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Electropolymerization of cobalt tetraamino-phthalocyanine at reduced graphene oxide for electrochemical determination of cysteine and hydrazine

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We described a simple and elegant electropolymerization method to prepare highly stable tetraamino functionalized cobalt phthalocyanine (pTACoPc) at electrochemically reduced graphene oxide (RGO). The described method efficiently bridges the excellent physicochemical properties of RGO with rich redox chemistry of TACoPc. Graphene oxide was electrochemically reduced to RGO at electrode surface along with concominent electropolymerization of TACoPc. The electrochemical studies showed that RGO on the pTACoP/GCE increased the effective surface area, reduced the charge transfer resistance of the electrode and enhanced the electrochemical signal. The RGO-pTACoPc film modified electrode exhibits excellent electrocatalytic ability to oxidize cysteine and hydrazine. To determine cysteine, RGO-pTACoPc sensor displayed linear concentration range of 50 nM–2.0 μ M, detection limit of 18.5 nM and sensitivity of 10.19 nAnM⁻¹ cm⁻². Besides, the sensor displayed linear concentration range of 50 nM–2.6 μ M, detection limit of 10 nM and sensitivity of 1.62 nAnM⁻¹ cm⁻² to determine hydrazine. The electrocatalytic ability of RGO-pTACoPc shows better performance over other cobalt phthalocyanine derivatives reported by other methods. Furthermore, the described sensor exhibited long-term storage stability, good repeatability and reproducibility. Practical applicability of the sensor has been assessed in biological and water

1. Introduction

Metal phthalocyanines are organic macrocyclic molecular catalysts with metal atoms at the center and possess rich redox chemistry.^{1,2} The central metal ion of phthalocyanines can reversibly bind with reactants and hence they exhibited good electrocatalytic activity to many important reactions. Cobalt phthalocyanine and its derivatives (CoPcs) are the most widely studied phthalocyanines as mediator/electrode modifiers attributed to their widespread electrochemical applications.³ However, there are two major limitations in using CoPcs at electrode surface: (1) physically adsorbed CoPcs on electrode surface is not stable and usually peel off from electrode surface; (2) They own low conductivity which reduces electron transfer rate and results in poor electrochemical activity.⁴ In order to overcome these issues, they require suitable support which should offer good conductivity, large surface area and good adhesion ability.⁵ Recently, reduced graphene oxide (RGO) attracted many interests due to its unique physicochemical properties such as

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Cysteine (CySH), an important biothiol plays crucial role in biological systems maintaining plenteous redox-related physiological process.¹² CySH is part of an endogenous antioxidant system that maintains cells' normal redox potential and it also a prominent biomarker.¹³ However, its deficiency is associated with slowed growth, hair depigmentation, edema, lethargy, liver damage, skin lesions and weakness and hence sensitive determination of CySH is important. Electrochemical methods are most preferable method to detect CySH attributed to its short reaction time, high sensitivity, and portability.¹⁴ From literature studies and our own previous reports; we have realized that CoPc derivatives are the most suitable electrocatalysts for the determination of CySH. Herein, we have developed a modified electrode based on pTACoPc at RGO for the sensitive determination of CySH. Besides, hydrazine is known for its widespread industrial applications.¹⁵ Despite its extensive

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large surface area, high conductivity, porosity and good electrocatalytic ability.⁶ The use of RGO as conducting support to stabilize electrocatalysts is widely acclaimed.⁷ A handful of reports are commenced to stabilize metal phthalocyanines at RGO, such as layer by layer,⁸ self-assembly,⁹ and chemical methods.² Electrochemical polymerization is an elegant, attractive and easy strategy to prepare stable and ordered films at electrochemical polymerization strategy to prepare highly stable electropolymerized TACoPc (pTACoPc) at RGO (Scheme 1).

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applications, it is highly toxic and hence its determination is of great importance.¹⁶ Electrochemical detection of hydrazine at bare electrodes suffers from high overpotential, low sensitivity and surface fouling issues. In the present work, we described a sensitive amperometric sensor based on pTACoPc decorated RGO to determine hydrazine. The main objective of the work is to describe a simple electropolymerization method to prepare stable pTACoPc at RGO for electrochemical sensing of CySH and hydrazine. The nanocomposite has excellent electrochemical properties such as large electrochemically active surface area, high capacitance current, wide potential window, high conductivity and large porosity and shown great electrocatalytic ability to CySH and hydrazine.

2. Materials and methods

2.1 chemicals and reagents

Graphite (powder, <20 µm), CySH and hydrazine were purchased from sigma-Aldrich and used as received. 4, 4', 4, 4"'-tetra-amino cobalt phthalocyanine (TACoPc) was synthesized according to established literature procedure.¹⁷ All other reagents were purchased from sigma-Aldrich and used without further purification. All electrochemical carried out using CHI measurements were 612D electrochemical work station at ambient temperature. Electrochemical studies were performed in a conventional three electrode cell using BAS GCE as a working electrode (area 0.071 cm²), saturated Ag|AgCl (saturated KCl) as a reference electrode and Pt wire as a counter electrode. 0.1 M tetrabutylammonium perchlorate (TBAP) dissolved in DMSO was used as supporting electrolyte for electrodeposition process, while phosphate buffer (PB) (pH 7) was used as supporting electrolyte for electrocatalytic applications. Prior to each electrochemical experiment, the electrolyte solutions were deoxygenated with pre-purified nitrogen for 10 min unless otherwise specified. Scanning electron microscopy (SEM) and Energy-dispersive X-ray (EDX) spectra were performed using Hitachi S-3000 H scanning electron microscope and HORIBA EMAX X-ACT (Sensor + 24V=16 W, resolution at 5.9 keV) respectively. Electrochemical impedance spectroscopy (EIS) studies were carried out using EIM6ex ZAHNER (Kroanch, Germany).

2.2 Electrodeposition of TACoPc at RGO

Graphite oxide was prepared from graphite by Hummers method.¹⁸ It was exfoliated to graphene oxide (GO) via ultrasonic agitation for 1 h with subsequent centrifugation at 3000 RPM for 30 min. Next, GCE surface was cleaned by polishing it with 0.05 μ m alumina slurry using Buehler polishing kit. About 5 μ l dispersion of GO (0.5 mg mL⁻¹) was dropped on pre-cleaned GCE and dried at ambient conditions. Then, GO modified GCE (GO/GCE) (3.52 μ g GO loading) was transferred to an electrochemical cell containing 1 mM TACOPc and 0.1 M TBAP in 5 mL DMSO. The electrodeposition was carried out by cyclic voltammetry at potential range between 1.0 V and – 2.6 V for 20 cycles, while scan rate was 0.1 Vs⁻¹.

Subsequently, the electropolymerized TACoPc at RGO (RGOpTACoPc/GCE) was carefully washed in DMSO and dried. As control, RGO/GCE and pTACoPc/GCE were also prepared.



Scheme 1 Preparation of graphene oxide and electropolymerization of TACoPc on RGO for electrocatalytic determination of CySH and hydrazine

3. Results and Discussions

3.1 Electrodeposition of TACoPc and simultaneous electrochemical reduction of GO

Fig. 1A displays the cyclic voltammograms (CVs) obtained for electrodeposition of TACoPc. The growth of peaks with increasing peak currents indicates the electropolymerization of TACoPc. The polymerization of TACoPc involves oxidation of amino group to form radicals which initiate condensation by attacking phenyl rings of neighbouring molecules. The CV obtained at RGO-pTACoPc/GCE features with three important redox couples at formal potentials ($E^{0'}$) of 0.002 V (1, 2), – 0.709 V (3, 4) and – 1.323 V (5, 6) and the redox process can be represented as follows (Fig. 1B),¹⁹

$$Co^{3+}/Co^{2+}$$
: $[Co^{3+}, Pc^{2-}]^+ + e^- \leftrightarrow [Co^{2+}, Pc^{2-}], E^{0'} = + 0.002 V (1, 2)$

$$Co^{2+}/Co^{+}$$
: $[Co^{2+}, Pc^{2-}] + e^{-} \leftrightarrow [Co^{+}, Pc^{2-}]^{-}, E^{0'} = -0.709 V (3, 4)$

$$Pc^{2-}/Pc^{3-}$$
: $[Co^{+}, Pc^{2-}]^{-} + e^{-} \leftrightarrow [Co^{+}, Pc^{3-}]^{2-}, E^{0'} = -1.323 V (5, 6)$

In general, the redox pair situated in most positive potential region is due to Co^{3+}/Co^{2+} , the middle pair is due to Co^{2+}/Co^{+} and the pair situated in most negative potential region is due to ring based redox process.²⁰ Here, the redox pair located at $E^{0'}$ of + 0.002 V is assigned to metal based electron transfer process (Co³⁺/Co²⁺) and the middle pair appeared at $E^{0'}$ of – 0.709 V is assigned to Co^{2+}/Co^{+} process. The redox pair appeared at $E^{0'}$ of -1.323 V is assigned to phthalocyanine ring based redox process (Pc²⁻/Pc³⁻).^{10,19} The lack of complete reversibility in Co^{3+}/Co^{2+} redox couple might effects.²¹ surface adsorption The be due to

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for

electropolymerization of TACoPc at bare GCE is unable to produce good peak growth indicating that the polymerization at bare GCE is scarcely formed (inset to Fig. 1A). However, the peak growth is uniform at GO/GCE which reveals successful polymerization of phthalocyanine. In our approach, both electrochemical reduction of GO and electrodeposition of TACoPc are occurring simultaneously which assists TACoPc to find fresh sites at RGO during each cycles. The pTACoPc acts as a spacer between RGO and makes both faces of RGO accessible. Additionally, a small cathodic peak was observed at the potential of -1.0 V during first cycle and disappeared in subsequent cycles which is characteristic behavior expected electrochemical reduction of GO.²² Also, the electrochemical reduction of GO/GCE (without CoTAPc) in DMSO exhibited typical voltammograms expected for reduction of GO (Fig. S1).



Fig. 1 (A) 20 consecutive CVs obtained at GO/GCE in DMSO containing 1 mM CoTAP and 0.1 M TBAP. Scan rate= 0.1 Vs⁻¹. Inset: Electropolymerization of TACoPc on unmodified GCE. (B) CV obtained at RGO-pTACoPC/GCE in DMSO containing 0.1 M TBAP, scan rate= 0.02 Vs⁻¹. (C) CVs obtained at RGOpTACoPc/GCE in DMSO containing 0.1 M TBAP at various scan rates a to j: 0.01 to 0.1 Vs⁻¹. (D) Electrochemical impedance spectra of pTACoPc (a), RGO (b) and RGO-pTACoPc (c) performed in 0.1 M KCl containing 5 mM Fe(CN)₆^{3-/4}-. Amplitude: 5 mV. Frequency: 0.1 Hz to 100 kHz.

3.2 Electrochemically active surface area, cyclic and storage stability and electrode kinetics

The electrochemically effective surface areas of RGO/GCE, pTACoPc/GCE and RGO-pTACoPc/GCE have been assessed using K₃[Fe(CN)₆] as a model redox mediator, while Randles-Sevcik equation was used to calculate areas.²³ The active surface areas of RGO/GCE, pTACoPc/GCE and RGOpTACoPc/GCE were calculated to be 0.103 cm², 0.076 cm² and 0.152 cm². Furthermore, the RGO-pTACoPc/GCE retained 97.05% of its initial response current even after one month of its storage period and also retained 96.22% of its initial peak current even after 300 successive cycles revealing its excellent storage and cyclic stability respectively. Fig. 1C portrays the CVs obtained at RGO-pTACoPc/GCE for different scan rates. The anodic (I_{pa}) and cathodic peak currents (I_{pc}) of all three redox couples were increased with scan rates (v). The peak

shape is sharp at lower scan rates, while changed to broader at higher scan rates due to slow kinetics at higher scan rates. The plots of I_{pa} and I_{pc} versus v for all three redox couples exhibits linear relationship which are characteristic of surface confined processes (Fig. S2). The loading of TACoPc was estimated using electrochemical quartz crystal microbalance studies and adopting well-known Sauerbrey equation which relates the change in mass (i.e. loading of electrodeposited film) at the quartz crystal with change in frequency. 24 About 0.312 $\mu g\ cm^{-2}$ of pTACoPc loading was estimated on the electrode. The loading of GO is 3.52 μ g cm⁻² and the weight ratio between RGO and pTACoPc is calculated to be about 11.28. The film thickness effect is studied on the electrocatalytic performance towards oxidation of CySH (inset to Fig. 3A) and hydrazine (inset to Fig. 4A). As shown in figures, the film produced at the electrodeposition cycle of 20 showed optimum catalytic performances. Further increase in the cycle numbers led to the decreased response current attributed to the formation of thick film. Therefore, we have chosen 20 cycles as the optimum cycle number for preparing pTACoPc film.

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Fig. 2 SEM of RGO (A) and RGO-pTACoPc (C). EDX of RGO (B) and RGO-pTACoPc (D)

3.3 Morphology and elemental analysis

The SEM image of RGO presents characteristic thin sheets like morphology with wrinkles and crumbles (Fig. 2A), while its EDX spectrum presents signals for carbon and oxygen with weight percentages of 89.54 and 10.46 respectively (Fig. 2B and Fig. S3A). The SEM image of RGO-pTACoPc nanocomposite exhibited similar morphology of RGO (Fig. 2C); however, EDX analysis indicates presence of cobalt and nitrogen signals which validated the formation of nanocomposite (Fig. 2D and Fig. S3B). EIS is an efficient tool to examine the electrical and interfacial properties of the modified films on the electrode surface. Fig. 1D shows the EIS obtained at pTACoPc (a), RGO (b), and RGO-pTACoPc (c) films modified GCEs in 0.1 M KCl containing 5 mM Fe(CN) $_{6}^{3-/4-}$. Randles equivalent circuit model has been used to fit the experimental data where, $R_{\rm s}$ is electrolyte resistance, R_{ct} is charge transfer resistance, C_{dl} is

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double layer capacitance and Z_w is Warburg impedance (inset to Fig. 1D). EIS measurements were represented as Nyquist plots, in which semicircles indicates the parallel combination of electron transfer resistance (R_{ct}) and double layer capacitance (C_{cll}) at electrode surface resulting from electrode impedance, while the linear portion represents diffusion limited process.

The size of curves (diameter of the semicircle portion) obtained was in the order of pTACoPc/GCE > RGO/GCE > RGO-pTACoPc/GCE. R_{ct} values of the electrodes were obtained by fitting the Nyquist plot results with Randles equivalent circuit model. The obtained R_{ct} values of pTACoPc/GCE, RGO/GCE and RGO-pTACoPc/GCE with respect to their effective area are 7.47, 1.94 and 0.3 K Ω cm⁻² respectively. The R_{ct} value obtained at RGO-pTACoPc/GCE is 25 and 6.5 times smaller than that of pTAcoPc/GCE and RGO/GCE, respectively. Thus, RGO-pTACoPc/GCE has significantly reduced charge transfer resistance over controls electrodes (RGO/GCE, pTACoPc/GCE).

3.4 Electrocatalysis of cysteine

The CVs obtained at unmodified GCE (a), RGO/GCE (b), pTACoPc/GCE (c) and RGO-pTACoPc/GCE (d) in PB (pH 7) containing 0.5 mM CySH were presented as Fig. 3A, while their corresponding electrochemical parameters such as, peak potential (E_p) and peak current (I_p) (with respect to their effective surface area) are given as Table S1. The electrocatalytic abilities of these electrodes are in the RGO-pTACoPc/GCE > pTACoPc/GCE > following order: RGO/GCE > unmodified GCE. The unmodified GCE and RGO/GCE were scarcely oxidizing CySH. The pTACoPc/GCE oxidizes CySH at overpotential of 0.30 V, however with smaller Ip. Among the electrodes, RGO-pTACoPc/GCE exhibited maximum electrocatalytic ability in terms of both peak current and overpotential. In comparison with pTACoPc/GCE, RGOpTACoPc/GCE exhibited 150 mV less overpotential and 5 fold increment in peak current which clearly indicate the enormous role of RGO in facilitating electrocatalytic ability. The large surface area of RGO provides sufficient anchoring sites to form electrochemically active pTACoPc film. Besides, the good conductivity of RGO provides significantly enhanced sensitivity.

The effect of scan rate was examined in PB (pH 7) containing 0.5 mM CySH at different scan rates ranges (Fig. 3B). The value of I_{pa} increases as the scan rate increases from 0.1 to 1 Vs⁻¹. A plot between square root of scan rates and I_{pa} exhibited linear relationship indicating diffusion controlled oxidation process (inset to Fig. 3B). Also, a plot of E_{pa} vs. log v displayed linear relationship with slope 89 mV/decade which indicating that two electrons were involved in the rate determining step (Fig. S4). E_{pa} can be represented by the Tafel eq. (1),²⁵

$$E_{pa} = [2.303RT/(1-\alpha) n_a F] \log v + K$$
(1)

Where, α and n_a (= 1) are representing electron transfer coefficient and number of electrons involved in the ratedetermining step. The constants *R*, *T* and *F* have their usual meanings (*R*=8.314 J K-1mol⁻¹, *T*=298 K, *F*=96485 C mol⁻¹). By substituting all the parameters in eq. (1), the value of α was Journal Name

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calculated as 0.66 which is close to 0.5 means that the activated complex is around halfway between reactants and products on the reaction coordinate.²⁶ The mechanism for the oxidation of CySH can be represented by eq. (2), (3) and (4).

$$Co^{2+}Pc \rightarrow [Co^{3+}Pc]^{+} + e^{-}$$
(2)

$$[Co^{3+}Pc]^{+} + RSH \rightarrow Co^{2+}Pc + RS^{\bullet} + H^{+}$$
(3)

$$RS^{\bullet} \rightarrow RSSR$$
 (4)

Here, RSH refers CySH, while RSSR refers cystine.

3.5 Determination of cysteine

Fig. 3C displays the CVs obtained at RGO-pTACOPc/GCE in absence (curve a) and presence of different concentration of CySH ranging from 50 μ M to 0.7 mM in PB (pH 7). The value I_{pa} increases linearly as the concentration of CySH increases. A plot between I_{pa} and [CySH] exhibited linear relationship (inset to Fig. 3C) with slope of 0.2106 μ A μ M⁻¹. The linear concentration range was observed from 50 μ M to 0.7 mM. Fig. 3D displays the amperometric i-t curves obtained at RGO-pTACOPc film modified rotating disc GCE for sequential addition of 100 nM CySH into PB. The rotation speed of electrode was 1500 RPM, while the applied potential (E_{app}) was + 0.20 V.



Fig. 3 (A) CVs obtained at unmodified (a), RGO (b), pTACOPC (c) and RGOpTACOPC composite (d) films modified electrodes in PB (pH 7) containing 0.5 mM CySH at scan rate 50 mVs⁻¹. Inset: optimization of cycle numbers for electrodeposition of pTACOPC at RGO/GCE. CVs were carried out at RGOpTACOPC/GCE (0, 5, 10, 15, 20, 25 and 30 cycles) in PB (pH 7) containing 0.5 mM CySH. (B) CVs obtained at RGO-pTACOPC/GCE in PB (pH 7) containing 0.5 mM CySH at different scan rates (a to j; 0.1 to 1 Vs-1). Inset: I_p vs. $v^{1/2}$; $I_{pa}/\mu A = 603.8$ $v^{1/2}$ ($\mu A/(Vs^{-1})^{1/2}$) – 116.75, R^2 =0.987 (C) CVs obtained at RGO-pTACOPC/GCE in PB (pH 7) containing CySH; a=0, b=0.05, c= 0.1, d= 0.15, e=0.20, f=0.25, g=0.30, h=0.35, i=0.40, j=0.45, k=0.50, l=0.55, m=0.60, n= 0.65 and o= 0.70 mM. Inset: [CySH] vs. I_p ; $I_p/\mu A = 0.211$ [CySH] ($\mu A/\mu M$) + 34.03. (D) Amperometric response of RGO-pTACOPC modified rotating disc GCE upon each sequential addition of 100 nM CySH into continuously stirred PB (pH 7) at rotation speed of 1500 RPM.

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 $E_{\rm app} = + 0.25 \text{ V. Inset: [CySH] vs. } I_p; I_p (\mu A) = 2.14 \text{ [CySH] (nA/nM)} + 0.0391, R^2 = 0.999.$

The RGO-pTACoPc film exhibited quick and sensitive amperometric response to each addition of CySH. The response current reached 95% steady-state current within 5s of CySH injection indicating rapid detection. The concentration dependent linear plot displayed good linearity with slope of 2.14 nA nM⁻¹ (inset to Fig. 3D). The working concentration range was linear from 50 nM – 2.0 μ M with sensitivity of 10.19 nAnM⁻¹ cm⁻². The limit of detection (LOD) was calculated as 18.5 nM (S/N=3). The LOD was calculated using the formula, LOD= 3 s_b/S where, s_b is the standard deviation of ten blank measurements and S is the sensitivity. The important parameters of sensor, such as LOD, linear range and sensitivity were compared with other modified electrodes available in literature (Table 1). The analytical performance of the RGOpTACoPc/GCE film is comparable with the previous reports. Remarkably, LOD of the sensor is much lower than the previously reported sensors.

Table 1 Comparison of electroanalytical parameters obtained at RGO-pTACoPc/GCE with previous reports for the determination of CySH $\,$

Electrodes	LOD/	Linear	Sensitivity/	Ref.
	μΜ	range/µM	μAμM cm ^{−2}	
^a FePc-based SAM	2	4-100	-	28
^b CoTPC/poly-l-lysine	0.15	0.50-216	0.157	29
Au NPs- ^b FePc	0.27	50-1000	57.2	30
Pectin/Au NPs/MWCNTs	0.019	0.1-1000	0.46	31
^c FeTSPc/MWCNTs	1	10-200	0.176	32
RGO-pTACoPc/GCE	0.018	0.05-2.0	10.19	This
				work

^aFePc= Iron phthlocyanine ^bCoTSPc= Cobalt tetrasulphonated phthalocyanine; ^cFeTSPc= Iron Tetrasulfonated Phthalocyanine

3.6 Repeatability, reproducibility, stability and selectivity

In order to calculate repeatability of the sensor, five repeated amperometric experiments were performed using RGOpTACoPc film modified electrode towards determination of CySH (100 nM). The relative standard deviation (R.S.D) between five repeatable measurements was calculated to be 2.14%. In order to calculate reproducibility of the sensor, five different RGO-pTACoPc films modified electrodes were fabricated and amperometric experiments were performed on each electrodes towards determination of CySH (100 nM). The R.S.D between five amperometric measurements was calculated to be 2.83%. The obtained R.S.D values are in the acceptable range and hence the sensor validated with good repeatability and reproducibility. Next, stability of the described film on the electrode surface was investigated. The sensor retained 94.28% of its initial response current even after one month storage period validated good storage stability. Only 6.4% of initial peak current was loosed even after 200 cycles indicating the excellent stability and antifouling ability of the modified electrode.

Selectivity study was carried out in order to investigate anti-interference ability of the sensor. The common interfering species in CySH analysis, such as, glycine, alanine, phenylalanine, lactic acid, L-tyrosine, glucose, uric acid, valin, and creatinine were tested. As shown in the fig. S5, 500-fold excess concentration of these species had no significance influences on the sensing of CySH which validated selectivity of the film.

3.7 Real-time application

Practical feasibility of the sensor has been assessed in human serum sample. Blood sample was collected from a healthy man and allowed to clot. Next, the clot was removed through centrifugation for 15 min at a speed of $2000 \times g$ and serum was separated and stored at -20° C. 1 mL serum sample was diluted with 50 mL PB. Next, known concentrations of CySH were injected and detection analyses were performed adopting similar conditions optimized for lab sample (Table S2). The described sensor has shown acceptable recoveries which validated practical applicability of the sensor.

3.8 Electrocatalysis of hydrazine at RGO-pTACoPc/GCE

Fig. 4A presents the CVs obtained at unmodified (a), RGO (b), pTACoPc (c) and RGO-pTACoPc (d) films modified GCEs in presence of 1 mM hydrazine. The unmodified GCE is unable to oxidize hydrazine, while RGO/GCE and TACoPc/GCE were shown poor electrocatalytic ability. However, RGOpTACoPc/GCE exhibited a sharp I_{pa} with significantly improved peak current. The overpotential obtained at RGOpTACoPc/GCE is + 0.17 V, which is significantly smaller than that obtained at other electrodes. Additionally, I_{pa} observed at RGO-pTACoPc is 5.6 fold higher than that observed at RGO/GCE. The obtained less overpotential and large Ipa revealed good electrocatalytic ability of the RGO-pTACoPc. The effect of applied scan rate to hydrazine oxidation was investigated (Fig. 4B). The I_{pa} increases as the scan rate increases from 0.05 to 0.5 Vs-1. A plot between $v^{1/2}$ and $I_{\rm p}$ exhibits good linearity indicating diffusion controlled process (inset to Fig. 4B). A plot between E_{pa} and log v displays good linearity with slope of 109.4 mV/decade which indicates that the rate determining step involves one electron process in (Fig. S6). The value of α was estimated to be 0.67 by substituting n_a (= 1) and slope of E_{pa} vs. log v in eq. (1). The value of α is close to 0.5 indicating that the activated complex is almost midway between reactant and product. The number of electrons (n) involved in the overall hydrazine oxidation process was calculated using eq. (5),²⁵

$$I_{\rm p} = (2.99 \times 10^5) n \left[(1 - \alpha) n_{\rm a} \right]^{1/2} A C_{\rm o} D_{\rm o}^{1/2} v^{1/2}$$
(5)

Here, D_0 (cm² s⁻¹) is diffusion coefficient (2.42×10⁻⁵ cm² s⁻¹, estimated via chronoamperometry), *A* is electrode area (0.071 cm²) and C_o is bulk concentration of hydrazine (1×10⁻³ mol cm⁻³), while other parameters represents their conventional meanings. By substituting all these parameters in eq. (5), the value of *n* was calculated as four which is consistent with previous reports.^{16,27} The first step involves slow one-electron

(8)

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transfer (eq. 6), while second step involves fast three-electron transfer (eq. 7).

$$N_2H_4 + H_2O \rightarrow N_2H_3 + H_3O^+ + e^- (slow)$$
 (6)

 $N_2H_3 + 3H_2O \rightarrow N_2 + 3H_3O + + 3e^{-}$ (fast) (7)

The overall reaction can be given as eq. (8) [27],

$$N_2H_4 + 4H_2O \rightarrow N_2 + 4H_3O + + 4e^-$$



Fig. 4 (A) CVs obtained at unmodified (a), RGO (b), pTACOPc (c) and RGO-pTACOPc (d) film modified GCEs in PB (pH 7) containing 1 mM hydrazine. Scan rate= 50 mVs⁻¹. Inset: optimization of pTACOPc electrodeposition cycles. CVs were carried out at RGO-pTACOPc/GCE (0, 5, 10, 15, 20, 25 and 30 cycles) in PB (pH 7) containing 1 mM hydrazine. (B) CVs obtained at RGO-pTACOPC/GCE in PB (pH 7) containing 1 mM hydrazine at different scan rates from 0.05–0.5 Vs⁻¹. Inset: $v^{1/2}$ vs. I_{pL}/μ A= 333.32 $v^{1/2}$ (μ A/(Vs⁻¹)^{1/2}) – 24.522. (C) CVs obtained at RGO-pTACOPC/GCE in PB (pH 7) containing hydrazine; a=0, b=0.2, c= 0.4, d=0.6, e=0.8, f=1.0, g=1.2, h=1.4, i=1.6, j=1.8, k=2.0, l=2.2, m=2.4, n=2.6 and o=2.8 mM. Inset: [hydrazine] vs. $I_{p,i}$ / μ /µA= 42.88 [hydrazine] (mM) + 6.45. (D) Amperometric response of RGO-pTACOPC film modified rotating disc GCE upon each sequential addition of 50 nM hydrazine into continuously stirred PB (pH 7) at rotation speed of 1500 RPM. $E_{app} = + 0.20$ V. Inset: [hydrazine] vs. $I_{p,i}$ / μ /µA= 0.341× 10⁻³ [hydrazine] (μ A/nM)–0.005.

Table 2 Comparison of electroanalytical parameters obtained at RGO-pTACoPc/GCE with reported works for the determination of hydrazine

Electrode	Detection limit/µM	Linear range/µM	Sensitivity /µAµM ⁻¹ cm ⁻²	Ref.
Curcumin/MWCNT	1.4	2–44	0.322	33
ZnO nanonail	0.2	0.1-1.2	8.56	34
C@ZnO nanorod	0.1	0.1-3.8	9.4	35
CuS/RGO	0.3	1-1000	0.008	36
Co-graphene	0.1	0.25-370	0.562	37
RGO-pTACoPc/GCE	0.01	0.05-2.6	1.624	This
				work

3.9 Determination of hydrazine

Fig. 4C shows the CVs obtained at RGO-pTACoPc/GCE in PB (pH 7) containing different concentration of hydrazine. The I_{pa}

increases as the concentration of hydrazine increases. A concentration dependent linear plot was obtained (inset to Fig. 4C). The concentration range was linear over 0.2 mM-2.8 mM with sensitivity of 42.88 μ A mM⁻¹. Fig. 4D presents the amperometric curves obtained at RGO-pTACoPc film modified rotating disc GCE for sequential addition of 50 nM hydrazine into PB (pH 7). The rotation speed was 1500 RPM, while E_{app} was + 0.25 V. The concentration dependent current plot displayed good linearity with slope of 0.341 nA nM⁻¹ (inset to Fig. 4D). The concentration range was linear from 50 nM to 2.6 μ M with sensitivity of 1.624 nA nM⁻¹ cm⁻², while LOD was calculated to be 10 nM (S/N=3). The important sensor parameters, such as α , D_{o} , LOD and linear range were compared with other sensors (Table 2). The described sensor exhibited quite comparable performance with reported sensors. Notably, LOD of the sensor is 10 nM which surpassed most of the reported metal phthalocyanines based sensors. Therefore, the described electropolymerization method has great advantage over other methods to fabricate highly sensitive hydrazine sensor.

3.10 Repeatability, reproducibility, stability, selectivity and real sample analysis

In order to calculate repeatability of the sensor, five repeated amperometric experiments were performed using RGOpTACoPc film modified electrode towards determination of hydrazine (100 nM). The R.S.D between five repeatable measurements was calculated to be 2.55%. In order calculate reproducibility of the sensor, five different RGO-pTACoPc films modified electrodes were fabricated and amperometric experiments were performed on each electrodes towards determination of CySH (100 nM). The R.S.D between five amperometric measurements was calculated to be 2.47%. The obtained R.S.D values are in the acceptable range and hence the sensor validated with good repeatability and reproducibility.

In order to determine storage stability, the electrocatalytic response of the sensor was monitored every day; 95.45% of initial I_p was retained over one month of its continuous use which revealed good storage stability. Stability of the sensor under hydrodynamic conditions was investigated. Only 5.4% of initial response current was decreased even after continuously operation for 3000 s.

Selectivity of the sensor was investigated in presence of likely interferences, such as, F^- , CI^- , Br^- , I^- , CO_3^{2-} , NO_3^- , NO_2^- , Na^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , NH_4^+ , glucose and fructose (1000-fold excess concentration than hydrazine) (Fig. S7). As shown in figure S7, the senor had not shown any quantifiable responses to these species; however it shows quick response to hydrazine which revealed its good anti-interference ability. Water samples collected from rain and tap were employed to evaluate practicality of the sensor, (Table S3). The spiked hydrazine concentrations were 50 mM and 100 nM. The found and recovery values are in acceptable range validated practical feasibility of the sensor.

4. Conclusions

summary, developed simple In we have а electropolymerization approach to prepare RGO-pTACoPc film modified electrode. The polymerized film was characterized by morphological, elemental and electrochemical methods. The presence of RGO on the electrode surface greatly improves the polymerization process and also significantly enhances conductivity and electrocatalytic ability as revealed by EIS data. The RGO-pTACoPc film modified electrode exhibited excellent electrocatalytic ability to CySH and hydrazine. The effects of film thickness, applied scan rate and analytes concentration were studied. The electrode detects CySH at wide linear range of 50 nM–2.0 μ M with LOD of 18.5 nM. The electrode detects hydrazine with wide linear range of 50 nM-2.6 μ M and low LOD of 10 nM. Besides, the electrode showed appreciable stability, repeatability, reproducibility and practicality. The easy preparation procedure, high stability and excellent electrocatalytic ability of RGO-pTACoPc revealed its great potential in electrochemical sensor applications.

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A simple electrodeposition route to prepare tetraamino phthalocyanine polymers on reduced graphene oxide for electrochemical determination of cysteine and hydrazine