



Review

New analytical applications of gold nanoparticles as label in antibody based sensors



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ARTICLE INFO

Article history:

Received 23 September 2012

Received in revised form

20 December 2012

Accepted 20 December 2012

Available online 3 January 2013

Keywords:

Gold nanoparticles

Label

Immunosensor

Antibody

ABSTRACT

Gold nanoparticles (AuNPs) with optical and electrochemical distinctiveness as well as biocompatibility characteristics have proven to be powerful tools in nanomedicinal application. This review article discusses recent advances in the application of AuNPs as label in bioanalytical devices, especially electrochemical immunosensors, rapid and point-of-care (PoC) tests. A crucial assessment regarding implementation of different formats of antibodies allowing rapid and sensitive analysis of a range of analytes is also provided in this study. In addition to this, different approaches to minimize antibodies into Fab, scFv or even single-domain antibody fragments like VHHs will be reviewed. Given the high level of target specificity and affinity, such biomolecules are considered to be excellent elements for on-site or PoC analysis.

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Abbreviations: ASV, anodic stripping voltammetry; BSA, bovine serum albumin; CDR, complementarity determining regions; CPE, carbon paste electrode; DPV, differential pulse voltammetry; ELISA, enzyme-linked immunosorbent assay; ENO1, α -enolase; Fab, fragment antigen-binding; Fc region, fragment crystallizable region; GCE, glassy carbon electrode; HCABs, heavy chain antibodies; hCG, human chorionic gonadotrophin; HER, hydrogen evolution reaction; HRP, horseradish peroxidase; HSA, human serum albumin; ITO, indium tin oxide; LoC, lab-on-a-chip; MCM, mobile crystalline material; PoC, point of care; PVA, poly vinyl alcohol; SPCSs, screen printed carbon strips; SPE, screen printed electrode; SWSV, square wave stripping voltammetry; TEM, transmission electron microscopy; VHHs, camelidae single-domain antibodies

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1. Introduction

Nowadays, biosensors are considered as supreme instruments, available in health care system, particularly for diagnosis and monitoring of disease.

The regular assessment of analytes in biological specimens is required to demonstrate the metabolic states of patients, particularly for those who have been admitted to intensive care (Choi et al., 2011; Omidfar et al., 2012a).

A large number of significant technological advances have been made in detection of biomolecules using principles of nanotechnology over the past decade. The biorecognition events have been successfully monitored on solid devices and in solution by means of different analytical techniques, such as optical and electrochemical methods.

Coupled with the development of such techniques, a great deal of effort has been devoted to realizing the robust, sensitive, selective and practical biosensing instrumentations as point of care (PoC) diagnostic tests in laboratories.

Biological samples have been usually taken to a medical laboratory for classical assessment of analytes. However, this would be accompanied with delay constraints of analysis, as most cases may not be completed for hours or even for days.

On-site or PoC biosensors, a kind of device independent of sophisticated instrumentation, are sensitive, rapid, specific, cheap, and easy to interpret at the same time. The possibility of achieving results in minutes through such on-the-spot diagnostic devices makes them ideal alternatives in emergencies (von Lode, 2005; Warsinke, 2009).

Advances in analytical technology and in the healthcare services have been focused on portable and reusable systems and most importantly on miniaturized devices. A near-patient testing instead of testing provided in the traditional environment of clinical laboratory can be feasible through such improvement.

Producing a prompt result is the main objective of PoC testing, by which suitable treatment approach can be applied in either the clinical or economic outcomes (Holford et al., 2012; Shetty et al., 2011).

Nanoparticles (NPs) with different characteristics are among particular nanomaterials extensively deployed in various kinds of analytical techniques.

Unique structural, electronic, magnetic, optical, catalytic and biocompatible properties can be attributed to their nanoscale dimension roughly in the range of 1–100 nm. Such splendid properties have specifically made them very attractive material, which can be used as a label for detecting analytes through colorimetric or diverse electrochemical techniques (Chen et al., 2008; Liu and Lin, 2007; Luo et al., 2006; Rusling, 2012; Seydack, 2005; Tansil and Gao, 2006).

Metal NPs have been known since ancient times. However, the systematic study on gold nanoparticles (AuNPs), known for the application in staining glass, was started with the pioneering work of Faraday on the color of colloidal gold in 1857 (Castañeda et al., 2007; Luo et al., 2006).

Compared with other metal NPs, AuNPs have found wide application in bioanalytical methods. Due to the high electron densities of AuNPs, they have been broadly used in immunocytochemistry, immunohistochemistry and immunoblotting for electron microscopy (EM) (Liu and Lin, 2007). Furthermore, the stability of AuNPs and their capability to combine with biomolecules can be regarded as other prominent properties of such particles.

The suspension of submicrometer-sized particles of gold in a solvent, usually water, is known as AuNPs. Based on the size of particles, different colors, commonly intense red (for particles smaller than 100 nm) or a dirty yellowish (for particles bigger

than 100 nm), is produced in AuNPs suspension. They are often generated in various shapes, including spheres, rods, cubes, triangles and ellipsoidal (Castañeda et al., 2007; Guo and Wang, 2007). Regarding size and properties, the particles are strongly dependent on the conditions in which they are prepared.

Given the outstanding characteristics of AuNPs and their potential applications in a wide variety of disciplines, synthesis of these particles has found a considerable interest in developing various kinds of detection methods, including optical, electrochemical and rapid PoC tests (Castañeda et al., 2007; Chen et al., 2008; Guo and Wang, 2007; Liu and Lin, 2007).

2. Synthesis and characterization

Several methods have been applied in order to synthesize well-defined AuNPs in solution with relatively narrow size distribution. The synthesis of AuNPs was reported firstly by Faraday in 1857. Afterwards, many different techniques have been developed to synthesize AuNPs. One of the most common approaches for the preparation of AuNPs in aqueous solution was introduced by Turkevich (Turkevich and Kim, 1970; Turkevich et al., 1951), which was subsequently improved by Frens (1973). In order to synthesize 10–100 nm AuNPs, generally, the tetrachloroauric acid (HAuCl_4) is reduced in the presence of different concentrations of trisodium citrate as a reducing agent. Briefly, HAuCl_4 is dissolved in deionized water (0.01%, w/v HAuCl_4) and the obtained solution is brought to boil. Finally, an aliquot of 1% trisodium citrate is added to the boiling solution while stirring constantly. Once mixing occurs, the color of the solution changes gradually from light yellow to wine red. In other word, the sodium citrate, first, behaves as a reducing agent and later on, the negative citrate ions adsorbed around the AuNPs provide the surface charge, which can repel the particles and restrain the formation of any aggregation. As a result, the colloidal solution of AuNPs is formed through reduction of trivalent gold (Au^{3+}) to zero-valent gold (Au^0) (Castañeda et al., 2007; Daniel and Astruc, 2004; Guo and Wang, 2007). The strength of the observed color of solution is highly dependent on the quality as well as the size of the colloidal gold particles. The size of the particles is directly determined by the initial concentration of reagents or employing a surfactant, such as tannic acid. In order to prepare AuNPs with different sizes and shapes, several other methods have been employed. Reduction of metal ions is mostly performed by commonly used reducing agents, including sodium borohydride, sodium cyanoborohydride, thiocyanate, white phosphorous, sodium citrate, ascorbic acid, aspartic acid, hydrazine hydrate, hydroxylamine, aminodextran, and tannic acid. Addition of the reducing agent to a solution containing the metal salt results in reduction of metal ions and nucleation of metallic solid particles (Castañeda et al., 2007; Daniel and Astruc, 2004; Gole and Murphy, 2004; Guo and Wang, 2007; He et al., 2005; Tabrizi et al., 2009). By adding ionic substance, on the other hand, the attracting force overcomes the counteraction, which leads to an aggregation and a change in color from red ($\lambda_{\text{max}} \approx 520 \text{ nm}$, A 520) to blue (λ_{max} , A 580) (Hermanson et al., 1992).

By coating the colloidal surfaces of gold particles with macromolecules, such as protein or DNA as well as electron donor or ligands, including phosphines, amines or thiols with the capability of stabilizing the particles electrostatically or satirically, such unexpected instability can be avoided (Castañeda et al., 2007; Daniel and Astruc, 2004; Guo and Wang, 2007; Omidfar et al., 2010).

Synthesis of AuNPs (1–10 nm) at room temperature employing tannic acid as the reducing agent has been carried out by Sivaraman et al. (2010). Ultrasound as an important tool for the synthesis of metal NPs (Zhu et al., 2005) has been deployed for

production of AuNPs, where hydroxyl and sugar pyrolysis radicals were used as reducing agents (Zhang et al., 2006). A new method for preparation of colloidal solutions containing AuNPs of various sizes (5–80 nm) was introduced by Šlouf et al. (2006). This method used a combination of the already described techniques (Turkevich et al., 1951) basically applying sodium borohydride ($\text{Na}(\text{BH}_4)$) and hydroxylamine (NH_2OH) solutions in order to perform several step reduction of HAuCl_4 water solution.

The controlled formation of AuNPs through reduction of amine has been recently reported by Newman and Blanchard (2006). The transfer of electrons from the amine to the metal ion resulted in the reduction of HAuCl_4 and the formation of Au^0 , which was subsequently converted to AuNPs. To explore the parameter space of reaction conditions, a detailed study regarding the growth of AuNPs through reduction by citrate and ascorbic acid was conducted by Kimling et al. (2006). Following the results of preceding study developed by Turkevich and Frens, it has been found that AuNPs can be formed in a broad range of sizes (9–120 nm) with distinct size distribution (Frens, 1973; Turkevich et al., 1951).

In order to obtain information about structure, morphology, size, composition and the electrochemical behavior of AuNPs, several optical (spectroscopic, microscopic, etc.) or electrochemical techniques has been applied to characterize such synthesized NPs.

Analysis of AuNPs can be performed by both optical and electrochemical methods. The UV–vis spectrophotometer has been the first optical technique employed for the characterization of Au-NPs. In this method, the peak of the spectrum resulted from the surface plasmon resonance (SPR) of colloidal AuNPs was observed at ~ 520 nm (Omidfar et al., 2010).

Both techniques of electron microscopy, including scanning electronic microscopy (SEM) and transmission electron microscopy (TEM) can be utilized to determine the size of the NPs. TEM is widely used, as the reference technique, to characterize AuNPs. Nonetheless, other methods, such as scanning tunneling microscopy (STM), atomic force microscopy (AFM), X-ray powder diffractometry (XRD) and Fourier transformed infrared spectroscopy (FT-IR) have also found broad application in characterization of such NPs (Castañeda et al., 2007; Guo and Wang, 2007).

3. Conventional and heavy chain antibodies

There are numerous methods applicable to rapid and quantitative measurement of those clinically important analytes. Many of these methods are based on the combination of physical separation and sensitive detection. However, from among them, it is only immunoassay which has the capability for direct and specific detection of biological samples, including serum and plasma or non-invasive samples, such as urine, saliva, sweat or other fluids excreted from the body. It has been about three decades since immunoassay methods have been employed and still they are considered among the most significant diagnostic instruments with an extensive application in clinical and research areas (Byrne et al., 2009; Holford et al., 2012; Peruski and Peruski Jr., 2003; Saerens et al., 2008).

Such assays benefit from specific interaction between antibodies and antigens, where the antigen is characterized as a foreign molecule. Polyclonal, monoclonal and recombinant antibodies (Byrne et al., 2009; Kashanian et al., 2002; Omidfar et al., 2007; Omidfar and Shirvani, 2012; Paknejad et al., 2003; Saerens et al., 2008) are among the most frequently used antibodies for development of immunodiagnostic tests like enzyme immunoassays (Mohammadnejad et al., 2006), immunochromatography (Omidfar et al., 2010, 2011b, 2012a), and immunosensors (Omidfar et al., 2011a, 2012b).

Polyclonal antibodies (PABs) showing heterogeneous immunological response to antigen and various antibodies with different affinities can be made very simple against different binding sites (epitopes) on the antigen. Because of batch-related differences, dissimilar affinity and poly-specificity, the PABs are capable of creating serious problems, particularly while they are applied as a probe in biosensors (i.e. reactivity with more than one epitope) (Byrne et al., 2009; Omidfar and Shirvani, 2012; Saerens et al., 2008).

One of the preferences in immunoassays is the application of antibodies with uniform characteristics and high specificity, i.e., monoclonal antibodies (MABs) typically produced in mice (murine Abs) by using hybridoma technology; these antibodies have optimized characteristics, such as high affinity or high specificity. MABs can be selected to be more specific for a unique epitope presenting on the protein and hapten. Also, they can be reproducibly produced in unlimited quantities and used as affinity reagents to identify, quantify, localize, analyze their functions and determine the validity of analytes (Killard et al., 1995; Omidfar and Shirvani, 2012; Pandey, 2010; Roitt et al., 1985).

The fundamental structural unit of the conventional antibody is comprised of two identical heavy and light chains interconnected by disulfide bridges (Fig. 1a). The chains entirely composed of constant and variable regions, combining one interaction site for the antigen. The antigen-binding site of these antibodies contains six complementarity determining regions (CDR). The conformation of amino acids of antibody in the CDR of hyper variable regions, on both heavy and light chains, determines its antigen binding activity (Butler, 1991; Killard et al., 1995; Stryer, 1988).

Since considerable advances have been recently made in the field of genetic engineering techniques, the size of MABs has been minimized and new antibody-based structures (Fab, scFv, and sdAbs) against various antigens, such as proteins, haptens and carbohydrate moieties were produced by means of phage display technology (Byrne et al., 2009; Filpula, 2007; Holliger and Hudson, 2005; Muyldermans, 2001; Omidfar and Shirvani, 2012; Saerens et al., 2008).

One of the main capabilities of the mentioned technology is to isolate specific antibodies from available phage libraries; this can provide the possibility of the selection and amplification of phage clones with specific binding activities (Byrne et al., 2009; Muyldermans, 2001; Omidfar and Shirvani, 2012; Saerens et al., 2008; Davies and Riechmann, 1996; De Genst et al., 2004; Li et al., 2009).

The Fab fragment consisting of the Fd (VH and CH1 domain), the complete light chain (VL and CL domain) and half a heavy chain can be regarded as the well-constituted smaller engineered form of MAB (Fig. 1a). This fragment includes the Ag-binding domain without the Fc part as the effectors function fragment.

The next designed fragment, referred to as single-chain Fv (scFv), is a small fragment composed of two variable domains. A small flexible polypeptide serves as a linker between VH and VL domain, one from a light and the other one from a heavy chain. The scFv fragment is generally more stable than the Fv fragment, which leads to higher functionality (Fig. 1a). Currently, cloning and engineering of fragments, such as Fab and Fv have been well-accomplished in bacteria, yeast, fungi, and plants (Filpula, 2007; Holliger and Hudson, 2005; Omidfar and Shirvani, 2012).

The production of scFv has yet been accompanied with some practical restrains, such as dimerization and aggregation. Such confines can be attributed to the presence of an oligopeptide linker susceptible to proteolytic cleavage and consequent unfolding of the antibody construction. The smallest possible antigen binding antibody fragment from a MAB is composed of only one variable domain e.g. the VH or VL (Muyldermans, 2001; Muyldermans et al., 1994).

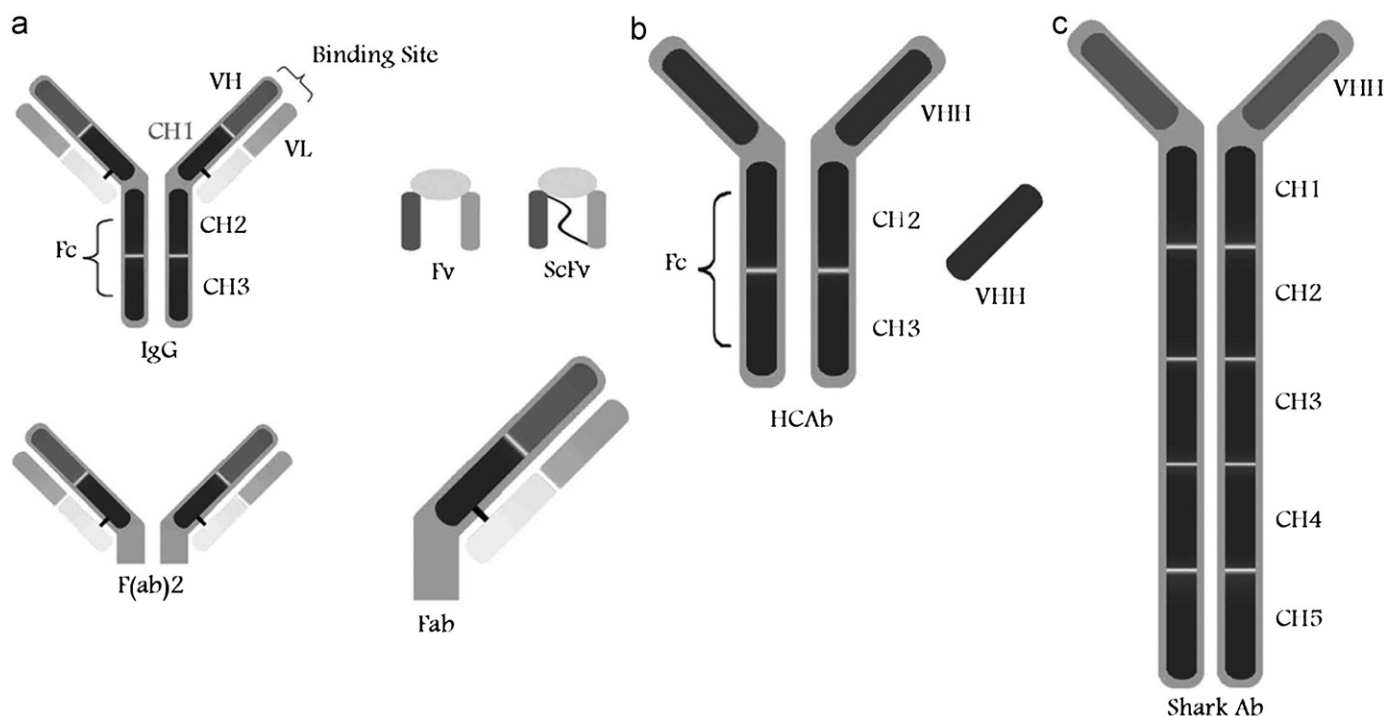


Fig. 1. Fragments from conventional antibodies (a), heavy-chain antibodies (b) and cartilaginous fish antibodies (c).

However, since these isolated single domains often expose large hydrophobic regions to the solvent, such fragments have a salient tendency to form aggregates. Serum of camelidae (camels, llamas, and alpacas) contains a substantial fraction of functional antibodies, known as heavy-chain antibodies (HCAbs), inherently devoid of light chains and first constant domain (CH1) (Fig. 1b) (Hamers-Casterman et al., 1993; Van der Linden et al., 1999).

The heavy-chain in the HCAbs encompasses three domains rather than having four globular domains. There are two constant domains with notable homology to the CH2-CH3 and Fc domains of classical antibodies. It is worthwhile to know that, HCAbs do not possess the domain corresponding to the CH1 domain of classical antibodies. Therefore, in the HCAb, the Ag-binding fragment of a classical antibody, the Fab, is diminished to a single variable domain referred to as VHH or nanobody. Lacking variable light-chain domain (VL), this domain is adapted to become functional in Ag-binding (Muyldermans, 2001; Nguyen et al., 2001; Omidfar and Shirvani, 2012).

It has been recently found that an alternate natural single domain antibody format can also be derived from the new antigen receptor antibodies (NARs) of the nurse sharks (*Ginglymostomacirraturum*). This new isotype is a heavy-chain homodimer alike the HCAbs in camelids. The immunoglobulin new antigen receptor (Ig-NAR) is a disulfide bonded homodimer of two identical H-chains lacking light chains. Every H-chain entails one variable (V-NAR) and five constant domains (Fig. 1c).

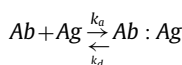
The camelid heavy-chain and shark Ig-NAR antibody fragments with decreased size, increased solubility and higher stability has become of special interest for biotechnological and medical applications, including biosensors (Bell et al., 2010; Greenberg et al., 1995; Muyldermans, 2001; Omidfar and Shirvani, 2012).

Biopanning is an affinity selection technique, by which binders is isolated from phage displayed antibody libraries. Selected binders are retained and subjected to additional screening to increase their specificity for the target (affinity maturation). This process can be monitored by enzyme-linked immunosorbent

assay (ELISA)-based analysis (Arbabi Ghahroudi et al., 1997; Deffar et al., 2010; Tillib, 2011; van der Linden et al., 2000).

Several non-covalent bonding, such as van der Waals interaction, non-polar hydrophobic interactions, London dispersion attractive forces; and steric repulsion forces contribute to create molecular interactions between the antibody-antigen.

This can be described with an association and a dissociation reaction rate constant k_a and k_d , respectively,



The association constant K_a , in the range of 10^5 – 10^{11} M⁻¹, can be depicted using the following equation:

$$K_a = \frac{k_a}{k_d} = \frac{[Ab : Ag]}{[Ab][Ag]}$$

where $[Ab]$, $[Ag]$ and $[Ab:Ag]$ respectively represent the concentrations of the antibody, antigen and complex in the solution (Butler, 1991).

4. Analytical applications of AuNPs as label in immunosensor

Accurate and sensitive detection of analytes is highly regarded as an important measure in preclinical and clinical research fields, including biomedicine and clinical diagnosis.

Considering the ability of nanomaterial to construct a sensitive and stable biosensor, they have been effectively employed for bioanalysis in general and immunosensing fields in particular. These benefits along with capacity of nanomaterials for improving cost optimality of biosensors, make them as one of the most interesting materials in development of biosensors.

Among numerous analytical methods developed for measuring the analytes, immunoassays play a significant role in the assessment of specific substances in biological fluids. Such assays, nowadays, have been widely applied in medical diagnosis, food

and consumer product safety as well as environmental analysis. However, developing new assays with high sensitivity and selectivity as well as low cost has been attracted much attention recently. Different substances, such as enzymes (horseradish peroxidase (HRP) (Du et al., 2003; Liu and Li, 2001), alkaline phosphatase (Duan and Meyerhoff, 1994), laccase (Kuznetsov et al., 2001) and glucose oxidase (Campanella et al., 1999)), nanomaterials, quantum dots, oligonucleotides, and dyes have been employed as a label for producing a detection signal (Liu and Lin, 2007; Rusling, 2012; Seydack, 2005).

Macromolecules labeling like metallic NPs conjugated to antibody plays an increasingly significant role in development of highly sensitive and specific bioassays, such as immunostrip and electrochemical immunosensors assays. Biomolecules which have been labeled with NPs are, not only capable of maintaining their bioactivity but also interacting with their counterparts. The amount or concentration of analytes can be detected based on the signal detection of such NPs. Metal NPs could be employed in both of immunosensors and DNA sensors, where AuNPs are the most often used labels among all currently available metal NPs (Castañeda et al., 2007; Liu and Lin, 2007; Omidfar et al., 2011a, 2011b, 2012a, 2012b).

The following provides a brief overview of the immunostrip assay, used in the measurement of interaction in several approaches. Turning over of immunosensors, special attention will be given to the electrochemical immunosensor, employing nanogold microsphere-labeled antibodies as electrochemical signal probes for detection of the analytes.

4.1. Immunostrip assay

By the late 1960s, the immunostrip assays or lateral flow assays or immunochromatography (ICG) assays were established, for the first time, and they were originally developed with the purpose of evaluating the existence of serum proteins (Kohn, 1968). In addition, the first home-made pregnancy strip assay was established in 1972 in order to determine the levels of human chorionic gonadotrophin (hCG) in urine specimen (Vaitukaitis et al., 1972). It is considered to be an ideal device for on-site detection

of target analytes in biological samples. In these kinds of immunoassays, the test sample flows along a solid substrate through capillary action. Taking approximately 10–15 min to run, immunostrip assays are regarded as rapid assays, which merely need several drops (~200 µl) of test agents diluted in a sample buffer to the test strip (Fig. 2) (Holford et al., 2012; Peruski and Peruski Jr., 2003).

During the past decades, a variety of immunostrip assays for detection of analytes in biological fluids have been reported. In a brand new way, the assay applies antigen–antibody interaction to provide a rapid detection of the analyte. Generally, colloidal gold has been employed as an immunospecific probe to develop one-step strip test. Since colloidal gold particles smaller than 15 nm were found to be too small for producing an intense color, usually nanocolloidal gold with a diameter of 20 nm was selected for developing immunostrip test. Moreover, colloidal gold particles larger than 60–70 nm self-aggregate after storage at 4 °C for several days (Gui et al., 2008; Omidfar et al., 2010; Shim et al., 2007). For conjugation, immunoglobulin is directly adsorbed on the surface of particles using non-covalent bonds, such as London–van der Waals force and hydrophobic interactions. The balance existing between electrostatic repulsion and London–van der Waals attraction among the particles contributes to the formation of the colloidal gold solution (Hermanson et al., 1992). There is a close relationship between the strength of the color and the size and quality of colloidal gold particles.

To detect high and low molecular weight antigen, the sandwich and competitive type of assay can be employed, respectively. In these kinds of assays, the antibody coupled with gold particle is often used, where antibody is used as a detector reagent and the gold particle as a tracer molecule. Usually, the capture zone on the nitrocellulose membrane comprises the analytes conjugated to a carrier or immobilized antibody. To detect the analytes in different samples, two types of assays have been defined. In one-step competitive test strip, as the test sample flows up through the absorbent device, the free analyte in the specimen competes with immobilized antigen at the test zone by binding to the MAb that has been conjugated with the colloidal AuNPs (Fig. 2a) (Laitinen and Vuento, 1996; Omidfar et al., 2010, 2011b).

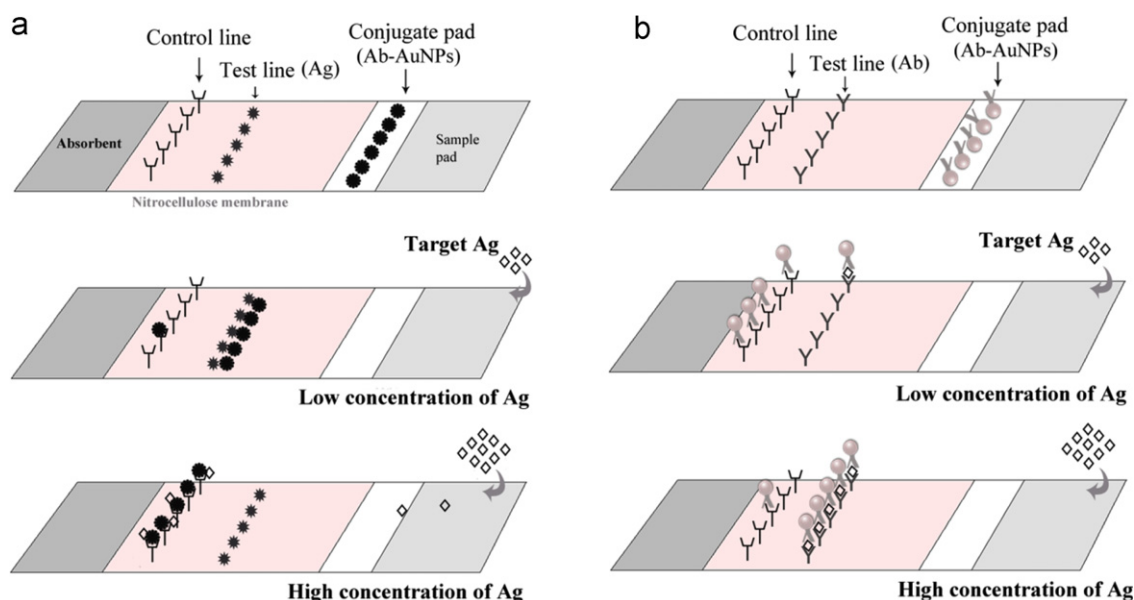


Fig. 2. Structure of immunostrip test and detection method. Schematic representation of the operation of a direct competitive (a) and sandwich (b) immunostrip test for the detection of analyte. The test strip showing its several components including a sample pad, a detector conjugated pad, an absorption pad, and one nitrocellulose membrane with test and control lines.

In the sandwich type of assay, the antibody–gold conjugate was attached to the antigen in samples and subsequently interacts with antibody immobilized on a test zone of membrane (Fig. 2b). Those assays which use this format have allowed a testing to be closer to the patient and at the same time, they can provide an easy-to read, rapid, simple, and convenient diagnostic method. Simplicity of the test, typically requiring little or no sample or reagent preparation, is another advantage gained by this test compared to other immunoassays (Nagatani et al., 2006; Tanaka et al., 2006).

Regarding speed and economic expenses, the feasibility of implementation of such diagnostic tests far from the laboratory could be highly pragmatic. Given the convenient use and visual end-point, recently, on-site strip test has been widely employed for the rapid detection of analytes. Such characteristics have brought some functional benefits in both of clinical and basic sciences and has been extensively employed as a widely popular diagnostic tool in clinical chemistry to detect tumor markers (Nagatani et al., 2006), hormones (Laitinen and Vuento, 1996; Tanaka et al., 2006), viruses (Louie et al., 2008; Nishizono et al., 2008; Reina and Ferrés, 2008), bacteria (Pastoor et al., 2008; Smits et al., 2003; Zhang et al., 2005), parasitic antigens (Wu et al., 2005; Zhang et al., 2006), and finally drug and toxins (Gandhi et al., 2009; Marchei et al., 2008; Omidfar et al., 2010; Tang et al., 2009).

Given the fact that sensitivity of conventional immunostrip assays is significantly lower than ELISA, some measures including use of colloidal AuNPs and liposome have been applied in order to enhance the sensitivity of these tests (Ho et al., 2008; Nagatani et al., 2006; Tanaka et al., 2006). Using colloidal AuNPs and mesoporous silica nanoparticles (MSN) our group successfully managed to develop a convenient detection method for a rapid measurement of urine albumin based on this assay (Omidfar et al., 2012b).

In a recent work, Parolo et al. (2013) discussed the development of an enhanced lateral flow immunoassay based on the use of AuNPs, not only as labels but also as carriers of enzymatic labels to achieve an improved optical immuostrip assay. AuNP labels were directly detected through production of red bands at control and detection lines. Additionally, they were also employed as carriers when coupled with a HRP-modified antibody. Therefore, two different detection strategies were allowed through these strips: the red color of the AuNPs was considered as the less sensitive one and the substrate of the HRP as the other more sensitive alternative. A detection limit of 310 pg mL^{-1} was achieved using this approach (Parolo et al., 2013).

4.2. Immunosensor

Immunosensors are considered as the miniaturized biosensor devices, integrating high specificity and selectivity of immunological assays with accuracy and sensitivity of detection techniques. Based on the method of signal transduction, immunosensors can be classified into four principal groups: electrochemical, optical, piezoelectric and thermal or calorimetric (Holford et al., 2012; Lippa et al., 2001). The advantages of electrochemical immunosensors, such as low cost, high sensitivity, simplicity of instrumentation, and easy signal amplification have distinguished them among other type of immunosensors.

As discussed above, among other kinds of NPs, AuNPs are commonly used as labels in development of electrochemical immunosensors.

Table S1 (supplementary material) outlines some of the recent approaches in this field.

The electrochemical immunosensors employing AuNPs as label can be developed through three types of chemical reactions.

The chemical and electrochemical oxidation of AuNPs occurring in Br_2 and HCl respectively produce electroactive AuCl_4^- , which can give a detectable electrochemical signal in reduced form. Additionally, the electrochemical signal can be also produced through catalytic reduction of *p*-nitrophenol by AuNPs (Castañeda et al., 2007; Liu and Lin, 2007).

4.2.1. AuNPs dissolving detection base

Indirect detection of antibody–AuNPs conjugate can be accomplished by oxidative dissolution of the AuNPs into aqueous metal ions targeted for electrochemical sensing. The chemical dissolution of AuNPs in a hydrobromic acid/bromine mixture followed by accumulation and analysis of the resulting Au^{3+} solution can be regarded as the basis of various electrochemical methods.

Application of antibody–AuNPs as label in acidic bromine–bromide solution has been reported by Dequaire et al. in a sensitive sandwich electrochemical immunosensor. In this method, the electrochemical reduction of solubilized gold ions and their accumulation were done on the carbon-based screen printed electrodes (SPE) and anodic stripping voltammetry (ASV) was used for the electrochemical sensing of amassed gold ions. A noncompetitive heterogeneous immunoassay of an IgG with the limit of detection as low as $3 \times 10^{-12} \text{ M}$ was evaluated based on this method. This assay can compete with the colorimetric ELISA or with immunoassays based on fluorescent europium chelated labels (Dequaire et al., 2000).

In another study by Authier et al. (2001), a similar electrochemical method was employed to develop a sensitive DNA sensor. This genosensor, based on the labeling of oligonucleotide with AuNPs, was capable of detecting the specific DNA sequence at a concentration of 5 pM.

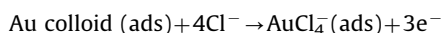
In a survey conducted by Mao et al. (2006), a new immunoassay method based on cyclic accumulation of AuNPs using ASV was introduced to detect human immunoglobulin G (IgG). The cyclic accumulation of AuNPs used for the final analytical measurement was highly dependent to the dissociation reaction between dethiobiotin and avidin in the presence of biotin. The increasing accumulation cycles led to gradual increase in anodic peak current. The assay could be adequately performed by five cycles of accumulation. The discrete advantage of the proposed strategy was in its low background, facilitating the precise determination of human IgG at low concentrations of at least 0.1 ng mL^{-1} .

Autocatalytic deposition of Au^{3+} onto AuNP labels was used for development of an amplified electrochemical immunoassay by Liao et al. (Liao and Huang, 2005). Using autocatalytic deposition along with square wave stripping voltammetry (SWSV), they managed to employ enlarged AuNPs as labels on goat anti-rabbit IgG. This method provided the possibility of quantitative determination of the rabbit IgG analyte with the detection limit of 0.25 pg mL^{-1} (1.6 fM). The detection limit yielded by this method was three orders of magnitude lower in comparison with those achieved by conventional immunoassay methods applying the same AuNP labels (Liao and Huang, 2005).

4.2.2. Direct detection

Considering the toxicity of hydrobromic acid/bromine solution, techniques based on direct electrochemical detection of AuNPs labels replacing the chemical oxidation agents are consequently indispensable. In electrochemistry, AuNPs which have also been employed as label can detect proteins and other target molecules based on monitoring the reduction current signal of gold in hydrochloric media (HCl).

The detection process is based on the following reaction: the oxidation of the AuNPs produces AuCl_4^- ions which were strongly absorbed on the electrode surface:



Then, AuCl_4^- ions were electrochemically reduced and produced a reduction peak in HCl solution roughly at 0.2–0.4 V: $\text{AuCl}_4^- (\text{ads}) + 3\text{e}^- \rightarrow \text{Au (ads)} + 4\text{Cl}^-$

The quantification of NPs and successively the analyte in the sample would be possible by employing the oxidoreduction characteristics of the AuNPs in acidic medium. In the following, several studies published in this regard will be discussed.

In a study by Gonzalez-Garcia and colleagues, the adsorptive electrochemical behavior of colloidal gold on carbon paste electrode (CPE) in HCl was investigated. The assay was performed based on the reduction process of AuCl_4^- ions, which have been generated through electrochemical oxidation of colloidal gold (Gonzalez-Garcia et al., 2000). Modified electrode prepared through adsorption of biotinylated albumin on the pretreated surface of a CPE was immersed in colloidal gold-streptavidin labeled solutions. To obtain a good reproducibility of the analytical signal, adsorptive voltammetry was used to monitor colloidal gold bound to streptavidin. This method was capable of monitoring the streptavidin–biotin interactions down to the 2.5 nM.

In another study, taking the advantages of AuNPs as an electrochemical label and SPCEs as a disposable electrode, a sandwich immunosensors array chip was described by Leng et al. (2010). Under optimal conditions, this method provided a linear range from 5.0 to 500 with low detection limit of 1.1 ng mL^{-1} , employing human IgG and goat IgG as model targets (Fig. 3).

Idegami et al. (2008) presented a highly sensitive immunosensor for detection of pregnancy marker, hCG, by means of electrochemical detection of AuNPs in connection with the disposable screen-printed carbon strips (SPCSs). The immunosensor demonstrated high sensitivity with a detection limit of 36 pg mL^{-1} . This approach provided cost-effective tests with the required antigen sample volume as small as 2 ml).

To directly determine AuNPs, a voltammetric method was reported by Pumera et al. (2005). This method which used the rough surface of graphite-epoxy composite electrode (GECE) was based on adsorption and electrochemical detection of colloidal gold. The provided differential pulse voltammetry (DPV) response demonstrated a linear range from 4.7×10^8 to $4.7 \times 10^{11} \text{ NPs mL}^{-1}$ having a detection limit of $1.8 \times 10^8 \text{ AuNPs mL}^{-1}$.

In addition, using colloidal gold as electrochemical label, Chen et al. (2007) described a sensitive sandwich immunosensor, in which the capture antibody was immobilized on a CPE using passive adsorption. By introducing antigen and colloidal gold labeled antibody to the medium, the amount of analytes was detected and adsorptive voltammetry was applied to determine the adsorbed AuCl_4^- ions. Detection of antigen concentration (human IgG) in a wide linear range from 10 to 500 ng mL^{-1} with a low detection limit of 4.0 ng mL^{-1} was possible using the immunosensor.

The electrochemical characteristics of AuNPs suspensions are strongly dependent on the size and hydrodynamic properties of the solvent. de la Escosura-Muñiz et al. (2011) investigated the effect of the AuNPs size, ranges from 5 nm to 80 nm, on the electrochemical response. They found that the most appropriate electrochemical response can be achieved by AuNPs suspension with 20 nm size. In addition to this, the effect of size on the role of AuNPs as electroactive labels was investigated in an immunosensor using magnetic beads as platforms of the bioreactions. The best response was achieved for the AuNPs with 5 nm size. A good explanation for the given result is that in the immunosensing conditions, since the NPs labels attracted to the electrotransducer surface upon application of a magnetic field, the Brownian motions are minimized as demonstrated by the increase of the voltammetric signal for the smallest NPs.

In order to detect the human lung cancer-associated antigen, α -Enolase (ENO1), Ho et al. (2010) presented a simple electrochemical immunosensing platform. In this study, the sensitivity of the immunosensor was enhanced by the poly ethylene glycol (PEG)-modified SPCE and anti-ENO1-tagged AuNP conjugate bioprobes deployed as the working electrode and signal amplifiers, respectively. After oxidation of the bound AuNP conjugates in 0.1 M HCl at 1.2 V for 120 s, followed by the reduction of AuCl_4^- in square wave voltammetry (SWV) mode, the electrochemical signal was produced. A linear range from 10^{-8} to $10^{-12} \text{ g mL}^{-1}$ and a low detection limit of 11.9 fg mL^{-1} were achieved for such immunosensor

A similar methodology was employed, in our lab, to develop a sensitive immunosensor for detection of analytes. A simple, yet novel, electrochemical immunosensor assay was developed by Omidfar et al. (2011a). Using antibody labeled AuNP and poly vinyl alcohol (PVA) modified electrode, the proposed assay was capable to determine human serum albumin (HSA) concentration at trace level (Fig. 4). Electrochemical measurements, including

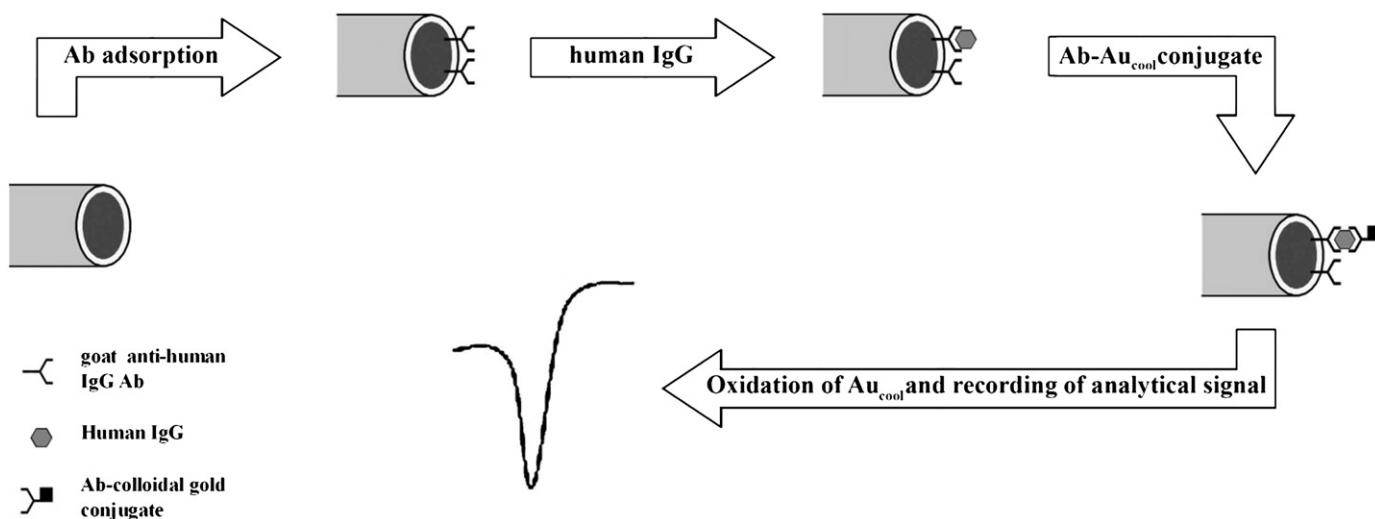


Fig. 3. Procedure of a sandwich electrochemical immunoassay based on AuNPs label.

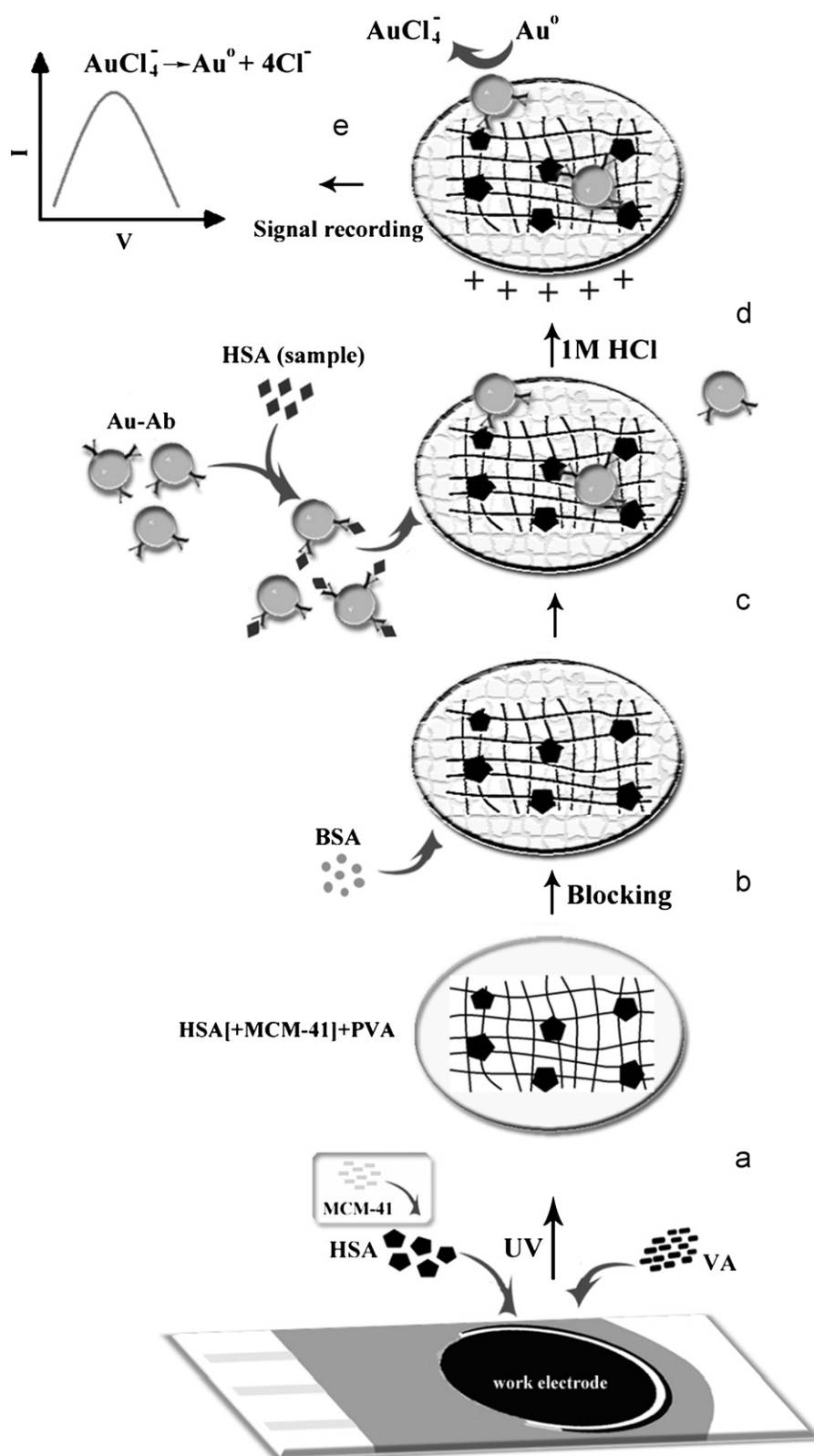


Fig. 4. Schematic representation of a direct competitive assay-based electrochemical immunosensor: (a) dropping of VA-HSA or MCM-41-HAS-VA on the working electrode of SPCE and photopolymerization of VA under UV exposure; (b) BSA blocking; (c) incubation of the patient/standard samples with Ab-AuNPs; (d) peroxidation of AuNPs at the constant potential in 0.1–1 M HCl; (e) voltammetric measurement.

DPV and SWV were used for quantitative detection of antigen by immunosensor. This method demonstrated a wide linear range from 2.5 to 200 $\mu\text{g mL}^{-1}$ with a low detection limit of 25 ng mL^{-1}

for HSA detection. Furthermore, our group showed that PVA layer as a novel support matrix for electrochemical immunosensor applications not only improves the stability and reproducibility

of the modified electrode but also successfully increases the binding efficiencies of the immobilized antigen on the electrode surface.

In another study by Omidfar et al. (2012b), a novel competitive immunosensor was introduced, in which HSA was applied as a model protein. In this assay, a novel inorganic nanostructured material, namely the mobile crystalline material (MCM-41) type silica NPS was employed for immobilization of HSA on the electrode surface (Fig. 4). MCM-41 has also the ability to promote electron transfer reactions. While MCM-41 was employed for the immobilization of HSA, efficient immobilization of MCM-41–HSA on the surface of SPCE was achieved by application of PVA as the sensor platform. This immunosensor could detect HSA in a high linear range ($0.5\text{--}200\text{ }\mu\text{g mL}^{-1}$) with a low detection limit of 1 ng mL^{-1} .

In addition, the catalytic properties of the metallic NPs, such as Au and platinum (Pt) NPs on hydrogen formation from hydrogen ions have been applied for electrochemical signal production in a limited number of reports.

The inertness of bulk gold is attributed to the repulsion between the filled d-states of gold and molecular orbitals of molecules like O_2 or H_2 . However, small AuNPs containing a large number of coordinative unsaturated atoms in edge positions show a different behavior.

The quantum effects can enable AuNPs to interact in electrocatalytic reactions. Such effects related with the shape and size of AuNPs are originated by d band electrons of the surface shifted towards the Fermi-level. These characteristics providing the occurrence of adsorption phenomena, have made such NPs attractive for

electrocatalyzed reactions. This approach is based on the catalytic reduction of protons to hydrogen in the presence of metallic NPs at an adequate potential in acidic medium.

An electrocatalytic device for the specific identification of tumor cells quantifying AuNPs coupled with an electrotransducing platform, was described by de la Escosura-Muñiz et al. (2009a). Proliferation and adherence of tumor cells were accomplished on the electrotransducer, consisting of a mass-produced SPCE. The device worked based on the cell surface proteins reaction with specific antibodies conjugated with AuNPs. Such a novel and selective cell-sensing device could provide in situ identification of tumor cells with a detection limit of 4000 cells per $700\text{ }\mu\text{L}$ of suspension. Taking advantage of the catalytic properties of AuNPs on hydrogen evolution, the final detection process could be performed only in a couple of minutes.

In another study, employing SPCEs as electrotransducers, the catalytic ability of AuNPs towards the formation of H_2 in the electrocatalyzed hydrogen evolution reaction (HER) was thoroughly investigated by Maltez-da Costa et al. The AuNPs on the surface of the SPCE provide free electroactive sites to the protons present in the acidic medium. The medium was then catalytically reduced to hydrogen through an adequate potential, with a resulting increase in the reaction rate of the HER (Fig. 5). Measurements, finally, were carried out by the generated catalytic current. Since the catalytic current was related to the concentration of AuNPs, the quantification of such particles (AuNPs) was possible. The electrocatalytic method for detection of AuNPs as labels in a magnetoimmunosandwich assay was applied for the first time in this survey. Benefitted from employing SPCEs as

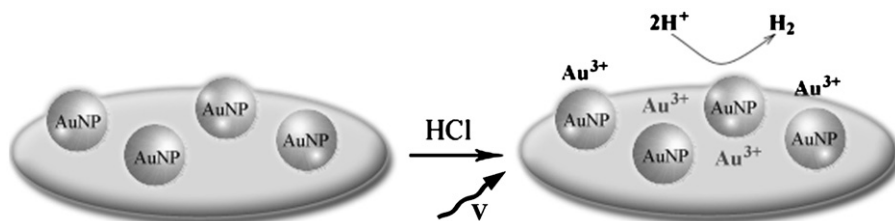


Fig. 5. Scheme of electrochemical HER induced by AuNPs.

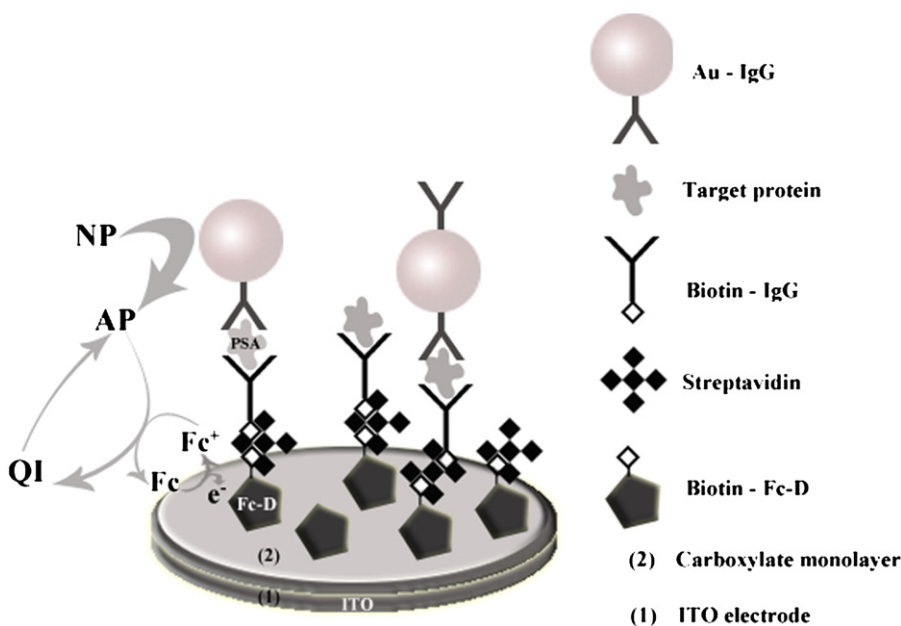


Fig. 6. Schematic representation of an electrochemical immunosensor for the detection of a target protein (mouse IgG or PSA) designed by Das et al. (2006). AP: *p*-aminophenol, NP: *p*-nitrophenol, QI: *p*-quinone imine.

electrotransducers, the determination of human IgG at levels of 1 ng mL^{-1} was feasible through this assay (Costa et al., 2010).

In addition, a novel signal amplification strategy to develop ultrasensitive electrochemical immunosensors for detection of prostate-specific antigen (PSA) was described by Zhang et al. (2010). The amplification process was based on platinum catalyzing a hydrogen evolution reaction. PSA capture antibodies covalently bound to the surface of a gold electrode were used in the immunosensing protocol. Subsequent to the binding of PSA to the electrode surface, a secondary antibody labeled by platinum NPs was used to complete the sandwich immunosensor. The signal readout was electrochemically obtained using a Pt-catalyzed hydrogen evolution reaction in 10 mM of HCl and 1 M of KCl solution. With a detection limit of 1 fg mL^{-1} , they successfully managed to detect the specific analyte.

Furthermore, nanomaterials and enzymes can be applied concomitantly for dual signal amplification in immunoassays. In a simple assay by Ambrosi et al. (2007), a double-codified nanolabel (DC-AuNP) based on AuNP modified with anti-human IgG- HRP conjugate was introduced. The enhanced spectrophotometric and electrochemical detection of human IgG as a model protein were provided through such labels. This approach was typically much more sensitive than ELISA tests. A detection limit of 52 and 260 pg mL^{-1} for human IgG were achieved in the spectrophotometric (HRP-based) and electrochemical (AuNP-based) detections, respectively.

4.2.3. AuNP labels as electrocatalysts

In an attempt towards a nanocatalyst-based electrochemical assay for proteins, an ultrasensitive and simple electrochemical method was developed by Das and coworkers for the fabrication of a sandwich-type heterogeneous electrochemical immunosensor. Through the signal amplification, accompanied with reduction of noise, the assay represented an ultrasensitive detection. In this method, briefly, biotinylated antibodies were immobilized on streptavidin-modified indium tin oxide (ITO) electrode. IgG was adsorbed on 10 nm AuNPs, and thereby an IgG nanocatalyst conjugate was prepared. Mouse IgG or prostate specific antigen was also employed as a target protein. It was sandwiched by the IgG-nanocatalyst conjugate and the immunosensing layer. The catalytic reduction of *p*-nitrophenol (NP) to *p*-aminophenol (AP) via gold nanocatalyst labels coupled with the chemical reduction of *p*-quinone imine (QI) by NaBH_4 were performed to achieve signal amplification (Fig. 6). Through application of an ITO electrode modified with a ferrocenyl-tethered dendrimer and a hydrophilic immunosensing layer, they succeeded in reducing noise in this assay. The assay was capable of very low limit of detection (1 fg mL^{-1}) to detect target analyte (Das et al., 2006).

4.2.4. Catalytic deposition of silver/copper on gold labels

The AuNP labeling coupled with the silver/copper enhancement technique holds great promise for signal amplification in antibody-based biosensors and DNA sensors labeled with AuNPs. The AuNPs can act as a catalyst and reduce copper and especially silver ions into metallic copper and silver in the presence of a reducing agent. The reduction reaction leads to the deposition of copper and silver on the gold surface as nucleation site and enlargement of the size of AuNPs resulting in remarkable signal amplification. Accordingly, several DNA and antibody based biosensors have been developed. Electrochemical detection of DNA hybridization with the detection limit of 50 pM of complementary oligonucleotides was carried out using gold labeled DNA probes and glassy carbon electrodes. Silver deposition onto AuNPs was the signal enhancement strategy, applied for DPV monitoring of hybridization event between target DNA and probe

(Cai et al., 2002). Using silver deposition strategy, Chu et al. (2005) has employed an AuNP labeled secondary antibody for detection of human IgG. This sensitive immunoassay was performed through ASV of the silver, deposited on the gold label at the surface of glassy carbon electrode (GCE). The human IgG was detected with the detection limit of $6 \times 10^{-12} \text{ M}$. An electrochemical metalloimmunoassay based on copper-enhanced AuNP label was introduced, by Dungchai et al. (2008), for determination of *Salmonella typhi* in real samples. MAbs for *S. typhi* were immobilized onto the microwells. Following the immunoreaction, a conjugate of PAb-AuNP and then, a copper-enhancer solution containing ascorbic acid and copper (II) sulfate were added. The reduction of copper ions by ascorbic acid led to the deposition of copper on gold surface. A detection limit of 98.9 cfu mL^{-1} was achieved using this immunoassay performed by ASV of copper ions on GCE. The silver enhancement strategy was applied in a magneto based immunoassay depending on the role of magnetic beads as immunoreactions platform. While primary antibodies were immobilized on microparamagnetic beads, secondary antibodies were modified with AuNPs. Using a built-in magnet carbon electrode and the catalytic deposition of silver on AuNPs, such sandwich type immunoassay was introduced for sensitive detection of model proteins as low as 23 fg mL^{-1} (de la Escosura-Muniz et al., 2009b).

5. Future perspectives

This review briefly dealt with the roles of AuNPs as label in analytical immunosensors. Particular attention, moreover, was given to the various forms of antibodies as the biorecognition elements significant for detection of analytes.

In general, NPs can have various functions in different analytical sensing systems. They can simultaneously play more than one role in an analytical system. For instance, AuNPs can be employed as substrates for immobilization of biomolecules in preparation of immunosensors with improved stability. Furthermore, considering the chemical and catalytic properties of AuNPs, they can be deployed as an excellent label for different sensing strategies in highly sensitive and selective immunosensors. The classification of immunosensors with NP labels is normally made based on the final detection of the NP. Given the importance of NPs, much attention should, therefore, be devoted to the preparation and application of special NP labels. The unique and attractive properties of AuNPs have paved the way for the development of measurement systems exhibiting attractive and promising analytical behaviors. Employing the special physical or chemical properties of AuNPs, fabrication of such improved analytical systems can be feasible.

The above mentioned studies evaluated the broad potential of AuNPs for signal amplification of antibody-antigen recognition events on immunosensors. Requiring simple instrumentation, the analytical detection can be remarkably easy through the physical and electrochemical characteristics of AuNPs. As a result, designing simple and inexpensive analytical systems for detection of ultrasensitive, multiplexed assays can be allowed by such properties of NPs.

The urgent need for smaller detection platforms with lower limits of detection is the motivation force for utilization of NPs in sensing devices. Further effort should be put into improvement approaches in order to take their advantages efficiently.

The remarkable sensitivity of sensing protocols based on the AuNPs label will open promising prospects for development of analytical strategies, concerning disease markers not detectable by conventional methods. Benefiting from such highly sensitive

biodetection plans, early diagnosis of diseases or the warning of a terrorist attack can be possible.

Since many of these immunosensors have been only developed in laboratory environments so far, it will be a major challenge to introduce a technology allowing rapid production of large numbers of sensors with relatively low cost and high quality specifications. Such technology is prerequisite for any successful commercial application.

The SPE, in recent years, have found considerably wide application in development of biosensors, particularly immunosensors. Disposability, a significant characteristic while working with real samples, and the small sensor dimension allowing their combination with a portable measuring system can be considered as the advantages of these electrodes.

Moreover, low cost and simplicity of use are among the other advantages of such electrodes manufactured by screen printing technology. In addition, the surface of electrode can be modified by NPs, polymers and biomolecules, making this type of sensor a good choice for biosensor construction.

Considering the importance of rapid management of a special medical, commercial, and environmental or security issue by non-trained personnel, the immunosensor research has been centered on developing on-site or PoCs assays. The ability to analyze the low volume of samples, particularly those non-invasive specimens, including saliva, urine, or other excreted fluids is another significant characteristic noticeable in designing PoC sensor. Constant efforts have been carried out in order to amend the reliability and diversity of PoC test devices, to reduce the detection limits of currently existing immunosensors and to develop new immunoassays used for clinically important targets.

The lab-on-a-chip (LoC) diagnostic biosensor also introduces a novel strategy for detecting diseases in real-time, representing high potential for application in the medical industry. Miniaturization of immunosensing devices into LoCs is being performed with the purpose of doing all testing in a single chip in one place, making continuous sampling feasible.

A growing trend in development of such improved devices, particularly PoC and LoC tests with greater abilities in high-throughput screening and multiple analyte analysis coincidentally is expected in the near future.

Acknowledgment

This work was supported by a grant from Endocrinology and Metabolism Research Center of Tehran University of Medical Sciences, Tehran, I.R. Iran.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bios.2012.12.045>.

References

- Ambrosi, A., Castañeda, M.T., Killard, A.J., Smyth, M.R., Alegret, S., Merkoçi, A., 2007. *Analytical Chemistry* 79 (14), 5232–5240.
- Arbabi Ghahroudi, M., Desmyter, A., Wyns, L., Hamers, R., Muyldermans, S., 1997. *FEBS Letters* 414 (3), 521–526.
- Authier, L., Grossiord, C., Brossier, P., 2001. *Analytical Chemistry* 73 (18), 4450–4456.
- Bell, A., Wang, Z.J., Arbabi-Ghahroudi, M., Chang, T.A., Durocher, Y., Trojahn, U., Baardsnes, J., Jaramillo, M.L., Li, S., Baral, T.N., 2010. *Cancer Letters* 289 (1), 81–90.
- Butler, J.E., 1991. *Immunochemistry of Solid-Phase Immunoassay*. CRC.
- Byrne, B., Stack, E., Gilmartin, N., O'Kennedy, R., 2009. *Sensors* 9 (6), 4407–4445.
- Cai, H., Wang, Y., He, P., Fang, Y., 2002. *Analytica Chimica Acta* 469 (2), 165–172.
- Campanella, L., Attioli, R., Colapicchioni, C., Tomassetti, M., 1999. *Sensors and Actuators B* 55 (1), 23–32.
- Castañeda, M.T., Alegret, S., Merkoçi, A., 2007. *Electroanalysis* 19 (7–8), 743–753.
- Chen, P.C., Mwakwari, S.C., Oyeler, A.K., 2008. *Nanotechnology, Science and Applications* 1 (2008), 45–66.
- Chen, Z.P., Peng, Z.F., Zhang, P., Jin, X.F., Jiang, J.H., Zhang, X.B., Shen, G.L., Yu, R.Q., 2007. *Talanta* 72 (5), 1800–1804.
- Choi, S., Goryll, M., Sin, L.Y.M., Wong, P.K., Chae, J., 2011. *Microfluidics and Nanofluidics* 10 (2), 231–247.
- Chu, X., Fu, X., Chen, K., Shen, G.-L., Yu, R.-Q., 2005. *Biosensors & bioelectronics* 20 (9), 1805–1812.
- Costa, M.M., la Escosura-Muñiz, A., Merkoçi, A., 2010. *Electrochemistry communications* 12 (11), 1501–1504.
- Daniel, M.C., Astruc, D., 2004. *Chemical Reviews—Columbus* 104 (1), 293.
- Das, J., Aziz, M.A., Yang, H., 2006. *Journal of the American Chemical Society* 128 (50), 16022–16023.
- Davies, J., Riechmann, L., 1996. *Immunotechnology* 2 (3), 169–179.
- De Genst, E., Handelberg, F., Van Meirhaeghe, A., Vynck, S., Loris, R., Wyns, L., Muyldermans, S., 2004. *The Journal of Biological Chemistry* 279 (51), 53593–53601.
- de la Escosura-Muñiz, A., Maltez-da Costa, M., Merkoçi, A., 2009a. *Biosensors & Bioelectronics* 24 (8), 2475–2482.
- de la Escosura-Muñiz, A., Parolo, C., Maran, F., Mekoçi, A., 2011. *Nanoscale* 3 (8), 3350–3356.
- de la Escosura-Muñiz, A., Sañchez, Espinel, C., Di'az, Freitas, B., González, Fernán, dez, Á., Maltez, da Costam, A., 2009b. *Analytical Chemistry* 81 (24), 10268–10274.
- Deffar, K., Shi, H., Li, L., Wang, X., Zhu, X., 2010. *African Journal of Biotechnology* 8, 12.
- Dequaire, M., Degrand, C., Limoges, B., 2000. *Analytical Chemistry* 72 (22), 5521–5528.
- Du, D., Yan, F., Liu, S., Ju, H., 2003. *Journal of Immunological Methods* 283 (1–2), 67–75.
- Duan, C., Meyerhoff, M.E., 1994. *Analytical Chemistry* 66 (9), 1369–1377.
- Dungchai, W., Siangproh, W., Chaicumpa, W., Tongtawe, P., Chailapakul, O., 2008. *Talanta* 77 (2), 727–732.
- Faraday, M., 1857. *Philosophical Transactions of the Royal Society of London* 147, 145–181.
- Filpula, D., 2007. *Biomolecular Engineering* 24 (2), 201–215.
- Frens, G., 1973. *Nature* 241 (105), 20–22.
- Gandhi, S., Caplash, N., Sharma, P., Raman Suri, C., 2009. *Biosensors & Bioelectronics* 25 (2), 502–505.
- Gole, A., Murphy, C.J., 2004. *Chemistry of Materials* 16 (19), 3633–3640.
- Gonzalez-Garcia, M., Fernandez-Sanchez, C., Costa-Garcia, A., 2000. *Biosensors & Bioelectronics* 15 (5–6), 315–321.
- Greenberg, A.S., Avila, D., Hughes, M., Hughes, A., McKinney, E.C., Flajnik, M.F., 1995. *Nature* 374 (6518), 168–173.
- Gui, W.-J., Wang, S.-T., Guo, Y.-R., Zhu, G.-N., 2008. *Analytical Biochemistry* 377 (2), 202–208.
- Guo, S., Wang, E., 2007. *Analytica Chimica Acta* 598 (2), 181–192.
- Hamers-Casterman, C., Atarhouch, T., Muyldermans, S., Robinson, G., Hammers, C., Songa, E.B., Bendahman, N., Hammers, R., 1993. *Nature* 363 (6428), 446–448.
- He, Y.Q., Liu, S.P., Kong, L., Liu, Z.F., 2005. *Spectrochimica Acta Part A, Molecular and Biomolecular Spectroscopy* 61 (13–14), 2861–2866.
- Hermanson, G.T., Mallia, A.K., Smith, P.K., 1992. *Immobilized Affinity Ligand Techniques*. Academic Press, San Diego.
- Ho, J.A., Chang, H.C., Shih, N.Y., Wu, L.C., Chang, Y.F., Chen, C.C., Chou, C., 2010. *Analytical Chemistry* 82 (14), 5944–5950.
- Ho, J.A., Zeng, S.C., Tseng, W.H., Lin, Y.J., Chen, C., 2008. *Analytical and Bioanalytical Chemistry* 391 (2), 479–485.
- Holford, T.R.J., Davis, F., Higson, S.P.J., 2012. *Biosensors & Bioelectronics* 34 (1), 12–24.
- Holliger, P., Hudson, P.J., 2005. *Nature Biotechnology* 23 (9), 1126–1136.
- Idegami, K., Chikae, M., Kerman, K., Nagatani, N., Yuh, T., Endo, T., Tamiya, E., 2008. *Electroanalysis* 20 (1), 14–21.
- Kashanian, S., Rasaee, M., Paknejad, M., Omidfar, K., Pour-Amir, M., Rajabi, B.M., 2002. *Hybridoma and Hybridomics* 21 (5), 375–379.
- Killard, A.J., Deasy, B., O'Kennedy, R., Smyth, M.R., 1995. *TrAC Trends in Analytical Chemistry* 14 (6), 257–266.
- Kimling, J., Maier, M., Okenve, B., Kotaidis, V., Ballot, H., Plech, A., 2006. *The Journal of Physical Chemistry B* 110 (32), 15700–15707.
- Kohn, J., 1968. *Immunology* 15 (6), 863.
- Kuznetsov, B.A., Shumakovich, G.P., Koroleva, O.V., Yaropolov, A.I., 2001. *Biosensors & Bioelectronics* 16 (1–2), 73–84.
- Laitinen, M.P.A., Vuento, M., 1996. *Acta Chemica Scandinavica* 50 (2), 141–145.
- Leng, C., Lai, G., Yan, F., Ju, H., 2010. *Analytica Chimica Acta* 666 (1), 97–101.
- Li, S., Zheng, W., KuoLee, R., Hiram, T., Henry, M., Makvandi-Nejad, S., Fjällman, T., Chen, W., Zhang, J., 2009. *Molecular Immunology* 46 (8–9), 1718–1726.
- Liao, K.T., Huang, H.J., 2005. *Analytica Chimica Acta* 538 (1), 159–164.
- Liu, G., Lin, Y., 2007. *Talanta* 74 (3), 308–317.
- Liu, Y., Li, Y., 2001. *Analytical Chemistry* 73 (21), 5180–5183.
- Louie, B., Wong, E., Klausner, J.D., Liska, S., Hecht, F., Dowling, T., Obeso, M., Phillips, S.S., Pandori, M.W., 2008. *Journal of Clinical Microbiology* 46 (4), 1494–1497.
- Luo, X., Morrin, A., Killard, A.J., Smyth, M.R., 2006. *Electroanalysis* 18 (4), 319–326.
- Luppa, P.B., Sokoll, L.J., Chan, D.W., 2001. *Clinica Chimica Acta* 314 (1–2), 1–26.

- Mao, X., Jiang, J., Chen, J., Huang, Y., Shen, G., Yu, R., 2006. *Analytica Chimica Acta* 557 (1), 159–163.
- Marchei, E., Colone, P., Nastasi, G., Calabro, C., Pellegrini, M., Pacifici, R., Zuccaro, P., Pichini, S., 2008. *Journal of Pharmaceutical and Biomedical Analysis* 48 (2), 383–387.
- Mohammadnejad, J., Rasaee, M.J., Saqafi, B., Rajabibazl, M., Rahbarizadeh, F., Omidfar, K., Paknejad, M., 2006. *Journal of Immunoassay & Immunochemistry* 27 (2), 139–149.
- Muyldermans, S., 2001. *Reviews in Molecular Biotechnology* 74 (4), 277–302.
- Muyldermans, S., Atarhouch, T., Saldanha, J., Barbosa, J., Hamers, R., 1994. *Protein Engineering* 7 (9), 1129–1135.
- Nagatani, N., Yuhi, T., Chikae, M., Kerman, K., Endo, T., Kobori, Y., Takata, M., Konaka, H., Namiki, M., Ushijima, H., Takamura, Y., Tamiya, E., 2006. *Nano BioTechnology* 2 (3), 79–86.
- Newman, J., Blanchard, G., 2006. *Langmuir* 22 (13), 5882–5887.
- Nguyen, V.K., Desmyter, A., Muyldermans, S., 2001. *Advances in Immunology* 79, 261–296.
- Nishizono, A., Khawplod, P., Ahmed, K., Goto, K., Shiota, S., Mifune, K., Yasui, T., Takayama, K., Kobayashi, Y., Mannen, K., 2008. *Microbiology and Immunology* 52 (4), 243–249.
- Omidfar, K., Dehdast, A., Zarei, H., Sourkhi, B.K., Larijani, B., 2011a. *Biosensors & Bioelectronics* 26 (10), 4177–4183.
- Omidfar, K., Kashanian, S., Paknejad, M., Larijani, B., Roshanfekar, H., 2007. *Hybridoma* 26 (4), 217–222.
- Omidfar, K., Khorsand, B., Larijani, B., 2012a. *Molecular Biology Reports* 39 (2), 1253–1259.
- Omidfar, K., Kia, S., Kashanian, S., Paknejad, M., Besharatie, A., Larijani, B., 2010. *Applied Biochemistry and Biotechnology* 160 (3), 843–855.
- Omidfar, K., Kia, S., Larijani, B., 2011b. *Hybridoma* 30 (2), 117–124.
- Omidfar, K., Shirvani, Z., 2012. *DNA and Cell Biology* 31 (6), 1015–1026.
- Omidfar, K., Zarei, H., Gholizadeh, F., Larijani, B., 2012b. *Analytical Biochemistry* 421 (2), 649–656.
- Paknejad, M., Rasaee, M., Tehrani, F.K., Kashanian, S., Mohagheghi, M., Omidfar, K., Bazl, M.R., 2003. *Hybridoma and Hybridomics* 22 (3), 153–158.
- Pandey, S., 2010. *Hybridoma* 1 (2), 017.
- Parolo, C., delaEscosura-Muñiz, A., Merkoçi, A., 2013. *Biosensors & Bioelectronics* 40 (1), 412–416.
- Pastoor, R., Hatta, M., Abdoel, T.H., Smits, H.L., 2008. *Diagnostic Microbiology and Infectious Disease* 61 (2), 129–134.
- Peruski, A.H., Peruski Jr, L.F., 2003. *Clinical and Diagnostic Laboratory Immunology* 10 (4), 506–513.
- Pumera, M., Aldavert, M., Mills, C., Merkoçi, A., Alegret, S., 2005. *Electrochimica Acta* 50 (18), 3702–3707.
- Reina, J., Ferrés, F., 2008. *Journal of Clinical Virology* 42 (3), 291–292.
- Roitt, I.M., Brostoff, J., Male, D.K., 1985. *Immunology*. Gower Medical Pub.
- Rusling, J.F., 2012. *Chemical Record* 12 (1), 164–176.
- Saerens, D., Huang, L., Bonroy, K., Muyldermans, S., 2008. *Sensors* 8 (8), 4669–4686.
- Seydack, M., 2005. *Biosensors & Bioelectronics* 20 (12), 2454–2469.
- Shetty, V., Zigler, C., Robles, T.F., Elashoff, D., Yamaguchi, M., 2011. *Psychoneuroendocrinology* 36 (2), 193–199.
- Shim, W.B., Yang, Z.Y., Kim, J.S., Kim, J.Y., Kang, S.J., Woo, G.J., Chung, Y.C., Eremin, S.A., Chung, D.H., 2007. *Journal of Microbiology and Biotechnology* 17 (10), 1629–1637.
- Sivaraman, S.K., Kumar, S., Santhanam, V., 2010. *Gold Bulletin* 43 (4), 275–286.
- Šlouf, M., Kužel, R., Matěj, Z., 2006. *Zeitschrift für Kristallographie Supplements (suppl. 23/2006)*, 319–324.
- Smits, H.L., Abdoel, T.H., Solera, J., Clavijo, E., Diaz, R., 2003. *Clinical and Diagnostic Laboratory Immunology* 10 (6), 1141–1146.
- Stryer, L., 1988. *Biochemistry*. W.H. Freeman, New York.
- Tabrizi, A., Ayhan, F., Ayhan, H., 2009. *Hacettepe Journal of Biology and Chemistry* 37 (3), 217–226.
- Tanaka, R., Yuhi, T., Nagatani, N., Endo, T., Kerman, K., Takamura, Y., Tamiya, E., 2006. *Analytical and Bioanalytical Chemistry* 385 (8), 1414–1420.
- Tang, D., Saucedo, J., Lin, Z., Ott, S., Basova, E., Goryacheva, I., Biselli, S., Lin, J., Niessner, R., Knopp, D., 2009. *Biosensors & Bioelectronics* 25 (2), 514–518.
- Tansil, N.C., Gao, Z., 2006. *Nano Today* 1 (1), 28–37.
- Tillib, S., 2011. *Molecular Biology* 45 (1), 66–73.
- Turkevich, J., Kim, G., 1970. *Science* 169 (3948), 873–879.
- Turkevich, J., Stevenson, P.C., Hillier, J., 1951. *Discussions of the Faraday Society* 11 (0), 55–75.
- Vaitukaitis, J.L., Braunstein, G.D., Ross, G.T., 1972. *American Journal of Obstetrics and Gynecology* 113 (6), 751–758.
- van der Linden, R., de Geus, B., Stok, W., Bos, W., van Wassenaar, D., Verrips, T., Frenken, L., 2000. *Journal of Immunological Methods* 240 (1), 185–195.
- Van der Linden, R., Frenken, L., De Geus, B., Harmsen, M., Ruuls, R., Stok, W., De Ron, L., Wilson, S., Davis, P., Verrips, C., 1999. *Biochimica et Biophysica Acta (BBA)—Protein Structure and Molecular Enzymology* 1431 (1), 37–46.
- von Lode, P., 2005. *Clinical Biochemistry* 38 (7), 591–606.
- Warsinke, A., 2009. *Analytical and Bioanalytical Chemistry* 393 (5), 1393–1405.
- Wu, Y., Lei, L.M., Li, M., 2005. *Academic Journal of the First Medical College of PLA* 25 (7), 761.
- Zhang, J., Du, J., Han, B., Liu, Z., Jiang, T., Zhang, Z., 2006. *Angewandte Chemie (International Edition in English)* 45 (7), 1116–1119.
- Zhang, J., Ting, B.P., Khan, M., Pearce, M.C., Yang, Y., Gao, Z., Ying, J.Y., 2010. *Biosensors & Bioelectronics* 26 (2), 418–423.
- Zhang, R., Jiang, Y., Chen, G.X., Yan, W.W., 2005. *Chinese Journal of Medical Laboratory* 8, 793–795.
- Zhu, S., Zhou, H., Hibino, M., Honma, I., Ichihara, M., 2005. *Advanced Functional Materials* 15 (3), 381–386.

Supplementary Material

New analytical applications of gold nanoparticles as label in antibody based sensors

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Table S1 Electrochemical immunosensors developed based on AuNPs as label.

Reaction	Type of sensor	Electrode	Detection limit	Size of AuNP	Detection method	Reference
Chemical oxidation of AuNP in Br ₂	Non-competitive immunosensor for detection of IgG	SPE	3pM	18 nm	ASV	Dequaire et al. (2000)
	Sandwich type genosensor	SPE	5pM	20 nm	ASV	Authier et al. (2001)
	Sandwich type immunoassay for human IgG detection	CPE	0.1 ng mL ⁻¹	~25 nm	ASV	Mao et al. (2005)
	Sandwich type immunoassay for detection of rabbit IgG analyte	Glassy carbon	1.6 fM	5 nm	SWSV	Liao et al. (2005)
Electrochemical oxidation of AuNP in HCl	Competitive immunosensor for monitoring the streptavidin–biotin interactions	CPE	2.5 nM	10 nm colloid al gold-str	Adsorptive voltammetry	Gonzalez-Garcia et al (2000)
	Sandwich type immunosensor for detection of human IgG and goat IgG	SPCE	1.1 and 1.6 ng mL ⁻¹	15 nm	DPV	Leng et al. (2010)
	Sandwich type immunosensor for detection of pregnancy marker (hCG)	(SPCS) Screen-printed carbon strip	36 pg mL ⁻¹ (612 fM, 3.6×10 ⁻⁴ IU mL ⁻¹)	40 nm	DPV CV	Idegami et al. (2008)
	Voltammetry method for determination of AuNP	Graphite-epoxy composite electrode (GECE)	1.8×10 ⁸ Au NPs mL ⁻¹	10 nm	DPV	Pumera et al (2005)
	Sandwich type immunosensor for detection of human IgG	CPE (carbon paste electrode)	4.0 ng mL ⁻¹	Approximately 20 nm	Adsorptive voltammetry	Chen et al (2007)
	Sandwich type immunosensor for detection of ENO1	Poly ethylene glycol (PEG)-modified SPCE	11.9 fg	33 nm	SWV	Ho et al. (2008)
	Direct competitive immunosensor for detection of HSA	PVA modified SPCE	25 ng mL ⁻¹	20 nm	DPV and SWV	Omidfar et al. (2011)

	Competitive immunosensor for detection of HSA as model	SPCE(PV A+MCM4 1)	1 ng mL ⁻¹	20 nm	DPV and CV	Omidfar et al. (2012)
	Electrochemical in situ identification of tumor cells (Ab+AuNP)	Mass-produced SPCE	4000 cells per 700 μ L of suspension	20 nm	CV and chronoamperometry	Escosura-Muniz et al. (2009)
	Magnetoimmunosandwich assay determination of human IgG (based on electrocatalyzed hydrogen evolution reaction)	SPCE	1 ng mL ⁻¹	20 nm	CV, Chronoamperometry, Electrochemical impedance spectroscopy (EIS)	Maltez-da Costa et al. (2010)
	Spectrophotometric and electrochemical detection of antigen human IgG	Graphite-epoxy composite-magnet electrode (GECE-M)	52 pg mL ⁻¹ HRP-based & 260 pg mL ⁻¹ for AuNP-based detection	13 nm	DPV	Ambrosi et al. (2007)
Catalytic reduction of <i>p</i> -nitrophenol by AuNP	Sandwich type immunosensor for detection of Mouse IgG or PSA	Streptavidin-modified indium tin oxide (ITO) electrode modified with ferrocenyl-tethered dendrimer and a hydrophilic immunosensing layer	1 fg mL ⁻¹	10 nm	CV	Das et al. (2006)
Catalytic deposition of silver/copper on gold labels	Sandwich immunoassay for detection of human IgG (silver-enhanced gold nanoparticle label)	Glassy carbon	6×10^{-12} M	20 nm $\pm$ 4.7 nm	ASV CV	Chu et al. (2005)
	An electrochemical metalloimmunoassay for <i>Salmonella typhi</i> determination (copper-enhanced gold nanoparticle label)	Glassy carbon	98.9 cfu mL ⁻¹	15 nm	Stripping voltammetry	Dungchai et al. (2008)
	A sandwich type	A	23 fg mL ⁻¹	15 nm	CV	De la

	magneto-based immunoassay (silver-enhanced gold nanoparticle label)	Graphite-epoxy composite electrode with a magnet inside (GECE-M)				Escosura-Muniz et al. (2009)
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