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Preparation, Physicochemical Characterization and Performance Evaluation of Gold Nanoparticles in Radiotherapy

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ABSTRACT

***Purpose*:** The aim of the present study was preparation, physicochemical characterization and performance evaluation of gold nanoparticles (GNPs) in radiotherapy. Another objective was the investigation of anti-bacterial efficacy of gold nanoparticle against E. coli clinical strains. ***Methods*:** Gold nanoparticles prepared by controlled reduction of an aqueous $HAuCl_4$ solution using Tri sodium citrate. Particle size analysis and Transmission electron microscopy were used for physicochemical characterization. Polymer gel dosimetry was used for evaluation of the enhancement of absorbed dose. Diffusion method in agar media was used for investigation of anti-bacterial effect. ***Results*:** Gold nanoparticles synthesized in size range from 57 nm to 346 nm by planning different formulation. Gold nanoparticle in 57 nm size increased radiation dose effectiveness with the magnitude of about 21 %. At the concentration of 400 ppm, Nano gold exhibited significant anti-bacterial effect against E. coli clinical strains. ***Conclusion*:** It is concluded that gold nanoparticles can be applied as dose enhancer in radiotherapy. The Investigation of anti-bacterial efficacy showed that gold nanoparticle had significant effect against E. coli clinical strains.

Introduction

Nanotechnology is beginning to show its impact on the way the health care is administered. These include new interventions in disease detection, treatment and prevention, which are collectively termed as nanomedicine.[1] The most well-studied nanoparticles include quantum dots, carbon nanotubes, paramagnetic nanoparticles, liposomes, gold nanoparticles (GNPs), and many others.[2] In recent years, gold nanoparticles have attracted much attention. They are agents with numerous applications in biomedicine like cancer research, diagnostic assay,[3-5] thermal ablation, gene and drug delivery,[6-8] etc. Nano gold have several unique properties, For example they are inert and nontoxic[9] and have good anti-bacterial,[10] anti-angiogenesis properties,[11] etc. GNPs have been prepared by both "physical" and "chemical" methods. For the "physical" preparation method, Au bulk is broken down by a strong attack force, for example, ion irradiation in air or arc discharge in water, to generate GNPs. Chemical method including chemical reduction of Au salts, electrochemical pathways and decomposition of organometallic compounds. Among them, the chemical reduction method is simple and controllable to prepare various sizes and shapes of GNPs.[12,13]

Today Cancer is the third leading cause of death in developed countries and the second leading cause of death in the United States.[2] Treatment of Cancer includes chemotherapy, surgery and radiotherapy. Although radiation therapy is one of the most preferred cure and has been practiced for about 100 years in cancer treatment, but this treatment has a lot of side effects. So scientists are looking for new ways to enhance effect of radiotherapy and lower damage to the normal cell.[14] The concept of using high-*Z* materials as dose enhancement in cancer radiotherapy has long been investigated. Several studies have focused on the potential application of GNPs in conjunction with radiation therapy.[15] The aim of this project was preparation, characterization of GNPs with the intention of absorbed dose enhancement in tumor cells. Anti-bacterial effect of prepared GNPs against clinical strains of E. coli was also investigated.

Materials and Methods

Materials

$HAuCl_4$ was purchased from Alfa Aesar (Great Britain). Tri sodium citrate was obtained from Scharlau (Spania). N,N'-methylenebis-acrylamide (bis) acrylamide (AA), Tetrakis (Hydroxymethyl)

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Phosphonium Chloride and Gelatin were obtained from Sigma chemical company. Mueller Hinton agar was purchased from Liofilchem. De-ionized water was used to prepare aqueous solutions.

Gold nanoparticles preparation

Gold nanoparticles were prepared by the classical citrate reduction (frens method). Briefly, 20ml of $HAuCl_4$ water solution (1 mM) was kept boiling. Various volume of 1% sodium citrate water solution was then added to the solution and stirred for about 10 min, until the formation of a colored gold nanoparticle suspension. Table 1 shows different citrate volume that use for preparation of GNPs.

Table 1. Different formulation for preparation of gold nanoparticle and their particle size and polydispersity index

Formulation No.	Volume of $HAuCl_4$ 1mM(ml)	Volume of citrate 1% (ml)	Particle size(nm)	Polydispersity Index
F_1	20	1.9	-	-
F_2	20	1.8	-	-
F_3	20	1.7	57	1.21
F_4	20	1.6	74	1.85
F_5	20	1.5	136	1.20
F_6	20	1.3	259	1.11
F_7	20	1.2	346	1.08

Characterization of GNPs

The mean particle-size values of GNPs were measured by using a laser diffraction particle-size analyzer (Sald 2101, Shimadzu, Japan) equipped with Wing software (version 1.20). The morphology of the nanoparticles was investigated by Transmission electron microscopy (TEM) (LEO906, Germany). Drops of the gold suspension (formulation F_6) were deposited and dried onto a Formvar-coated copper grid. The UV–visible absorption spectra of the one of the prepared colloidal solutions recorded using a spectrophotometer (Shimadzu, Japan), from 400 to 900 nm.

Gel dosimetry

Gel fabrication

The gel solution consists of water (89 % of total mass), acrylamide (3 %), N,N-methylene-bisacrylamide (3 %) and gelatin (5 %). The gel components were mixed together at 35-40 °C in a 500 ml beaker. An oxygen scavenger, Tetrakis (hydroxyl methyl) phosphonium chloride (THPC), was added to the gel mixture at a concentration of 10 mM as anti-oxidant. Nano gold (formulation F_3) was used as a part of water in gel preparation to fabricate gel with GNPs batch. The GNPs were observed to mix homogeneously in the gel. Another batch of gel without GNPs served as a control. The gel was then quickly poured into separate tubes.

Irradiation

The tubes of the both batches were irradiated with CT scanner after put them in a head and neck phantom. The gel samples were exposed to radiation doses of 40, 80 and 120 Gy. Irradiation of the gel samples was carried out at Day CT scanner center with following parameters: slice thickness=1 cm, t=0.8 s/turn, mA=200, kVp =140.

Magnetic Resonance Imaging (MRI)

Irradiated and non-irradiated gel samples were scanned using a 1.5 T MRI scanner (GE Sigma, Milwaukee, USA), to measure spin–spin relaxation time of the free protons using a head coil. A fast-spin echo sequence was used with following parameters: field of view =105 × 120 mm^2, slice thickness = 5 mm (kV X-ray beams), effective echo time TE = 22 ms, turbo factor = 14, repetition time (TR) = 5,710 ms, the field of view = 128 ×128 matrix, total imaging time = 20 min. At least 24 h elapsed after irradiation prior to imaging to allow for polymerization. All the samples were scanned at room temperature.

Data analysis

Analysis of the image was performed using MATLAB software (version 3.5.7) (The Math Works Inc, Natick, Massachusetts, USA). The program examined the data before analyzing it to determine the region of interest. T_2 values were calculated and formed T_2 maps on a pixel-by-pixel basis. The levels of the polymerization of the irradiated gels with and without GNPs were compared by calculating the R_2 ($1/T_2$).

Anti-bacterial test

Antibacterial activity was studied by the agar-well-diffusion method, wherein 100 µl bacterial suspension was added to 20-mL sterile nutrient Mueller Hinton agar at 45 °C and the mixture was solidified on a Petri dish. After the medium had solidified, 7-mm-diameter wells were made in the agar (three wells per dish) that were equidistant from one another and from the dish edge. The wells received 150 µL of different concentration of GNPs from formulation F_2 (400 ppm, 200 ppm, 100 ppm). The petri dishes were incubated in a thermostat at 37 °C for 24 h. After incubation, the diameter of the zone of bacterial-growth inhibition was measured. All experiments were done for five clinical strains of E. coli and repeated thrice.

Results and Discussion

Physicochemical properties of GNPs

The influence of citrate volume in particle size is shown in Table 1. This data indicated that as citrate volume increase, the particle size of gold nanoparticle get smaller. TEM image show that synthesized GNPs are spherical in shape (Figure1). Narrow range of sizes was achieved using reduction method (Figure 2). The formation of GNPs was confirmed from an absorption maximum at 532 nm. The absence of absorbance at wavelengths greater than 600 nm confirmed their well dispersed state in solution[16] (Figure 3).

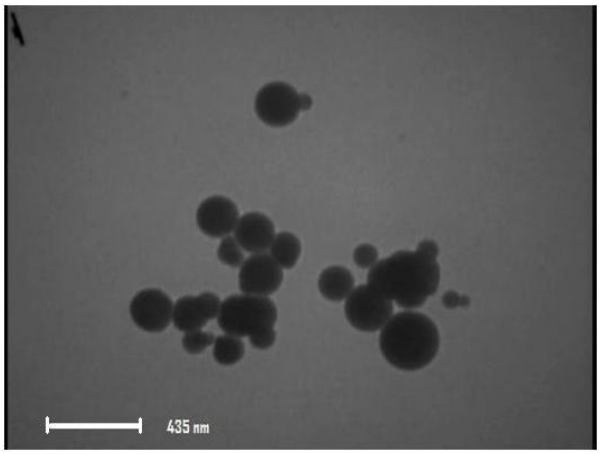

Figure 1. TEM image of gold nanoparticle (formulation F_6)

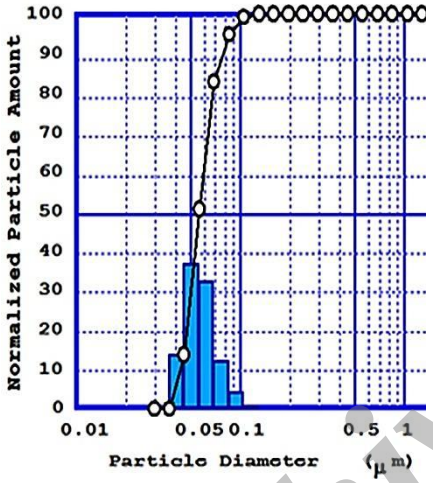

Figure 2. Particle size distribution of gold nanoparticles (formulation F_1)

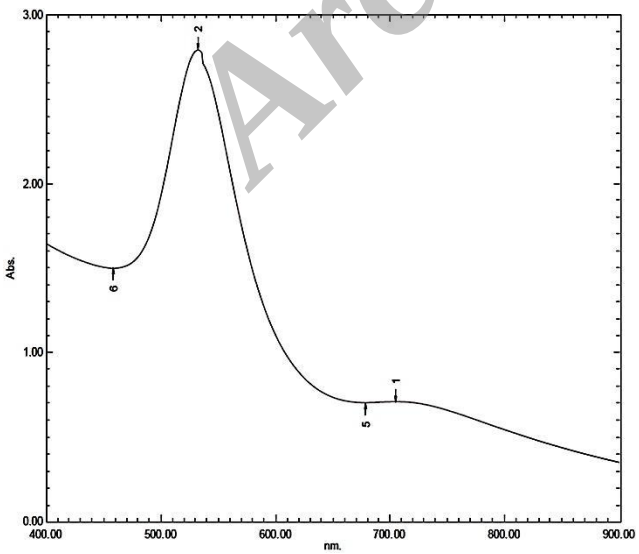

Figure 3. UV–Vis spectra of 250 nM gold nanoparticles (formulation F_3)

Gel dosimetry

The relationship between the delivered X-ray dose and the R_2 (spin–spin relaxation rate) was investigated to characterize the effect of GNPs using polymer gel. R_2 is equal to $1/T_2$ (spin–spin relaxation time) and is a function of dose (dose delivered to water). A linear relationship is found between delivered dose and R_2 (Figure 4). The dose–response slopes for R_2 versus delivered X-ray dose for gel–GNP and pure gel were calculated. The ratio of these slopes was taken as the dose enhancement factor (DEF). The DEF of 1.21 was obtained for the dose–response relationship. Dose enhancement by high Z material is believed to be caused predominantly by enhancing the likelihood of the photoelectric interaction. When GNPs are added to the gel prior to irradiation and bombarded with kilovoltage X-rays, the photoelectric interaction cross section will increase. This can be clearly inferred from the interaction probability of these X-ray photons with gold atoms compared to their interaction with the tissue equivalent medium such as water.

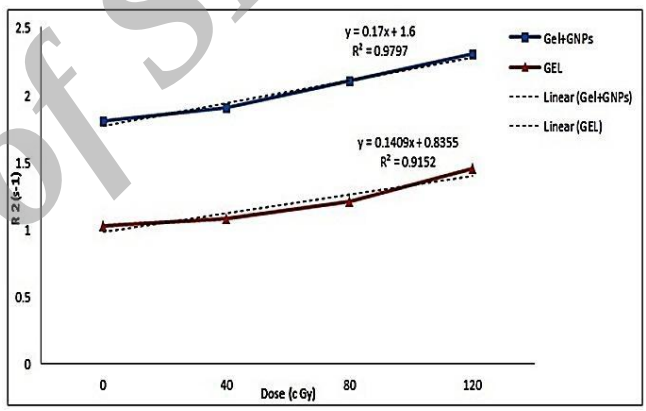

Figure 4. dose–response curve for pure gel and gel–GNPs

Antimicrobial activity of GNPs against E. coli clinical strains

Table 2 indicates Mean inhibitory diameters related to different concentrations of GNPs (formulation F_2). At the concentration of 400 ppm GNPs exhibited good effect against E. coli clinical strains. The concentration 200 ppm had a little effect and 100 ppm almost had no effect. GNPs exert their antibacterial action mainly by two ways: one is to change membrane potential and inhibit ATP synthase activities to decrease the ATP level, indicating a general decline in metabolism. The other mechanism is to inhibit the subunit of ribosome for tRNA binding, indicating a collapse of biological process.[17] Nishat et al. reported a simple one step microwave irradiation method for the synthesis of GNPs using citric acid as reducing agent and cetyl trimethyl ammonium bromide (CTAB) as binding agent. They investigated antibacterial efficacy of the nano gold against E. coli standard strain and reported high antibacterial activity with zone of inhibition of about 22 mm against E. coli.[18] This result showed more anti-bacterial effectiveness of nano gold against E. coli.

CTAB is a potent anti-microbial agent, so this difference may be related to use of this material.

Table 2. Inhibition zone diameter (mm) of different concentration of nano gold loaded in plates with *E. coli* clinical strains inoculums

Strain	100 ppm	200 ppm	400 ppm
1	0	8.33	10
2	5.5	9	11
3	0	8.66	11.33
4	0	5.33	11.33
5	0	2.66	8.16

Conclusion

We reported here the measurement of radiation dose enhancement generated by GNPs using polymer gel dosimeters as a phantom. This study has found a significant dose enhancement from the inclusion of the GNPs within polymer gels irradiated with kilovoltage X-rays beams from a therapy machine. Besides, GNPs exhibited a good anti-microbial effect against E. coli clinical strains at 400 ppm concentration.

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Conflict of Interest

The authors report no conflicts of interest.

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