IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
August 2016 Vol.:7, Issue:1

Gold Nanoparticles: Preparation, Application and Toxicological Effects of Gold Nanoparticles

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Dr. Awani kumar rai, Dr.Ruchi Tiwari, Dr. Gaurav Tiwari, Priyanka maurya*, Pooja yadav

Pranveer Singh Institute of Technology, Kanpur, India.

Submission: 5 August 2016
Accepted: 10 August 2016
Published: 25 August 2016

Keywords: Nanoparticles, gold, applications, synthesis, toxicity, characterization

ABSTRACT

Gold has been used as a therapeutic agent to treat a wide variety of rheumatic diseases including psoriatic arthritis, juvenile arthritis and discoid lupus erythematosus. Although the use of gold has been largely superseded by newer drugs, gold nanoparticles are being used effectively in laboratory based clinical diagnostic methods while concurrently showing great promise *in vivo* either as a diagnostic imaging agent or a therapeutic agent. For these reasons, gold nanoparticles are therefore well placed to enter mainstream clinical practice in the near future. Hence, in the present review we are discussing the synthesis and applications of gold nanoparticles in the field of medicine and targeted drug delivery. Nanotechnology has become one of the most interesting and advanced areas of research in this field. Among nanoparticles, gold nanoparticles demonstrate special advantages in this field due to their unique properties, small size and high surface area-to-volume ratio. These particles have been widely used in various biomedical applications and drug delivery systems due to their inert nature, stability, high dispersity, non-cytotoxicity and biocompatibility. The importance of controlling the size and shape of gold nanoparticles to minimize any potential toxic side effects was also discussed.

INTRODUCTION

Nanotechnology is an anticipated manufacturing technology that allows the long established trend toward smaller, faster, cheaper materials and devices. Gold nanoparticles (GNPs) are the most compatible nanomaterial for preparation of engineered nanoplatforms in smart sensing devices.[1] Nanogold have several unique properties, for example, they are inert and nontoxic[2] and have good anti-bacterial,[3]anti-angiogenesis properties,[4]etc. One of the outstanding achievements at the end of the last century is the studies on properties of biological and synthetic materials in nanometer. The rapid development of nanoscience has caused the formation of fundamentally new directions for biotechnology research nano-objects, which are characterized by peculiar, often unexpected properties that are different from the properties of both macro and micro scale particles[5].

APPLICATION

Surface Plasmon resonance property of GNP makes them most suitable engineered nanomaterial for bioimaging, biomedical therapeutics and biodiagnostic tools[1]. GNPs, also named as gold colloids, have attracted increasing attention due to their unique properties in multidisciplinary research fields [6,7]. Although GNPs are defined by tiny size, significant quantities of GNPs are likely required in many commercial and industrial applications.

Shapes of gold nanoparticles and their applications[8]

Shape	**Size(nm)**	**Application**
Nano-rod	2-5	Drug delivery and photothermal therapy
Hollow particle	25	Photo-electronics, catalysis and cancer therapy
Triangular particle	3.85-7.13	Highly effective against E. coli and K. Pneumonia
Faceted particle	50-100	Effective, reproducible, and stable large area substrates for NIR SERS [near infra-red surface enhanced Raman spectroscopy
Nanocube	50	Field enhancement applications and refractive-index sensing
Nanocage	50	Effective molecular contrast agent for nonlinear endomicroscopy imaging and *in vivo* medical applications
Nanobelt	Thickness, ~80 nm, Width, ~ 20 µm, Length, ~0.15 m	Strain sensors
Branched particle	90 nm	Substrates for SERS-based imaging of kidney cells

a) Biopolymer-conjugated GNPs are largely used as biomarkers and biodelivery vehicles in the medicine/pharmacy and in cosmetic products. GNPs are employed as anti-aging components for skin protection [9].

b) GNPs are used to treat wool or cotton fibres for a permanent coloration of value textiles.

c) Various polymer/gold nanocomposites display a high potential for novel coatings and paintings [10,11].

d) GNPs are used to enhance the performance of non-volatile memory devices[12] and low temperature printing metal inks in electronics[13]

f) Nanotechnology and nanomaterials have to find a wide application in cardiology and vascular therapy in the treatment of patients with venous and arterial thrombosis, the manufacture of intravascular and intra-cardiac implants, the creation of vascular tissue,etc[14-18.]

g) Gold nanoparticles (AuNPs) are used in vaccine delivery[19], as they can be easily fabricated into different shapes (spherical, rod, cubic, etc.)[20] with a size range of 2–150 nm[21], and can be surface-modified with carbohydrates[22].

h) Gold nanorods have been used as a carrier for an antigen derived from respiratory syncytial virus by conjugating the antigen to the surface[23]. Other types of gold nanoparticles have been used as carriers for antigens derived from other viruses such as influenza[24] and foot-and-mouth disease.

i) Gold nanoparticles and biosensors: The primary principle involved in the design of a biosensor based on gold nanoparticles is that the gold nanoparticles are functionalized with a thiolated biomolecule which upon recognizing the perfecting biomolecule brings about change in the optical absorption of gold nanoparticles.[25]

j] Antimicrobials: Although silver has a long history of being used as an antimicrobial, in recent years gold has also become a good rival for silver. For example, gold nanoparticles can fight against '*E. coli*' bacteria.[25]

k] Gold Nanoparticles in Cancer Diagnosis and Therapy: The main problem with many currently available cancer treatments is that they cannot be precisely targeted. As it is very

hard to get an effective drug, such as paclitaxel, directly to the tumour, large doses are needed in the hope that enough of the drug will reach the diseased cells where it is needed. Recently gold nanoparticles have found a role to deliver drug easily. Cancer therapy has various routes such as chemotherapy, photo-thermal therapy and radiotherapy.[25]

METHODS OF PREPARATION OF GOLD NANOPARTICLES

1] Turkevich method

The Turkevich method was first described in 1951[26] and is one of the most commonly used methods for synthesis of spherical AuNPs in the size range of 10 nm-20 nm (Figure 1). The principle of this method involves reduction of gold ions (Au^{3+}) to gold atoms (Au^{0}) in the presence of reducing agents like citrate[26-28], amino acids, ascorbic acid or UV light [29-32]. Size of AuNPs is further stabilized using various capping/stabilizing agents. Initially, the Turkevich method was limited by the narrow range of AuNPs that could be generated by this method. However, several advances in the original method have allowed for researchers to expand the size range of particles that can be generated via this method. In 1973, Frens found that by varying the ratio of reducing to stabilizing agents, AuNPs of specific size, ranging from 16 nm-147 nm can be achieved[33-35]. Later, the roles of pH, temperature and sodium citrate concentration were better understood, allowing for the generation of a particle growth model[36-39].

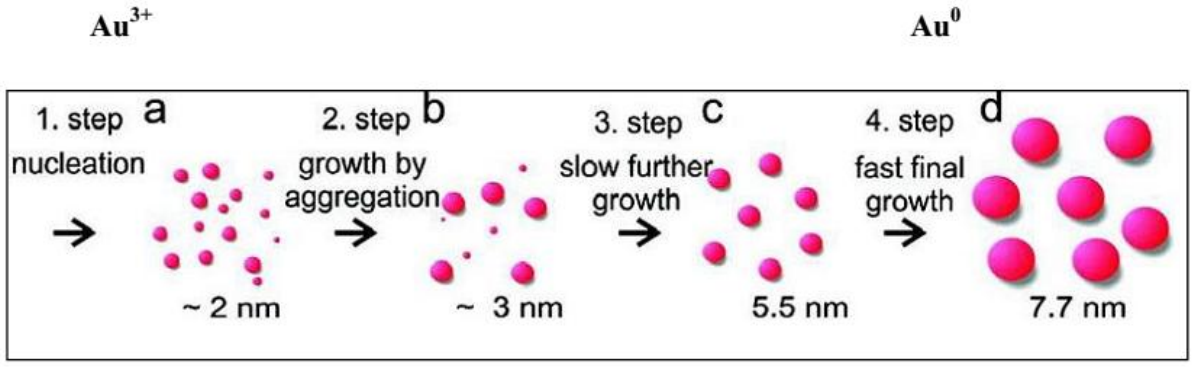

Figure 1: Preparation of Gold Nano-Particles by Turkevich method

2] Brust method

The Brust method was first described in 1994[40]. This method is a two phase process to generate 1.5. nm to 5.2. nm AuNPs using organic solvents (Figure 2) and by varying the ratio of thiol to gold. The Brust method was inspired from Faraday's two phase system. The

method involves transfer of gold salt from aqueous solution to an organic solvent (e.g. toluene) using a phase transfer agent (e.g., tetraoctylammonium bromide (TOAB). The gold is then reduced using sodium borohydride in presence of an alkanethiol. The alkanethiols stabilize the AuNPs[42], resulting in a color change of the reaction from orange to brown[40,41]. Purification of AuNPs stabilized with dodecanethiol from TOAB was reported by Schriffin[43].

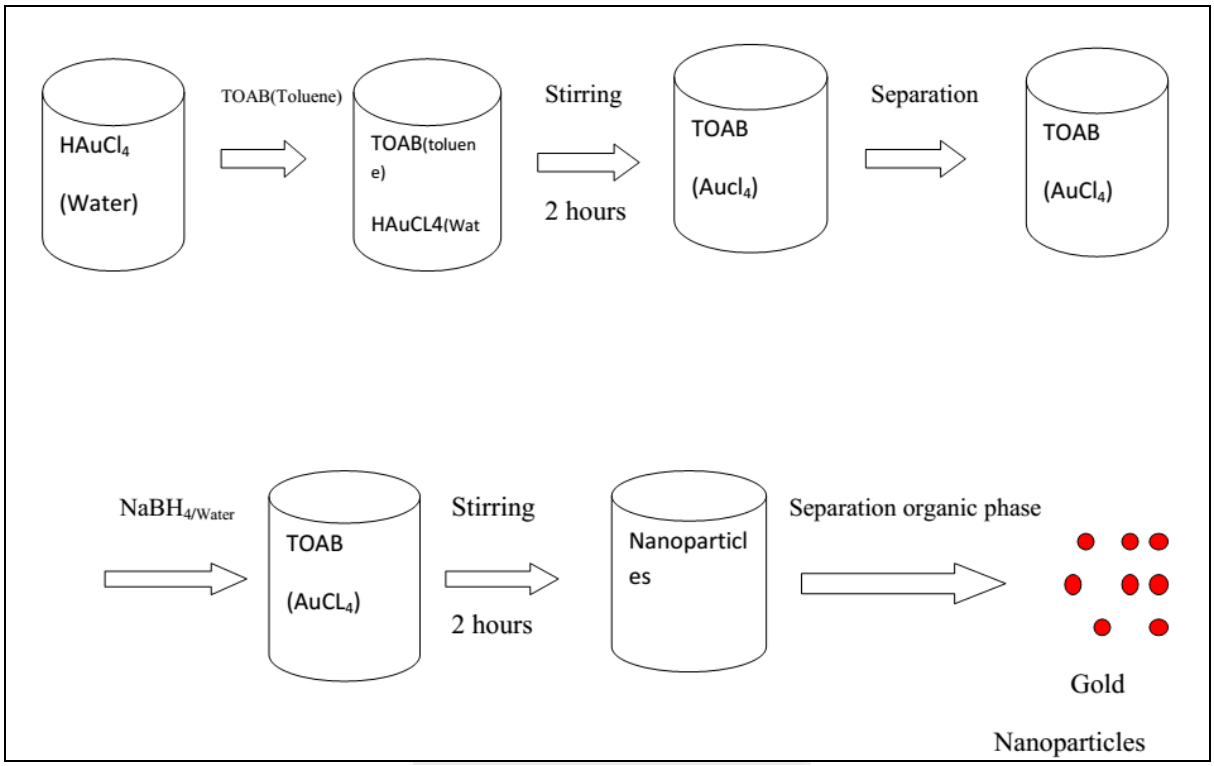

Figure 2: Preparation of Gold Nano-Particles by Brust method

3] Seeding Growth Method

While the Turkevich and Brust methods can generate spherical AuNPs, AuNPs can also exist in variety of nanostructures such as rods, cubes, tubes etc[8]. The most widely preferred technique to obtain AuNPs in other shapes is seed mediated growth[44] (Figure 3). The basic principle of this technique is to first produce seed particles by reducing gold salts with a strong reducing agent like sodium borohydride. The seed particles are then added to a solution of metal salt in presence of a weak reducing agent (ascorbic acid) and structure directing agent to prevent further nucleation and accelerate the anisotropic growth of AuNPs. Geometry of gold nanostructures can be altered by varying the concentration of seeds, reducing agents and structure directing agents.[45]

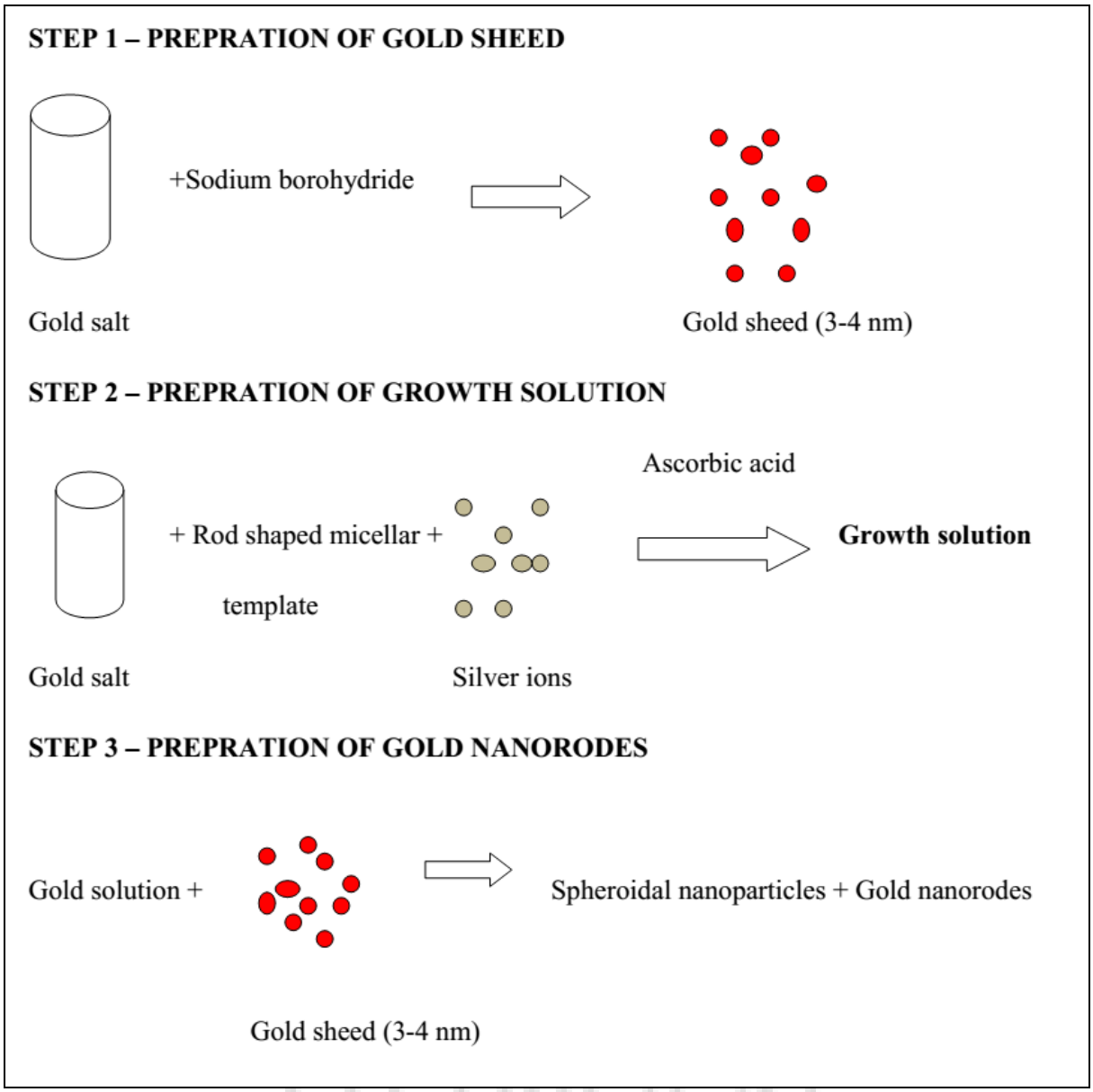

Figure 3: Preparation of Gold Nano Particles by Seeding Growth Method

4] GREEN METHOD

Green chemistry synthesis routes are environment friendly and non-toxic. A facile green biosynthesis method for the preparation of gold nanoparticles of size 25 + 7 nm was reported by using natural biomaterial eggshell membrane (ESM). In this method ESM was immersed in aqueous solution of $HAuCl_4$ without using any reductant[46]. Another green synthetic approach was developed to synthesize gold sononanoparticles of size 5 - 17 nm by using high-power ultrasounds and sodium dehydrate[47]. Gold nanoparticles were successfully synthesized by adopting sunlight irradiation method and were modified with folic acid and capped by 6-mercaptopurine. In this method, solar energy was used to reduce the gold salt [48].

A new green chemistry method for the preparation of gold nanoparticles has been reported, in which gold nanoparticles were formed in aqueous NaCl solution from the bulk gold substrate by natural chitosan without using any external stabilizer and reductant[49]. Gold nanoparticles of size 15 - 80 nm are also synthesized via another green synthetic route. In this method $HAuCl_4$ was reduced by using citrus fruits juice extracts [*Citrus limon, Citrus reticulate* and *Citrus sinensis*] [50]. Edible mushroom was also used for the synthesis of gold nanoparticles via sunlight exposure[51].

TOXICOLOGICAL PROFILE OF GOLD NANOPARTICLES IN INVIVO AND INVITRO STUDIES

AuNPs Target on dermal				
Conjugation	**Concentration (time/size) /route of administration**	**Cellular target**	**Animal target**	**Major outcomes**
	95, 142 and 190 µg/mL (13 nm)13, 20 and 26 µg/mL (45 nm) (3 or 6 days)	CF-31 (human dermal fibroblasts)		Cytotoxicity was size and dose dependent. Larger particles (45 nm) exhibited greater toxicity at smaller doses (10µg/mL) compared to smaller ones (13 nm) which only exhibited cytotoxicity at a concentration of 75 µg/mL.[52]
	0.8-15 nm in size (48h)	SK-Mel-28 (melanoma cells), L929 mouse fibroblasts		Maximum cytotoxicity with smaller NP (1.4 nm) characterized by apoptosis and Necrosis[52]
Citrate	0-0.8 µg/mL (14 nm in size) (2, 4 or 6 days)	Human dermal fibroblasts		Dose-dependent reduction in cell proliferation[52]
	15, 102 and 198 nm in size		Excised abdominal skin of Wistar rats	Size-dependent permeation through rat skin with smallest NP having deeper tissue penetration[52]
AuNPs Target on Liver				
Conjugation	**Concentration (time/size)**	**Cellular target**	**Animal target**	**Major outcomes**

	/route of administration			
Immunogenicpeptides: • pFMDV • pH5N1	8mg/kg/week (3-100 nm in size) (4 weeks) Intraperitonea		BALB/C mice	Naked NP: severe adverse effects with resultant death with particles ranging from 8 to 37 nm in diameter. Microscopically, Kupffer cell activation in the liver and lung parenchymal destruction was observed. Surface modified NP: elicited increased host immune response and improved Cytocompatibility[52]
PEG	0.17, 0.85 and 4.26 mg/kg body weight (13 nm in size) (30 min after injection for 7days) Intravenous		BALB/C mice	NPs were found to accumulate in liver and spleen. Significant up regulation of inflammatory cytokines (IL-1, 6, 10 and TNF) with subsequent apoptosis of hepatocytes at highest concentrations (4.26 mg/kg). No significant changes in the liver at lower doses[52]
PEG	4.26 mg/kg (4 and 100 nm in size) (30 min) Intravenous		BALB/C mice	Both 4 and 100 nm sized gold NP up regulated genes responsible for inflammation, apoptosis and cell cycle[52]
	0.14-2.2 mg/kg(13.5 nm in size) (14-28 days) Per oral, intraperitneal or intravenous			Highest toxicity was found with oral and i.p. administration whereas lowest toxicity was seen with tail vein injection.[52]
AuNPs Target on Brain				
Conjugation	**Concentration (time/size) /route of administration**	**Cellular target**	**Animal target**	**Major outcomes**

	0.8-50 µg/mL (3, 5, 7, 10, 30 and 60 nm) (24 h)	rBMEC (primary rat brain microvessel endothelial cells		No morphological changes could be detected after 24 h suggesting cytocompatibility of the NP tested. Only the smallest NP tested (3 nm) induced mild signs of cellular toxicity.[52]
	(12.5 nm in size)(40, 200 or 400g/kg/day for 8 days)Intraperitoneal		C57/BL6 mice	Small amounts of NP were able to cross the BBB but did not induce evident neurotoxicity.[52]

CHARACTERIZATION

The morphology, size and shape of synthesized gold nanoparticles were characterized using transmission electron microscopy, zeta size and spectrophotometer.

Transmission electron microscopy

The size of gold nanoparticles has been determined by measuring the diameter of whole particles on TEM images. It is a microscopic method in which the sample was suspended in ethanol and homogenized using a sonicator for 15 min. One drop of unsettled suspension was placed on a copper grid and the solvent was allowed to dry at room temperature. The average diameter of particles was calculated by measuring 100-300 individual particles with a software[53].

UV-VIS spectrophotometry: Spectrophotometry is another important aspect for characterization of gold nanoparticles. With increase in particle size, the absorption peak shifts to longer wavelength and the width of absorption spectra is related to the size distribution range. Generally, gold nanospheres display a single absorption peak in the visible range between 510-550 nm, because of surface Plasmon resonance and show heavy absorption of visible light at 520 nm. This gives brilliant red colour to Gold Nanoparticle (GNP), which varies according to their size[54].

Zeta size and zeta potential distribution

The Zeta study is conducted for particle size, size distribution as well as for zeta potential measurement of both bare and antibody conjugated gold nanoparticles. In transmission

electron microscopy (TEM) study, images show particles with lower and higher size range. To obtain uniformity of size, colloidal gold solution is centrifuged (at 8000 rpm) for 10 minutes. The supernatant is collected and characterized for further use. For zeta potential distribution study, peak number and peak area give important explanation. Three cycles of different counts are run and average of the counts is taken. The peak mean gives the mean diameter of particle and peak area give the percentage of mean diameter according to intensity. The graphs is plotted using the means of all peaks mean diameter and the intensity of peak area[53].

CONCLUSION

Gold nanoparticles have, in some ways, revolutionized the field of medicine because of its widespread applications in targeted drug delivery, imaging, diagnosis and therapeutics due to their extremely small size, high surface area, stability, non-cytotoxicity and tunable optical, physical and chemical properties. Au-NPs were proven to be a promising vehicle for drug delivery. Side effects of conventional drugs have been minimized by conjugation with gold nanoparticles and they increase the quality life of patients.

REFERENCES

1. Jain, P .K., Lee, K.S., El-Sayed, I.H. and EL-Sayed, M.A. Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: application in biological imaging and biomedicine. J. Phys. Chem. B.,2006; 110: 7238-7248.
2. Patra CR, Bhattacharya R, Mukhopadhyay D, Mukherjee P. Fabrication of gold nanoparticles for targeted therapy in pancreatic cancer. Adv Drug Deliv Rev 2010;62(3):346-61.
3. Dastjerdi R, Montazer M. A review on the application of inorganic nanostructured materials in the modification of textiles: focus on antimicrobial properties. Colloids Surf B Biointerfaces2010;79(1):5-18.
4. Mukherjee P, Bhattacharya R, Wang P, Wang L, Basu S, Nagy JA, et al. Anti-angiogenic properties of gold nanoparticles. Clin Cancer Res 2005;11(9):3530-4.
5. Lanza GM, Winter PM, Caruthers SD, Hughes MS, Cyrus T, Marsh JN,Neubauer AM, Partlow KC, Wickline SA: Nanomedicine opportunities for cardiovascular disease with perfluorocarbon nanoparticles. Nanomedicine 2006,1:321–329.
6. Daniel, M.C. and Astruc, D. Gold nanoparticles: Assembly, supramolecular chemistry, quantum-size-related properties and applications toward biology , catalysis, and nanotechnology . Chem. Rev.,2004; 104: 293-346.
7. Sardar, R., Funston, A.M., Mulvaney, P . and Murray, R.W . (2009) Gold Nanoparticles: Past, Present, and Future. Langmuir.,2009; 25: 13840-13851.
8. AK Khan, R Rashid, G Murtaza and A Zahra, Gold Nanoparticles: Synthesis and Applications in Drug Delivery, Trop J Pharm Res, July2014; 13(7): 1169-1177.
9. Boisselier, E. and Astruc, D. Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity . Chem. Soc. Rev .,2009; 38: 1759-1782.
10. Freudenberger, R., Zielonka, A., Funk, M., Servin, P ., Haag, R., V alkova, T . and Landau, U. Recent developments in the preparation of nano-gold composite coatings. Gold Bull.,2010; 43: 169.
11. Jans, H., Jans, K., Lagae, L., Borghs, G., Maes, G. and Huo, Q. Poly (acrylic acid)-stabilized colloidal gold nanoparticles: synthesis and properties. Nanotechnol.,2010; 21: 455702.
12. Li, D.X., Li, C.F., Wang, A.H., He, Q. and Li, J.B. Hierarchical gold / copolymer nanostructures as hydrophobic nanotanks for drug encapsulation. J. Mater . Chem.,2010; 20: 7782.

13. Bishop, P .T ., Ashfield, L.J., Berzins, A., Boardman, A., Buche, V ., Cookson, J., Gordon, R.J., Salcianu, C. And Sutton, P .A. (2010) Printed gold for electronic applications. Gold Bull.,2010; 43: 181.
14. Lanza GM, Winter PM, Caruthers SD, Hughes MS, Cyrus T, Marsh JN,Neubauer AM, Partlow KC, Wickline SA: Nanomedicine opportunities for cardiovascular disease with perfluorocarbon nanoparticles. Nanomedicine 2006;1:321–329.
15. Juenger J, Schellberg D, Kraemer S, Haunstetter A, Zugck C, Herzog W,Haass M:Health related quality of life in patients with congestive heart failure: comparison with other chronic diseases and relation to functional variables. Heart2002;87(3):235–241.
16. Hobbs FD, Kenkre JE, Roalfe AK, Davis RC, Hare R, Davies MK: Impact of heart failure and left ventricular systolic dysfunction on quality of life: a cross-sectional study comparing common chronic cardiac and medical disorders and a representative adult population. Eur Heart J2002; 23(23): 1867–1876.
17. Neubauer S:The failing heart—an engine out of fuel.N Engl J Med2007; 356(11):1140–1151.
18. Krumholz HM, Chen YT, Wang Y, Vaccarino V, Radford MJ, Horwitz RI:Predictors of readmission among elderly survivors of admission with heart failure.Am Heart J 2000; 139(1 Pt 1):72–77.
19. Peek LJ, Middaugh CR, Berkland C. Nanotechnology in vaccine delivery. Advanced Drug Delivery Reviews 2008;60:915–28.
20. Niikura K, Matsunaga T, Suzuki T, Kobayashi S, Yamaguchi H, Orba Y, et al.Gold nanoparticles as a vaccine platform: influence of size and shape on immunological responses in vitro and in vivo. ACS Nano 2013;7:3926–38.
21. Gregory AE, Titball R, Williamson D. Vaccine delivery using nanoparticles. Frontiers in Cellular and Infection Microbiology 2013;3:13.
22. Marradi M, Chiodo F, Garcia I, Penades S. Glyconanoparticles as multifunctional and multimodal carbohydrate systems. Chemical Society Reviews 2013;42:4728–45.
23. Stone JW, Thornburg NJ, Blum DL, Kuhn SJ, Wright DW, Crowe Jr JE. Gold nanorod vaccine for respiratory syncytial virus. Nanotechnology 2013;24:295102.
24. Tao W, Ziemer KS, Gill HS. Gold nanoparticle-M2e conjugate coformulated with CpG induces protective immunity against influenza A virus. Nanomedicine 2013:1–16.
25. Adeleh Granmayeh Rad, Hamed Abbasi ,Mohammad Hossein Afzali, Gold Nanoparticles: Synthesising, Characterizing and Reviewing Novel Application in Recent Years, Physics Procedia,2011;22: 203 – 208.
26. J. Turkevich, P.C. Stevenson and J. Hillier: A study of the nucleation and growth process in the synthesis of colloidal gold. Discuss. Faraday Soc.,1951; 11:55-75.
27. B.V. Enustun and J. Turkevich: Coagulation of colloidal gold. J. Am. Chem. Soc.,1963; 85:3317- 3328.
28. S.K. Sivaraman, S. Kumar and V. Santhanam: Monodisperse sub-10 nm gold nanoparticles by reversing the order of addition in turkevich method- The role of chloroauric acid. J. Colloid Interface Sci., 2011: 361(2): 543-547. .
29. Y. Niidome, K. Nishioka, H. Kawasaki and S. Yamada: Rapid synthesis of gold nanorods by the combination of chemical reduction and photoradiation processes; morphological changes depending on the growing processes. Chem. Commun., 2003;2376-2377.
30. Y. Shao, Y. Jin and S.Dong: Synthesis of gold nanoplates by aspartate reduction of gold chloride. Chem Commun.,2004; 1104-1105.
32. D.V. Goia and E. Matijevic: Preparation of monodispersed metal nanoparticles. New J. Chem.,1998; 22, 1203-1215.
32. N.R. Jana, L. Gearheart and C.J. Murph: Seed mediated growth approach for shape controlled synthesis of spheroidal and rod like gold nanoparticles using a surfactant template. Chem. Mater.,2001; 12: 1389-1392.
33. G. Frens: Controlled nucleation for the regulation of particle size in monodisperse gold suspensions. Nature: Phys. Sci.,1973; 241: 20-22.
34. T. Yonezawa and T. Kunitake: Practical preparation of anionic mercapto ligand stabilized gold nanoparticles and their immobilization. Colloids Surf. A: Physicochem. Eng. Asp.,1999; 149: 193-199.
35. K.J. Watson, J. Zhu, S.B.T. Nguyen and C.A. Mirkin: Hybrid nanoparticles with block copolymer shell structures. J. Am. Chem. Soc.,1999; 121: 462-463
36. M.K. Chow and C.F. Zukoski: Gold sol formation mechanisms- role for colloidal stability. J Colloid Interface Sci,1994; 165: 97.
37. X.H. Ji, X.N. Song, J. Li, Y.B. Bai, W.S. Yang and X.G. Peng: Size controlled of gold nanocrystals in citrate reduction: the third role of citrate. J. Am. Chem. Soc.,2007; 129: 13939- 13948.
38. S.C. Yang, Y.P. Wang, Q.F. Wang, R.L. Zhang and B.J. Ding: UV irradiation induced formation of Au nanoparticles at room temperature: the case of pH values. Colloid Surf A- Physicochem Eng. Asp.,2007; 301: 174-183.
39. S. Kumar, K.S.Gandhi and R. Kumar: Modeling of formation of gold nanoparticles by citrate method. Ind. Eng. Chem. Res.,2007; 46: 3128-3136..

40. M. Brust, M. Walker, W. Bethell, D.J. Schriffin, R. Whyman: Synthesis of thiol-derivatised gold nanoparticles in a two phase liquid system. J. Chem. Soc.,1994; 801-802.
41. M. Faraday: The Bakerian Lecture: Experimental relations of gold to light. Philos. Trans. R. Soc. Lond.,1857; 147: 145-181.
42. M. Giersig and P. Mulvaney: Preparation of ordered monolayers by electrophoretic deposition. Langmuir,1993; 9: 3408-3413.
43. C.A. Waters, A.J. Mills, K.A. Johnson and D.J. Schriffin: Purification of dodecanethiol derivatized gold nanoparticles. Chem. Commun.,2003; 540-541.
44. X. Zhi-Chuan, S. Cheng-Min, X. Cong-Wen, Y. TianZhong, Z. Huai-Ruo, L. Jian-Qi and G. Hong-Jun: Wet chemical synthesis of gold nanoparticles using silver seeds: a shape control from nanorods to hollow spherical nanoparticles. Nanotechnol.,2007; 18, 115608.
45. 78. C.J. Murphy, T.K. Sau, A.M. Gole, C.J. Orendorff, J. Gao, L. Gou, S.E. Hunyadi and T. Li: Anisotropic Metal Nanoparticles: Synthesis, Assembly, and Optical Applications. J. Phys. Chem. B.2005; 109, 13857–13870..
46. Murawala P, Tirmale A, Shiras A, Prasad BLV. In situ synthesized BSA capped gold nanoparticles: Effective carrier of anti-cancer drug Methotrexate to MCF-7 breast cancer cells. Mater Sci Engineer C 2014; 34: 158-167.
47. Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA. Gold nanoparticles for biology and medicine. Angewandte Chem 2010; 49: 3280- 3294.
48. Ghosh R, Singh LC, Shohet JM, Gunaratne PH. A gold nanoparticle platform for the delivery of functional microRNAs into cancer cells. Biomater 2013; 34: 807- 816.
49. Namazi H, Fard AMP. Preparation of gold nanoparticles in the presence of citric acid-based dendrimers containing periphery hydroxyl groups. Mater Chem Physic 2011; 129: 189-194.
51. Tarnawski R, Ulbricht M. Amphiphilic gold nanoparticles: Synthesis, characterization and adsorption to PEGylated polymer surfaces. Colloid Surface A: Physicochem Engineer Aspects 2011; 374: 13-21.
52. Lara Yildirimer, Nguyen T.K. Thanh, Marilena Loizidou, Alexander M. Seifalian, Toxicological considerations of clinically applicable nanoparticles, Nano Today 2011; 6: 585—607.
53. Harihar Nath Verma , Praveen Singh and R. M. Chavan, Gold nanoparticle: synthesis and characterization, Veterinary World, EISSN: 2231-0916,Feb-2014; 7: 72-77.
54. Verma HN, Singh P and Chavan RM (2014) Gold nanoparticle: synthesis and characterization, Veterinary World, 2014; 7(2): 72-77.