Nanocomposite Gold Nanoparticles and Hyaluronic acid synthesis using the atmospheric air jet plasma

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Abstract

The atmospheric cold plasma has been used in the synthesis of gold nanoparticles using different molar concentration from Aqueous gold tetrachloride salts HAuCl4.4H2O mixed with Hyaluronic acid (HA) using a low-cost and simple method in order to create a cold plasma at exposure time of 6 min. The effect of mixing with different concentrations ratio of gold salts with 1mM of hyaluronic acid with ratio Gold salt:HA (10:1) were studied with the same exposure time which stated above to determine the best concentration for nanoparticles of gold. X-ray diffraction (XRD), UV-Visible spectra were used to characterize the nanoparticles. The synthesis of AuNPs indicated to surface Plasmon resonance (SPR) at 540, 544, and 550 nm for samples that prepared. The strong intense peaks that showed in the XRD patterns for all prepared samples is indicating to the face centered cubic structure and nature of crystalline of nanoparticles. The average size of crystallite were from 20 to 40 nm for the AuNPs, and the FESEM showed the AuNPs morphology, and the synthesize of AuNPs were showed by observations are have spherical shape and have diameters sizes from 16 to 38 nm.

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:

Nanotechnology is a technique that has the ability to fulfil a high level degree of accuracy in size, functions and materials forms and components by controlling the reaction of molecules and directing the atoms involved in the reaction with specific guidance from the production the most accurate and refined
materials of traditional manufacturing as well as a decrease in energy consumption[1]. The word 'Nano' originated from a Greek word that means too small or the little thing infinitely [2].

Depending on ASTM criterion definition, Nanoparticles consider a particles has 2 or 3 dimensions and has range of length from 1 nm to 100 nm [3]. The biological and chemical nanomaterials properties in this scale is vary in basic ways that are characteristic of both atoms/individual molecules and corresponding materials. [4]

Nanoparticle allow or cause to be fully optimized or new properties, like distribution, size and molecules morphology [5]. Gold nanoparticles are important because of their unique properties that able to having been integrated with applications of antimicrobial, composite fibres, electronic components and materials of biological sensor[6]. Several physical and chemical methods have been used to synthesize and stabilize gold nanoparticle [7]. Size of particle and nanoparticles hardly affect the electrical, physical, chemical, electrical, physical and optical nanomaterials of properties [8]. At now days, gold and silver nanoparticles are used in vastly biomedical range such as catalytic, drug delivery, anticancer antibacterial, anti-fungal, anti-biofilm, agriculture antioxidant, entomological and parasitological applications [9,10].

Hyaluronic Acid (HA) discovered by the scientist K. Meyer and his assistant, J. Palmer during 1934[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 physical treatment by cold plasma consider a green technique, this technique lately has become an attraction for metal ions efficient reduction [16,17].

The metal nanoparticles which prepared by cold plasma was found has specifications that high dispersion, smaller particle size and thereby exhibiting high selectivity and activity. Furthermore, frequently thought that the electrons which have high-energy is being agents of reducing in these studies [18]. The synthesis of Plasma-assisted nanomaterial is now a substitutional to synthesis of purely aqueous[19]. In the present work, Synthesize gold nanoparticles (AuNPs), by atmospheric pressure plasma jet as a function of the molar concentration of gold salt mixed with constant ratio of Hyaluronic acid (HA) at exposure time of 6 min.

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:
Argon gas bottle which is affordable available in the market, and contain an gas outlet containing a regulator to control the speed of exit gas.
Air flowmeter with a calibrator of (1-10) minutes / liter to control the air intake which connects to the hollow metal tube.
Aqueous gold salts (HAuCl4.4H2O) with a molecular weight of 411.8 g / mol dissolved in ionic water at a concentration of 0.4, 0.6, 0.8, 1 mM.
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 air regulator, which in turn connects by a rubber tube to the air compressor. 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](image)

**Figure 1 illustrates the plasma system generating nanomaterials**

(a) Connecting the system with the air generator (B) illustrates plasma generation to produce nanomaterials.

**Preparation of the solution**

The salts of aqueous tetrachloride HAuCl4.4H2O which used has range of purity (99%) is produced by SKMA (German company). The prepared quantity is 20 ml volume while the required weight is compute by the following equation (1):

\[
\text{Concentration (mole)} = \frac{\text{(mass (gram))}}{\text{(Molecular weight (g / mol) } \times \text{ volume (litter))}} \ldots (1)
\]
-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.

![Figure 2](image)

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

Gold nanoparticles prepared using plasma jet system with argon gas was first diagnosed by changing the color of the prepared gold solutions from transparent to purple (Figure. 2). Presence the gold particles in a color other than silver 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 [20]. The colored images and UV-vis spectra of Au NPs/HA for the samples that prepared with different concentrations of HClAu are presented in Fig. 2. The SPR band blue shifts from 540 to 550 nm with gold salt concentration increased from 0.4 to 1 mM. The reaction mixture colour changed from purple to dark purple with increasing Gold salt concentration. The optical properties of the prepared gold nanoparticle solutions as a function of gold salt molar concentration has been investigated. Accordingly, it can be concluded that the increase molar concentration of Gold salt lead to increases the concentration of the metal ion and then increases the size of the nanoparticles [16].

![Figure 3 Visible ultraviolet spectra of gold nanoparticles prepared using jet plasma as a function of gold salt molar concentration](image-url)
In addition, besides the absorption peak of surface Plasmon resonance at $\lambda = 545$ 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 [21–24]. Consequently, the results showed that the full width at half maximum for AuNPs/HA is relatively narrower (circa 66nm) for sample prepared at 1mM molar concentration compared to AuNPs/HA synthesized at 04.mM conjugates (circa 75 nm), which show more size distribution of monodispersed nanoparticle.

Figure 4 explain the diffraction patterns of X-ray for dried particles of gold which prepared using Jet Plasma. The tests of XRD patterns showed a diffraction pattern with face center cubic for the gold nanoparticles. The XRD peak correspond to the angles $38.29^\circ$ degrees, $44.41^\circ$ degrees and $77.67^\circ$ degrees. It can be traced as 111, 200, 220 and 311 crystalline levels of face-centered cubic (FCC) planes from metallic gold. It was noted that the sample was prepared with a concentration of 1, 0.8 mM have four peaks without any other peak that attributed for the impurities [25]. The vertices in 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 concentration 0.6, and 0.4 mM have just small two peaks. Due to the ubiquitous specimens pattern presence, the intensity of diffraction peak (111) that located at $38.18^\circ$ was stronger too much compared with the peak (200) which located at $44.44^\circ$. By the equation of Debye-Scherrer the AuNPs average volume has been calculated through determining the strongest width of peak. Through depending on the system, the diameter the crystals size average of crystals of 17 nm and a size ranging from 18 to 29 nm.
Figure 4 explain the X-ray pattern of gold nanoparticles that prepared using jet plasma as a function of gold salt molar concentration.

The results of the FESEM microscopy showed that the gold particles produced are irregularly shaped spheres with sizes of particle which ranging from 4 to 66 nm, which approximately like that values which obtained from the measurements of X-ray diffraction. It is common that the electronic and optical properties of metal particles are greatly influenced by the nanoparticles shape[26]. The result confirms that spherical-shaped gold nanoparticles can be produced in diameters within the nanoscale, as shown in Figure 5:
Figure (5) images of the electron microscope FESEM gold nanoparticles prepared using jet plasma as a function of gold salt molar concentration

**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 different molar concentration of gold tetrachloride salts with constant 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.

References:

[1] Gang W., (2018), Nanotechnology: The New Features, esarXiv: 1812.04939v1 [cs.ET].

[2] Gnach A., Lipinski T., Bednarkiewicz A., Rybka J., Capobianco, J.A. (2015), Upconverting nanoparticles: Assessing the toxicity. Chem. Soc. Rev, 44, 1561–1584.

[3] Samer B., Muhammad A., Tiziano T., Marco C. and Flavio R. (2020), The History of Nanoscience and Nanotechnology: From Chemical–Physical Applications to Nanomedicine, Molecules, 25, 112.
[4] Iqra Z. A., Hussain S.B., ul Haq A. and Abdul Qadeer K. (2016), Wondrous Nanotechnology, J. Chem. Soc. Pak, Vol. 38, No. 06.

[5] Filipponi L. and Sutherland D. (2013), Nanotechnologies; Principles, Applications, Implications and Hands-on Activities, European Union, EUR 24957.

[6] Wilson M., Kannangara K., Smith G., Simmons M., and Raguse B. (2002). Nanotechnology: basic science and emerging technologies. CRC Press.

[7] Hornyak G. L., Tibbals H.F., Dutta J., Moore J.J. (2008), Introduction to nanoscience and nanotechnology, CPR press, 1st edition.

[8] Kandasamy S. and Sorna R. P. (2011), Methods of synthesis of nanoparticles and its applications, J Chem Pharm Res, 7(3), pp.(278-285).

[9] Iravani S. (2011), Green synthesis of metal nanoparticles using plants, Green Chem, 13, 2638–2650.

[10] Mansoori G. A. (2005), Principles of Nanotechnology: Molecular Based Study of Condensed Matter in Small Systems, World Scientific Pub, New York, 2st Edition.

[11] Natalia M. S., Katarzyna A. B., Dominika A. Z., Dominika L. W.-D. (2016), Physiochemical properties and application of hyaluronic acid: a systematic review, Journal of Cosmetic Dermatology, 15, 520-526.

[12] Shyam V., Pratik K., Deepak R. (2020), Hyaluronic acid: A review on its biology, aspects of drug delivery, route of administrations and a special emphasis on its approved marketed products and recent clinical studies, International Journal of Biological Macromolecules 151, 1012–1029.
[13] Wenwei H., Lili S., Yingdi W., Youjing Lv, Xiangyan C. and Xia Z. (2019), Preparation, Characterization, and Inhibition of Hyaluronic Acid Oligosaccharides in Triple-Negative Breast Cancer, Biomolecules, 9, 436; doi:10.3390/biom9090436.

[14] Ramesh C. G., Rajiv L., Ajay S. and Anita S. (2019), Hyaluronic Acid: Molecular Mechanisms and Therapeutic Trajectory, Frontiers in Veterinary Science, Volume 6, Article 192.

[15] Litwiniuk M., Krejner A., Speyrer M.S., Gauto A.R., Grzela T. (2016), Hyaluronic acid in inflammation and tissue regeneration. Wounds, 28:78–88.

[16] Ismail E., Saqer A., Assirey E., Naqvi A., and Okasha R. (2018). Successful green synthesis of gold nanoparticles using a corchorus olitorius extract and their antiproliferative effect in cancer cells. International journal of molecular sciences, 19(9), 2612.

[17] Ban A., Ahmed M., and Murbat H. (2019). Cold Atmospheric Plasma generated by FE-DBD Scheme cytotoxicity against Breast Cancer cells.

[18] Hoffmann C., Berganza C., and Zhang J. (2013). Cold Atmospheric Plasma: methods of production and application in dentistry and oncology. Medical gas research, 3(1), 21.

[19] Bisht A., and Talebitaher A. (2011). Synthesis of Nanoparticles using Atmospheric Microplasma Discharge. In FPPT5 Conf.

[20] Omar M. H., Ibraheem J.I., Ban. H. A., Obaid A.S., Thaher A. S., (2020), Synthesis of Silver Nanoparticles by ecofriendly environmental method using Piper nigrum, Ziziphus spina-christi, and Eucalyptus globulus extract, Journal of Physics: Conference Series 1530, 012139.
[21] Amendola V., Meneghetti M., Phys J. (2009). Size evaluation of gold nanoparticles by UV-vis spectroscopy, J.Phy Chem. C 113, 4277–4285.

[22] Haiss W., Thanh N.T.K., Aveyard J., Fernig D.G., (2007), Determination of size and concentration of gold nanoparticles from UV-vis spectra, Anal. Chem. 79 4215–4221.

[23] Capanema N.S.V., Mansur A.A.P., Carvalho S.M., Mansur L.L., Ramos C.P., Lage A.P., Mansur H.S., 2018, physicochemical properties and antimicrobial activity of biocompatible carboxymethylcellulose-silver nanoparticle hybrid for wound dressing and epidermal repair, J. Appl. Polym. Sci. 135, 45812.

[24] Amendola V., Pilot R., Frasconi M., Maragão O.M., Iatì M.A., 2017, surface plasmon resonance in gold nanoparticles: a review, J. Phys. Condens. Matter 29, 203002.

[25] Thamer N.A., Adil B.H., and Obaid A. S., (2020, Gold Nanoparticles Synthesis Using Environmentally Friendly Approach for Inhibition Human Breast Cancer. International Journal of Nanoscience, p. 1950040.

[26] Kelly K. L., Eduardo C., Lin L. Z., and George C. S., (2003), The Optical Properties of Metal Nanoparticles: The Influence of Size, Shape, and Dielectric Environment J. Phys. Chem. B, 107, 668-677