IJBPAS, October, 2020, 9(10): 2666-2675

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ELECTROCHEMICAL ANALYSIS AND CHARACTERIZATION OF EXTRACELLULARLY SYNTHESIZED GOLD NANOPARTICLES BY *E. COLI*

AGGARWAL K, BHATIA D AND MALIK DK*

Department of Biotechnology, U.I.E.T, Kurukshetra University Kurukshetra, Haryana-136119

***Corresponding Author: Malik DK: E Mail: deepmolbio@rediffmail.com**

Received 9th Feb. 2020; Revised 6th March 2020; Accepted 9th April 2020; Available online 1st Oct. 2020

https://doi.org/10.31032/IJBPAS/2020/9.10.5222

ABSTRACT

The biogenic synthesis of gold nanoparticle is reliable, nontoxic, economical and ecofriendly in comparison to the conventional synthesis of gold nanoparticles. The synthesis of AuNPs by *E. coli* MTCC40 supernatant was confirmed by absorption spectrums and electron microscopy. The synthesized NPs showed red color and absorption peak at 549 nm. On the basis of transmission electron microscopy analysis, size of gold nanoparticles was estimated 5-20 nm. In Fourier Transform Infra red spectroscopy (FTIR), observed absorption peaks corresponding to amine, amide and carbonyl group were believed to be present on the amino acid residue of protein and may be responsible for reduction and formation of AuNPs. The extracellular AuNPs were further utilized for surface modification by Self assembled monolayer of L cysteine in order to immobilize them on the gold electrode. Cysteine Modified gold nanoparticles and gold electrode were characterized by cyclic voltammeter study**.** Electrochemical impedance spectroscopy analysis confirmed the increase impedance with gold nanoparticles for the detection of *E. coli*.

Keywords: Gold nanoparticles, Characterization, TEM, Biogenic

1. INTRODUCTION

Green nanotechnology utilizes biological systems like bacteria, fungi, actinomycetes and plants for nanoparticle synthesis **[1]**. Nanoparticles exhibit size and shape-dependent properties which are of interest for applications in biotechnology, industries, electrical, pharmaceutical, medical, agricultural and many other fields **[2]**. By using physical, chemical, biological and hybrid methods synthesis of different types of nanoparticles like gold, silver, platinum, palladium, titanium, titanium dioxide, magnetite, cadmium sulphide, and so forth can be done **[3]**. AuNPs are more explored because of their excellent optoelectronic properties **[4]**. Tremendous work has been published on AuNP synthesis by plant extracts. Therefore researchers shifted their focus on microorganisms for AuNP synthesis **[5]**. Nanoparticles produced by microbes either intracellularly or extracellularly, according to the location where nanoparticles are formed **[6]**. Nanoparticles are also known to have excellent antimicrobial activity against various microorganisms. Keeping in view the vast applications and unique properties of gold nanoparticles, objectives of our work was the biogenic synthesis of gold nanoparticles and electrochemical analysis of modified gold nanoparticles attached on gold electrode.

2. MATERIALS AND METHODS

2.1 Gold nanoparticles synthesis from *E. coli* supernatant

The 0.034 g hydrogen tetrachloroaurate was added in 50 ml double distilled water. After mixing the 50 ml of 1 mM concentration of gold ions in the solution with 50 ml supernatant, the resulting solution was incubated at 37 °C. The control (without the gold ions only supernatant) was also incubated under same conditions. The samples of the reaction mixtures were visualized for a possible color change after 12, 24, 48 and 72 hrs of incubation. The change in color from pale yellow to a purple was checked for the formation of AuNPs. The UV- Visible spectrum of the samples was monitored at different time intervals to confirm the synthesis of nanoparticles. The synthesized gold nanoparticles were further characterized by FTIR and TEM.

2.2 Surface modification of nanoparticle by cysteine and gold electrode

The modification of L-cysteine self-assembled monolayer (SAM) on Au nanoparticles was performed by incubating the nanoparticles overnight in 10 mM L-cysteine solution made in PBS at room temperature. Activation was performed where solutions of 2 mM EDC and 5 mM NHS were added onto the SAM-modified Au nanoparticles and kept for 1 hr **[7, 8]**. After changing the colloids with L-cysteine, the surfaces were covered with carboxylic acid and amine groups. The cleaned gold film electrode was immersed into 11-mercaptoundecanoic acid (MUA) solution in ethanol for over 17 hrs. MUA modifies the gold electrode where thiol group was directly attached to the gold surface and carboxyl group protrudes outward. The gold electrode was carefully taken out from MUA solution. The excess of MUA was removed by rinsing with a large amount of absolute ethanol and deionized water. It was added into the solution of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydrosuccinimide (NHS) for 1 hour which activates the carboxylic group. The modified gold electrode was kept in the cysteine modified nanoparticle solution containing carbodiimide cross linkers 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) along with N-hydrosuccinimide (NHS), which work as a catalyst. The EDC reacts with activated carboxyl (-COOH) groups on the gold electrode and then with the amine group of cysteine modified nanoparticle thus forming a strong covalent bond which was the amide (-CONH) bond. The gold electrode was taken out carefully from nanoparticle solution mixture after 1 hr.

2.3 Electrochemical characterization and cyclic voltammeter

The cyclic voltammeter was checked for gold electrode modification with gold nanoparticles. The MUA modified gold electrode was immersed in L- cysteine modified AuNP solution. The modified gold electrode was carefully plugged with crocodile clip in order to connect with the electrochemical analyzer system. The reference electrode and counter electrode was also adjusted with the working gold electrode and cyclic volatammogram was analyzed for surface modification of gold electrode with the gold nanoparticles at scanning rate 100mV/s.

2.4 Electrochemical impedance spectroscopy

The electrochemical analysis of AuNPs modified gold electrode was checked against PBS and *E.coli* (dilution 10^{-4}), whereas gold electrode without any surface modification was also checked against the solution containing only bacterial dilution (10^{-4}) in order to draw comparison between the impedance curves. The reference electrode and counter electrode was also adjusted with working gold electrode. The electrochemical cell assembly was set up by carefully adjusting, AuNP modified gold electrode with the crocodile clips in order to connect with electrochemical analyser system. Then AuNP modified gold electrode was immersed first in PBS and after that checked against 50 ml of 10^{-4} dilution solution of *E.coli*. Then the top of the cell assembly chamber was covered after noting that the electrodes must partially immerse in the solution inside and then electrochemical impedance spectrum was checked.

3. RESULTS

3.1 Synthesis of gold nanoparticles by *E. coli*

The *E. coli* was found to synthesize nanoparticles extracellularly. The AuNPs formation was visually observed on the basis of color of the reaction mixture changing from pale yellow to purple, resulting from the incubation of Au^{+} with the cell free supernatant for 24 hrs. The color change indicates AuNPs synthesis. UV-Vis absorption spectrum of gold nanoparticles, synthesized after 24 hr and 48 hr of reaction with *E. coli* supernatant has an absorption maximum at 549 nm and 551 nm respectively. The UV–Vis spectrum was also observed after 96 hrs, 154 hrs and 15 days and sharp peaks were obtained at 553 nm, 556 nm and 562 nm respectively. On the basis of the Transmission electron microscopy, extracelluarly synthesized AuNPs were of average 10 nm size and spherical shape as shown in **Figure 1**. In the case of FTIR analysis, the spectrum of synthesized nanoparticles shows the presence of absorption peaks located at about 3399 cm^{-1} , 2135 cm^{-1}, 1644cm^{-1} and 1037 cm^{-1} shown in **Figure 2**. The small band at 2,138 cm^{-1} arises from the -C=C stretching vibrations and the peak at 1,033 cm^{-1} may be arise due to C–O stretching vibration.

3.2 Cyclic voltammeter of AuNPs modified gold electrode

The steady state cyclic voltammogram for AuNPs modified on gold electrode**.** L-cysteine modified gold nanoparticles covalently adsorbed on MUA modified gold electrode due to surface group activation by EDC/NHS. The voltammogram result shows at -1 V to +1 V as shown in **Figure 3**. The cyclic voltammeter was scanned at sweep rate of 100 mV/s for the range of frequency from 10 Hz to 1 MHz.

3.3 Electrochemical Impedance Spectroscopy

The electrochemical analysis of AuNP modified gold electrode was checked against *E.coli* (dilution $10^{-4)}$ by EIS. The AuNP modified gold electrode was immersed in 50 ml 10^{-4} dilution solution of *E.coli*. The electrochemical impedance graph shows different curves as showm in **Figure 4**. The red dotted curve represents impedance of modified gold electrode without nanoparticles against solution containing bacterial dilution 10^{-4}. The blue dotted curve represents impedance for AuNP modified gold electrode in PBS solution. The pink dotted curve represents impedance of gold nanoparticles modified gold electrode against the 10^{-4} *E. coli* dilution. It is clearly seen that impedance occurred on the nanoparticle interface was due to the interaction between AuNPs and bacterial surface. The gold electrode without nanoparticles has low impedance value compared to the gold nanoparticle modified electrode. The impedance value was the lowest in case of AuNP modified gold electrode in the presence of *E. coli* dilution 10^{-4}. The range of AC frequency was from10 Hz to1 MHz.

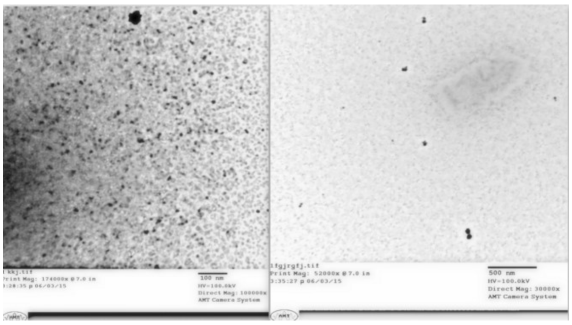
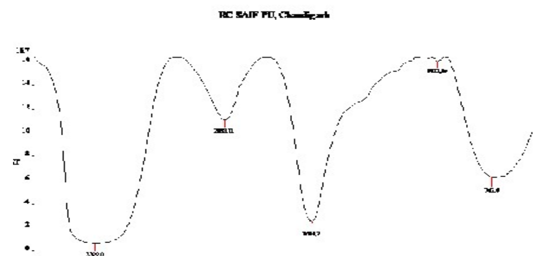


Figure 1: The TEM image of the extracellular AuNPs synthesized by the *E. coli.* batch ES1.



KUK--5.sp - 6/3/2015 - GS-1

Figure 2: The FTIR spectra of the extracellular AuNPs synthesized by the *E.coli*).

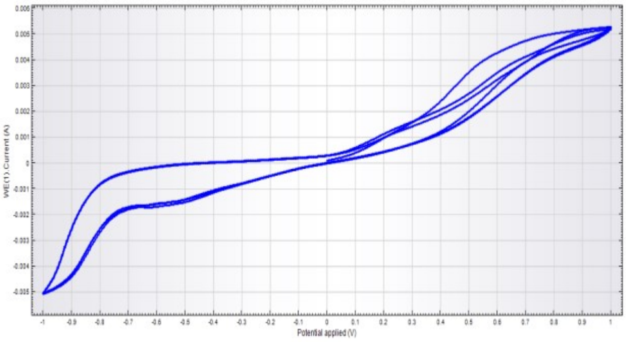


Figure 3: The Cyclic Voltammograms of AuNPs modified gold electrode at sweep rate of 100 mV/s with number of repeated scan, n=3

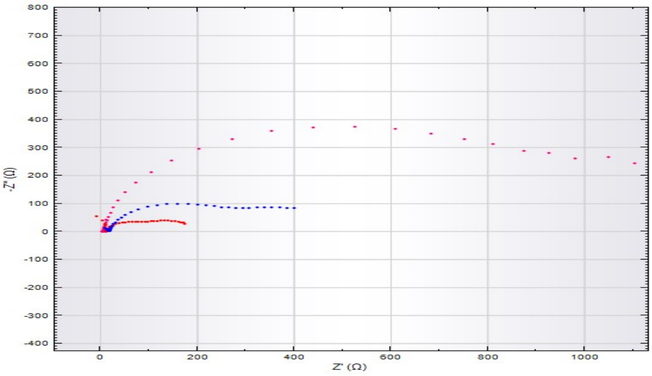


Figure 4: The electrochemical impedance analysis, red dots represent for gold electrode without nanoparticle modification and blue represents AuNPs modified gold electrode and pink represents AuNPs modified gold electrode against 10^{-4} *E. coli* dilution

4. DISCUSSION

The extracellularly synthesized gold nanoparticles synthesis showed characteristic color and peak. The Surface Plasmon Resonance was analyzed by UV–visible absorption spectra in order to check the formation and stability of the synthesized AuNPs. The absorption spectrum for AuNPs (ranges from 510 to 560 nm) in aqueous solutions depends on the shape and size of nanoparticles. In this study AuNPs synthesized by *E. coli* supernatant clearly shows one absorption peak at 549 nm. The TEM image indicates that the majority of nanoparticles were of monodispersed nature.

For extracellular AuNP synthesis, *E. coli* was grown in nutrient broth and later centrifuged at 5000 rpm for 6 min. The similar method was already adopted for synthesis of AuNPs of size 15-30 nm by *Pseudomonas aeruginosa* which is almost consistent with our results **[9]**. The TEM image of extracellular AuNPs reveals the spherical shaped nanoparticles with size range between 5 to 20 nm. Oza *et al*. (2012) **[10]** worked on *Salmonella typhi,* resulted in spherical nanoparticles in 10-15 nm size range. The FTIR absorption peak of extracellular AuNP showed major peak at 3,399 cm^{-1} which corresponds to stretching of primary amine (N-H) functional group and peak situated at 1,644 cm^{-1} was identified as the amide group I, form due to carbonyl stretch vibrations in the amide linkage of proteins **[11]**. These functional groups can be used in immobilization and bio conjugation.

The FTIR spectra of *Enterobacter aerogenes* **[12]** and *Alcaligenes Faecalis* **[13]** also showed similar functional groups which were primary amine and amide group I at 3440 cm^{-1}, 1644 cm^{-1} and 3047 cm^{-1}, 1633 cm^{-1} respectively. This is already reported that because of the presence of carbonyl group, reduction and stabilization is possible in the case of cell free extract of *E. aerogenes* **[13]**. In our study, reduction and stability of AuNPs synthesized by *E. coli* supernatant is offered by the carbonyl group present. In case of synthesized nanoparticles, amine and amide groups were indicated by FTIR analysis and it can be interpreted that these free amine groups are the part of protein which can bind to AuNPs and provide stabilization.

The extracellular AuNPs were further utilized for surface modification by Self assembled monolayer of L cysteine in order to immobilize them on the gold electrode. Gold electrode is modified by MUA finally modified gold electrode was added in L-cysteine modified AuNP solution containing EDC and NHS, due to which covalent bond may have formed between amine and carboxyl groups. The electrochemical analysis by cyclic voltammogram showed possible adsorption of gold nanoparticles on the surface of gold electrode.

To explore surface modification capability of gold nanoparticle, Self assemble monolayer of L- cysteine was done on gold nanoparticle surface in the same manner as already done by **[14]**. But, they performed this AuNP surface modification to capture target DNA whereas in this study modification of AuNP surface was done so that gold nanoparticle can be immobilized on the surface of gold electrode. Although groups were generated on the surface of gold but they require activation by EDC and NHS. We have adopted protocol for the activation of groups by adding solutions of EDC and NHS onto the SAM-modified AuNPs for 1 hour **[13, 14]**. In this solution, MUA modified gold electrode was immersed, due to which covalent bond was formed between carboxyl groups of MUA modified gold electrode and amine group of L-cysteine modified gold nanoparticles. This is suggested that for the activation of carboxyl group, EDC works as carbodiimide cross-linkers and NHS as a catalyst, which helps in the formation of more stable complex **[15]**. The adsorption of modified AuNPs on the surface of modified gold electrode due to the covalent bonding results in the immobilization of AuNP on the surface of gold electrode.

The electrochemical analysis by cyclic voltameter (CV) shows immobilization of gold nanoparticle has occurred on the surface of gold electrode. The Electrochemical impedance spectroscopy (EIS) generated a graph for the AuNP modified gold electrode in the presence of *E. coli* dilution. The graph shows that the impedance has occurred on the nanoparticle interface between AuNP and bacterial surface. These results of EIS and CV were compared with results work on construction of Impedimetric biosensor based on self assembled hybrid cystein gold nanoparticles and Cramo LL lectin for bacterial lipopolysaccharide recognition of *E. coli* and other gram negative bacteria **[16]**. In that study, CV and EIS were evaluated for cysteine modified gold nanoparticles and for modified gold electrode, their results are in accordance with our result of EIS and CV.

5. CONCLUSION

The method adopted for gold nanoparticles synthesis is ecofriendly and economic. Synthesized nanoparticles were stable and monodispersed and can be utilized in different applications. The results of electrochemical impedance spectroscopy show impedance at the interface of nanoparticle between nanoparticle and bacterial surface. Electrochemical analysis results confirm their potential in biosensor applications.

REFERENCES

[1] Begum, N.A., Mondal, S., Basu, S., Laskar, R. A. and Mandal, D.: Biogenic synthesis of Au and Ag nanoparticles using aqueous solutions of Black Tea leaf extracts Colloids. Surf B: Biointerfaces 71,113 (2009).

[2] Salata, O. V.: Applications of nanoparticles in biology and medicine. Journal of Nano-biotechnology 2(1), article 3 (2004).

[3] Mohanpuria, P., Rana, N. K. and Yadav, S. K.: Biosynthesis of nanoparticles: technological concepts and future applications. Journal of Nanoparticle Research 10(3), 507–517 (2008).

[4] Huang, X., Jain, PK., El-Sayed, I. H. and El-Sayed, M. A.: Gold nanoparticles: interesting optical properties and recent applications in cancer diagnostics and therapy. Nanomedicine 2(5), 681 (2007).

[5] Bhattacharya, D. and Gupta, R. K.: Nanotechnology and potential of microorganisms. Critical Reviews in Biotechnology 25(4), 199-204 (2005).

[6] Nangia, Y., Wangoo, N., Sharma, S., Wu, J. and Dravid, V. J.: A Facile biosynthesis of phosphate capped gold nanoparticles by a bacterial isolate *Stenotrophomonas maltophilia*. Phys Lett. 94, 233901 (2009).

[7] Millan, K. M., Spurmanis, A. J. and Mikkelsen, S. R.: Covalent immobilization of DNA onto glassy carbon electrodes. Electroanalysis 4,929-932 (1992).

[8] Gooding, J. J., Praig, V. G. and Hall, E. A. H.: Platinum catalyzed enzyme electrodes immobilized on gold using Self-Assembled layers. Anal. Chem. 70, 2396- 2402(1998).

[9] Husseiny, M. I., El-Aziz,M. A., Badr,Y. and Mahmoudb, M. A.: Biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa*. Spectrochimica. Acta. Part A 67, 1003–1006 (2007).

[10] Oza, G., Pandey, S., Mewada, A. and Sharon, M. Extracellular Biosynthesis of Gold Nanoparticles using *Salmonella typhi*. Der. Chemica. Sinica. 3(5), 1041-1046 (2012).

[11] Sathyavathi, R., Krishna, M. B., Rao, S. V., Saritha, R. and Rao, D. N.: Biosynthesis of silver nanoparticles using *Coriandrum Sativum* leaf extract and their application in nonlinear optics. Adv Sci Letters 3, 01–06 (2010).

[12] Srinath, B. S. and Rai, R. V.: Biosynthesis of gold nanoparticles using extracellular molecules produced by enterobacter aerogenes and their catalytic study. Journal of Cluster Science1-12 (2015).

[13] El-Deeb, B., Mostafa, N.Y., Tork, S. and El-Memoni, N.:.Optimization of green synthesis of gold nanoparticles using bacterial strain *Alcaligenes Faecalis.* Nanoscience Nanotechnology Letters 6, 1–13 (2014).

[14] Yang, L. and Bashir, R.: Electrical/electrochemical impedance for rapid detection of foodborne pathogenic bacteria. Biotechnology Advances 26,135–150 (2008).

[15] Baird, C. L. and Myszka, D. G.: Current and emerging commercial optical biosensors. J. Mol. Recognit. 14, 261-268 (2001).

[16] Oliveira, M. D. L., Andrade, C. A. S., Correia, M. T. S., Coelho, L. C. B., Singh, P. R. and Zeng, X. Q.: Impedimetric biosensor based on self assembled hybrid cystein gold nanoparticles and Cramo LL lectin for bacterial lipopolysaccharide recognition. J. Col. Int. Sc. 362,194–201 (2011).