Nanomed Res J 9(2):103-119, Spring 2024

REVIEW ARTICLE

New Approaches in Electrochemical Nanosensors and Biosensors for Intracellular Thiols Detection

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ARTICLE INFO

Article History:
Received 14 Apr 2024
Accepted 25 May 2024
Published 01 Jul 2024

Keywords:
Biothiols
Electrochemical
Biosensor
Nanosensor
Aptamer

ABSTRACT

Biothiols are the main part of different proteins and matabolites which have tremendous impact on the neutralization of oxidative stress in the cell and stability of the intracellular environment. Metabolite perturbation of these compounds can be indicative of many serious diseases such as weight loss, liver damage, cardiovascular diseases and Alzheimer's. Thus, tremendous researches have been devoted on the detection of thiols via biossensors and nanosensors using electrochemical methods. Higher sensitivity, selectivity, catalytic activity, conductivity, and biocompatibility are the advantage of nanomaterial-based electrochemical sensors. With recent advances in nanotechnology, creating new electrode materials with novel chemical and physical properties has become more accessible using nanoparticles (NPs). The progress of NP-based electrochemical sensors and its applications for biothiols detection was reviewed in this article. The aim of this effort is to provide the reader a view of the main functions of NPs in conventional and miniaturized electrochemical sensors. As well as, the novel and significant development of biorecognition elements used in conjunction with nanosensors were reported, which motivate more interests in targeted detection of thiols in further studies. The references selected based on the following characteristics: appropriate signal amplification, minimum detection limit, and simultaneous-detection capabilities toward thios detection.

How to cite this article
Ebrahimiasla S., Zamanfar M., Badr A. New Approaches in Electrochemical Nanosensors and Biosensors for Intracellular Thiols Detection. Nanomed Res J, 2024; 9(2): 103-119. DOI: 10.22034/nmrj.2024.02.001

INTRODUCTION

Biothiols including Homocysteine(Hcy), Cysteine (Cys) and Glutathione (GSH) have many significant functions in the biological systems and involved in a variety of diseases and medical conditions[1]. Cysteine is an essential amino acid that plays role in protein synthesis, metabolism, and detoxification. It is also a biomarker for diagnosis of several disease associatedphysiological regulator[2]. Glutathione is the most abundant intracellular thiol that play a crucial role in cellular defense[3] and related with several disease including AIDS[4], liver damage [5], cancer [6] and neuro-degenerative diseases [7-8]. Homocysteineis an amino acid that formed during methionine metabolism[9]. Increase in homocysteine concentration is associated with cardiovascular disease such as coronary artery[10]. So identification of the biothiols and its concentration in blood serum is very important in medical diagnosis.

There are several analytical techniques have been reported for the detection of biothiols. These contains mass spectrometry, gas chromatography and high performance liquid chromatography [11] capillary electrophoresis [12], immunoassay[13], fluorescent methods [14-15] and other methods which are commonly time consuming and costly. Amongthem, electrochemical and fluorescent methods were considered in recent studies for simple operation and low cost.[16-17].In electrochemical methods, potential versus current was measured

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during an electrochemical reaction. Regarding different aspects of measuring or controlling of the cell, it divided to potentiometry, coulometry and voltammetry [18]. Electrochemical methods have more advantages such as being portable, easy-to-operate, sensitive, economical, and simple-to-construct and recently attracted more attention for biomedical application.

This review concentrated in recent developments in electrochemical biosensors and nanosensors for detection of biothiols. It can provide a comprehensive and vivid view of new strategies for modification of electrodes to improve sensitivity and selectivity of biothiols using nanostructures.The results of studies are summarizes for mechanism, limit of detection (LOD), dynamic range and fordiagnosis of biothiols to open up newavenues and methods for future research.

Electrochemical nanosensors for biothiols detection

To promote sensitivity and selectivity of electrochemical sensors for biomoleculs detection, modification of electrodes using nanostructures have been widely expanded. Due to antibacterial activity, high thermal and electrical conductivity of nanoparticles, their use in electrochemical sensors has increased in recent years. The electrode surface modify with nanostructures to detect the desired analytes[19-22]. Different structures of these materials such as nanoparticles, nanosheets, nanocomposites and CNTs are developed for biological sensors. Chengzhou et al. reviewed electrochemical nanosensors and nanobiosensors [23]. The perspectives and current advances of AuNPs in biology and medicinewere discussed by Dykman et al.[24] Siangproh et al. reviewed electrochemical detection methods for conventional and miniaturized systems based on nanoparticles used in bioanalytical applications [25]. Following are some examples of electrochemical nonosensorsincluding CNT, AuNP, nanocomposte, quantum dot, AgNp and so on for detection ofbiothiols.

Carbon nanotube(CNT)

Carbon nanotubes are commonly classified as single-walled (SWNT) and multi-walled nanotubes (MWNT). A single-walled carbon nanotube is just like a uniform fibre. The electrochemical and adsorption properties of nanotubes are origin of their uniquelylarge unit surface and weight concentrated in its surface layer which makes them a promising primary ingredient for the development of biological sensors. [26-27]Various techniques and different electrodeswereused to study novel and sensitive carbon nanotube modified electrochemical sensors for biothiols detection which are presented in Table 1 in summery. Chronoamperometric, cyclic voltammetric, and square wave voltammetric methods were used by Gholami et al. to expolre the suitability of chlorpromazine as a intermediary at the surface of MWCN paste electrode for the electrically oxidation of Hcy in aqueous solutions. The detection limit and dynamic range for this modified electrode were reported as 0.08 μM and (0.1–210.0 μM) respectively[28].Silva et al. reported a nano hybrid structure modified with MWCNs and Au nanorods based on GCE for detection of Cys.The result show a LOD of 8.25 nmol L^{-1}, a sensitivity of 120 nA L $\mu mol^{-1,}$ and a linear response range of 5.0 up to 200.0 μmol L^{-1} for detection of Cys [29]. Deng et al., was developed B-doped CNT-modified glassy carbon electrode (GCE) for electrochemical detection of (L-cys teine) L-cys . A comparison of detection response for this electrode to bare GCE or the CNT/GCE (within 7 s) was faster and demonstrated a low LOD of 0.26 ± 0.01 μM and a high sensitivity of 25.3 ± 1.2 nA mM^{-1} [30].

In the following different redox mediators were used to improve electron transfer reaction of the thiols. Modification of electrodes using such mediators improved the detection limit of biothiols as revealed in Table 1 and (Fig. 1).[31-33] Pretreatment of electrodes was the next strategy to improve nanosensors sensitivity toward biothiols detection [34].Functionalization of CNTs is remarkably important for the improvement of performance, sensitivity and selectivity of electrochemical biosensors. Zhang et al. described on the efficiently improvement of electrocatalysis of glutathione by functionalization of CNTs [35].

Gold nanoparticle (AuNP)

Gold nanoparticle,isone of the most commonly used metal nanoparticles, which have been applied in the identification of chemical and biological agents.Gold nanoparticles have unique surface plasmon resonance, narrowsize distribution and convenient labeling of biomolecules. Regarding this properties it can bind to amine and thiol groups of biomolecules in surface modified electrodes and biosensors [43]. Liu et al**.** reported a novel Au-NPs/

Table 1. Summaryof the recent researches on the use of CNT to promote sensitivity of biothiols detection using electrochemical methods.

Analytes	Electrodes*	Methods**	Materials	Modifier (mediator)	LOD	LDR	Ref.
Cys	GCE	CV, AMP	Boron doped CNT	-	0.26±0.01μM	7.8×10^{-7} to 2×10^{-4} M	[30]
Hcy	CPE	CV, CA	MWCNT	-	3.3μM	5.0-800μM	[36]
	CPE	CV, CA,SWV	CNT	Benzoylferrocene	50 nM	0.1-10μM	[31]
	GCE	CV, SWV	CNT	Catechol	660 nM	-	[16]
GSH	GCE	CV, DPV	MWCNT	Poly(bromocresol green)	1.0×10^{-8} M	2×10^{-7} to 5×10^{-6} M	[33]
	GCE	CV	Functionalized CNT	-	0.2 pM	-	
	CPE	CV, CA	MWCNT	3,4-Dihydroxycinnamic acid	0.1μM	0.5-400μM	[37]
	CPE	CV, DPV	CNT	(E)-3-((2-(2,4 dinitrophenyl)hydrazono)methyl) benzene-1,2-diol	70nM	0.4-700mM	[38]
	CPE	CV, DPV	MWCNT	Ferrocenedicarboxylic acid	0.2mM	0.5-24mM	[39]
	SPE	CV, SWV	CNT	-	-	-	[35]
Cys+Hcy	GCE	CV, SWV	CNT	Catechol	11nM-Hcy 5mM-Cys	0-0.1mM	[40]
	Preheated GCE		CNT	-	25nM	0.1-0.8μM	[34]
GSH+Hcy	SPE	CV	CNT	Catechol	1.2μM-Hcy 0.11μM –GSH	-	[32]
	SPE	CV	MWCNT	Catechol	0.9 M -Hcy 2M- GSH	-	[41]
Cys+GSH	GCE	CV	CNT	-	0.89±3.53M-Hcy	2.5M-10M	[42]

*Type of electrodes were used; (ITOE): Indium Tin Oxide Electrode, (SPCE): Screen Printed Carbon Electrode, (GE): Graphite Electrode, , (GF): Graphite Felt, (CPE): Carbon Paste Electrode.
Metods; (CV): Cyclic Voltammetry, (DPV): Differential Pulse Voltammetry, (AMP): Amperometry,(SWV): Square Wave Voltammetry.

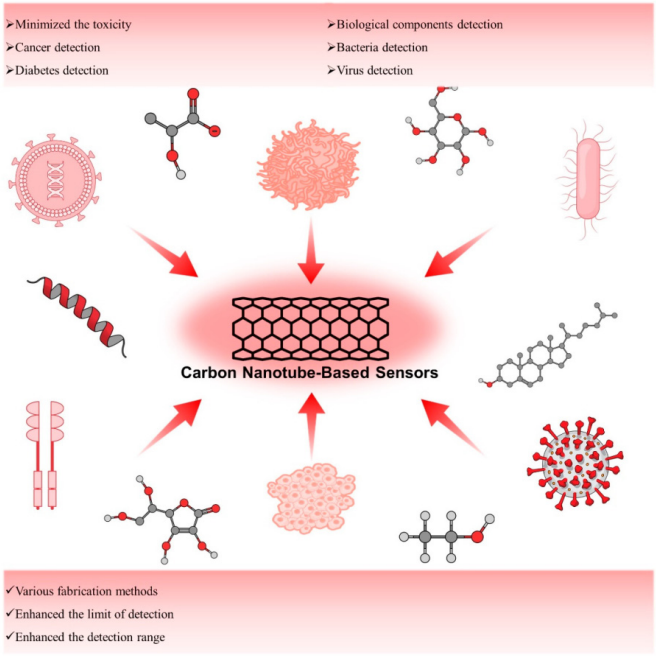

Fig. 1. Schematic of CNT-based biosensors[113].

poly-eriochrome black T film modified GCE for the characterization of L-cys . They used FTIR spectra and electrochemical impedance spectroscopy to investigated polymerizationof Au-nanoparticles/ poly-eriochrome black T film on the surface of glassy carbon electrode. The results indicated decrease in the charge transfer resistance value of electrode and improve in the electron transfer kinetic between analytes and electrode. The oxidation currents of analytes increased four times in modified electrode compared to bare glassy carbon electrode. A linear increase was observed in the electrocatalytic currents with L-cys concentrationsin the range of 0.05–100 µM. The LOD of 8 nM was detected for modified GCE [44]. Taei et al., was used AuNPs/ poly(E)-4-(p-tolyldiazenyl) benzene-1,2,3-triol (PTAT) film for modification of GCE. FE-SEM images of the nanogold modified GCE obtained by electrodeposition of 0.4 g L^{-1} $HAuCl_4$ at the voltage of −200 mV are presented in (Fig. 2). The result of electrochemical impedance spectroscopy indicated that the charge transfer resistance of bare electrode increased by modification of PTAT on the surface of electrode. Electrodeposition of AuNPs enhanced kinetics of electron transfer between analytes and electrode. A linear changes in electrocatalytic current was detected in the ranges of 2–540 µmol L^{-1} with a LOD of 0.04 µmol L^{-1} for Cys [45]. Devasenathipathy et al. reported a GCE modification with AuNPs. They used calcium-crosslinked pectin for stabilization and electrodeposition of AuNPson multiwalled carbon nanotubes. They used cyclic voltammetric (CV) method for electrodeposition and used the modified electrode for the selective characterization of L-cys . The result demonstrated that calcium-crosslinked pectin significantly increase stability, electrochemical activity and uniformity of the AuNPssuch as a scaffold. The modified glassy carbon electrode showed a minimum overpotential with a maximum oxidation peak current and optimum electrocatalytic activity for oxidation of L-cys . A linear range (LR)of 0.1 to 1,000 µM, high sensitivity of 0.46 µA μM^{-1} cm^{-2} and a LOD of 19 nM was measured for this optimized electrochemical sensor. The diffusion coefficient of 3.0×10^{-6} cm^2 s^{-1} was determined for the oxidation of L-cys . This amperometric sensor was able to specifically characteriz L-cys in the presence of high volume of interferes. Good repeatability and

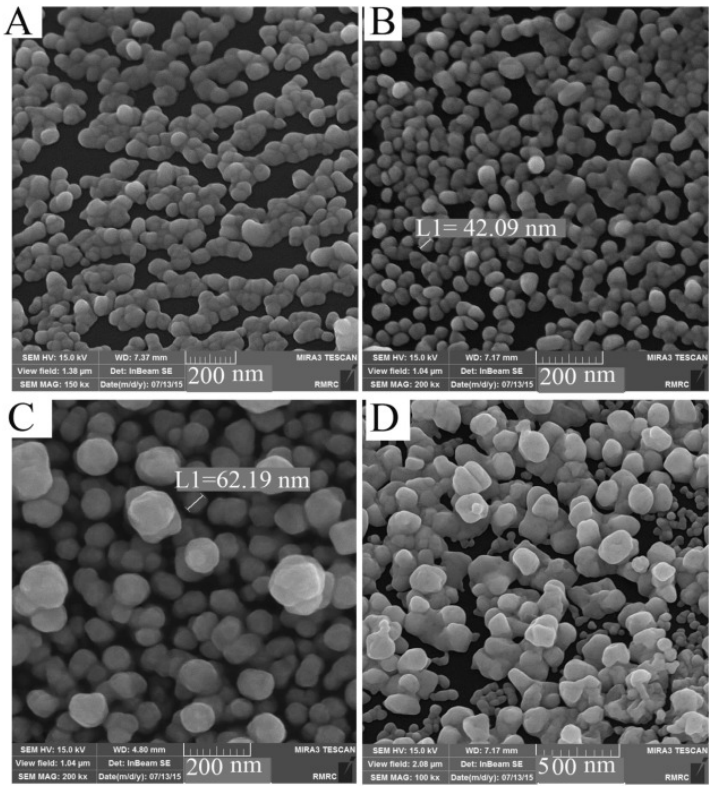

Fig. 2. FE-SEM images of the electrodes: the AuNPs coated GCE achived by electrodepositing at the potential of −200 mV in 0.1 mol L^{-1} KNO_3 and 0.4 g L^{-1} $HAuCl_4$:(A) 300s; (B) 250s; (C) 200s; and (D) 100s [45].

Table 2. Summaryof the recent reports on the application of gold nanoparticles to improve sensitivity of biothiols detection using electrochemical methods

Analytes	Electrodes*	Methods***	Materials**	LOD	LDR	Ref.
Cys	GCE	CV, AMP	AuNPs/MWCNT-Calcium-crosslinked pectin	19nM	0.1 to 1,000 μM	[46]
	GCE	AMP	AuNPs/PEBT	8 nM	0.05–100 μM	[44]
	GCE	CV, DPV	AuNPs/PTAT	0.04 μM	2–540 μM	[45]
	GE	CV	AuNPs	0.6 pM	1 to 14 pM	[47]
	Nanoporous gold	CV, AMP	-	50nM	1 -400 μM	[49]
Hcy	CPE	SWV	AuNPs/MWCNT	-	-	[48]
GSH	GE	CV	AuNPs	15 ·10-3 M	-	[50]
	CPE	DPV	AuNPs- MWCNT	-	25-800μM	[51]
Cys+GSH	Nano-Zeolite	CV, DPV	AuNPs	0.3nM-Cys 0.6nM-GSH	2nM-800μM-Cys 3nM-800 μM-GSH	[52]

*Type of electrodes were used; (GCE): Glassy Carbon Electrode, (GE): Graphite Electrode, (SPCE): Screen Printed Carbon Electrode, (MWCNTPE): Multi Walled Carbon Nanotube Paste Electrode, (CPE): Carbon Paste Electrode,
**Metods; (CV): Cyclic Voltammetry, (DPV): Differential Pulse Voltammetry, (AMP): Amperometry,(SWV): Square Wave Voltammetry.
**Materials; (PEBT): Poly-eriochrome black T, (PTAT):poly(E)-4-(p-tolyldiazenyl)benzene-1,2,3-triol, (NPG): Nanoporous gold

reproducibity and stability were obtained for this modified sensor. Application of this new sensor in spiked speciemens of L-cys in blood serum was successful [46]. Graphite electrode modification with AuNPs was reported by Perevezentseva et al., in electrochemical study of Cys using CV method. The electrocatalytic properties regard the oxidation of Cys was observed in 0.1 M of NaOH. A revers maximum was located in the cathodic voltammogram of Cys at voltage of 0.05. A linear response in the range of 1 to 14 pM was observed for the peak current of the revers maximum versus cysteine volume. The low LOD of 0.6 pM offers this easy, fast and sensitive method as a good candidate for cysteine detection [47].

Hung et al., reported electrodeposition of AuNPs on MWCNs on a carbon paste electrode for Hcy characterization. Homocysteine oxidation signal significantly increase in modified electrode. The constructed nanosensor offers a high sensitive model for biosensingthiol-containing molecules. The result indicated that modification of electrode using AuNPs increase the electrode's active surface area and consequently higher sensitivity. Three times larger signal was obtained for modified carbon paste electrode. Square wave voltametric measurements represented average peak potential of 8.2×10^{-1} V, 7.7×10^{-1} V and 6.2×10^{-1} V for carbon past electrode, CNT/carbon paste electrode and gold electrodeposited CNT/carbon paste electrode in continous [48]. A review of the recent reports on the application of AuNPs for the detection of biothiols by electrochemical methods are presented in Table 2.

Nanocomposite material

A multiphase solid material with dimensions less than 100 nm in one, two or three direction or nano-scale repeating distance in different phases named as nanocomposite. Three different categories wereconsidered for nanocompoite based on their matrix materials. Polymers, metals and ceramics are three types of matrix for nanocomposites[43]. Deng et al. used a two-dimensional ternary nanocomposite of magnetite, , reduced graphene oxide (GO) and platinum for modification of GCE. They were used amperometric, cyclic voltammetric, differential pulse voltammetric methods and double potential step chronoamperometric in electrochemical detection of L-cys . Diffusion coefficient and reaction rate constant were calculated as 9.96×107 cm^3 mol^{-1} s^{-1} and 7.41×10^{-7} cm^2 s^{-1} respectively. The best voltage for the operation of developed sensor was obtained at 0.65V versus saturated calomel electrode in 0.1 M of NaOH solution. The LOD for L-cys was 1.0×10^{-5} M. Due to the application of various components and their synergistic effect constructed sensor shown acceptable stability, selectivity, repeatability and reproducibility and could be extensively

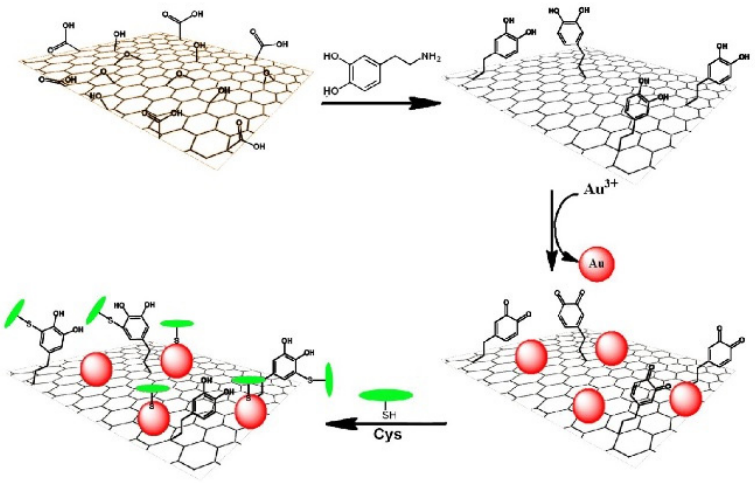

Fig. 3. Cys based in-situ synthesis of AuNps/reduced GO nanocomposites [53].

explored in the future studies[30].Zhang et al. reported modification of GCE using in situ synthesis of AuNP/reduced GO nanocomposites. (Fig.3) shows schematic in situ synthesis of AuNP/ reduced grapheme oxidenanocomposites.They concluded that Au-S bond between sulphydryl group of cysteine and AuNPwas increased the detection sensitivity(DS) regarding Cys. Detection limit and (LR)of detection for modified GCE was obtained 1.0×10^{-10} M (S/N = 3) and 1.0×10^{-9} to 3.0×10^{-8} M. The calculated linear coefficiency was 0.991[53]. Majd et al. was casted a thin film of manganese oxide nanoparticles /graphene nanosheets on GCE surface. Then MnOx was electrodeposited on modified electrode at applied constant potential. Catalytic activity of above mentioned modified electrode toward oxidation of L-cys was higher. A linear relationship with that of the L-cys concentration was observed in the range up to 120 mM with a detection limit of 75 nM and sensitivity of 27 $nAmM^{-1}$. The modified electrode was sensitive, stable and reproducible and open up new avenues for improvement of electrochemical sensors[54]. In the following Xiao et al. was synthesized one-dimensional (1-D) caterpillar-structure manganese dioxide–carbon nanocomposite by co-deposition with a chitosan hydrogel for development of GCE. The synthesized MnO_2–C nanocomposite had a good microstructure, conductivity and high specific surface area. The specific interaction between the solid MnO_2 and –SH group of L-cys was led to excellent catalytic activity and fast detection response (within 7 s). An optimum linear relation in the range of 0.5–680 μM was detected for logarithm of catalytic currents versus L-cys concentration. The LOD of 22 nM with good stability and reduced glutathione interference or other electroactive substances were the advantage of this developed electrode[55]. Hsiao et al. used poly(3,4-ethylenedioxythiophene (PEDT)) and AuNPs nanocomposite for modification of screen-printed carbon electrode. A thin layer of PEDT and AuNPs were electrochemically deposited on bare electrode in two consecutive stages. An excellent catalytic activity for the oxidation of Cys in different pH buffer solutions was observed for the modified electrode.A linear calibration curve with a slope of 0.115 μA/μM was observed for 0.5–200 μM of Cys in pH 4.0 buffer solutions using flow-injection amperometry. The LOD of 0.05 μM was observed for constructed nanosensor [56]. Hou et al, reported synthesis of encapsulated CoO nanoparticles on the surface of the ordered mesoporous carbon. They nanocompositewas utilized for modification of the GCE. This nanosensor exhibited strong and stable electrocatalytic activity towards glutathione, which concluded that the cobalt oxide nanoparticlesremarkably increase electrocatalytic properties of modified electrode.Amperometric determination of GSH gave linear responses over a concentration range up to $28\mu mol^{-1}$ with a sensitivity of $17.4\mu AmM^{-}$ and ^{1} LOD of 0.14 nM. They concluded that the result was more executive compare to previous reports which can be related to the acceptable combination of ordered mesoporous carbon and CoO nanoparticles [57]. Ethynylferrocene and NiO/MWCN nanocomposite

Table 3. Summary of the recent researches on the application of nanocomposites to enhance sensitivity of biothiols detection using electrochemical methods

Analytes	Electrodes*	Methods**	Materials	LOD	LDR	Ref.
Cys	GCE	CV	Pt-Fe_3O4/RGO	10 μM	0.10 to 1.0 mM	[60]
	GCE	DPV, CV	AuNPs/RGO	1.0×10^{-10} M	1.0×10^{-9} to 3.0×10^{-8} M	[53]
	GCE	CV	GNSs/MnOx	75 nM	Up to 120 mM	[54]
	GCE	AMP	MnO_2–C/Chitosan	22 nM	0.5 to 680 μM	[55]
	SPCE	FIA	PEDOT/AuNPs	0.05 μM	0.5 to 200 μM	[56]
	GCE	CV	GO–Au NCs	0.02 M	0.05 to 20.0 M	[61]
	GCE	CV	GO/CCNTs/AuNPs@MnO_2	3.4nM	1.0 × 10– 8 to 7.0 × 10– 6M	[62]
	GCE	CV	MoN/N-MWNTs	3.64 μM	5 μM– 0.79 mM an	[63]
	GCE	CV, DPV	GNR-Nafion	100 μM	25 to 500 μM	[64]
	GCE	AMP	YHCFNP/MWNT/Nafion	0.16 μM	0.20–11.4μM	[65]
	CPE	CV	PB–AuNP–Pd	0.18 μM	0.3 and 400 M	[66]
GSH	GCE	CV, AMP	OMC–Co	0.14nM	4 to 28 μM	[57]
	CPE	SWV	EF/NiO/MWCNT	0.006 M	0.01 to 200 μM	[58]
	CPE	SWV, CV	FePt/CNTs/DEDED	0.05μM	0.08 to 220 M	[59]
	GCE	CV, AMP	pCAF-NC	500 nM	-	[67]
	GCE	I-V	CdO.CNT NC	30.0 pM	0.1 nM to 0.01 M	[68]
	CPE	SWV, CV, CA	DMBQ/ZnO/CNTs	0.0008μM	0.002–720 μM	[69]
	CPE	SWV	ZnO/CNTs/BCB	0.002 μM	0.006–161μM	[70]
	CPE	SWV, CV, CA	NHPDA/FePt/CNTs	1.0 nM	0.004–340μM	[71]
	GCE	CV	CCNN	-	-	[72]
	ITOE	CV, AMP	annealed-Ni	5 μM	5 - 840 μM	[73]
	CILE	CV	NCHC	30 nM	-	[74]
	ALE	I–V	BT-NH_2 NPs	10 μM	10 μM–1mM	[75]

*Type of electrodes were used; (GCE): Glassy Carbon Electrode, (GC): Graphite Carbon, (SPCE): Screen Printed Carbon Electrode, (GF): Graphite Felt, (ITOE): Indium Tin Oxide Electrode, (MWCNTPE): Multi Walled Carbon Nanotube Paste Electrode, (CPE): Carbon Paste Electrode, (CILE): Carbon Ionic Liquid Electrode, (ALE): Aluminum Electrode.

**Methods were applied; (CV): Cyclic Voltammetry, (DPV): Differential Pulse Voltammetry, (AMP): Amperometry, (FIA): Flow-Injection Analysis, (SWV): Square Wave Voltammetry, (LSV): Linear Sweep Voltammetry, (DPSC): Double Potential Step Chronoamperometry, (ASV): Anodic Stripping voltammetry, (I-V):I-V Technic.

***Materials;

was utilized for modification of carbon paste electrode by Shahmiri et al. The modified electrode display a unique electrocatalytic oxidization of GSH. A linear increase at the ranges of 0.01–200 μM was detected for peaks current versus concentration with the LOD of 0.006 μM [58]. A high sensitive sensor of FePt/CN nanocomposite was synthesized by Moradi et al. for the detection of GSH in laboratory and actual specimen. A well-separated oxidation peak of glutathione dependent on its concentration was observed. The conversion in the peak currents value were linear with the concentration of GSH in the range from 0.08–220 M with a LOD of 0.05μmolL^{-1}[59].We reviewed some of the recent researches in biothiols detection using nanocomposites. In continues, summary of the further analyticalfigures for biothiolsdetectionare presented in Table 3, when using nanocomposites in voltammetry.

Quantum dot

Quantum dots are colloidal nanocrystalline semiconductorswith unique tunable photoluminescenceproperties due to quantum confinement effect with great potentials for advancement of novel chemical sensors and biosensors.Wang et al. was grafted a nanocomposite of polypyrrole and grapheme quantum dots@ Prussianblue on a geaphite substrate for electrochemical determination of L-cys . The study confirmed that electro-polymerization of polypyrrolewas improved the electrochemical

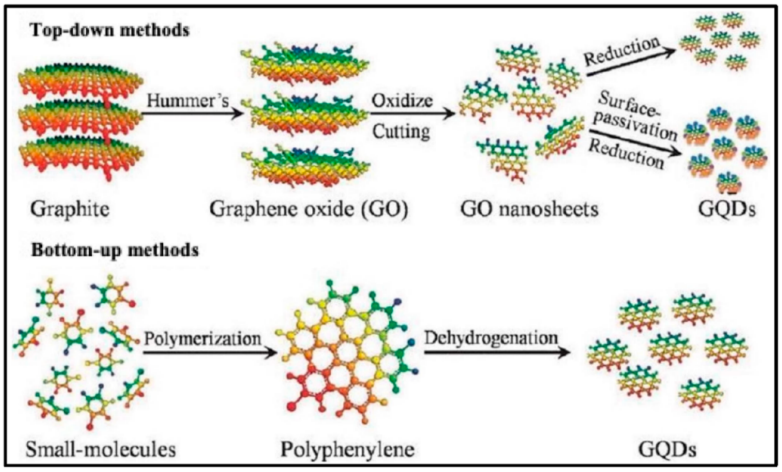

Fig. 4.Schematic of GQD synthesis using the top to down and bottom to up approach [114]

stability of the modified electrode. TEM, SEM, XRD, infrared spectroscopy(IR), and electrochemical methods were used for characterization of modified electrode.This electrode shows a considerable stability with lower response to interferences. The DL of 0.15 μM and DS equal to 0.41A $molL^{-1}$for a concentration range of 0.2–50 μmol L^{-1}, and equal to 0.15Amol L^{-1} for a concentration range of 50–1000 $\mu molL^{-1}$ was obtained for modified electrode, (Fig. 4)shows the quantum dot synthesis methods[76].

Silver nanoparticle (AgNP)

Recently, AgNP have been employed as enormous electrodes for energy and sensing applications taking advantages of its unusual features, electronic and physiochemical properties and vast specific surface area[77]. Sensitive and stable electrochemical biothiolssensing methods have been improved by monitoring the electrochemical current changes of the AgNP. The electrochemical current decreasedbybiothiol-induced aggregation of AgNP. In this regard Hu et al. reported on simple designed and fast operated optical and electrochemical detection of biothiols based on aggregation of AgNP. They concluded that the electrochemical method was more sensitive than that of colorimetric method (2 orders) for the detection of glutathionewith detection limit of 200 nM. They offered that it can be used in other target-induced aggregation assaysin biosensors [78]. The interaction of cysteine with citrate capped AgNP was formed cysteine capped nanoparticles by replacing cysteine to citrate capping agent on the AgNP. As a result cyclic voltamogram exhibited a decrease in oxidative peakcurrent with no changes in UV-vis spectra. So it was proposed that the silver surface was inactivated or AgNPwere detached from the electrode surface[79].A polydopamine capped AgNPwas used by Thota et al. for modification of indium tin oxide (ITO) electrode. Higher and larger selectivity was obtained by AgNP-polydopamine core–shell nanoparticles for cysteine in the presence of interferences. It was obtained dueto specific steric barrier, appropriate synergistic and redox properties of AgNP-polydopamine core–shell nanoparticles. The electrochemical properties of the modified electrode were determined using cyclic voltammetry and linear sweep voltammetry. Field emission scanning electron microscopy result revealed appropriate establishment of AgNP-polydopaminenanocomposite on the ITO electrodes. Electrochemical detection of cysteine was performed for modified electrode in 0.1 M phosphate buffer solution which was exhibited a linear calibration plot for cysteine in the concentration range between 0.05 μM and 300 μM.The sensitivity and detection limit were obtained as 0.023 μA μM^{-1} and 0.02 μM. The use of this biosensor in biological samples to determine cysteine showed suitable and promising results for future applications [80].

We reviewed all the recently used nanoparticles to notice biothiols such as glutathione، Hcy and cysteine.

In Table 1 and (Figure 5), recent reports on the use of nanosensors to enhance sensitivity for the detection of biothiols by electrochemical methods are summarized.The main disadvantage of nanosensors is presence of interferences and necessity of a dedicated agent to identify compounds

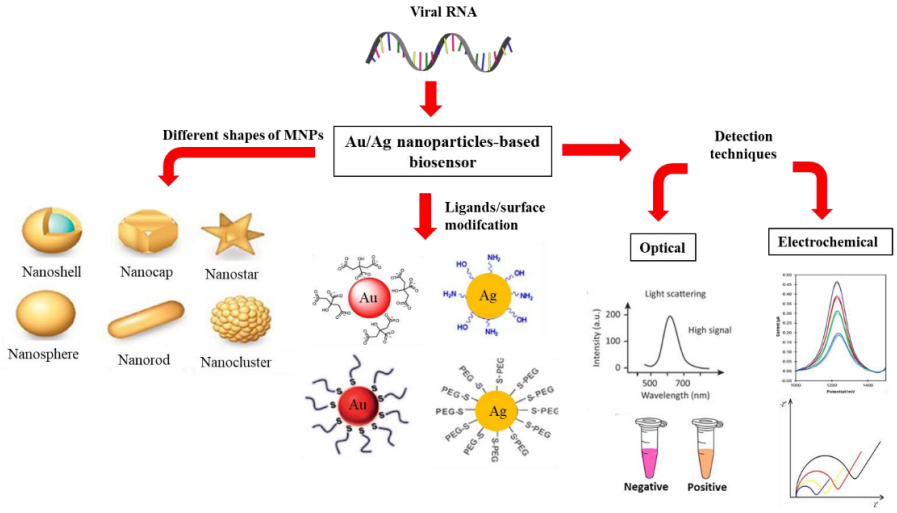

Fig. 5. Schematic form of two types of MNPs (AuNPs or AgNPs) and the use of ligands in their stabilization [115].

Table 4. Summary of the recent researches on the use of further nanoparticles to promote sensitivity of biothiols detection using electrochemical methods

Analytes	Electrodes*	Methods**	Materials***	LOD	LDR	Ref.
Cys	GCE	CV, AMP	Sb-doped ZnO NWs	0.025 mM	0.075 to 100 mM	[81]
	GCE	CV	$CuGeO_3$ nanowire	9×10^{-7}M	1×10^{-3} to 1×10^{-6} M	[82]
	GCE	CV	Au-SH-SiO_2@Cu-MOF	0.008 µM	0.02–300 µM	[89]
	CPE	AMP, FIA	n-Fe_2O_3@NaCo[$Fe(CN)_6$]	40nM	3.0–37 µM	[90]
	GC	CV	Pt/CNT	0.3 M	0.5 M–0.1mM	[83]
	GCE	CV	MF	7.4 nM	0.04–0.20 ppm	[91]
	PB	CV	FTO	25mM	200pM	[84]
HCY	GCE	DPV	N-GN–PtNPs	200 pM	-	[85]
	GCE	CV, AMP	ATD	51 pM	100 to 1400 nM	[92]
GSH	GCE	DPV	nickel(II) oxide	5 mM	0.2 to 6.0 mM	[86]
	GCE	CV, AMP	Cu2O/NiOx/GO	-	-	[94]
	CPE	CV, DPV	TiO2/ FCCa	0.098µM	0.1–12µM	[87]
	CPE	SWV	Ag–ZnO/CBF	20 nM	5.0×10^{-8} to 2.0×10^{-4} M	[93]
	CPE	CV	AF/NiO/NPs	0.09 µM	0.2 µM to 350.0 µM	[88]

*Type of electrodes were used; (GCE): Glassy Carbon Electrode, (GC): Graphite Carbon, (CPE): Carbon Paste Electrode.
**Methods were applied; (CV): Cyclic Voltammetry, (DPV): Differential Pulse Voltammetry, (AMP): Amperometry, (FIA): Flow-Injection Analysis, (SWV): Square Wave Voltammetry.
***Material were used to modified: (Sb-doped ZnO NWs): Sb-doped ZnO nanowires, (Au-SH-SiO_2@Cu-MOF): Au-SH-SiO_2 nanoparticles supported on metal-organic framework, (n-Fe2O3@NaCo[Fe(CN)6]): cobalt hexacyanoferrate nanoparticles with a core–shell structure (iron(III) oxide core–cobalt hexacyanoferrate shell, (Pt/CNT): platinum (Pt)/carbon nanotube, (MF): mercury film, (FTO): F-doped tin oxide, (N-GN–PtNPs): graphene supported platinum nanoparticles and nitrogen doped graphene supported platinum nanoparticles, (ATD):2-amino-1,3,4-thiadiazole,(nickel(II) oxide): nickel(II) oxide nanoparticles, (Cu_2O/NiOx/GO): Cu2O/NiOx/graphene oxide, (TiO_2/ FCCa): TiO_2 nanoparticles and ferrocene carboxylic acid, (Ag–ZnO/CBF):Ag–ZnO nanoplates and 2-chlorobenzoyl ferrocene, (AF/NiO/NPs): incorporating acetylferrocene (AF) and NiO nanoparticles.

containing thiols. Enzymes, aptamers, molecularly imprinted polymers and other elements have potential application as recognition elements.

Further nanomaterials

Other naomaterials, including Sb-doped ZnO NWs,[81]$CuGeO_3$ nanowire,[82] platinum (Pt)/carbon nanotube [83]F-doped tin oxide, [84] Pt nanoparticles reinforced with graphene and Pt nanoparticles with graphene doped with nitrogen,[85]nickel(II) oxide [86] and TiO_2 nanoparticles and ferrocene carboxylic acid[87] and incorporating acetylferrocene (AF) and NiO nanoparticles,[88] also have good potential in biosensor development due to goodsensitivity, stability and selectivity. The details of these electrodes are presented in Table 4 for a glimpse. For example, a new high-sensitivity nanosensor for detecting

electrocatalytic oxidation and determination of L-cys was developed by Hosseini et al. In this study, Au-SH-SiO_2 nanoparticles supported on metal-organic framework (Au-SH-SiO2@Cu- MOF) is used with microstructural and morphological studies.The modified electrode revealed a very efficient electrocatalytic activity for the oxidation of L-cys with low oxidation over-potentials and high oxidation peak currents. The (LR) of detection was observed in the range of 0.02–300 μM and the DL was 0.008 μM. Application of the sensor in water and biological samples was also examined [89]. Also for oxidation of L-cys Saadttarahmy et al. were used cobalt hexacyanoferrate nanoparticles with a core–shell structure (iron(III) oxide core–cobalt hexacyanoferrate shell). They observed that the anodic peak current of the Fe(II)/Fe(III) transition was increased and the cathodic peak current reduced in the presence of L-cys . But the peak current of the Co(II)/Co(III) transition remained constant. This method was ultrasensitive and time-saving designation procedures with DL of 40 and 20 nm in batch and flow systems. This sensor was also used to the analysis of L-cys in human urine and serum blood samples [90]. Farias et al. reported on the adsorption-controlled accumulation of Cys at the Hg film electrode in the presence of sub-micromolar concentration levels of Cu. The amperometric current was increased linearly from 0.04–0.20 ppm L-cys and achieved the DL of 0.9 ppb for an accumulation time of 15 minutes. The application of this method in the presence of casein and ATP was also investigated [91].

For selective determination of Hcy , a film of2-amino-1,3,4-thiadiazole with a thickness of 25 nm was electropolymerized on GCF by Kalimuthu et al. The proposed electrode was used at physiological pH and in the presence of ascorbic acid as one of the very important interferents. The modified electrode was able to split the voltammetric signals of ascorbic acid and Hcy with a peak separation of 490 mV. The amperometric peak current of Hcy was increased linearly with Hcy concentrations in the range of 100 to 1400 nMwith method detection limit of 51 pMwas obtained for Hcy . Better recoveries was observed for spiked Hcy in human blood serum samples [92].

A glutathione electrochemical sensor was developed using Ag–ZnO/CBF modified carbon paste electrode. The square wave voltammetric current of glutathione was increased linearly from 5.0×10^{-8} to 2.0×10^{-4} M and achieved the DL of 20.0 nM.A good resolution of the voltametric peaks of GSH was observed in the presence of tryptophan as a main interference in real samples [93].

Nanobiosensors and Recognition Elements

In the previous section, we reviewed several reports on the application of nanoparticles to enhance the performance of sensors in the detection of biothiols. Depends on the type of materials used to design sensors the efficiency was changed. But in a biosensor, a biorecognition strategy provides analytical quantitative or semi-quantitative information. In fact, what increases sensors performanceis biorecognition element which almost acquired from living systems. Although many diagnostic elements are synthetic and obtained in biological science laboratories. For this reason, different types of biomolecules have been considered in recent studies include antibodies, enzymes, receptors, lectins, nucleic acids, aptamers, nucleic acids, and molecularly imprinted polymers. In the following we refer to the sensors that recently developed for recognition of biothiolsandtheir importance in design and diagnosis[95].Stability of nanosensors and selectivity of biosensors comes together in nanobiosensors to improve electrochemical sensors performance. In continues different types of nanobiosensors were discussed for enzyme, AuNP, AgNP, molecular imprinted polymer, aptamer and further nanoparticles.

Enzyme

Enzymes are catalytic protein with specificity for particular molecule which has been attracted more attention as a recognition element over the last few decades. Easy miniaturization, robustness and operation with small specimen volumes of even complex matrices are some advantages of enzyme type recognition elements. Enzyms are also able to conjugate to a secondary recognition element and amplify received signals.[95]The target analyte and the complexity of the matrix should be considered in biosensor designing. Cytochrome c and D-amino acid oxidase were studied in nanobiosensors for Hcy detection[96-98].

Gold nanoparticle(G-NP)

Madasamy et al. was immobilized cytochrome c on G-NP modified screen printed carbon electrode for electrochemical detection of Hcy . This new miniaturized electrochemical sensor revealed

partly reversible redox peaks at the potentials 0 and −0.2 V. The Fe^{3+}/Fe^{2+} splittedoutcytochrome c were oxidized Hcy at a potential of +0.56 V.Amperometric determination revealed the DL of 0.3 ± 0.025 μM and a LR of detection between 0.4 μM and 700 μM. A sensitivity of 3.8 ± 0.12 nA μM^{-1} cm^{-2} along with good repeatability (2.85% SD) and high stability (83% of initial stability after 4 weeks) were obtained for the developed nanobiosensor. They were studied the effect of common interfering by the measurement of Hcy in blood plasma samples[99].For GSH detection, Noh et al covalently immobilized glutathione reductase and -b-nicotinamide adenine dinucleotide phosphate on the surface of AuNPs. Then the resulting material was deposited on poly[2,20:50,200-tertthiophene-30-(p-benzoic acid)] and the results were determined using SEM, TEM, XPS and QCM. Analytical affecting parameters such as temperature, pH and two enzyme ratio were optimized in terms of applied potential. The LDR of detection was obtained between 0.1 mM and 2.5 mM with a detection limit of 12.5- 0.5 nM and stability of up to 10 weeks. In vivo application of present biosensor microbiosensor to detect the oxidative stress arises by diquat and t-butyl hydroperoxide was successful[100].Chauhan et al. developed an amperometric method for biosensor to determination of GSH. For this purpose, Pt electrode was modified using gold coated magnetic nanoparticles (Fe@AuNPs). Then a GSH oxidase was covalently immobilized on the surface of modified electrode. High enzyme loading and long shelf life comes from covalent linkage and morphology of glutathione oxidase. Maximum response of electrode was obtained within 4 s during polarization at +0.4 V. A linear relation was detected for electrode response versus GSH concentrations in the range 5.0–4000 μM. The DL of 0.1 μMalong with analytic recoveries of 97.5 ± 1.7 and 96.1 ± 1.3 for 50 μMand 100 μM of glutathione were determined respectively. Analytical recovery of <2.14% and <2.39% were determined in blood serums respectively. After 150 uses over 4 months, 50% of initial activity of the biosensor was lost. GSH concentration in hemolysated erythrocytes as measured by the present biosensor was 2.8 mmol L^{-1} in apparently healthy persons[101].

Silvernanoparticle

Narang et al. co-electrodeposited AgNP/ carboxylatedmultiwalled carbon nanotubes / polyaniline film on Au electrode. In the following, the enzyme GSH oxidase was immobilized covalently on the surface of the modified electrode. The optimum response within 4 s at +0.4 V versus Ag/AgCl was obtained for constructed nanobiosensor. Linear working range and detection limit of 0.3–3500 μM and a 0.3 μM was observed in the optimum condition. GSH content in hemolysated erythrocyte was measured by this GSH biosensor. The initial activity of the enzyme electrode was decreased to 50% after 300 uses for 3 months[102].

Molecularly Imprinted Polymer (MIPs)

In molecular imprinted polymer, a synthetic polymer was formed in a molecular template to assemble selective binding sites. Then a suitable solvent and a chemical reaction provided detachment of the template molecule from the polymeric matrix. Thus, a cavity was created within the polymeric matrix with ability to trap the target analyte. Molecular imprinted polymers have remarkable sustainability and a proper potential for production of synthetic recognition elements[95]. Prasad et al. reported the fabrication of (MIPs) including graphite/multiwalled carbon nanotubes/ AuNPs/sol-gel on the surface of a sandpaper electrode. Potassium ferricyanide was used as an external probe for measurement of differential pulse anodic signal. The (DL) of 0.26–0.30 ng mL^{-1} was obtained without any cross-reactivity or interference effect in pharmaceutical samples and blood serum [103].MIP electrochemical sensor for detection of (GSH) was improved by Aswini et al. trough chemical oxidative polymerization and intercalation of Fe_3O_4/polyaniline into the graphene oxide layers. GSH, Fe_3O_4/polyaniline reduced graphene oxide and pyrrole was arranged as template molecule, functional monomer and cross-linker respectively. Then it was coated onto the surface of magnetic glassy carbon electrode and promoting network for electron transfer. Appropriate stability and reproducibility with the (DL) of 3 nmol $L^{-1}$for the determination of glutathion using assembled sensor was obtained[104].

Some limitation of enzymatic sensors such as instability, complicated modification procedures, and critical microenvironmentalfactorsaffected its advantages. Thus, development of nonenzymatic electrochemical sensors with high stability and simple modification is essential for determination of biothiols.The use of nanoparticles in sensor design

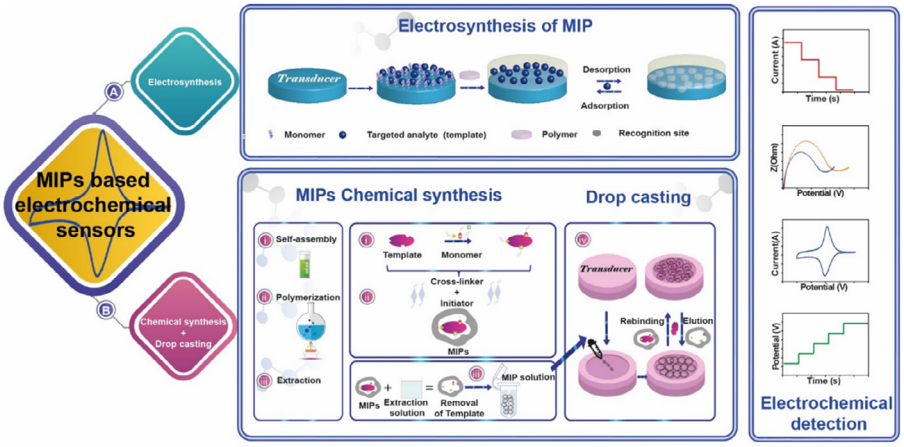

Fig. 6.Scheme of electrochemical and chemical synthesis MIP-based preparations electrochemical sensors [116].

and construction causes stability and sensitivity improvement as observed in previous examples. Chen et al.recentlyreviewed advantages and current challengesof enzyme-free electrochemical sensors[105].Following are some examples of nonenzymaticnanobiosensors for the detection ofsmall molecules(Fig. 6).

Aptamer

Aptamer nucleic acid ligands with high affinity for a specific target are synthesized in vitro through a process called sequential evolution of ligands by exponential enrichment (SELEX)[95]. Improved stability, low cost, easy regeneration and ability to modification at precise locations are some more advantages of aptamers[106-107]. Keague et al reported on the selecting and application of high affinity binding and selective aptamerfor L-Hcy detection. After 8 rounds of selection, two novel aptamer with high affinity binding and selectivity to Hcy were selected. A AuNP solution was added and by creating a vortex, it was possible to react with the selected aptamer. The modified sensor demonstrated a (LOD) of 0.5 μM and a (LR) of 0.5–3.0 μMfor (Hcy) detection in human serum[108].

Other nanobiosensors

Lv et al used the high-association binding effect of AgNPand glutathione for the electrochemical detection of intracellular glutathione. AgNPwere constructed by reduction of silver ions on the surface of a DNA template. In the presence of glutathione the ultra-sensitive Ag-S interaction occurred and due to AgNP released from the template DNA. Silver ions can be released and produce a detectable electrochemical response. The linear detection range of 0.1 nm to 1 μM with a low (DL) of 2.3×10^{-11} M confirmed the successful application of the modified nanobiosensor for the detection of intracellular glutathione [109]. Sharifi et al reported about the voltammetric test of L-cys and Hcy compounds simultaneously without any separation or pretreatment.They electrodeposited nickel oxide nanoparticles and deoxyribonucleic acid complex osmium (III) entrapped on (GCE). Chronoamperometric method was used to detect sensitivity(DS) and (LOD)and obtained as 44 μAmM^{-1} and 0.07 μMrespectively. The application of modified electrode for the analysis of L-cys and Hcy concentration in complex serum samples was successful. The proposed DNA based biosensor has more advantages including excellent electrocatalytic activity, stability and high selectivity[110]. In the study of Miao et al., two gold electrodes and two complementary thiolated oligonucleotides modified on AuNPs were used to determine the electrochemical performance of (GSH). One of the two oligonucleotides was settled on the gold electrode and immersed in the (GSH)solution. The oligonucleotides released and replaced by glutathione because of the ligand release effect. Then the second gold electrode was immersed in the glutathione solution and the released oligonucleotide molecules were placed on the electrode surface. Presence of AuNPsincrease the number of electrochemical species localized onto the electrode surface. Thus, the detection signal was amplified and ultrasensitive detection of glutathione achieved in the range of 1×10^{-12} to 1×10^{-10} M. The features of this method were (DL)

of 4×10^{-13} M and suitable application in detection of glutathione in fetal calf serum [111].In continues Stobiecka et al. was studied the effect of buried potential barrier in label-less electrochemical immune-detection of glutathione and glutathione-capped AuNPs. They concluded that studies with monoclonal anti-glutathione antibody-based sensors using a ferricyanide ion probe, in addition to the sensor showing a stronger response to layer components, indicated that films with a positive potential barrier compared to The negative barrier has been identified as AuNPacting (GSH) capping antigen[112].

CONCLUSIONS

Electrochemical sensors and biosensors have been widely considered and developed due to their simplicity, speed and intrinsic sensitivity quality and reasonable cost. Various mechanisms have been used to improve the performance of sensor components and elements. Recent advances in the construction of novel ultrasensitive electrochemical assays based on nanomaterials and nanobiomaterials reviewed here. The use of NPs as catalysts,electronic conductorsfor signal amplification with effective surface area was developed engineering of recognition components based on nanoparticles. In this recognition componentsthe synergy of multifunctional materials was improved the selectivity, stability, and reproducibility of biosensors and nanosensors. The progress of these systems has brought the sensors to the point where they can simultaneously check the levels of cysteine, Hcy and glutathione. Studies have shown that there is still room for further research into the development of nanobiosensors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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