



Application of Gold Silica Nanocomposites in Electrochemical Biosensors: A Review



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ABSTRACT

Gold silica nanocomposite-based biosensors are performing well in sensor technology for biosensor development. The gold silica nanocomposite-based biosensor has good selectivity, excellent conductivity, large surface area, efficient enhancement of electron transfer between enzymes and electrodes and good biocompatibility. Therefore, gold silica nanocomposite can be an ideal matrix for immobilization of biomolecules. This review describes the method of synthesizing gold silica nanocomposite and their characterization, interaction with biomolecules and application of gold silica nanocomposite in electrochemical biosensors.

Keywords: Gold silica nanocomposite, electrochemical biosensor, application.

INTRODUCTION

The nanoparticle scale size of a particle is often used in analytical chemistry including biosensors because it has different and unique properties, structure and surface area. Nanoparticles have a wide application in sensor manufacturing (Rao et al., 2013). A nanoparticle is a particle with a nanoscale size between 1-100 nanometers. Nanoparticles can have several geometric shapes such as round (nanosphere), nanorod, nanosheet, nanotube, and nanowire (Famia & Muldarisnur, 2019).

Among the types of nanoparticles used in the biomedical and bioanalytical fields, silica is highly regarded because of its nano-sized and mesoporous properties. In addition, silica nanoparticles include high surface area, good stability, large pore volume, controllable morphology and size, ease of preparation and biocompatibility (Bagheri et al., 2020). The silica nanoparticles based on their shape are non-porous, hollow / hollow, mesoporous / porous, amorphous, rod, core-shell, yolk / shell and janus SiNPs. Mesoporous silica materials have characteristics, high surface area, specificity, and regular pores (Rao et al., 2013).

The characteristics of silica can be a barrier to the immobilization of biomolecules because of its poor conductivity, so that the synthesis of silica composites with metal ions that have good conductivity can be carried out. One of them is that gold nanoparticles have been used to immobilize biomolecules but are easy to agglomerate, so the synthesis of silica and gold nanocomposites can reduce the drawbacks of each. In addition, AuNP can be used as a limiting agent that prevents the leakage of cargo out of mesoporous silica (MSN) nanoparticles and can be opened and closed under controlled conditions to release the load (Bagheri et al., 2020). To determine the success of the synthesis, characterization of UV-Vis spectrophotometry, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) was carried out (Bai et al., 2007).

Electroanalytic sensors have advantages over other methods, namely their high sensitivity, accuracy, and precision as well as a large linear dynamic range, relatively cheap instrumentation, and a short analysis time (H. X. Zhang et al., 2006). The electrode surface is modified silica gold nanocomposite, so that the target compound is selectively adsorbed and enriches the surface which allows ultra-trace detection. So silica is

considered attractive for electroanalytic applications (Hasanzadeh et al., 2012). This review describes the synthesis and characterization of gold silica nanocomposites and their application to electrochemical biosensors.

SYNTHESIS AND CHARACTERIZATION OF GOLD SILICA NANOCOMPOSITES

Bai *et al* (2007) and Yu *et al* (2004) synthesized gold silica nanocomposite using SBA-15 mesoporous silica via the Sol-Gel method. Briefly P123 and potassium chloride under acidic conditions at 38°C. TEOS was added using a variation of the final reactant molar composition (0.02: 3: 6: 166: 1) P123 / KCl / HCl / H₂O / TEOS. After heating, filtered and dried. Then the addition of the -NH₂ group to be able to bind with emans, by dispersing the APTS solution and reflux. Product (H₂N-SBA-15), gold loading from HAuCl₄. After centrifugation, the original yellow HAuCl₄ aqueous solution became colorless, while the white mesoporous silica powder obtained became yellowish. Reduction of Au (III) with NaBH₄. Then red wine solid GNPs-SBA-15 was obtained (Bai et al., 2007).

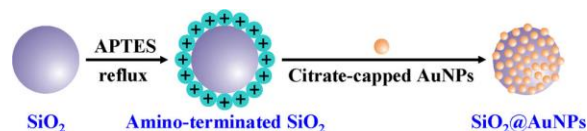


Figure 1. The formation of gold silica nanocomposites (Sun et al., 2018).

Sun *et al* (2018) sun forms silica gold nanocomposites by synthesizing their respective precursors; nanosilica and nano gold. The formation of the silica gold nanocomposite is shown in Figure 1. The synthesis of silica nanoparticles using the stober method and gold nanoparticles were synthesized using the Turkevich method. First, monodispersed SiO_2 was synthesized using the Stober method modified with ammonia dissolved with ethanol under ultrasonic. Then, a mixture of ethanol and TEOS is added by heating. By hydrothermal method, the product is annealed at 500 ° C. Furthermore, $\text{SiO}_2\text{-NH}_2$ is prepared with the product dispersed with isopropanol and APTES and ultrasonic and reflux, to obtain the product centrifuged. Furthermore, citrate-covered AuNP

is synthesized with $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ reduced by sodium citrate or NaBH_4 , as shown in Figure 2. Then the citrate-covered AuNP was anchored to the SiO_2 surface at the NH_2 end by the following procedure. So that the SiO_2 @AuNP particles are obtained (Sun et al., 2018).

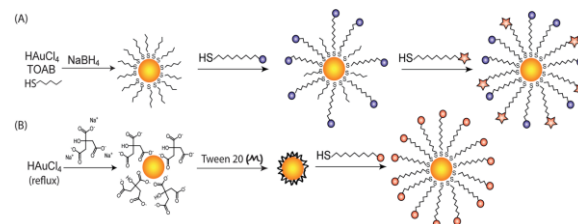


Figure 2. Schematic of the formation of gold nanoparticles (Yeh et al., 2012).

According to Firdaus *et al* (2020), to obtain silica nanoparticles, extraction methods from natural sand can be obtained. The beach sand is filtered using a 100 mesh sieve followed by immersion in acid to remove unwanted minerals other than silica. Then wash it so that the pH is neutral. Addition of base and heating for the alkaline fusion reaction with the K_2SiO_3 product. Filtration and addition of acid to obtain a white gel, then filtered, made the pH neutral and dried (Firdaus et al., 2020) Previous research by Eddy *et al* (2015), powder silica particles with a sieving machine obtained 325 mesh then soaking with acid, washing until there is no yellowish color. Drying at 110° C and reacted with alkaline, then filtered and allowed to stand the gel for 18 hours. Purification by distillation, finally drying (Eddy et al., 2015).

The stages after synthesis are characterization of size and morphology. In general, gold silica nanocomposites are characterized using UV-VIS Spectroscopy, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM). UV-Vis spectroscopy is light absorption, light radiation or electromagnets can be considered to resemble waves. When light falls on a compound, part of the light is absorbed by the molecules according to the structure of the compound's molecule. The absorption of light by molecules in the UV-Vis spectrum depends on the electronic structure of the molecules (Day & Underwood, 2002). Gold silica nanocomposites are formed from AuCl_4 absorbed in $\text{SiO}_2\text{-NH}_2$, where reduction occurs so

that it is identified through UV-Vis spectrophotometric analysis indicated by the presence of a maximum wavelength of around 500-600 nm originating from AuNP. The wavelength also depends on the particle size as can be seen in Figure 3.

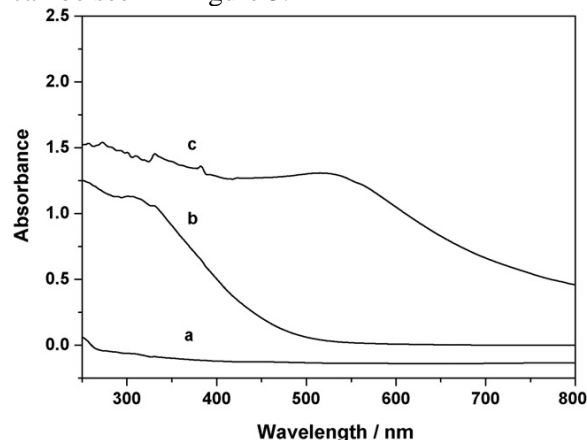


Figure 3. (a) The results for the $\text{SiO}_2\text{-NH}_2$ sample there is no absorbance in the 250-800 nm range, (b) After the addition of AuCl_4^- there is a peak at 305 nm, and (c) after the addition of the reducing agent so that the peak appears at 520 nm from AuNP (Bai et al., 2007).

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) characterization were performed as standards for identifying nanoparticles. TEM or SEM is a class of electron microscopy that uses electrons as a substitute for light, to study morphology, structure, particle size and distribution, and various forms including granules, phases, attached phases, and attached particles. SEM is also used to study the detailed structure of the cell surface, observe material in the macro and submicron regions, and observe objects in three dimensions. Meanwhile, TEM is used to observe the details of the cell's internal structure. SEM is an electron microscope that is capable of producing high resolution images of solid surfaces (Prasetyoko et al., 2016). The TEM morphologies of gold silica nanocomposite was showed in Figure 4.

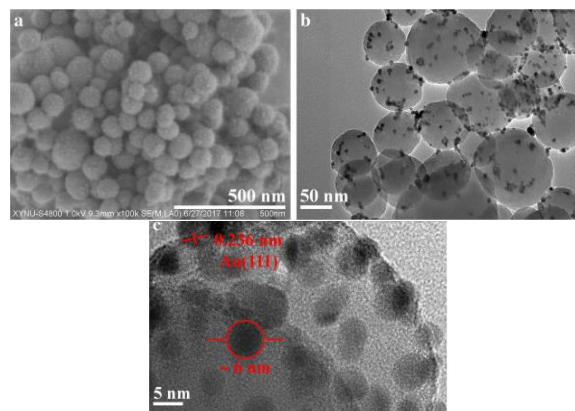


Figure 4. (a) TEM results show that the morphology of gold silica nanocomposites is uniform in size, (b) SEM results show that there are 95 nm of silica nanoparticles (light color) and 6 nm gold nanoparticles (dark color) which are uniformly distributed, and (c) results of HRTEM so that the interplanar distance is 0.236 nm (Sun et al., 2018).

The color difference between SiO_2 and Au is due to the difference in electron density between the two. Compared to Au, SiO_2 has a lower electron density, allowing more electrons to transmit. Characterization was carried out with high resolution TEM (HRTEM) as well as this can determine the location of the atoms in the sample.

CONJUGATION OF GOLD SILICA NANOCOMPOSITES WITH BIOMOLECULES

The conjugation between nanocomposites and biomolecules is also called bionanocomposite. In general, bionanocomposites are formed by a combination of two or more phases of different properties. The formed bionanocomposite acts not only as an immunological support agent but also a transducer. The binding agent is essential in the formation of bionanocomposites. Apart from facilitating the conjugation of biomolecules, the function of forming composites is to support the interaction of electrons between the solution and electrodes through the intervals. For the development of a competitive type electrochemical immunoassay, the preparation of bionanotags is essential. Gold silica nanocomposites interact strongly through gold and protein nanoparticles (Liu et al., 2011).

The ability of nanoparticles to be stabilized in water with size and shape is easily controlled because they are easy to function with biomolecules such as proteins, peptides, enzymes, antibodies and DNA. Gold nanoparticles that are reduced by citrate and react with peptides have biomedical applications such as diagnosis and therapy. The gold nanoparticle conjugation has good function and ability, with the biomolecules bind with a high sensitivity tinggi (Ojea-Jimenez & Puentes, 2010). The functionalization process of nanoparticles can improve their stability, functionality, and biocompatibility. The functionalization effect can also stabilize the properties of gold nanoparticles which are easy to aggregate due to the bound biomolecules (Mahon et al., 2012).

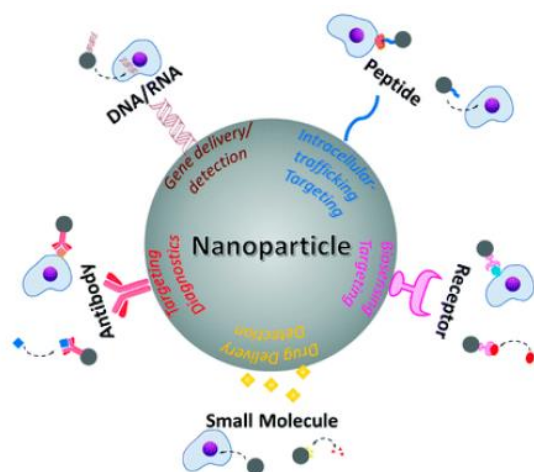


Figure 5. Biomedical application through the conjugation of nanoparticles with biomolecules (Graczyk et al., 2020).

There are two different strategies for protein conjugation to nanoparticles, first adsorption of biomolecules on nanoparticles by electrostatic / hydrophobic interactions or secondly directional binding using a linker (Figure 6a, b). In the case of the directed strategy, the hetero-bifunctional linker, hydrazide-polyethylene glycol-dithiol, is used to control the orientation of the molecules on the surface of the nanoparticles. Hydrazides are able to react with aldehyde groups which can be produced by oxidizing carbohydrate glycosylated proteins, such as antibodies. For this purpose, HRP antibody and carbohydrates are periodically

oxidized to attach the mentioned linker in the Fc region of the antibody. HRP and AuNP-modified antibodies, trigger covalent binding (Ciaurriz et al., 2017).

In Figures 6c and 6d, one approach is covalent conjugation in which the antibody and HRP are covalently bound to the surface using a PEG linkage via their free amine group using the EDC / NHS carbodiimide method. The second is the incorporation of a previously tested procedure, combining targeted binding of the antibody with HRP adsorption to the AuNP surface (alignment and adsorption procedure). For the covalent strategy we prepared a conjugation procedure according to previous work and DOE results. In the case of the directed / adsorption procedure, the protocol for directed Ab loading and the HRP concentration of the adsorption method are applied. As a result, ELISA was assessed to compare the proposed new strategies as well as the direct adsorption that has given good results (Figure 6c) (Ciaurriz et al., 2017).

The adsorption conjugation produced a better S / N response than Ab. However, the new conjugation strategy (covalent and directed / adsorption) yields worse S / N values than direct adsorption and even more than Ab-HRP, although it is explained that covalent and site-specific immobilization leads to a more stable and clearer composition conjugate. So it was found that the HRP molecule (Uniprot accession number P80679) showed a lower number of free amine groups (less lysine amino acid residues) than the antibody molecule. The lower availability of free amino groups may inhibit peroxidase adhesion in the covalent strategy (Figure 6c), although more experiments should be carried out to confirm this. Consequently, this will lead to lower peroxidase coverage and thus decreased ELISA increases. The combination of directional and adsorption strategies will be presented as the best alternative according to this. Considering that, the total amount of protein bound to AuNP might be higher by direct adsorption methods than other strategies, resulting in lower protein denaturation and higher S / N ratio (Ciaurriz et al., 2017).

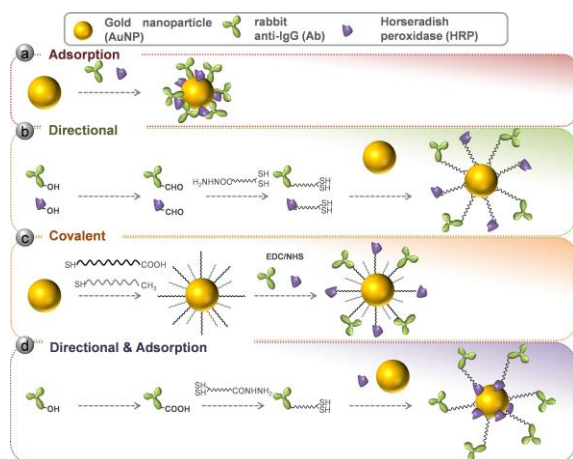


Figure 6. There is a scheme of 4 functionalization methods. (a) direct adsorption, (b) targeted conjugation with antibody control and HRP orientation, (c) covalent conjugation via antibody and HRP amine groups, and (d) combined directional and adsorption strategies for antibody and HRP (Ciauriz et al., 2017).

According to the thiol group linker, the Au-NP-DNA conjugation can also occur through the addition of the streptavidin-biotin linker. The streptavidin-biotin interaction is the strongest non-covalent interaction in nature, because it has a high affinity for biotin. Where streptavidin-biotin as a linker to DNA or aptamer, the stability of AuNP increases. Streptavidin binds to AuNP through adsorption because of the negative charge of AuNP which has been stabilized by citrate so that it binds to the positive charge of the amino acid streptavidin residue. Therefore, AuNP can bind to DNA or aptamer which biotinylated streptavidin-biotin bonds specifically such as ligands (Zhou & Rossi, 2014).

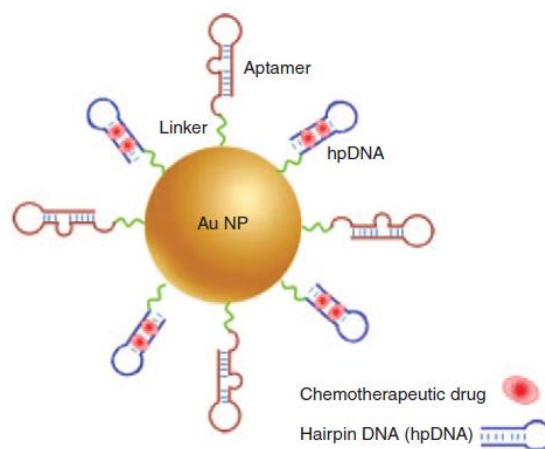
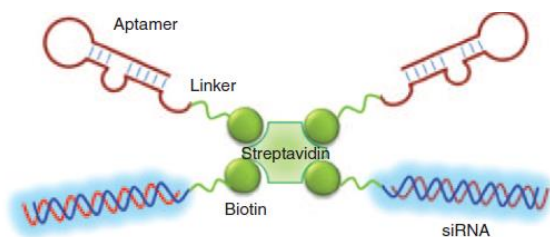


Figure 7. An AuNP-streptavidin-biotin-aptamer conjugation occurred (Zhou & Rossi, 2014).

Characterization of AuNP showed that gold nanoparticles were successfully synthesized. The color of the gold nanoparticles depends on the size of the nanoparticles, and the wavelength of the surface plasmon absorption increases with increasing nanoparticles. There is a linker that can connect the AuNP with the target. The $-SH$ of the linker through the interaction between the $-SH$ terminal group and the Au and directly emulsified on the AuNP surface. Furthermore, the other end of the $-COOH$ group can form a peptide bond with the target group $-NH_2$, thus forming a bioconjugate. Modification and characterization of surface electrode morphology with gold silica nanocomposite and bioconjugate by SEM by comparing before and after modification. Voltammetry characterization will show changes in the peak of the modified electrode current, due to the electron transfer from the redox system (can be from $[Fe(CN)_6]^{4- / 3-}$). Due to the presence of bioconjugates, the peak currents decrease due to the biomolecules covering the surface of the electrodes inhibiting electron transfer. So compared to the bare electrode peak, there will be a decrease in the peak current, as well as when the target biomolecule is added (Hartati et al., 2020).

APPLICATION OF GOLD SILICA NANOCOMPOSITE IN ELECTROCHEMICAL BIOSENSORS

Electrochemical biosensors can be classified into amperometric, impedimetric, potentiometric and conductometric biosensors

according to the observed data type, such as current, impedance, potential and conductance, respectively. The biosensor has the principle of utilizing a self-assembled monolayer (SAM) modified electrode surface, because with a favorable substrate and site it can bind to biological recognition elements via chemical groups (such as salines, thiols, acid, disulphides, or amines) on the electrode surface of electrode (Z. Zhang et al., 2019). The operating principle of electrochemical biosensor is shown in Figure 8.

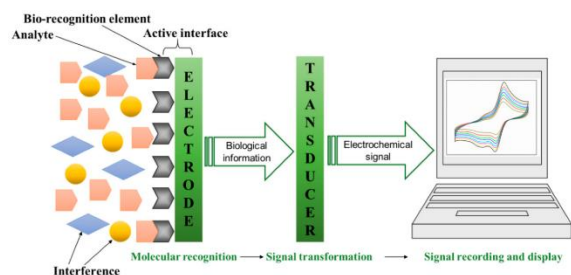


Figure 8. Schematic of an electrochemical biosensor (Z. Zhang et al., 2019).

The bio-recognition element is a core component of the electrochemical biosensor on the surface of the electrode. Biosensors can selectively identify target molecules and capture them to the electrode surface, due to the specific recognition of biological elements. The electrode obtains an identification signal from the surface of the electrode which is converted into an electrical signal, in the form of current, voltage, and resistance, which can be measured and analyzed for qualitative or quantitative analysis of the analysis target. (Z. Zhang et al., 2019).

Voltammetry is an electrochemical method in which a current is observed at a given potential. Cyclic voltammetry is a qualitative and quantitative analysis technique that can provide information about the results of reactions that occur in electrochemical cells. In cyclic voltammetry, the current response is measured as a function of potential (voltage), where the potential application is done back and forth, so that information on reduction and oxidation can be observed properly (Gosser, 1993).

The types of silica based on their biosensing application is shown in Figure 9. That Section (1) Types of SiNP for delivery of biologically active agents and drugs, (2)

Targeting parts on the surface of a SiNP or magnetic composite, (3) SiNPs respond to stimuli eg pH, glutathione, magnetic field, light and temperature, (4) SiNP for optical, magnetic resonance, and other bioimaging applications (Mebert et al., 2017).

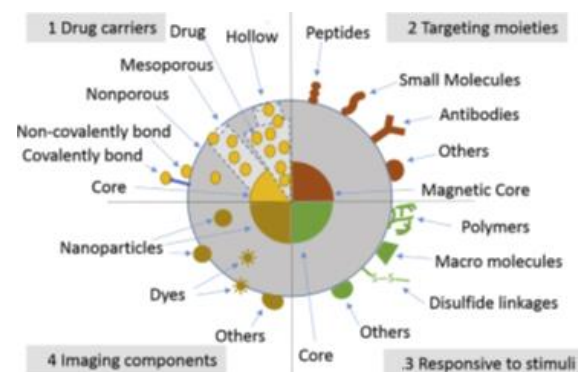


Figure 9. The types of silica based on their biosensing application (Mebert et al., 2017).

The application of gold silica nanocomposites in electrochemical biosensors has been developed as enzymatic biosensor for glucose detection the enzyme IO_4^- - oxidized-glucose oxidase (IO_4^- - oxidized-GOD) immobilized onto a gold electrode modified 2-aminoethanethiol as a cross-linker. It was analyzed using cyclic voltammetry (CV) and amperometry, so as to know the catalytic behavior of glucose oxidation (Bai et al., 2007). This mechanism can be shown in Figure 10.

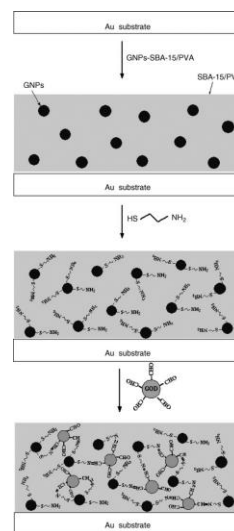


Figure 10. Biosensor Construction (Bai et al., 2007).

According to Figure 10, the activated Au electrode is then rinsed clean, then dripped with gold silica nanocomposites. After drying, dipped in 2-aminoethanethiol for 2 hours to add the amine group and dried. After the modified electrode, dipped in PBS solution and IO_4^- -oxidized-GOD resulting in covalent bonds and schiff-bases bonds of the -CHO group with $-\text{NH}_2$ from 2-aminoethanethiol efficiently increase the stability of the enzyme biosensor. Then formed, a gold electrode / nanocomposite $\text{SiO}_2 @ \text{Au} / \text{IO}_4^-$ -oxidized-GOD is adsorbed using a cross- GOD resulting in covalent bonds and schiff-bases bonds of the -CHO group with $-\text{NH}_2$ from 2-aminoethanethiol efficiently increase the stability of the enzyme biosensor. Then formed, the $\text{SiO}_2 @ \text{Au} / \text{IO}_4^-$ -oxidized-GOD electrode gold / nanocomposite was adsorbed using the 2-aminoethanethiol cross-linker (Bai et al., 2007).

As a result, the biosensor showed a very good bioelectrocatalytic response to glucose with a fast response time of less than 7 seconds, a wide linear range of 0.02–14 mM, a high sensitivity of $6.1 \mu\text{A mM}^{-1} \text{cm}^{-2}$, and long term stability and reproducibility. the good one. The performance of gold silica nanocomposites makes for excellent conductivity, large surface area, efficient enhancement of electron transfer between enzymes and electrodes and good biocompatibility. Thus, these nanocomposites are considered to be the ideal matrix for immobilization of biomolecules (Bai et al., 2007).

Furthermore, Wang *et al* (2017) used an aptamer RNA-based electrochemical aptasensor to detect C-reactive protein using gold silica nanocomposites as an immunoprobe. Biosensors based on aptamer as a recognition element are called aptasensors. Aptamer is synthetic oligonucleotide RNA or single-chain DNA selected through an in vitro method known as Systematic Evolution of Ligands by EXponential enrichment (SELEX) which can bind to its target with high affinity and specificity due to its 3-dimensional structure (Rajabnejad et al., 2020). Immunoprobe is an antibody that is conjugated with an enzyme to become an Ab-enzyme conjugate. C-reactive protein (CRP) is a widely

accepted biomarker of cardiovascular disease and inflammation.

The RNA applicator is specific to CRP on the glassy carbon electrode (GCE) surface modified with gold nanoparticles. The affinity bonding occurs via the gold-sulfur nanoparticles from aptamer. With the sandwich-type aptasensor method, after the aptamer was specific for CRP then the addition of the immunoprobe. The immunoprobe content is an anti-CRP (Ab) dispersion with gold silica nanocomposites to form a bioconjugate, BSA to block the inactive side and Zn^{2+} (Wang et al., 2017). This mechanism can be shown in Figure 11.

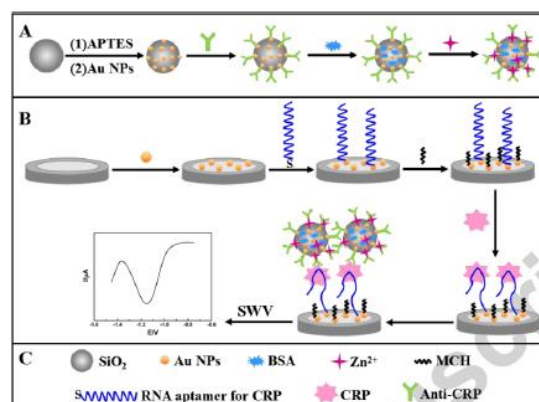


Figure 11. Biosensor Construction (Wang et al., 2017).

According to Figure 10, prior to modification GCE bare was activated. Electrodeposition of the electrodes with gold nanoparticles (AuNPs / GCE). Then immersed in aptamer, this process aptamer specific RNA CRP occurs affinity bond S-Au with gold nanoparticles denoted as RNA / Au NPs / GCE. The addition of MCH, so that the electrodes are modified MCH / RNA / Au NP for fabrication. Then incubated for the concentration of CRP or sample. After that, it was incubated again with immunoprobe (Zn^{2+} / Ab / Bioconjugate AuNPs / SiO_2). Recording the response signal from aptasensor with voltammetry (Wang et al., 2017).

Gold silica nanocomposites are used as carriers and signal enhancers. Sensing performance including sensitivity, selectivity and reproducibility were analyzed. Square wave voltammetry (SWV) is used to record the sensing

signal, and clearly the reductive peak corresponds to Zn^{2+} around -1.16 V (vs. SCE). Under optimal conditions, the aptasensor shows a wide linear range (0.005 ng mL^{-1} to 125 ng mL^{-1}) and the lowest detection limit ($0.0017 \text{ ng mL}^{-1}$). This aptasensor has good selectivity so that real serum samples show good results. that the sensor has potential real application capabilities (Wang et al., 2017).

Following are some of the reported applications of gold silica nanocomposites for various analyte targets in the electrochemical biosensor are shown in Table 1. Based on Table 1, it is known that gold silica nanocomposites can bind well with various sensing elements or increase the biosensor signal, so that it has good sensitivity.

Table 1. Use of SiO_2 / AuNP nanocomposites in electrochemical biosensors in 2007-2019

Electrode	Nanocomposites	Bioreceptor	Target	LOD (CFU/mL)	References
Screen-Printed Electrode (SPE)	AuNPs / silica nanoparticles with amine function	Aptamer	Tryptophan	Tryptophan	(Hashkavayi et al., 2017)
Glassy Carbon Electrode (GCE)	AuNPs-GO modified GCE SiO_2 @Ag/DNA	DNA	Breast cancer susceptibility gene (BRCA-1)	2.53 fM	(You et al., 2018)
Gold Electrode (GE)	Mesoporous silica thin films (MSF) Au electrode	DNA aptamer	Prostate Specific Antigen (PSA)	280 pg mL^{-1}	(Argoubi et al., 2018)
Glassy Carbon Electrode (GCE)	SiO_2 @Au	Enzyme	Diuron	51.9 nmol/L	(Sun et al., 2018)
Glassy Carbon Electrode (GCE)	SiO_2 @MoSe ₂ AuNPs	GO- DNA	DNA	0.068 fM	(Shuai et al., 2017)
Glassy Carbon Electrode (GCE)	MCM-41-Au	Biotinylated peptide	Caspase-3	5 fM	(Khalilzadeh et al., 2016)
Gold Electrode (GE)	nanocomposite SiO_2 @Au/ IO_4^- -oxidized-GOD/2-aminoethanethiol	Enzyme	Glukosa	15 μM	(Bai et al., 2007)
Glassy Carbon Electrode (GCE)	MCH / RNA / Au NP/ immunoprobe (Zn^{2+} /Ab/ Bioconjugate AuNPs/ SiO_2)	RNA aptamer	CRP	$0.0017 \text{ ng mL}^{-1}$	(Wang et al., 2017)
Screen-Printed Electrode (SPE)	DNA/HSM/AuNPs/SP E	DNA	<i>E. coli</i>	$1.95 \times 10^{-15} \mu\text{M}$	(Ariffin et al., 2020)
Screen-Printed Gold Electrode (SPGE)	SiNWs-DTPA-AuNPs	DNA oligomers	DNA oligomers in the dengue virus	$1,63 \times 10^{-12} \text{ M}$	(Rashid et al., 2015)
Gold Electrode (GE)	AuNP/ Silica/ Biotin/ Streptavidin/ aptamer	Aptamer	adenosine triphosphate (ATP)	400 μM	(Carrasquilla et al., 2011)

Glassy Carbon Electrode (GCE)	MSN/AuNP/Aptamer	Aptamer	Thrombin	0,5 pg mL ⁻¹ (≈15 fM)	(Tang et al., 2012)
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CONCLUSION

The gold silica nanocomposite is excellent for applications in electrochemical biosensors, and is still being developed to date. This shows that the potential use of silica gold nanocomposites is good enough to continue to grow. From this reviews, there are improvements in the synthesis and construction methods of biosensors. Judging from the advantages of complementing each other in gold and silica nanoparticles, its application to electrochemical biosensors can continue to increase.

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