Silver nanoparticles as free radical scavengers for protection from nuclear radiation hazards

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Abstract

Silver nanoparticles have found tremendous applications in the field of diagnostics and therapeutics. This work was aimed to study the antioxidant activity of Ag nanoparticles in water by scavengers the free radicals produced by radiation. The antioxidant properties of silver nanoparticles prepared by wire explosion technique have been evaluated using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay. Ag nanoparticles were characterized by UV-Visible absorption and the results showed surface Plasmon resonance absorption peaks around 397nm. The nanoparticle average size and its size distribution were determinate by Atomic Force Microscopy (AFM). The results showed that the absorption intensity varying systematically with increasing silver nanoparticles concentration with certain limits. So Ag nanoparticles were found to have significant antioxidant capacity and thus can be used as potential radical scavenger against deleterious damages caused by the free radicals.

Keywords: Silver nanoparticles, radical scavenger, DPPH assay.

دقائق الفضة النانوية النانوية كمضادات للأكسدة للحماية من اضرار الاشعاع النووي

الخلاصة

قد وجدت للفضة النانوية تطبيقات هائلة في مجال التشخيص والعلاج. ويهدف هذا العمل إلى دراسة فعالية الفضة النانوية كمضادات للأكسدة في الماء عن طريق كسح الجذور الحرة التي تنتجها الإشعاع . تم تقييم الخصائص المضادة للأكسدة للفضة النانوية المحضرة بواسطة تقنية انفجار السلك وذلك باستخدام فحص 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH). ميزت جسيمات الفضة النانوية بواسطة طيف الامتصاص UV-Visible وأظهرت النتائج ذروة امتصاص للرنين البلازمي السطحي حول 397nm . تحدد معدل الحجم والتوزيع الحجمي للجسيمات النانوية بواسطة مجهر القوة الذرية (AFM). أظهرت النتائج ان شدة الأمتصاصية تتغير بصورة منتظمة مع زيادة تركيز الفضة ولمدى محدد. لذلك فان الفضة النانوية لها القدرة الكبيرة كمضادات للاكسدة ويمكن استخدامها كاسحات جيدة للجذور ضد الضرر الكبير الناتج من الجذور الحرة.

INTRODUCTION

Nanotechnology is unique in that it represents not just one specific area, but a vast variety of disciplines ranging from basic material science to personal care applications [1]. The development of nanoparticles for the delivery of therapeutic agents has introduced new opportunities for the improvement of medical

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treatment [2]. Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures [3], they exhibit completely new or improved properties bases on specific characteristics such as size, distribution and morphology [4] than compared to the bulk materials. Nanomaterials are present in some sunscreens, toothpastes, sanitary ware coatings and even food products [6]. Nanoparticles of noble metals, such as gold, silver, and platinum, are widely applied in products that directly come in contact with the human body, so there is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals [7]. Among the various inorganic metal nanoparticles, silver (Ag) nanoparticles have received substantial attention for various reasons. Silver is an effective antimicrobial agent, exhibits low toxicity and has diverse *in vitro* and *in vivo* applications [8]. The most widely used and known applications of silver and silver nanoparticles are in the medical industry [7].

The inorganic nanoparticles are found to be effective in scavenging oxygen based free radicals [9]. A free radical is defined as a molecular species capable of independent existence and which contains one or more unpaired electrons [10]. Reactive oxygen species (ROS) are small, highly reactive, oxygen-containing molecules that are naturally generated in small amounts during the body's metabolic reactions and damage complex cellular molecules such as fats, proteins, or DNA [11]. Antioxidants are the substances which act as free radical scavengers by preventing and repairing damages caused by reactive oxygen species, and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases [12].

The synthesis of noble metal nanoparticles attracts an increasing interest due to their new and different characteristics as compared with those of macroscopic phase, that allow attractive applications in various fields such as antimicrobials, medicine, biotechnology, optics, microelectronics, catalysis, information storage and energy conversion[13]. Among all the known chemical and physical preparation methods, the exploding wire technique is one of the newest and simplest methods for producing metal nanoparticles [14, 15].The explosion is achieved when a very high current density is applied to a thin metal wire, causing the wire to explode to very small fragments. This process involves wire heating and melting followed by wire evaporation, formation of a high-density core surrounded by low-density ionized corona, coronal compression, and fast expansion of the explosion products [16].

The most effectively studied nanoparticles today are those made from Noble metals, in particular Ag [17], Au [18], Pt [19] and Palladium. Metal nanoparticles find vast applications in various fields ranging from medical to physical fields [20,21,22].

Among the four, silver nanoparticles play a significant role in the field of biological system, living organism and medicine [23-25].Conventionally, nanomaterials are synthesized using either chemical or physical methods which include micelles[26] sol process, chemical precipitation, mechanical shaking, hydrothermal method and chemical vapour deposition method [27].

Ionizing radiation was divided into directly and indirectly ionizing for the understanding of biological effects. Electromagnetic radiations, x and γ rays, are indirectly ionizing because they do not produce chemical and biological damage themselves but produce secondary electrons (charged particles) after energy absorption in the material.

Free radicals are generated in great number by ionizing radiation due to the process of energy absorption and breakage of chemical bonds in molecules. Then they play a major role in radiation effects on biological tissues and organisms. These radicals are highly reactive and found in a number of biological processes, metabolism, oxidation, reduction, and pathological diseases and cancer induction. The radicals formed after passage of radiation and water radiolysis, namely the hydrated electron (eaq -), the hydrogen atom (H.) and the hydroxyl radical (.OH) contribute in causing damage to biological systems[28].

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry, so it can be useful to assess various products at a time.

This is known as a standard 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay. The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. The antioxidant effect is proportional to the disappearance of DPPH in test samples. Monitoring DPPH with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH showed a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm [29].

The aim of the present work was used silver nanoparticles (AgNPs) as free radicals scavengers and protects water samples from gamma irradiation.

Experimental details

Preparation of silver nanoparticles

Electrical explosion of wire (EEW) in gas has been applied to synthesize many kinds of nanomaterials including metal and compounds .More recently, this method has been developed to synthesize metal nanoparticles in a solution [30]. Compared with the EEW in gas, EEW in liquid has been less investigated. It has become one of promising method for synthesis metal nanoparticles because of its simplicity, effectiveness and low cost. Synthesis of nanoparticles in liquid does not needs vacuum system. In addition, nanoparticles can synthesize in water without impurities or in any arbitrary solution. In the explosion process, several parameters such as voltage, current pulse, material type and their wire dimension, and the medium in which the explosion is performed these effects the properties of product. Silver nanoparticle was produced by electrical explosion of wire in deionized water (DIW) as a medium of explosion. A thin silver wire (0.4 mm in diameter, 50 mm in length) was used.

Determination of free radical scavenging activity

The free radical scavenging capacity of silver nanoparticles was determined by using DPPH assay. DPPH (2, 2-diphenyl-1- picrylhydrazyl) is a stable free radical and has been used as a model free radical compound to evaluate the effectiveness of antioxidant. Ethanoic solution of DPPH (0.1 mM) was prepared and incubated at ambient temperature. The absorbance of all samples was measured by UV- VIS

Spectrophotometer. The percentage inhibition of DPPH was calculated according to the formula:

$$\% \text{ Inhibition } (I\%) = [(A_{ref} - A_s)/A_{ref}] \times 100 \quad \ldots(1)$$

Where:

A_{ref} is the absorbance of irradiated water samples as a reference, and A_s is the absorbance of samples with different concentration of silver nanoparticle.

Material:

In this work, deionized water was used as a sample for the study of scavenging free radicals because it represents the largest proportion of the human body (75%), furthermore ionizing radiation affects a severe impact on the water and leads to the formation of free radicals. Water samples were irradiated by ^{137}Cs gamma source at a dose rate of 0.2 rad / hr.

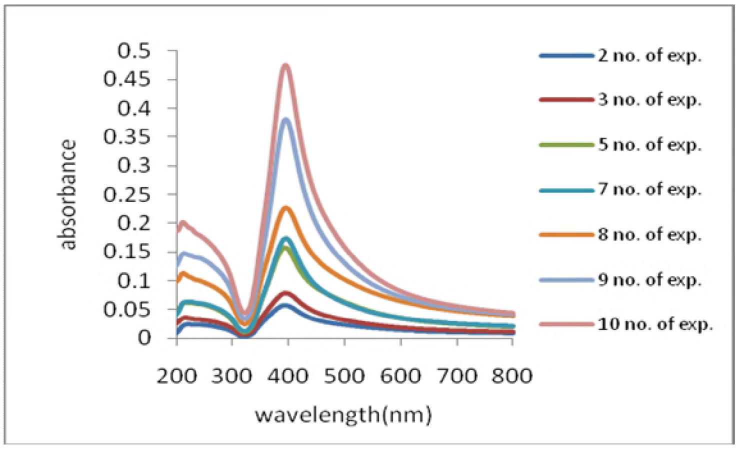
Silver nanoparticles were added with different concentration (0.008, 0.0126, 0.0256, 0.0387, 0.0661, 0.0825gm/l) to the water samples before and after irradiation, and then mixed with fixed amount of ethanoic DPPH solution to assess the inhibition of free radical.

Results and discussion

Characterization of Silver Nanoparticles

Characterization of silver nanoparticles by UV-VIS Spectroscopy.

The absorption spectrum of prepared silver nanoparticles was investigated by UV-vis method in the wavelength range of 200–800 nm. The peak of absorption was around 395nm for most samples. Fig.1 shows the absorbance of silver nanoparticles with increasing the silver nanoparticles concertation.



Figure(1) UV-VIS absorption spectra of silver nanoparticles functions of its concentration

The concentration of Ag NPs was calculated using standard solution, according to calibration curve of silver nanopoweder.

Atomic Force Microscopy (AFM): The morphology and the size of the prepared silver nanoparticles were determined by atomic force microscopy.

Fig. 2 shows the histogram of Ag NPs size, Fig.3_a two dimensions image and Fig 3_b three dimensions image.

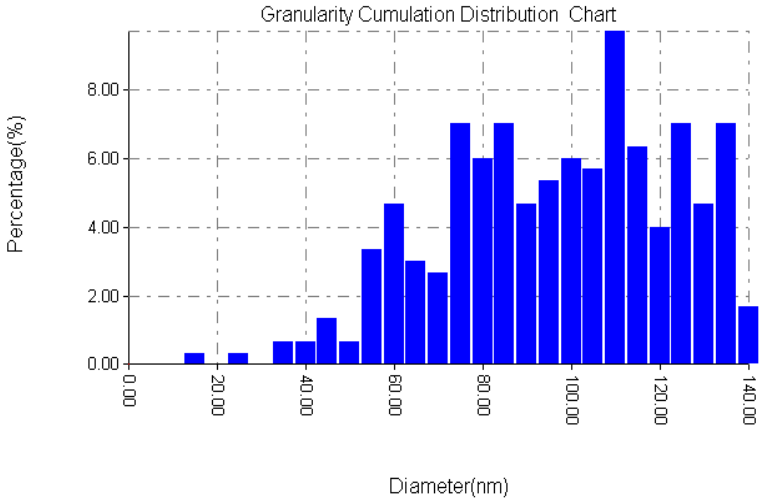


Figure (2):The histogram of Ag NPs size.

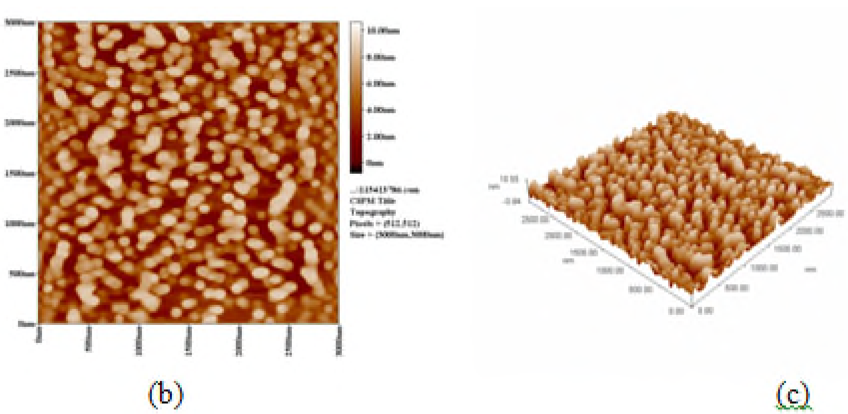


Figure (3) 3_a and 3_b: AFM image of Ag NPs in two dimensions and three dimensions respectively

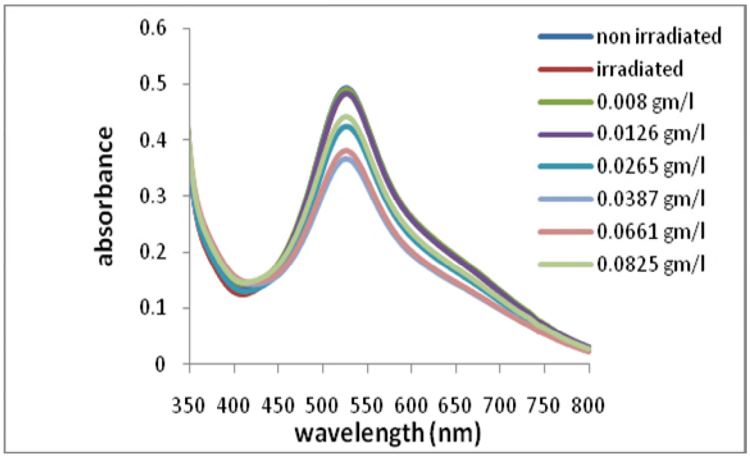
DPPH free radical scavenging activity of silver nanoparticles

The absorbance of DPPH at 526 nm for normal samples (not irradiated), irradiated samples and the samples with addition the different concentration of silver nanoparticles before and after irradiation were summarized in Table 1.

Table (1) Values of the DPPH absorption intensity at 526 nm before and after irradiation

Samples	Absorption	
	Before irradiation	After irradiation
Normal (not irradiation)	0.486	0.496
Irradiation	0.494	0.502
Ag NPs concentration (gm/l)		
0.04	0.493	0.447
0.06	0.484	0.453
0.13	0.426	0.399
0.19	0.367	0.356
0.33	0.382	0.368
0.41	0.443	0.538

The absorbance spectra of DPPH at 526 nm for the samples with Ag NPs before irradiation and the samples after irradiation were showed in Figs. 4_a and 4_b.



Figure(4)a: DPPH absorbance at 526 nm before irradiation.

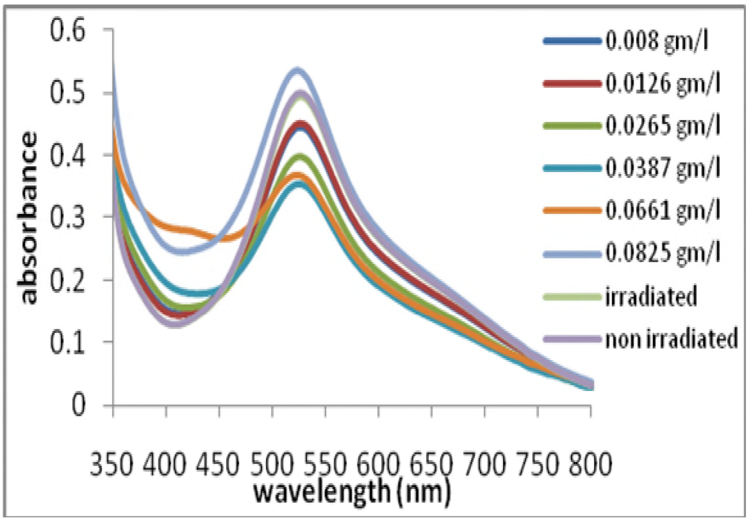
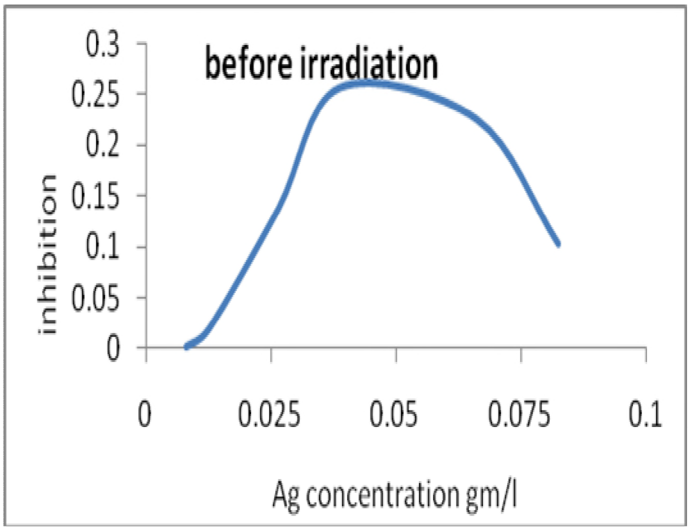
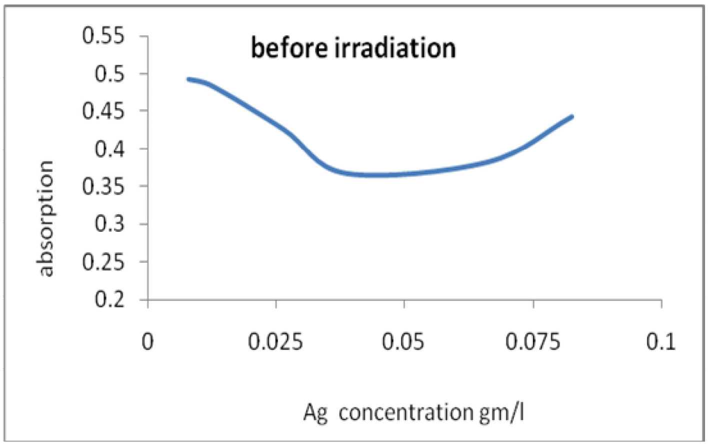


Figure (4)b : DPPH absorbance at 526 nm after irradiation

When silver nanoparticles were added with different concentrations to the water sample before the irradiation, the intensity of the DPPH absorbance decreases with the increasing of Ag concentration in finite range (0.0265- 0.0661 gm/l) and then the absorption increases with increasing Ag concentration as shown in Figs. 5_a.In Fig.4_b free radical inhibition were increased with increasing Ag concentration in the same finite range. The behavior of absorption and inhibition were due to the Ag nanoparticles interaction with free radicals and equivalent to become neutral molecules.

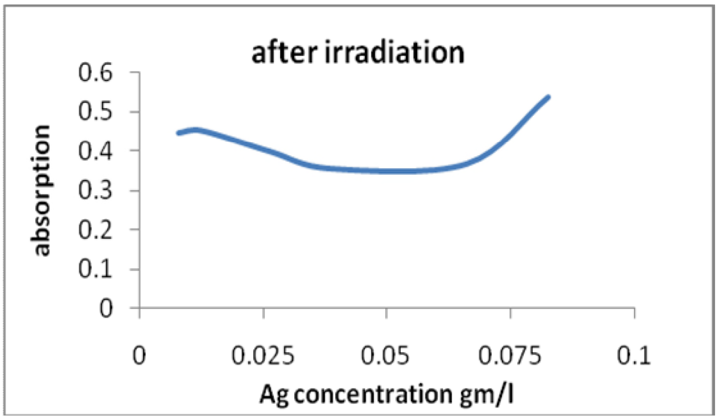


Figure(5)$_a$: Inhibition of free radical as a function of Ag NPs concentration.

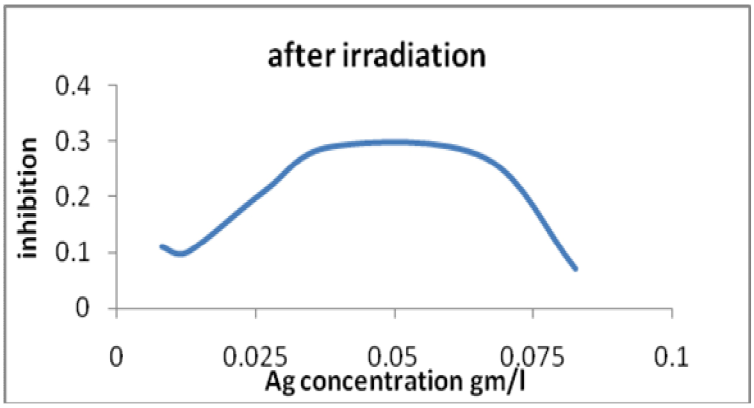


Figure(5)$_b$:Absorption of DPPH as a function of Ag NPs concentration

When the addition of Ag NPs with the same concentration to the water sample after irradiation, the behavior of absorption and inhibition is approximately the same with the behavior when the addition before irradiation, as shown in Fig.s 6_a and 6_b .



Figure(6)$_a$:The absorbance as a function of Ag NPs concentration.



Figure(6)$_b$: Inhibition of free radical as a function of Ag NPs concentration

Conclusions

The present results suggest the possibility of using Ag NPs as an anti-oxidant agent by inhibition the formation of free radicals and scavenging all species of (ROS) from the irradiation water samples in certain range of concentration of Ag NPs. And addition of Ag NPs before irradiation water samples leads to greater scavenged free radicals efficient than if nanoparticles added after irradiation.

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