Synthesized Gold Nanoparticles Using *Pseudomonas* Supernatant and Study the Physical Characterization– Antproliferative Activity of Breast Cancer Cells (MCF-7)

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Abstract

In this paper we used a biological method to prepare gold nano particles as a simple technique, environmentally friendly and limited toxics compared with chemical and physical method. Where used *Pseudomonas* bacteria supernatant to prepare gold nano particles and use this supernatant to reduction gold salt ($HAuCl_4.4H_2O$) and converted to AuNPs. XRD measurement showed the average the crystallite size ranging from (9.2-11.4) nm, UV-VS spectrum exhibited the maximum absorbance peak at (530)nm and optical energy gap (E_g = 1.88)e.v, FESEM image showed the shapes of AuNPs as (spherical and semispherical), TEM image showed the formation of particles as clusters, spherical and hexagonal shapes. AuNPs was used as anticancer cell line where testing against breast cancer line (MCF-7) and interested result indicated that can be used AuNPs in reliably as an active treatment to those have breast cancer (MCF-7).

Keywords: *Gold Nanoparticles, MCF-7, Anticancer.*

Introduction

Gold is one of precious metals that was discovered in nature by human and used in jewelry making Gold is also used not only for jewelry, but also for industrial uses and applications in the fields of biology and medicine etc.(1)(2)(3)(4)(5)

Physical and chemical method succeeded in the manufacture of many nanomaterials that are high purity, but in return there are negative sides such as the high cost and risks of chemical reactions The biological method has no chemical risks as well as is less costly and environmentally friendly. Biosynthesis of gold is being different size from Several nanometer to hundreds of nanometers and different shapes.(6)(7)(8)

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AuNPs are formed by the reduction of Gold salts in the solution through various reduction agents in the solution.(9)(10)

Rapid reduction results NPs in large particles and spherical shape in general as for slow reduction results NPs in small particles.(7)(9)

Safety aspect of NPs: The properties of Many Materials change when they are reduced to nanoscale as the surface area of the particles increased resulting in interactions with environment. Nanomaterials are of limited toxicity and therefore have the advantage in medicine, biochemistry and nanotechnology.(11)(12)(13)(14)

Materials and Method

To preparing the Nutrient broth medium (NBM) we suspended 0.8 gm of the medium in 100 ml deionized water. Mix well and dissolve with frequent agitation for one minute until complete dissolution. And finally sterilizes it in Autoclave for 15 min in 15 lb and 121 C.(15)

***Pseudomonas* culture:** To preparing of *Pseudomonas* culture solution, under sterile environment, we were

taken a full loop of new activated *Pseudomonas* bacteria (which provided from biological department- Science Faculty/University of mustansiriyah) and cultured in 100 ml flask of nutrient broth medium then incubated for 24hr under 37 ºC.

Preparation of bacterial supernatant: After 24hr of culturing, we centrifuge the culture tube at 5000 rpm, and take the supernatant in quiet and kept it in +4 ºC, until preparing gold nanoparticles.

Preparation 0.02 M of gold (III) chloride tetrahydrate ($HAuCl_4.4H_2O$) stock solution:

To preparation of a stock solution of gold salts, dissolved 1gm of ($HAuCl_4.4H_2O$) in 100ml of deionized water. Then we calculated the stock concentration according to the following equation:-[16]

$$M = \frac{Wt}{Mwt} \times \frac{1000}{V}$$

M=Molarity,

Wt=weight of ($HAuCl_4.4H_2O$) (1)gm

Mwt= Molar mass of $HAuCl_4.4H_2O$ is 411.8476 g/mol

V=Volume of deionized water=100ml

So: $$M = \frac{1gm}{411.8476\ gm/mol} \times \frac{1000}{100ml}$$

$$= 0.02\ Molar$$

Synthesis of (Au-NPs) by using *Pseudomonas* bacteria: To synthesis of Au-NPs, 0.5 ml of $HAuCl_4.4H_2O$ stock solution which prepared was added dropwise in 25 ml glass tube with screw cupcontains of 2ml of bacterial supernatant and 7.5 ml deionized water as shown (figure 1) a,b,c, the final volume will be 10ml. The concentration of $HAuCl_4.4H_2O$ in this solution calculated according to the following equation:-[17]

$$C1 \times V1 = C2 \times V2$$

$$0.02 \times 0.5 = C2 \times 10$$

$$C2 = \frac{0.02 \times 0.5}{10} = 0.001\ Molar$$

Then, the glass tube was placed in the heating mantle with inserting the thermometer and thermostat, the temperature was raised until 80-85ºC for 30 min, the color of the solution will change to dark red which indicated to synthesized the gold nanoparticles. Also, we controlled the pH value (7.5-8) of this reaction by added 0.25 ml of NaOH at concentration 0.1 Molar.

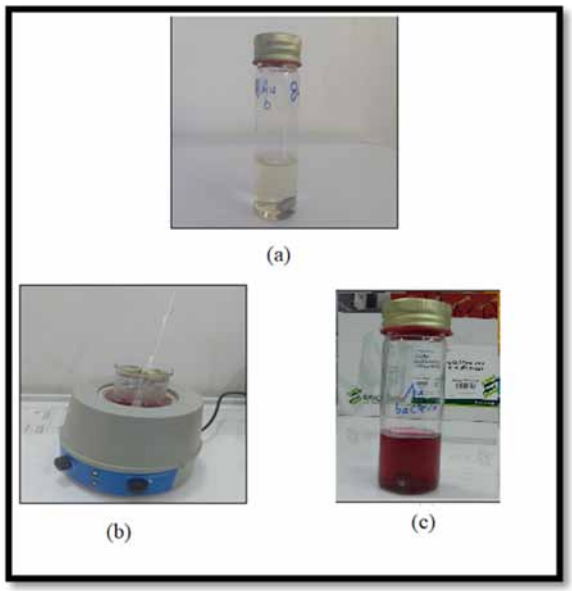


Figure (1) prepared gold nanoparticles from bacteria supernatant

(a) Solution contain of (0.5 ml of HAuCl4.4H2O+ 2 ml of bacteria supernatant+ 7.5 mlD.W)

(b) Solution of Au NPs during preparation

(c) Solution of Au NPs after preparation

Results and Discussion

XRD analysis: Formation of gold nanoparticles by biological method using Bacterial supernatant was assured by XRD spectrum as shown in figure (5) peaks (111) (200), (220), and (311) point out FCC phase of gold nanoparticle, the peak broadening in XRD pattern indicates that small nanocrystallite are work out in specimens.

The average crystallite size was calculated using scherrer's equations and its values was found to be in the range (9.2 to 11.4) nm. Result of figures (2)a are agreement with[18]

UV-Vis measurements: The absorbance spectra for sample of gold NPs solution prepared from bacteria supernatant was investigated by uv-vis spectroscopy. (Figure 2) b shows absorbance spectra of Au NPs solution at concentration (100 ppm) with images of collides. Investigation shows presence of absorbance peak at 530

nm for NPs prepared from bacteria supernatant. Which corresponding to surface plasmon resonance (SPR) of Au NPs, and this result is agreement with[19][20][21][22]

The optical energy gap (E_g^{op}) of AuNPs is calculated from the following equation:

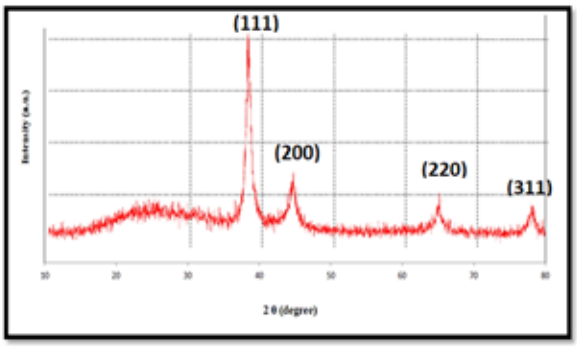
$$\propto h\upsilon = B(h\upsilon - Eg)^n$$

Where ($h\upsilon$) is incident photon energy

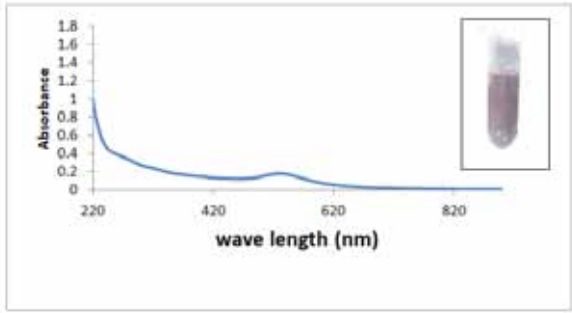
(B) is constant, (n) is constant take Value (1/2, 3/2, and 3) depend on type of the optical transitions (direct or indirect)

where the values of energy gap for AuNPs solution prepared from bacterial supernatant (E_g^{op} = 1.88) e.v, the reason for appearance band gap energy due to quantum size effect.

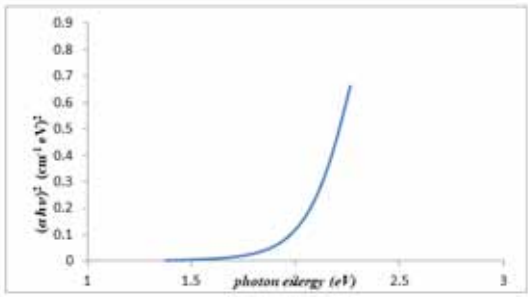
The figures (2)a,b,c show XRD pattern, Absorbance and optical energy gap for gold nanparticle



(a)



(b)



(c)

Figure(2): a) XRD pattern of gold nanoparticles prepared by Biological method using Bacteria supernatant, b) Absorbance as a function of wavelength for gold colloids nanoparticles prepared by Biological method using Bacteria supernatant, c) Variation of ($\alpha h\nu$)2 with hv for direct transition in Au NPs prepared from Bacteria supernatant

FE-SEM: By examining the FE-SEM of gold nanoparticles prepared from Bacteria supernatant, as shown a (figure 3 a) imagewith magnification (100 kx). the shapes were semispherical and spherical with random shapes. and it can be note that the agglomeration percentage is high in the image. The average of grain size was found in the range from (12.13 – 37.8)nm.

TEM: Figure (10b,c) show the TEM image of Gold nanoparticles - prepared from bacterial supernatantat (pH = 7.5-8) and concentration (100ppm), with (125000x) magnification, the image reveal formation of gold nanoparticles with spherical,semispherical and hexagonal shapes. The average particle diameter between (10 to 40) nm, which agreement with FE-SEM result and very closely with XRD result.(figure b,c)

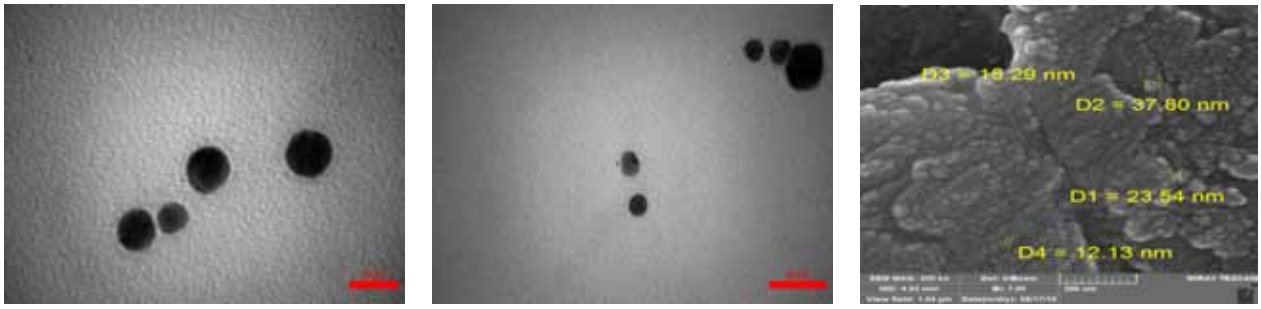


Figure (3)a: FE-SEM images recorded by deposited gold-DW nanoparticles on pure glass slide; b,c: TEM image of gold nanoparticles prepared by Biological method using Bacteria supernatant

Cytotoxic Effect of synthetic Au-NPs using *Pseudomonas sp.supernatant* on MCF-7 Tumor Cell Lines using MTT colorimetric assay: The cytotoxic activity of Au-NPs which synthesized via using *Pseudomonas sp.* supernatant was determined by MTT colorimetric assay. As shown in Table 1 and (figure 4), the cytotoxic (Viability percentage) of created Au-NPs was not affected significantly (p>0-05) with 6.25 and 12.5 µg/mL concentrations. While, the cytotoxicity was increasing significantly with increasing of synthesized Au-NPs concentrations (P<0.05). Moreover, this assay showed that the minimum inhibitory concentration of MCF-7cells and WRL68 by reaction with synthesized Au-NPs were obtained at 6.25 µg/mL (95.602±1.505 and 95.833±0.579 respectively). However, the maximum inhibition concentration at 400 µg/mL (54.707±1.124 and 77.585±1.238 respectively; Table (1). Also, when reacted of synthesized Au-NPs with the normal cell line cells, the median lethal dose (IC50) was 117.4, while it was reduced to 51.66 in MCF-7 cells (figure 11)and this result agree with[23].

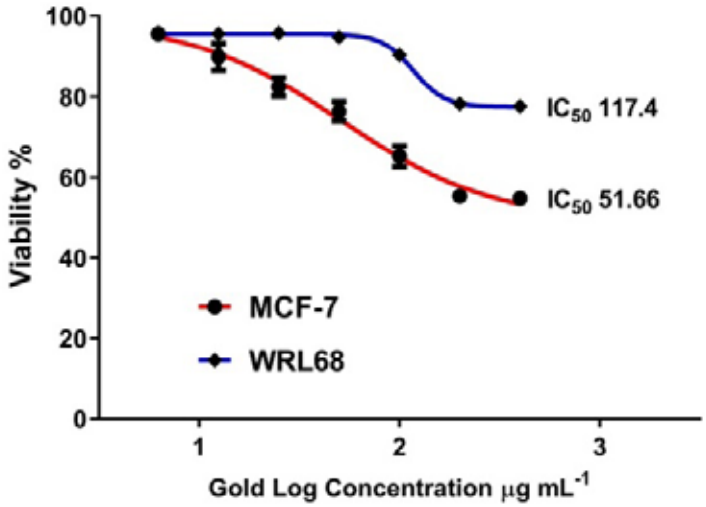


Figure. 4 Comparison of the viability percentage of MCF-7 cells and WRL68 versus the logarithm of the concentration of synthetic Au-NPs using *Pseudomonas* sp. supernatant.

Table 1: Viability percentage of MCF-7 and WRL68 tumor cell line, and degree of freedom (Df) regarding to different concentrations of synthesized Au-NPs using *Pseudomonas sp.*supernatant

Mean percentage (%) for each cell line					
Row stats	Au-NPs Concentration (ppm)	MCF-7		WRL68	
		Mean±SD	Df (n-1)	Mean±SD	Df (n-1)
1	400	54.707±1.124	2	77.585±1.238	2
2	200	55.324±1.219	2	78.241±1.632	2

Mean percentage (%) for each cell line					
Row stats	**Au-NPs Concentration (ppm)**	**MCF-7**		**WRL68**	
		Mean±SD	**Df (n-1)**	**Mean±SD**	**Df (n-1)**
3	100	65.201±2.604	2	90.316±1.772	2
4	50	76.350±2.401	2	94.753±0.788	2
5	25	82.407±2.259	2	95.795±0.372	2
6	12.5	98.892±3.323	2	95.602±0.531	2
7	6.25	95.602±1.505	2	95.833±0.579	2

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.

Conflict of Interest: None

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References

1. Salata OV Application of nanoparticles in biology and medicine. J Nanobiotechnol (2004) 2:3–9
2. Sperling RA, Gil PR, Zhang F, Zanella M, Parak WJ Biological application of gold - nanoparticles. Chem Soc Rev (2008) 37:1896–1908
3. Cai W, Gao T, Hong H, Sun J Applications of gold nanoparticles in cancer nanotechnology, Nanotechnol Sci Appl (2008) 1:17–32
4. Chen PC, Mwakwari SC, Oyelere AK, Gold nanoparticles: from nanomedicine to nanosensing. Nanotechnol Sci Appl,(2008) 1:45–66
5. Vieira RHSH, Volesky B, Biosorption: a solution to pollution. Int Microbiol,(2000), 3:17–24
6. Brust M, Walker M, Bethell D, Schiffrin DJ and Whyman R Synthesis of thiol derivatised gold nanoparticles in a two-phase liquid/liquid system. Chem Commun:(1994), 801-802
7. Selvakannan PR, Mandal S, Phadtare R, Pasricha R, Sastry M, Capping of gold nanoparticles by the amino acid lysine renders them water-dispersible. Langmuir,(2003),19:3545–3549
8. Daniel M-C, Astruc D, Gold nanoparticles: assembly, supramolecular chemistry, quantum size-related properties, and applications toward biology, catalysis and nanotechnology. Chem Rev,(2004), 104:293–346
9. Handley DA, Method for synthesis of colloidal gold, Chap. 2. In: Hayat MA (ed) Colloidal gold: principles, method and application, vol 1. Academic, San Diego, (1989), pp 13–33
10. Masala O, Seshadri R, Synthesis routes for large volumes of nanoparticles. Annu Rev Mater Res, (2004), 34:41–81
11. Gwinn MR, Vallyathan V, Nanoparticles: health effects: pros and cons. Environ Health, (2006), Persp 114:1818–1825
12. Akerman ME, Chan WC, Laakkonen P, Bhatia SN, Ruoslahti E, Nanocrystal targeting in vivo. Proc Nat Acad Sci USA,(2002),99:12617–12621
13. Cao YC, Jin R, Mirkin CA, Nanoparticles with Raman spectroscopic fingerprints for DNA and RNA detection. Science,(2002),297:1536–1540
14. Georganopoulou DG, Chang L, Nam J, Thaxton CS, Mufson EJ, Klein WL, Mirkin CA From the cover: nanoparticle based detection in cerebral spinal fluid of a soluble pathogenicbiomarker for Alzheimers disease. Proc Natl Acad Sci,(2005), 102:2273–2276
15. Bruchez J, Moronne MM, Gin P, Weiss S, Alivisatos AP Semiconductor nanocrystals as fluorescent biological labels. Science, (1998), 281:2013–2016.
16. MacFaddin J. F., Media for the Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore,1985.
17. Chris Hren (Author), Peter J. Mikulecky,Chemistry Workbook For Dummies with Online Practice,For Dummies; 2017,3 edition.
18. Vo, T. T., Nguyen, T. T. N., Huynh, T. T. T., Vo, T. T. T., Nguyen, T. T. N., Nguyen, D. T., Nguyen, T. D. Biosynthesis of silver and gold nanoparticles using aqueous extract from crinum latifolium leaf and their applications forward antibacterial effect and wastewater treatment. Journal of Nanomaterials, 2019. https://doi.org/10.1155/2019/8385935

19. Kumar PV. Kala SMJ. Prakash K.S. Green synthesis of gold nanoparticles using Croton Caudatus Geisel Leaf extract and their biological studies. Materials Letters,(2018),236: 12
20. Chen MN, Chan CF, Huang SL, Lin YS. Green biosynthesis of gold nanoparticles using Chenopodium formosanum shell extract and analysis of the particles' antibacterial properties. J Sci Food Agric.(2019). 99(7):3693-3702
21. Ben Tahar, I., Fickers, P., Dziedzic, A., Płoch, D., Skóra, B., & Kus-Liśkiewicz, M., Green pyomelanin-mediated synthesis of gold nanoparticles: modelling and design, physico-chemical and biological characteristics. Microbial Cell Factories,(2019), 18(1), 210
22. Manar E Selim 1, Awatif A Hendi, "Gold nanoparticles induce apoptosis in MCF-7 human breast cancer cells", Asian Pac J Cancer Prev . 2012;13(4):1617-20.
23. Manar E Selim, Awatif A Hendi, Gold nanoparticles induce apoptosis in MCF-7 human breast cancer cells, Asian Pac J Cancer Prev . 2012,13(4):1617-20.