

Chapter 3

Gold Nanostructure in Sensor Technology: Detection and Estimation of Chemical Pollutants

Peuli Nath, Nivedita Priyadarshni, Soumen Mandal, Preeti Singh,
Ravi Kumar Arun and Nripen Chanda

Abstract Nanosensors have been proven to be a powerful tool in sensing various targeting analytes such as proteins, DNA, and RNA and small molecules such as toxins, drugs, metabolites, biomarkers, and environmental pollutants with high specificity and selectivity. Among various environmental pollutants, pollution by contamination of heavy metal is one of the most serious issues in current global scenario because of its potential toxicity toward human and aquatic life. Conventional methods of detecting such toxic ions include inductively coupled plasma mass spectroscopy (ICP-MS) and atomic absorption spectroscopy (AAS). These methods are accurate in minute-level detection, but still possess some drawbacks such as high time consumption, involvement of toxic chemicals, and requirement of sophisticated laboratory setup. Therefore, there is a need for inexpensive, user-friendly, quick, and portable methods for detection of these toxic ions. Efforts are being made in developing gold nanosensors for easy monitoring of heavy metal toxins in environmental samples. Due to unique optical, electrical, and mechanical properties, gold nanoparticles render improved performance as sensor probe for better sensitivity, selectivity, portability, and multi-load detection capability. During sensing process, the nanoparticles aggregate in the presence of specific metal ions and show visible color change from red to blue to colorless. The qualitative color change detected using naked eyes shows the presence of targeted heavy metal ions. Apart from the qualitative analysis, the quantitative estimation can be achieved with the help of gold nanoparticles by various techniques such as CCD or CMOS sensors, photodetectors, and color light sensors. This chapter deals with various synthesis processes, potential colorimetric-based sensing applications of gold-based nanosensor, and associated electronic circuitry, which could be employed for detection and quantification of various heavy metal toxins.

P. Nath · N. Priyadarshni · S. Mandal · P. Singh · R. K. Arun · N. Chanda (✉)
Micro System Technology Laboratory, CSIR-Central Mechanical Engineering Research
Institute, Mahatma Gandhi Avenue, Durgapur 713209, India
e-mail: n_chanda@cmeri.res.in

P. Nath · N. Priyadarshni · S. Mandal · P. Singh · R. K. Arun · N. Chanda
Academy of Scientific and Innovative Research (AcSIR), CSIR Campus, CSIR Road,
Taramani, Chennai 600113, India

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1 Introduction

Gold is always considered as a special element both historically and economically. Bulk gold is shiny yellow metal widely used in jewelry and dentistry and for decoration in paintings, in ceramics, and in tapestries. However, when bulk gold is broken down to nanometer size range, the yellow metal color is converted into red-colored suspension in aqueous medium termed as colloidal gold (Fig. 1). Michael Faraday first chemically synthesized it in 1847 by reduction of aqueous chloroaurate ions using phosphorous in carbon disulfide (CS_2) (Heiligtag and Niederberger 2013). Since then, it gained profound interest as an important nanostructure material. Colloidal gold of different shapes and sizes possesses strong vibrant color and thus introduced as a colorant for decorative paintings and in stained glass preparation used in windows. The colloidal gold contains gold element in a nanoscale form called “gold nanoparticle” (AuNP) and remains in highly dispersed phase in colloidal state (Mody et al. 2010). Among various noble metal nanoparticles, gold nanoparticles (AuNPs) provide an excellent platform for designing novel analytical sensors having a wide range of applications from sensing biological to chemical analytes (Saha et al. 2012). AuNPs are extensively studied due to their unique tunable optical, electronic, catalytic, biocompatibility, and thermal properties, unlike bulk gold which is inert in nature (Yeh et al. 2012). The unique optical properties of AuNPs come from the collective oscillation of electrons at their surface called surface plasmon resonance (SPR) which is responsible for its vibrant color in colloidal state. As AuNPs of size 1–100 nm have a high surface-to-volume ratio, their SPR frequency is highly sensitive to the dielectric

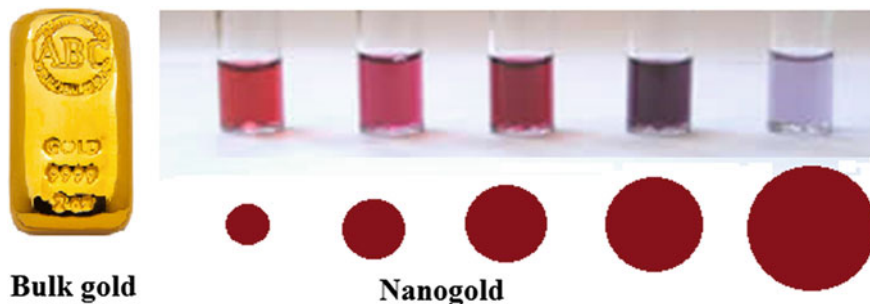


Fig. 1 Yellow-colored metal gold in bulk form (left) and color from red to blue of gold nanoparticle respective to their increasing size

nature of its surrounding medium which makes them a suitable candidate for sensing various analytes such as DNA, RNA, biomolecules, proteins, and heavy metal ions (Fig. 2) (Amendola et al. 2017).

Gold nanoparticle-based sensors can be classified into optical sensor (Yuan et al. 2016), electrochemical sensor (Shams et al. 2016) and piezoelectric sensor (Ding et al. 2014), among which AuNP-based optical sensor gained immense attention due to its interesting optical properties and has been used as colorimetric sensor material (Jongjinakool et al. 2014; Chah et al. 2005; Hung et al. 2010; Kumar et al. 2016). The optical and electronic properties of gold nanoparticles arise from their size confinement of electrons. As the size of gold nanoparticles increases, the SPR frequency redshifts to higher wavelength and the color changes from red (~ 15 nm) to pink (~ 40 nm) and to blue (~ 100 nm) (El-Brollosy et al. 2008) as shown in Fig. 1. AuNPs are synthesized by reduction of gold salts using reducing agents in the presence of appropriate capping agents to prevent aggregation (Kumar and Ganesan 2011). The size of AuNPs can be varied by altering the salt concentration, temperature and rate of addition of reductants. The most common method of gold nanoparticle synthesis is reduction of gold salts using trisodium citrate in aqueous medium. AuNPs of size range from 10 to 25 nm are formed in this method. Other methods of preparation include phase transfer using tetraoctylammoniumbromide as phase transferring agent and sodium borohydride as reducing and stabilizing agent in organic medium like toluene (Cheng and Wang 2004). Gold nanoparticles formed by this method are insoluble in water and often require stronger capping agents such as thiol for their stabilization. AuNPs of size less than diameter 3.0 nm

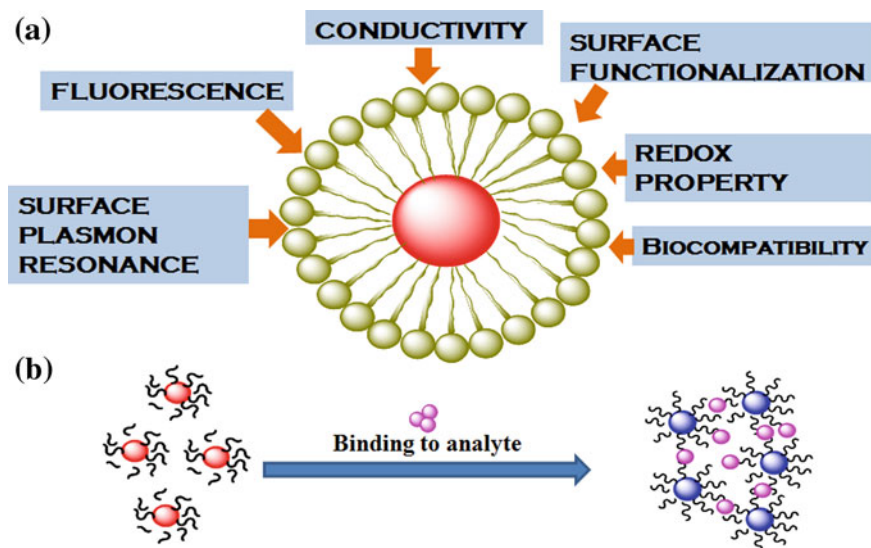


Fig. 2 Schematic illustrations showing **a** various physical properties of gold nanoparticle and **b** AuNP-based detection system

exhibit excellent fluorescence properties and are called fluorescent gold nanoclusters (AuNCs), also known as gold nanodots (Sumi et al. 2015; Chen et al. 2013). AuNCs are highly stable, non-toxic, and biocompatible and can easily enter into various cells, which make them suitable candidature for detection of metal ions inside cell due to metal–ligand interaction leading to either enhancement or quenching of fluorescence (Qu et al. 2015; Durgadas et al. 2011). Fluorescent gold nanoparticles can be encapsulated and stabilized by polyamidoamine (PAMAM) dendrimers or polyethyleneimine (PEI) (Huang et al. 2007) to produce strong fluorescence with high quantum yields. Several methods are applied for synthesis of fluorescent gold nanocluster such as template-based method with DNA, dendrimers, proteins, and polymers and ligand-protected method with thiol or phosphine ligand (Govindaraju et al. 2017). Most common method for preparation of AuNCs is with bovine serum albumin where Au^{3+} is converted into Au^+ with the help of tyrosine, aspartate, asparagines present in the protein (Xie et al. 2009). For gold nanoparticles to remain stable and dispersed in colloidal state, various stabilization agents such as citrate, borohydride, phenols, and alkenethiols (Comeau and Meli 2012) are used in their synthesis process or surface functionalization is done with suitable coordinating species such as thiol (Moon et al. 2010), PEG (polyethylene glycols) (Manson et al. 2011), or surfactants (Benkovičová et al. 2013). Stabilized tiny gold nanoparticles have electric double layer, which provide strong repulsive force to maintain the nanoparticle in dispersed phase without aggregating in solution (Polte 2015). Thus, stable gold nanostructures with proper surface modification can find several applications from drug delivery (Ghosh et al. 2008) to cell imaging and photothermal therapeutics (McQuaid et al. 2016). In this chapter, the main focus has been given to highlight the synthesis, properties, and the importance of functionalization of gold nanoparticles toward sensing of heavy toxic metal ions which is one of the main sources of the environmental pollution and discuss the recent advances in developing gold-based nanosensors in environmental pollutants monitoring for the benefit of the society.

Chemical pollution in water is one of the major environmental problems nowadays as it possesses threats to human health and welfare and has harmful impact on both society and economy. Chemical pollutants such as chemical toxins, heavy metal ions, and other organic and inorganic pollutants pollute water by various natural or artificial processes that need to be monitored regularly for safe supply of drinking water and also to clean our environment and ecosystem. Heavy metal ions such as arsenic, lead, copper, and mercury are highly toxic in nature. Consumption of these heavy metal toxin-contaminated water leads to several life-threatening diseases such as liver and kidney disease, skin disease, renal failure, cancer, and even death (Moreira and Moreira 2004; Hong et al. 2014; Desai and Kaler 2008). Conventional methods of detecting toxic metal ions are based on chromatographic or spectroscopic techniques such as inductively coupled plasma mass spectroscopy (ICP-MS), atomic absorption spectroscopy (AAS), and high-performance liquid chromatography (HPLC). Though these methods are highly sensitive and provide accurate measurement, they require highly sophisticated instruments, technical expertise, long time for analysis, handling of toxic

chemicals, dedicated laboratory setup, and periodical electrode maintenance making the analysis highly expensive (Yuan et al. 2011). To overcome these challenges, there is a need of inexpensive, portable, simple, rapid user-friendly technique for detection of these toxic ions. In this context, the colorimetric methods are promising as they are simple and inexpensive and need less detection time. Colorimetric sensors are generally based on dyes or involve the use of surface-modified nanoparticles (Yan et al. 2014). Gold nanoparticle-based colorimetric sensor for metal ion detection requires the incorporation of chelating agent on its surface (Priyadarshini and Pradhan 2017). The presence of metal ions induces nanoparticle aggregation by forming complex with the chelating agent, and the color of stabilized nanoparticle changes from red to blue. Recently, many researchers have explored functionalized gold nanoparticles as potential sensors in both colorimetric and fluorometric assays exploiting their unique photophysical properties which make them sensitive to binding with toxic metal ions (Chai et al. 2010; Fang et al. 2010; Jongjinakool et al. 2014; Nath et al. 2014). Detailed study of gold nanoparticle's property and their applications is discussed in consequent segments.

2 Properties of Gold Nanoparticles Toward Sensing Applications

Gold nanoparticles are of great interest because of their size-dependent photophysical properties as compared to its bulk (Link and El-Sayed 1999; Eustis and El-Sayed 2006). The properties of gold nanoparticles change as size decreases, because the number of active atoms on their surface increases unlike present in interior of the particles. They have large surface area that helps them to form suspension (colloidal gold) within the solvent which is strong enough to exceed density differences and otherwise could result in either floating of materials or precipitate formation in the medium (Nguyen Ngoc et al. 2009).

2.1 Photophysical Properties

The photophysical properties of gold nanoparticles arise due to localized surface plasmon resonance phenomenon (LSPR), i.e., collective oscillations of free electrons at a metal–dielectric interface when the frequency of incident light coincides with the frequency of electron oscillations and thus highly influenced by nanoparticle's size, shape, dispersion, and composition (Fig. 3) (Hutter and Fendler 2004). Among noble metal nanoparticles, gold nanoparticles show strong SPR band than any other metal nanosuspension and possess unique optical, physical, and electronic properties, which makes them ideal tool for sensing, imaging, and therapeutic applications in sensitive, easy, simpler, and cost-effective ways

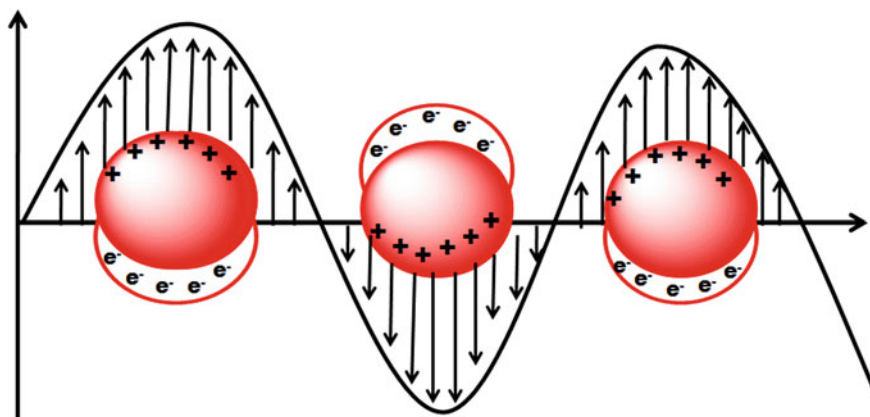


Fig. 3 Schematic representation of the SPR phenomenon showing interactions of the incident electromagnetic wave with the electrons at the surface of the gold nanoparticles

(Homola 2008). The surface plasmon resonance (SPR) of gold nanoparticles primarily depends on the factors such as particle size, shape, composition, and physical–chemical environment including the interparticle distance and dielectric constant of the surrounding medium, which affect electron charge density on the particle surface. Spherical gold nanoparticles exhibit a vibrant range of colors (brown, orange, red, purple) in colloidal state as its size increases from 1 to 100 nm and show a size-relative absorption peak maximum in the span of 520–550 nm (Fig. 4) (Kelly et al. 2003; He et al. 2005). The absorption band at ~ 520 nm is highly uncertain for particles less than 10 nm and almost absent for particles less than 3.0 nm (fluorescent gold nanoparticles) and for bulk material. This is due to increase rate of electron–surface collisions compared to larger particles. This phenomenon is well described by Mie theory (Huang and El-Sayed 2010). The effects of size on SPR are divided into intrinsic and extrinsic ones. The extrinsic size effect is an electromagnetic phenomenon, which appears in the optical absorption spectrum due to broadening or redshift of SPR on increasing the gold nanoparticle size that causes the change of color from red to blue, also called aggregation of nanoparticle or interparticle plasmon coupling. Intrinsic size effect is due to the modification of metal optical constants when size is below 30 nm (Ghosh and Pal 2007).

The shape of the nanoparticle as explained by Richard Gans in 1912 also determines the number of SPR bands, its width, and position. Gans theory states that absorption is only dependent on the aspect ratio of the particle, not on the absolute dimensions. For example, spherical gold nanoparticles have one SPR band at ~ 520 nm, while gold nanorods have two plasmon bands. A strong band in NIR region referred to the longitudinal band and a weak band at visible region similar to that of spherical gold nanoparticles called as transverse band resonating at different

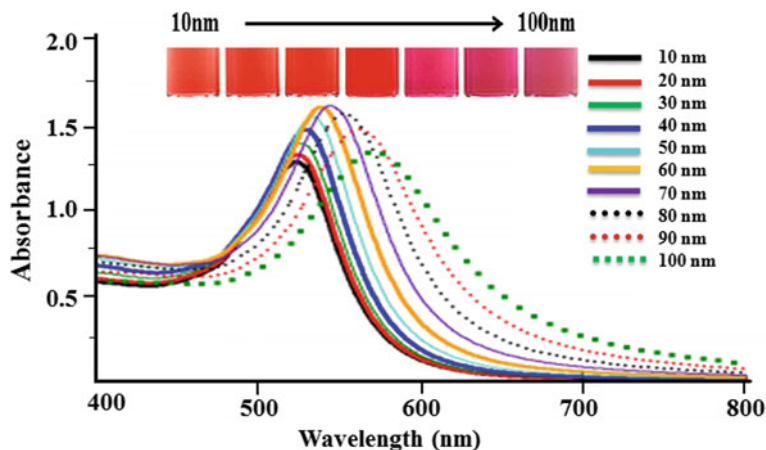


Fig. 4 Variation of UV-Vis absorption spectra of gold nanoparticles with increasing size and respective color change (inset)

wavelengths, which correspond to the collective excitation of conduction electrons along the two axes of nanorod (Hu et al. 2006; Zeng et al. 2010). By further changing the symmetry of nanoparticles, color may change according to the length of the nanorod (Fig. 5) and new plasmon bands may arise which is redshifted compared to that of spheres.

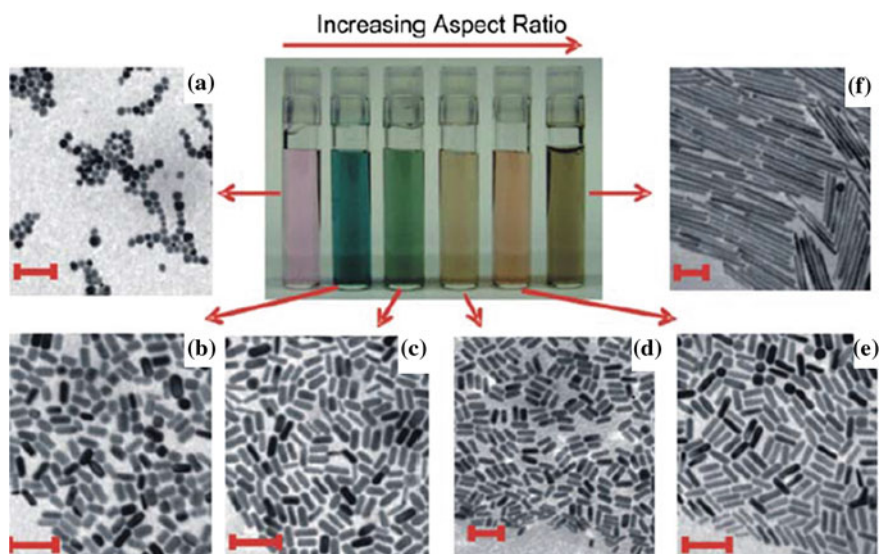


Fig. 5 a–f TEM images of gold nanorods respective to their increasing aspect ratios. *Photograph* Showing different color of aqueous solution of gold nanorods with different aspect ratios. All scale bars are 100 nm. Reprinted with permission from Zeng et al. (2010)

The physical–chemical environment influences the SPR bands in many ways: One such mechanism is chemical interface damping (CID). CID is widening of SPR band due to the presence of adsorbates on particle's surface. The photophysical properties of gold nanoparticles are therefore highly dependent on its physical parameters as electronic structure of gold nanoparticles changes with shape and size (Douglas-Gallardo et al. 2016). From this, it is clear that gold nanoparticles exhibit strong SPR absorption band compared to other metal nanoparticles, which makes it a suitable candidate for chemical and biological sensing due to its sensitive spectral response to the surrounding environment. The spectral sensitivity is evident by the relative shift of resonating wavelength with respect to the change in refractive index of the surrounding materials, which depends on the type of metal, its SPR position, width, and shape of the particles (Ghosh and Pal 2007). One of the important features of the sensor is its selectivity, which can be conferred to gold nanoparticle functionalization. The surface of gold nanoparticles can easily be engineered with thiols or disulfides or with a suitable receptor for targeting molecules, enough to produce an appreciable redshift of the SPR band once the nanoparticle binds to the target molecules. All these properties make gold nanoparticle a suitable colorimetric sensor for detection of analytes.

2.2 Colloidal Stability

Nanoparticles' behavior in suspension is highly dependent on particle size, surface composition, and density of the materials used. In nanoscale range below 100 nm, the Brownian motion causes water molecules to collide with particles with high enough force-to-mass ratios, which prevent them from settling, and thus, a true colloidal suspension occurs (Michaelides 2015). However, a particle at such nanometer range becomes highly unstable and tends to aggregate because of their short interparticle distances. At such distances, van der Waals, electrostatic, and magnetic forces come into play and particles are attracted to each other (Rance et al. 2010). Synthesis of metal nanoparticles such as gold is often performed by reduction of gold salts with reducing agents such as sodium citrate, sodium borohydride, and ascorbic acid that also act as stabilizing agents to restrict the aggregation behavior of nanoparticles. The stabilizing agent is adsorbed on the surface and provides a repulsive force for an electrostatic stabilization or steric stabilization. The electrostatically stabilized nanoparticles have one electric double layer due to surface charge (zeta potential) which prevents the nanoparticles from coagulation if the repulsion between the particles is sufficiently high. For preparation of stable nanoparticles, a repulsive interparticle force is required to oppose van der Waals force of attraction, which is provided by the surface charge of the particle.

In solvation state, ions of the solvent surround the ions or molecules of solute to shield their surface charge, which is described by Stern–Gouy–Chapman theory where the surface potential decreases as the ions from first layer move away from the surface of the bulk fluid, and these layers are collectively known as electric

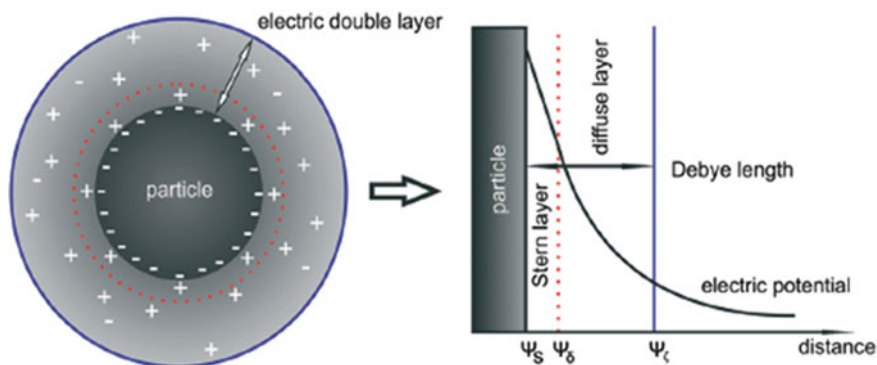


Fig. 6 Electric double layer (EDL) around nanoparticle consisting of inner Stern layer and outer diffuse layer according to Gouy–Chapman model. Reprinted with permission from Polte (2015)

double layer (EDL)—one is the inner compact Stern layer and the other is outer diffused layer. The thickness of EDL is defined as Debye length λ (k^{-1}) and can be quantified using simple electrostatics. A detailed understanding of the distribution of electric surface potential $\psi(x)$ is required to describe the electric double layer effect in colloidal phase (Fig. 6) (Polte 2015). This is well described by two Russian scientists, Derjaguin and Landau, and two Dutch scientists, Verwey and Overbeek, in their stability model known as DLVO theory. The basic assumption of this theory is that the total forces acting on colloidal are the addition of van der Waals force (attraction) and the EDL (repulsion) force acting in between the particles (Polte 2015).

The stability of the nanoparticle depends on the types of ion and their concentration, value of charge density on surface, and size of the particle. When the surface charge (zeta potential) increases, repulsion between the particles is higher. The stable colloidal suspension has zeta potential more than +30 mV or more negative than -30 mV. The van der Waals interaction is relatively independent of ion concentration, but repulsive force depends on it since the counterions are dominant in the Stern and diffused layers. On the other hand, the particle size influences both attractive and repulsive forces of the total interaction energy. As the particle size increases, the total interaction potential (TIP) increases proportionally which is represented in DLVO theory (Polte 2015).

3 Synthesis and Characterization of Gold Nanoparticles

Design and synthesis of nanoscale materials is an important aspect for any sensing applications. Synthesis of nanomaterials involves two main approaches: top-down and bottom-up approaches (Wang and Xia 2004). Top-down approach involves breaking down of bulk material to nanometer scale and is mainly accomplished by

lithography techniques such as electron-beam lithography or soft lithography. Humans have been following this approach since the beginning of civilization and mastered the art of breaking materials to sub-micron size, although physical constraint has limited the applications of this approach to nanorange. The bottom-up approach relies on conjugation of several molecules to build nanoconstruct. Various techniques are employed in bottom-up synthesis such as chemical synthesis or chemical self-assembly, where different atoms and molecules are conjugated to form a well-defined stable nanostructure. The most common example of such chemical syntheses is the synthesis of noble metal nanoparticle from their precursor salts. Some common chemical routes for nanoparticles synthesis are the sol-gel method (Epifani et al. 2000), solvothermal synthesis (Choi et al. 2013), micelles-based synthesis (Liebig et al. 2016), and galvanic replacement (Ramos et al. 2011), but the most popular route is by chemical reduction, which provides nanoparticles with controlled size and shape. Controlled size, shape, and stability of nanoparticles are achieved by using different capping agents, solvents, and templates. Although, over the past few decades, physical and chemical methods have dominated the synthesis of nanoparticles, nowadays synthesis by using the biological system has gained great attention. For example, magnetite nanoparticles are found in magnetosomes of the magnetic bacteria which are functional nanoparticles used by the microorganisms to navigate the Earth's geomagnetic field (Yan et al. 2012). This has arisen interest among researchers to understand the underlying mechanism used by the microorganism and to explore the biomimetic approach toward the synthesis of nanoparticles. To date, several bacteria, algae, fungi, and plant materials have been used for the synthesis of nanoparticles including gold one. The details of few well-known bottom-up synthesis procedures of gold nanoparticles are discussed below.

3.1 Turkevich–Frens Method

Turkevich et al. described the first method for gold nanoparticle synthesis in 1951. In this method, gold chloride salt is reduced by sodium citrate forming monodispersed gold nanoparticle suspension in an aqueous medium (Fig. 7) (Kimling et al. 2006). This method is later modified by Frens who produces gold nanoparticles of different sizes from 10 to 100 nm by varying the ratio of gold chloride salts and sodium citrate solution, which is being used as both reducing agent and electrostatic stabilizer. The reaction temperature is kept optimum at ~ 90 °C, which is important for the formation of gold nanoparticles as both reaction kinetics and oxidation potential are dependent on temperature. The citrate anions form a complex multi-layer around nanoparticles preventing it from aggregation and providing a net negative charge. The advantage of citrate layer is its weak coordination with the gold nanoparticle that makes it amenable to functionalization with thiols (Zhu et al. 2003), proteins, or other biomolecules (Thi Ha Lien et al. 2010). Citrate-stabilized gold nanoparticles are more sensitive to change in pH, ionic strength of the

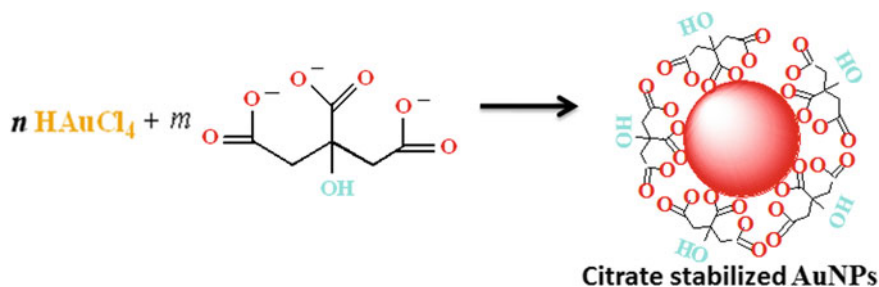


Fig. 7 Reaction mechanism for formation of citrate-stabilized gold nanoparticles

medium, and the presence of other organic and inorganic molecules and thus can directly be used as sensor materials for various sensing applications (Nam et al. 2009).

3.2 Brust–Schiffrin Method

Brust and Schiffrin first explored in 1994 that gold nanoparticle can be produced in an organic medium, which is not miscible in water (Perala and Kumar 2013). The synthesis involves the reaction of the chloroauric acid with tetraoctylammonium bromide (TOAB) solution in toluene and sodium borohydride as reducing and anti-coagulant agent. Tetraoctylammonium bromide is used as phase transfer catalyst and as the stabilizing agent. The gold salt is transferred from aqueous medium to organic medium using TOAB followed by reduction with sodium borohydride in the presence of alkanethiols. The reactions involved are shown in Fig. 8.

Gold nanoparticles formed by this method are of the 5–6 nm size range. One of the disadvantages of this method is that the formed gold nanoparticles tend to aggregate over the course of time, as TOAB does not bind to the nanoparticles strongly. In addition, some of the phase transfer agents may remain bound to the nanoparticles, which may affect the physical properties of the gold nanoparticles like solubility.

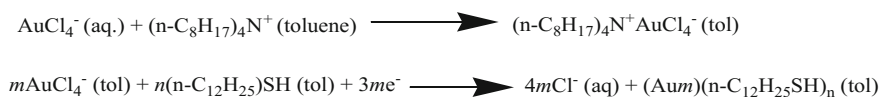
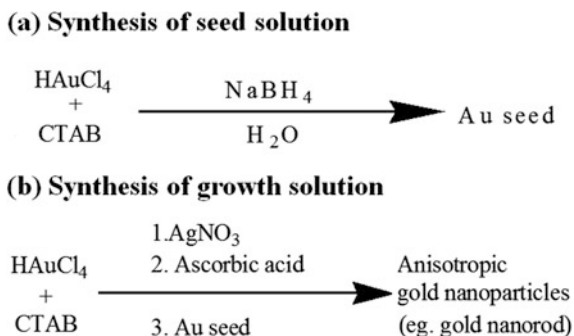


Fig. 8 Reaction mechanisms for formation of gold nanoparticles in organic medium

Fig. 9 Reactions illustrate synthesis of gold nanoparticles by seed-mediated growth method



3.3 Seed-Mediated Growth Method

The seed-mediated method is a widely preferred technique for fabricating gold nanoparticle of different shapes. The basic principles of this technique are first to prepare seed particles by reducing gold salts with a strong reducing agent such as sodium borohydride. Then, this seed solution is added to the solution of metal salt in the presence of weak reducing agent (ascorbic acid) and surfactant agent (cetrimonium bromide) to prevent nucleation and accelerate the anisotropic growth of gold nanoparticles (Fig. 9) (Leng et al. 2015).

3.4 Sodium Borohydride Reduction Method

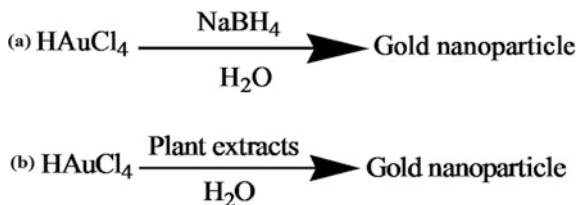
This is “one-pot” synthesis method, which involves reduction of gold salts by sodium borohydride. The method generally produces particle of size range between 4 and 25 nm (Fig. 10a). Sodium borohydride is a strong reducing agent capable of producing small nanoparticles (Deraedt et al. 2014).

3.5 Biosynthesis of Gold Nanoparticles

Chemically synthesized gold nanoparticles generate toxic byproducts and may be harmful for biological applications. Therefore, nowadays, new strategies to synthesize gold nanoparticles without toxic chemicals are developed which attract significant attention among the researchers. This new procedure of producing gold nanoparticle, also referred as “GREEN chemistry,” involves the use of biodegradable agents, low toxicity of the final products, and synthesis at ambient temperature and pressure. Different plant extracts are being used to synthesize AuNPs in a clean, reliable, and biofriendly way. Various biocomponents present in plants such as flavonoids, phytosterols, and quinones possess functional groups for

Fig. 10 a Reaction mechanism of formation of sodium borohydride-induced gold nanoparticle;

b biosynthesis of gold nanoparticles using plant extracts



reducing gold salts and also provide stability to the nanoparticle (Lee et al. 2016; He et al. 2007). The procedure involves mixing of gold salts with plant extracts in ambient temperature, pH, conditions to produce AuNPs of different shapes and sizes (Fig. 10b). Biosynthesis of AuNPs using carbohydrate (Shervani and Yamamoto 2011), proteins (Shi et al. 2015), lipids (Nam et al. 2016), and nucleic acid is a fast growing research area as it produces gold nanoparticles in a non-toxic, clean, and eco-friendly way.

3.6 Synthesis of Fluorescent Gold Nanoclusters (AuNCs)

Gold nanoclusters of sub-nanometer size range emit strong fluorescence and are thus suitable for biosensing inside living cells. Several methods such as template-based method, i.e., with polymers, proteins, DNA, dendrimers, and ligand-protected method with phosphine or thiol, are used to synthesize AuNCs. Among these, the most common method of AuNCs synthesis involves bovine serum albumin protein (BSA). The glutamate, aspartate, asparagines, and tyrosine of BSA convert Au^{3+} to Au^+ ions. Sodium hydroxide (NaOH) is then added which reduces the Au^+ ions to Au^0 to form clusters, which is confirmed by the color change from yellow to dark brown (Fig. 11) (Aziz et al. 2015; Xie et al. 2009). In this process, BSA acts as reducing and stabilizing agent. NaOH increases the pH, which further improves the reducing power of BSA. The concentration of gold atoms increases during the reaction, and homogeneous nucleation occurs at the supersaturating level of gold atoms leading to the formation of BSA-stabilized AuNCs. TEM image shows about 1–3 nm size AuNPs are formed in this method.

3.7 Characterization of Gold Nanoparticles

Determination of size, shape, and presence of surface functional molecules on AuNPs is required before any sensing applications and is achieved by several techniques such as atomic force microscopy (AFM), transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), UV–Vis absorption

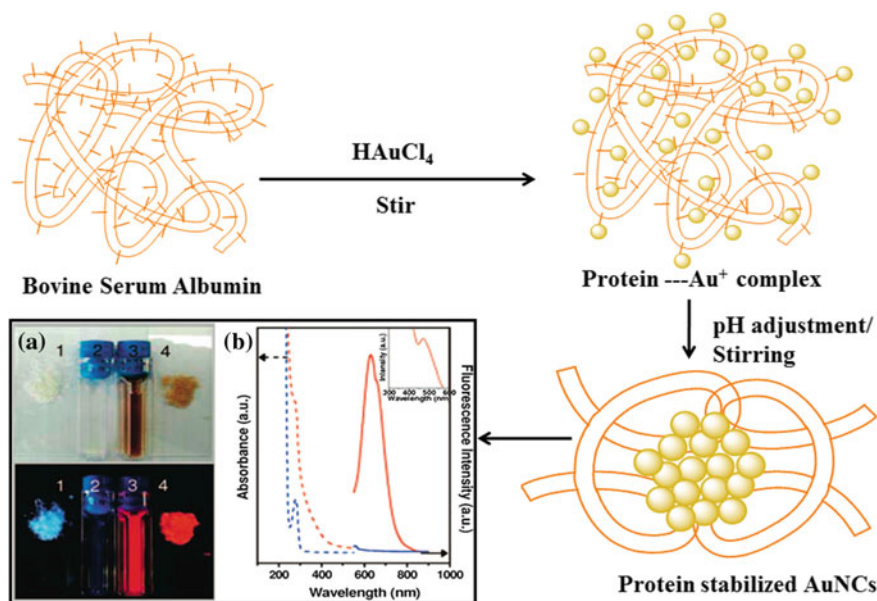


Fig. 11 Schematic illustration of synthesis of fluorescent gold nanoclusters (AuNCs) using protein BSA as reducing agent. Inset (a) represents the AuNCs under visible light (above) and shows red fluorescence of AuNCs under UV light of 360 nm wavelength (below). Inset (b) demonstrates the UV-Vis and fluorescence graphs in aqueous medium. Reprinted with permission Xie et al. (2009)

spectroscopy, FT-IR spectroscopy, Raman spectroscopy, dynamic light scattering (DLS), electrochemistry and fluorescence spectroscopy. AFM permits for 3D visualization of nanoparticles with sub-nanometer resolution. TEM image allows seeing any self-organization and core-to-core spacing in thin nanoparticle assembly, also interparticle edge-to-edge distance that is ideally equal to the length of the capping agent. X-ray diffraction (XRD) measurements provide information on the crystallographic structure of the nanoparticles. The Fourier transform-infrared spectroscopy is performed to establish the interaction between gold nanoparticles and surface molecules. The electronic states and chemical composition of the nanoparticles are determined using cyclic voltammogram and X-ray photoelectron spectroscopy. The UV-Vis absorption spectra determine the position and the intensity of the SPR band that serves as evidence for changes in the gold nanoparticle structure. Among the various methods, the Raman spectroscopy deserves special mention as it indicates the structure of molecules on the surface of modified gold nanoparticles. For functionalization of gold nanoparticles with either individual organic ligands or coordination compounds, the surface Raman scattering is noticeably enhanced due to surface-enhanced Raman scattering (SERS) from the molecules that are adsorbed on the AuNP surface (Philip 2008; Singh et al. 2016; Nath et al. 2015).

4 Functionalization of Gold Nanoparticles

Surface coating of gold nanoparticles with capping ligands is necessary for stabilization of gold nanoparticle and for functionalization of the particles enabling them to use as sensors by targeting specific analytes. Functionalization can modify the surface, dispersibility, and size of the nanoparticles. Above all, surface modification provides a protective layer on the surface, thus increasing the overall stability of the nanoparticles.

4.1 Thiol-Functionalized Gold Nanoparticles

Thiol ($-SH$) group binds to gold nanoparticles with high affinity (126–146 kJ/mol) by forming covalent bonds. Organic thiolates such as dihydrolipoic acid have been widely used as surface functionalization ligands because of high stability of Au–S bonds. For example, gadolinium chelates are attached to gold nanoparticles via dithiolated derivatives of diethylenetriaminepenta acetic acid (Spampinato et al. 2016). It provides a protective monolayer around the AuNPs. The exact nature of bond between the Au–S was not known until 2007. A report of single-crystal X-ray diffraction study of a sample of monodispersed gold particles with mercaptobenzoic acid (MBA) reveals a core containing 102 gold atoms that is surrounded by 44 MBA ligands in $Au_{102}(p\text{-MBA})_{44}$ (Levi-Kalisman et al. 2011). The gold core comprises of 89 Au atoms, 49 of which are arranged in Marks decahedron with two 20-atoms caps. The remaining 13 atoms form a band around the equator of the core forming a protective shell with the thiolates. The shell consists of $RS(AuSR)_n$ units with $n = 1, 2$ generally indicated as “staples.” Monomeric staples if $n = 1$ ($-RS-Au-SR-$) or dimeric staples $n = 2$ ($-RS-Au-SR-Au-SR-$) are bonded to gold through the terminal sulfur atom. A significant finding is that the MBA ligands not only bind to the gold through Au–S bonds but also interact with each other through a series of face-to-face and face-to-edge interactions between adjacent phenyl rings and also by phenyl sulfur interactions. These findings are also in line with recent advances on the study of gold sulfur interfaces in 2D-SAMs of alkanethiols on Au (111), to which the gold–sulfur interaction contributes with about 208 kJ/mol and the van der Waals interaction between methylene groups contributes with an average energy of 6.24 kJ/mol (Bourg et al. 2000). One of the important chemical properties of Au–S bond is the possibility to undergo an exchange reaction, i.e., replacing a ligand with another without affecting the structural integrity of the particle. Compared to other compounds, thiol compound efficiently improves the stability and dispersibility of AuNPs in aqueous medium. Currently used thiol compound includes glutathione (GSH) (Chai et al. 2010), mercaptopropionic acid (MPA), cysteine, cystamine, dihydrolipoic acid (DHLA), thiol ending PEG (PEG-SH) for various applications including sensing of metal toxins (Fig. 12a) (Gao et al. 2012).

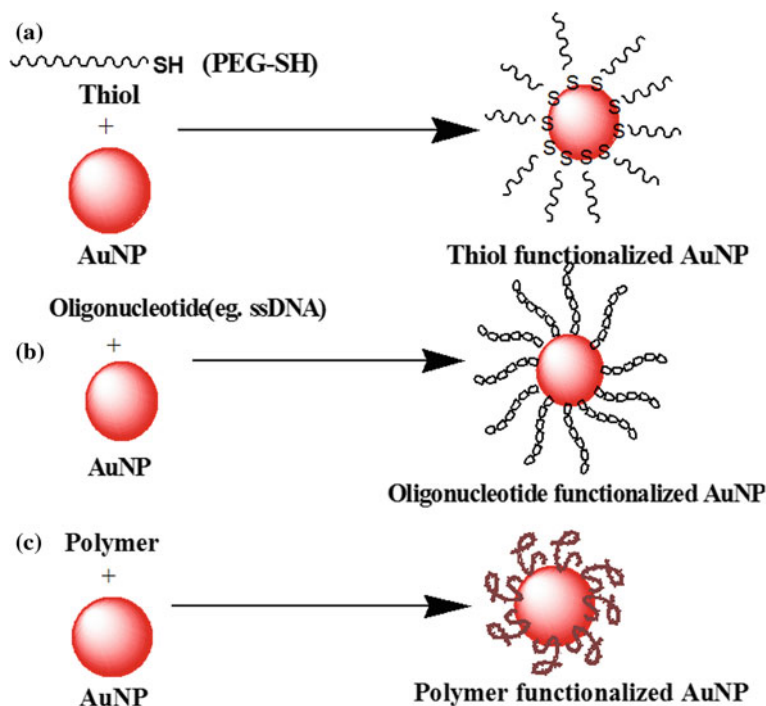


Fig. 12 Schematic presentation of gold nanoparticle functionalization with various moles such as thiols, oligonucleotide (ssDNA), and polymers

4.2 Oligonucleotide Functionalized Gold Nanoparticles

Functionalization of gold nanoparticles with oligonucleotide is another approach toward sensing applications. The gold nanoparticles are generally functionalized with oligonucleotides like DNA in a controlled manner either by attaching modified single-stranded DNA molecules through thiol groups or by saturating the surface of AuNPs with DNA molecules (Fig. 12b). Thermodynamics and kinetics study of DNA-conjugated AuNPs have shown that ssDNA first attaches to the AuNPs and then slowly get adsorbed on its surface (Tan et al. 2011). The DNA hairpin secondary structure inhibits interaction between AuNPs and DNA further thus increasing the overall stability of DNA-functionalized gold nanoparticles. Recently, Mirkin et al. developed DNA-functionalized AuNPs to selectively detect Hg^{2+} ion with high sensitivity. In this study, thiolated DNA-based AuNPs exploit the coordination of thymidine- Hg^{2+} -thymidine complex and related melting temperature (T_m) of the DNA aggregates is monitored (Lee et al. 2007). In this experiment, AuNPs are functionalized with two different DNA probes modified with different thiol groups (probe 1 and probe 2). Probe 1 and probe 2 form aggregates when mixed leading to lower T_m value which corresponds to the mismatch in T - T base

pair sequences. However in the presence of mercury in the system, the T_m of the AuNP aggregates increases through selective coordination forming stable $T\text{-Hg}^{2+}\text{-T}$ base pairs. This system successfully detects mercury up to 100 nM concentration. Major drawback of this system is the incorporation of an electronic heating device attached with the sensor part for close monitoring of T_m during the detection process. To eliminate this disadvantage, Liu and co-worker investigate and optimized the number of T -units to be incorporate on DNA strands so that the system works in ambient temperature (Liu et al. 2013). Recently, a much simpler thymine functionalized AuNPs are designed for colorimetric detection of mercury. The sensor works on the principle of specific interaction of mercury with thymine residue forming the aggregation of AuNPs and indicates corresponding color change. Liu et al. demonstrated DNAzyme base AuNP biosensor for sensitive detection of Pb^{2+} ion. In this sensor, Pb^{2+} ion-specific DNAzyme is chosen as probe for target recognition and AuNPs as the signaling element (Liu and Lu 2004b). The Pb^{2+} -specific DNAzyme comprises of an enzyme strand and its corresponding substrate strand; in the presence of Pb^{2+} ion, the enzyme strand undergoes catalytic reaction cleaving the substrate strand hydrolytically. Incubation of enzyme strand with DNAzyme, DNA-functionalized AuNPs form aggregates according to Watson–Crick base-pairing model giving blue color solution. In the presence of Pb^{2+} ion, DNAzyme is activated further cleaving the substrate strand, thus disassembling AuNPs which results in blue to red color change. The sensor detects up to 100 nM Pb^{2+} ion in less than 10-min time. Other competent metal ions such as Ca^{2+} , Co^{2+} , Ni^{2+} , and Cd^{2+} do not show any color change proving selectivity toward Pb^{2+} ion. Recently, DNA-based AuNPs for uranyl ion detection in living cells is reported. In this experiment, uranyl ion-specific DNAzyme with 3' thiol end for conjugation with AuNP and Cy3 fluorophore at 5' end and a molecular quencher at 3' end is fabricated. In the absence of uranyl, the fluorophore is quenched by both AuNPs and molecular quencher, while in the presence of uranyl ion, the DNAzyme cleaves the fluorophore-based molecular strand releasing shorter fragments of Cy3, thus increasing fluorescence intensity. This DNA probe can readily enter living cells making it suitable for intracellular metal ion sensing system (Wu et al. 2013). Such methods are used to detect several metal ions using DNA-functionalized AuNPs in in vivo and in vitro environment.

4.3 Polymer-Functionalized Gold Nanoparticle

Dispersed gold nanoparticles are mostly thermodynamically unstable due to high surface energy and tend to form precipitation under the influence of external stimuli. In order to prevent the self-assembly of AuNPs, conjugation with water-soluble polymer decreases the surface energy of the particles and inhibits interparticle interaction (Fig. 12c) (Iatridi and Bokias 2009). Faraday prepared red-colored gold nanoparticle which is stabilized by gelatin, a water-soluble biopolymer (Neupane et al. 2011). Since then, the polymer is used as an effective

agent to functionalize gold nanoparticles. Polymer-functionalized AuNPs are investigated by many research groups and can be achieved by three techniques:

- (a) “Grafting from” fabrication technique: In this method, polymer chains are attached to gold nanoparticles (Huo and Worden 2007; Popelka et al. 2007). This technique provides specific control over the molecular weight of the polymer, flexibility in structural design of polymer layer, and effective introduction of high density polymer. Mostly, artificial polymers are introduced by this technique, like thiol-attached primers, ssDNA. For example, to grafting peptides on AuNPs surfaces, sulfhydryl amines are introduced onto nanoparticle surface followed by elongation of peptide chain with ring opening polymerization.
- (b) “Grafting to” fabrication technique: In this technique, the gold salt is reduced in polymer aggregates forming polymer-modified gold nanoparticles (Liu et al. 2007; Liang et al. 2010). This is one-pot synthesis process. Polymer ending with sulfur-containing group such as dithioester, thioether, trithioester, and thiol and with sulfur-free group is used in this method. The thiol-containing polymer forms a shell layer well bounded around the gold core.
- (c) Post-modification technique: Here as synthesized gold nanoparticles and as synthesized polymer are mixed together, eliminating uncertain factors such as dispersion of AuNPs and molecular weight of the polymer. Polymer with thiol group is covalently attached to the AuNP surface, while polymer without thiol group is physically adsorbed in the gold nanocomposite.

Hydrophilic polymers such as polypeptides, nucleic acids and polysaccharides, exhibit various unique analytical properties that play important roles in specific detection of homeostasis processes (Siigur and Siigur 2000). Thermoresponsive polymers such as poly(*n*-isopropyl acrylamide) and poly(methyl vinyl ether) are used as functional materials in many chemical analyses. They exhibit a conformational change when subjected to different solution temperatures. This thermoresponsive property facilitates the use of these polymers as key materials in fabricating actuators, micro-TAS, drug delivery systems, and sensors. Conjugation of AuNPs with functional polymers is used to recognize analytes which results in a morphological change in the gold cores and a colorimetric change (Sugunan et al. 2005).

Destabilization of polymer-functionalized AuNPs is a potential approach for developing colorimetric sensors. The negative charge stabilizes the gold nanoparticle which upon binding to metal ions causes the cancellation of negative charge surrounding the AuNPs or shrinkage of electric double layer and resulted in spontaneous aggregation of AuNPs (Uehara 2010). Chen and co-worker synthesize chitosan-functionalized gold nanoparticles that act as signaling probe for metal ion detection. Metal ion Hg^{2+} induces aggregation of AuNPs through a chelation reaction between chitosan and Hg^{2+} ion leading to a strong decrease of the absorbance as the color changes from red to blue. The sensing system is quite sensitive and detects Hg^{2+} ion over other metal ions as low as 1.35 μM which is lower than the WHO level of safe limit for mercury in drinking water (Chen et al. 2015).

5 Applications of Gold Nanoparticles in Sensing Heavy Metal Ions

Functionalized gold nanoparticles are used in wide range of sensing applications because of its unique size-dependent optical properties, SPR. During sensing process, the shift in SPR band happens due to the formation of aggregates of AuNPs that leads to a visual color change in the solution. The benefit of AuNP-based optical colorimetric detection is that a rapid visual assays can be performed which require less or no instrumentation for detection of biomolecules, small molecules including various toxic metal ions. AuNPs have been exploited in the development of biosensors for the detection of specific biomolecules that are significant in disease etiology (Tansil and Gao 2006). For example, uric acid is an important end product of purine metabolism. Abnormal level of UA indicates various metabolic diseases such as gout, hyperuricemia, pneumonia, kidney damage (Kutzing and Firestein 2008). Several methods including colorimetric (Lu et al. 2015), electrochemical (Sakamoto et al. 2011), and enzymatic methods (Cunningham and Keaveny 1978) have been developed for uric acid detection in human fluids. Functionalized AuNPs can also calorimetrically detect uric acid in blood serum and urine with a detection limit as low as 8 ppm (Kumar et al. 2015). Similarly, bimetallic gold–silver nanoparticle-based sensor is developed for glucose detection in blood sample. In this work, silver ions are reduced on gold nanoparticle surface in the presence of glucose forming a new bimetallic gold–silver nanosystem which leads to a direct SPR shift in the UV–Vis spectrum (Li et al. 2017). Correspondingly, another colorimetric-based gold nanosensor is developed using gelatin-coated AuNPs with 6-mercaptohexane-1-ol (MCH) for proteinase activity assay where gelatin serves as proteinase substrate. Proteinase digestion separates the gelatin and brings the nanoparticles closer due to the presence of MCH, therefore forming an aggregation of AuNPs, and change in surface plasmon resonance band is observed which is determined by the change in absorbance ratio (van Hengel et al. 2017). There are many reports on sensing biomolecules using gold nanoparticles; however, as this chapter is focused on detection of chemical pollutants, the following sections are emphasized only on toxic metal ion detections using functionalized AuNPs as sensor probes.

5.1 Detection of Heavy Metal Ions Using Gold Nanoparticle

Arsenic—Arsenic is a heavy metal toxin responsible for causing chronic diseases in human including skin cancer, abdominal pain, renal failure, lungs, and liver diseases (Hong et al. 2014). Colorimetric-based detection of As^{3+} ion using AuNPs is mainly based on the intrinsic property of strong binding affinity of As^{3+} ion toward thiol compounds attached to AuNPs (Al-Sid-Cheikh et al. 2015). Kalluri and co-workers develop gold nanosensor functionalized with thiol-based compound

glutathione (GSH), dithiothreitol (DTT), and cysteine (CYST) for colorimetric detection of As^{3+} ion. The minimal detection is reported 5 ppb for DTT-capped AuNPs, 20 ppb for GSH-capped AuNPs, and 25 ppb for CYST-conjugated AuNPs. The comparative lower detection in DTT is because of binding of As^{3+} ion to DTT via As–S linkage unlike other where As^{3+} ion binds to ligand via As–O linkage. Dynamic light scattering analysis further improves the detection up to 10 ppt limit, suggesting DLS technique as a more sensitive detection method (Fig. 13) (Kalluri et al. 2009). Recently, citrate AuNPs functionalized with GSH-DTT-Cys in one setup and GSH-DTT-Cys-PDCA in another are demonstrated for As^{5+} ion detections. PDCA is used to avoid interference with Hg^{2+} ion. DTT converts As^{5+} to As^{3+} , which binds to AuNP leading to aggregation followed by a color change and SPR shift. It is observed that GSH-DTT-Cys-PDCA is more sensitive in detecting $\text{As}^{3+/5+}$ than GSH-DTT-Cys-functionalized AuNPs with a detection limit of 2 ppb which is lower than WHO level of 10 ppb (Dominguez-Gonzalez et al. 2014). Wu et al. detect As^{3+} ion using an As^{3+} ion-specific aptamer and a water-soluble cationic polymer poly(diallyldimethylammonium) (PDDA). In the absence of As^{3+} ion, aptamer forms a duplex complex with PDDA preventing its binding to AuNPs; thus, stabilized AuNPs remain red in color in the colloidal state. However, in the presence of As^{3+} ion, the aptamer forms a complex with the ion leaving PDDA molecules free which binds to the surface of AuNPs causing aggregation and corresponding color change from red to purple (Wu et al. 2012). Similarly, Zhan et al. developed aptamer-based detection method without the involvement of any polymer. Here, the aptamer-functionalized AuNPs remain stabilized and red in color in ionic solution. In the presence of As^{3+} ion, As–aptamer complex is formed which destabilize the AuNPs, resulting in an aggregation with a detection limit of 1.26 ppb (Yu 2014). Xia and co-workers reported a novel detection system using unmodified AuNPs and a phytochelatin-like peptide (*g*-Glu-Cys)₃-Gly-Arg (PC3R) for the detection of As^{3+} ion. PC3R is an oligomer of glutathione, and in the presence of As^{3+} ion, it forms a chemical complex as arsenic has a strong affinity toward thiol compound, thus preventing the aggregation of AuNPs and retaining its red color in solution. In the absence of As^{3+} , the peptide molecules bind to AuNPs surface forming aggregates and change in color from red to blue is observed (Saha et al. 2012).

Copper—Copper is another vital biologically important element, but elevated concentration has an adverse effect on human health. Its accumulation causes reactive oxidation stress and several neural diseases (Desai and Kaler 2008). Various AuNP-based colorimetric methods have been employed in the detection of Cu^{2+} ion. Ye and co-workers use AuNP-stabilized with polyvinylpyrrolidone (PVP) for copper ion detection in solution. In the detection process, 2-mercaptobenzimidazole (MBI) is used to cause aggregation of PVP-stabilized AuNPs by the formation of mercapto-ligand self-assembly of AuNPs leading to a color change from red to blue. However, Cu^{2+} ion, which has an exclusive affinity toward MBI prevents the mercapto-ligand self-assembly of AuNPs and the color remains red due to monodispersity of AuNPs in solution. The detection limit of this colorimetric “purple-to-red” sensor is 5 μM through naked eye and 0.5 μM through

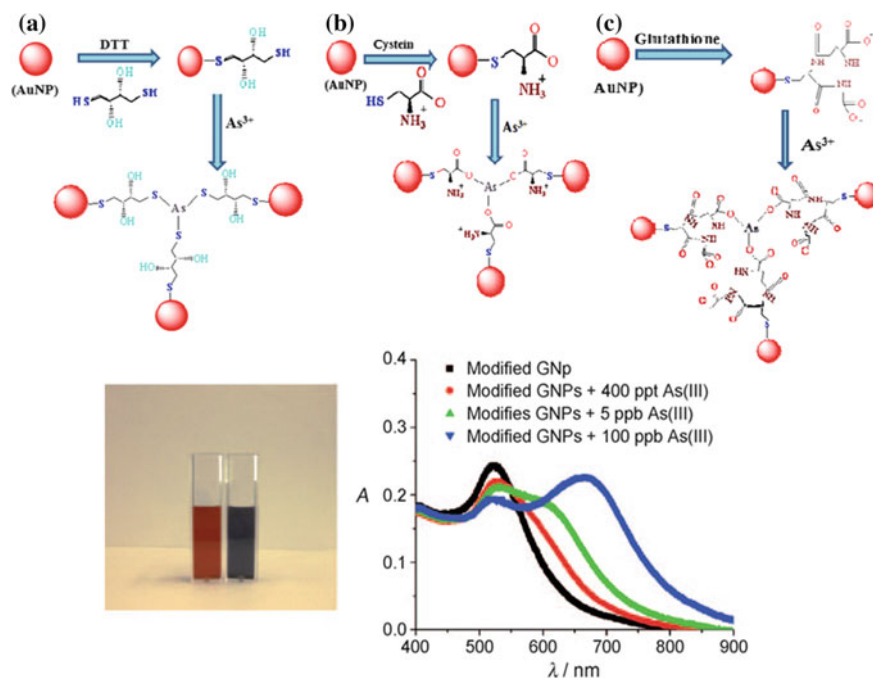


Fig. 13 (Above) Schematic illustration of DTT-AuNPs (a), Cys-AuNPs (b), GSH-AuNPs (c), and their respective interaction with As^{3+} ions. (Below) Image showing colorimetric change of gold nanosensor before and after addition of arsenic ions from red to blue and their corresponding UV-Vis absorption peak. Reprinted with permission from Kalluri et al. (2009)

UV-Vis spectroscopy (Fig. 14) (Yen et al. 2015). Wang et al. synthesize cysteamine-functionalized AuNPs for Cu^{2+} ion detection based on aggregation technique. AuNPs are conjugated with thiol compound cysteamine via Au-S linkage, and thereafter, salicylaldehyde is linked to cysteamine with the help of Schiff base reaction. The Schiff base reacts specifically to Cu^{2+} ion forming aggregation of AuNPs followed by a color change of the sensor from red to blue and SPR shift from 520 to 625 nm (Wang et al. 2012). Another group, Yang, and co-workers develop L-cysteine-functionalized AuNPs for rapid visual detection of copper ion in aqueous solution. In the presence of Cu^{2+} ion, functionalized AuNPs aggregate with a change in color from red to blue and corresponding SPR absorption shift. The Cu^{2+} ion coordinates with the $-COOH$ and $-NH_2$ functional group of L-cysteine molecule forming the aggregates. The minimal detection limit (MDL) of this sensor is up to $10^{-5}M$ (Yang et al. 2007). Copper-specific DNAzyme-functionalized gold nanoparticles is also fabricated by Wang et al. for colorimetric biosensing of copper. In the presence of Cu^{2+} ion, the Cu^{2+} -specific DNAzymes are cleaved to short ssDNA, which are easily adsorbed on AuNP surface enhancing the stability of AuNPs against salt-induced aggregation; thus, the color remains red in solution. However, in the absence of Cu^{2+} ion long strand of

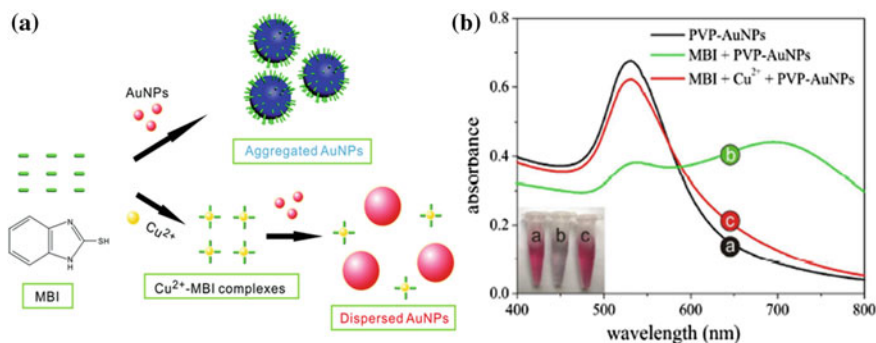


Fig. 14 **a** Schematic showing colorimetric detection of Cu^{2+} ions using PVP-stabilized gold nanoparticles, **b** UV-Vis spectrum of PVP-AuNPs (*a*), MBI-PVP-AuNPs (*b*), MBI + Cu^{2+} + PVP-AuNPs (*c*). Inset photograph showing corresponding color changes. Reprinted with permission Ye et al. (2015)

DNA is slowly adsorbed on AuNP surface leading to aggregation on addition of salt due to diminishing electrostatic repulsion of AuNPs and the color changed to blue. The MDL of this method is as low as 290 nM against other common metal ions (Wang et al. 2010).

Lead—Contamination of water with lead ion is a common problem having adverse effects on living beings. It causes anemia, high blood pressure, and renal disease and affects the central nervous systems (CNS) (Moreira and Moreira 2004). Colorimetric sensor for detection of lead is developed by Chai et al. GSH-functionalized gold nanoparticles are synthesized which show color change from red to blue due to aggregation in the presence of Pb^{2+} ion with the lowest detection limit of 100 nM over other metal ions (Fig. 15) (Chai et al. 2010). Highly sensitive colorimetric sensor and maleic acid-functionalized gold nanoparticles for detection of Pb^{2+} ion are developed by Nalin and co-workers. Maleic acid has $-\text{COOH}$ groups that are present on AuNP surface and have a strong affinity toward Pb^{2+} ion forming an aggregation of AuNPs, and color changes from red to blue. The detection limit is 0.5 ppb through naked eye within a period of 15 min (Ratnarathorn et al. 2015). There is a huge development for detection of Pb^{2+} ion using DNAzymes and unmodified gold nanoparticles. DNAzymes are specific to metal ions and form the core of many detection studies. Liu et al. use lead-dependent DNAzyme-functionalized AuNPs for sensitive and selective detection of lead ion in an aqueous environment. In this method, gold nanoparticles are cross-linked by DNAzyme substrates through DNA hybridization forming blue color aggregates with absorption at 700 nm. In the presence of lead, the DNA substrates are cleaved and dispersed gold nanoparticle shows red color with an absorption peak at 522 nm. However, the sensor is time-consuming and requires 2 h to observe the color change (Liu and Lu 2004a).

Mercury and other toxic metal ions—Mercury (II) is the most toxic ion among all heavy metal-based pollutants. Aqueous mercury solution is easily absorbed by

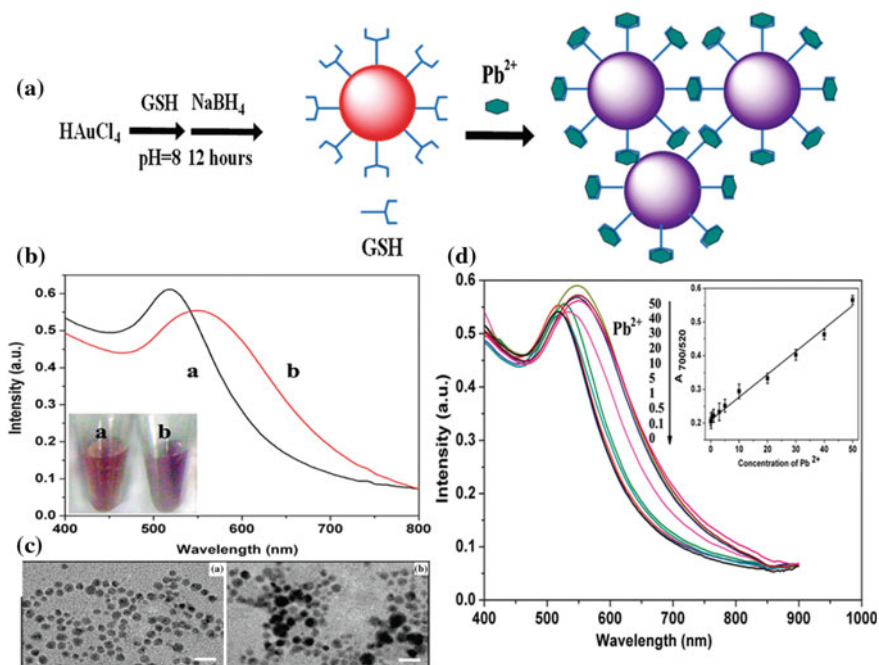


Fig. 15 **a** Binding mechanism of Pb²⁺ ion with GSH-functionalized gold nanoparticles. **b** UV-Vis spectra of GSH-functionalized gold nanoparticle with (b) and without (a) Pb²⁺ ion and their respective TEM images (c). **d** UV-Vis spectra of gold nanosensor with increase concentration of Pb²⁺ ions. Reprinted with permission Chai et al. (2010)

fish or shell fish which then enter human body via food chain causing severe brain, liver, and kidney damage. Several AuNP-based colorimetric methods are employed for mercury (II) detection (Wu and Sun 2016). Among them, DNA-functionalized gold nanoparticles are successfully used in mercury ion detection which are discussed in Sect. 4.2 (Fig. 16) (Lee et al. 2007). Among non-DNA-functionalized gold nanosensor, Kim and co-workers develop 11-mercaptopundecanoic acid (MUA) capped gold nanoparticles using Au-S chemistry for binding 11-MUA with AuNPs which have absorption peak at 526 nm. In the presence of mercury (II) ion, the color of the nanosensor is changed from red to blue due to aggregation. Similarly, 3-mercaptopropionic acid-functionalized AuNPs are developed with 2, 6-pyridinedicarboxylic acid (PDCA) for detecting mercury (II) ion with a minimal detection limit up to 20 ppb (Huang and Chang 2007). L-cysteine-stabilized gold nanoparticles are synthesized to detect Hg²⁺ ion up to a concentration less than 20 μM forming analyte-induced aggregation (Fang et al. 2010). Su et al. report the synthesis of thioctic acid-modified AuNPs for mercury (II) ion detection. Thioctic acid binds to AuNPs via strong Au-S bond and Hg²⁺ ion by free carboxyl groups forming aggregation of nanoparticles and showing redshift of SPR with a minimum detection limit of 10 nM (Su et al. 2013). Fu et al. fabricated array of 3D gold micro-/

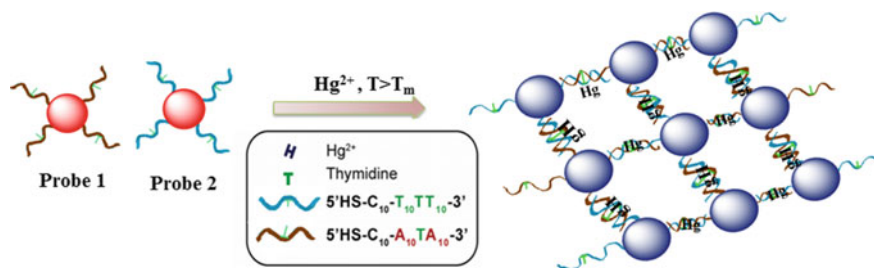


Fig. 16 Colorimetric detection of Hg^{2+} using DNA-functionalized AuNPs exploiting thymidine– Hg^{2+} –thymidine coordination chemistry. Reprinted with permission from Lee et al. (2007)

nanopore structures on gold substrate for the electrochemical determination of mercury in water. Gold is electroplated onto the SiO_2 spheres followed by etching of the template in hydrogen fluoride forming three-dimensional gold microarray electrodes. For selective detection of mercury ion, 2-mercaptobenzothiazole is adsorbed on the electrode surface which precisely detects Hg^{2+} ion up to a trace level of 0.02 nM using square wave anodic stripping voltammetry (Fu et al. 2010). In similar fashion, other toxic species such as chromium and cadmium including some anions such as AcO^- and HPO_4^{2-} are detected with functionalized gold nanoparticles (Liu et al. 2011). It is reported that Cr^{3+} ion have strong affinity toward citrate, and based on this observation, Liu and Wang et al. demonstrate Cr^{3+} ion detection using citrate-capped gold nanoparticles which aggregate in the presence of Cr^{3+} and exhibit a clear-cut color change from red to blue (Wang 2015). AuNPs co-conjugated with L-Cysteine and 6-mercaptopnicotic acids are studied for sensitive colorimetric detection of Cd^{2+} ion. Co-functionalization with these two ligands, L-Cysteine and 6-mercaptopnicotic acid, shows better sensitivity toward Cd^{2+} ion detection forming aggregation and SPR shift to 620 nm (Xue et al. 2011).

5.2 Heavy Metal Ions Detection Using Fluorescent Gold Nanoclusters (AuNC)

Detection of heavy metal ions using fluorescent gold nanoclusters has gained attention nowadays. A fluorescent gold nanocluster emits strong fluorescence with high quantum yields, which are increased by tenfolds with the use of alkanethiols, dendrimers, or polyethyleneimine as stabilizing agent. Chang et al. synthesize fluorescent gold nanoparticle using 11-mercaptopundecanoic acid, 2-mercaptoethanol, and 6-mercaptophexanol for selective detection of Hg^{2+} ion. Among all fluorescent AuNPs, 11-MUA-stabilized AuNPs is more sensitive toward Hg^{2+} ion with a minimal detection limit of 5 nM (Fig. 17) (Huang et al. 2007). Guo et al. demonstrate the synthesis of glutathione-capped fluorescent gold nanoparticle, which is highly sensitive for detection of copper ions. In both the cases, the fluorescent gold nanoclusters

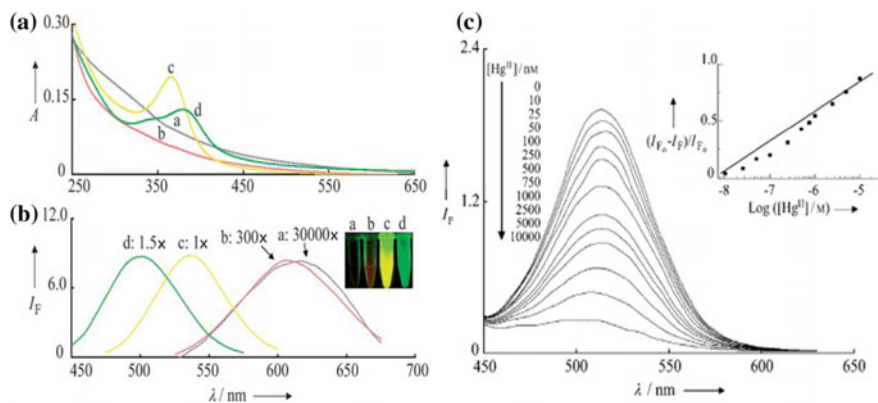


Fig. 17 **a** UV-Vis absorbance spectra and **b** fluorescence spectra of AuNPs (a), 2-ME-Au NPs (b), 6-MH-AuNPs (c), and 11-MU-AuNPs (d). Photograph of the fluorescence of the various AuNPs upon excitation under a handheld UV lamp (365 nm) (inset). **b** Their respective fluorescence intensities excited at wavelength 365 nm. **c** Fluorescence spectra showing quenching effect of 11-MUA-functionalized AuNPs on addition of increase concentration of Hg²⁺ ion. Reprinted with permission from Huang et al. (2007)

show quenching effect in the presence of mercury and copper ions up to a low limit of 5 and 8 nM, respectively (Chen et al. 2009).

All these above methods based on either colorimetric or fluorometric approach are exploiting the optical properties of gold nanoparticles, but they are time-consuming and requires handling of toxic chemicals or proper safety for biological substrate for any detection. Recently, there is a thrust for developing miniature devices for detection of these heavy metal ions (Zhao et al. 2016), wherein nanoparticles are immobilized on some substrates like paper and used for sensing applications. Developing such “lab-on-chip” devices for sensitive detection of heavy metal ions opens up new areas toward sensing applications that also allow quantitative analysis of metal ions for monitoring environmental toxicity and providing safety consciousness to human health and welfare (Am et al. 2011).

5.3 Heavy Metal Ion Detection on Paper Substrate

As discussed in Sect. 1, traditional methods for the detection of heavy metal ions include ICP-MS, AAS, X-Ray diffraction, and other electrochemical techniques. These methods are highly sensitive, precise, and specific giving accurate result. However, the major drawbacks of these methods include requirement dedicated laboratory setup with trained personnel, sophisticated instruments, involvement of toxic chemicals, and laborious operations which make the analysis highly expensive (Zhao et al. 2016). In developing countries, there is a high demand of simple, rapid, cost-effective sensor device as they lack infrastructure, professional experts

for detection of heavy metals in metal toxin monitoring. In last two decades, microfluidics has emerged as a promising technology in providing rapid “point-of-care” sensing applications. Recently, paper has been explored as a potential candidate for fabricating “lab-on-chip” sensing device (Yang et al. 2017). “Paper-based microfluidic” device works on the capillary flow of actions on the paper substrate. The use of paper also makes the cost of detection device extremely low. Chromatography paper, filter paper, and nitrocellulose paper are used as substrate for paper-based microfluidic device fabrication. In this section, several paper-based microfluidic devices are demonstrated for the detection of heavy metal toxins that require environment monitoring. Conventional colorimetric paper-based kits for detection of metal ions involve the release of toxic gases during operation (Das et al. 2014; Wang et al. 2017). So researchers have come up with the idea of immobilizing colorimetric AuNP-based sensor on paper substrate. Recently developed paper-based device immobilized with gold nanosensor for detection of heavy metal ions is listed in Table 1 (Fig. 18) (Elavarasi et al. 2013; Nath et al. 2014, 2015; Fang et al. 2015; Vijitvarasan et al. 2015). The embedded microstructures of the paper substrate help to pump the fluid due to capillary action. As the sensor comes in contact with the analytes, there is a visual color change of the immobilized gold nanosensor providing a semiquantitative result (Nath et al. 2014). It is worth noting that such paper-based materials have extensively been investigated in microfluidic research on various sensing applications and widely used in point-of-care (POC) diagnostics.

5.4 Quantification of Heavy Metal Ions Using AuNP-Based Electronic Color Sensor Module

As discussed in previous section, exposure to heavy metal ions shows distinct change in color of gold nanosensor adsorbed on paper-based substrates. The change in color renders a qualitative detection of particular heavy metal ion in sample. In case of real-time applications such as testing the quality of drinking water or monitoring the amount of pollutants in industrial wastewater, quantification of heavy metal ions is essential. This is owing to the limits set by the WHO and other regulatory bodies in order to check the amount of toxins in the sample. Precise quantitative detection with respect to change in color can be conducted employing color or image sensors along with proper calibration and display module. In one of the arsenic detection techniques, μ PAD (microfluidic paper-based analytical device) housing AuNPs functionalized with α -lipoic acid and thioguanine is developed. The change in color of the μ PAD on arsenic exposure is recorded using a desktop scanner, and the image is further processed to extract the intensities of red, green, and blue colors. However, quantification of arsenic is not conducted in this work (Chowdury et al. 2017).

Table 1 Recently developed paper-based microfluidic device for sensing heavy metal toxins as chemical pollutants

Nanoconstructs	Metal ions	Paper-based detection techniques	References
Citrate AuNPs	Chromium (III)	Colorimetric (red to blue)	Elavarasi et al. (2013)
DNAzyme-functionalized AuNPs	Lead(II)	Scanometric assay Colorimetric	Vijitvarasan et al. (2015)
Fluorescent AuNCs	Copper(II)	Fluorescent quenching	Fang et al. (2015)
Dansylhydrazine-functionalized AuNPs (Au-TA-DNS)	Lead(II) and Copper(II)	Fluorescent quenching colorimetric	Nath et al. (2015)
Thioguanine-functionalized AuNPs (Au-TA-TG)	Arsenic(III)	Colorimetric	Nath et al. (2014)

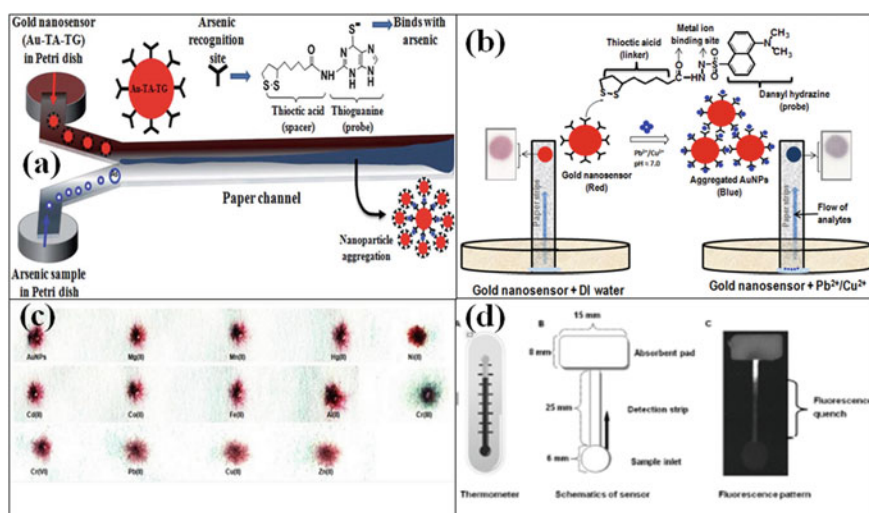
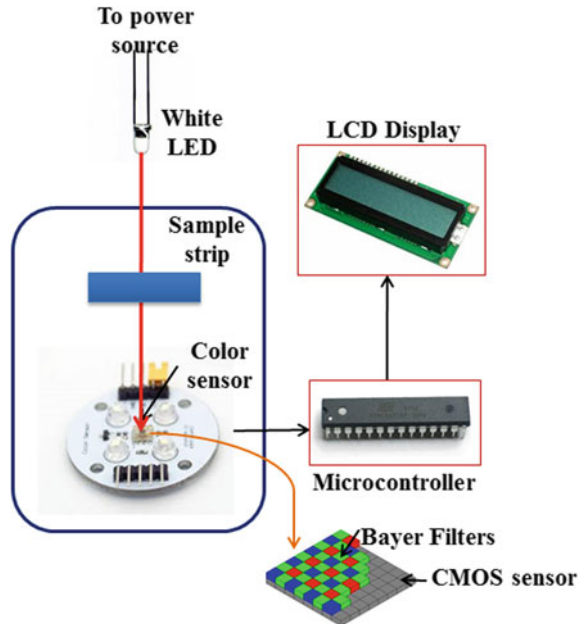


Fig. 18 a Schematic showing paper-based microfluidic device for arsenic detection using gold nanosensor Au-TA-TG. b Schematic illustration of lead detection in paper-based channel using Au-TA-DNS gold nanosensor. c Photograph showing paper strips with gold nanosensor spots for selective detection of chromium ion. d Thermometer-like biosensor for detection of copper ion using quenching mechanism of fluorescence gold nanosensor. Reprinted with permission from Nath et al. (2014, 2015), Elavarasi et al. (2013), Fang et al. (2015)

Though systems pertaining precise quantification of heavy metal toxins are limited, approach toward quantitative estimation employing color sensors has been initiated for other chemicals and biomolecules (Shen et al. 2011, 2012; Cho et al. 2010). The generic schematic of the devices used for color-based sensing is shown in Fig. 19. As shown in Fig. 19, the device consists of a color sensor module, a light source, and associated microcontroller-based circuitry for processing,

Fig. 19 Generalized schematic for color sensor module used for detecting heavy metal ion induced color change in test strip



inference, and display. The light source incidents white light on the strip. The color of the transmitted light is dependent on the color of the strip. The color sensor module consists of a CMOS-based image sensor, which houses array of Bayer filters. Bayer filters are array of red-, green-, and blue-colored filters, which can absorb a specific wavelength of light and can transmit the rest. It is obvious that the filter will block specific color according to the array configuration and will allow others leading to variation in intensity of the transmitted light based on variation in color. A signal proportional to light intensity will be generated by the CMOS sensor, which is further processed to map the amount of color on the incident light. An approach to quantify heavy metal ions by observing change in color of a test strip is conducted by Ruiz et al. (Cho et al. 2010). In this research, the authors develop a handheld colorimeter for determination of heavy metal concentrations. A microcamera is used to capture the image of the test strip. The HSV (hue, saturation, value)-based color model is used for analysis of the image. The hue component for every image is recorded pixel by pixel. Color change in the test strip due to exposure to particular heavy metal ion renders a different value of hue. The test strips are also scanned for their images employing a desktop scanner. The scanned images are subjected to HSV processing using MATLAB. The hue values obtained from camera images and scanner images are matched for verification.

6 Conclusion

The unique photophysical properties of gold nanoparticles ease of their synthesis and functionalization have made gold nanoparticles a versatile platform for designing various chemical sensors. The optoelectronic properties of AuNPs make them highly selective and sensitive to any binding analytes such as heavy metal toxins. Functionalization of AuNPs improves the sensor performance as they act as both molecular receptor and signal transducer in a single unit.

Detection of heavy metal toxins such as arsenic, mercury, lead, and copper to minute level is mandatory to monitor environmental toxicity, thus helping in providing safe water for consumption which otherwise could have a deleterious effect on human health. Traditional methods of detection of these toxic metal ions are expensive, especially for developing countries. Nanoparticle-based detection system provides simple, rapid, low-cost identification of these toxic ions. AuNP-based colorimetric sensor could provide detection limit from micromolar to picomolar depending on the target species and sensor design. In order to develop efficient “point-of-testing” device, paper-based microfluidics has emerged as a growing platform. In this technique, functionalized gold nanoparticles are immobilized on paper substrate and analytes are passed through exploiting the capillary flow actions of the paper. The paper-based device increases the efficiency of the colorimetric method as miniaturization increases the number of recognition elements. Quantitative analysis of heavy metal ions using gold nanoparticle-based sensor is still in demand and encourages researchers for further innovations for real sample analysis around the corner.

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