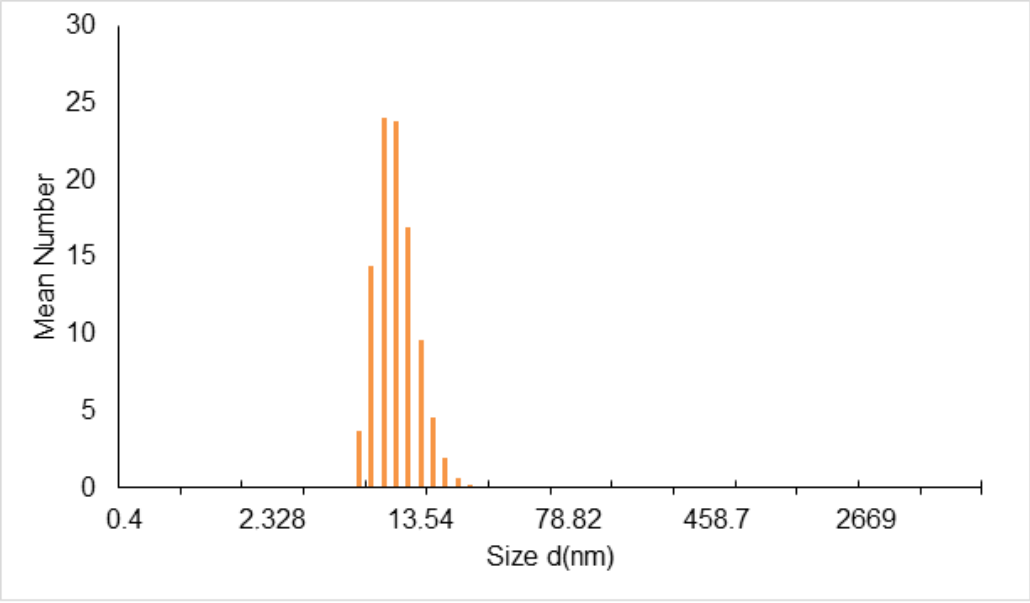

Figure 3: DLS Results. Average size: 8.721 nm.

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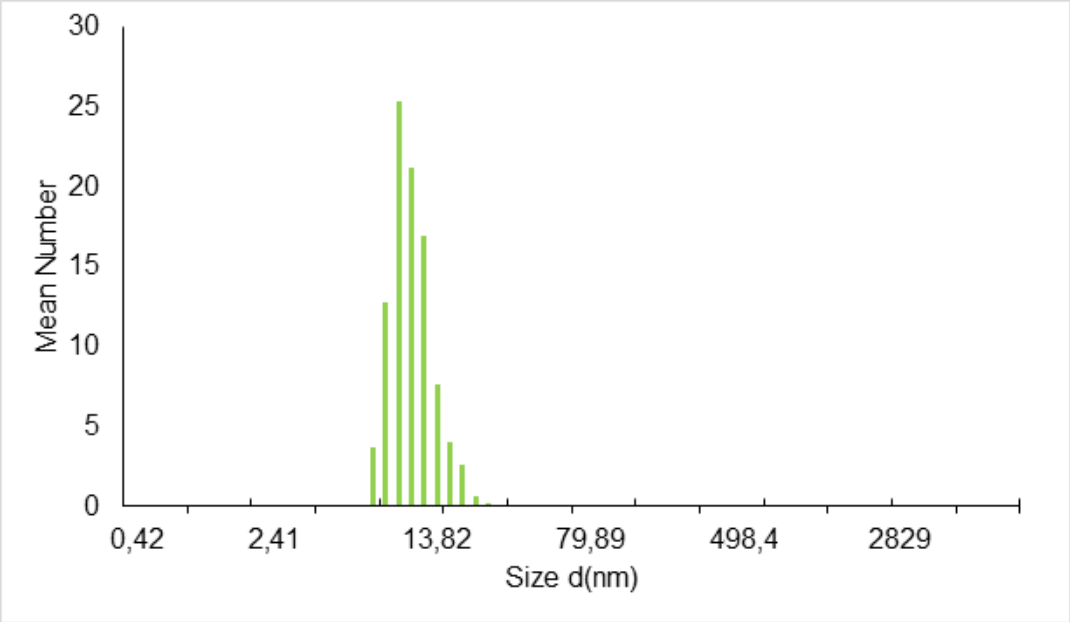

Figure 4: DLS Results after decay. Average size: 8.95 nm.

TEM was performed after radioactive decay (figure 5). Size was calculated by the ImageJ software. The size difference when compared to DLS is acceptable.

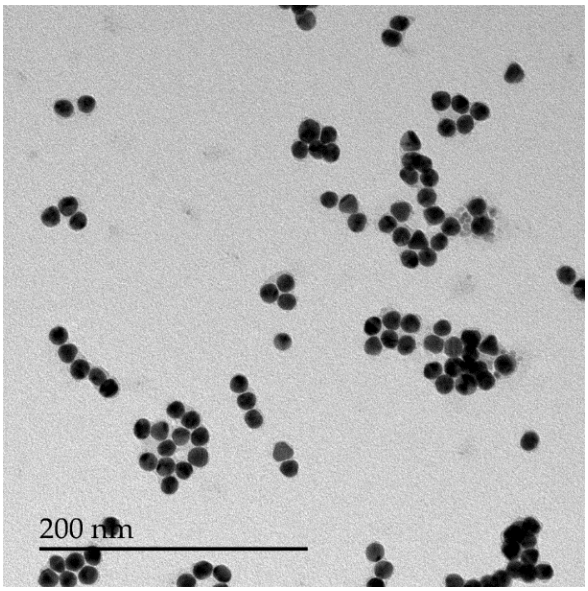

Figure 5: TEM images. Results show a good distribution and size (11 nm average).

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4. CONCLUISION

This paper presented a synthesis and evalutaion of radioactive gold-198 nanoparticles. Nuclear activation in IPEN IEA-01 reactor, prepartion of $HAuCl_4$, and synthesis by using sodium citrate as a reducing agent were presented.

Evaluation were performed. Gold-198 presence was confirmed by a HPGe detector. The size of nanoparticles for the radioactive and decaied particles were measured by DLS and yielded, repectvelly, 8.721 nm and 8.95 nm. The results confirm size maintenance after the decay. TEM image of the decaied particles showed a good size distribuition and average size of 11 nm.

The next step of the project is stabilization evaluation in biological fluids, coating, in-vivo and in-vitro studies.

ACKNOWLEDGMENTS

The authors with to thank IAEA and FAPESP por funding equipment's and fellowships that allowed the work to be made.

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