Analyst

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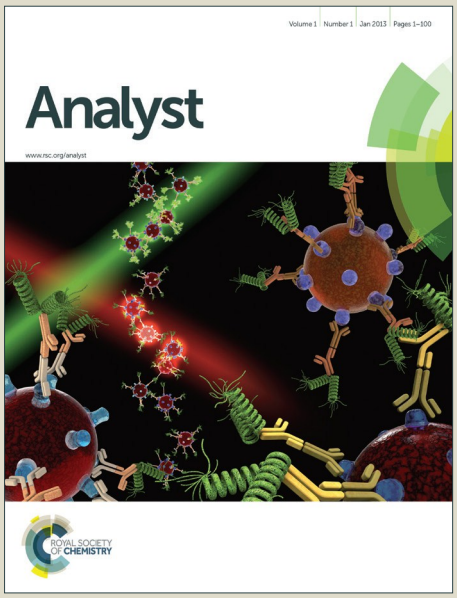

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DOI: 10.1039/C6AN00550K

Voltammetric detection of glutathione; an adsorptive stripping voltammetry approach

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Keywords: copper(II), glutathione, glassy carbon electrode, thiols, cyclic voltammetry

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Abstract

A simple, sensitive, and rapid detection of glutathione by cyclic voltammetry using a bare glassy carbon electrode is reported in which glutathione forms a 1:1 complex compound with copper(II) ion. This complex compound is adsorbed onto the electrode surface and undergoes electrochemical oxidation at a characteristic oxidation potential of ca. – 0.20 V *vs* standard mercury/mercurous sulphate reference electrode, which is used to detect the glutathione concentration. The linear dynamic range is obtained for a glutathione concentration of 1 μM to 12.5 μM, and the sensitivity is found to be 0.1 ± 0.002 μA μM^{-1}. The low limit of detection (n=3) of 0.14 μM and the precision of 1.8% are achieved using a simple, unmodified electrode. The robustness of the present methodology is demonstrated by the successful quantitative analysis of glutathione in the presence of cysteine.

1. Introduction

Glutathione (γ-glutamyl-cysteinyl-glycine, GSH) is the most common low-molecular-weight thiol in the animal cells at the concentration range of 0.5 to10 mM.[1] This is known to decrease in response to protein malnutrition and oxidative and nitrosative stresses.[2,3] GSH is also found in the physiological fluids at the concentration of around 2–12 μM for a healthy individual;[3] the concentration is reportedly lower for patients with a number of pathological conditions such as diabetes, macular degeneration, and HIV disease.[4,5,6] For these reasons, GSH is considered as a promising biomarker for various diseases. Measurement of GSH concentration is however complicated by its instability in solution as it is readily oxidized to form disulphide.[7] For this reason, conventional methods derivatize GSH to form a stable compound before it is analysed by high performance liquid chromatography[8-14] or capillary electrophoresis[15] , that are coupled with fluorescence[8-13], UV-visible spectroscopy[14,15],

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mass spectrometry[16], and electrochemical detectors.[17] These approaches are generally time-consuming and expensive, and it is rather more desirable to develop a system that is simple, rapid, and low cost for long-term continuous monitoring of the GSH concentration.

Electrochemical sensors are a promising alternative to the conventional approach. The thiol group of GSH undergoes oxidative dimer formation at an electrode surface allowing the reagent to be quantitatively analysed using various voltammetric and amperometric techniques. [18-39] Table 1 summarises previously reported approaches to the electrochemical detection of glutathione. Most techniques involve modification of the electrode surface to improve the electrode-substrate affinity or the use of pulse voltammetric techniques to reduce capacitive charge. While the techniques listed in Table 1 have successfully detected GSH, the electrode modification can increase the operating cost and complicate the use of the electrochemical sensors. Recently, it was shown that captopril, a thiol containing therapeutic drug for hypertension, can be electrochemically detected at an unmodified glassy carbon electrode (GCE) in the presence of copper (II) ion.[40] This was related to complex formation between the molecule and copper (II) ion which facilitated the oxidative dimer formation of the thiol functional group. This system is applied herein to detect GSH, a thiol containing amino acid, which is reportedly forms a complex compound with copper (II) ion in aqueous media.[7,39,40] Successful application of this work can greatly simplify the electrochemical detection of the physiologically important molecule and make it more cost-effective by avoiding the need for any electrode modification and employing a conventional GCE.

Table 1: Summary of electrochemical techniques reported for glutathione detection.

Electrode	**Modification**	**Method**	E_{det}	**LDR (μM)**	**Sensitivity (μA μM^{-1})**	**LOD (μM)**	**Ref.**
GC	No	CV	−0.2 V *vs*. MSE	1-10	0.10	0.14	This work
GC	DEPD	CV	+0.09 V *vs*. SCE	20-100	0.044	6.2_4	[18]
GC	DEPD	CV	+0.17 V *vs*. SCE	40-160*	0.12_{5*}	0.55_{2*}	[19]
GC	Catechol	CV	+0.11 V *vs*. SCE	1-80	0.029	0.93	[20]

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GC	Catechol	CV	+0.2 V *vs.* SCE	6-59	0.01	1	[21]
SP	Ferrocene carbaldehyde	CV	+0.53 V *vs.* SCE	0.148-2×10^3	N/S	0.86_7	[22]
GC	Enzyme/Os-PVP	CV	+0.02 V *vs.* Ag/AgCl	1-200	1.2×10^{-3}	0.5	[23]
BDD	5-thio-2-nitrobenzoic acid	CV	+0.85 V *vs.* SCE	10-100	0.022_9	5.8	[24]
EPPG	No	CV	+0.65 V *vs.* SCE	10-80	0.039	2.7	[25]
GC	SWCNT/TOQ	CV	+0.0 V *vs.* Ag/AgCl	0.07-500	6.5×10^{-3}	0.07	[26]
ABPP	No	CV	+0.05 V *vs.* SCE	100-280	0.57	10	[27]
SP	Prussian blue	CV	+0.2 V *vs.* Ag/AgCl	2-500	2.6×10^{-4}	2.0	[28]
CP	TTF-TCNQ	CV	+0.2 V *vs.* SCE	5-340	0.090_1	0.3	[29]
GC	CoTSPc-PLL	CV	+0.2 V *vs.* Ag/AgCl	0.05-2.16	1.5	0.02	[30]
CP	2,7-bis(ferrocenyl ethyl) fluoren-9-one	CV	+0.41 V *vs.* Ag/AgCl	52.9-4.19×10^3	1.9_9	14	[31]
GC	Cafeic acid	CV	+0.22 V *vs.* SCE	0.3-5×10^3	0.21_3	2.2	[32]
SP	Catechol	CV	–0.22 V *vs.* SCE	0-60	1.9×10^{-3}	0.11	[33]
Au	MWCNT	CV	+0.08 V *vs.* Ag/AgCl	99-8.8×10^3	1.22×10^{-3}	99	[34]
GC	Hg(II)-DPTA -Pb(II)	DPV	+0.45 V *vs.* Ag/AgCl	0.032-32.5	N/S	0.032	[35]
CP	TiO_2-ferrocene carboxylic acid	DPV	+0.33 V *vs.* Ag/AgCl	0.1-12	0.99_4	0.1	[36]
BPPG	Nitrophenyl	SWV	–0.1 V *vs.* SCE	8.1-1×10^3	0.051	8.1	[37]
BPPG	MWCNT/FeT4MPyP	SWV	–0.025 V *vs.* Ag/AgCl	1.5-5×10^3	0.703	0.5	[38]
CILC	$Cu(OH)_2$	SWV	+0.15 V *vs.* Ag/AgCl	1-50	N/S	0.03	[39]

ABPP: acetylene black-packed powder; BDD: Boron doped diamond; GC: Glassy carbon; CP: Carbon paste; CILC: Carbon ionic liquid composite; SP: Screen printed; BPPG: Basal plane pyrolytic graphite; EPPG: Edge plane pyrolytic graphite; BDD: Boron doped diamond; CV: Cyclic voltammetry; SWV: Square wave voltammetry; LOD: Limit of detection; LDR: Linear dynamic range; SWCNT: Single-walled carbon nanotubes; MWCNT: Multi-walled carbon nanotubes; DEPD: N,N-diethyl-p-phenylenediamine; Os-PVP: Osmium-Polyvinylpyridine; TOQ: Synthetic triptycene orthoquinone; TTF-TCNQ: Tetrathiafulvalene-tetracyanoquinodimethane; CoTSPc-PLL: Cobalt tetrasulfonated phthalocyanine immobilised in poly(L-lys); Pip-Buff: piperazine-*N,N'*-bis(2-ethanesulfonic acid) buffer; FeT4MPyP: Fe(III) tetra-(*N*-methyl-4-pyridyl)-porphyrin; RPMI-1640: Bicarbonate system produced by Roswell Park Memorial Institute; PBS: Phosphate-buffered saline. N/S: Not specified. SCE: Saturated Calomel Electrode. (*) Derived from the figure given in the reference.

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2. Experimental

2.1 Chemicals and Materials

Potassium nitrate (KNO_3, 99+%, but containing around 0.2 ppm of the copper(II)) was purchased from Fisher Scientific (Loughborough, UK). Copper(II) nitrate trihydrate ($Cu(NO_3)_2.3H_2O$, 99+%), sodium bicarbonate ($NaHCO_3$, 99+%) and hexaaminеruthenium(III) chloride ($[Ru(NH_3)_6]Cl_3$, 99+%,), Glutathione (GSH, $C_{10}H_{17}N_3O_6S$, 98+%) and Cysteine ($C_3H_7NO_2S$, 97+%) were obtained from Sigma-Aldrich (Gillingham, UK). Aqueous stock solutions of Glutathione and Cysteine were prepared freshly for every experiment. All solutions were prepared with ultrapure water from Millipore SimPak® 1 purification pack (lot# F5BA50456) with resistivity not less than 18.2 MΩ cm. Alumina powders (1.0, 0.3 and 0.05 μm) were obtained from Buehler (Coventry, UK). Oxygen free N_2 gas (99.998%, BOC Gases plc, Guildford, UK) was humidified by passing through a trap containing ultrapure water. A standard mercury/mercurous sulphate reference electrode (MSE, [Hg/Hg_2SO_4, saturated K_2SO_4], + 0.64 V *vs*. standard hydrogen electrode) was purchased from BASi (West Lafayette, USA). A platinum wire (diameter 1 mm, Goodfellow Cambridge Ltd, Huntingdon, UK) was used as a counter electrode.

2.2 Voltammetry

All electrochemical experiments were performed in a glass electrochemical cell (volume 10 mL) using a three-electrode system in a Faraday cage with a PGSTAT 101 potentiostat/galvanostat from Metrohm-Autolab BV (Utrecht, The Netherlands).

A glassy carbon working electrode (GCE, 0.0707 cm^2 in area) was purchased from CH Instruments (Austin, USA). The area of the electrode was confirmed by conducting cyclic voltammetry in aqueous 1 mM hexaammineruthenium(III) chloride at several different scan rates.[42] Prior to experiment, a bare GCE was polished to a mirror finish using aqueous

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slurries of 1.0, 0.3 and 0.05 μm alumina in descending order of size and rinsed thoroughly with ultrapure water in between. The GCE was then placed in deaerated 0.1 M KNO_3 and the potential was cycled between – 0.5 V and + 0.1 V *vs.* MSE at the scan rate of 100 mV s^{-1} until voltammograms became reproducible (ca. 5 cycles). Note that it is important to avoid alumina residues which interfere with the electrochemical measurement by providing an absorption platform for and/or catalysing the oxidation of the reagent. [43,44] Thereafter, the cleaned GCE was activated by being immersed in deaerated 0.1 M $NaHCO_3$ solution, and the potential was cycled between – 1.6 V and + 1.6 V *vs.* MSE at a scan rate of 100 mV s^{-1} until stable voltammetric response was obtained (also normally 5 cycles). Without going through this pre-treatment, the oxidation of copper-GSH complex compound can still be observed at GCE; the precision of the voltammetric response is however found to be very poor (around 32 %). Carboxyl functional groups are reportedly generated at the GCE surface[45] during the pre-treatment, by which adsorption of the copper(II)-GSH complex compound is improved and the voltammetric response is stabilized. The treated GCE was thereafter rinsed thoroughly with ultrapure water and stored under the ambient conditions. Solutions containing copper(II), GSH, and 0.1 M KNO_3 separately were mixed in the following manner immediately prior to each electrochemical measurement: A pre-determined amount of copper(II) was first pipetted into a deaerated 0.1 M KNO_3 solution followed by the addition of GSH. The final mixture was stirred vigorously for 4 min to allow the formation of copper(II)-GSH complex. GCE was then inserted and rested in the solution for 60 s before cyclic voltammograms were recorded between – 0.5 V to + 0.5 V *vs.* MSE at a scan rate of 50 mV s^{-1}. Stable cyclic voltammograms were observed after the third scan, and the scan was triplicated thereafter. For the investigation of the interference from cysteine, a mixture of GSH and cysteine (5 μM each) was added to the above mentioned solution and waited for additional 20 min. before immersing GCE in order to allow the system to reach equilibrium.

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All experiments were performed in solutions degassed with N_2 and at 25 °C. All experiments were carried out in triplicate.

3. Results and discussion

In the following, the electrochemical analysis of GSH in 0.1 M KNO_3 in the presence and absence of copper(II) is first conducted. Then, the formation of copper(II)-GSH complex is investigated using UV-Visible spectroscopy and cyclic voltammetry. The mechanism of electro-oxidation of the copper(II)-GSH complex compound at the GCE surface is thereafter carefully examined. Subsequently, a calibration plot is constructed for GSH and analytical parameters are determined, and the possible interference of cysteine is investigated.

3.1 Glutathione oxidation in presence of copper(II)

Electro-oxidation of 10 μM GSH in the absence of copper (II) was first investigated by recording a cyclic voltammogram of a bare GCE in 0.1 M KNO_3 at the scan rate of 50 mV s^{-1}. The resulting voltammogram represented in Figure 1 (blue line) shows a small increase in current at around + 0.4 V during anodic scan in comparison to the voltammogram recorded in only 0.1 M KNO_3 (Figure 1, black dash line). The direct oxidation of GSH is likely responsible for this current increase; a similar result with a slightly cathodically shifted peak has been observed in 0.1 M phosphate buffer solution containing 1 mM GSH using a specially prepared carbon paste electrode using an ionic liquid as a pasting liquid.[39] Another small anodic peak, which appears at ca. – 0.20 V *vs.* MSE (Figure 1, blue line) is attributable to the oxidation of a copper(II)-GSH complex compound. This is due to the presence of copper(II) impurity in the KNO_3 salt (4.3 μM as determined in our previous study[40], and the copper (I) ion is not stable under the experimental conditions). To confirm this, we observed

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that the anodic peak becomes more prominent as the copper concentration is increased to 14.3 µM (Figure 1, red line). The redox activity of the uncomplexed copper ion itself is negligible at that concentration (Figure 1, pink line).

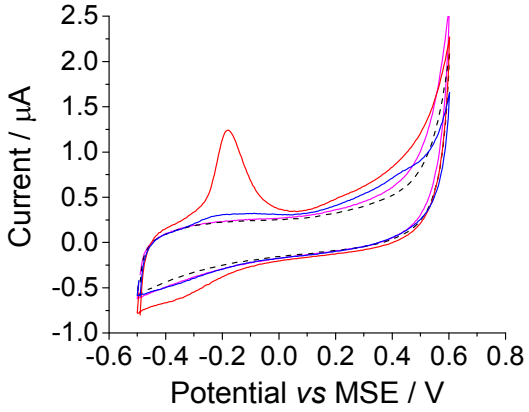

Fig. 1. Cyclic voltammograms of a bare GCE in 0.1 M KNO_3 (black dash line), in 0.1 M KNO_3 with 10 µM GSH (blue line), in 0.1 M KNO_3 14.3 µM copper(II) ion (pink line), and in 0.1 M KNO_3 14.3 µM copper(II) ion with 10 µM GSH (red line). All voltammograms are recorded at the scan rate of 50 mV s^{-1}. The results shown are from the third cycle.

To further investigate the presence of a copper(II)-GSH complex compound, a mixture of 0.05 M copper(II) and 0.2 M GSH was analysed by UV-Visible spectroscopy. Figure 2 shows that GSH nor copper alone absorb between 400 nm and 250 nm, whereas the mixture has a maximum absorption at 293 nm. This absorption profile is consistent with that of the copper(II)-GSH complex compound reported in the literature.[39]

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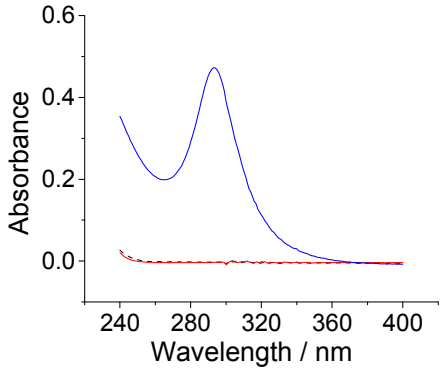

Fig. 2. UV-Vis spectra of 0.4 mM GSH (black dashed), 0.1 mM copper(II) (red) and, 0.2 mM GSH + 0.05 mM copper (II) mixture (blue) in water. Analyte was contained in a quartz cuvette with light path length of 10 mm. Spectra were recorded using a Shimadzu UV-Vis Spectrophotometer, UV-1800. All experiments were performed using ultra-pure water as a reference. A molar extinction coefficient found from the figure is 4700 L mol^{-1} cm^{-1}.

3.2 Effect of scan rate on the peak current

To gain deeper understanding of the mechanism of the oxidation of copper(II)-GSH complex compound at the GCE surface, cyclic voltammograms were recorded in 0.1 M KNO_3 with 10 μM GSH and 14.3 μM of copper(II) at scan rates between 0.025 V s^{-1} and 0.50 V s^{-1}. The resulting voltammograms are presented in Figure 3A which shows an increase in current with the scan rate. It is moreover found that there is a direct correlation between the anodic peak current and the scan rate as illustrated in Figure 3B. This behaviour is indicative of that the oxidation process is of a surface-bound species. The surface coverage, which is determined by integrating the area under the oxidation peak, suggests that 1.5 (± 0.2) $\times 10^{-10}$ mol cm^{-2} of copper(II)-GSH complex compound is adsorbed on the electrode surface. Furthermore, the surface coverage does not depend on the scan rate as shown in Figure 3C; this is consistent with the proposed surface controlled process.[46]

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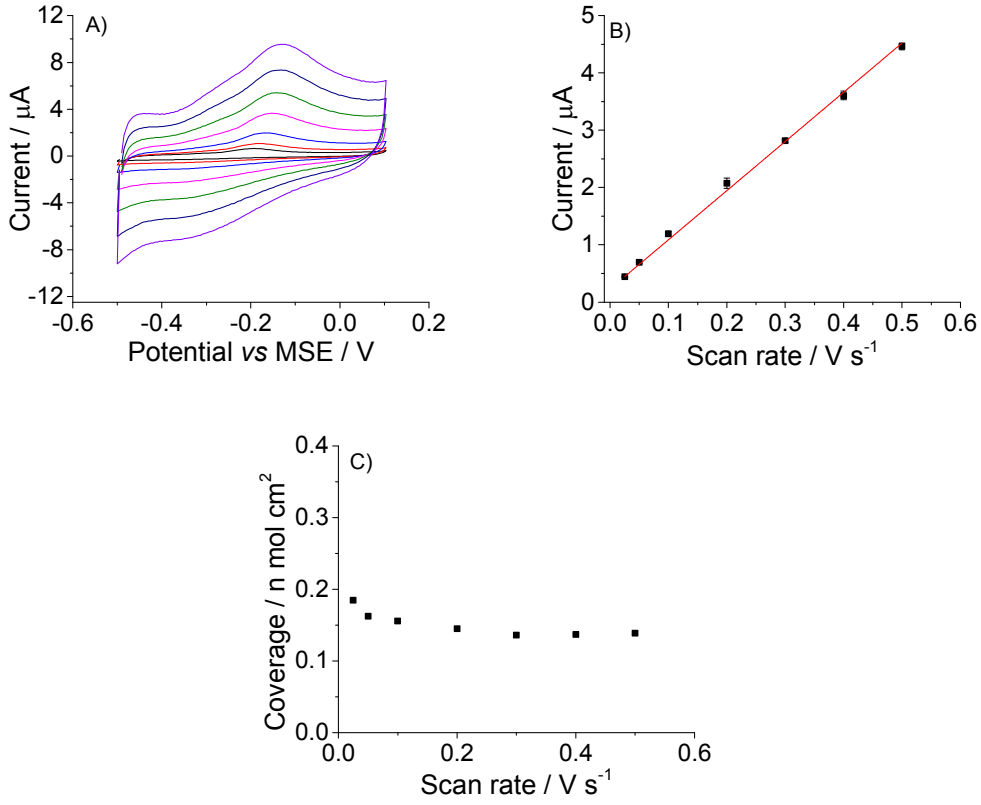

Fig. 3. (A) The cyclic voltammogram of a bare GCE in in 0.1 M KNO_3 with 10 µM GSH and 14.3 µM copper(II) at different scan rates. Black: 0.025 V s^{-1}; red: 0.050 V s^{-1}; blue: 0.10 V s^{-1}; pink: 0.20 V s^{-1}; green: 0.30 V s^{-1}; dark blue: 0.40 V s^{-1} and, violet: 0.50 V s^{-1}. The results shown are from the third cycle. (B) Peak current as a function of the scan rate. (C) The surface coverage of GSH as a function of the scan rate.

3.3 Reaction mechanism

A previous report[47] shows that oxidized glutathione (GSSG) forms a very stable complex compound with copper(II) ($\beta = 1.66\times10^{16}$) at the stoichiometric ratio of 1:2. This suggests that the copper(II)-oxidized glutathione complex, Cu_2(GSSG), is the product of the oxidation of GSH under the current experimental condition. In contrast, while the formation of the copper(II)-GSH complex has been reported,[7,39,41] stoichiometry of this complex has not been fully investigated. Accordingly further voltammetry was carried out to explore these possibilities.

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Cyclic voltammograms were recorded at GSH concentrations of between 1.0 μM to 25 μM in the presence of 14.3 μM of copper(II) in 0.1 M KNO_3. The resulting peak current is plotted against the GSH concentration in Figure 4B. Under the excess copper concentration, the anodic peak current indicating the oxidation of copper-GSH complex increases with the concentration of the GSH ligand, as the number of the redox active species in the solution increases.[48] The peak current increases with the GSH concentration until 14.3 μM. Thereafter, the peak current appears to be relatively stable, as the GSH concentration surpasses that of copper. The intercept of the two extrapolated best-fit lines of the peak current versus the GSH concentration (Figure 4B) is found at 16.0 μM, indicative of that the stoichiometric ratio of the complex compound is 1:1. Therefore, it can be concluded that the copper(II)-GSH complex compound ($[Cu–SG]^+$,) is likely oxidized at the electrode surface according to the following reaction:

$$2[Cu–SG]^+ \rightarrow Cu_2(GSSG) + 2e^- \quad (1)$$

3.4 Calibration Plot for Glutathione

Calibration curve was derived from Figure 4B using the GSH concentration range of 1.0 μM to 12.5 μM. The best-fit line of Figure 4B for the concentration range up to 12.5 μM reveals the following relationship (n = 3):

$$I_p/\mu A = (0.10 \pm 0.002)[Glutathione/\mu M] \qquad R^2 = 0.995 \quad (2)$$

where I_p is the peak current. Physiological fluids, such as plasma and urine contain roughly 2.0 μM to 12 μM of GSH.[9,49,50] Therefore, the linear dynamic range obtained in this study,

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i.e., from 1.0 μM to 12.5 μM, suggests that samples can be analysed without pre-treatment such as dilution or pre-concentration. This is particularly important in order for clinical analysis laboratory application to be cost- and time- effective as well as to reduce the chemical waste. The reproducibility of the anodic peak current determined after triplicated collection of cyclic voltammograms at 10 μM GSH was 1.8%. The limit of detection (LOD) was calculated using the following formula: $LOD = \frac{3\sigma}{S}$ where σ is the standard deviation and S is the sensitivity and found to be 0.14 ± 0.004 μM. This value is one of the lowest among the presently reported methods (Table 1).

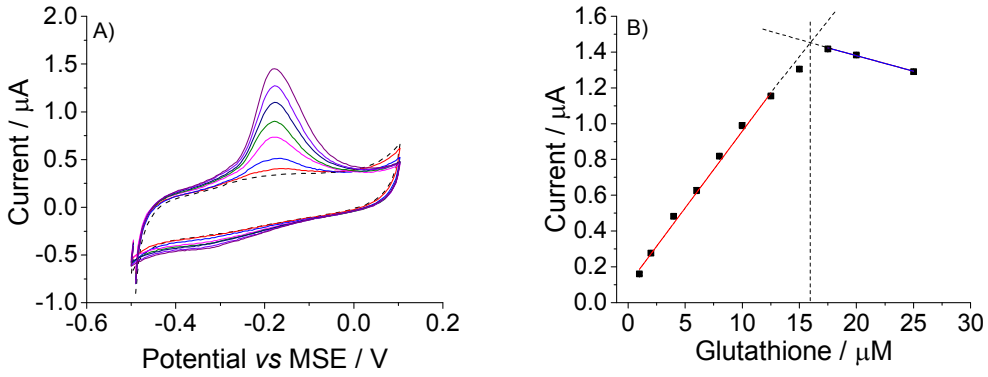

Fig. 4. (A) Cyclic voltammograms of a bare GCE in 0.1 M KNO_3 in presence of 14.3 μM of copper(II) and various GSH concentrations. Black dashed: No glutathione; red: 1.0 μM; blue: 2.0 μM; pink: 4.0 μM; green: 6.0 μM; dark blue: 8.0 μM; violet: 10.0 μM; brown: 12.5 μM. All voltammograms were recorded at 50 mV s^{-1}. The results shown are from the third cycle. (B) Plot of the peak currents obtained from the Figure 4A as a function of glutathione concentration. The calibration curve (red line, R^2=0.995) was obtained from glutathione concentration range from 1.0 μM to 12.5 μM. The limit of detection was 0.14 ± 0.004 μM. The blue line is the best-fit of data points obtained from glutathione concentration range from 17.5 μM to 25.0 μM. The extrapolation of the two lines gives the stoichiometric concentration of glutathione (16.0 μM), indicating that the stoichiometric ratio of the glutathione to copper(II) is 1:1.

3.5 Glutathione selectivity in presence of Cysteine

Cysteine is a thiol containing amino acid that exists in physiological fluids at 5.0–15 μM alongside of GSH.[10] Due to the structural similarity, it may interfere with the

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electrochemical detection of GSH. Cyclic voltammogram recorded in 0.1 M KNO_3 in the presence of 10 μM cysteine and excess copper shows an anodic peak which appeared at ca. – 0.20 V *vs.* MSE (Figure 5, pink line). As the direct oxidation of this molecule is at much more anodic potential [39], the peak observable in the figure is likely of the copper(II)-cysteine complex.[51,52] In comparison to the voltammogram recorded in the solution containing the equivalent amount of GSH (Figure 5, blue line), the anodic peak for cysteine is broader and less intense. When a voltammogram is recorded in a solution that contained equi-molar concentrations of GSH and cysteine, the anodic peak current showed, at first, significant increase above that of the system which contained GSH alone (result not shown). Thereafter, successive scans showed gradual decrease of the peak current. It was found that reproducible voltammograms can be obtained after the reaction mixture was equilibrated for 20 min prior to the electrochemical analysis (Figure 5, line green). Accordingly this equilibration time was included in the procedure to determine glutathione concentration in the presence of cysteine.

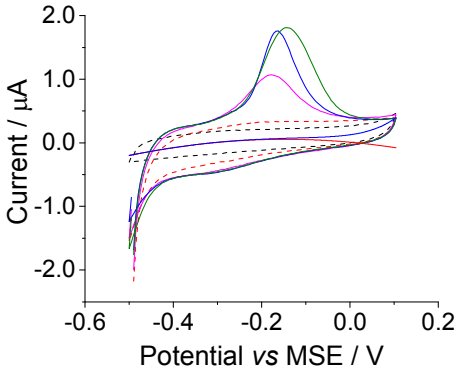

Fig. 5. Cyclic voltammograms of a bare GCE in 0.1 M KNO_3 with 44.3 μM copper(II) ion recorded at 50 mV s^{-1} in presence of 10 μM GSH (blue line), 10 μM cysteine (pink line), and a mixture of 10 μM glutathione and 10 μM cysteine (green line). The dashed black line is recorded in 0.1 M KNO_3 alone, and the red line is recorded in absence of the thiol compounds. The results shown are from the third cycle.

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3.6 Determination of Glutathione in presence of Cysteine

The detection of glutathione in presence of cysteine was carried out by the standard addition. A mixture of 5 μM GSH and 5 μM cysteine was prepared as an analyte and added to four standard solutions that contained 3.0 μM to 10.0 μM of GSH standard and 44.3 μM of copper(II) in 0.1 M KNO_3. A standard solution was prepared 4 min prior to the addition of an analyte, and voltammograms were recorded after the wait period of 20 min.

Figure 6A shows the anodic peak current increases with respect to the concentration of the GSH standard. The voltammograms were highly reproducible with around 1.7 % error after triplicated collection. There is a linear correlation between the peak current, I_p, and the concentration of the GSH standard as shown in Figure 6B. The relation between the peak current and the GSH concentration can be expressed as (n=3):

$$I_p/\mu A = (0.10 \pm 0.001)[Glutathione/\mu M] \qquad R^2 = 0.999 \qquad (3)$$

The gradient in the above equation is closely similar to that observed from the calibration curve of GSH (Eqn. 1) indicating that presence of cysteine does not interfere with the electrochemical detection of copper(II)-GSH complex compound at a bare GCE surface. Extrapolating the best-fit line to the zero current, this analysis confirms a concentration of 5.3 μM of glutathione for the analyte, which is in good agreement with the original amount of the reagent (5.0 μM).

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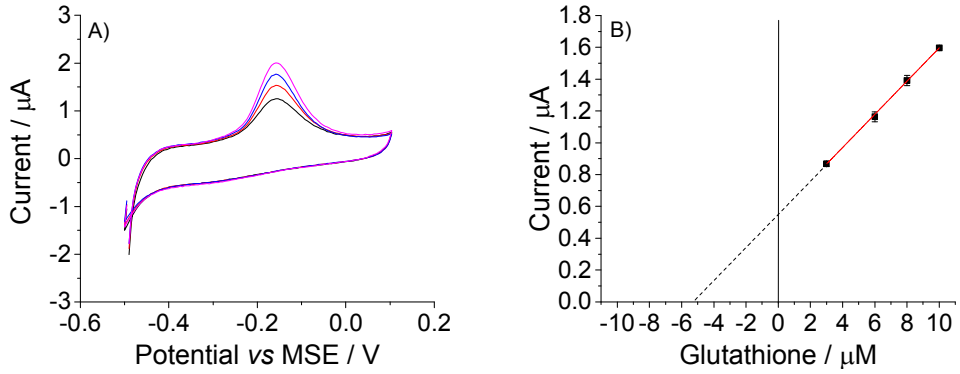

Fig. 6. (A) Cyclic voltammograms of a bare GCE recorded at 50 mV s^{-1} in 0.1 M KNO_3 containing 44.3 μM of copper(II) and various amount of GSH standard (Black: 3.0 μM; red: 6.0 μM; blue: 8.0 μM and pink: 10.0 μM) in presence of an analyte (5 μM glutathione + 5 μM cysteine). The results shown are from the third cycle. (B) Plot of the peak currents obtained from the Figure 6A as a function of the concentration of the GSH standard. The red line is the best-fit of data points between 3.0 μM to 10.0 μM. The extrapolation of the red line shows the GSH concentration in the analyte (5.3 μM).

4. Conclusions

We demonstrate in this work the electrochemical detection of glutathione based on the complex formation with copper(II) on an unmodified glassy carbon electrode. The copper(II) forms a 1:1 complex compound with GSH, which gives a characteristic voltammetric response of a surface bound species that undergoes oxidation on the electrode surface and at ca. – 0.20 V *vs.* MSE ($2[Cu–SG]^+ \rightarrow Cu_2(GSSG) + 2e^-$). The limit of detection obtained in this work is one of the lowest among the various voltammetric techniques that has been reported for this reagent. Furthermore, the detection technique described in this work has the sensitivity of 0.10 ± 0.002 μA μM^{-1} and the dynamic linear range up to 12.5 μM. It is highlighted in the manuscript that the current method of the GSH detection is not interfered by the presence of cysteine as the sensitivity (0.10 ± 0.001 μA μM^{-1}) obtained from the standard addition is identical to that from the calibration curve of the GSH only system. The

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low overpotential, a sufficient dynamic linear range, the high sensitivity, and no necessity of electrode surface modification make this approach a highly compatible clinical application.

Acknowledgements

MCCA thanks the granting authority of Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq for a fellowship. KS is supported by a Marie Skłodowska-Curie Intra-European Fellowship within the 7th European Community Framework Programme.

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