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# Studying the effect of exposure time for Nanocomposite Gold Nanoparticles with Hyaluronic acid synthesis using the atmospheric air jet plasma

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**Abstract:** The atmospheric air cold plasma has been used in the manufacture of gold nanomaterials. Where plasma was generated non-thermal atmospheric pressure using diameter of 1 mm. Aqueous gold tetrachloride salts HAuCl4.4H2O is used for production of gold nanoparticles with Hyaluronic Acid (HA).
The experiment was conducted in parameter of time, where the use aqueous gold tetrachloride salts HAuCl4.4H2O in concentration of 1 mM with Hyaluronic Acid (HA) in concentration of 1mM in a different exposure time ( 3, 4, 5, 6 ) min.
To insure the typical preparing of the nanoparticles, UV-visible and X-ray have been conducted for that purpose. Morphology of the AuNPs were carried out by FESEM.
The outcome of the study revealed that the atmospheric air cold plasma is a promising technique to be used in production of the nanoparticle's materials for the medical application.

***Key word:*** Cold Plasma, Gold Nanoparticles, Plasma-Liquid Interactions.

**Introduction:**

Besides to the solid state, liquid and gas, the Plasma is one of the four fundamental states of matter, both of them are closely or appropriate to the modern industry and human life. There is an equal number of positive and negative ions in plasmas resulting from ionization of neutral gases generally[1]. It's consider one of the main a component parts of the universe: interstellar and stellar matter is regard a plasma state which is widely spread in nature.

Plasmas are consider an ionized gas that habitually consist of reactive species, electrons, photons of UV, neutrals, etc. The cold atmospheric plasma habitually is generated at temperature of room [2]. The plasmas are produces by chambers of internal vacuum at low pressures also so-called quasi equilibrium plasmas or low-temperature thermal plasmas [3]. Because temperatures of heavy and light species are same approximately. Dielectric barrier discharges (DBD) can be produced at high pressure by the gas ionization between two electrodes tight in the ambient environment that means the plasma generation requires no expensive vacuum equipment.

cold plasma can be generated with similar technique by one electrode and the second one be living tissue. This scheme type called "floating electrode dielectric barrier discharges" (FE-DBD) [4,5].

There is an increasing attention electrical discharges production on and inner the liquid surfaces beneath cold atmospheric plasmas conditions (atmospheric conditions).

Plasma of atmospheric pressure is nonequilibrium or non-thermal because the temperature of ions is much lower than electron, in addition to that, the temperature of room is stay similar to species of gas. It is can use thermal and non-thermal plasma (both kinds) for synthesis of nanomaterial. Using different gases like argon and hydrogen gas, synthesize nanomaterials by cold plasma due to its unparalleled characteristics that regarding to their bulk equivalents [5].

In the last years, nanomaterials have much attention due to their tiny size and extremely high surface area. And explanated in human body many biological activities, which plays in the biomedicine an important roles in several applications such as delivery of drug[16], cancer inhibition, disinfect bacteria [7] and usage of nanomaterials in water to eliminate some contaminants [8,9]..ect.

In literature, some studies alleged that there is a harms can accrue for living organisms as a result of using the nanomaterials. And the approach of chemical conventional for synthesis of nanomaterial that required using oxidants toxic or reductions fundamental for stabilization and formation of nanoparticle, and this

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could be considerd the clarification reasonable for that problem. Therefore, the alternative synthesis of free of toxic chemicals are important for development of nanotechnology with regard to applications of biomedical.

Lately, technology of plasma is acquisiting for nanomaterials a great attention as a prominent "green" synthesis method, that are consider a characteristic properties when it compared to gas, liquid and solid phase synthesis approaches, on the other hand, in applications of biomedical the combining of the plasma and nanomaterials are demonstrates many synergistic effects and efficiency of treatment.

Hyaluronic Acid (HA) discovered by the scientist K. Meyer and his assistant, J. Palmer during 1934[10,11]. This acid has unique properties as high molecular weight lipopolysaccharide [12]. This acid consider a biopolymer that is naturally occurring, has an important multiple biological functions as in humans beings, animals and bacteria [13].

HA is available in great amount of connective tissues and settled specially in the vitreous fluid of the eye, fluid of synovial, chicken combs and umbilical cords [12,14]. Its created naturally through some types of integral membrane proteins which calls "hyaluronan synthases", that degraded by enzymes family so called hyaluronidases [15].

The physico-chemical properties of this polymer are important from both scientific and practical point of view.

UV-irradiation can modify several properties of natural polymers. Natural polymers are widely applied in several fields, such as biomedical and cosmetic ones. They are also widely used in packaging and textile industry but after sterilization for products made of natural polymer for biomedical applications.

For the sterilization of materials made of polymers, both high temperature and UV radiation can be used [11]. UV light can be also considered as a tool for surface modification of polymeric biomaterials.

Regarding the requirement of UV sterilization of biomaterials, there is a need to investigate the properties of materials after UV-irradiation and to understand photochemical processes in the materials [14].

## Material And Methods

### Linking the system

Normal atmospheric air cold plasma is used in the synthesis gold nanomaterials.

The plasma system consists of the following parts:

Aqueous gold salts ($HAuCl_4.4H_2O$) with a molecular weight of 411.8 g / mol dissolved in ionic water at a concentration of 1 mM. Capillary (with diameter 1mm ) in which the gas flowed through it and connected to the negative terminal of the power supply, and placed at 1 cm above the liquid. Stainless steel conductive length7 cm and width 5 mm strip ends with a 1 x 1 flat end that connects to the anode inside the aqueous solution. DC Power supply with high voltage about 6 KV. Argon gas bottle which is affordably available in the market, and contains a gas outlet containing a regulator to control the speed of exit gas. Flowmeter meter with a calibrator of (1-5) minutes/liter to control the Argon intake which connects to the hollow metal tube, we use a calibrator of 2.5 minutes/liter. A glass vessel is used in this work as a cell. The stainless steel foil is placed in the glass vessel filled with 25 ml of solution. The depth of the stainless steel foil under the solution is about 2 cm.

Hole metal tubes of stainless steel with a length of (10 cm) that connect to the cathode of the power supply, equipped with continuous and intermittent high voltage, manufactured for this purpose. It is capable of processing voltage up to (25) kV, and cutting (25) kHz, stainless steel conductive length 7 cm and width 5 mm strip ends with a 1 x 1 flat end that connects to the anode, Metal tube holder, holder to carry the glass beaker containing the solution aqueous gold salts.

The metal tube is fixed vertically by the catcher, and its upper end is connected by a rubber tube to the gas regulator, which in turn connects by a rubber tube to the argon gas bottle. The brine of gold is placed in a small flask (a capacity of 25 mm) and the beaker is placed on a movable holder under the metal tube. The anode (anode) of the voltages is equipped as shown in Figure 1.


**Figure 1 illustrates the plasma system generating nanomaterials(a) Connecting the system with the argon generator (B) illustrates plasma generation to produce nanomaterials.**

**Preparation of the solution**

HAuCl4.4H2O aqueous tetrachloride salts is used (a partial weight of 411.8476 g and a 99% purity) manufactured by the German company SKMA. A volume of 20 mL of 0.4 mM was prepared and the following equation (1) was used to calculate the required weight:

Concentration (mole) = (mass (g)) / (Molecular weight (g / mol) × volume (liter)) … (1)

HAuCl4.4H2O aqueous tetrachloride salts in concentration 1M and volume 10 ml are mixed with Hyaluronic Acid (HA) in concentration 1M and volume 1ml.

**Preparation of nanoparticles**

To prepare the gold nanoparticles, we follow these steps:

The argon gas tube is opened, the 1 mm diameter metal tube is fixed vertically by the catcher, after the process of the gold salts solution preparing with the demanded volume and concentration, after that it mixing with 1 ml of hyaluronic acid in concentration 1 mM, the prepared form is placed on the stand under the metal tube as mentioned in detail.

The form which is produce by the preparing process is located on the holder beneath the metal tube. The distance between the tube nozzle and liquid surface becomes 1mm when the beaker getting close from the metal tube.

The gas quantity which inter inside of the metal tube is organized by flow meter which can be controlled from control of speedometer and the gas tube. The voltage that produced by the system that gradually increases till the case of the plasma generated between the surface of fluid and the tube.

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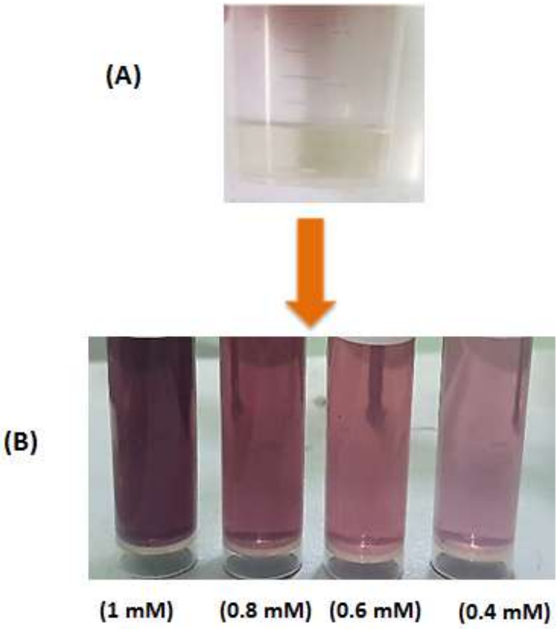


**Figure 2 shows the gold salt solution (A) before exposure to plasma (B) after 6 minutes exposure to plasma for different diameters**

The materials were prepared in the above method for the parameters was consist a constant concentration and volume of gold salts with constant concentration and volume of hyaluronic acid (HA) in different exposure times. In this parameter: HAuCl4.4H2O aqueous tetrachloride salts in concentration 1M and volume 10 ml are mixed with Hyaluronic Acid (HA) in concentrations 1 M and volume 1ml in exposure times of 4, 5, 6 min.

**Results and discussion**

**1- UV spectra measurement**

Gold nanoparticles prepared using a plasma jet system with argon gas were first diagnosed by changing the color of the prepared gold solutions from transparent to purple. The presence the gold particles in a color other than gold is due to the phenomenon of surface Plasmon resonance (SPR) which occurs in metals such as silver and gold as a result of their nanometer particles reaching the nanometer scale. Therefore, spectroscopy at visible wavelengths is used in order to prove the formation of the gold nanoparticles [12]. The colored images and UV-vis spectra of Au NPs/HA The UV-vis spectra of Au NPs/HA for the samples that are prepared with concentration 1mM of HA and 1mM of Au are presented in Figure 4.5. As the plasma treatment time increased, the solution gradually became a red color. The shift in color was basically due to light absorption, depending on the particle size. When the size of the nanoparticles decreased, smaller wavelengths would be absorbed, and, so, a red color would be reflected. The absorption peak showed a similar trend: the absorption peak shifted towards lower wavelengths as the process time increased. The absorption peaks were centered at 540 to 530 nm for the exposure time increased from 6 to 3 min. Piotr Cyganowski et al [13] study the Hydrogel-based nanocomposite catalyst containing uncoated gold nanoparticles synthesized using cold atmospheric pressure plasma for the catalytic decomposition of 4-nitrophenol . they found that The optical properties of the resultant uncoated AuNPs were immediately assessed using UV/Vis absorption spectrophotometry. The characteristic localized surface plasmon resonance (LSPR) absorption band at 546 nm was observed in the spectra of the solution containing the raw-AuNPs, which agreement with Our result. The reaction mixture color changed from purple to dark purple with increasing exposure time for Gold salt. The optical properties of the prepared gold nanoparticle solutions as a function of gold salt molar concentration has been investigated. Accordingly, it can be

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concluded that the increases in the exposure time of the metal ion leads to a decrease in the size of the nanoparticles.

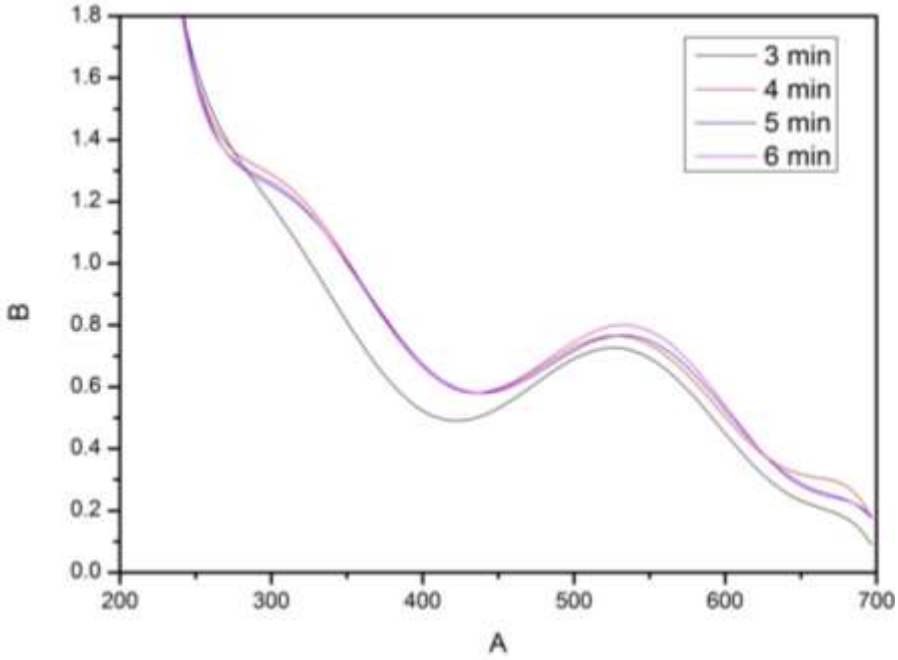


**Figure (3) Visible ultraviolet spectra of gold nanoparticles prepared using jet plasma as a function of exposure time .**

In addition, besides the absorption peak of surface Plasmon resonance at λ = 540 nm ( which consider maximum value) for AuNPs which consider to some extent constant (i.e., the range of AuNPs from 10 to 30 nm), the distribution of their size can be evaluated by measuring the full width at half maximum ( which is symbolized by FWHA) of the UV–vis spectra. Consequently, the results showed that the full width at half maximum for AuNPs/HA is relatively narrower (circa 72nm) for the sample prepared at 6 min compared to AuNPs/HA synthesized at 3 min conjugates (circa 98 nm), which show more size distribution of monodispersed nanoparticle.

**2− X-ray Diffraction measurement**

The tests of XRD patterns showed a diffraction pattern with face center cubic for the gold nanoparticles. The XRD peak corresponds to the angles 38.29° degrees, and 44.41° degrees, 64.10° degrees and 77.67° degrees. It can be traced as 111, 200, 220 and 311 crystalline levels of face-centered cubic (FCC) planes from the metallic gold. XRD patterns of gold nanoparticles correspond to the standard gold JCPDS (file 00-004-0784). It was noted that the sample was prepared with an exposure time of 6 min have four peaks without any other peak that attributed to the impurities [15]. The vertices in the pattern of XRD for the gold are due to the structure of cubic (face center cubic) which centered around the face. While samples prepared at lower exposure time 3,4 and 5 min have just small two peaks. Due to the ubiquitous specimen pattern presence, the intensity of diffraction peak (111) located at 38.18 ° was stronger too much compared with the peak (200) which is located at 44.44°. By the equation of Debye-Scherrer, the AuNPs average volume has been calculated by determining the strongest width of the peak. Through depending on the system, the diameter of the size of the crystals average of crystals of 20 nm and a size ranging from 22 to 34 nm, and figure (4) explain the diffraction patterns of X-ray for dried particles of gold which prepared using Jet Plasma. M. F. Al-Halbosiy et al [16] study the Effect gold nanoparticles generated by cold plasma for mineral blood, they found that the average crystal size was 17nm which agreement with our result.

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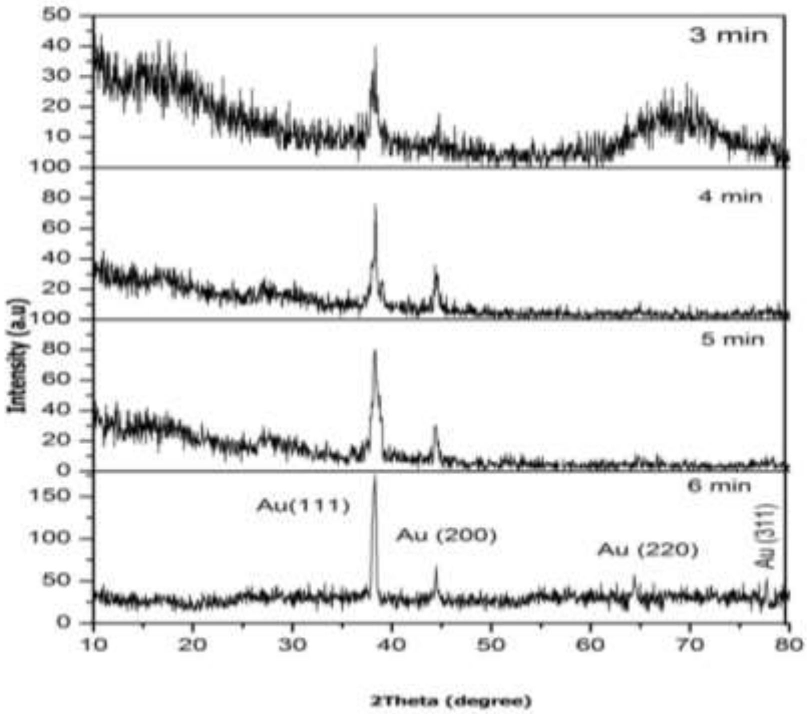
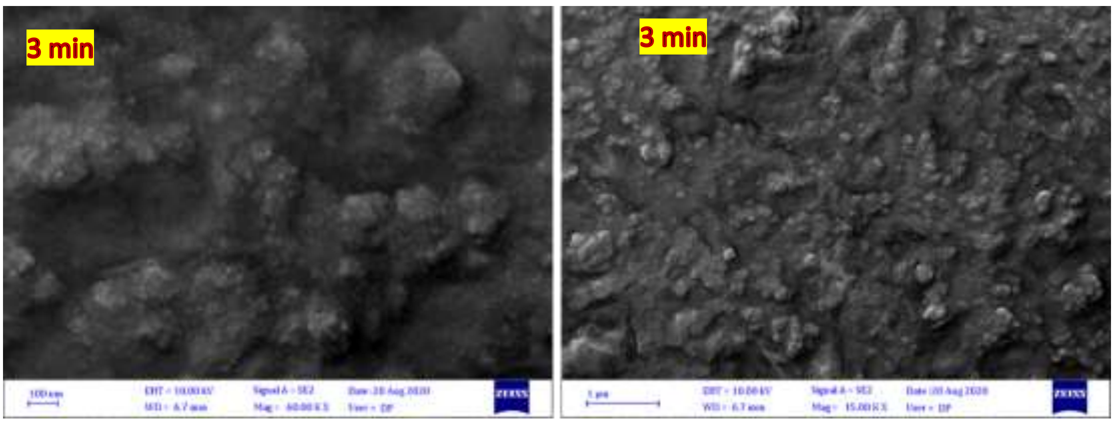



**Figure (4) explain the X-ray pattern of gold nanoparticles prepared using jet plasma as a function of gold salt times.**

3- **FESEM microscopy measurement**

The gold particles produced are showed in FESEM as spheres have irregularly shaped with sizes ranging from 15 to 60 nm, which is almost similar to the values measurements of X-ray diffraction. It is common that the electronic and optical properties of metal particles are greatly influenced by the shape of the nanoparticles [15]. The result confirms that can produce spherical-shaped gold nanoparticles in diameters within the nanoscale, the images showed that the gold prepared with a time of 3 minutes was spherical, the images were more blurry compared to the rest of the models, and this was confirmed by the X-rays, as the crystallization of the prepared model with a time of 3 minutes was not clear compared with the rest of the models. And in the pictures of the sample that prepared with a time of 5 minutes, the spherical formation was clearer in it than the rest, with the appearance conglomerates in all models, which refer to the method of sedimentation, as shown in Figure (5):



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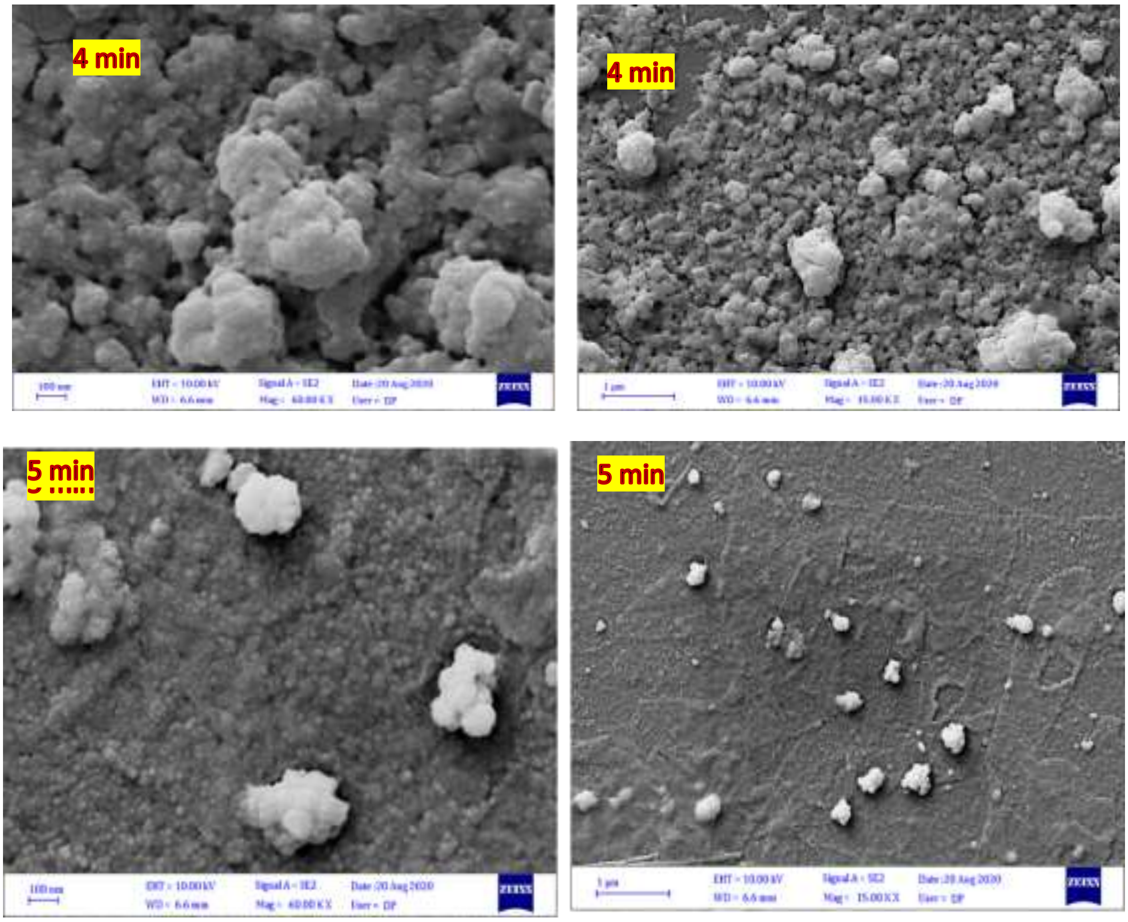


**Figure (5) images of the electron microscope FESEM gold nanoparticles prepared using jet plasma as a function of gold salt times.**

**Conclusion:**

Many researchers are shedlights on nanoparticles and its fields application diversity as medicine, electronics, farming and photonics. The system of plasma jet offers easy, safe and simple way of preparation that has non-toxic properties, strong ,don't need a long time to preparation, it has potential commonly use of medical procedures for techniques of bacteria and fungi, unlike physical and chemical conventional processes that often use substances has the ability to cause a toxic in cellular and environmental, in addition to the initiation of cancer formation in cells.

Using a salt of gold as a function, AuNPs has been synthesized: HA volume ratio. In UV spectra the band of absorption from 544 to 550 nm and that is what distinguishes surface Plasmon resonance of AuNPs.

In XRD pattern the prepared sample was at 1mM concentration of gold tetrachloride salts with 1mM molar concentration of HA without any other peak that regard to impurities. The vertices in pattern of XRD for the gold are due to the structure of cubic (face center cubic) which centerd around the face. FESEM gives an idea about the morphology of AUNPs, where shaped spheres with particle sizes have a range from 4 to 66 nm, which approximately like that values which obtained from the measurements of X-ray diffraction. Moreover, the low cost of cold plasma technology is comparable to other technologies.

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