

UV-VISIBLE SPECTROSCOPY AND ATOMIC FORCE MICROSCOPY ANALYSIS OF GOLD NANOPARTICLES EXPOSED TO VARYING DOSES OF IONIZING RADIATION

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Abstract

Gold nanoparticles (AuNPs) exhibit strong X-ray absorption, making them promising candidates for enhancing medical diagnostics and X-ray imaging. However, understanding their behaviour under ionizing radiation is crucial for their safe and effective clinical translation. This study examines the impact of varying X-ray irradiation doses on the morphology and physicochemical properties of AuNPs. AuNPs synthesized via the classical Turkevich method were characterized using UV-Vis spectroscopy, atomic force microscopy (AFM) and transmission electron microscopy (TEM) before and after irradiation. Our findings demonstrate dose-dependent alterations in the optical and structural characteristics of the nanoparticles, providing valuable insights into their radiation-induced transformations and potential implications for their biomedical applications.

Key words: gold nanoparticles, medical linear accelerator (LINAC), X-ray irradiation doses, UV-Vis and AFM

Introduction. Gold nanoparticles (AuNPs) have a rich history in medicine, dating back to their intravenous use in the late 19th century for treating alcoholism

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and cancer [1,2]. They have also shown promise in facilitating specific biomolecular interactions in bodily fluids [3]. Leveraging gold's high X-ray absorption, AuNPs have emerged as valuable contrast agents for medical imaging [4]. Compared to traditional iodine-based agents, spherical AuNPs offer higher absorption coefficients, enabling enhanced image clarity with reduced interference from surrounding tissues and lower radiation doses [5]. Their high atomic number and low viscosity further facilitate their intravenous administration [6]. AuNPs absorb X-rays effectively in the clinical range (around 100 keV), making them suitable for various imaging modalities, including CT, fluoroscopy, and mammography [7]. Moreover, their high X-ray absorption properties also make them promising candidates for radiosensitizing agents in radiotherapy, enhancing the efficacy of cancer treatment at low concentrations (below toxic thresholds) [8]. The interaction of AuNPs with ionizing radiation, particularly the potential for morphological and physical changes, is crucial for their safe and effective clinical application. Previous studies have shown that the radiation enhancement mediated by AuNPs is influenced by their size and shape [9]. Optimizing nanoparticle morphology is essential to maximize therapeutic benefits while minimizing collateral damage during radiotherapy (RT) [10]. TARANTINO et al. [11] hypothesized that AuNPs, both spherical and star-like, retained their morphology under therapeutic irradiation doses of 1.8–2 Gy from a medical linear accelerator (LINAC). To further investigate the effects of radiation on AuNPs, we refined our irradiation protocol. This involved using a water phantom to handle the AuNPs' solutions and applying higher irradiation doses, up to 100 Gy, in addition to the standard therapeutic range. We then used UV-Vis spectroscopy, atomic force microscopy (AFM) and transmission electron microscopy (TEM) to analyze the radiation's impact on the AuNPs and observed an increase in the plasmon maxima in the UV-Vis spectra of the AuNPs, accompanied by evidence of alterations in their shape and size. Hence, our study aims to demonstrate that even at low therapeutic doses, radiation exposure leads to an increase in the plasmon maxima in the UV-Vis spectra of AuNPs, accompanied by observable morphological changes.

Materials and methods. Chemicals and reagents. Gold(III) chloride hydrate (auric acid, HAuCl_4 , MW = 339.79 g/mol) and trisodium citrate dihydrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$, MW = 294.1 g/mol) were of analytical grade, sourced from Merck (Germany). All solutions were prepared with deionized water.

Synthesis procedure. Gold nanoparticles were synthesized using the TURKEVICH method [12,13]. A 10 mL 2.5 mM auric acid solution, containing 5 mg of gold, was mixed with 85 mL of deionized water, stirred at 350 rpm, and heated to 90 °C, then a 5 mL 1% trisodium citrate solution was added. The solution transitioned from blue to cherry red, completing the reaction after 30 min.

Irradiation at the medical LINAC suite. Gold nanoparticle solutions were contained within 1.5 mL Eppendorf tubes and subjected to varying doses of high-energy photon ionizing radiation. A Versa HD LINAC (Elekta, UK) equipped

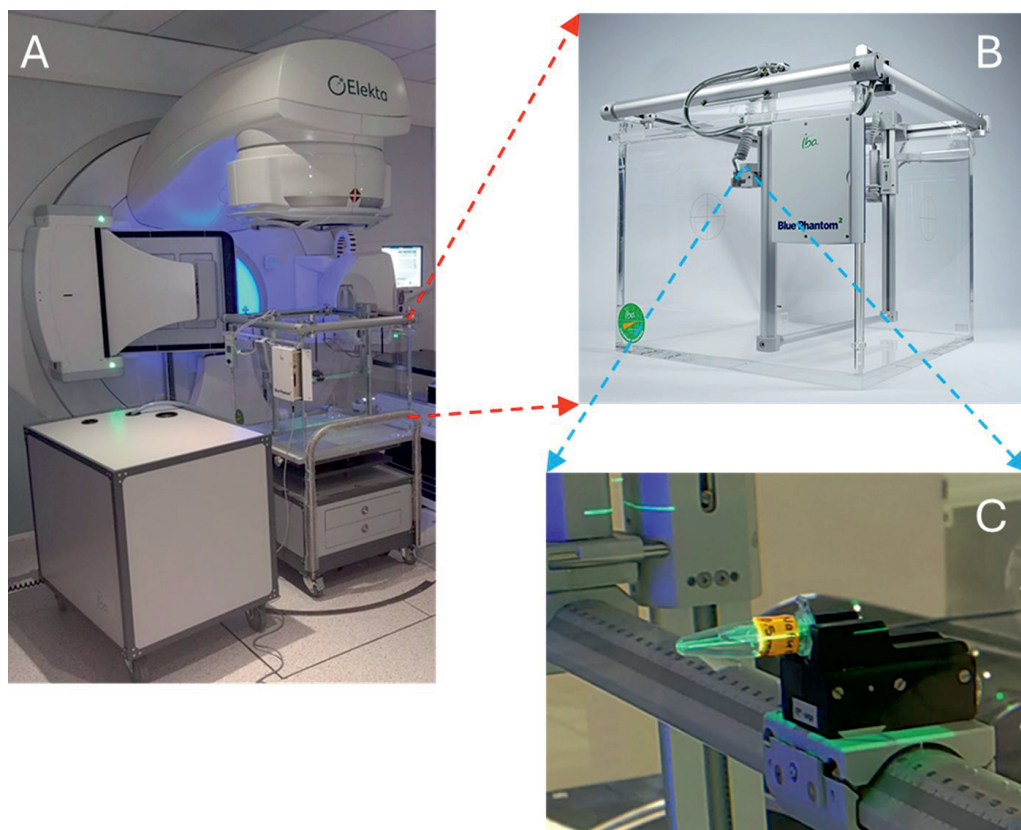


Fig. 1. (A) LINAC suite utilized for the irradiation of AuNP solutions. The setup includes a water phantom (B), filled with distilled water, which ensures uniform radiation distribution. A sample of the AuNP solution, contained in a 1.5 mL Eppendorf tube, is positioned securely on a support (C) within the water phantom. This arrangement facilitates controlled and precise irradiation under consistent conditions

with 6 MV flattened beams operating in service mode was utilized for irradiation (Fig. 1A).

To ensure accurate dose delivery, the Eppendorf tubes were submerged 10 cm deep within a BluePhantom2 water phantom (IBA Dosimetry, Germany) (Fig. 1B). The irradiation setup was positioned at the LINAC isocenter, maintaining a 90 cm virtual source distance (Fig. 1C).

Dose planning was meticulously carried out using the Monaco 5.51 system. Subsequently, the planned doses were verified through independent measurements using an FC65-G Farmer chamber and Dose1 electrometer (both from IBA Dosimetry).

UV-Vis absorption spectroscopy. UV-Visible absorption spectra of the gold nanoparticle dispersions were recorded using an Evolution 300 spectrophotometer (Thermo Scientific, USA). Measurements were conducted approximately

one hour after irradiation. Samples were transported from the hospital, where the LINAC facility is located, to the laboratory for subsequent spectral analysis.

Deposition of gold nanoparticles, AFM imaging and TEM imaging.

For AFM imaging, gold nanoparticle solutions (both non-irradiated and irradiated with specified doses) were deposited onto mica substrates. Following spin-coating, the samples were dried under a stream of nitrogen gas and subsequently transferred to the AFM system. Imaging was performed using a NanoScope V system (Bruker Ltd, Germany) in tapping mode at room temperature. Silicon cantilevers with aluminum reflex coatings, a tip radius of less than 10 nm, and a resonance frequency of approximately 150 kHz were employed. Images were acquired at a resolution of 512×512 pixels and subsequently flattened using the NanoScope software, which was also utilized for subsequent section analysis. The TEM sample preparation involved depositing gold nanoparticle solutions onto copper grids. After the liquid evaporated, the samples were analyzed for morphology and size using a JEM-2100 LaB₆ Transmission Electron Microscope (JEOL Ltd, Japan).

Results and discussion. Previous studies have reported that AuNPs synthesized using the Turkevich method and exhibiting a size of approximately 20 nm demonstrated minimal changes in their properties when irradiated with doses around 2 Gy [11]. However, as confirmed by UV-Vis spectroscopy and AFM imaging, our findings show that much smaller AuNPs (approximately 8 nm in diameter, 2.5 times smaller) synthesized using the same method exhibit significantly altered optical properties and morphology after irradiation.

The UV-Vis spectra (Fig. 2A) and the absorbance maximum as a function of irradiation dose (Fig. 2B) are shown in Fig. 2. Irradiation, even at the lowest dose, causes a small ($\sim 10\%$) increase in absorbance. This increase becomes substantial ($\sim 45\%$) at 1 Gy. However, further increases in dose lead to a decrease

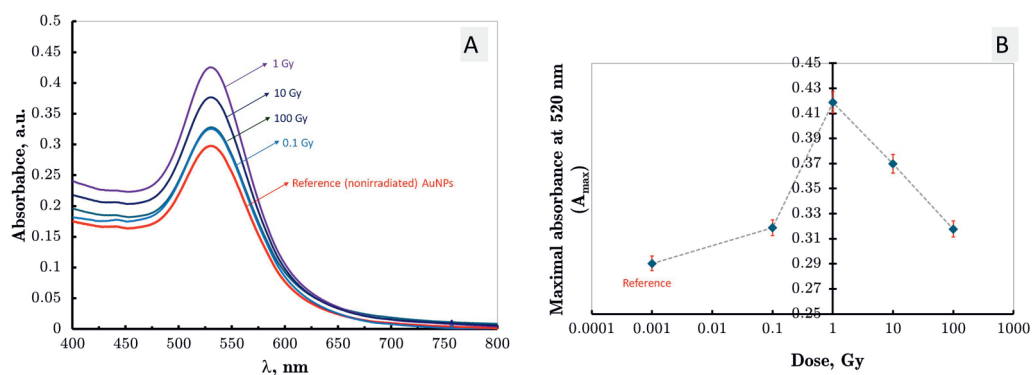


Fig. 2. (A) UV-Vis spectra of AuNP solutions showing the non-irradiated sample (red curve) and irradiated samples with increasing doses as indicated by the arrow. (B) Plot of the maximal absorbance values from the spectra in (A) against dose, with the x-axis presented on a logarithmic scale. The dotted line is included to illustrate the trend

in absorbance, though it remains elevated ($\sim 27\%$ above the control) compared to the non-irradiated sample. At the highest doses, the absorbance approaches a value $\sim 10\%$ greater than the control. The changes in absorbance observed for gold nanoparticles in the 400–450 nm range are likely not due to changes in the quantity of material, as has been reported for the growth of nanosized gold clusters in glass [14]. Instead, the increased intensity and slight broadening of the 520 nm peak are likely a result of increased AuNP size, potentially accompanied by changes in shape.

To further explore this unusual behaviour of the AuNPs we deposited their solutions on mica solid supports. The topography of the samples which correspond to different X-ray irradiation doses (0.1 Gy, 1.0 Gy, 10 Gy and 100 Gy) were scanned at areas $5 \times 5 \mu\text{m}$ and corroborates these changes in UV-Vis spectra showing significant alterations in their morphology. For a clearer visualization of these changes in Fig. 3 (upper images in each panel), AFM images are also presented in a 3D format. Four of these images correspond to AuNPs samples irradiated with a specific dose, alongside the reference sample. In the image of the reference sample, individual nanoparticles can be easily distinguished and measured, exhibiting a spherical shape, monodispersity, and an absence of surface aggregates, consistent with previously reported results [13].

To compare the sizes of synthesized AuNPs irradiated with varying X-ray doses, cross-section analysis data are also presented in Fig. 3. From the 2D-

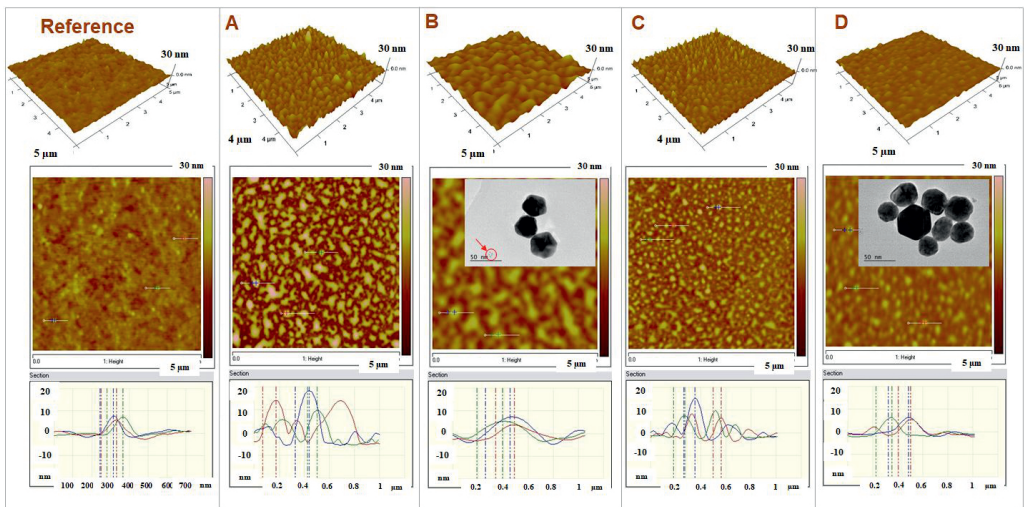


Fig. 3. AFM images of gold nanoparticles on mica at varying X-ray irradiation doses: (A) 0.1 Gy, (B) 1.0 Gy, (C) 10 Gy, and (D) 100 Gy, compared to a reference sample (Reference). Each panel shows a 3D image (top) and a 2D image with a cross-sectional profile. All images are $5 \times 5 \mu\text{m}$ with a 30 nm z-range. Panels (B) and (D) include TEM images of the 1.0 Gy and 100.0 Gy samples, respectively, with a scale bar of 50 nm. In image (B), the arrows indicate the presence of small-sized nanoparticles

AFM image and cross-section analysis of the reference sample, it is apparent that the AuNPs exhibit spherical shapes with diameters ranging from 8 to 9 nm, as determined by z -height measurements. The surface of the sample irradiated with a dose of 0.1 Gy displays increased complexity. While many AuNPs retain near-spherical shapes with sizes between 11 and 13 nm, a notable presence of larger, irregularly shaped particles with dimensions up to 25 nm is observed. The surface characteristics of the 1 Gy sample are even more intricate, featuring a variety of irregularly shaped particles. These include nanoparticles with heights around 10 nm, alongside smaller particles measuring between 4 and 6 nm. As seen in the inset of Fig. 3B, the TEM image confirms the presence of these small nanoparticles (indicated by arrows), while also revealing the existence of larger particles exceeding 50 nm.

At higher irradiation doses of 10 Gy and 100 Gy, the AuNPs exhibit polydispersity but appear more uniformly dispersed compared to the samples irradiated at 0.1 Gy and 1 Gy. The 10 Gy sample primarily consists of spherical nanoparticles with diameters ranging from 8 to 11 nm, whereas the 100 Gy sample contains particles measuring between 6 and 14 nm. TEM images further confirm the presence of larger, irregularly shaped AuNPs, as exemplified by the TEM image of the 100 Gy-irradiated sample (inset of Fig. 3D).

For better visualization, a comprehensive summary of the sizes and shapes of the AuNPs at each irradiation dose is presented in Fig. 4. Due to the pronounced polydispersity observed in the 0.1 Gy and 1 Gy samples, establishing a

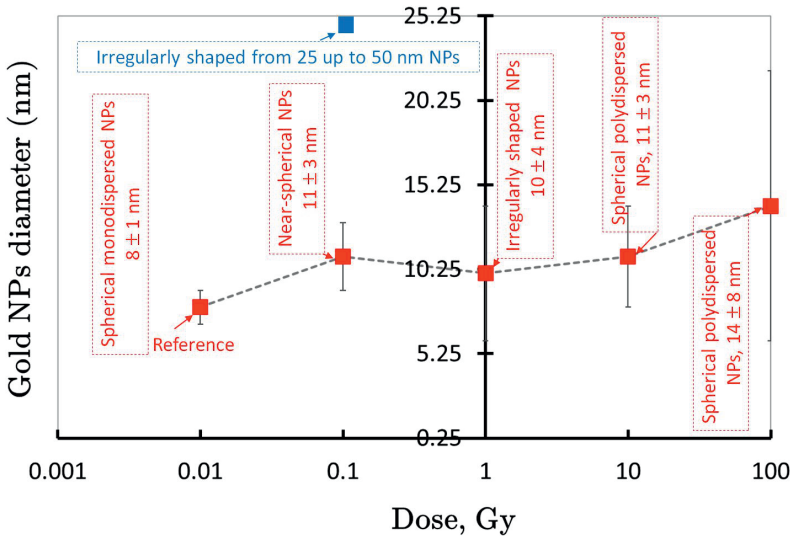


Fig. 4. Gold nanoparticle (AuNP) diameter as a function of irradiation dose (logarithmic scale), measured and statistically analyzed from AFM images. The corresponding nanoparticle shapes for each sample are also indicated on the graph

direct correlation between the UV-Vis spectra and AFM data would not be reliable. However, it is evident that X-ray irradiation influences the properties of AuNPs and further experimental and analytical methods are needed to elucidate the mechanisms driving these alterations.

Conclusions. This study investigates the effects of ionizing radiation generated by a medical LINAC on AuNPs synthesized using the classical Turkevich method. Changes in the size, shape, and optical properties of AuNPs exposed to varying X-ray irradiation doses were assessed using UV-Vis spectroscopy and AFM. An increase in the plasmon maxima of the AuNPs was observed with increasing irradiation dose, reaching a maximum of 44% at 1 Gy relative to non-irradiated samples. This increase was also accompanied by significant morphological alterations, with the AuNPs transitioning from monodispersed and spherical shapes to more complex and irregular structures. At higher irradiation doses, the plasmon maxima in the UV-Vis spectra decreased, with the 100 Gy-irradiated AuNPs exhibiting a maximum that was only 10% higher than that of the non-irradiated sample, similar to those irradiated at 0.1 Gy. Nevertheless, at these higher doses, the nanoparticles are larger than the nonirradiated ones and exhibit more regular close-to-spherical shapes. In conclusion, this study supports the hypothesis that ionizing radiation from a medical linear accelerator (LINAC) alters both the physicochemical properties and morphology of gold nanoparticles (AuNPs). These results demonstrate the need for expanded research utilizing advanced nanoscale analytical methodologies. Future experimental designs should incorporate pH monitoring and the characterization of biotinylation or PEGylation effects to optimize the biocompatibility and efficacy of gold nanoparticles in radiotherapy and other medical applications.

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