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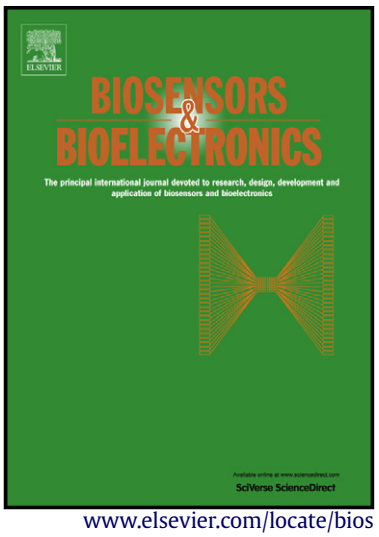

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A novel bioelectrochemical sensing platform based on covalently attachment of cobalt phthalocyanine to graphene oxide

Hadi Hosseini, Mojtaba Mahyari, Akbar Bagheri*, Ahmad Shaabani

Department of Chemistry, Shahid Beheshti University, G. C.; P. O. Box 19396-4716, Tehran, Iran

* Corresponding author. Tel.: +98 21 29903251; fax: +98 21 22431661.

E-mail address: akbr_bagheri@yahoo.com (Akbar Bagheri).

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Abstract

Graphene oxide-cobalt phthalocyanine (GO–PcCo) hybrid material as a new electrocatalyst was synthesized and used successfully to fabrication of new biosensor for the electrooxidation of L-cysteine (CSH) in aqueous media. Fourier transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS), Thermogravimetric analysis (TGA), and transmission electron microscopy (TEM) images revealed that cobalt phthalocyanine is covalently attachment on graphene oxide sheets as single layers GO–PcCo. Cyclic voltammetric studies showed that the GO–PcCo/glassy carbon electrode (GO–PcCo/GCE) improves electrochemical behavior of CSH oxidation, as compared to the GO and PcCo. In addition, the results indicated that GO and PcCo have a synergic effect in the electrooxidation of CSH. The catalytic oxidation responses were studied and the reaction mechanisms were discussed. The electrocatalytic behavior is further developed as a new detection scheme for CSH by chronoamperometry method and under optimized conditions, excellent analytical features, including high sensitivity and selectivity, low detection limit and satisfactory dynamic range, were achieved.

Keywords: Graphene oxide; Cobalt phthalocyanine; Covalently attachment; Electrochemistry; Biosensors.

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1. Introduction

Detection of biomolecules at low concentrations is critically important to the early diagnosis and successful treatment of diseases (Claussen et al., 2009; Drummond, 2003; Patolsky et al., 2006). In addition, there have been continuous researches for fabrication of new electrode materials for developing new sensitive and selective electrochemical biosensors for the detection of the trace amounts of biomolecules (Claussen et al., 2009; Drummond, 2003; Patolsky et al., 2006). Cysteine (CSH) is one of the important amino acids and biomolecules that plays an important role in living systems, acting as an active site in biological processes because of the high nucleophilicity of thiol function (Su and Cheng, 2008). Also, CSH has an application in the food, pharmaceutical and medicine industries. Abnormal levels of CSH are related to many diseases, such as slowed growth, Alzheimer's disease and cardiovascular disease (Refsum et al., 1998; Seshadri et al., 2002; Ueland et al., 2004; Wang et al., 2005). Elevated levels of CSH are considered to be associated with cardiovascular disease (van Meurs et al., 2004). Therefore, it is important to develop the simple, rapid and low-cost method for the detection and quantification of CSH in physiological media for academic research and clinic applications. Various chemically modified electrodes (CMEs) with electrocatalytic properties have been fabricated and applied in the detection of CSH (Chen et al., 2008; Hosseini et al., 2013; Ge et al., 2012; Zhou et al., 2007). The reported CMEs have their advantages and limitations in linear dynamic range, selectivity, sensitivity, detection limit, and other characteristics. So, it is necessary to have further efforts to fabricate simple, low cost, stable, sensitive and selective CMEs that can improve the characteristics of electrocatalytic activity for determination of CSH.

Graphene, the two-dimensional sp^2-hybridized carbon, is a novel material that has emerged as a rapidly rising star in the field of electrochemistry due to its remarkable physicochemical

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properties (Chen et al., 2012; Georgakilas et al., 2012). One specific branch of graphene research deals with graphene oxide (GO) and has great potential for providing new approaches and critical improvements in the field of electrochemistry (Chen et al., 2012; Georgakilas et al., 2012). GO-based materials consist of a 2D layered structure with a large surface area and also possess a large number of oxygen-containing functional groups, such as hydroxyl, carboxyl, and epoxy groups. Such properties make it possible to functionalize such GO-based materials using either covalent or noncovalent chemistry in order to modulate the electrode's structural architecture and intrinsic properties (Chen et al., 2012; Georgakilas et al., 2012). These generally exhibit novel, interesting properties and hold promise for design and prepare GO-based electrodes for a wide range of applications in the field of electrochemistry, due to their high electrical conductivity, chemical stability, tunable modification, and multifunctional structures.

Electrocatalysis lies at the heart of the chemical phenomena that take place at electrochemical interfaces (Markovic, 2013) and represents one of the most important areas for the application of GO-based electrodes in electrochemistry. First of all, GO itself possesses excellent electrocatalytic activities toward some important species (He et al., 2010; Kim, et al., 2010; Lin et al., 2009; Tan et al., 2010; Tang et al., 2009; Wang et al., 2010; Wang et al., 2009; Yang et al., 2010;). Furthermore, the introduction of electrocatalytic centers such as inorganic catalysts to GO may offer GO-based electrodes novel electrocatalytic properties due to the excellent catalytic activities of such GO and electrocatalytic centers. In this case, the GO generally acts as a support for the linking of the inorganic catalysts and it facilitates or mediates the charge/electron transfer between the electroactive species and the electrode surface of the GO supported electrocatalysts and modulates the electrochemical reactions in a controlled fashion. Phthalocyanines (Pc) constitute a remarkably versatile and the best class of compounds with diverse technological

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applications (Darwent et al., 1982; Leznoff et al., 1996). Cobalt phthalocyanines (PcCo) have been used as efficient inorganic electrocatalysts for the construction of new electrode material for the detection of the trace amounts of biologically and environmentally important compounds [Barrera et al., 2006; da Silva et al., 2011). Recently, the graphene modification with the metal phthalocyanines (MPc) has received enormous attention due to their unique properties and few reports were published which describe the synthesis and characterization of metal phthalocyanines (MPc) on graphene. For example, Zhu and co-workers have reported functionalizing the graphene oxide (GO) with zinc phthalocyanine for broadband optical limiting (Zhu et al., 2011). Ragoussi et al. demonstrated that phthalocyanine can be covalently attached to the surface of graphene by photo- and redoxactive linking method (Ragoussi et al., 2012), and Mensing and co-workers prepared graphene–MPc hybrid material by electrolytic exfoliation (Mensing et al., 2012).

Based on the told preface, the covalent attachment of cobalt phthalocyanines (PcCo) to the surface of graphene is a good new strategy used to fabrication of new electrochemical biosensors with unique properties for the detection of biomolecules. Hence, in this work, Graphene oxide-cobalt phthalocyanine hybrid material (GO–PcCo) was synthesized and used successfully as a new electrocatalyst in the fabrication of new electrochemical biosensor for detection of CSH. To the best of our knowledge, this is the first report that the GO covalently with cobalt phthalocyanine (PcCo) was used as a catalyst for the electrooxidation of biomolecules.

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2. Materials and methods

2.1. Apparatus and reagents

All chemicals were purchased from Aldrich and used without further purification. FT-IR spectra were recorded on a Bomem MB-Series FT-IR spectrophotometer. X-ray photoelectron spectroscopy (XPS) analysis was performed using a VG multilab 2000 spectrometer (ThermoVG scientific) in an ultra high vacuum. Transmission electron microscopy (TEM) analyzes were performed by LEO 912AB electron microscope. Ultrasonic bath (EUROSONIC® 4D ultrasound cleaner with a frequency of 50 kHz and an output power of 350 W) was used to disperse materials in solvent. Voltammetric experiments were performed using a μAutolab Type III electrochemical system. A conventional three-electrode cell consisting of a glassy carbon working electrode (modified and unmodified), a platinum wire counter electrode and a saturated Ag/AgCl reference electrode were used for voltammetric experiments. A digital pH-meter (Ion Analyzer 827, Metrohm) with precision of ±0.001 was used for pH measurements. All electrochemical experiments were done at room temperature (25.0±1 °C).

2.2. Preparation of GO

GO was prepared using Hummer's method (Hummers and Offeman, 1958) and describes in supporting data.

2.3. Preparation of 2,9,16,23-tetranitro-PcCo

PcCo was synthesized by a template method (Safari et al., 2004; Shaabani, 1998; Shaabani et al., 2007; Shaabani and Rezayan, 2005) and describes in supporting data.

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2.4. Preparation of 2,9,16,23-tetranitro-PcCo functionalized by 3-(trimethoxysilyl)propane-1-thiol

Under a blanket of nitrogen, 3-(trimethoxysilyl)propane-1-thiol (0.19 g, 1.0 mmol) and PcCo (0. 75 g, 1.0 mmol) were dissolved in dry DMSO (5.0 mL). Anhydrous K_2CO_3 (0.20 g, 1.4 mmol) was added to this suspension and the mixture stirred at 80-100 ^oC. After 48 h the mixture was filtered and dried under vacuum at 25 ^oC for 24 h (89%). The product was confirmed by FT-IR (Figure 2S).

2.5. Preparation of GO–PcCo

In a typical run, GO (0.05 g) and DMF (5.0 mL) in a round flask was subjected to ultrasonic pulse for 30 min, and then PcCo functionalized (0.25 g) was added to the solution under nitrogen. The reaction mixture was heated at temperature 80-110 ^oC for 40 h. For coupling reaction 20 μL of triethylamine was typically added to the reaction mixture to promote the graft reaction. Then, the mixture was subjected to centrifugation and thoroughly washed with toluene and THF for at least five times. The product was carefully collected and dried under vacuum at 60 ^oC until constant weight. GO grafted with PcCo was obtained as a black powder and its dispersity in solvents is higher than GO. The procedures for GO–PcCo preparation are shown in Schema 1.

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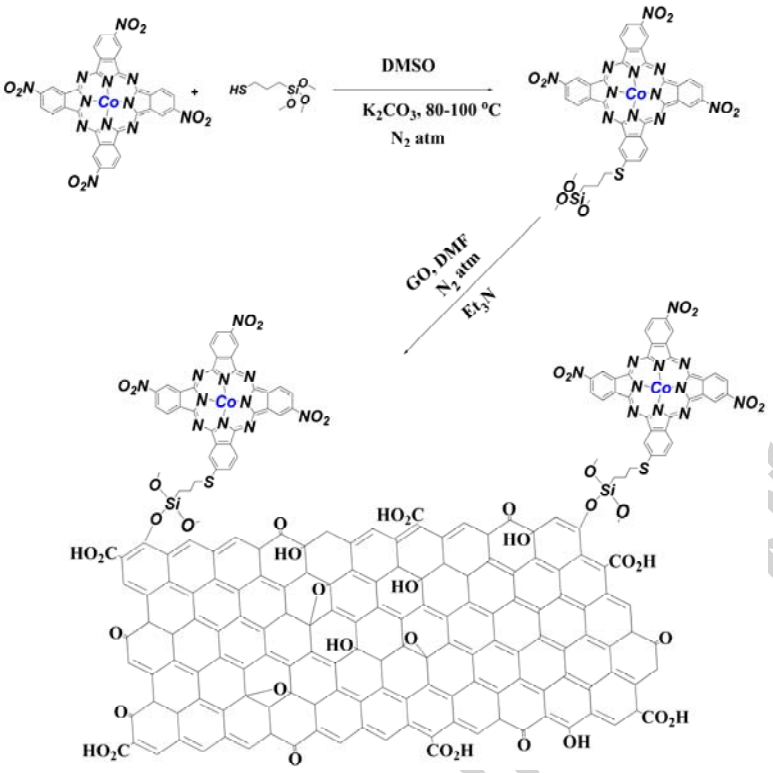

Schema 1. Synthesis of GO–PcCo.

2.6. Preparation of the GO–PcCo/GCE

Prior to the electrode modification process, a glassy carbon electrode (GCE) (2mm diameter) was washed with deionized water and ethanol after polishing was performed with 0.05 μm alumina slurry on a polishing cloth. Typically, a stable suspension of GO–PcCo containing 2.0 mg ml^{-1} in DMF using 20 min ultrasonic agitation was prepared. After the electrode surface was air dried, 5.0 μL of this suspension was cast onto the surface of the pretreated GC electrode with a microsyringe and then it was dried in air. Furthermore, the suspensions of GO and PcCo were prepared and used with the same procedure.

2.7. Analytical procedure

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After fabricating each electrode in order to obtain reproducible current-potential curves, cyclic voltammetry was performed at scan rate of 100.0 mV s^{-1} between -0.8 and 1.0 V for 10.0 times in 0.1 M in robinson buffer solution. In addition, the buffer solution was purged with high-purity nitrogen for at least 10.0 min prior to each electrochemical measurement, because CSH can be oxidized by atmospheric oxygen, particularly in basic solutions.

2.8. Sample preparation

Human urine samples were prepared according to references (Chen et al., 2007; Ge et al., 2012) and describes in supporting data.

3. Results and discussion

3.1. Characterization of GO–PcCo hybrid materials

To confirm covalently functionalized graphene oxide with cobalt phthalocyanine, XPS analysis was performed (Figure 1A). GO-PcCo sample has N 1s peak at 401.3 eV for metal–N bonding in the metal centered phthalocyanine. And O1s peak was appeared at 531.4 eV. The oxidation state of the Co(II)Pc was confirmed with X-ray photoelectron spectroscopic measurements. The $2p_{3/2}$ peak located at 780.6 eV is due to the Co(II) oxidation states (Ivill et al., 2008; Wagner, et al., 1978; Yang et al., 2010). The Si 2p located at 103.9 eV (Barreca et al., 2007), were confirmed the covalently bonding silicium on graphene oxide.

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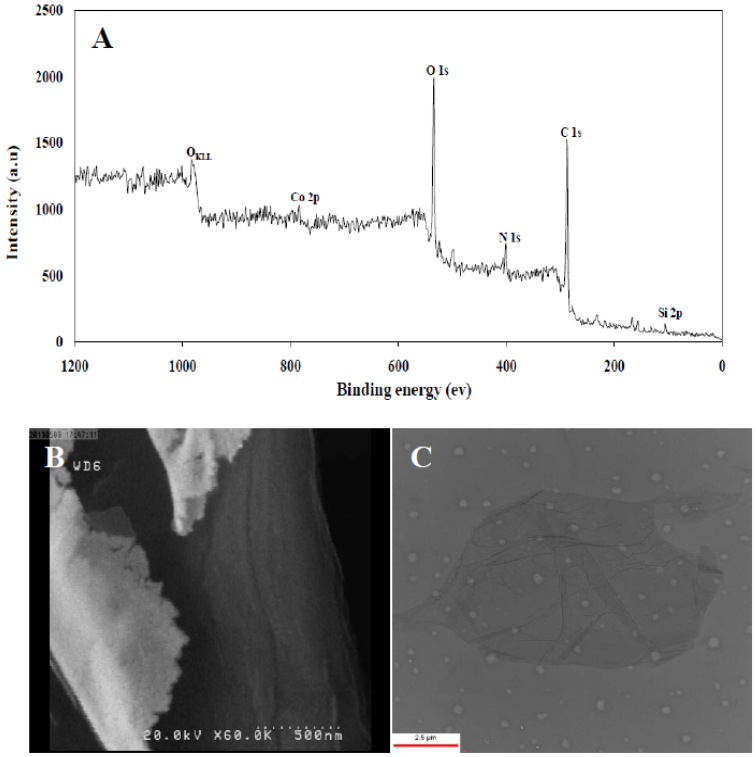

Figure 1. XPS spectrum (A), SEM (B) and TEM (C) images of GO–PcCo hybrid materials.

TEM and SEM images of synthesized GO-PcCo were shown after purification that clearly deduced the single layers and very high quality of GO hybrid material sheets (Figure 1B and C). Furthermore, the resulting GO-PcCo was also characterized by TGA and FT-IR and the results are describes in supporting data.

3.2. Electrochemistry of modified electrodes

Figure 3S (Supplementary information) shows cyclic voltammograms of GO-PcCo/GCE in robinson buffer solution (pH 3) at potential sweep rate of 50 mV s^{-1}. The GO-PcCo exhibits two redox transitions denoted as transitions I/I' and II/II' which the anodic peaks located at 370 (I') and 670 mV (II') that are attributed to Co(II)/Co(III) and Co(III)/Co(IV) oxidation transition on the electrode surface (Hasanzadeh et al., 2009; Ozoemena et al., 2005). The cathodic peaks at 45

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(I') and 520 (II') mV correspond to the reduction of different cobalt species formed during the anodic sweep (Hasanzadeh et al., 2009; Ozoemena et al., 2005).

3.3. Electrochemistry of CSH on the surface of modified electrodes

To address the analytical applicability of the GO-PcCo, The electrocatalyst ability of the GO-PcCo was evaluated for electrooxidation of CSH. At first, the electrochemistry of CSH on the surface of different modified GCEs was studied using cyclic voltammetry. Figure 2 shows the typical cyclic voltammograms of the GO/GCE (blue line), PcCo/GCE (red line), and GO–PcCo/GCE (green line) in the presence of 0.5 mM CSH. As seen, a weak irreversible oxidation peak was noticed at GO/GCE and PcCo/GCE at 0.73 and 0.64 V, respectively. When the surface of the electrode was modified with the GO–PcCo/GCE films, a well-defined anodic peak at 0.52 V with a remarkable increase in oxidation current was achieved for the oxidation of CSH, indicating a significant electro-catalytic effect and a good electrochemical response of catalyst to CSH.

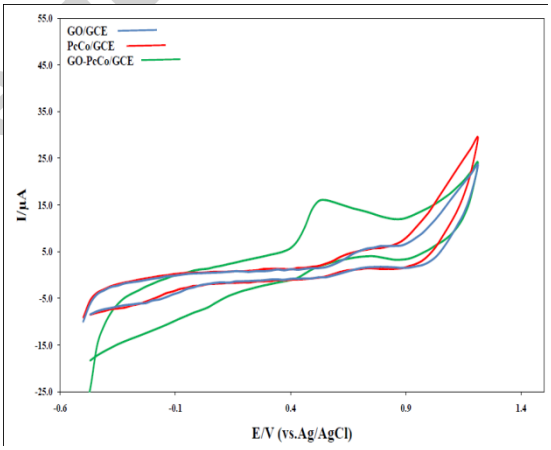

Figure 2. Cyclic voltammograms of the GO/GCE (blue line), PcCo/GCE (red line), and GO–PcCo/GCE (green line) catalysts in the presence of 0.5 mM CSH in 0.1 M robinson buffer solution (pH 3) with a scan rate of 50 mV s^{-1}.

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In this case, the electrocatalytic activity of GO–PcCo/GCE catalyst can be considered to result from the combination of two factors: one is the catalytic activity of the PcCo, and the other one is the excellent electrocatalytic activity of the GO which it can facilitates or mediates the charge/electron transfer between CSH, PcCo, and the electrode surface and modulates the electrochemical reactions in a controlled fashion. In addition, the high density and well-distributed PcCo on the surface of GO would induce more active sites for the catalytic redox reaction and bring about an efficient electrical network through their direct binding with the GO, which would greatly increase the electrocatalytic activity (Chen et al., 2012). Based on above explanation, both GO and PcCo clearly plays an important role and synergistic effect in the oxidation of CSH at pH 3.0.

3.4. pH effect

The pH is an important factor in the electrochemical behavior of CSH and its impact on the electrochemical reaction of CSH was studied by using different buffer solutions ranging from pH 2.0–12.0. Figure 3A depicts the dependence of the voltammetric response of CSH at GO–PcCo/GCE in some pH values. The results showed that the anodic peak current decreased with pH increasing from 2.0 to 8.0, and its value is very low from 8.0 to 10.0 and disappeared when

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the solution pH was further increased from 10.0 to 12.0 (Figure 3B).

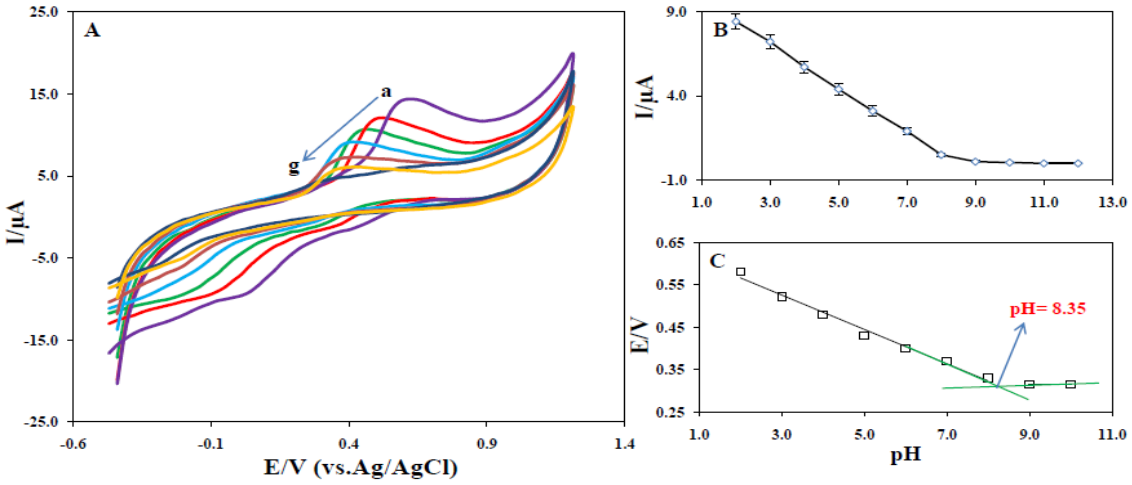

Figure 3. (A) Cyclic voltammograms of the GO–PcCo/GCE catalyst in the presence of 0.5 mM CSH with a scan rate of 50 mV s^{-1} in 0.1 M robinson buffer solution under some pHs: (a→g) 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0. (B): Variation of the anodic peak current with pH. (C): Plot of variation of oxidation peak potential with pH.

These results indicating that in alkaline media the GO–PcCo/GCE has no catalytic effect to CSH oxidation. CSH has three pKa values as 1.71, 8.36, and 10.77, which correspond to various functional groups of COOH, SH, and NH_2, respectively, which the SH group possesses the strongest oxidizing tendency among the three functional groups and can form the disulfide (CS-SC) bond easily. In addition, CSH has different chemical species (four species) against pH (Figure 4A). As can be seen, the dominant species change with pH and can be used to explain the experimental results. As we mentioned, the GO–PcCo/GCE shows electrochemical responses to CSH in pH lower than 8.0 and shows maximum peak current with well-shaped voltammetric curve at pH 2.0. On the basis of the distribution diagram (Figure 4B), the cationic [H_3A^+] (i.e., CSH_2^+) or the neutral [H_2A] (i.e., CSH) is predominated in pH <8.0 and thus [H_3A^+] and [H_2A]

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are responsible for irreversible oxidation reaction of CSH and the disulfide formation which occurred at GO–PcCo/GCE in pH 2.0–8.0 buffer solutions.

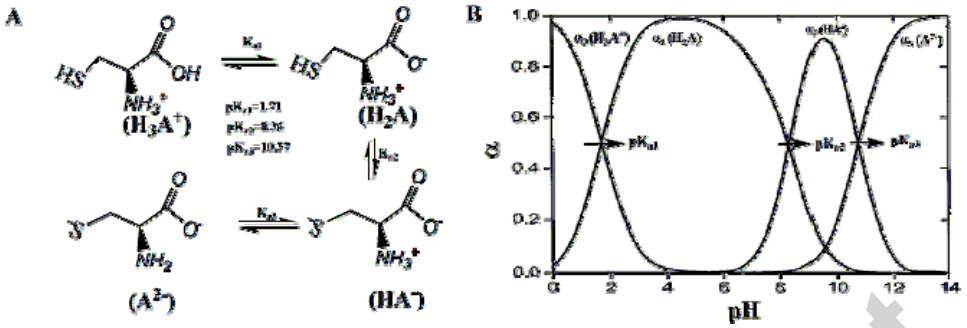

Figure 4. (A): Various forms of CSH with respect to its pK_a value. (B): Distribution fraction (α) for various CSH species vs. its pH.

Furthermore, the previous studies conducted on the surface of various modified electrodes showed that the electrochemical reaction of CSH versus pH was complicated and not always predictable and its behavior was dependent on the electrode characteristics and the CSH species (Hsiao et al., 2011; Spataru et al., 2001; Su et al., 2008; Zen et al., 2001; Zhou et al., 2007; Zhao et al., 2003). For example, The Au-SH-SiO_2 nanoparticles immobilized on the metal□organic frameworks shows maximum current values at pH 5.0 for CSH oxidation and H_2A species predominated in the irreversible oxidation of CSH in pH 4.0–7.0 buffer solutions (Hosseini et al., 2013). In the surface of Lead Ruthenate Pyrochlore Modified Electrode, maximum current values was observed at pH <5.0 for CSH oxidation and the cationic [H_3A^+] and the neutral [H_2A] species are electroactive in this range of pHs (Zen et al., 2001). The oxidation of the HA^- electroactive species was observed in pH 9.0 solutions at conductive boron-doped diamond electrodes (Spataru et al., 2001). The two species of CSH (H_3A^+ and HA^-) were electroactive at mesoporous carbon modified glassy carbon electrodes (Zhou et al., 2007). In the case of CSH oxidation on conducting polymers/gold nanoparticles hybrid nanocomposites (Hsiao et al.,

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2011), the H_2A species were electroactive in pH 2.0–8.0 buffer solutions. At Screen-printed electrode/poly (3, 4-ethylenedioxythiophene), Three forms of CSH (H_2A, HA^- and A^{2-}) were electroactive in pH 6.0–12.0 buffer solutions (Su and Cheng, 2008). Protonated CSH (H_3A^+) was electroactive at carbon nanotube powder-modified electrodes in acidic sulfuric acid solution (Zhao et al., 2003). The relationship between the peak potentials and pH is shown in the Figure 3C. As it can be seen, in the pH ranges from 2.0 to 8.0, the anodic peak potential linearly shifts to the less positive values. The results indicate that the protons are participated in the oxidation of CSH, it can be seen that, at pH values between 8.0 and 10.0, the peak potential is rather constant, indicating that in alkaline media the process is not pH dependent. Also, at pH values higher than 10.0, CSH oxidation is obscured by oxygen evolution and reliable data for E_p could not be obtained for a 0.5 mM CSH concentration. In the Ep-pH diagram deviation from linearity is observed at about 8.35, which is near to pKa values of the dissociation of the proton from the thiol group in CSH. In the pH ranges from 2.0 to 8.0, the peak potential shifts linearly toward more positive potentials with a slope dEp/dpH of 40.0 mV, which it is smaller than the nernstian value for an expected $2e^-$ and $2H^+$ transfer reaction equilibrium and does not correspond strictly to the overall equilibrium (Moore et al., 2004):

$$\mathbf{2CSH \longrightarrow CS{-}SC + 2H^+ + 2e^-} \qquad (1)$$

This behavior could be due to the complicated electron transfer reactions and/or the co-existence of other species, which will obviously perturb the overall equilibrium in neutral and acidic media (Spataru et al., 2001).

3.5. Kinetic study of CSH on the surface of GO–PcCo/GCE

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The oxidation of CSH at the GO–PcCo/GCE catalyst electrode is a completely irreversible kinetic process. For further study, the effect of scan rate on the electrochemical behavior of CSH at the GO–PcCo/GCE electrode was studied by cyclic voltammetry (Figure 4S (A), Supplementary information). For an irreversible reaction, the relationship between the peak current density (i_p) and scan rate (υ) can be expressed as:

$$i_p=2.99\times10^5\, n(\alpha n\square)^{1/2}AC_0D^{1/2}\upsilon^{1/2} \qquad (2)$$

Where i_p (A cm^{-2}) refers to the peak current, n is the number of electron transfer, $n\square$ is the electron number transferred in the rate-determining step, α is the charge transfer coefficient, A (cm^2) is the surface area of the electrode, C_0 (mol cm^{-3}) is the concentration of CSH, D_0 (cm^2 s^{-1}) is diffusion coefficient, and υ (V s^{-1}) is the scan rate. Figure 4S (B) (Supplementary information) shows the curve of peak current vs. the square root of the scan rate. As it can be seen, the peak current associated to the CSH electro-oxidation increases linearly with the square root of scan rate, which indicates that the electrocatalytic process under study is controlled by diffusion. The equation related to peak currents and the scan rate can be represented as $i_p(\mu A)= 44.04\upsilon^{1/2}(Vs^{-1})^{1/2}+ 0.627$, $R^2= 0.992$. In addition, a good linearity between logarithm of anodic peak current and logarithm of scan rate with a slope of 0.502 was also found (Figure 4S (C), Supplementary information), which is close to the theoretical value of 0.5 for the diffusion-controlled electrode process. The linear equation obtained was:
$log I_{pa}(\mu A) = 0.502 \log \nu(V\ s^{-1}) + 1.626$, $R^2 = 0.990$. Furthermore, by increasing the scan rate, the peak potential shifts to more positive values, which this behavior was expect for an irreversible electrode process (Figure 4S (D), Supplementary information). For a diffusion-controlled and irreversible electrode process, according to Brett and Brett (Wang et al., 2009), the relationship between the peak potential (E_p) and scan rate (υ) is defined by the following (Bard and Faulkner, 2001):

$$\left| dE_p/d \log \upsilon^{1/2} \right| = 0.0592/(1-\alpha n\square) \qquad (3)$$

The value of $\alpha n\square$ can be easily estimated from the slope of E_p vs. $\log \upsilon^{1/2}$. In our system, the slope is 0.190. Generally, $n\square$ is assumed (Bard and Faulkner, 2001) to be 1.0 in the rate-determining step. Thus, α was calculated to be 0.69.

3.6. Determination of CSH on the surface of GO–PcCo/GCE

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Considering the information obtained by the cyclic voltammetry studies and in order to determination of CSH and characterizes the electrodes response, a chronoamperometry was conducted at 0.52 V during 100 s. Figure 5S (A) (Supplementary information) shows the typical current–time response of the GO–PcCo/GCE towards CSH. As it can be seen, the GO–PcCo/GCE showed good linear response to the changes of CSH concentrations. The calibration curve for the CSH sensor (Figure 5S (Inset), Supplementary information) showed that the modified electrode gives a linear dependence in the CSH concentration range of 0.03 to 200 μM with a correlation coefficient of 0.994, and a detection limit of 5.0 nM. The sensor has a wide linear range and low detection limit as compared to some reported CSH sensors which indicated the high electro-catalytic activity of GO–PcCo/GCE toward CSH oxidation (Table 1S, Supplementary information) (Hosseini et al., 2013; Hsiao et al., 2011; Sattarahmady and Heli, 2011; Spataru et al., 2001; Su et al., 2008; Zhou et al., 2007) and this sensor can be used to readily detect CSH within the range of its physiological levels. To characterize the reproducibility of the GO–PcCo/GCE, ten repetitive measurement regeneration cycles were carried out for 10 and 100 μM CSH (Table 2S, Supplementary information). The electrode renewal gives a good reproducible surface since the relative standard deviation obtained was 3.56 and 2.45%, respectively, suggesting that the electrode does not undergo surface fouling during the measurements. In addition, the electrochemical activity of the GO–PcCo/GCE remained 96% after 2 days storage in the doubly distilled water and remained 90 % after 4 days storage in the doubly distilled water, suggesting that the stability of the modified electrode was good. The relative standard deviations obtained by proposed method are comparable with standard method (ranging from 1.7 to 19 %) (Commission Regulation (EC) NO 152/2009). In this method the CSH was analyzed using amino acid analyzer or high performance liquid

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chromatography (HPLC) which these methods need expensive equipments, are time-consuming, and require complex sample pretreatment, which make them unsatisfactory for routine or cost-effective screening. Uric acid (UA) and ascorbic acid (AA) present in physiological fluids are the most important interferences for direct electrochemical detection of CSH on different electrodes. Therefore, the interference effect of AA (0.1 mM) and UA (0.1 mM) on the chronoamperometric response of 10 μM CSH was studied (Figure 5S (B), Supplementary information). As shown, in the presence of AA and UA, the chronoamperometric response of CSH is almost constant, which demonstrate excellent selectivity and stability of sensor toward the CSH detection. In addition to AA and UA, the effect of other possible interferences such as Homocysteine (HCy) and glutathione (GTh), Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe^{3+}, Na^{+}, K^{+}, SO_4^{2-}, Cl^{-}, glucose, sucrose, lactose, nitrate, citric acid, and dopamine on the determination of CSH were investigated by using chronoamperometry. The results indicated that no interference was observed on the signals of CSH in the presence of interfering substances with concentration of 100.0 fold interfering substance to CSH with a deviation below 5.0%. It was observed that the method can be safely applied for determination of CSH in biological samples. To evaluate the capability of the sensor, the proposed amperometric method was applied to detect CSH in biological fluids. The amounts of both free reduced and total CSH in three human urine samples were estimated by using the GO–PcCo/GCE, and the results obtained are shown in Table 1. To confirm the validity of the results, the recovery tests of CSH were carried out by comparing the results obtained before and after the addition of standard CSH to the diluted uric samples. The results are shown in Table 1. The recoveries were determined in the range of 93.15 %–108.27 % with RSD (%) below 6.2%, which validated the reliability and practicality of this method.

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Table 1. Determination of CSH in urine samples using the GO–PcCo/GCE (n = 5).

Sample	f-CSH measured (μM)[a]	RSD (%)	Amount added (μM)	Recovery (%)	t-CSH measured (μM)[a]	RSD (%)	Amount added (μM)	Recovery (%)
			10	94.32			10	104.55
1	9.64+0.60	6.2	20	97.27	70.57+4.16	5.9	50	103.25
			30	93.15			100	97.32
			10	105.81			10	102.75
2	13.2+0.48	3.7	20	95.35	92.47+4.35	4.7	50	103.78
			30	93.94			100	106.12
			10	97.46			10	95.31
3	11.7+0.50	4.3	20	103.68	81.40+4.31	5.3	50	97.77
			30	107.15			100	103.56
			10	95.26			10	93.64
4	15.1+0.47	3.1	20	102.40	98.63+3.85	3.9	50	95.57
			30	108.27			100	98.26

[a]Mean value±S.D.

4. Conclusion

A new electrocatalyst based on covalently attachment of cobalt phthalocyanine to graphene oxide (GO–PcCo) was synthesized and used to fabrication of new biosensor for the electrooxidation of L-cysteine (CSH) in aqueous media. Electrochemical studies showed that the new sensor exhibits excellent electrocatalytic behavior toward CSH oxidation in aqueous robinson buffer solution and indicated that GO and PcCo have a synergic effect in the electrooxidation of CSH. The catalytic oxidation responses were studied and the reaction mechanisms were discussed. The sensor exhibited wider linear range of concentration change and lower detection limit compared with some previously reported.

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Highlights

Graphene oxide-cobalt phthalocyanine (GO–PcCo) hybrid material as a new electrocatalyst was synthesized.► The GO–PcCo used successfully as a new biosensor for the electrooxidation of L-cysteine.► The GO–PcCo showed good electroanalytical performance toward the L□cysteine detection.

Graphical abstract

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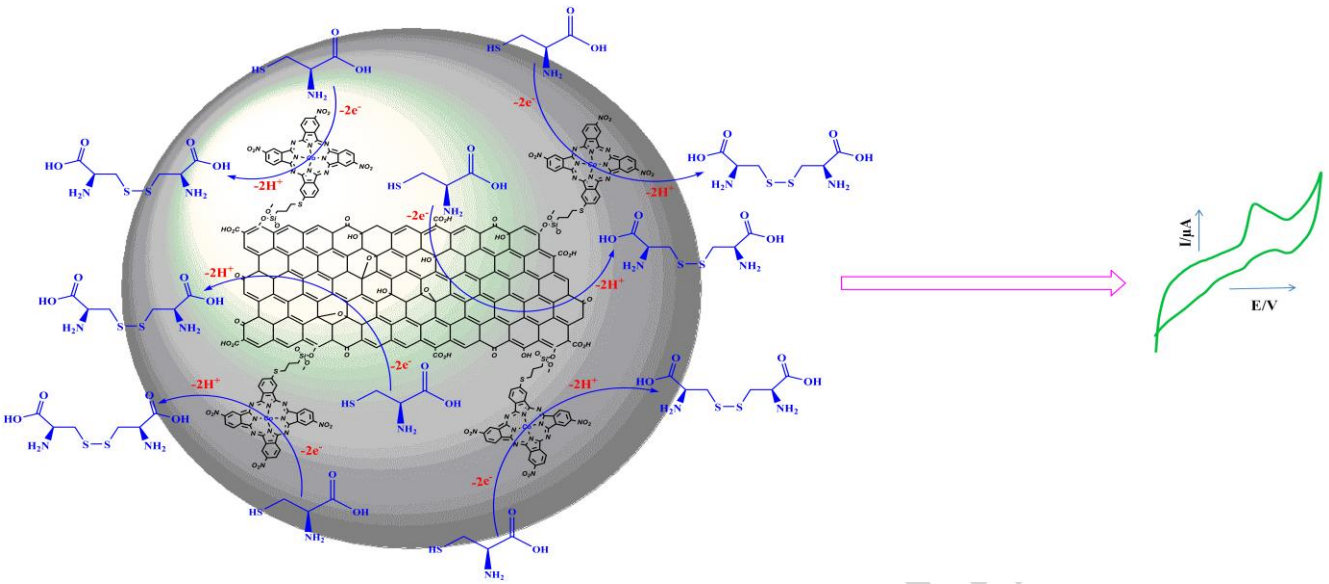

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