THE STRUCTURES, PROPERTIES, SYNTHESIS, AND INNOVATIVE APPLICATIONS OF GOLD NANOPARTICLES IN LABORATORY MEDICINE: A REVIEW

Ishak N[1], Zabidi MA[2], and Noordin SS[1,3].
[1]Clinical Diagnostic Laboratory, Pusat Perubatan Universiti Sains Malaysia Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia
[2]Department of Dental Science, Institut Perubatan dan Pergigian Termaju, Universiti Sains Malaysia, Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia
[3]Department of Clinical Medicine, Institut Perubatan dan Pergigian Termaju, Universiti Sains Malaysia, Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia

Correspondence:
Siti Salmah Noordin,
Department of Clinical Medicine,
Institut Perubatan dan Pergigian Termaju,
Universiti Sains Malaysia, Bertam,
13200 Kepala Batas, Pulau Pinang, Malaysia
Email: ssalmah@usm.my

Abstract

Nanoparticles technology has gained a great deal of interest in medical and non-medical fields. Gold nanoparticles (AuNPs) which are one of the most attractive metal nanoparticles are widely used in many biomedical fields either for therapeutic or diagnostic purposes. The properties of AuNPs, such as their stability, high biocompatibility, large surface area, relatively inert element, and tunable chemical and physical characteristics have enabled a wide range of applications and discoveries. The AuNPs can be synthesised chemical, physical, or biological synthesis. It is prepared through top-down or bottom-up methods depending on the starting material preparation. AuNPs are used in assays to detect diagnostic markers and specific nucleic acid sequence. Additionally, it is also used in assays to improve bacterial species identification. This review article provides a general overview of the structures, properties, synthesis, and various innovative applications of AuNPs in laboratory medicine such as in chemical pathology, immunology, haematology, molecular, cytogenetics, and transfusion medicine laboratories.

Keywords: Gold Nanoparticles, Laboratory, Medicine, Diagnostics

Introduction

Nanoparticle technology advancements, particularly the dynamicity of nanoparticle synthesis, offer significant advantages in many disciplines, including laboratory medicine. Nanoparticles (NPs) refer to material between one and 100 nm in size, and the term "nano" is derived from the Greek word "small"(1, 2). Notably, gold nanoparticles (AuNPs) are recognised for their unique properties, such as being stable, having high biocompatibility, optically electronic, good conductivity, and having a large surface area (1). Additionally, AuNPs required a simple synthesis mechanism, are easily reactive with many molecules and are simply detected and quantified. Hence, AuNPs have been widely applied in various fields, especially in the laboratory medicine since early 2000s as an analytical tool (3). Besides AuNPs, silver, nanoparticles (AgNPs) are also commonly used in laboratory medicine. As compared to AuNPs, AgNPs have higher cytoxicity and generate more reactive oxygen species (ROS), and leakage of lactate dehydrogenase (4). This review aimed to provide a general overview of the structures, properties, synthesis, and examples of AuNPs application in laboratory medicine.

Gold nanoparticles structures and properties

The AuNPs can be divided into three main groups according to their size and surface functionality: gold colloids, monolayer-covered clusters (MPCs), and small gold clusters (5). Gold colloids are large particles with a size range between 10 and 100 nm and are usually formed after the reduction synthesis process. Monolayer protected clusters (MPCs) are 1 to 10 nm particles surrounded by a highly organic ligand monolayer, such as thiols. Meanwhile, small gold clusters correlated to the lower bound of MPCs and consist of a few tens of atoms that are normally purely monodispersed (6).

The AuNPs are exceptionally stable nanomaterials with a

unique combination of physical and chemical properties that have been widely recognised and applied. The AuNPs are wine red, whereas gold particles are yellow. The particles are available in a variety of shapes and sizes ranging from 1 to 8 µm, including spherical, nanorods, irregular shape, sub-octahedral, octahedral, icosahedral multiple twined, decahedral, tetrahedral, nanotriangles, hexagonal platelets, and nanoprisms (Figure 1) (7).

Most AuNPs synthesis methods are rapid, highly sensitive, and need only a simple mechanism with monodisperse sizes. Given that AuNPs react easily with numerous molecules due to their relatively inert element and beneficial electronic, catalyst, chemical, and optical properties, AuNPs are useful in many scientific domains (3). The AuNPs also have tunable chemical and physical properties, allowing interaction with different biological macromolecules and organic compounds (1). Besides, the high electron density in AuNPs enhanced protein agglutination by increasing cellular uptake upon interaction with cell membranes (8, 9). AuNPs can generate a strong linkage between nanoparticle-protein interaction, immunogenicity, and cytotoxicity when they react with human serum (10). The AuNPs are an ideal probe element in signal, sensory, and molecular applications since they are easily detected and quantified (11).

Surface Plasmon Resonance (SPR), which is correlated to the cumulative absorption of conduction electrons, is the most outstanding optical feature of AuNPs. The SPR can be found in the broad optical region, depending on the size, shape, and environment of AuNPs, including surface chemistry, aggregation phase, and surrounding medium properties (12). The SPR can detect variations in the AuNPs aggregation phase and display specific colours. For example, a solution containing spherical AuNPs that is red-purple may change slightly when the shape of AuNPs or the surrounding medium is modified (6). Due to these unique properties, AuNPs have been studied and utilised in the high-tech laboratory medicine field.

Gold nanoparticles synthesis method

The approach for AuNPs synthesis involves chemical, physical, and biological mechanisms (13). The AuNPs can be prepared through top-down or bottom-up methods depending on the starting material preparation (13–15). The main factors that can affect the characteristics of the final product are the strength of the reductant, the action of the stabiliser, and the step of the synthesis procedure (16). In the top-down method, the bulk gold material is broken down to generate AuNPs, while atoms or molecules are used as starters to form nano-size particles in the bottom-up method (Figure 2) (17). The comparison between three methods in term of their advantages and disadvantages is summarised in Table 1.

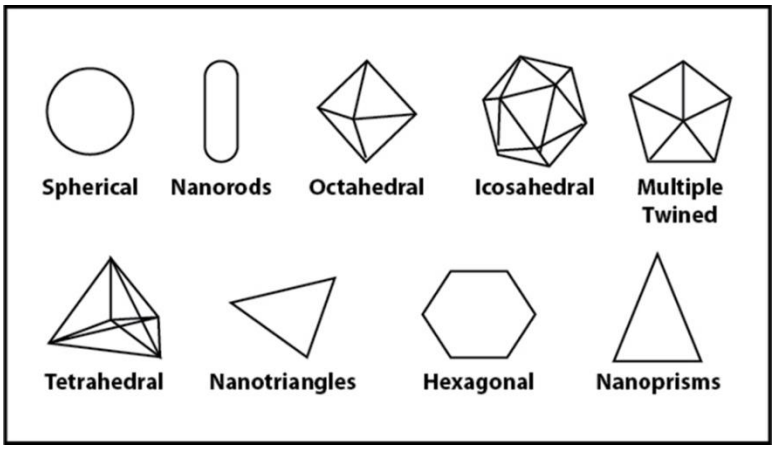

Figure 1: Various shapes of AuNPs

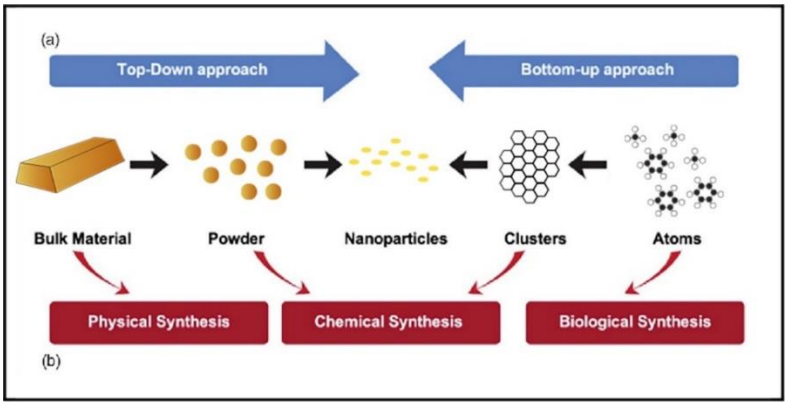

Figure 2: AuNPs synthesis methods: (a) top-down or bottom-up approach (b) chemical, physical, and biological synthesis mechanisms

Table 1: Comparison between three types of synthesis methods for AuNPs

Synthesis Methods	Techniques	Advantage	Disadvantage
Chemical	Turkevich's	Produce tunable particles with diameters ranging from 15-150 nm (18).	Produce limited number of AuNPs and difficult to interpret the surface chemistry of NPs (20, 21).
	Brust–Schiffrin method or two-phase method	Produce smaller and stable AuNPs (23).	Complicated preparation method.
	Seed-mediated growth	Produce various size and shape and prevent further aggregation and nucleation of AuNPs (18, 19).	Various factors may affect the size of AuNPs including gold concentration, reaction temperature, and number of seeds added into reaction (25).
Physical	Laser ablation	Simple and effective in producing a large amount of AuNPs (27).	AuNPs produced can aggregate, is homogeneous, and has a wide size distribution (29).
	Ion implantation	Physical and chemical properties of AuNPs could be maintained (30).	High capital cost.
Biologi-cal	Bioreduction and biosorption mechanisms	Cost-effective, eco-friendly, easy to regulate the shape and size of NPs, and mass production of AuNPs (33, 34).	Requires a long time, delicate construction steps, and detection of organic compounds involves in reduction and stabilization of AuNPs (35).

i. Chemical synthesis method

The chemical method is the most common approach to AuNPs synthesis. This method comprises of two critical components: reductant and stabiliser (18). The reductant serves as a chemical reducer, while the stabiliser prevents particle accumulation. The most popular reductant agents are sodium citrate, sodium borohydride, and diborane (12).

The first AuNPs were developed using Turkevich's chemical synthesis method in 1951 and were spherical with a diameter of about 10 to 20 nm (19). This well-established method known as nucleation-growth or single-phase synthesis involved the reduction of gold chloride by sodium citrate (reductant) and stabiliser agents for AuNPs processing in boiling water (Figure 3) (16). The method was then improved by regulating the gold to sodium citrate ratio and producing tunable particles with diameters ranging from 15-150 nm (18). Nevertheless, the disadvantages of this method include it produces limited number of AuNPs, only small-size nanoparticle is prepared, and the surface chemistry of NPs was difficult to interpret (20, 21).

In 1994, Brust and Schiffrin invented the two-phase synthesis method that can yield smaller spherical AuNPs with a diameter of 1.5 to 5.2 nm and were able to combine with a stabiliser, such as organic solvents and thiolate molecules (15, 20). This method used toluene as an organic solvent, tetraoctylammonium bromide (TOAB) as a phasetransfer agent, and alkanethiole as a stabiliser (22). This approach had the advantage of producing smaller and stable AuNPs but the preparation method is complicated (23).

In 2015, Wuithschick and colleagues also proposed a different chemical synthesis approach for AuNPs namely the seed-mediated method, whereby the AuNPs could be enlarged and produced in various shapes (24). This method used sodium borohydride ($NaBH_4$) as a strong reducing agent, ascorbic acid as a weak reducing agent, and a structure-directing agent to prevent further aggregation and nucleation of AuNPs. Interestingly, this method improved the growth and control of the structural properties of AuNPs, including size and morphology, by adjusting the concentration of seed, reducing agents, and structural-directing agents (18-19, 25).

ii. Physical synthesis method

Another preferred method for producing AuNPs is physical synthesis. Predominantly, the preparation method involves reducing and trapping agents. Physical synthesis techniques include γ-irradiation, microwave irradiation, sonochemical, thermolytic, photochemical, ion implantation, electron beam, optical lithography, and the most prevalent being laser ablation and ion implantation (26). The laser ablation technique is simple and effective in producing a large amount of AuNPs (27). The surface area, aggregation, shape, size, and properties of AuNPs were controlled by choosing the appropriate parameter and type of aqueous solution (28).

Nevertheless, AuNPs synthesis using laser ablation technique in the alkanes solvent had been shown to be not soluble and stable, having a wide size distribution, aggregated, and was homogenous (29). Contrarily, ion implantation involved injecting the gold element into the near-surface area in a vacuum chamber. Using this process, the physical and chemical properties of AuNPs could be maintained (30).

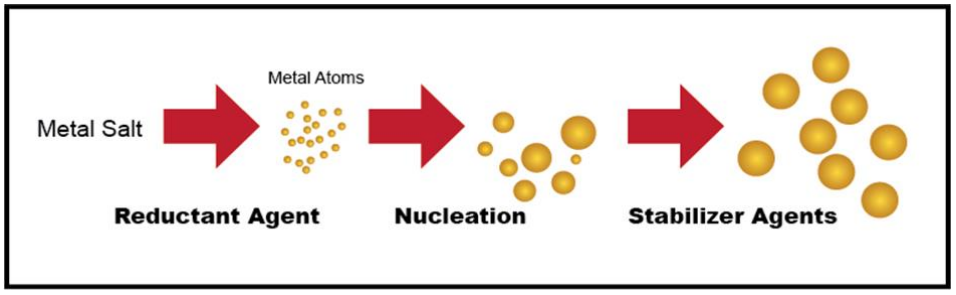

Figure 3: The nucleation-growth or single-phase synthesis involving reductant and stabiliser agents

iii. Biological synthesis method

Biological synthesis, also known as biosynthesis, biomimetic, or green synthesis, is an environmentally sustainable method as it utilises natural and non-toxic products, such as plants and microorganisms (bacteria, fungi, algae, and yeast). This synthesis method is divided into bioreduction and biosorption mechanisms (31). Bioreduction uses microorganism enzymes to reduce the gold ions into a stable and inactive form, whereas in biosorption, the cell wall of the organism fastens the gold cations in media to form stable AuNPs (32). The biological synthesis method offers the advantage of easing in regulating the shape and size of the NPs, more cost-effective for mass production of AuNPs, and is more environmental friendly (33, 34).

Application of gold nanoparticles in laboratory medicine

Research on the application of AuNPs in laboratory medicine has recently received increased interest. The AuNPs are utilised as a sensor or a protein target for in vitro laboratory analysis. Table 2 summarises the application of AuNPs in laboratory medicine.

i. Gold nanoparticles application in chemical pathology laboratory

In chemical pathology analysis, AuNPs were used to detect lung, head, and neck cancer whereby electrochemical sandwich immunosensor method of detection using immunorecognition by congregation of alpha-Enolase (ENO1) tagged with AuNP was performed. In this 2010 study by Ho (36), they had shown that AuNP congregate-based assay was able to provide a detection limit of ENO1 at trace level which was as low as 11.9 fg (equivalent to 5 µL of a 2.38 pg/mL solution). Additionally, AuNPs were also used as a microelectronic transducer sensor to detect volatile organic compounds in breath samples to diagnose and monitor chronic kidney disease (CKD). This study, which was conducted in 2012 by Marom (37) had found that there was 77% sensitivity, 80% specificity, and 79% accuracy in detecting early-stage CKD. Recently in 2020, a dual-color aptasensor system was developed through a mix of salt-induced AuNPs aggregation that promotes fluorescence of DNA-silver nanoclusters. Thus, the dual cancer markers, carcinoembryonic antigen (CEA) and carbohydrate antigen 125 (CA125) can be monitored as low as 7.5 pg/mL and 0.015 U/mL detection limit (38).

ii. Gold nanoparticles application in clinical microbiology laboratory

The clinical microbiology laboratory is crucial to identify microorganisms and antimicrobial drug activity. Therefore, a rapid, accurate, and sensitive testing activity is of importance. In a study to enhance the identification of *Mycobacterium tuberculosis* (MTB) and *Mycobacterium tuberculosis* complex (MTBC) species in clinical sputum samples, AuNPs conjugated with thiol-modified oligonucleotides were used as probes in the polymerase chain reaction (PCR) and immunochromatography test (ICT) (39). In addition, the use of AuNPs in combination with specific peptide ligands via colorimetric method enabled the detection of *Aspergillus niger* spores with high sensitivity and fast detection time (40). Furthermore, AuNPs coupled with other compounds such as *Curcuma pseudomontana* showed a 94% inhibition in antimicrobial drug activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* (41). Similarly, AuNPs capped with cefaclor had demonstrated excellent antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* during the synthesis process (42).

In a more recent study conducted by Abdou Mohamed (43), AuNPs combined with multicomponent nucleic acid enzyme had shown 90% clinical sensitivity and 95% clinical specificity respectively in detecting antibiotic resistance in methicillin-resistant Staphylococcus aureus in patients swab and mecA resistance genes in uncultured wound swabs. In addition, AuNPs colorimetric reagent was also developed for rapid detection of SARS-CoV-2 virus antibodies in human blood using lateral flow immunoassay. This method detects anti-SARS-CoV-2 IgM and IgG in the specimen through the conjugation of SARS-CoV-2 recombinant antigen with labelled AuNPs. This method demonstrated high sensitivity (88.7%) and specificity (90.6%) (44).

iii. Gold nanoparticles application in cytogenetics laboratory

Cytogenetics laboratory involves chromosome structure analysis and detection of genetic disorders. The AuNPs-based genomagnetic sensor was designed to detect DNA hybridization in breast cancer and cystic fibrosis genes.

The AuNPs were tagged on the magnetic graphite-epoxy composite electrode to induce direct electrochemical detection of DNA hybridization (45). Moreover, the AuNPs reflected three beneficial impacts in a nanomaterial-assisted polymerase chain reaction (nanoPCR). Firstly, AuNPs displayed high adsorption capability in polymerase and can modulate the amount of active polymerase in PCR, allowing for case-by-case PCR regulation. Furthermore, AuNPs could adsorb primers and adjust the melting temperatures during the annealing process to improve PCR specificity. Finally, AuNPs could adsorb PCR products and promote dissociation in the denaturing stage, improving PCR efficiency further (46).

Table 2: Application of gold nanoparticles in laboratory medicine

Laboratory Medicine	Year	Development of the AuNPs
Chemical Pathology	2010	Detection of alpha-Enolase (ENO1) or a tumor suppressor of lung, head, and neck cancer (36).
	2012	Sensor to diagnose and monitor chronic kidney disease (37).
	2020	Dual-Color Aptasensor System to monitor cancer markers: carcinoembryonic antigen and carbohydrate antigen 125 (38).
Clinical Microbiology	2009	Probes in the polymerase chain reaction and immunochromatography test to identify *Mycobacterium species* in sputum samples (39).
	2021	Detection of *Aspergillus niger* spores (40).
	2020	Rapid detection of IgM-IgG SARS-CoV-2 infection (44).
	2010, 2021	Inhibition of antimicrobial drug activity (41)(42).
	2021	Detection of antibiotic resistance in methicillin-resistant *Staphylococcus aureus* and *mecA* resistance genes (43).
Cytogenetic	2007	Detection of DNA hybridization in breast cancer and cystic fibrosis genes (45).
	2013	Genetic analysis using nano polymerase chain reaction (PCR) (46).
Immunology	2016	Detection of DNAse I activity in systemic lupus erythematosus (50).
	2019	Detection of rheumatoid arthritis antibody (IgM) (42).
	2019	Detection of antibodies towards casein, ovalbumin, and hazelnut allergenic proteins (48).
	2020	Rapid detection of β-conglycinine (soybean allergen) (47).
Haematology	2011	Detection of coagulation proteins reactions (51).
	2014	Detection of glucose-6-phosphate dehydrogenase (G6PD) gene mutations (53).
	2020	Detection of G6PD variants (52).
Transfusion Medicine	2007	Agglutination reaction enhancement in detecting weak B subgroups (54).
	2016	Agglutination reaction enhancement for red cells antibody detection in pre-transfusion testing (55).
Molecular Diagnostic	2003	Detection of Factor V Leiden mutation (56).
	2012	Detection of DNA sequence by asymmetric PCR (57).
	2016	Detection of KRAS Gene using high-throughput DNA array (58).
	2018	Rapid colorimetric detection of DNA sequence (59).

iv. Gold nanoparticles application in immunology laboratory

The immunology laboratory is mainly involved in the testing of allergies, autoimmune diseases, immunodeficiency, and immunoproliferative disorders. Using the sandwich lateral flow immunochromatographic detection method, AuNPs in combination with p-aminothiophenol were used to enhance signal detection of β-conglycinine, a protein in soybeans that may cause food allergies. This developed method was able to quantitatively detect β-conglycinin concentrations between 160 ng/mL to 100 µg/mL (47). Besides, by using AuNPs in combination with silver nanoparticles, a qualitative multicolour multiplex lateral flow immunoassay was developed to detect antibodies directed towards casein, ovalbumin, and hazelnut allergenic proteins (48). This method used two different colours of AuNPs (magenta and cyan) with different SPR bands at ca 525 and 620 nm, and yellow silver nanoparticles with SPR of ca 420 nm. Through visual observation, the presence of allergens was identified by the different colour code. Moreover, AuNPs colorimetric immunosensor had also been developed to detect immunoglobulin M (IgM) in rheumatoid arthritis (49) and to detect the reduction or absence of DNAse I activity in systemic lupus erythematosus patients (50).

v. Gold nanoparticles application in haematology laboratory

In the haematology laboratory, AuNPs had been used as a sensor to detect coagulation factors. For example, in a study by Chen (51) in 2011, thrombin-conjugated AuNPs were utilised to analyse the fibrinogen levels in plasma samples via fibrinogen-induced aggregation, which resulted in fast and easy visual detection. In addition, AuNPs were also used to differentiate glucose-6-phosphate dehydrogenase (G6PD) deficiency variants through the colour modification of the G6PD enzymes reaction caused by the unique plasmon absorbance properties of AuNPs (52). In addition, AuNPs were able to detect mutations in the G6PD gene by combining several sizes of AuNPs with a single-strand DNA (ssDNA) sequence specific to G6PD gene mutation (53).

vi. Gold nanoparticles application in transfusion medicine

Transfusion medicine is a constantly evolving discipline that entails precision and personalised medicine. Consequently, there are various challenges in providing safe blood in a timely and cost-effective manner. The difficulties include pre-transfusion testing that involves the detection of clinically significant red cell antibodies. Wiwanitkit (54) had demonstrated that the developed AuNPs solution was able to enhance agglutination reaction in detecting weak B subgroups. Another report in 2016 by Chowtaweesak (55) had shown that AuNPs solution improved the agglutination reaction in red cells antibody detection testing by approximately 37.2% compared to the standard enhancement reagent, low ionic strength solution (LISS). Therefore, the potential of developed AuNPs solution had provided an alternative approach in immunohaematology work up.

vii. Gold nanoparticles application in molecular diagnostic laboratory

In the molecular diagnostic laboratory, AuNPs were used as it can be functionalised with oligonucleotide. In 2003, Ozsoz (56) had demonstrated Factor V Leiden mutation detection using oxidation signal of Au in PCR amplicons. In this method, appearance of Au oxidation signal following hybridization, shortened the assay time, eased the detection, and discriminates between the homozygous and heterozygous mutations of Factor V Leiden. In addition, Deng (57) had developed a colorimetric analysis of a specific DNA sequence by integrating asymmetric polymerase chain reaction (As-PCR) with AuNPs. In this process, the oligonucleotides on the surface of AuNPs were selectively hybridised to construct complementary sequences of the single-stranded DNA (ssDNA) target generated by As-PCR. During the DNA hybridization, AuNPs self-assembled and aggregated, resulting in a color shift from ruby red to purple-blue. As a result, the analysis of specific nucleic acid sequences was made easy and had high specificity and sensitivity with minimal PCR product cross-contamination (57). Subsequently, AuNPs were also used in high-throughput DNA array for single nucleotide polymorphism (SNP) detection of KRAS gene using microfluidic device (58). Recently, rapid colorimetric detection of DNA sequence by using two DNA-functionalised AuNP was constructed. This method was capable to detect wide range of target DNA (from 16 fM to 1.6 nM) and had had shown low limit of detection (LOD) of 4.3 fM (59).

Conclusion

Gold nanoparticles have a wide range of potential applications in laboratory medicine due to ease of manufacture and high biocompatibility. The nanoparticles can be utilised in assays to detect diagnostic markers and enhance the identification of bacteria species, protein agglutination, and specific nucleic acid sequence, making AuNPs a promising material for laboratory diagnostics. Despite the extensive nanotechnologies applications explored, further research is needed to fully optimise the usage of AuNPs in laboratory medical settings and to address potential health and environmental risks.

Acknowledgements

The authors would like to express their gratitude towards the Advanced Medical and Dental Institute, Universiti Sains Malaysia (USM) for the opportunity to publish the present paper. Part of the work was supported by Short Term Grant USM (304/CIPPT/6315165).

Conflict of interest

The authors declare no conflict of interest.

References

1. Khan AK, Rashid R, Murtaza G, Zahra AJ. Gold nanoparticles: synthesis and applications in drug delivery. Tropical journal of pharmaceutical research. 2014;13(7):1169-77.
2. Nasimi P, Haidari M. Medical use of nanoparticles: Drug delivery and diagnosis diseases. International Journal of green nanotechnology. 2013;30;1.
3. Azzazy HM, Mansour MM, Samir TM, Franco R. Gold nanoparticles in the clinical laboratory: principles of preparation and applications. Clin Chem Lab Med. 2011;50(2):193-209.
4. Bahadar H, Maqbool F, Niaz K, Abdollahi M. Toxicity of Nanoparticles and an Overview of Current Experimental Models. Iran Biomed J. 2016;20(1):1-11.
5. Louis C, Pluchery O. Gold nanoparticles for physics, chemistry and biology. London: Imperial College Press, London. 2014.
6. Amendola V, Meneghetti M, Stener M, Guo Y, Chen S, Crespo P, *et al*. Physico-Chemical Characteristics of Gold Nanoparticles. Comprehensive Analytical Chemistry. 2014;66:81–152.
7. Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA. Gold Nanoparticles for Biology and Medicine. Angewandte Chemie (International ed in English). 2010;49(19):3280–94.
8. Jazayeri MH, Amani H, Pourfatollah AA, Pazoki-Toroudi H, Sedighimoghaddam B. Various methods of gold nanoparticles (GNPs) conjugation to antibodies. Sensing and Bio-Sensing Research. 2016;9:17–22.
9. Thanh NT, Rosenzweig Z. Development of an aggregation-based immunoassay for anti-protein A using gold nanoparticles. Anal Chem. 2002;74(7):1624–8.
10. Ajdari N, Vyas C, Bogan SL, Lwaleed BA, Cousins BG. Gold nanoparticle interactions in human blood: a model evaluation. Nanomedicine: Nanotechnology, Biology, and Medicine. 2017;13(4):1531–42.
11. Dreaden EC, Alkilany AM, Huang X, Murphy CJ, El-Sayed MA. The golden age: Gold nanoparticles for biomedicine. Chemical Society Reviews. 2012;41(7):2740–79.

12. Liu Z, Zhao F, Gao S, Shao J, Chang H. The Applications of Gold Nanoparticle-Initialed Chemiluminescence in Biomedical Detection. Nanoscale Research Letters. 2016;11(1):1–8.
13. Elahi N, Kamali M, Baghersad MH. Recent biomedical applications of gold nanoparticles: A review. Talanta. 2018;184:537–56.
14. Jamkhande PG, Ghule NW, Bamer AH, Kalaskar MG. Metal nanoparticles synthesis: An overview on methods of preparation, advantages and disadvantages, and applications. Journal of Drug Delivery Science and Technology. 2019;53:101174.
15. Zhao P, Li N, Astruc D. State of the art in gold nanoparticle synthesis. Coordination Chemistry Reviews. 2013;257(3–4):638–65.
16. Kimling J, Maier M, Okenve B, Kotaidis V, Ballot H, Plech A. Turkevich method for gold nanoparticle synthesis revisited. J Phys Chem B. 2006;110(32):15700–7.
17. Kumari Y, Kaur G, Kumar R, Singh SK, Gulati M, Khursheed R, *et al*. Gold nanoparticles: New routes across old boundaries. Advances in Colloid and Interface Science. 2019;274:102037.
18. Herizchi R, Abbasi E, Milani M, Akbarzadeh A. Current methods for synthesis of gold nanoparticles. Artificial Cells, Nanomedicine and Biotechnology. 2016;44(2):596–602.
19. Shah M, Badwaik V, Kherde Y, Waghwani HK, Modi T, Aguilar ZP, *et al*. Gold nanoparticles: Various methods of synthesis and antibacterial applications. Frontiers in Bioscience - Landmark. 2014;19(8):1320–44.
20. Stiufiuc R, Iacovita C, Nicoara R, Stiufiuc G, Florea A, Achim M, *et al*. One-step synthesis of PEGylated gold nanoparticles with tunable surface charge. Journal of Nanomaterials. 2013;2013:230–6.
21. Hammami I, Alabdallah NM. Gold nanoparticles: Synthesis properties and applications. Journal of King Saud University-Science. 2021;33(7):101560.
22. Giersig M, Mulvaney P. Preparation of Ordered Colloid Monolayers by Electrophoretic Deposition. Langmuir. 1993;9(12):3408–13.
23. Gu X, Xu Z, Gu L, Xu H, Han F, Chen B, Pan X. Preparation and antibacterial properties of gold nanoparticles: A review. Environmental Chemistry Letters. 2021;19(1):167-87.
24. Wuithschick M, Birnbaum A, Witte S, Sztucki M, Vainio U, Pinna N, *et al*. Turkevich in New Robes: Key Questions Answered for the Most Common Gold Nanoparticle Synthesis. ACS nano. 2015;9(7):7052–71.
25. Ward CJ, Tronndorf R, Eustes AS, Auad ML, Davis EW. Seed-mediated growth of gold nanorods: limits of length to diameter ratio control. Journal of Nanomaterials. 2014;2014.
26. Ramalingam V. Multifunctionality of gold nanoparticles: Plausible and convincing properties. Advances in Colloid and Interface Science. 2019;271:101989.
27. Simakin AV, Voronov VV, Kirichenko NA, Shafeev GA. Nanoparticles produced by laser ablation of solids in liquid environment. Applied Physics A: Materials Science and Processing. 2004;79(4–6):1127–32.
28. Correard F, Maximova K, Estève MA, Villard C, Roy M, Al-Kattan A, *et al*. Gold nanoparticles prepared by laser ablation in aqueous biocompatible solutions: Assessment of safety and biological identity for nanomedicine applications. International Journal of Nanomedicine. 2014;9(1):5415–30.
29. Dheyab MA, Aziz AA, Khaniabadi PM, Jameel MS, Ahmed NM, Ali AT. Distinct advantages of using sonochemical over laser ablation methods for a rapid-high quality gold nanoparticles production. Materials Research Express. 2021;8(1):015009.
30. Fnu N. Synthesis and Characterization of Ion-Implanted Gold Nanoparticles. Masters Theses. Michigan:Western Michigan Unniversity. 2019;5109.
31. Okuyama K, Lenggoro WW. Preparation of nanoparticles via spray route. Chemical Engineering Science. 2003;58(3–6):537–47.
32. Pantidos N. Biological Synthesis of Metallic Nanoparticles by Bacteria, Fungi and Plants. Journal of Nanomedicine & Nanotechnology. 2014;05(05).
33. Karnani RL, Chowdhary A. Biosynthesis of silver nanoparticle by eco-friendly method. Indian Journal of Nanoscience. 2013;1(1):25–31.
34. Hu X, Zhang Y, Ding T, Liu J, Zhao H. Multifunctional gold nanoparticles: a novel nanomaterial for various medical applications and biological activities. Frontiers in Bioengineering and Biotechnology. 2020;8:990.
35. Mikhailova EO. Gold Nanoparticles: Biosynthesis and Potential of Biomedical Application. Journal of Functional Biomaterials. 2021;12(4):70
36. Ho JA, Chang H-C, Shih N-Y, Wu L-C, Chang Y-F, Chen C-C, *et al*. Diagnostic Detection of Human Lung Cancer-Associated Antigen Using a Gold Nanoparticle-Based Electrochemical Immunosensor. Anal Chem. 2010;82(14):5944–5950.
37. Marom O, Nakhoul F, Tisch U, Shiban A, Abassi Z, Haick H. Gold nanoparticle sensors for detecting chronic kidney disease and disease progression. Nanomedicine. 2012;7(5):639–50.
38. Xu X, Ji J, Chen P, Wu J, Jin Y, Zhang L, *et al*. Salt-induced gold nanoparticles aggregation lights up fluorescence of DNA-silver nanoclusters to monitor dual cancer markers carcinoembryonic antigen and carbohydrate antigen 125. Analytica Chimica Acta. 2020;1125:41–9.
39. Soo PC, Horng YT, Chang KC, Wang JY, Hsueh PR, Chuang CY, *et al*. A simple gold nanoparticle probes assay for identification of Mycobacterium tuberculosis and Mycobacterium tuberculosis complex from clinical specimens. Molecular and Cellular Probes. 2009;23(5):240–6.
40. Lee JI, Jang SC, Chung J, Choi WK, Hong C, Ahn GR, *et al*. Colorimetric allergenic fungal spore detection using peptide-modified gold nanoparticles. Sensors

and Actuators B-chemical. 2021;327:128894.
41. Muniyappan N, Pandeeswaran M, Amalraj A. Green synthesis of gold nanoparticles using Curcuma pseudomontana isolated curcumin: Its characterization, antimicrobial, antioxidant and anti-inflammatory activities. Environmental Chemistry and Ecotoxicology. 2021;3:117–24.
42. Rai A, Prabhune A, Perry CC. Antibiotic mediated synthesis of gold nanoparticles with potent antimicrobial activity and their application in antimicrobial coatings. Journal of Materials Chemistry. 2010;20(32):6789–98.
43. Abdou Mohamed MA, Kozlowski HN, Kim J, Zagorovsky K, Kantor M, Feld JJ, *et al*. Diagnosing Antibiotic Resistance Using Nucleic Acid Enzymes and Gold Nanoparticles. ACS nano. 2021;15(6):9379-90.
44. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, *et al*. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. Journal of Medical Virology. 2020;92(9):1518–24.
45. Castañeda MT, Merkoçi A, Pumera M, Alegret S. Electrochemical genosensors for biomedical applications based on gold nanoparticles. Biosensors and Bioelectronics. 2007;22(9–10):1961–7.
46. Lou X, Zhang Y. Mechanism studies on nanoPCR and applications of gold nanoparticles in genetic analysis. ACS Applied Materials and Interfaces. 2013;5(13):6276–84.
47. Xi J, Yu Q. The development of lateral flow immunoassay strip tests based on surface enhanced Raman spectroscopy coupled with gold nanoparticles for the rapid detection of soybean allergen β-conglycinin. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy. 2020;241:118640.
48. Anfossi L, Di Nardo F, Russo A, Cavalera S, Giovannoli C, Spano G, *et al*. Silver and gold nanoparticles as multi-chromatic lateral flow assay probes for the detection of food allergens. Analytical and Bioanalytical Chemistry. 2019;411(9):1905–13.
49. Veigas B, Matias A, Calmeiro T, Fortunato E, Fernandes AR, Baptista PV. Antibody modified gold nanoparticles for fast colorimetric screening of rheumatoid arthritis. The Analyst. 2019;144(11):3613–9.
50. He Y, Cheng F, Pang DW, Tang HW. Colorimetric and visual determination of DNase I activity using gold nanoparticles as an indicator. Microchimica Acta. 2016;1(184):101–6.
51. Chen YY, Tseng CW, Chang HY, Hung YL, Huang CC. Gold nanoparticle-based colorimetric assays for coagulation-related proteins and their inhibition reactions. Biosensors and Bioelectronics. 2011;26(7):3160–6.
52. Boonyuen U, Praoparotai A, Chamchoy K, Swangsri T, Warakulwit C, Suteewong T. Controlled reversible assembly of gold nanoparticles as a new colorimetric and sensitive detection of glucose-6-phosphate dehydrogenase deficiency. Analytica Chimica Acta. 2020;1122:61–9.
53. Seow N, Lai PS, Yung LYL. Gold nanostructures for the multiplex detection of glucose-6-phosphate dehydrogenase gene mutations. Analytical Biochemistry. 2014;451(1):56–62.
54. Wiwanitkit V, Seereemaspun A, Rojanathanes R. Increasing the agglutination reaction in slide test for weak B blood group by gold nanoparticle solution: The first world report. Journal of Immunological Methods. 2007;328(1–2):201–3.
55. Choktaweesak N, Krasathong P, Ammaranond P. Development of enhancing agglutination reaction using gold nanoparticle for pre-transfusion testing. Transfusion Medicine. 2016;26(5):360–4.
56. Ozsoz M, Erdem A, Kerman K, Ozkan D, Tugrul B, Topcuoglu N, Ekren H, Taylan M. Electrochemical genosensor based on colloidal gold nanoparticles for the detection of Factor V Leiden mutation using disposable pencil graphite electrodes. Anal Chem. 2003;75(9):2181-7.
57. Deng H, Xu Y, Liu Y, Che Z, Guo H, Shan S, *et al*. Gold Nanoparticles with Asymmetric Polymerase Chain Reaction for Colorimetric Detection of DNA Sequence. Anal Chem. 2012;84(3):1253–1258.
58. Sedighi A, Li PC. High-Throughput DNA Array for SNP Detection of KRAS Gene Using a Centrifugal Microfluidic Device. Methods Mol Biol. 2016;1368:133-41.
59. Zou L, Shen R, Ling L, Li G. Sensitive DNA detection by polymerase chain reaction with gold nanoparticles. Analytica Chimica Acta. 2018;1038:105–11.