BJRS
BRAZILIAN JOURNAL OF RADIATION SCIENCES

doi.org/10.15392/2319-0612.2024.2753
2024, 12(4B) | 01-13 | e2753
Submitted: 2024-08-31
Accepted: 2025-05-08

Synthesis and characterization of Au-198 nanoparticles for radiotherapy

Rigoa*, M. E. Z.; Barbezan, A. B.; Talacimon, C.; Teodoro, L. E. H.; Rodrigues, P. S.; Rosero, W. A. A.; Nogueira, T.C.; and Rostelato, M. E. C.

Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN), Avenida Professor Lineu Prestes, 2242, Cidade Universitária, São Paulo SP, CEP 05508000, Brazil

*Correspondence: rigomaria110@gmail.com

Abstract: In recent years, rapid progress in nanotechnology has stimulated growing interest in nanoparticle research, particularly in the fight against cancer, a leading cause of death worldwide. Currently, gold nanoparticles (AuNPs) are being studied as an alternative to conventional cancer treatments, which, despite their effectiveness, face challenges such as side effects due to low selectivity. This work focuses on the synthesis of AuNPs functionalized with polyethylene glycol (PEG) in both their non-radioactive and radioactive forms and on their physicochemical properties. The AuNPs functionalized with PEG were produced using the adapted Turkevich method, and their physicochemical characteristics were analyzed using Dynamic Light Scattering with Zeta Sizer (DLS), UV-Vis spectroscopy, and transmission electron microscopy (TEM), assessing the effect of the amount of sodium citrate, as a reducing agent, on the size of the nanoparticles. The results showed a hydrodynamic diameter of 22.62 nanometers, a surface charge of - 0.1269mV, and an average size of 12.12nm as measured by TEM. The studies were conducted with radioactive gold-198 nanoparticles, and their presence was confirmed by an HPGe detector.

Keywords: Gold nanoparticles, Nanobrachytherapy, Polyethylene glycol (PEG)

BJRS
BRAZILIAN JOURNAL OF RADIATION SCIENCES

doi.org/10.15392/2319-0612.2024.2753
2024, 12(4B) | 01-13 | e2753
Submitted: 2024-08-31
Accepted: 2025-05-08

Síntese e caracterização de nanopartículas de Au-198 para radioterapia

Resumo: Nos últimos anos, o rápido progresso na nanotecnologia tem estimulado um crescente interesse na pesquisa de nanopartículas, particularmente na luta contra o câncer, uma das principais causas de morte em todo o mundo. Atualmente, as nanopartículas de ouro (AuNPs) estão sendo estudadas como uma alternativa aos tratamentos convencionais de câncer, que, apesar de sua eficácia, enfrentam desafios como efeitos colaterais devido à baixa seletividade. Este trabalho foca na síntese de AuNPs funcionalizadas com polietilenoglicol (PEG) tanto em suas formas não radioativas quanto radioativas, e em suas propriedades físico-químicas. As AuNPs funcionalizadas com PEG foram produzidas utilizando o método adaptado de Turkevich, e suas características físico-químicas foram analisadas usando Dispersão Dinâmica de Luz com Zeta Sizer (DLS), espectroscopia UV-Visível e microscopia eletrônica de transmissão (TEM), avaliando o efeito da quantidade de citrato de sódio, como agente redutor, sobre o tamanho das nanopartículas. Os resultados mostraram um diâmetro hidrodinâmico de 22,62 nanômetros, uma carga superficial de -0,1269 mV e um tamanho médio de 12,12 nm mensurado por TEM. Os estudos foram realizados com nanopartículas de ouro-198 radioativas, e sua presença foi confirmada por um detector HPGe.

Palavras-chave: Nanopartículas de ouro, Nanobraquiterapia, Polietilenoglicol (PEG).

SBPR

1. INTRODUCTION

Nanomedicine (the medical application of nanotechnology) holds incredible potential to revolutionize cancer therapy and diagnosis by developing ingenious biocompatible nanocomposites [1]. In recent years, the use of gold nanoparticles (AuNPs) has stood out due to their unique physical and chemical properties, manifested mainly in the following aspects: they are relatively safe, stable, and easy to prepare, and they possess many unique characteristics, such as small size effects, surface effects, quantum size effects, electrical, and optical properties [2-4].

AuNPs are widely used for drug delivery after modifying their surfaces by attaching biomolecule ligands. Modeling studies have shown increasing interest in AuNPs, and the investigation of their toxicity, size, shape, and surface modifications has been proposed [5]. Polyethylene glycol (PEG) coatings on AuNPs protect the surface from aggregation, opsonization, and phagocytosis, thereby prolonging circulation time [6].

Radioactive nanoparticles, a new frontier in cancer therapy, offer a refined approach to radiotherapy, a common technique in fighting this disease. These nanoparticles have the remarkable ability to enhance radiation effectiveness by precisely targeting tumors, which is essential for minimizing damage to nearby healthy tissues. This advanced method has the potential to overcome significant challenges encountered in conventional treatments, including cancer cell resistance to radiation and reduction of adverse side effects, making the treatment more tolerable for patients [7].

This paper will present a methodology and characterization for the standardization of gold-198 nanoparticle production for future evaluation as a source of nanobrachytherapy. The methodology is described step by step with all material quantities properly detailed, facilitating reproducibility.

2. MATERIALS AND METHODS

The work was developed for the production of gold nanoparticles functionalized with polyethylene glycol, based on a widely accepted and well-documented method in the literature by Turkevich [8], which uses sodium citrate as a reducing agent. Using this methodology, characterization techniques were employed to assess the physical and chemical properties of the nanoparticles produced in their radioactive form.

2.1. Nuclear Activation

Au-198 was produced by neutron capture in the IEA-R1 reactor at the Nuclear Research Center (CERPq) at IPEN, a pool-type research reactor. Au-197, with a neutron capture cross-section of 98.65 ± 0.3 barns, has a high probability of transforming into Au-198 when irradiated with neutrons.

Au-198 decays to Hg-198 with a half-life of approximately 2.69 days, primarily through β^- emission, although γ-ray emission also occurs. Confirmation of Au-198 was carried out using an HPGe detector (ORTEC, GEM-C5970-B, Coaxial Detector System).

2.2. Synthesis

For the dissolution of gold and synthesis of Au-198 nanoparticles, a closed system was developed to prevent dust entry and to avoid the release of acidic gases and potential radioactive particulates into the environment.

The main step in the synthesis of gold nanoparticles (AuNPs) is the reduction of Au^{+1} or Au^{+3} to Au^{0} using a reducing agent. In this case, $HAuCl_4$ with gold in the form of Au^{+3} was used. To prepare the gold reagent, all glassware was cleaned with aqua regia, rinsed with nanopure water, and dried to prevent residual particle aggregation and unwanted nucleation.

Gold is oxidized by aqua regia, a mixture of hydrochloric acid (HCl) and nitric acid (HNO_3). HCl provides chloride ions (Cl^-) that react with gold ions (Au^{3+}) dissociated by HNO_3 to form tetrachloroaurate (III) anions, favoring the formation of chloraurate anions ($AuCl_4^-$).

The synthesis involved adding freshly prepared 0.055 mM $HAuCl_4$ to 3 ml of ultrapure water, prepared in a 50 ml round-bottom flask equipped with a condenser. The solution was kept boiling (100 °C) while stirred with a magnetic stir bar for two minutes, or until the solution became homogeneous and temperature stabilized. Then, 0.12 mM PEG was added and kept under the same stirring and temperature conditions for an additional three minutes. Next, 39 mM Na_3Ctr was added rapidly. After the addition of the reducing agent, the solution was left under stirring for 3 minutes, during which a color change was observed. The flask was removed from the heating plate and allowed to cool for 10 minutes while still under reflux. After this period, the reflux system was turned off, and characterization analyses were initiated.

2.3. Characterization of Nanoparticles

To assess the success of the reaction, the size of the PEG-functionalized nanoparticles is measured using techniques such as Dynamic Light Scattering (DLS) (Anton Paar Litesizer 500), Ultraviolet-Visible (UV-Vis) spectroscopy (Shimadzu UV- 1800), and Transmission Electron Microscopy (TEM) (JEOL JEM 2100 TEM, HAADF ("High Angle Annular Dark Field") detector). DLS measures the hydrodynamic radius of the sample, UV-Vis spectroscopy can estimate the core size of the nanoparticles based on the interaction of light with the gold core, while TEM evaluates the projected diameter of the particles. DLS provides information on Brownian motion, UV-Vis measures the plasmon resonance absorbance of the AuNPs based on the interaction of light with the gold core, and TEM allows direct visualization and identification of particle shape and aggregation. Differences in sizes measured by these techniques may indicate changes in the surface of the nanoparticles.

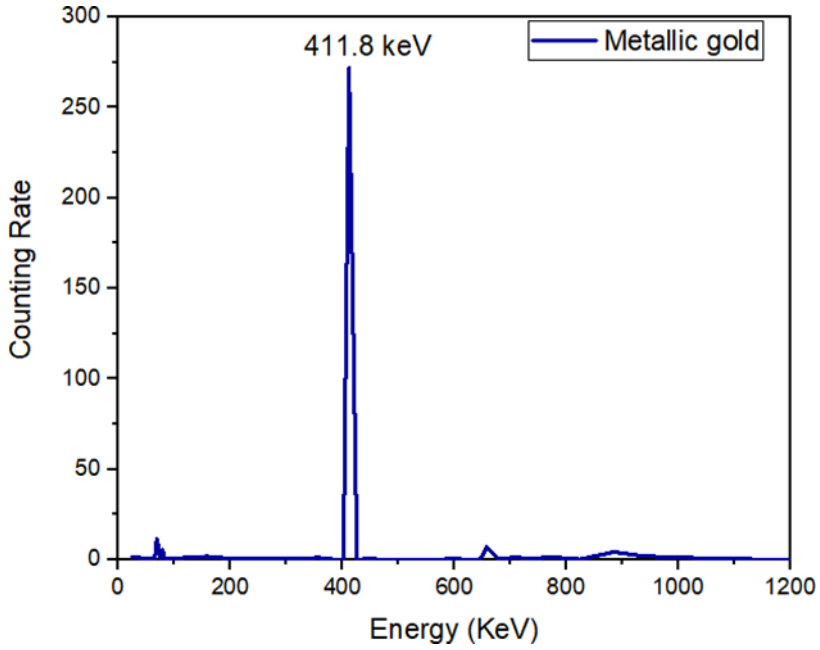
The stability and shelf life of the particles were confirmed with Zeta potential measurements, which assess the surface charge through the magnitude of repulsion and

attraction forces between the particles, and DLS. In the current reaction, measurements were taken at 0.5 hours, 24 hours, and 10 days after production.

3. RESULTS AND DISCUSSIONS

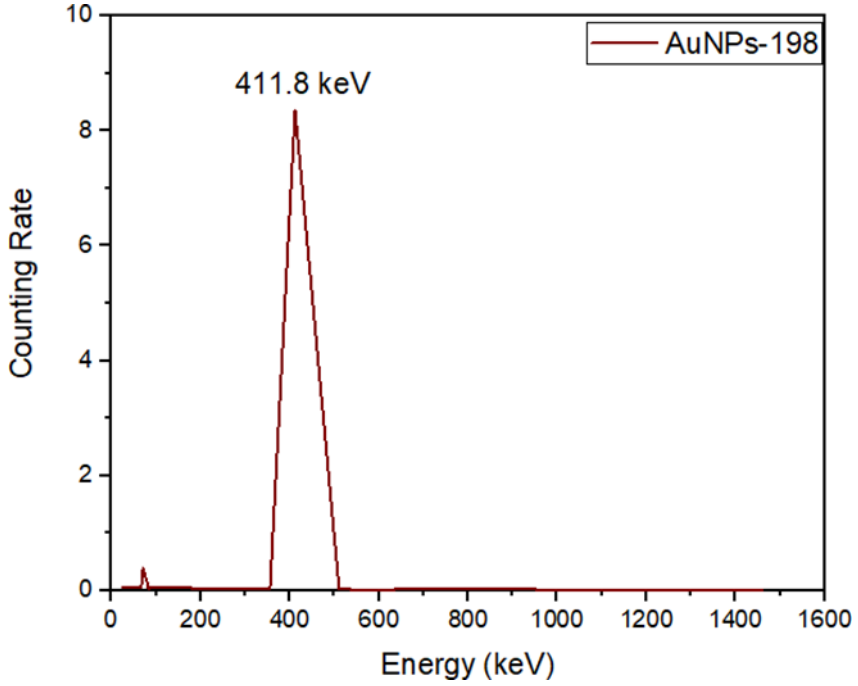
The reactions for obtaining AuNPs-198 showed an activity of 671 μCi ± 5 μCi (for a final volume of 4.25 mL for each reaction). After the activation of Au-197 to Au-198, the purity of the radionuclide was confirmed with gamma spectrometry using an HPGe detector. The live time for reading each sample was 7200 seconds. The spectra obtained for metallic gold and AuNPs-198 are presented in Figures 1 and 12, respectively.

Figure 1: Gamma radiation spectrum obtained from metallic Au-198.



Source: Prepared by the author.

Figure 2: Gamma Radiation Spectrum of AuNPs-198.



Source: Prepared by the author.

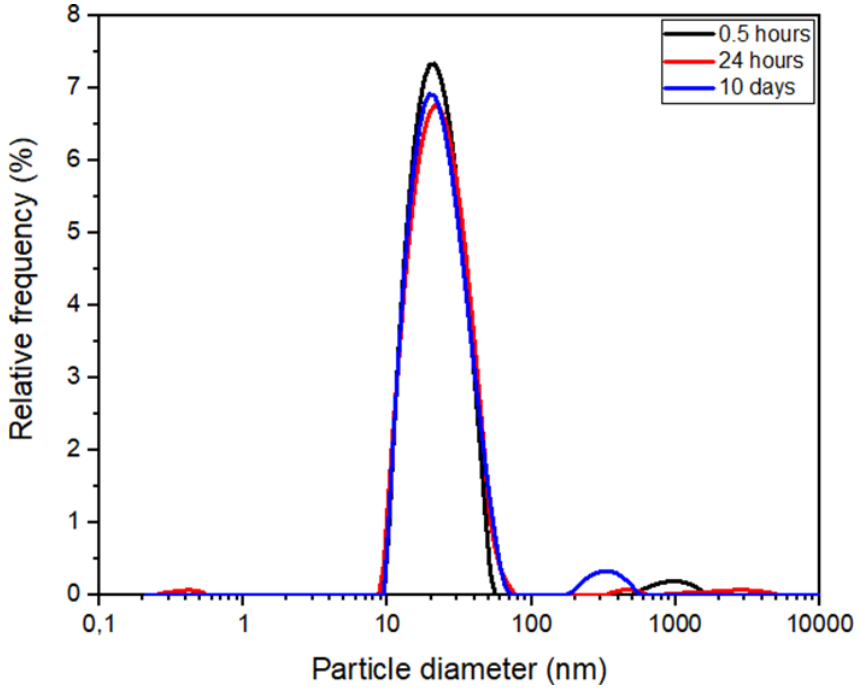
As observed, both in the spectrum of metallic gold and in the spectra of the AuNPs, the presence of characteristic peaks of Au-198 can be verified, confirming that both the metallic gold was activated and the obtained AuNPs retained the characteristic peaks of Au-198.

The results from the DLS analyses provided the size distribution profile of the nanoparticles, their stability, and surface charge when tested at 0.5 hours, 24 hours, and 10 days after production. Table 1 shows the average values of the hydrodynamic diameters of the AuNPs and their respective standard deviations. Figure 3 illustrates the monodisperse distribution profile of the NPs over time.

Table 1: Hydrodynamic Diameter Obtained by DLS of PEG-AuNPs-198 Over Time.

	Time After Preparation	Hydrodynamic Diameter (nm)	Polydispersity Index (%)	Standard Deviation (nm)	Standard Deviation (%)
Au-198 Radioactive	0.5 hour	21.6326	18.6310	0.3327	3.579
	24 hours	21.8454	19.0791	0.2749	1.5697
	10 days	24.3283	24.3783	0.4796	0.2352

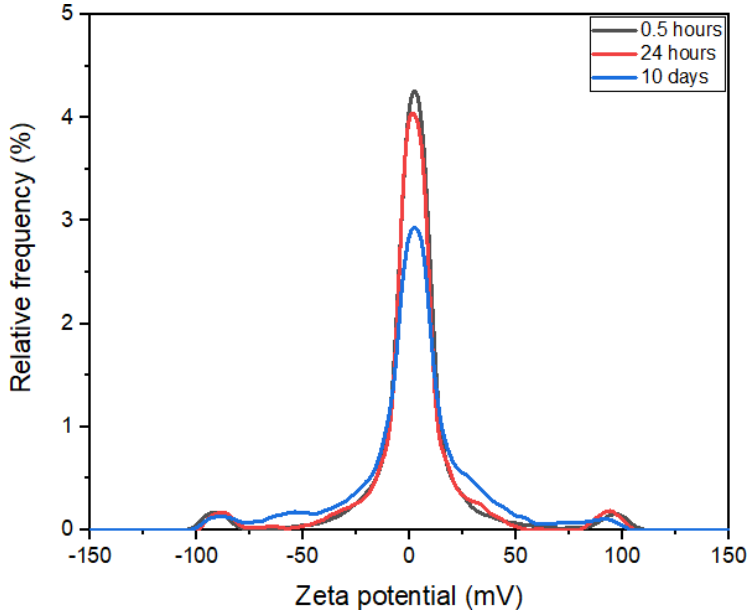
Figure 3: Legend style. Style name (MS Word taskbar): Distribution profile of AuNPs-198 (radioactive) tested in three periods of 0.5 h, 24 h and 10 days after being produced.



Source: Prepared by the author.

The average Zeta potential of the AuNPs-197 was -0.1269 mV with a standard deviation of 0.0634 mV. This result can be explained by the fact that the PEG coating, which is electrically neutral, reduces the negative charges of the sodium citrate reducer, since the measurement is taken on the surface of the nanoparticles [9-11]. Literature also indicates that changes in Zeta potential may suggest a strong interaction of PEG with the AuNPs. This result is supported by the maintenance of the hydrodynamic size of the AuNPs over time, as evidenced by Figure 4 [9].

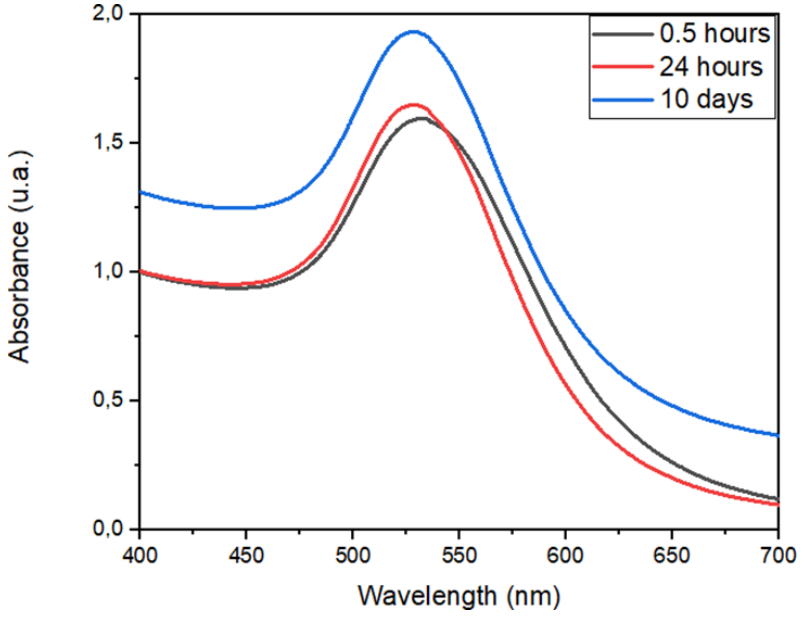
Figure 4: Zeta potential distribution function at three time points for AuNPs-198.



Source: Prepared by the author.

Figure 5 shows the UV-Visible spectra obtained for the non-radioactive AuNPs. The results indicated that the AuNPs-197 exhibited an average absorbance peak at 530 nm. The calculated core size of the nanoparticles was 12.61 nm. There was a slight variation in the absorbance peak at 10 days, but this variation was not statistically significant ($p>0.05$).

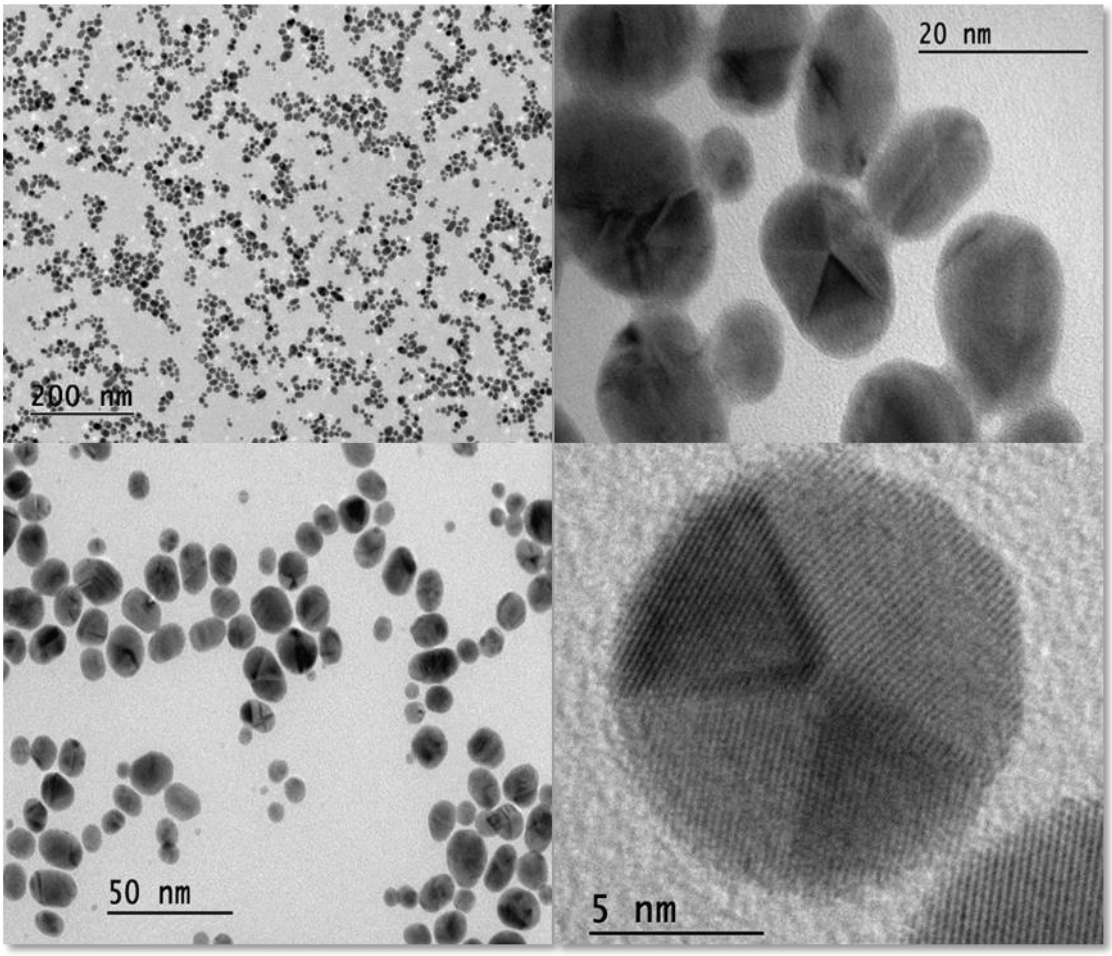
Figure 5: UV-Visible spectra of AuNPs-198 over time.



Source: Prepared by the author

To evaluate the shape and size of the gold nanoparticles produced by the synthesis developed in this work, TEM analysis was performed after the decay of AuNPs-189 with PEG. Figure 6 presents the micrographs obtained at different magnifications.

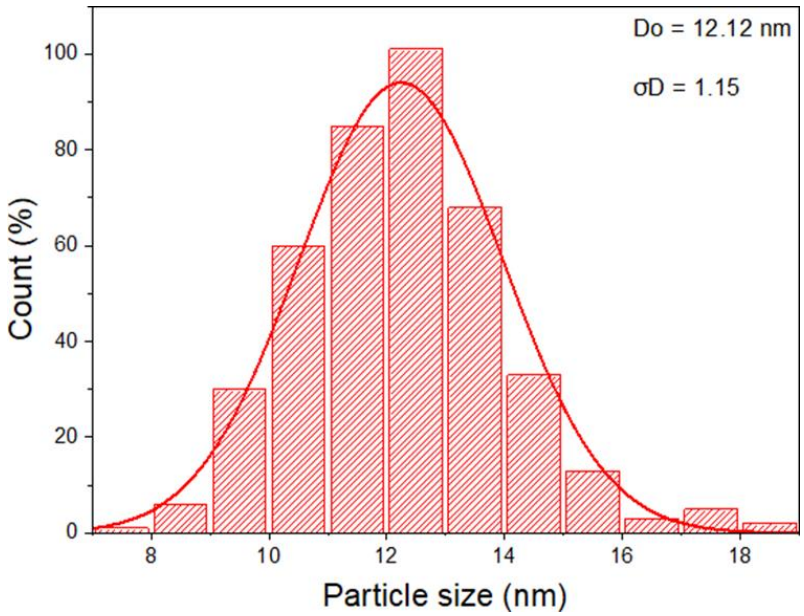
Figure 6: TEM micrographs of AuNPs-198 functionalized with PEG 5000 after decay, at different magnifications.



Source: Prepared by the author.

The analysis of the size distribution of AuNPs-197 from the micrographs (Figure 6) revealed that the average diameter of the nanoparticles was 12.12 nm. Figure 7 further shows that the highest frequency of the diameter of AuNPs-197 is between 11 and 12 nm. Here, D0 corresponds to the average particle size and σD is the standard deviation.

Figure 7: Particle size distribution with log-normal distribution fit.



Source: Prepared by the author.

4. CONCLUSIONS

In this work, a specific synthesis method was developed for the production of gold nanoparticles, considering the need to handle radioactive material and aiming to mitigate the risk of contamination for operators and the work area. Apresentou a síntese e avaliação de nanopartículas radioativas de ouro-198. Foram descritos a ativação nuclear no reator IPEN IEA-R1, a preparação de $HAuCl_4$ e a síntese utilizando citrato de sódio como agente redutor. As avaliações foram realizadas, e a presença de ouro-198 foi confirmada por um detector HPGe.

TEM revealed that the AuNPs-198 (after decay) produced for this work are spherical in shape and suggest good dispersion in the AuNPs suspension, without excess coating agent. The characterization of the radioactive AuNPs by DLS showed that the obtained nanoparticles have an average hydrodynamic size of 22,6 nm, and this size remained consistent over time for both types of nanoparticles. Besides having a desirable hydrodynamic size, the stability of the AuNPs are favorable factors for biomedical applications. The size distribution results of

the AuNPs-198 cores after decay, obtained from transmission electron microscopy images, indicated that over 80% had core diameters of 12.12 nm.

FUNDING

Our gratitude to the National Council for Scientific and Technological Development (CNPq), Brazil, for their support of our research through project number CNPq 302135/2024-3. Additionally, we acknowledge the support from the INCT - INTERAS project (CNPq process 406761/2022-1), the Intercentros 2020.06IPEN37 project, and the FAPESP project 2020/07065-4. These contributions have been invaluable to the advancement of our research.

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

REFERENCES

[1] MISRA, R.; ACHARYA, S.; SAHOO, S. K. Cancer nanotechnology: application of nanotechnology in cancer therapy. Drug Discovery Today, v. 15, n. 19-20, p. 842-850, 2010.

[2] BLANCO, E.; SHEN, H.; FERRARI, M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nature Biotechnology, v. 33, n. 9, p. 941-951, 2015.

[3] SINGH, R. K.; KNOWLES, J. C.; KIM, H.-W. Advances in nanoparticle development for improved therapeutics delivery: nanoscale topographical aspect. Journal of Tissue Engineering, v. 10, p. 2041731419877528, 2019.

[4] MOGHIMI, S. M.; HUNTER, A. C.; ANDRESEN, T. L. Factors controlling nanoparticle pharmacokinetics: an integrated analysis and perspective. Annual Review of Pharmacology and Toxicology, v. 52, p. 481-503, 2012.

[5] ZHANG, N.; XIONG, G.; LIU, Z. Toxicity of metal-based nanoparticles: Challenges in the nano era. Frontiers in Bioengineering and Biotechnology, v. 10, p. 1001572, 2022.

[6] KLIBANOV, A. L.; MARUYAMA, K.; TORCHILIN, V. P.; HUANG, L. Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. FEBS Letters, v. 268, n. 1, p. 235-237, 1990.

[7] MUHAMMAD ARIF, A.; FAZAL NAWAZ, A.; KHAN, S. U.; MUEEN, H.; RASHID, F.; A. HEMEG; RAUF, A. Nanotechnology-based radiation therapy to cure cancer and the challenges in its clinical applications. Heliyon, v. 9, n. 6, 2023.

[8] TURKEVICH, J.; STEVENSON, P.; HILLIER, J. A study of the nucleation and growth process in the synthesis of colloidal gold. Discussions of the Faraday Society, v. 11, p. 55, 1951.

[9] FERREIRA, M. et al. Técnicas de Nanocaracterização. 1ª. ed. Rio de Janeiro: Elsevier, v. 3, 2015. ISBN 978-85-352-8091-3.

[10] CALLISTER JR., W. D. Materials Science and Engineering: An Introduction. 7ª. ed. New York: John Wiley & Sons, 2007.

[11] ROEBBEN, G.; RAUSCHER, H. Towards a review of the EC Recommendation for a definition of the term "nanomaterial", Part 2: Assessment of collected information concerning the experience with the definition. JRC Scientific and Policy Reports. Luxembourg. 2014.

LICENSE