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Voltammetric Determination of Glutathione Using a Modified Single Walled Carbon Nanotubes Paste Electrode

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Abstract- In this study, a carbon paste electrode modified with carbon nanotubes and benzoylferrocene (BF) was used to prepare a novel electrochemical sensor. The objective of this novel electrode modification was to seek new electrochemical performances for the detection of glutathione. The response of catalytic current with glutathione concentration showed a linear relation in the range from 1.0×10^{-7} to 1.0×10^{-4} M with a detection limit of 3.0×10^{-8} M. Finally, this method was used for the determination of glutathione in real samples.

Keywords- Glutathione, Carbon nanotubes paste electrode, Voltammetry, Electrochemical sensor

1. INTRODUCTION

Electrochemical sensors and biosensors for pharmaceutical, food, agricultural and environmental analyses have been growing rapidly, due to advances in electrochemical measuring systems [1-12]. The merger between fast, sensitive, selective, accurate, miniaturizable and low-cost electrochemistry-based sensing and fields like proteomics, biochemistry, molecular biology, nanotechnology and pharmaceutical analysis leads to the evolution of electrochemical sensors [12-25].

Nanostructured materials particularly carbon nanomaterials including nanoparticles, nanowires and nanotubes have attracted considerable interests and have become a vast area of research owing to their unique physical and chemical properties which can provide an important and feasible platform for electroanalysis particularly in the design of modified electrodes for electrochemical sensing. Applications of carbon nanoparticles (CNPs) in electroanalytical studies display extraordinary advantages over conventional electrodes including enhanced mass transport and catalysis, highly effective surface areas, high porosity, more absorption and reactisites and control over the electrode macro-environment [26-31].

The key advantages of carbon nanotube modified electrodes are their small diameter but long length, their electro-activity which appears to be as good as or better than any of the other carbon based electrodes and their high surface area [32, 33]. Generally there are two ways to fabricate CNTs based electrodes. One way is to cast CNTs suspension on the surface of solid electrodes such as Pt, Au and glassy carbon electrodes to make CNTs film modified electrodes [34]. Another method is to mix CNTs with bonds such as nujol, bromoform or mineral oil, and then pack the mixture into a pipe to prepare paste electrodes [35]. Naturally, the characteristics of CNTs paste electrodes depend on the type of CNTs and the bonds used. Especially, the bonds show greater influence on the accumulation efficiency of electroactive species and blank current due to their different hydrophobicity. Every bond has its characteristics and suits for some purposes. Paraffin oil is commercially available and paraffin oil based paste electrode exhibits some characteristics; hence it is frequently used as bond [36, 37].

Glutathione is the most abundantly found non-protein thiol in living organisms with numerous important roles in protein and DNA synthesis, transport, catabolism, and metabolism [38]. It provides living cells with protection against toxicity, hypoxia, or mutagenicity, and the effects of many carcinogens [39]. Changes in its concentration levels are possible indications of certain diseases such as premature arteriosclerosis, occlusive vascular, leukaemia, diabetes, acquired immunodeficiency syndrome (AIDS), and cataract, among others [40]. Studies have shown that the total GSH present in cells may be either free or bound to proteins. The amount of free glutathione in blood is indicative of cell protection

against oxidative and free radical-mediated cell injury. In addition, glutathione levels in blood samples help in diagnosis of c-glutamyl cycle disorders. Its precise determination is, therefore, of utmost importance for diagnostic purposes. A number of methods have been proposed for the determination of glutathione that include titrimetry, spectrophotometry, spectrofluorimetry, high performance liquid chromatography (HPLC), capillary zone electrophoresis, proton nuclear magnetic resonance (^{1}H NMR), enzymatic method, flow injection analysis, and electrochemical methods [41-55].

To our knowledge, no study has reported the electrocatalytic oxidation of glutathione by using benzoylferrocene (BF) modified single walled carbon nanotube paste electrode (BFCNPE). Thus, in the present work, we described initially the preparation and suitability of a BFCNPE as a new electrode in the electrocatalysis and determination of glutathione in an aqueous buffer solution. Then, in order to demonstrate the catalytic ability of the modified electrode in the electrooxidation of glutathione in real samples, we examined this method for the voltammetric determination of glutathione in human erythrocyte, tablet, and urine samples.

2. EXPERIMENTAL

2.1. Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302 N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three electrode cell was used at 25±1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and the BFCNPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was used for pH measurements.

All solutions were freshly prepared with double distilled water. Glutathione and all other reagents were of analytical grade from Merck (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-11.0. Single walled carbon nanotubes with diameter between 1.2 and 1.5 nm, and tube length from 2.0 to 5.0 μm were prepared from Sigma Aldrich Company (Fig. 1). Benzoylferrocene was synthesized in our laboratory as reported previously [56]. The prepared electrodes were characterized by scanning electron microscopy (SEM), Philips, Model XLC.

2.2. Preparation of the electrode

The BFCNPEs were prepared by hand mixing 0.01 g of BF with 0.89 g graphite powder and 0.1 g CNTs with a mortar and pestle. Then, ~ 0.7 mL of paraffin oil was added to the above mixture and mixed for 20 min until a uniformly-wetted paste was obtained. The paste was then packed into the end of a glass tube (ca. 3.4 mm i.d. and 15 cm long). A copper wire

inserted into the carbon paste provided the electrical contact. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing with a weighing paper.

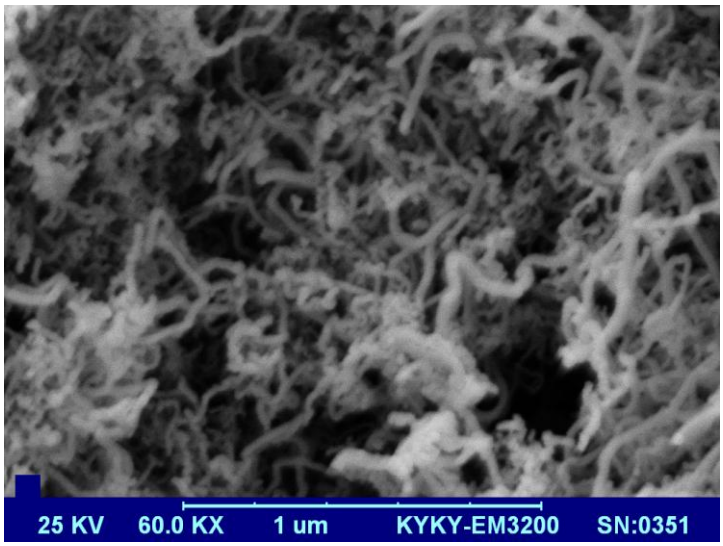

Fig. 1. SEM image of single walled carbon nanotubes

For comparison, BF modified CPE electrode (BFCPE) without CNTs, CNTs paste electrode (CNPE) without BF, and unmodified CPE in the absence of both BF and CNTs were also prepared in the same way.

2.3. Preparation of real samples

Human whole blood samples were obtained from the local labs. Erythrocytes were separated from whole blood samples by removing the plasma. The sample thus obtained (3.0 ml) was first centrifuged for 10 min at 3000 rpm. The supernatant (plasma) was discarded and the rest was mixed with 7.5 ml of 0.9% NaCl solution. The solution was centrifuged for another 5 min at 3000 rpm and the supernatant (diluted plasma) was again discarded. The washing procedure with NaCl solution was repeated three times in order to remove almost all the plasma. Erythrocyte pellets were haemolysed with water (1:1, v/v). For protein precipitation, the haemolysate was mixed with 5–sulfosalysilic acid (10%, w/v) at a ratio of 2:1 (v/v). The mixture obtained was centrifuged under the same conditions described above.

Urine samples were stored in a refrigerator immediately after collection. Ten millilitres of each sample was centrifuged for 15 min at 1500 rpm. The supernatant was filtered using a 0.45 mm filter and then diluted five times with PBS (pH 7.0). The solution was transferred into the voltammetric cell to be analyzed without any further pretreatment. Standard addition method was used for the determination of glutathione in real samples.

Tablet solution was prepared by completely grinding and homogenizing ten tablets of glutathione, labelled 100 mg per tablet (Chongqing Yaoyou Pharmaceutical Co., Ltd., Chongqing, China). Then, 10 mg of each tablet powder was accurately weighed and dissolved in 100 ml water by ultrasonication. After mixing completely, the mixture was filtered on an ordinary filter paper, 10 ml of which was subsequently transferred into a 100-ml volumetric flask and diluted to the mark with water. Then, 1.0 ml of the solution plus 4.5 ml of the buffer (pH 7.0) was used for analysis using the standard addition method.

3. RESULTS AND DISCUSSION

3.1. Electrochemical properties of BFCNPE

BFCNPE was constructed and its electrochemical properties were studied in a 0.1 M PBS (pH 7.0) using CV. The experimental results show well-defined and reproducible anodic and cathodic peaks related to benzoylferrocene/benzoylferricenium ion redox system, which show a quasireversible behavior in an aqueous medium. The electrode capability for the generation of a reproducible surface was examined by cyclic voltammetric data obtained in optimum solution pH 7.0 from five separately prepared BFCNPEs (Table 1).

Table 1. Cyclic voltammetric data obtained for constructed BFCNPE in 0.1 M PBS (pH 7.0) at 100 mV s^{-1}

E_{pa} (V)[a]	E_{pc}(V)	$E_{1/2}$ (V)	E_p (V)Δ	I_{pa} (μA)	I_{pc} (μA)
0.645±1.3	0.540±1.4	0.592±1.3	0.105±1.4	5.2±1.7	-2.4±1.6

[a]Versus Ag/AgCl/KCl (3.0 M) as reference electrode

b All the '±' values are RSD% (n=5)

3.2. Electrocatalytic oxidation of glutathione at a BFCNPE

Fig. 2 depicts the CV responses for the electrochemical oxidation of 50.0 μM glutathione at unmodified CPE (curve c), CNPE (curve d), BFCPE (curve a) and BFCNPE (curve b). As it is seen, while the oxidation glutathione does not takes place at the surface of a CNT and unmodified CPE up to +850 mV the corresponding potential at BFCNPE and BFCPE is ~645 mV. These results indicate that the peak potential for glutathione oxidation at the BFCNPE and BFCPE electrodes shift toward negative values compared to CNPE and unmodified CPE. However, BFCNPE shows much higher anodic peak current for the oxidation of glutathione compared to BFCPE, indicating that the combination of CNTs and the mediator (BF) has significantly improved the performance of the electrode toward glutathione oxidation. The BFCNPE, in 0.1 M PBS (pH 7.0) and without glutathione in solution, exhibited a well-behaved redox reaction and with addition of 50.0 μM glutathione,

increased the anodic peak current (Fig. 2 curve b), indicating a strong electrocatalytic effect [57].

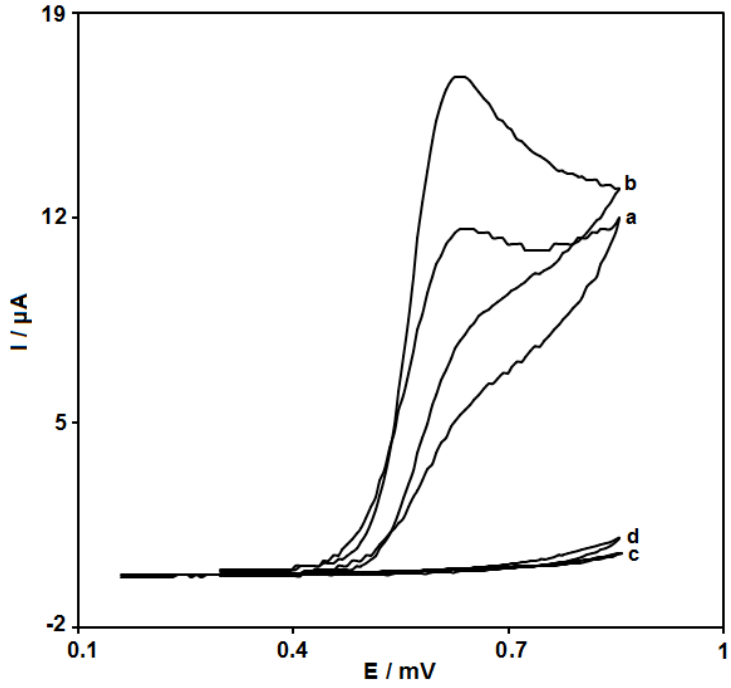

Fig. 2. CVs of BFCPE (a) and BFCNPE (b) in 0.1 M PBS (pH 7.0) containing 50.0 µM glutathione. (c) and (d) are CVs of CPE and CNPE in 0.1 M PBS (pH 7.0) containing 50.0 µM glutathione. In all cases scan rate is 10 mV s^{-1}

The effect of scan rate on the electrocatalytic oxidation of glutathione at the BFCNPE was investigated by CV (Fig. 3). As can be observed in Fig. 3, the oxidation peak potential shifted to more positive potentials with increasing scan rate, confirming the kinetic limitation in the electrochemical reaction. Also, a plot of peak height (I_p) *vs*. the square root of scan rate ($v^{1/2}$) was found to be linear in the range of 5-30 mV s^{-1}, suggesting that, at sufficient overpotential, the process is diffusion rather than surface controlled [57] (Fig. 3A).

A plot of the scan rate-normalized current ($I_p/v^{1/2}$) *vs*. scan rate (Fig. 3B) exhibits the characteristic shape typical of an EC´ process [57].

The Tafel slope (b) can be obtained from the slope of E_p *vs*. log v using Eq. (1) [57]:

$$Ep = b/2 \log v + constant \qquad (1)$$

The Tafel slope was found to be 0.108 V (Fig. 3C), which indicates that a one-electron transfer process is the rate limiting step assuming a transfer coefficient (α) is about 0.45.

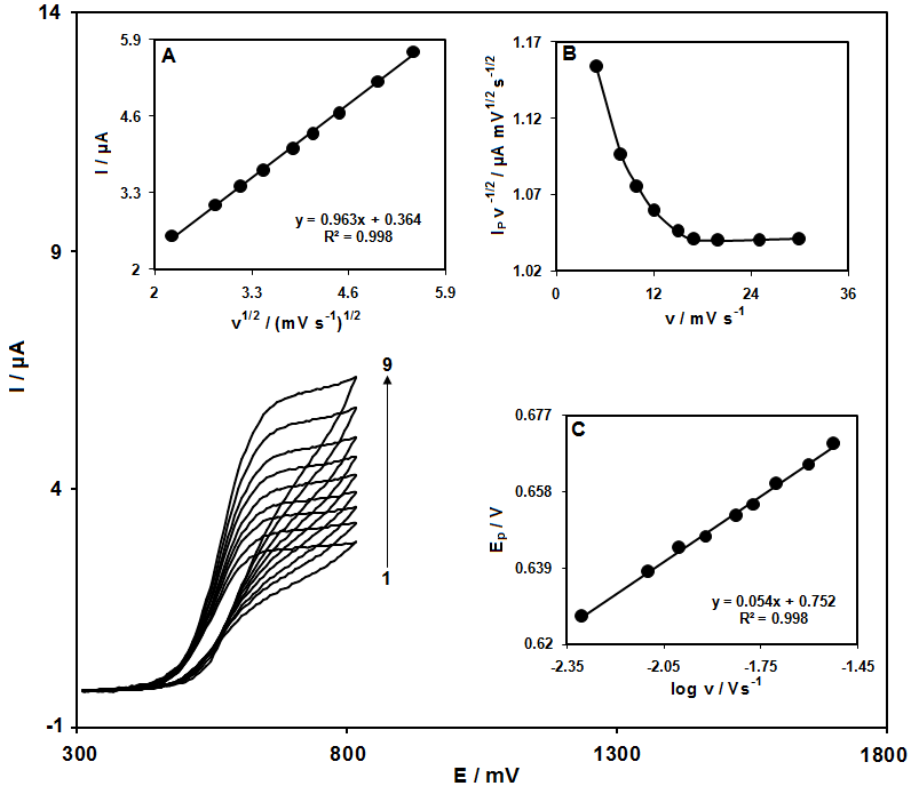

Fig. 3. CVs of BFCNPE in 0.1 M PBS (pH 7.0) containing 50.0 µM glutathione at various scan rates; numbers 1-9 correspond to 5, 8, 10, 12, 15, 17, 20, 25 and 30 mV s^{-1}, respectively. Insets: Variation of **(A)** anodic peak current *vs*. $v^{1/2}$; **(B)** normalized current ($I_p/v^{1/2}$) *vs*. v **(C)** anodic peak potential *vs*. log *v*

3.3. Chronoamperometric measurements

Chronoamperometric measurements of glutathione at BFCNPE were carried out by setting the working electrode potential at 0.7 V (at the first potential step) and at 0.5 V (at second potential step) *vs*. Ag/AgCl/KCl (3.0 M) for the various concentration of glutathione in PBS (pH 7.0) (Fig.4). For an electroactive material (glutathione in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [57]. Experimental plots of I *vs*. $t^{-1/2}$ were employed, with the best fits for different concentrations of glutathione (Fig. 4A). The slopes of the resulting straight lines were then plotted *vs*. glutathione concentration (Fig. 4B). From the resulting slope and Cottrell equation [57]:

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \qquad (2)$$

Where *D* and C_b are the diffusion coefficient (cm^2 s^{-1}) and the bulk concentration (mol cm^{-3}), respectively, the mean value of the D was found to be 1.15×10^{-5} cm^2/s.

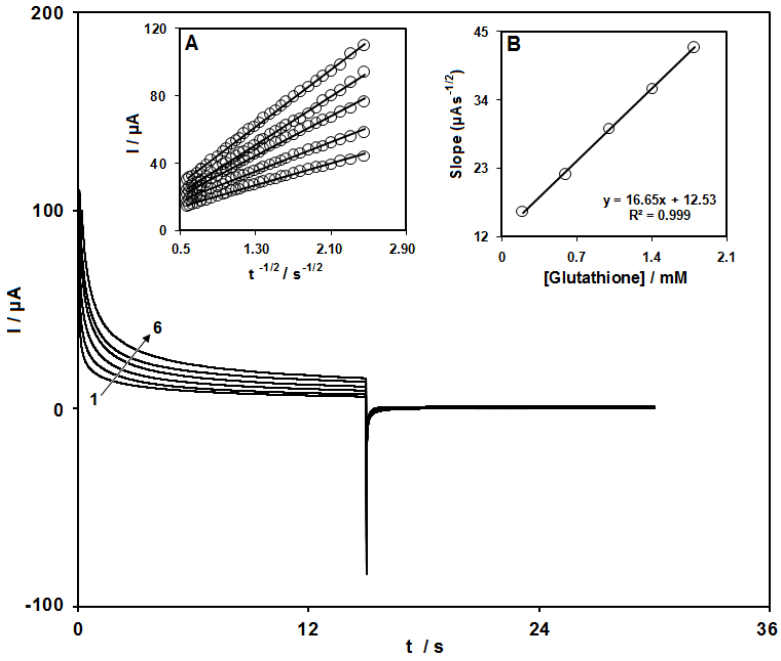

Fig. 4. Chronoamperograms obtained at BFCNPE in 0.1 M PBS (pH 7.0) for different concentration of glutathione. The numbers 1–6 correspond to 0.0, 0.2, 0.6, 1.0, 1.4 and 1.8 mM of glutathione. Insets: **(A)** Plots of I *vs*. $t^{-1/2}$ obtained from chronoamperograms 2–6. **(B)** Plot of the slope of the straight lines against glutathione concentration

3.4. Calibration plot and limit of detection

SWV method was used to determine the concentration of glutathione (Fig. 5). The plot of peak current *vs*. glutathione concentration consisted of two linear segments with slopes of 0.22 and 0.05 µA μM^{-1} in the concentration ranges of 0.1 to 10.0 µM and 10.0 to 100.0 µM, respectively. The decrease in sensitivity (slope) of the second linear segment is likely due to kinetic limitation [57]. The detection limit (3σ) of glutathione was found to be 30.0 nM. These obtained values are comparable with values reported by other research groups (Table 2).

3.5. Determination of glutathione in real samples

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of glutathione in glutathione tablet, haemolysed erythrocyte and urine samples. Results are given Table 3. Satisfactory recovery of the experimental results was found for glutathione. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

Table 2. Comparison of the efficiency of some modified electrodes used in the electrocatalysis of glutathione

Electrode	Modifier	pH	Method	LOD (M)	LDR (M)	Ref.
Carbon paste	Ferrocene	7.0	Voltammetry	2.1×10^{-6}	$2.2\times10^{-6} - 3.5\times10^{-3}$	43
Carbon paste	Ferrocene carboxylic acid	7.0	Voltammetry	9.8×10^{-8}	1.0×10^{-7}- 1.2×10^{-5}	51
Carbon paste	2,7-BFEF	7.0	Voltammetry	5.0×10^{-7}	9.2×10^{-7}-1.1×10^{-5}	53
Carbon paste	TTF-TCNQ	7.0	Voltammetry	3.0×10^{-7}	5.0×10^{-6}–3.4×10^{-4}	54
Carbon paste	p-aminophenol	5.0	Voltammetry	9.0×10^{-8}	2.0×10^{-7}–1.0×10^{-4}	55
Carbon paste	Benzoylferrocene	7.0	Voltammetry	3.0×10^{-8}	1.0×10^{-7} - 1.0×10^{-4}	This Work

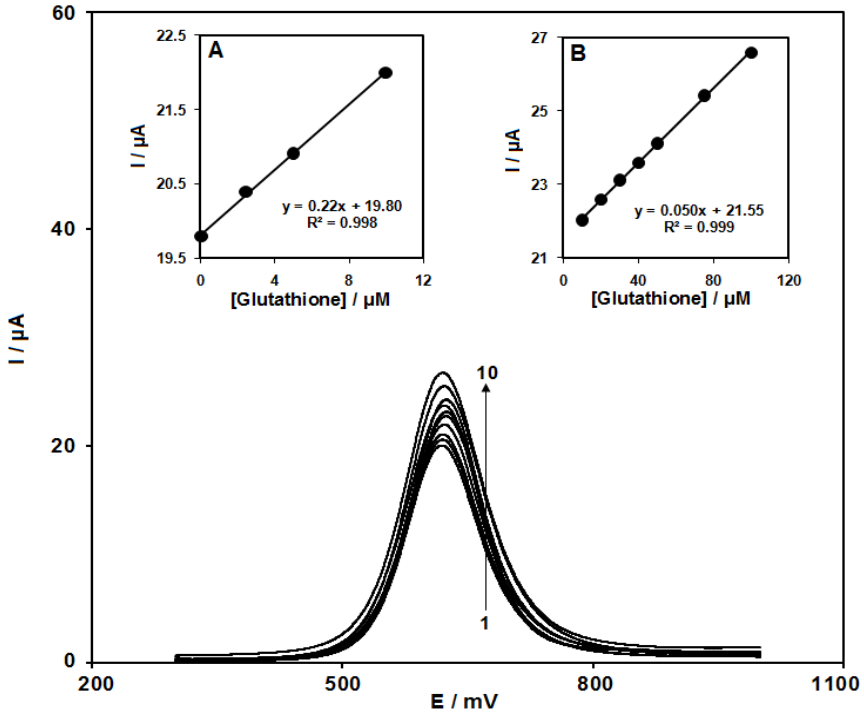

Fig. 5. SWVs of BFCNPE in 0.1 M PBS (pH 7.0) containing different concentrations of glutathione. Numbers 1-10 correspond to 0.1, 2.1, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 75.0 and 100.0 µM of glutathione. Inset: plots of the electrocatalytic peak current as a function of glutathione concentration in the range of **(A)** 0.1 to 10.0 µM and **(B)** 10.0 to 100.0 µM

Table 3. The application of BFCNPE for determination of glutathione in tablet, haemolysed erythrocyte and urine samples (n=5)

Sample	Sample No.	Original content (μM)	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
Tablet	1	5.0	5.0	9.9	99.0	3.1
	2	10.0	5.0	15.3	102.0	2.4
	3	15.0	5.0	20.6	103.0	1.6
	4	20.0	5.0	24.6	98.4	1.9
	5	25.0	5.0	30.2	100.7	3.2
Haemolysed erythrocyte	1	50.0	10.0	60.2	100.3	1.9
	2	60.0	10.0	68.6	98.0	2.2
	3	70.0	10.0	79.1	98.9	3.3
	4	80.0	10.0	91.3	101.4	2.7
	5	90.0	10.0	99.5	99.5	2.6
Urine	1	ND	10.0	10.3	103.0	2.3
	2	ND	20.0	19.8	99.0	3.4
	3	ND	30.0	31.2	104.0	2.1
	4	ND	40.0	41.1	102.7	1.7
	5	ND	50.0	48.9	97.8	2.3

4. CONCLUSION

The BFCNPE was prepared and used for the investigation of the electrochemical behavior of glutathione. The BFCNPE showed excellent electrocatalytic activity for the redox of glutathione. Compared with the bare electrode, the oxidation current of glutathione increased greatly and the oxidation peak potential shifted toward negative potentials. The SWV currents of glutathione at BFCNPE increased linearly with the glutathione concentration in the range from 1.0×10^{-7} to 1.0×10^{-4} M with a detection limit 3.0×10^{-8} M. Finally, this method was used for the determination of glutathione in real sample. The high sensitivity and low detection limit, together with the ease of preparation and surface regeneration and high repeatability and stability of the modified electrode, are the advantages of the studied modified electrode.

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