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Determination of L-cysteine at Iron Tetrasulfonated Phthalocyanine Decorated Multiwalled Carbon Nanotubes Film Modified Electrode

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A sensitive amperometric sensing platform has been developed for the determination of L-Cysteine (L-Cys) at iron tetrasulfonated phthalocyanine (FeTsPc) decorated multiwalled carbon nanotubes (MWCNT) composite film modified electrode. Surface morphological studies revealed uniform decoration of the FeTsPc at the surface of MWCNT. MWCNT-FeTsPc composite modified GCE has been prepared which exhibited prominently enhanced electrocatalytic performance to the oxidation of L-Cys with two oxidation peaks that are associated with FeTsPc mediated electrocatalysis. The overpotential of the oxidation peaks were greatly reduced to – 0 .1 V and + 0.22 V, while the peak currents were significantly improved in comparison with control electrodes. An amperometric sensor was fabricated which exhibited excellent electroanalytical parameters for the determination of L-Cys including wide linear range (i0 µM to 0.2 mM) and very low detection limit (1 µM). Practical feasibility of the sensor has been assessed in human urine samples. Moreover, the sensor exhibited excellent stability, repeatability and reproducibility results.

Keywords: MWCNT, iron phthalocyanine, L-Cysteine, electrochemistry, amperometry.

1. INTRODUCTION

Iron tetrasulfonated phthalocyanine (FeTsPc) is a two dimensional organic macrocyclic electrocatalyst with iron atom at the center and consisting of 18 π electrons with conjugated planar structure [1-3]. FeTsPc has excellent electrocatalytic ability and unique physicochemical properties and therefore it finds widespread application in electrochemistry as a mediator/electrode modifier for the fabrication of sensors and biosensors [4-6]. However, there are two main limitations in the use of FeTsPc at the electrode surface: (1) physically adsorbed FeTsPc on the electrode surface is unstable and hence they tend to peel off from the electrode surface. (2) FeTsPc has low electrical conductivity and reduces the electronic transfer rate; as a result the electrode has been limited to poor electrochemical activity [7]. Therefore, FeTsPc is requiring suitable conducting support. Multiwalled carbon nanotube (MWCNT) is a highly versatile conducting support attributed to its excellent physicochemical properties such as large surface area, excellent electronic and mechanical properties [8-11]. In the present work, we have described a simple method for the stabilization of FeTsPc at MWCNT surface aiming towards the preparation of highly stable MWCNT-FeTsPc composite.

L-Cysteine (L-Cys) is an amino acid plays an important role in biological systems and widely used in food and pharmaceutical applications [12-14]. Nevertheless, its deficiency is associated with several health issues such as hair depigmentation, lethargy, muscle and fat loss, liver damage, weakness and skin lesions [15-17]. Therefore, sensitive determination of L-Cys is highly important [18-21]. In comparison with other analytical methods, electrochemical methods offer great advantages such as simplicity, short reaction time, direct use in point-of-care assays and portability [8, 22-24]. However, electrochemical detection of L-Cys at bare electrodes suffers from high overpotential, high level of interference from other analytes, low sensitivity and fouling effect issues [25]. Therefore, chemically modified electrodes are fabricated to avoid the aforementioned issues associated with the bare electrodes [26, 27].

The main objective of the work is to prepare highly stable MWCNT- FeTsPc composite and develop a sensitive determination platform for the detection of L-Cys. The preparation of composite and fabrication of the modified electrode involve very simple and easily adoptable protocols. The nanocomposite exhibited excellent electrocatalytic activity to the determination of L-Cys and presented outstanding electroanalytical parameters such as wide linear range, low detection limit and high sensitivity.

2. EXPERIMENTAL

2.1. Reagents and apparatus

FeTsPc was purchased from Porphyrin Products Inc, Utah, U.S. MWCNT (bundled > 95%, O.D × I.D × length of 7-15 nm × 3-6 nm × 0.5-200 μm) and all other chemicals were purchased from Sigma-Aldrich. All the chemicals used were of analytical grade and used without further purification. 0.1 M of phosphate buffer solution (PBS) was prepared using Na_2HPO_4 and NaH_2PO_4. Double distilled water with conductivity ≥18 MΩ was used for all the experiments. A stock solution of L-Cys was prepared in PBS (pH 7).

Electrochemical measurements were carried out using CHI 611A work station in a conventional three electrode cell with modified GCE as a working electrode (area 0.071 cm^2), saturated Ag|AgCl (saturated KCl) as a reference electrode and Pt wire as a counter electrode. All the electrochemical experiments were carried out at ambient temperature. Amperometric measurements were performed with analytical rotator AFMSRX (PINE instruments, USA) and rotating disc glassy carbon electrode (RDE, area 0.21 cm^2). Scanning electron microscopy (SEM) was performed using Hitachi S-3000 H scanning electron microscope.

2.2. Preparation of MWCNT-FeTsPc composite and fabrication of MWCNT-FeTsPc/GCE

1 mL of MWCNT (2 mg/ml) and 1 mL of FeTsPc (0.5 mg/ml) were mixed together and ultrasonicated for 2 hours. The resulting residue was washed several times with deionized water to remove excess FeTsPc and the final composite was dried at oven. The final composite (MWCNT-FeTsPc) was redispersed in DMF and used for the fabrication of electrode. The glassy carbon electrode (GCE) surface was polished with 0.05 μm alumina slurry using a Buehler polishing kit, then washed with water, ultrasonicated for 5 min and allowed to dry. Then, 5 μl dispersion of MWCNT-FeTsPc nanocomposite was drop casted on the GCE surface and dried at ambient conditions. As a control, MWCNT/GCE and FeTsPc/GCE were also prepared accordingly. All the electrochemical experiments were carried out at room temperature.

3. RESULTS AND DISCUSSIONS

3.1 Surface morphological characterization of MWCNT-FeTsPc composite

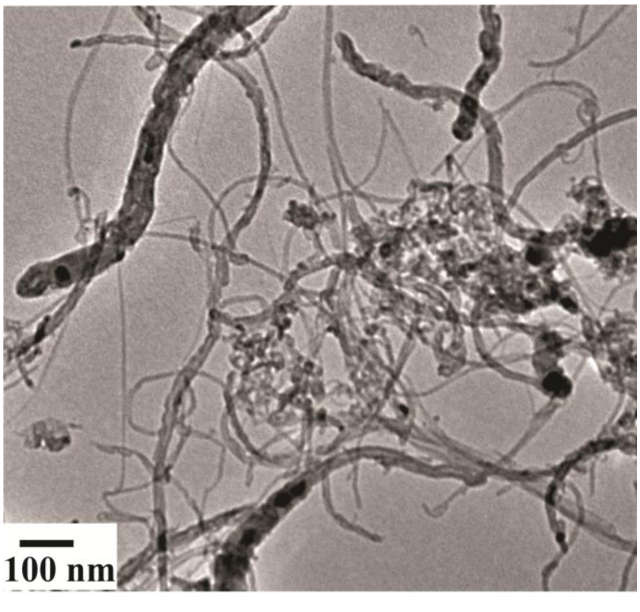

Figure 1. TEM image of the MWCNT-FeTsPc composite

Transmission electron microcopy (TEM) image of the MWCNT-FeTsPc composite has been shown in figure 1. The TEM image presents the characteristic tubular network of MWCNTs along with the decoration of FeTsPc at the tips and walls of the MWCNTs confirmed the successful formation of MWCNT-FeTsPc composite. The composite in the DMF dispersion is highly stable for more than one month without aggregation which revealed the appreciable stability of the composite.

3.2 Electrocatalysis of MWCNT-FeTsPc composite film modified electrode to the oxidation of L-Cys

Fig. 2 shows the CVs obtained at FeTsPc (A), MWCNT (B) and MWCNT–FeTsPc (C) films modified GCEs in the absence (curve a) and presence of 1 mM L-Cys (curve b) at the scan rate of 50 mVs^{-1}. The electrocatalytic behavior of these electrodes follows the order: FeTsPc/GCE< MWCNT/GCE< MWCNT–FeTsPc/GCE. Both, FeTsPc/GCE and MWCNT/GCE have exhibited poor electrocatalytic ability to the oxidation of L-Cys.

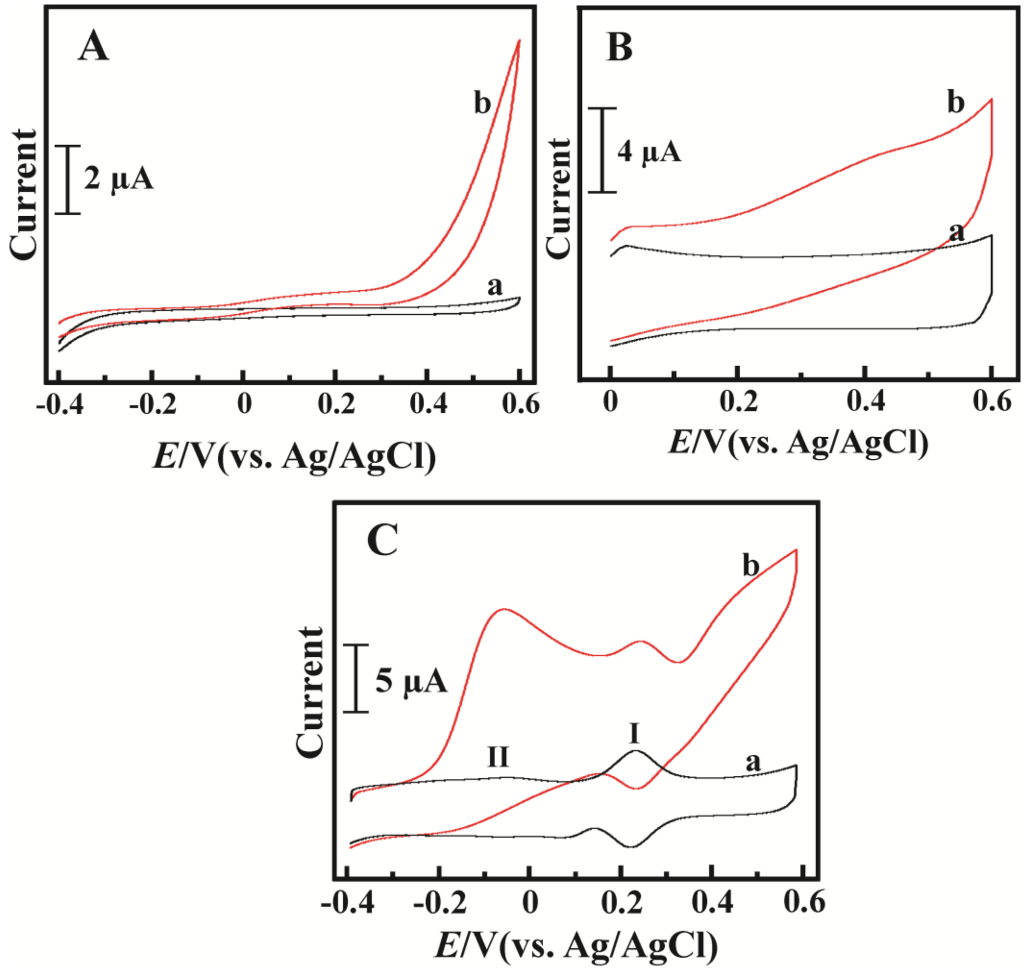

Figure 2. CVs obtained at FeTsPc (A), MWCNT (B) and MWCNT–FeTsPc (C) films modified GCEs in PBS (pH 7) in the absence (a) and presence of 1 mM L-Cys (b) at the scan rate of 50 mVs^{-1}.

In these electrodes, the oxidation occurs at high overpotential, while no obvious oxidation peak was observed. The CV of MWCNT–FeTsPc/GCE presented a sharp reversible redox couple (I) at the formal potential of + 0.221 V which is assigned to the one electron redox reaction involving iron and phthalocyanine $[Fe(III)Pc(-2)]^{+}/Fe(II)Pc(-2)$ [28]. Additionally, a quasi-reversible redox couple (II) was observed at the formal potential of - 0.1 V [4]. In the presence of 1 mM L-Cys, the oxidation peak currents of these two redox couples (I and II) were greatly increased at the potentials of +0.22 V and -0.1 V respectively responsible for the FeTsPc mediated electrocatalytic oxidation of L-Cys. The large decrease in overpotential and substantial increase in the oxidation peak currents at the MWCNT–FeTsPc/GCE is indicating the excellent electrocatalytic ability of the modified electrode towards oxidation of L-Cys. The superior electrocatalytic ability of the composite should be attributed to the large surface area and high electrical conductivity of the MWCNT and good electrocatalytic behavior of the FeTsPc. Additionally, there might be strong synergy between MWCNT and FeTsPc to enhance the electrocatalysis of the composite.

3.3 Different pH studies

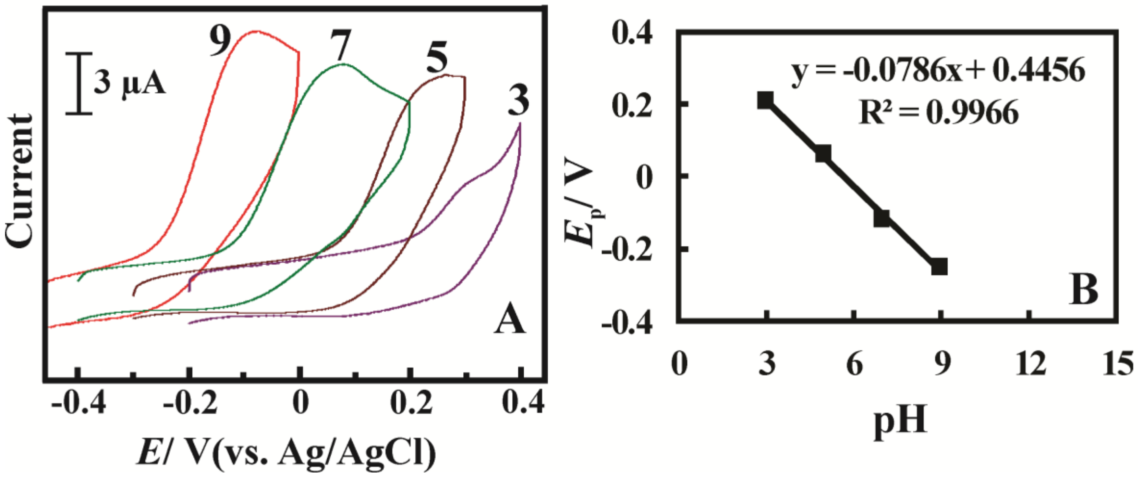

Figure 3. (A) CVs obtained at MWCNT–FeTsPc/GCE in PBS of various pH (pH 3–9) containing 1 mM L–Cys at the scan rate of 50 mV s^{-1}. (B) Plot of different pH vs. $E_{p.}$

The effect of pH of the supporting electrolyte has been investigated to understand the influence of various pHs towards oxidation of L-Cys. The electrocatalytic behavior of the MWCNT–FeTsPc/GCE towards oxidation of L-Cys was studied in PBS with different pH values (pH 3 to 9) containing 1 mM L-Cys (Fig. 3A). As shown in the plot, the L-Cys oxidation peak was significantly affected in different pH solutions. The oxidation peak current increases with increasing pH from 3 to 9, while the peak potential varies drastically. Depending on the electrolyte pH, L-Cys has existed in various forms, such as, H_3A^+ (amino group protonated), H_2A (uncharged), HA^- (carrying a COO^- group) and A^{2-} (carrying COO^- and S^-). A^{2-} is present at pH ≤12, HA^- is present at pH = 9.0–12.0, H_2A (in the form of zwitterion) is present at the pH ranges from 4.0–9.0. The protonated species, H_3A^+ is largely present at pH values < 4.0. In our experimental results, the maximum electrocatalytic response for the oxidation of L-Cys was observed at the pH of 9 which indicating that H_2A form of L-Cys (in the form of zwitterion) is more likely presents in the electrolyte solution. Despite the maximum performance at pH 9, the peak current has shown significant enhancement at pH 7. Also, considering practical feasibility, we choose pH 7.0 as the working pH for the determination experiments [29]. The plot between pH and peak potential has exhibited linear relationship (Fig. 3B). However, considering the practicality of the electrode, we choose pH 7 as the working condition for all the electrochemical experiments.

3.4 Different scan rates

The effect of scan rate (v) towards oxidaiton of L-Cys at the MWCNT–FeTsPc/GCE was examined in PBS (pH 7) at the scan rate ranges from 0.1 to 01 V s^{-1} (Fig. 4A). Both the anodic peak currents (I and II) assigned to the oxidation of L-Cys are increased linearly with increase in the scan rates from 0.1 to 1 Vs^{-1}. Moreover, the peak potentials of both the peaks were shifted to positive potential values upon scan rate increases. A plot of square root of scan rates ($v^{1/2}$) and anodic peak current exhibited linear relationship indicating that the L-Cys oxidation occured at the MWCNT–FeTsPc/GCE is a diffusion controlled electron transfer process (Fig. 4B) [30].

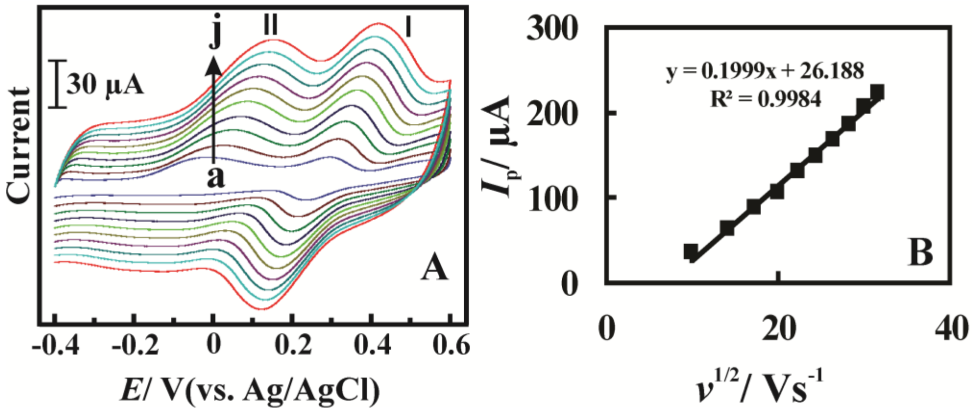

Figure 4. (A) CVs obtained at MWCNT-FeTsPc/GCE in pH 7 containing 1 mM L-Cys at different scan rates from 100 to 1000 mV s^{-1}. (B) Plot of $v^{1/2}$ vs. I_p.

3.5 Determination of L-Cys

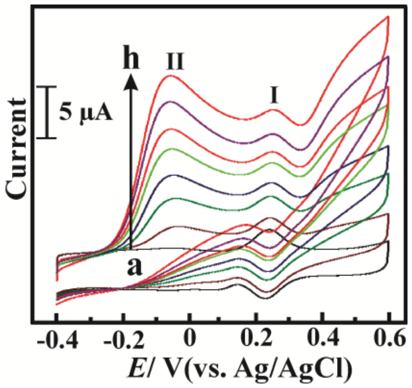

Figure 5. CVs obtained at MWCNT–FeTsPc/GCE in the absence (a) and presence of 0.2 to 1.4 mM L–Cys (curves b to h; each addition of 0.2 mM) in PBS (pH 7) at the scan rate 50 mVs^{-1}.

Fig. 5A show the CVs obtained at MWCNT-FeTsPc/GCE in the absence (curve a) and presence of L-Cys (curves b to h; each addition of 0.2 mM)) in PBS (pH 7).

The potential range is applied between -0.4 V and 0.6 V and the scan rate is hold at 50 mV s^{-1}. The anodic peak currents (I and II) responsible for the oxidation of L-Cys have significantly increased upon addition of 0.2 mM concentration of L-Cys. The peak current increased linearly upon further additions of L-Cys. The linear increase in the oxidation peak currents shows the efficient electrocatalytic ability of the electrode without any fouling effect. Therefore, the MWCNT-FeTsPc/GCE has been used to fabricate an amperometric sensor for the sensitive determination of L-Cys.

Fig. 6A shows the amperometric i-t response of MWCNT–FeTsPc nanocomposite film modified rotating disc GCE upon each addition of 10 µM L-Cys into continuously stirred PBS (pH 7) at the rotation speed of 1500 RPM. Applied potential (E_{app}) of the electrode was hold at -0.10 V. The sensor exhibited quick and sensitive amperometric responses towards each addition of L-Cys. The response current reaches its 95% steady-state current within 4 s indicating fast electrocatalysis at the MWCNT–FeTsPc/GCE. A calibration plot was made between concentration of L-Cys versus response current (Fig. 6B) and the respective linear regression equation can be expressed as I_p (µA) = 0.037 [L-Cys] (µA/µM) + 0.134. The response current for the each addition increases linearly as the concentration of L-Cys increases over the linear range between 10 µM and 200 µM. The sensitivity of the sensor has been estimated to be 0.176 µA μM^{-1} cm^2, while the limit of detection (LOD) was calculated to be 1 µM. The proposed sensor exhibited quite comparable performance with other sensors in terms of wide linear range, low detection limit and high sensitivity [31-33].

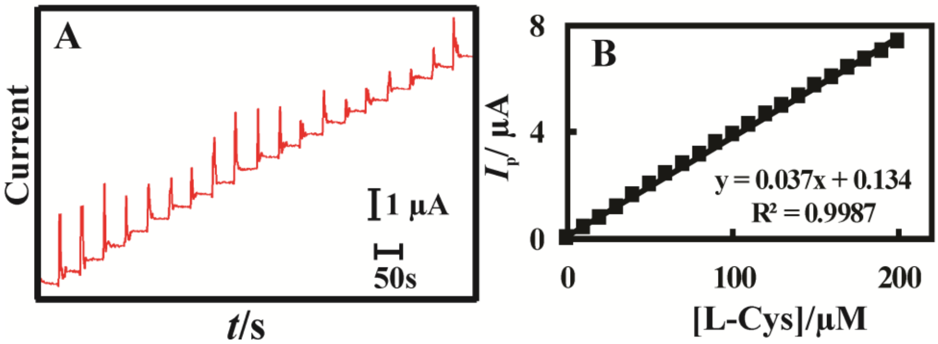

Figure 6. (A) Amperometric i-t response of MWCNT–FeTsPc nanocomposite film modified rotating disc GCE upon each addition of 10 µM into continuously stirred pH 7 at the rotation speed of 1500 RPM. E_{app} = -0.1 V. (B). Plot of [L–Cys] vs. peak currents.

3.6 Determination of L-Cys in real samples

The practicality of the fabricated sensor has been demonstrated in human serum samples. The MWCNT–FeTsPc was utilized for the determination of L-Cys present in the serum samples. The experimental conditions are similar to the lab sample analysis. The spiked L-Cys concentrations were of 10 and 30 µM (Table 1). An appreciable electrochemical response was observed for the detection of

L-Cys in serum samples. The obtained found and recovery results are acceptable and therefore the fabricated sensor could be a promising sensor for the analysis of real samples.

Table 1. Determination of L-Cys at MWCNT–FeTsPc/GCE in human blood serum samples

Sample	Added (µM)	Found (µM)	Recovery (%)	RSD (%)
1	10	9.7	97	2.6
2	30	29.3	97.6	2.4

3.7 Repeatability, reproducibility and stability studies

Repeatability and reproducibility of the proposed MWCNT–FeTsPc/GCE have been evaluated in PBS (pH 7) containing 1 mM L-Cys at the scan rate of 50 mVs^{-1}. The sensor exhibited appreciable repeatability with relative standard deviation (R.S.D) of 2.74% for six repeatitive measurements carried out using single electrode. The sensor presented acceptable reproducibility of 2.41% for six independent measurments carried out in six different electrodes. In order to determine storage stability of the electrode, the electrocatalytic response of the MWCNT–FeTsPc/GCE towards oxidation of 1 mM L-Cys was monitored every day. The modified electrode was stored in PBS (pH 7) at 4°C when not in use. During one month stroge period, the sensor exhibited well defined electrocatalytic response towards oxidation of L-Cys. 95.16% of the initial response current was retained over one month of its continuous use which revealing the excellent storage stability of the sensor. The stability of the MWCNT–FeTsPc/GCE under hydrodynamic conditions is mandatory for the amperometric sensing applications. We have investigated operational stability of the MWCNT–FeTsPc/GCE in the presence of 50 µM L-Cys. The MWCNT–FeTsPc/GCE was continously rotated at 1500 RPM. Stable amperometric response was observed and only 6.3% of the initial response current was decreased even after continously rotated for 3500 s indicating excellent operational stability of the modified electrode.

4. CONCLUSIONS

In summary, we successfully prepared MWCNT–FeTsPc/GCE and employed it for the electrochemical determination of L-Cys. The MWCNT–FeTsPc/GCE has exhibited superior electrocatalytic abilty for the oxidatio of L-Cys. The developed amperometric sensor exhibited excellent analytical parameters for the determination of L-Cys; wide linear range (10 µM - 200 µM), low LOD (1 µM) and high sensitivity (0.176 µAµM^{-1} cm^{2}). The practical feasibility of the sensor has been assessed in human urine samples. The sensor exhibited appreciable stability, repeatability and reproducibility results. The outstanding electrocatalytic ability, good stability, fast response, and sensitivity of the sensor revealed that the MWCNT–FeTsPc composite could be a promising electrode material for the fabrication of efficient electrochemical sensors and biosensors.

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