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SYNTHESIS OF GOLD NANO PARTICLES USING POLY ACRYLIC ACID AS REDUCING AGENT - CHARACTERIZATION AND IN VITRO STUDY OF ANTICANCER CERVIX (HELA) ACTIVITY

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ABSTRACT

Chemical reduction method for the synthesis of metallic nanoparticles has been playing effectual role in the development of nanotechnology and was contributed in improving other related synthesis methods. Recently, many chemical methods are being conducted based on the use of various chemicals as reducing agents to synthesis gold nanoparticles (AuNPs). In this study, gold nanoparticles (AuNPs) is synthesized applying a new method, which is inverse to the general (Turkevich΄s Method) and so it is called (Reverse Method). This method involves reduction of Au^{+3} ions by poly acrylic acid as reducing and stabilizing agent. Au^{+3} ions are present in the form of chloroauric acid ($HAuCl_4.3H_2O$). The appearance of a ruby-red color solution by the addition chloroauric acid to poly acrylic acid solution is a clear indication to the formation of (AuNPs) due to Surface Plasmon Resonance (SPR). AuNPs is characterized by different techniques, such as UV-Vis absorption spectroscopy, depicting an absorbance band at 524 nm. Measurements of size and shape of particles are conducted using AFM and TEM techniques. The shapes appeared as variable and wear mostly spherical and their sizes were in the range (14-25) nm. Zeta potential showed that AuNPs nanoparticles were stable and their application on cervix (Hela) cancer cells gave fine results.

KEYWORDS: Inverse Method, Gold Nanoparticles, Poly Acrylic Acid, Atomic Force Microscopy, Transmission Electron Microscopy, Cervix (Hela) Cancer Cells

INTRODUCTION

Metallic nanoparticles (NP) are attracting attention of chemist due to their novel properties including high surface area and exceptional surface activity, which provide excellent catalytic optical and electrical properties. This is especially with NPs, which are obtained from the noble metals, such as, gold and silver. Gold nanoparticles ($AuNP_S$) generally exhibit various Plasmon absorption bands depending on their size and shape. These nanoparticles have many applications for example, effective drug delivery to cancerous tumors, cosmetics, catalysis, and biomedical/biosensors applications [1-3]. Several physical and chemical methods have been employed in the preparation of AuNPs. Chemical reduction, for instance, the Turkevich route is still considered one of the most applied procedures in the synthesis of AuNPs. Reduction of (Au^{+3}) ions using sodium citrate in hot aqueous solution gives gold metal (Au^0) colloid [4]**.** Another route to synthesis AuNPs is known as Green Synthesis, which is environment-friendly and non-toxic. In this facile green biosynthesis method, plants' extracts are used as reducing agent for the preparation of gold nanoparticles [5-7]. In general, AuNPs are prepared by chemical reduction, typically performed by reducing $HAuCl_4$ in aqueous solutions using inorganic reducing agents, such as sodium citrate, sodium borohydride or organic compounds, such as, polyvinyl alcohol (PVA), polyethylene glycol (PEG), polyvinylpyrrolidone (PVP). These compounds may also function as stabilizers in preventing agglomeration of the AuNPs [8-12].

In this work, poly acrylic acid is used as reducing and stabilizer agent. Polymers of acrylic acid are applied in various fields such as automotive, construction, electronic, cosmetic and pharmaceutical industries, with its advantageous material properties. The presence of carboxylic groups in poly acrylic acid work in fictionalization of bioactive molecules [13-15].

EXPERIMENTAL SECTION

Chemicals

Research chemicals were supplied by HIMEDIA company-India and MERCK Company-Germany.

Instruments

UV-Vis spectroscopy (Shimadzu, Japan), Atomic force microscope (AFM); (SPM AA 3000, USA); Transmission electron microscope (TEM); (Philips CM 100, Holland), and Zeta potential analyzer (Brook haven ,USA) are used for the characterization of AuNPs.

Preparation of Aqueous Gold Nanoparticles

0.25 gm of poly acrylic acid was dissolved in 250 ml distilled water and the solution was heated up to the range 60 -70 ^{0}C. The pH of solution was adjusted to 7-8 by adding few drops of 5% sodium hydroxide solution. To this mixture 3.6 mL of 10 mM $HAuCl_4.3H_2O$ (99%, HIMEDIA, India) were added drop wise with continues stirring. After 20 minutes the color of the solution was changed from pale yellow to ruby-red color, indicating the formation of AuNPs.

Characterization of Gold Nanoparticles

AuNPs were characterized by UV-Vis spectroscopy (Shimadzu, Japan), Zeta potential analyzer (Brook haven, USA), Atomic force microscope (AFM) - (SPM AA 3000, USA), Transmission electron microscope (TEM), and (Philips CM 100, Holland).

The effect of aqueous solution of AuNPs on cervix (Hela) cancer cells was studied in vitro.

RESULTS AND DISCUSSIONS

In this research, a new, easy and economical method has been developed by adding the gold ion (Au^{3+}) to the reducing agent as opposed to the common procedure of Turkevich [4], and so we call it "Inverse Turkevich Method". This inverse method provides a much better control on the added amount of gold salt solution, which easily monitored by observing the formation of red color as indicative to the formation of gold nanoparticles (AuNPs). The poly acrylic acid is used as a reducing agent and at the same time as a stabilizing agent. The produced solution was stable for more than nine months.

UV-Vis Spectra

UV-Vis spectroscopy is one of the important techniques utilized in qualitative and quantitative analysis and also in determination of AuNPs formation. Theoretically, AuNPs absorb visible light between (500-600 nm) due to surface plasmon resonance. AuNPs produced from reduction of gold ions by poly acrylic acid, were characterized by UV-Vis spectra as giving absorption peak at 524 nm. This is identical to what has been reported elsewhere [16]. No change was observed in the original absorption spectra of the prepared AuNPs solution for as long as nine months.

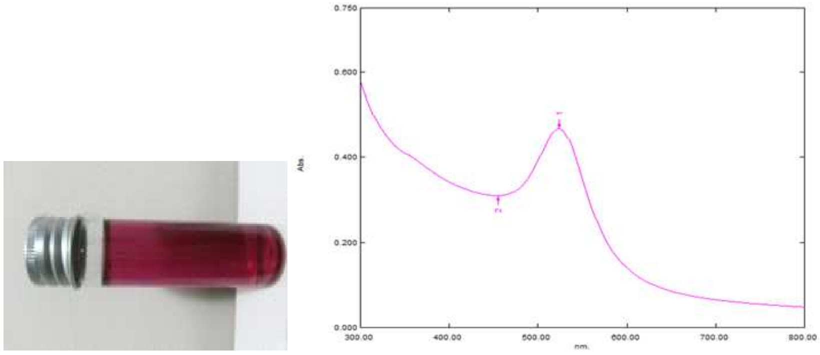


Figure 1: UV-Vis Spectra of Synthesized AuNPs using Poly Acrylic Acid as Reducing Agent

Zeta Potential (ζ)

The zeta potential of a colloidal solution is a tool used to measure the stability of such solutions. The colloidal solution is considered to be unstable, if its recorded zeta potentials were in the range -30 mV and +30 mV. High values, positive or negative, of zeta potential mean a higher repulsion between the particles. Therefore, colloidal suspensions are considered stable when their zeta potentials are more positive than +30 mV or more negative than -30 mV [17]. The more negative zeta potential value of our synthesized AuNPs solution (-40.7 mV) indicates its stability and coinciding with other works.

The peak of the measured zeta potential for the AuNPs solution is shown in Figure 2.

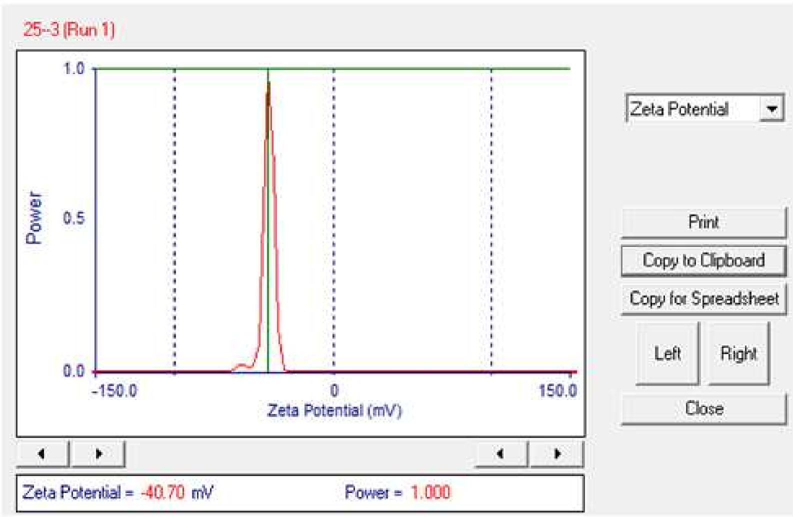
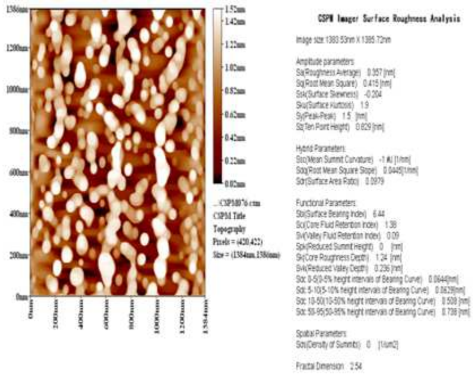


Figure 2: Zeta Potential Value for $AuNP_S$ using Poly Acrylic Acid as Reducing Agent

Atomic Force Microscopy (AFM)

The atomic force microscope (AFM) is suited for characterizing nanoparticles. It offers the capability of 3D visualization and both qualitative and quantitative information on many physical properties including size, morphology, surface texture and roughness. Statistical information, including size, surface area and volume distributions can be determined as well. A wide range of particle sizes can be characterized in the same scan, from 1 nanometer to 8 micrometers [18]**.** The particle size distribution for the synthesized gold nanoparticles was (72 nm) as show in **Figure 3**.



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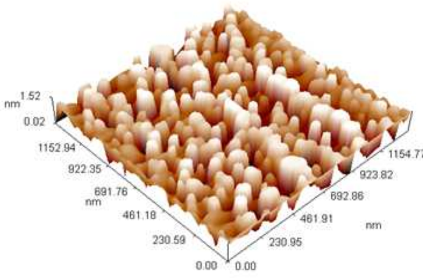

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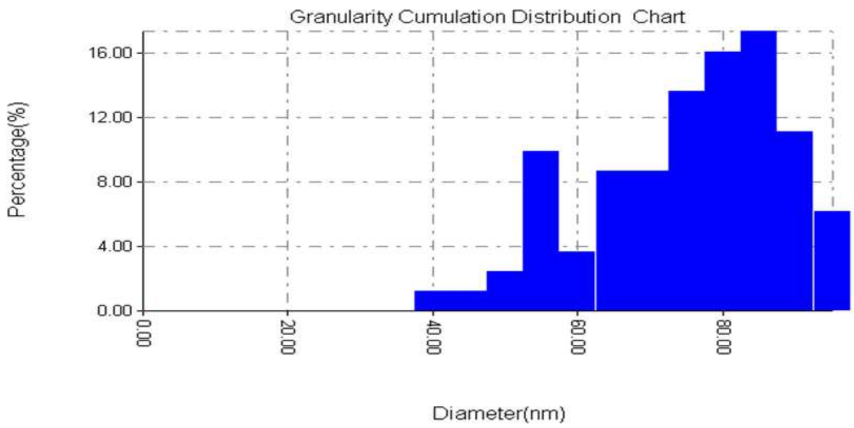

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Figure 3: AFM Image of AuNP$_S$ Synthesized by using Poly Acrylic Acid as Reducing Agent, (A) 2D, (B) 3D, (C) Average Diameter (72 nm)

Transmission Electron Microscopy (TEM)

TEM is the one of the most popular techniques for the characterization of nanoparticles. In this technique, a real image of nanoparticles is taken with different magnifications to develop a more detailed or general shape of nanoparticles [19]. The TEM images (Figure 4) show the AuNPs in variable shapes. The size of the particles ranged from 14-30 nm.

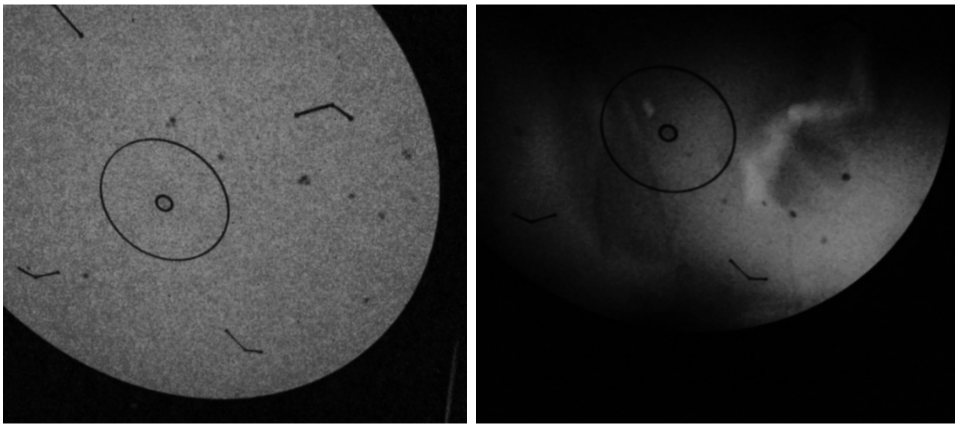
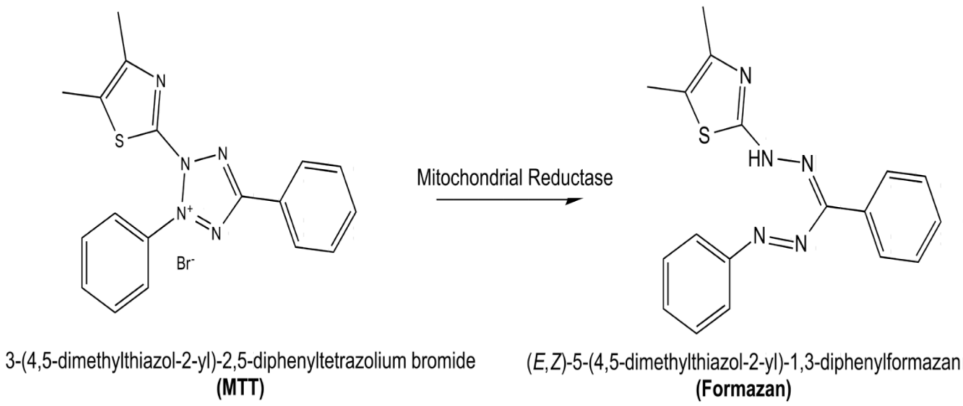


Figure 4: TEM Image Shows Particles Size of the Synthesized AuNPs at 14.5 nm and 25 nm

The Effectiveness of the Synthesized Aunp$_s$ on Cervix (Hela) Cancer Cells

Cervix (Hela) cell cancer is a second most common cause of death among women with various cancers [20].

The effectiveness of the gold nanoparticles to kill cancer cells is studied according to the following mode of action. MTT (3-(4, 5-dimethylthiazole-2-yl)-2,5-diphenyltetrazoliumbromide) tetrazolium salt is reduced to insoluble purple formazan in the mitochondria of living cells**.**



Cell viability assay was conducted on 96-well plates, cervix (Hela) cells were seeded at 7000-10000 cells/ well after 24 hr, cells were treated with AuNPs in serial dilution from 0.01 to 0.00125 mg/mL. Cell viability was measured at 24, 48, 72, and 96 hr of exposure by removing the medium, adding 2 mL solution of tetrazoluim dye, MTT, and incubating for 4 hr at 37 ^{0}C in 5% CO_2. After removing the MTT solution. The crystals remaining in the wells were dissolved by adding 1 mL of DMSO followed by 37 ^{0}C incubation for 15 min with shaking. This works to dissolve crystals of the purple formazan. Absorbency was measured at 550 nm, that are calculated for the cervix (Hela) cell line include the inhibiting rate of cell growth (GI). The percentage of cytotoxicity was calculated as (A-B)/Ax100, where A is the mean optical density of untreated wells and B is the optical density of treated wells. The selected nano particles were taken from the product of using our reducing agent because of its high stability [21, 22].

This study investigated the cytotoxic effect of gold nanoparticles on cervix (Hela) cancer cells in vitro. After the cancer (Hela) cells were exposed to various concentrations of gold nanoparticles for different periods. The AuNPs were found to reduce cell viability in a concentration dependent manner. The cytotoxicity of cancer cell line at concentrations (0.00125, 0.0025, 0.005, 0.01) mg/mL and exposure times were 24, 48, 72, 96 hr. The results varied according to the concentration and exposure time and the inhibition percentage was found to be in the range (46%-87%).

Table 1: Shown Inhibition Percentages of Cervix Cancer Cells through Different Periods and at Different Concentrations of AuNPs

Conc. (mg/ mL)	Time (hr)			
	24	48	72	96
0.01	75	87	84	77
0.005	61	61	76	78
0.0025	47	49	76	76
0.00125	46	56	48	76

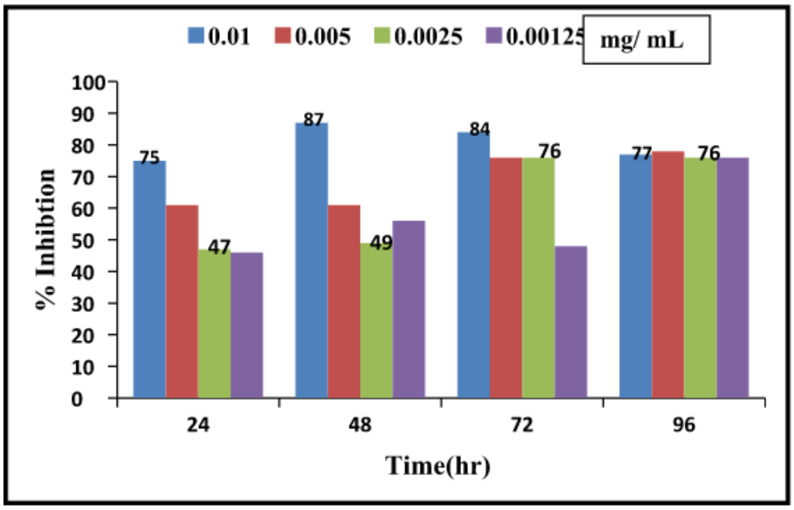


Figure 5: Shown a Scheme (Graphical Representation) of Cervix Cancer Cells Inhibition Percentages over different Periods and Concentrations of AuNPs

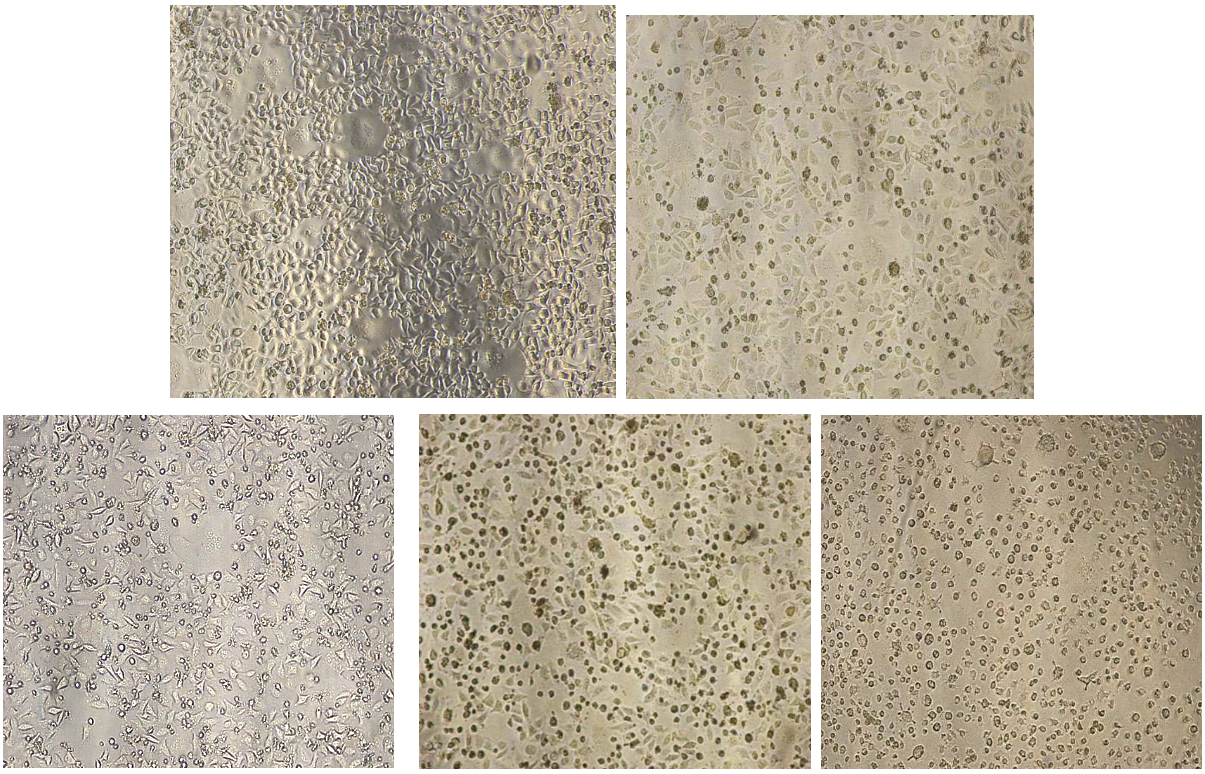


Figure 6: Morphologic Appearances of Cervix (Hela) Cancer Cells after Incubation with Various Concentrations of AuNPs: (A) Control, (B) (0.00125) mg/mL, (C) (0.0025) mg/mL, (D) (0.005) mg/mL, (E) (0.01) mg/mL Respectively at 48 hr

Results indicate a significantly different impact of time and concentration on the inhibition percentage of cancer cell. The highest inhibition percentage was 87% at AuNPs concentration of 0.01 mg/mL over 48 hr, whereas the lowest inhibition percentage was 46% at AuNPs concentration of 0.00125 mg/mL over 24 hr. Gold nanoparticles have been observed as dark spots aggregation in cervix cancer cell under light microscope after treatment with AuNPs. This study has proven that AuNPs not only possess specific imaging properties that can be observed under light microscope but also are cytotoxic to cervix cell at high concentration [23, 24].

CONCLUSIONS

This work describes the facile and rapid synthesis of gold nanoparticles by a novel chemical route. The new method (Reverse Turkevich Method) was used by adding the Au^{3+} solution to the reducing agent with heating and stirring. Stable gold nanoparticles for a longer time were obtained through this easy method. From the results of the experiments, it was concluded that poly acrylic acid reduces Au^{+3} ions to nanoparticles of gold metal (Au^0 NPs). UV-Vis, zeta potential, AFM and TEM studies were used to characterize the synthesized gold nanoparticles. The UV-Vis spectra showed a maximum absorption at 524 nm. The AFM showed an average size value of 72 nm diameter, whereas the size values measured by TEM image data were 14 and 25nm. The AuNPs show enhanced anticancer activity at low concentrations and display effectiveness in killing cervix cancer (Hela) cells. Finally, this study provides an eco-friendly route for the synthesis and application of AuNPs.

REFERENCES

1. P. Pimpang, W. Sutham, N. Mangkornton, P. Mangkorntong, S. Choopun **(2008)**" *Effect of Stabilizer on Preparation of Silver and Gold Nanoparticle Using Grinding Method"* Chiang Mai J. Sci. **35(1): 250-257.**

2. K. L. Kelly, E. Coronado, L .L. Zhao, G. C. Schatz **(2003)**" *The Optical Properties of Metal Nanoparticles: The Influence of Size, Shape, and Dielectric Environment* "J. Phys. Chem. **107(3): 668-677.**

3. E. C. Dreaden, A. M. Alkilany, X. Huang, C. J. Murphy, M. A. El- Sayed **(2012)** *"The golden age: gold nanoparticles for biomedicine*" Chem. Soc. Rev. **41: 2740−2779.**

4. J. Turkevich, P. C. Stevenson, J. Hillier **(1951)** "A study of the nucleation and growth processes in the synthesis of colloidal gold" Discuss. Faraday. Soc. **11: 55–75.**

5. D. Nath, P. Banerjee **(2013)** "*Green nanotechnology – A new hope formedical biology"* Environm. Toxicology and pharmacology **36: 997–1014.**

6. B. S. Bhau, S. Ghosh, S. Puri, B. Borah, D. K. Sarmah, R. Khan **(2015)***"Green synthesis of gold nanoparticles from the leaf extract of Nepenthes Khasiana and antimicrobial assay*" Adv. Mater. Lett**. 6(1): 55-58.**

7. W. Yan, C. Chen, L. Wang, D. Zhang, AJ. Li, Z. Yao, LY. Shi **(2016)** *" Facile and green synthesis of cellulose nanocrystal-supported gold nanoparticles with superior catalytic activity"* Carbohydr Polym. **140: 66-73.**

8. X. Liang, ZJ. Wang, CJ Liu **(2010)** *"Size-controlled synthesis of colloidal gold nanoparticles at room temperature under the influence of glow discharge"* Nanoscale Res Lett. **5:124–129.**

9. T. Sakai, P. Alexandridis **(2005)** *"Size and shape-controlled synthesis of colloidal gold through autoreduction of the auric cation by poly (ethylene oxide)-poly(propylene oxide) block copolymers in aqueous solutions at ambient conditions"* Nanotechnology **16: S344–S353.**

10. X. Tang, P. Jiang, G. L. Ge, M. Tsuji, S. S. Xie, Y. J. Guo **(2008)** "*Poly (N-vinyl-2-pyrrolidone) (PVP)-Capped Dendritic Gold Nanoparticles by a One-Step Hydrothermal Route and Their High SERS Effect"* Langmuir. **24: 1763-1768.**

11. G. Jiang, L. Wang, T. Chen, H. Yu, C. Chen **(2006)** **"***Preparation of Gold Nanoparticles in The Presence of Poly(Benzyl Ether) Alcohol Dendrons "* Mater. Chem. and Phys. **98: 76-82.**

12. A. A. Bogdanov, S. Gupta, N. Koshkina, S. J. Corr, S. Zhang, S. A. Curley, G. Han **(2015)** *" Nanoparticles Stabilized with MPEG Grafted Poly(L-lysine): in Vitro and in Vivo Evaluation of a Potential Theranostic Agent*" Bioconjugate Chem. **26: 39−50.**

13. V. Vetriselvi, R. Jaya Santhi R **(2014)***" Synthesis and Characterization of Poly Acrylic Acid Modified with Dihydroxy Benzene-Redox Polymer"* Res. J. Chem. Sci**. 4(5): 1-9.**

14. N. Hadjichristidis , M. Pitsikalis, S. Pispas, H. Iatrou **(2001)** "*Polymers with Complex Architecture by Living Anionic Polymerization* "Chem. Rev. **101: 3747-3792.**

15. W. Van Camp, V. Germonpre, L. Mespouille, Ph. Dubois, E. Goethals, F. Du Prez **(2007)** *"New poly(acrylic acid) containing segmented copolymer structures by combination of ''click" chemistry and atom transfer radical polymerization"* Reactive & Functional Polymers. **67(11) : 1168–1180** .

16. A. Choudhury, A. Malhotra, P. Bhattacharjee, G. S. Prasad **(2014)** *"Facile and rapid thermo- regulated biomineralization of gold by pullulan and study of its thermodynamic parameters"* Carbohydrate Polymers. **106(5) : 154-159.**

17. V. Raj, K. Sreenivasan **(2010)** *"Selective detection and estimation of C-reactive protein in serum using surface – functionalized gold nano – particles"* Anal. Chim. Acta, **662*(2)*: 186–192**.

18. J. Vasenka, S. Manne, R. Giberson, T. Marsh, E. Henderson **(1993)** *"Colloidal Gold Particles as an Incompressible AFM Imaging Standard for Assessing the Compressibility of Biomolecules"* Biophys J**. 65: 992-997.**

19. Z. Wang, M. Mohamed, S. Link, M. El-Sayed **(1999)** *"Crystallographic facets and shapes of gold nanorods of different aspect ratios"* Surf. Sci. **440(1): 809- 814.**

20. M. Karimi, N. Behtash, Z. Chiti, S. Kargar **(2009)** *"Cervical cancer and HPV vaccines in developing countries"* Asian Pac J Cancer Prev. **10 (6): 969-974**.

21. E. Ulukaya, F. Ozdikicioglu, A. Yilmaztepe, M. Demirci **(2008)** **"***The MTT assay yields a relatively lower result of growth inhibition than the ATP assay depending on the chemotherapeutic drugs tested"* Toxicology in Vitro. **22(1): 232–239**.

22. X. Wang, X. Cai, J. Hu, N. Shao, F. Wang, Q. Zhang, J. Xiao, Y. Cheng **(2013)** *"Glutathione-triggered "off-on" release of anticancer drugs from dendrimer-encapsulated gold nanoparticles*" J. Am. Chem. Soc. **135: 9805−10**.

23. W. Ngwa, R. Kumar, S. Sridhar, H. Korideck, P. Zygmanski, R.A. Cormack, R. Berbeco, G. M. Makrigiorgos**(2014)** *"Targeted radiotherapy with gold nanoparticles: current status and future perspectives*" Nanomedicine **9 (7): 1063−1082.**

24. MY. Lan, YB. Hsu, CH. Shu, CY. Ho, JC. Lin, SW. Lee **(2013)** *"Induction of apoptosis by high-dose gold nanoparticles in nasopharyngeal carcinoma cells"* Auris Nasus Larynx. **40 (6): 563-568.**