Trends in Applied Sciences Research

ISSN 1819-3579

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OPEN ACCESS Trends in Applied Sciences Research

ISSN 1819-3579
DOI: 10.3923/tasr.2020.151.167

Review Article
Synthetic Applications of Gold Nanoparticles in Research Advancement of Electrochemical Immunosensors

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Abstract
The convergence of new technologies, including nanotechnology, biotechnology and information technology has opened new horizons in electrochemical immunosensors. Electrochemical immunosensors has attracted numerous interests due to its inherent benefits over the other transduction schemes, such as a high sensitivity, ease of use, a possible automation and integration in compact diagnostic systems and analytical systems which must be mainly low cost and use of simple technology of their designing and production as compare to the existing technology. This review covers the synthetic applications of gold nanoparticles (AuNPs) in electrochemical immunosensors. This review is divided in the following sections: (i) Synthesis of AuNPs (ii) Unique properties of AuNPs (iii) Role of Au NPs in electrochemical immunosensors and (iv) Application of AuNPs and future trends. The addition of AuNPs in modified electrodes itself increases the electron transfer between the electrode and biomolecules which directs to advanced bioanalytical devices. Electrochemical immunosensors developed in laboratory are very sensitive, hand-held, user-friendly devices that can provide new analytical approaches for the infield detection of various biological warfare agents.

Key words: Gold nanoparticles, electrochemical immunosensors, biomolecules, synthesis of AuNPs, electrodes

Citation: Arun Sharma and Vepa Kameswara Rao, 2020. Synthetic applications of gold nanoparticles in research advancement of electrochemical immunosensors. Trends Applied Sci. Res., 15: 151-167.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In the last two decades an intensive effort has been done in the field of bioanalytical chemistry for the aim to develop metal nanomaterials having unique physical, chemical, optical and electronic properties[1]. When the crystallite size of the materials are reduced at nanometers dimensions two different phenomena can be observed, the first is called quantum size effect which happens when physical-chemical properties of the material is changed drastically[2]. The second phenomenon is attributed due to the enormous correlation between the surface area and volume. The second one is more interesting for electroanalytical purposes[3]. Nanotechnology is the branch of science which deals with the study of nanoparticles and their unique properties. When the size of the material is reduced to the nano-meter level the properties of the materials may drastically changed[4]. The size of the nanoparticles can be easily tuned which makes them perfect for the development of various sensing devices[5].

Electrochemical immunosensors are particularly used in food safety, environment, pharmaceutical, chemistry and clinical diagnostics etc. because of their simplicity, rapid response, low-cost, high speed and ease of assimilation with electronic devices[6-11]. Since, for early detection of diseases, terror detecting, pollution control and in Point of Care (POC) diagnostics electrochemical immunosensors are mostly used[12-15]. Numerous efforts have been put in the development of electrochemical immunosensors during last decades. Different amplification strategies and fabrication methods have been employed to reach the high sensitivity and efficiency.

In this region, nanotechnology and material science has been involved which present great superiority in the fabrication and signal amplification of electrochemical immunosensors. Nanoparticles synthesized by chemical or physical methods are mainly classified into five categories viz. magnetic nanoparticles, semiconductor quantum dots, carbon-based nanostructures, polymeric nanoparticles and metallic nanoparticles. Since, large number of various nanomaterials has been applied in the fabrication of electrochemical immunosensors to achieve the high sensitivity and enormous review articles are already been reported in this field[16-19]. Moreover, the electrochemical immunosensors are highly sensitive detection tools which can easily identify the presence of toxins and pathogens specifically at very low concentration. Various enzymes-coupled carbon nanotubes[20], metal nanoparticles[21,22], magnetic nanoparticles[23] and quantum dots are used as labels for the signal enhancement and to improve the detection limits[24,25].

Gold Nanoparticles (AuNPs) are widely used in the various applications of molecular biology like genomics, drug delivery, medicinal chemistry, immunoassays and clinical applications etc. The information of size dependency of AuNPs and its application is given in the Table 1.

Now these days, gold nanoparticles (AuNPs) are playing an important role in the field of immunosensors for human welfare and disease diagnosis[26]. However, the systematic study for the use of AuNPs in the development and fabrication of electrochemical immunosensors is still not discussed. In this paper, recent research reports published on the AuNPs based electrochemical immunosensors are reviewed. For last two decades, AuNPs have been attracted much attention in biological studies because of their high affinity towards biomolecule, low toxicity, unique electrochemical and optical properties[27]. AuNPs based detection technologies are becoming more promising approaches in electrochemical immunosensing that is reflected in the large number of papers which are published during the last 5 years. In this sense, the utilization of AuNPs brought a new and exciting outlook for analytical sciences.

This review is focused on the unique characteristic properties of AuNPs and the exploitation of their significant contribution in the development of electrochemical immunosensors. The surface atoms of AuNPs are highly active because of their high surface to volume ratios and high surface energies which make them outstanding candidates to work like an excellent electrocatalyst and nanocatalyst[28]. AuNPs are used as labels in the immunosensing to enhance the voltammetric signals[29]. Since, the AuNPs are also used for the immobilization of biomolecules on their surface[30]. As compare to the ELISA and optical methods, the signals obtained by electrochemical immunosensors are not affected by the turbidity if present in the sample and the interference caused by the fluorescent compounds. The analysis of food samples is also possible by electrochemical techniques which

Table 1: Application or use of AuNPs with respect to their particle size

Particle size range of AuNPs (nm)	Application
2-15	Biomarkers, Immunohistochemistry, light microscopy, high resolution TEM
20-60	Environmental pollution detection and purification, Drug delivery, DNA-Sensors
80-250	Electronic device, Optical mammography, Forensic science and manufacture

are failed in case of other analytical methods. Fabrication and operation principle of the electrochemical immunosensor is based on sandwiched ELISA method in which the event of antigen-antibody immunocomplex formation is converted into an electrical signal which can be easily measured by electrochemical immunosensors.

In this review, the recent advances of AuNPs in electrochemical immunosensing technology which can be achieved by the conjugation of biomolecules with the AuNPs in analytical devices have been discussed. This study divided in the following sections (i) Materials and methods in which the reagents and apparatus used for the preparation of AuNPs, characterization, formation of bioconjugates and optimization are mentioned, (ii) Unique properties of AuNPs, (iii) Role of AuNPs in electrochemical immunosensors which including bioconjugation, AuNPs as signal tags and electrode modification in various schemes and (iv) Application of AuNPs and future trends. Since, this review article explore the new horizons of biomedical research based on the use of AuNPs in the field of electrochemical immunosensing to enhance the detection limits for the early diagnosis of disease and take precautionary measures for their treatment.

Reagents and apparatus: In the reduction of Au III ions to Au 0, various reducing agents like sodium borohydride, presence of sodium hydroxide, monosodium glutamate, ascorbic acid, sodium citrate, trisodium citrate and hydrogen peroxide have been used in the presence of one or more water-soluble polymers, surfactants or capping agents. All other chemicals and reagents like Tetraoctylammonium Bromide (TOAB), Potassium ferricyanide i.e., $K_3[Fe(CN)_6]$ and potassium ferrocyanide i.e., $K_4[Fe(CN)_6]$, Bovine Serum Albumin (BSA), chloroauric acid ($HAuCl_4$), 1-Ethyl-3-(3-(dimethylamino) propyl) carbodiimide (EDC), N-hydroxysuccinimide ester (NHS), were of Analytical Reagent (AR) grade and used without further purification. Other reagents and chemicals were all of analytical reagent grade. Aqueous solutions were prepared with Triple Distilled Water (TDW) (18 MO cm resistivity, Milli-Q).

Electrochemical measurements were performed by using a conventional three-electrode system comprised of platinum wire as the counter electrode, saturated Ag/AgCl electrode (Reference electrode) and a GCE/SPE as the working electrode. SEM/EDX measurements were performed with a Quanta 400 ESEM (Netherlands). UV-Vis spectra were recorded on a PerkinElmer Lambda 35. Photoluminescence spectra were obtained using a PerkinElmer LS55 fluorescence spectrometer. Zeta-potential and Dynamic Light Scattering (DLS) analysis of the AuNPs was measured by Malvern Instruments (Malvern, UK) Zetasizer Nano-ZS. A magnetic stirrer controller (model no. TH100) and a pH meter (Eutech Instruments, Singapore) were utilized in this study. All electrochemical experiments were performed at room temperature.

Synthesis and characterization of AuNPs: Many synthetic procedures are reported in the literature to synthesize the AuNPs of different size, shapes, morphology and dimensions[31,32]. Still, it is a great challenge to obtain the particular size and shape of AuNPs by the chemical synthesis methods. A little change in the concentration of reactants, pH and temperature and stirring speed etc. may change the dimensions of the prepared particles. The effect of all these parameters and the various synthetic procedures are reported in this section.

AuNPs can be easily prepared in the laboratories by using the principle of top-down and bottom-up approach. In a typical "top down" approach, the bulk gold is gradually reduced until its size or dimension will come to the nanometer regime. These methods are generally called as physical methods. Simply, in top down method, bulk material was first grind mechanically and nanosized particles were obtained. These particles were made to be stabilized by the addition of protecting agents. Photolithography, high energy ball milling, melt mixing, physical vapour deposition, laser ablation, laser pyrolysis, sputtering, ion beam techniques and electron beam lithography are the commonly used top down techniques used for the synthesis of nanomaterials.

In the "bottom up" approach, the gold atoms which are produced by reduction of the gold ions are assembled in controlled conditions to produce nanostructured materials. Bottom up method basically includes collecting, consolidating and arranging individual atoms and molecules into different structures, which can be carried out by a sequence of chemical reactions with or without catalysts. There are various bottom-up techniques such as chemical bath synthesis, electrochemical deposition, sonochemical approach, hydrothermal method and photochemical reduction method. Bottom up techniques usually followed by the addition of capping agents during the growth of nanomaterials to prevent aggregation and precipitation of the AuNPs out of solution. A capping agent may be a unifunctional, bifunctional ligand, polymer and a surfactant which is used to stabilize the nanomaterials. Size and shape of the AuNPs can be controlled and it is basically depend on the technique which is used for the synthesis, reaction temperature and the choice of capping material. Table 2 represents the AuNPs synthesis using different chemical methods.

AuNPs can be synthesized of various shapes i.e., spheres, rods, cubes, disks, wires, tubes, triangular prisms, flowers, stars and tetrahedral nanoparticles which is depend on the varying

Table 2: Synthesis of AuNPs by various chemical methods

Method of synthesis of AuNPs	Reactants or precursors used	Size of obtained AuNPs (nm)
Citrate capped method	$Na_3C_6H_5O_7$, $HAuCl_4$	10-20
Brust method	$NaBH_4$, Tetraoctylammonium bromide (TOAB)	1.5-5.2
Seeded growth method	$Na_3C_6H_5O_7$, $HAuCl_4$ and $NaBH_4$	30-300
Biosynthesis method	Plant extract or microorganisms	4-200
Oleyl amine reduction method	$HAuCl_4$, Oleyl amine	9-10
Glutamate reduction method	$HAuCl_4$, NaOH, monosodium glutamate (MSG)	15-60
Gallic acid reduction method	$HAuCl_4$, NaOH, gallic acid	13.7-76.7
Gamma irradiation using sodium alginate as stabilizer	$HAuCl_4$, sodium alginate	5-40

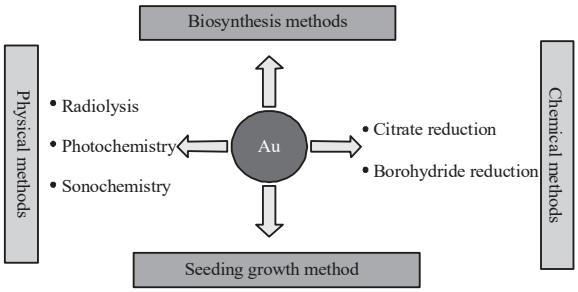

Fig. 1: Various methods for AuNPs synthesis

multiple parameters such as the concentration of reactants, reaction conditions (like, pH, temperature, stirring speed etc.) nature of solvent and the method used for the synthesis[33]. Figure 1 illustrates various methods for the synthesis of AuNPs.

Turkevich method: It is the well known citrate capped method which is the most commonly used method for the synthesis of AuNPs in the range of 10-20 nm. Various capping/stabilizing agents are used to stabilize the AuNPs in their colloidal suspension[34].

Brust method: It is used to generate the AuNPs in the size range of 1.5-5.2 nm. In this method, sodium borohydride is used as reducing agent and tetraoctylammonium bromide (TOAB) is used as phase transfer catalyst[35].

Seeded growth method is preferred to obtain the AuNPs of various shapes like rods, cubes, tubes etc.[36]. The geometry and shape of AuNPs is controlled by reducing agents, structure directing agents, seed concentration etc. Besides of these others miscellaneous methods like digestive ripening, solvothermal method, electrochemical deposition method, photochemical reduction etc., can be used. But, all these methods described above required toxic chemicals and solvents. Since, it is necessary to develop a method which does not require the toxic chemicals.

Biosynthesis method: It is a clean, eco-friendly and non-toxic method in which biodegradable chemicals are used[37]. Plant constituents and microorganisms are safe, easily available and do not pollute the environment since these are the excellent candidates used for the AuNPs biosynthesis. In this category, various parts of plants like fruits, seeds, leaf, fruit pericarp, bean extract, petals, flower extract etc., can be used as well as bacteria, yeast, fungi, algae, sponge etc., can also be used for the biosynthesis of AuNPs. Some biomolecules like amino acids, nucleic acids, carbohydrates, lipids etc. are also used to produce stabilized AuNPs by the addition of suitable capping agents.

In DRDO laboratory, citrate capped AuNPs, gold nanoseeds and gold nanorods were synthesized by the colloidal method. For the colloidal synthesis of citrate capped AuNPs, heated solution of $HAuCl_4$ (1 mM, 20 mL) is taken in the conical flask and add 2 mL of 1% trisodium citrate drop by drop in this flask with vigorous stirring. After some time, the color of the solution in the flask is changed from yellow to wine red. Hence, the citrate capped gold nanoparticles were synthesized in the colloidal solution in which the citrate ions provide the electrostatic stability via capping the AuNPs as well as study like a reducing agent during synthesis. The scheme of synthesis of citrate capped AuNPs is shown in Fig. 2a.

In the synthesis of Au nanoseeds, $HAuCl_4$ and sodium citrate ($Na_3C_6H_5O_7$) were mixed in 20 mL TDW in appropriate concentrations. After some time, drops wisely add the freshly prepared solution of ice-cooled $NaBH_4$ (0.1 M, 60 µL). Continuously stir the solution and the solution of Au nanoseeds appears to give pink color which became red after keeping it in dark chamber for overnight. Scheme of synthesis of Au nanoseeds is shown in Fig. 2b.

Seed mediated growth method is used for the synthesis of CTAB capped gold nanorods (Au NRs) in large amount. In this method, cetyltrimethylammonium bromide (CTAB), ascorbic acid and silver nitrate was added in appropriate concentration in the previously prepared solution of Au nanoseeds. Figure 2c illustrates the synthesis of Au nanorods. CTAB act like a capping agent, ascorbic acid work as a reducing agent and silver nitrate is responsible for the shape of nanorods. Gold nanorods (Au NRs) can be synthesized in different aspect ratio by varying the volume of seed solution used during the synthesis[38]. The schematic diagram of synthesis of citrate capped AuNPs, Au nanoseeds and AuNRs are given in Fig. 2a-c.

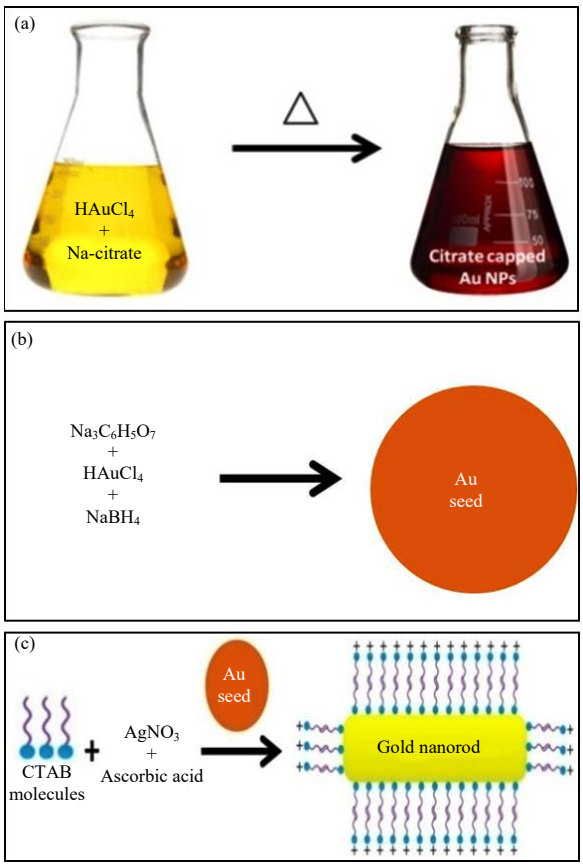

Fig. 2(a-c): Schematic diagram of synthesis of citrate capped (a) Colloidal synthesis of citrate capped-AuNPs, (b) Colloidal synthesis of Au nanoseeds and (c) Synthesis of Au nanorods

Scanning Electron Microscopy (SEM) and UV-V is spectroscopy method is used for the characterization of synthesized citrate capped AuNPs, Au nanoseeds and Au NRs. The obtained characterization data is given in Fig. 3a-c.

Size of the synthesized gold nanoparticles and gold nanoseeds is found in the range of 10-50 nanometers as shown in Fig. 3a, b, respectively. Stability of the synthesized gold nanomaterials is one of the most important factors and to prevent them from precipitation or aggregation various capping agents are used to modify the surface. In the colloidal synthesis of citrate capped-AuNPs monodisperse particles are obtained having protected by negatively charged citrate groups and can be characterized by UV-Vis spectroscopy. The band observed at 520 nm indicates that AuNPs are having 15 nm diameter as shown in Fig. 3c. The borohydride reduction of $HAuCl_4$ in the presence trisodium citrate gives rise to small gold nanoseeds (50-80 nm) as shown in Fig. 3d. Au nanorods show strong and tunable absorption band that is corresponding to the longitudinal plasmon oscillation which appears due to the possibility of unidirectional plasmon propagation. Au nanorods exhibit 2 distinct plasmon bands, one associated with the transverse ($\lambda = 520$ nm) mode and the other with the longitudinal mode (usually $\lambda \geq 600$ nm) as shown in Fig. 3e. These properties of Au nanorods motivate the researchers to take interest in controlled assembly of Au nanorods into functional architectures as well as for analyte-sensing applications[39].

Formation of bioconjugates and optimization: Conjugation of biomolecules with AuNPs is called "Bioconjugation". Bioconjugation is a process of interaction which may be possible either by covalent mode or non-covalent mode. Covalent mode includes basically the chemical interactions such as chemisorptions via thiol derivatives, bifunctional cross linkers e.g., EDC/NHS. On the other hand non-covalent mode includes the physical interactions such as electrostatic interactions or ionic interactions, hydrophobic forces, dative binding etc. Figure 4 illustrates the possible interactions of bioconjugate formation.

One of the major drawbacks of non-covalent interaction is that the antibodies are the random oriented at AuNPs surface and large amount of antibodies are required to prepare such nanobioconjugates. As well as these antibodies may be easily substituted by other biomolecules if present in the biological samples and the electrostatic charges on antibodies may also affect their efficiency in detection of the target species. On the other hand the covalent binding is much more efficient to retain the biological activity of antibodies during the immunosensing and also enhance the stability of the prepared nanobioconjugate without effecting their orientation and also do not require their big amount for immobilization. Before going for the electrochemical immunosensing of any target antigen the prepared bioconjugate must be properly optimized such as the amount of biorecognition species loaded or immobilized on the nanoparticles surface, pH of the medium in which the bioconjugate is prepared, stability of the prepared bioconjugates which including their self-life, function and orientation.

The amount of biomolecule immobilized on the fixed amount of AuNPs can be estimated by bicinchoninic acid (BCA) assay or Lowry protein assay or Bradford protein assay using spectrophotometric method in which the Bovine Serum Albumin (BSA) is used as standard. For this purpose, BCA working reagent was prepared by mixed with 50 parts of reagent A containing bicinchoninic acid, sodium carbonate, sodium tartrate and sodium bicarbonate in 0.1 N NaOH, pH 11 with 1 part of reagent B containing 4% (w/v) copper (II) sulfate

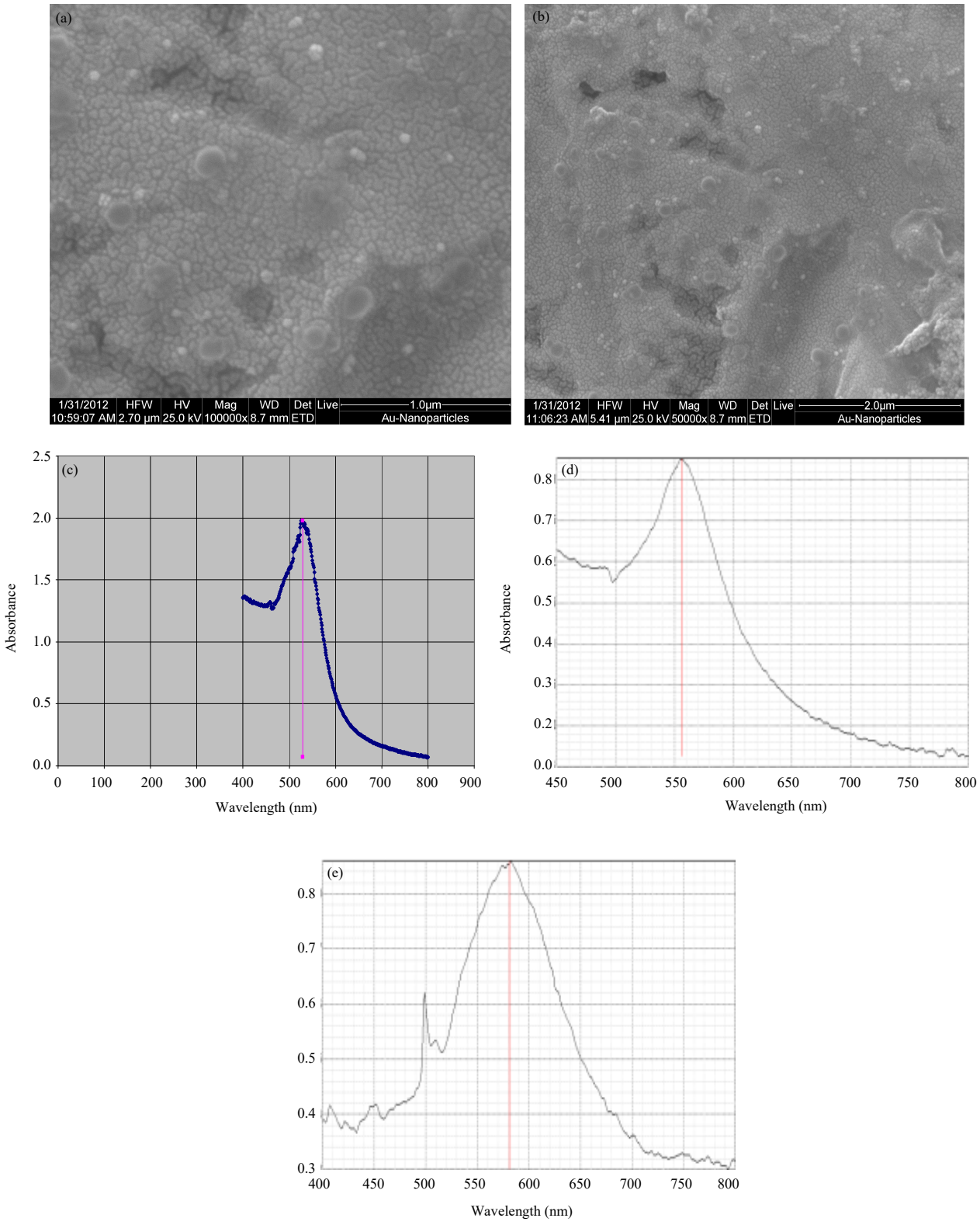

Fig. 3(a-e): Scanning electron microscopy results (a) SEM-image of colloidal synthesis of citrate capped-AuNPs, (b) SEM-image of colloidal synthesis of Au nanoseeds, (c) UV-Vis spectra of citrate capped-AuNPs, (d) UV-Vis spectra of Au nanoseeds and (e) UV-Vis spectra of Au nanorods

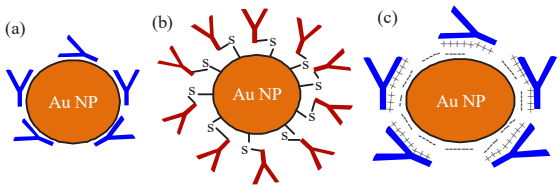

Fig. 4(a-c): (a) Hydrophobic, (b) Covalent and (c) Ionic interactions between antibodies and AuNPs

pentahydrate. In the standard procedure, BCA working reagent was taken in 200 µL are mixed with 0, 2, 4, 6, 8, 10 µL part of a standard protein (BSA) solution of known concentration. Water (10 µL) without protein was used as blank. Unknown purified detection IgG immobilized AuNPs sample was assayed with the known concentration of BSA protein standard. After that, the plate was incubated at 37°C for 30 min. In the next step, absorbance was recorded at 562 nm and the unknown protein concentration was determined by comparison to the standard curve.

The detection antibodies which were immobilized on AuNPs can be estimated by this standard protocol. The amount of AuNPs bind with the biomolecules can be optimized by ultraviolet-visible (UV-Vis) spectrophotometric analysis and the ratio of gold nanoparticles-detection antibodies i.e., [Au:Ab_d] by weight in the 5 µL of the nanobioconjugate is determined. For the quantitative determination of AuNPs present in the 5 µL of detection antibodies [Ab_d] bioconjugates which can be used for the electrochemical immunoassay of the target antigen. Amount of AuNPs detection antibodies [Au:Ab_d] was determined by UV-Vis analysis.

For this purpose, perform the UV-Vis experiment of various dilutions of AuNPs and the standard graph is plotted between the obtained absorption spectrum with their corresponding respective dilution. It can be easily found that the large amount of specific detection antibodies (Ab_d) are present on AuNPs surface and high sensitivity for the electrochemical detection of the antigens can be achieved. Several other techniques can also be used to determine the amount of nanobioconjugates such as colorimetry, Dynamic Light Scattering (DLS), Atomic Force Microscopy (AFM), confocal microscopy, Transmission Electron Microscopy (TEM), gel electrophoresis, critical flocculation concentration analysis and Carbon Rod Atomic Absorption Spectrophotometry (CRAAS). All these techniques are widely used to check the binding efficiency of AuNPs with various biomolecules both qualitatively and quantitatively.

The performance of the analytical electrochemical immunosensor is depending on various factors. These parameters are mainly including amount of capturing antibodies which is immobilized on to the electrode surface, spatial alignment of the immobilized antibodies, incubation period, pH of the medium and amount of detection antibodies. It is observed that the voltammetric response is increased with increasing the concentration of the immobilized capturing antibodies at a certain level and after that the response is saturated and remains as such. The amount of antibodies captured is different for different types of electrode used for the experiment like screen printed electrode, carbon paste electrode, glassy carbon electrode etc. It is because of their different electro-active surface areas which can be determined by using the Randles-Sevcik equation:

$$i_p = 2.69\times10^5\, n^{3/2}\, A\, C\, D^{1/2}\, \upsilon^{1/2}$$

where, i_p is the peak current, n is the number of electrons involved in the reaction, A is the electroactive surface area, C is the concentration of the reactant, D is the diffusion coefficient of the reactant species and υ is the scan rate. In case of large amount of capturing antibodies may randomly oriented on the electrode surface and do not enhance the signal because of spatial hindrance. Since, it is mandatory to optimize the amount of capturing antibody to get the reproducibility in the obtained results. The formation of stable immunocomplex due to the specific reaction between antibody and antigen is mainly depending on the pH of the medium which provides the necessary environment for the immunoreactions.

The configuration of the proteins, samples bioactivity, loading capacity of antibody and their electroactive states directly affected by the pH of the medium. It is observed that the pH 7.2-7.4 is optimum because at this pH the biomolecules preserve optimum bioactivity. It is also found that the voltammetric peak current was increased with increasing the incubation time and after a certain period the voltammetric signals are not enhanced and remain fixed because the biomolecules fully covered the adsorption sites on the effective electroactive surface area that is limited for the particular electrode. Since, after formation of bioconjugate and their proper optimization, these can be further used for the electrochemical sensing of the target antigen.

Unique properties of AuNPs: AuNPs have unique chemical (e.g., its electronic configuration represented by [Xe] $4f^{14}$ $5d^{10}$ $6s^1$ and it is least chemically active element as compare to the bulk gold etc.) and physical properties (e.g., melting point, fluorescence, malleability, electrical conductivity, magnetic permeability etc.). Gold in the bulk shows the golden colour but there is drastic change in its colour when the size is

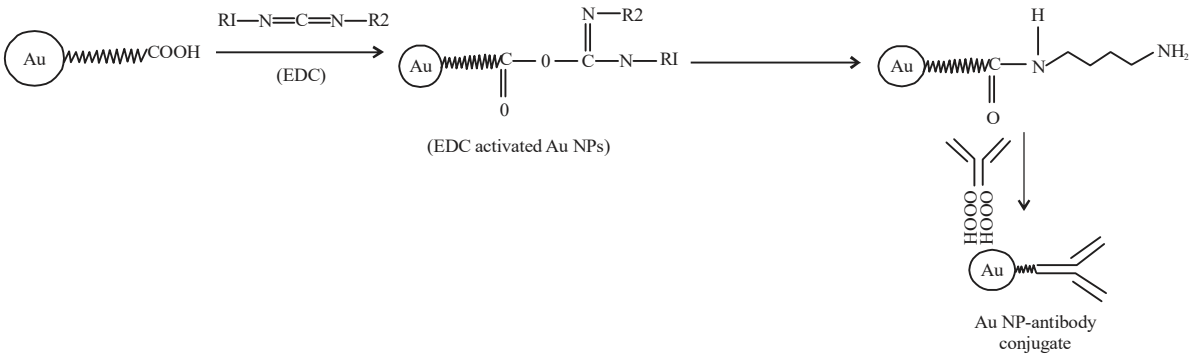

Fig. 5: Formation of AuNPs antibody conjugates

reduced to nanolevel. One can easily determine the size of the spherical shape AuNPs in their colloidal solution by knowing the value of λ_{max} in the UV-Vis spectrum. It is reported in the literature that the λ_{max} of the 5-10 nm size AuNPs is around 515-520 nm. When the size of AuNPs is increased the λ_{max} value for the maximum adsorption is also increased. The λ_{max} value is 524, 526, 530, 535, 540, 553, 572 nm for the size of AuNPs 20, 30, 40, 50, 60 and 80 nm, respectively[31]. Surface Plasmon Resonance (SPR) is found in AuNPs which is induced resonance between the electromagnetic radiation and the excited plasmon at the nanoparticles surface. These oscillations are called SPR and it gives strong optical absorbance in the visible region. This phenomenon can be measured by UV-Vis spectrometer. The intensity and wavelength of the SPR band is mainly depend on the size and shape of the nanoparticles. AuNPs have unique chemical and physical properties and due to the size effect these particles show various potential interests in applications of physics, chemistry, biology, medicine and material science.

Role of AuNPs in electrochemical immunosensors: To enhance the sensitivity, AuNPs are extensively used for the detection of various analytes in the electrochemical immunosensing strategy because of their high conductivity, high affinity, good biocompatibility and excellent electroactivity. AuNPs in combination with graphene, MWCNTs and biopolymers allows the large number of antibodies to capture onto the electrode surface as well as promotes the electron transfer rate to the electrode. Now these days, to improve the synergistic properties hybrid electrodes are used based on AuNPs in association with other compounds like calcium carbonate, amino-functionalized cuprous oxide and ceric dioxide (Cu_2O at CeO_2-NH_2), carbon nanospheres and silicon oxides to increase the sensitivity of analytical electrochemical detection with high efficiency[40-43]. In the construction of competent electrochemical immunosensors, colloidal gold have been used as electroactive nano carriers and nano tracers along with functional electrodes[44]. In this section, bioconjugation of antibodies with AuNPs, use of AuNPs as electroactive labels or signal tags for generating the electrochemical signals which can also be used to increase the intensity of the peaks and modification of the various types of electrode surfaces in various immunosensing schemes are discussed.

Bioconjugation with AuNPs: AuNPs exhibit excellent affinity to bind with various biomolecules such as antibodies, plasmids, aptamers, peptides, DNA, RNA etc., because of their surface can be easily modified by various cross-linkers and after immobilization the AuNPs retain the good electrical properties, high surface area to volume ratio and high electrocatalytic activities. These biomolecules can also directly attach with the AuNPs. In a published report, glutathione coated AuNPs were used for the preparation of conjugate with antibodies using N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and diaminohexane (DAH)[45]. Figure 5 illustrates the formation of AuNPs-antibody conjugates.

The antibodies attach with the surface of AuNPs can be distinguished by colour change that can be visually observed in their Surface Plasmon Resonance (SPR) band as shown in Fig. 6. SPR phenomenon of AuNPs bound to antibodies or targeted sites leading to color[27]. Typically, in case of AuNPs Surface Plasmon Resonance (SPR) is occurred when light wave is incident on the particles surface. SPR technique is widely used in the application of disease diagnosis, pharmaceuticals, therapy, enzyme-substrate interaction, detection of toxins, DNA hybridization, antigen-antibody interaction, identification of protein conformation and label free immunoassays.

Particularly in electrochemical immunosensors, AuNPs are having good biocompatibility and bioconjugation properties. These properties make Au NPs one of the most

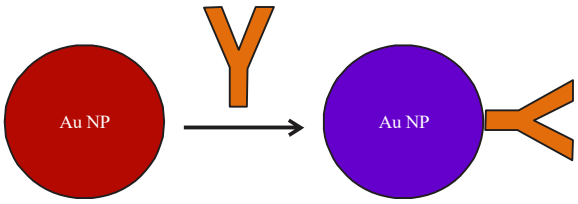

Fig. 6: Color change of AuNPs when bind with antibodies (SPR-effect)

suitable candidates for the immobilization of various biomolecule. Numerous electrochemical immunosensors have been developed earlier in which the antibodies are directly conjugated with AuNPs. After conjugation of biomolecules on AuNPs surface their bioactivity do not be effected and also improve their self life and to provide more stability[46]. Antibodies assemble near the sides of catalytic AuNPs for immobilization purpose and directly bind to it with the covalent bond without affecting the function, structure, orientation and shape of the antibody. When the surface of AuNPs is modified by thiol ended molecules makes them suitable to immobilize large number of biomolecules. Electrostatic bioconjugation is also possible because at a particular pH some biomolecules may have the positive charge. Since, they can easily bind via electrostatic attractive forces with the negatively charged AuNPs. To improve the sensitivity AuNPs can be associated with graphene sheets and carbon nanotubes for signal amplification in the electrochemical detection. Conjugated AuNPs are excellent nanobiomaterials which can be explored to new horizons of immunosensing strategies.

AuNPs as signal tags: In the field of electrochemical immunosensing, AuNPs is the most widely used nanoparticles as signal tags[47]. A novel potentiometric immunosensor for hepatitis B surface antigen (HBsAg) has been developed with a detection limit of 1.3 ng mL^{-1} using AuNPs-based biomolecular immobilization method[48]. Au-enhanced antibody immobilization and antibody-antigen reactions were used for the detection[49-51] of Hepatitis B virus surface antigen with a limit of 50 ng L^{-1}. Some of the latest advances in the detection of antigen are discussed by using AuNPs and biomolecules assemblies for the immunosensor development. AuNPs were used as labels for the electrochemical sensing of target c-Myc oncoprotein (C_{Ag}) antigen[50]. Using this signal enhancement strategy, C_{Ag} can be detected with a limit of 1.5 pmol L^{-1}. In another study, silver-enhanced colloidal gold electrochemical stripping technique and gold nanoclusters (AuNCs) labels were used for the detection of human IgG by Anodic Stripping Voltammetry (ASV) with the detection[51] limit of 0.5 pg mL^{-1}. For this purpose, gold nanocrystals tagged secondary antibodies were used as detection antibodies in the sandwiched immunoassay. This is a direct, rapid and simple approach of detection without using multiple separations and labeling steps.

In this study, the authors synthesized colloidal AuNPs having a large surface area to increase the immobilization of large amount of antigen on their surface and then used to monitor the impedance change during the antibody-antigen interaction. Polyamidoamine (PAMAM) dendrimers coated fullerene (C_{60}) and (AuNPs) labeled with detection antibody were used for alpha fetoprotein (AFP) detection via a sandwich-type immunoreactions with a detection[52] limit of 0.03 pg mL^{-1}. Interferon-gamma (IFN-γ) is main cause of mycobacterium tuberculosis (MTB) infection which has been detected by using novel signal tags of horseradish peroxidase (HRP)-labeled antibody-conjugated AuNPs (HRP-Ab2-AuNP) bioconjugates and under optimized conditions 0.048 pg mL^{-1} detection limit was achieved[53]. This electrochemical immunosensor is useful in early diagnosis and control of tuberculosis.

Electrochemical detection of Mycobacterium lipoarabinomannan antibody on a disposable chip i.e., Screen Printed Carbon Electrode (SPCE) was developed in a study using gold nanoparticles (AuNPs) labeled Staphylococcal protein A (Au-SPA) as the electrochemical signal tags and under optimized conditions 5.3 ng mL^{-1} detection limit for mycobacterium lipoarabinomannan antibody was achieved[54]. This strategy has good performance, simplicity, sensitivity, selectivity, stability, easy rapid operation and also very much promising tool that can be used in early diagnosis of tuberculosis. Gold nanoparticles can be easily oxidized in presence of dilute HCl to give $AuCl_4^-$ and the obtained acid dissolved gold ions (Au^{3+}) gives the oxidation peak current at +0.3V using Differential Pulse Voltammetry (DPV) technique from +0.6-0.0 V, with a step potential of 4 mV, a pulse amplitude of 50 mV and a pulse period of 0.2 sec because electro oxidation of AuNPs occurs at constant high peroxidation potential i.e., +1.3 V for 30 sec. The value peroxidation potential was also optimized because at higher values of peroxidation potential i.e., more than +1.3 V may cause to damage the surface of SPCE, decrease the electrochemical signal, poor reproducibility, decrease peak current and repeatability is also adversely affected. Redundant time (30 sec) is also optimized that is sufficient for complete oxidation of AuNPs more the value of redundant time would

Table 3: Popular research articles using AuNPs and their nanocomposites as signal tags or electroactive labels

Type of signal tags or labels of gold nanocomposites	Antigen or target analyte	Limit of detection
AuNPs	c-Myc oncoprotein (C_{Ag}) antigen	1.5 pmol L^{-1}
Au Nanoclusters (NCs)	Human IgG	0.5 pg mL^{-1}
AuNPs	Hepatitis B surface antigen (HBsAg)	1.3 ng mL^{-1}
AuNPs	Hepatitis B virus surface antigen	50 ng L^{-1}
Polyamidoamine (PAMAM) dendrimers coated fullerene (C_{60}) and AuNPs labels	Alpha fetoprotein (AFP)	0.03 pg mL^{-1}
Horseradish peroxidase (HRP)-labeled antibody-conjugated AuNPs	Interferon-gamma (IFN-γ)	0.048 pg mL^{-1}
AuNPs labeled Staphylococcal protein A	Mycobacterium lipoarabinomannan antibody	5.3 ng mL^{-1}
Double-codified AuNPs	HBsAg	10 pg mL^{-1}
Ionic liquid AuNPs-graphene nanosheets	Human apurinic/apyrimidinic endonuclease 1	0.04 pg mL^{-1}
AuNPs	Nuclear matrix protein 22	3 pg mL^{-1}
Poly (o-phenylenediamine) AuNPs	Carcinoembryonic antigen (CEA)	5.0 pg mL^{-1}
Sodium nano-ontmorillonite-polyaniline-Au	Squamous cell carcinoma antigen	0.3 pg mL^{-1}
Single domain antibody-conjugated AuNPs	Clostridium difficile toxin A	0.61 pg mL^{-1}
	Clostridium difficile toxin B	0.60 pg mL^{-1}
AuNPs-horseradish peroxidase	Pantoea stewartii subsp. stewartii-NCPPB44	7.8×10^3 CF U mL^{-1}
AuNPs-hybrid graphene nanocomposites	Salbutamol	40 pg mL^{-1}
Functionalized Au nanorods	Ofloxacin	30 pg mL^{-1}
Prussian blue-Au hybrid nanostructures	Alpha-fetoprotein (AFP)	40 pg mL^{-1}
Glucose oxidase (GOD)-Au and Ag mesoporous NPs	CEA	1.0 pg mL^{-1}
Mesoporous carbon foam (MCF)-AuNPs	CEA	0.024 pg mL^{-1}
AuNPs-graphene nanocomposites	CEA	0.12 pg mL^{-1}

LOD: Limit of detection, CEA: Carcinoembryonic antigen

not increase the value of electrochemical current signal and an insufficient time may cause incomplete oxidation of AuNPs. Hence, in the reported strategy, +1.3V preoxidation potential and 30 sec preoxidation time was optimized to achieve the high detection sensitivity and rapid response[54,55]. Double-codified AuNPs were utilized as signal tags for the detection of hepatitis B surface antigen (HBsAg) using a conductometric immunosensor with limit of detection or detection limit (LOD) obtained[56] at 10 pg mL^{-1}. Ionic liquid AuNPs-graphene nanosheets were used as labels for the detection of human apurinic/apyrimidinic endonuclease 1 at LOD of 0.04 pg mL^{-1} using electrochemical amperometric technique[57].

Poly (o-phenylenediamine) AuNPs were used as labels for the detection of carcinoembryonic antigen (CEA) at LOD of 5.0 pg mL^{-1} using DPV-technique[58]. AuNPs were used as signal tags for the detection of nuclear matrix protein 22 at the detection limit[59] of 3 pg mL^{-1}. Sodium nano-montmorillonite-polyaniline-AuNPs were used as signal tags for the detection of squamous cell carcinoma antigen at LOD of 0.3 pg mL^{-1} using amperometric technique[59]. Single domain antibody-conjugated AuNPs were used as signal tags for the detection of Clostridium difficile toxin A and Clostridium difficile toxin B at LOD of 0.61 and 0.60 pg mL^{-1} using impedmetry[60]. AuNPs-horseradish peroxidase were used as signal probes for the detection of Pantoea stewartii subsp. stewartii-NCPPB44 at LOD of 7.8×10^3 CFU mL^{-1} using linear sweep voltammetry[61]. AuNPs-hybrid graphene nanocomposites were used as labels for the detection of Salbutamol at LOD of 40 pg mL^{-1} using amperometric technique[62].

Functionalized Au nanorods were used as labels for the detection of Ofloxacin at LOD of 30 pg mL^{-1} using cyclic voltammetric technique[63]. Prussian blue-Au hybrid nanostructures were used as labels for the detection of alpha-fetoprotein (AFP) at LOD of 40 pg mL^{-1} using linear sweep voltammetry[64,65]. Glucose oxidase (GOD)-Au and Ag mesoporous NPs were used as nanocatalytic labels for the DPV based detection of CEA at the LOD[66] of 1.0 pg mL^{-1}. Mesoporous Carbon Foam (MCF)-AuNPs were used in a published article as a nanocarrier and nanocatalyst for the detection of CEA at the LOD of 0.024 pg mL^{-1} using stripping DPV method[67]. AuNPs graphene nanocomposites were used as electroactive tracers for the detection of CEA at the LOD of 0.12 pg mL^{-1} using anodic stripping triple signal amplification strategy[68].

Multistep-enhancement strategies were used for the detection of Prostate Specific Antigen (PSA) using the nanomaterials of AuNPs-modified Prussian blue onion like mesoporous graphene sheets and AuNPs modified nickel hexacyanoferrate NPs-decorated onion like mesoporous graphene nanosheets at the LOD of 7 pg mL^{-1} and 3 pg mL^{-1} respectively[69]. Gold nanomaterials are excellent signal generators who can replace the enzymes in the sandwich immunoassays based protocols. These nanocarriers can immobilize detection antibodies and glucose oxidase (GOD) in a study reported by Cheng *et al.*[65]. The glucose oxidase (GOD) is responsible for the catalytic deposition of AuNPs on to the Au nanorods. This scheme was suitable for the detection of carcinoembryonic antigen (CEA) at the LOD of

4.2 pg mL^{-1} using stripping by DPV. All of these published works based on AuNPs and their nanocomposites as signal tags or labels are summarized in the Table 3. In summary, for accurate determination of antigens, AuNPs labeled antibody-based electrochemical immunosensors provides high sensitivity, selectivity and reproducibility, low-cost and miniaturize sensing platforms.

Use of AuNPs for electrode modification: In this section, some of the recent advances in electrode modification using AuNPs are presented. Generally, AuNPs are used to modify the surface of the electrodes. Different bifunctional crosslinking reagents like 1,6-hexanedithiol[70,71], 4-thiophenol[72] and thiourea[73] etc. are used as mediator for the attachment of AuNPs on to the surface of gold electrode. The fabrication of electrochemical immunosensors based on AuNPs modified electrode is a typical procedure in which the AuNPs are firstly attached on the electrode surface and then capturing antibodies are immobilized on the modified electrode. The capturing antibodies immobilized on the electrode surface are randomly oriented.

However, the AuNPs provide large surface area for the immobilization of capturing antibodies which can easily bind with the target antigen in higher concentrations to give the lower limit of detection. Numerous strategies have been reported for the attachment of capturing antibodies onto the surface of AuNPs which including, MSA[75], glutathione (GSH) as a spacer arm[74] physical adsorption[75,76]. Other researcher found to conjugate the AuNPs with the primary capturing antibodies and then subjected this bionanoconjugate to the electrode surface for immobilization by the physical adsorption. In the preparation of this bionanoconjugate sometimes carbon nanotubes (CNTs) and graphene can be also used to improve the sensitivity[9].

To enhance the signal AuNPs based electrochemical immunosensors, some scientists were focused on the possible arrangements of AuNPs on the electrode surface. Rusling's group reported that response is improved when using the densely packed AuNPs for the fabrication of electrochemical immunosensors[74]. Chen *et al.*[75] reported direct electrode position of 3D ordered gold nano-prickle clusters on GCE utilizing the spatial obstruction/direction of the polycarbonate membrane. Ou *et al.*[76] fabricated a label-free amperometric immunosensor using mercaptopropane sulfonic modified gold electrode surface based on covalently bound posterior anti-CEA via layer-by-layer (LbL) assembly of Au NPs, multi-walled carbon nanotubes-thionine (MWCNT-Thi) and chitosan (Chit), $\{AuNPs/MWCNT\text{-}Thi/Chit\}_n$. Different current response is observed in case of absence and presence of the antigen. Immunocomplex is formed and the immunoreactions are possible only in the presence of antigen. The electron-transfer mechanism is inhibited due to the formation of the antigen-antibody immunocomplex. Since, the amperometric current signal is decreased with increasing the concentration of antigen and the amount of antigen captured can be easily quantified.

AuNPs have a special role in providing the stable immobilization of biomolecules to retain their bioactivity as well as high selectivity, stability, reliability and low detection limits are achieved. In another work, nano-Fe_3O_4 modified carbon paste electrodes were used to detect CEA by an amperometric immunosensor. The Fe_3O_4 nanoparticles show the magnetic behavior and their surface is modified with (3-mercaptopropyl) trimethoxysilane in order to attach the AuNPs with biomolecules[77]. To improve the electrochemical signal, AuNPs provide a high superficial biocompatible platform for CEA immobilization. In this aspect, the AuNPs work like a mediator for the direct electron transfer between redox biomolecules and electrode surfaces which make this process more effective and efficient.

In one of the most recent works describes a simple mixing of gold colloidal nanoparticles and carbon paste, followed by the adsorption of glucose oxidase[78] or HRP[79] which provides fast electron transfer between the electrode and biomolecules. In another study AuNPs were linked via sulfur bond with the gold electrode, then a biocompatible cystamine monolayer was chemisorbed onto AuNPs surface and glucose oxidase could be covalently attached to the gold electrode[80]. This biosensing interface provides the stability for more than 30 days. Table 4 describes all of the popular and recently published electrochemical immunosensing reports based on AuNPs and their nanocomposites modified various electrodes for the sensitive detection of various target analytes or antigens.

Nano gold-chitosan film modified Glassy Carbon Electrode (GCE) was used for the LSV based detection[81-84] of AFP at the LOD of 40 pg mL^{-1}. Electrodeposited nano gold on GCE was used for the DPV based detection[85] of CEA at the LOD of 0.5 pg mL^{-1}. AuNPs deposited on polydopamine film modified electrode surface was used for the DPV based detection[86] of CEA at the LOD of 1.0 pg mL^{-1}. AuNPs-multi walled carbon nanotubes (MWCNTs)-chitosan modified electrode surface was used for the amperometric detection[87] of ricin at the LOD of 2100 pg mL^{-1}. AuNPs mercapto-functionalized graphene sheet modified electrode was used for the amperometric detection[88] of squamous

Table 4: Selected examples of use of AuNPs and their nanocomposites for electrode modification

Materials used for electrode modification	Antigen or target analyte	Limit of detection
Graphene-chitosan-gold nanoparticles-capturing antibodies	Staphylococcal enterotoxin B (SEB)	5.0 ng mL^{-1}
Multi-walled carbon nanotubes-chitosan-gold nanoparticles-capturing antibody		10 ng mL^{-1}
Three-dimensional (3D) gold (Au) nano-prickle clusters	Picloram	0.0021 μg mL^{-1}
Layer-By-Layer (LBL) assembly of AuNPs, multi-walled carbon nanotubes-thionine and chitosan on 3-mercaptopropane sulfonic, sodium salt-modified gold electrode surface	CEA	0.01 ng mL^{-1}
AuNPs-(3-mercaptopropyl) trimethoxysilane/nano-Fe_3O_4	Carcinoembryonic antigen	0.13 ng mL^{-1}
Glucose oxidase (GOD)-colloidal AuNPs	Glucose	0.01 mM
Glucose oxidase (GOD)-AuNPs monolayer modified gold electrode	Glucose	8.2 μM
Nano gold-chitosan	AFP	40 pg mL^{-1}
Electrodeposited AuNPs	CEA	0.5 pg mL^{-1}
Polydopamine-AuNPs	CEA	1.0 pg mL^{-1}
AuNPs-MWCNTs-chitosan	Ricin	2100 pg mL^{-1}
AuNPs mercapto-functionalized graphene sheet	Squamous cell carcinoma antigen	0.18 pg mL^{-1}
AuNPs-graphene-chitosan	CEA	20 pg mL^{-1}
Polypyrrole film-Au nanoclusters	Ofloxacin	30 pg mL^{-1}
AuNPs modified planar gold electrode	Mouse IgG	200 pg mL^{-1}
Polythionine (PTH)-AuNPs	Aflatoxin B1 (AFB1)	0.07 ng mL^{-1}
Protein A tagged AuNPs human serum antibodies	Japanese encephalitis virus (JEV)	------
AuNPs-Colloidal carbon sphere	Human IgG	1.8 ng mL^{-1}
OTA-BSA-AuNPs	Mycotoxin ochratoxin A (OTA)	0.20 ng mL^{-1}
Polythionine-Au nanocomposites	Carbohydrate antigen 19-9	0.26 U mL^{-1}
PDDA-functionalized Graphene-AuNPs	Inflammatory Cytokine Interleukin-22	0.5 pg mL^{-1}
Toluidine blue-AuNPs-Fe_3O_4 NPs-Graphene nanocomposites	Alpha Fetoprotein	2.7 fg mL^{-1}

LOD: Limit of detection, CEA: Carcinoembryonic antigen

Table 5: Various applications of AuNPs

Fields/Areas	Expected applications
Sensors	AuNPs are widely used in the field of electrochemical immunosensors for the detection of various pathogens, toxins, proteins, environmental pollutants etc
Electronics	In electronic devices, conductors, electronic components, electronic chip design these particles are used to make the world of electronics smart and portable which is termed as nanoelectronics
Probes	AuNPs are currently used for biological imaging, dark-field microscopy and also helpful as probes for transmission electron microscopy
Photodynamic therapy	AuNPs exhibit excellent therapeutic effect and can be used to kill tumor cells. In photodynamic therapy, the targeted tumors can be destroyed by light waves containing AuNPs in the selected region. Since, the tumor cells are easily burn when excited them at wavelength from 700-800 nm because AuNPs absorb light and produce heat. This treatment is also called hyperthermia therapy
Diagnostics	AuNPs are used in clinical and medical diagnosis, lateral flow immunoassays, home pregnancy test etc
Therapeutic agent delivery	AuNPs having size in the range of 120-140 nm can be used in the therapeutic and imaging purpose
Fluorescence properties	In the presence of light, AuNPs show anti-photo bleaching effect. Fluorescence Linked Immunosorbent Assay (FLISA) based techniques are used in the sensitive detection of various analysis
Catalysis	AuNPs are excellent electro catalyst used to catalyze various chemical reactions. In fuel cells, display, automotive industry etc. Au NPs are widely used

cell carcinoma antigen at the LOD of 0.18 pg mL^{-1}. AuNPs-graphene-chitosan modified electrode surface was used for the Cyclic Voltammetric (CV) based detection[89] of CEA at the LOD of 20 pg mL^{-1}.

Polypyrrole film-Au nanoclusters modified electrode surface was used for the detection of Ofloxacin at the LOD of 30 pg mL^{-1} using CV-technique[63]. AuNPs modified planar gold electrode was used for the potentiometric detection[90] of mouse IgG at the LOD of 200 pg mL^{-1}. In another strategy, AuNPs were attached to hollow porous thiol-functionalized polymeric nanospheres providing an active matrix for further immobilization of HRP[81]. Sol-gel science is used to prepare 3D network for the encapsulation of AuNPs and enzymes in order to improve the spatial arrangement and rate of electron transfer[82,91]. AuNPs based electrochemical immunosensors are also used in agriculture field and food analysis because of their high sensitivity. An interesting work describes in the literature for the detection of herbicides, pesticides and the detection of *Escherichia coli* in milk samples[83,92-94]. In this work, disposable and cheaper SPE was used and its surface was modified with AuNPs in order to achieve the high sensitivity and excellent electrochemical behavior.

Application of AuNPs and future trends: In this section, various applications of AuNPs in various regions have been addressed. Au NPs play a very important role for the

development of nanoscience and nanotechnology because of their good optical, electronic, magnetic, catalytic and biomedical applications. These properties depend on the size, shape and morphology of the gold nanomaterials which can be easily tuned by using large number of synthetic methods. AuNPs are specially used in the field of electrochemical immunosensors, environmental and clinical applications because of their good biocompatibility and excellent physical-chemical properties. Since, the future of AuNPs is very bright in the application of point of care testing and disease diagnosis and with high sensitivity and low detection limits. Table 5 gives the brief information about the various applications of AuNPs in various fields.

Ultrapure AuNPs-DNA assemblies are very much promising for future biomolecular applications and manipulations such as detection, labeling and delivery of drugs, including transfer of genetic materials. In these days, AuNPs have drawn considerable attention of researchers to look their use in the field of nanomedicine, nanocardiology and neuroscience. Further studies are based on the mechanism of cardioprotective effects of AuNPs. Various factors will be studied for delivering drugs and testing are going on different animal models with relation to the age of the animals. Furthermore studies are required to clear the molecular mechanism and to develop new methods of diagnosis and treatment of heart related diseases that will be cheaper and efficient comparatively to the existing tools. AuNPs are truly revolutionized and will definitely serve as potential candidates for future innovation and development.

Since AuNPs possess unique, beneficial chemical, physical and mechanical properties, they can be used for a wide variety of applications. These applications include: next-generation computer chips, better insulation materials, phosphors for high-definition TV, low-cost flat-panel displays, elimination of pollutants, high energy density batteries, high-power magnets, high-sensitivity sensors, automobiles with greater fuel efficiency, aerospace components with enhanced performance characteristics, better and future weapons platforms, longer lasting satellites, large electrochromic display devices etc. AuNPs offer some attractive possibilities in the field of biomedicine and bioanalysis. They have controllable sizes ranging from a few nanometers up to hundreds of nanometers, which place them at dimensions that are smaller than or comparable to those of a cell (10-100 μm), a virus (20-450 nm), a protein (5-50 nm) or a gene (2 nm wide and 10-100 nm long)[91]. This means that they can get close to a biological entity of interest and allows an integration of nanotechnology and biotechnology.

In addition to the advantage in their attractive size, special physicochemical properties of AuNPs that are very different from those of bulk materials accelerate their bioanalytical and medical applications such as multiplexed bioassays, drug delivery, biomedicine, clinical diagnostics, ultra-sensitive biodetection and bioimaging. AuNPs are very attractive and most widely used nanomaterials for various biological applications including nanobiotechnology, genomics, bionanomedicine, immunoassays, biophotonics and clinical chemistry because of its unique physical and chemical properties. Lot of study has already been done on *in vitro* diagnostics and in the next 5 years, intensive research activity will be mainly focused on the *in vivo* uses of AuNPs is imagined. In future, various studies such as specific detection, cancer, imaging etc., will be performed as a function of AuNPs size, shape and surface coating on various animal models before subjected to human beings. The feasibility of constructing immunosensors based on AuNPs with electrochemical approach presents a very promising avenue for future research.

CONCLUSION

The future of electrochemical immunosensor significantly affected by AuNPs, which opens the new horizons to improve the sensitivity and performance of immunosensors. Since, multi-analyte detection, lab-on-a-chip, point of care diagnosis, treatment and prevention of diseases, health-care assessment and many more applications are expected in this field. AuNPs-based electrochemical immunoassay is easy, faster and cost-effective that can be used for the detection of various analytes in the field.

SIGNIFICANCE STATEMENT

This study discovers that AuNPs are suitable candidates with unique structural, electronic, magnetic, optical and catalytic properties shows biocompatibility that can be beneficial for biosensor and chemical sensor. In the developing countries, more and more research is required in the field of immunosensors that will greatly improve the present scenario of health caring situation and more efforts should be dedicated to cure the infections and autoimmune diseases. Moreover, excellent results can be obtained with high recoveries and reproducibility in real sample analysis using AuNPs based immunosensors. AuNPs in combination with other bionanomaterials are excellent composites/ nanohybrids in point-of-care diagnosis and cost-effective ultrasensitive devices for practical usage. It is hoped that the

AuNPs are one of the most attractive nanomaterials and one of the most studied in the electro bioanalytical field of research.

This study will help the researcher to uncover the critical areas of fundamental and practical highlights to understand the behavior of Au NPs in biological systems as well as their engineering related to the currently developed electrochemical immunosensors that many researchers were not able to explore. Thus a new theory on electrochemical techniques are very much promising to achieve such type of useful results and more efforts are required to reach the goal which makes this technique more suitable for point-of-care detection, prevention and treatment of many illnesses, including infectious and autoimmune diseases may be arrived at.

ACKNOWLEDGMENT

The author would like to thanks the Trends in Applied Sciences Research for publishing this article FREE of cost and to Karim Foundation for bearing the cost of article production, hosting as well as liaison with abstracting & indexing services, and customer services.

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