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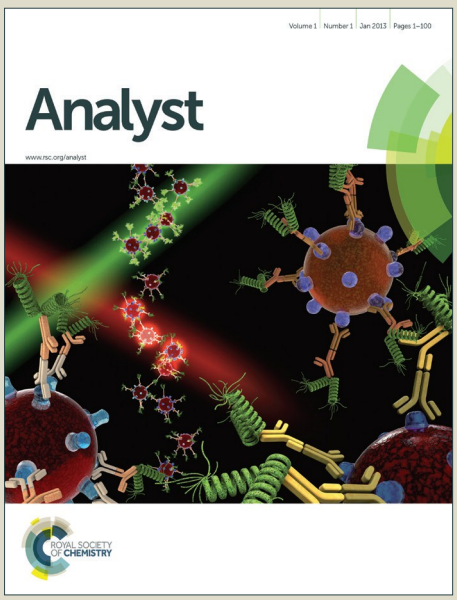

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Cysteine Determination via Adsorptive Stripping Voltammetry Using a Bare Glassy Carbon Electrode

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Abstract

The electrochemical determination of cysteine is investigated by adsorptive stripping voltammetric detection of copper-cysteine complex compound using a bare glassy carbon electrode. In acidic 0.1 M KNO_3 solution (pH 4), electrochemical oxidation of this complex compound generates a characteristic anodic peak ca. – 0.17 V *vs* standard mercury/mercurous sulphate reference electrode. The voltammetric response is highly reproducible within 2.1 % error (n=3). A linear dynamic range is obtained for a cysteine concentration of 1.0 μM to 10.0 μM. The sensitivity of 0.18 ± 0.006 μA μM^{-1} and the limit of detection of 0.03 μM (n=3) make our methodology highly applicable for practical applications. Successful determination of cysteine concentration in presence of glutathione has also been demonstrated by sequential determination of total thiol and the tripeptide.

1. Introduction

Cysteine (2-amino-3-sulfhydrylpropanoic acid) is a sulphur-containing amino acid which arises in the human body from methionine metabolism via the trans-sulfuration pathway.[1] In mammals, this compound is considered as non-essential as it is synthesized by the organism although methionine must be ingested. Amino acids are commonly regarded as the building blocks of proteins that are of significant importance in maintaining regular body functions. This thiol compound is also a key component in the synthesis of glutathione, which is an antioxidant and removes reactive oxygen species from physiological fluids.[2–4] Deficiency of cysteine hinders production of the tripeptide, and may increase risk of number of diseases including cancer, neuropsychiatric and immune dysfunctions, as well as aging.[2,4,5] Continuous monitoring of the physiological cysteine level in blood plasma and urine can be used to detect Vitamin B deficiency[6], oxidative stress[7], and inflammatory conditions and metabolic syndrome.[8,9] In addition, detection of cysteine along with glutathione may be

beneficial as reduced activity of thiol metabolism has been found among in patients with Parkinson's disease, Alzheimer's disease, and Motor neurone disease.[10] Hence, there is a need for a cysteine sensor that can be easily and frequently operated.

Quantitative analysis of cysteine has been reported using variety of analytical methods; many require intensive sample pre-treatments, costly and sophisticated instrumentation, additional chemical reagents, and/or a long analysis time. For instance, in the spectrophotometric determination of cysteine as reported by Zaia, *et al.*[11] it is necessary to chemically react cysteine with *p*-benzoquinone to form a complex which is UV/Vis active. The determination of cysteine via quenching is performed using Cd(II)-8-hydroxyquinoline-5-sulphonic acid by Wang *et al.*[12] This technique utilizes the property of cysteine to form a stable complex with Cd(II); however the fluorimetric response appears non-selective and moreover interference from other amino acids is severe. The electrophoretic analyses reported by Jin and Wang[13] and Zeng *et al.*[14] require sophisticated instrumentation to improve the selectivity. Furthermore, the determination of cysteine using high performance liquid chromatography as described by Amarnath *et al.*[15] requires a time consuming sample preparation including derivatization of cysteine to 2-thioxothiazolidine-4-carboxylic acid in order to extend shelf life of analyte and to improve UV absorption.

Electrochemical analysis provides a promising alternative for the determination of cysteine as it is cost effective, requires only a short operation time, involves little chemical waste, can show sufficient sensitivity, and has potential for miniaturization.[16] There are diverse articles which discuss the electrochemical detection of cysteine. Table 1 provides a summary of the electrochemical techniques which have utilized voltammetric or amperometric methods. The table shows that, hitherto, electrode surfaces have usually been modified to improve the electrode-substrate affinity. While such an approach has successfully

detected cysteine, the electrode modification can markedly increase the operating cost and/or complicate the use of the electrochemical sensors.

Our previous works[17,18] on the detection of captopril and glutathione show that thiols can be electrochemically detected at an unmodified glassy carbon electrode (GCE) after complex formation with a suitable metal ion. Captopril and glutathione form stable coordinate compounds with copper(II) ions.[17,18] Moreover the complexes can be adsorbed onto a bare glassy carbon electrode for subsequent electrochemical analysis. Herein we detail this approach to detect cysteine which also forms a complex compound with copper(II) ion in aqueous media.[19–22] Quantitative analysis of copper(II)-cysteine coordinate complex using absorptive stripping voltammetry is investigated. Furthermore, the possibility of determining the cysteine concentration in the presence of glutathione is explored. The application of this approach greatly simplifies the electrochemical detection of the physiologically important thiol and makes it cost-effective by avoiding the need for any electrode modification as well as employing a conventional GCE.

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

Electrode	Modification	Method	Medium	E_{det}	LDR (μM)	Sensitivity (μA μM^{-1})	LOD (μM)	Ref.
GC	--	CV	KNO_3/HNO_3, pH 4	–0.17 V *vs*. MSE	1.0–10.0	0.18	0.03	This work
EPPG	--	CV	Phosphate buffer, pH 7	0.58 V *vs*. SCE	17–208	0.07	2.6	[23]
CC	Nafion/[Ru(bpy)(tpy)Cl]PF6	CA	Phosphate buffer, pH 5	0.6 V *vs*. Ag/AgCl	0.1–100	0.05	0.02	[24]
Pt	CNT	CA	Phosphate buffer, pH 7.4	0.6 V *vs*. SCE	0.5–100	$0.80_7\times10^{-3}$	0.3	[25]
GC	OMC	CV	Phosphate buffer, pH 2.0	0.35 V *vs*. Ag/AgCl	$18–2.5\times10^3$	$23._6$	0.02	[26]
GC	BCNT	CA	Phosphate buffer, pH 7.4	0.47 V *vs*. SCE	0.78–200	$2.5_3\times10^{-5}$	0.26	[27]
CP	CNF	CA	Phosphate buffer, pH 7.0	0.75 V *vs*. Ag/AgCl	$0.15–63._8$	$15._9$	0.1	[28]
Au	GaNNW	CV	Phosphate buffer, pH 7.4	0.76 V *vs*. Ag/AgCl	0.5–75	0.042	0.5	[29]
CP	PDMA/FNC	CA	Phosphate buffer, pH 6.0	0.2 V *vs*. Ag/AgCl	$80–2.2_5\times10^3$	0.025_4	61.7	[30]
GC	Hg	CA	Phosphate buffer, pH 7.5	–0.7 V *vs*. Ag/AgCl	5–120	0.011	N/S	[31]
SPC	PEDOT/AuNP	CA–FIA	Citrate buffer, pH4.0	0.55 V *vs*. Ag/AgCl	0.5–200	0.11_5	0.05	[32]
CP	n–Fe_2O_3@NaCo[$Fe(CN)_6$]	CA	Phosphate buffer, pH 7.4	0.9 V *vs*. Ag/AgCl	3–37	0.10_8	0.04	[33]
CF	Au	CV	Acetate buffer, pH 4.65	2.0 V *vs*. Ag/AgCl	4.1–33	0.02	0.49_6	[34]
GC	Caffeic acid	CV	Phosphate buffer, pH 7.0	0.22 V *vs*. SCE	$1\times10^3–5\times10^3$	4.9	99	[35]
GC	Au	CA	Phosphate buffer, pH 7.0	0.65 V *vs*. SCE	1–400	0.37_4	0.05	[36]
GC	CTC	CV	Phosphate buffer, pH 7.0	0.10 V *vs*. SCE	0–40	0.023	0.6	[37]

GC: glassy carbon; EPPG: edge plane pyrolytic graphite; CC: carbon ceramic; CNT: carbon nanotube; OMC: ordered mesoporous carbon; BCNT: boron-doped carbon nanotube; CP: carbon paste; CF: carbon fiber; CNF: carbon nanofiber; GaNNW: gallium nitrite nanowire; PDMA/FNC: poly N,N-dimethylaniline/ferrocyanide; Ct-Cu: chitosan-copper; SPC: screen-printed carbon; PEDOT/AuNP: poly(3,4-ethylenedioxythiophene)/gold nanoparticle; CTC: cyclotricatechylene; FIA: flow inject analysis; CA: Chronoamperometry; N/S: not specified

2. Experimental

2.1 Chemicals and Materials

Potassium nitrate (KNO_3, 99+%, containing around 0.2 ppm of the copper(II)) and nitric acid (HNO_3, 70+%, <0.005 ppm copper(II)) were purchased from Fisher Scientific (Loughborough, UK). Copper(II) nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$, 99+%), sodium bicarbonate ($NaHCO_3$, 99+%), glutathione ($C_{10}H_{17}N_3O_6S$, 98+%) and cysteine ($C_3H_7NO_2S$, 97+%) were obtained from Sigma-Aldrich (Gillingham, UK). Aqueous stock solutions of glutathione and cysteine were prepared fresh daily. Ultrapure water with resistivity not less than 18.2 MΩ cm (25 ^oC) which was used throughout this work was generated from Millipore SimPak® 1 purification pack (lot. F5BA50456). Alumina polishing powders were from Buehler (Coventry, UK). Oxygen free N_2 gas (99.998%, BOC Gases plc, Guildford, UK) was humidified before use. A glassy carbon working electrode (GCE, 3 mm in diameter) was purchased from CH Instruments (Austin, USA). The surface area of the electrode (= 0.0707 cm^2) was measured by conducting cyclic voltammetry in aqueous 1 mM hexaammineruthenium(III) chloride (99+%, Sigma-Aldrich) at various scan rates.[38] 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). The choice of reference electrode does not affect the result of this study. A platinum wire (1 mm in diameter, Goodfellow Cambridge Ltd, Huntingdon, UK) was flame cleaned before it was used as a counter electrode.

2.2 Voltammetry

All electrochemical measurements were carried out at 25 °C under N_2 atmosphere using a conventional three-electrode system in a Faraday cage with a PGSTAT 101 potentiostat/galvanostat (Metrohm-Autolab BV, Utrecht, The Netherlands) and NOVA software (v. 1.11.2) as an operating interface.

A bare GCE was cleaned in the following manner before use. It was polished to a mirror finish using aqueous slurries of 1.0, 0.3, and 0.05 μm alumina in descending order of size. The electrode was thereafter rinsed thoroughly with ultrapure water. Note that alumina can interfere with electrochemical measurements by providing an adsorption platform for and/or catalysing reactions.[39,40] Then, the GCE was conditioned in deaerated 0.1 M $NaHCO_3$ solution by cycling the potential between – 1.6 V and + 1.6 V *vs.* MSE at a scan rate of 0.1 V s^{-1} for 5 cycles or until a stable voltammetric response was obtained. This pre-treatment reportedly increases the amount of hydroxyl functional groups at the GCE surface[41], which may enhance adsorption of the copper(II)-cysteine complex compound as well as stabilizing the voltammetric response. The treated GCE was subsequently rinsed thoroughly with ultrapure water and stored under ambient conditions.

A test solution was prepared immediately prior to electrochemical measurement by a successive addition of pre-determined amounts of nitric acid, copper(II), and cysteine in deaerated 0.1 M KNO_3 solution. Cyclic voltammograms were recorded at GCE between – 0.5 V to + 0.1 V *vs.* MSE at a scan rate of 0.05 V s^{-1} after the working electrode was immersed in a test solution for 120 s. The stability of the electrode response was ensured by cycling the potentials for three times; thereafter the analytical measurements were carried out in triplicate. Between experiments, any Cu(II)-cysteine complex residue adsorbed onto the GCE surface was electrochemically removed by cycling potential between – 0.5 V to + 0.1V *vs.* MSE in deaerated 0.1 M KNO_3 solution for around 10 cycles. For the detection of cysteine in

the presence of glutathione, pre-determined amounts of cysteine and glutathione (5.0 μM each or 10.0 μM and 5.0 μM, respectively) were added to the above mentioned test solution. Various amounts of cysteine standard solution were then added to the test solution and cyclic voltammograms were recorded. All experiments were carried out in triplicate.

3. Results and discussion

In the following, the effect of copper(II) ions in the detection of cysteine was first examined by carrying out cyclic voltammetric analysis of a cysteine containing test solution in the presence and absence of copper(II). UV-Visible spectroscopy was then employed to probe the formation of a copper(II)-cysteine complex compound in an aqueous solution. Cyclic voltammetry was then employed to explore electro-oxidation of the complex compound. Thereafter, the detection of cysteine in absence and presence of glutathione is demonstrated through the construction of a calibration curve and through a two-step determination, from which the cysteine concentration was derived via two separate standard addition experiments carried out at pH 4.0 and 7.0, respectively.

3.1 Cysteine oxidation in presence of copper(II)

In our previous studies[17,18], it was observed that the oxidations of copper(II)-captopril and of copper(II)-glutathione complexes occur at much more lower overpotential than that of the thiol compounds alone. To investigate if such a potential shift also occurs for cysteine, cyclic voltammograms were recorded in the presence and absence of the copper(II). The result (Figure 1 pink line) shows that the oxidation of cysteine in the absence of copper(II) ion has two anodic peaks at around + 0.3 V *vs.* MSE and + 0.4 V *vs.* MSE. These peaks are attributable to a series of oxidations in which the sulphydryl group of cysteine is first converted to sulfenic acid and subsequently to sulfinic acid.[42] A pair of anodic and cathodic peaks is also observable in the voltammogram at ca. – 0.13 V *vs.* MSE and ca. – 0.21 V *vs.*

MSE, respectively. This is attributable to the redox reaction of surface quinone groups[43], which are likely generated during the pre-treatment of GCE. The signal is also visible from the voltammogram recorded in an electrolyte solution in the absence of cysteine (Figure 1 red dash). Upon addition of 14.3 μM copper(II) an anodic oxidation peak is appeared at ca.– 0.17 V *vs.* MSE (Figure 1, blue line) suggesting the oxidation of copper(II)-cysteine complex.

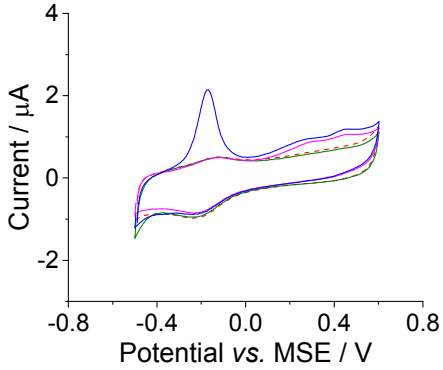


Fig. 1. Cyclic voltammograms of a bare GCE recorded in the 0.1 M KNO_3/4.0 mM HNO_3 electrolyte solution only (red dash), with 10.0 μM cysteine alone (pink), with 14.3 μM copper(II) ion alone (green), and with both 14.3 μM copper(II) ion and 10.0 μM cysteine (blue). All voltammograms are recorded at the scan rate of 0.05 V s^{-1} at 25 °C under N_2 atmosphere.

To further investigate the formation of the complex, a mixture of copper(II) and cysteine in a dilute nitric acid solution was analysed by a double bean Shimadzu UV-1800 UV-Visible spectrophotometer. Background subtracted absorption spectra of 50.0 μM cysteine with various concentrations of copper(II) are shown in Figure 2A. It is found that the absorbance at around 280 nm increases with an increase in the copper(II) concentration. A similar broad absorption spectrum as in the figure has been reported by Rigo *et al.*[21] who conducted spectrophotometric analysis of a phosphate buffered solution containing 0.33 mM copper(II) and 1.13 mM cysteine. The weak absorption maximum at 261 nm additionally reported by Rigo *et al.*[21] is not observed in the present study likely because the concentrations of the

analytes are much lower in the present study. Figure 2B illustrates the background subtracted absorbance at 280 nm as a function of the copper(II) concentration. The figure shows a linear increase in the UV–Vis absorption maximum with the concentration of the copper(II) while there is an excess cysteine concentration, indicating the formation of copper(II)–cysteine complex. Once the copper(II) concentration surpasses that of cysteine, the absorption becomes independent of the amount of the metal cation in the solution. It is found that the two extrapolated best-fit lines to the curve intercept at a copper(II) concentration of ca. 60 μM. This result suggests that the stoichiometric ratio of the complex compound is ca. 1:1 as reported by Smith *et al*.[19]

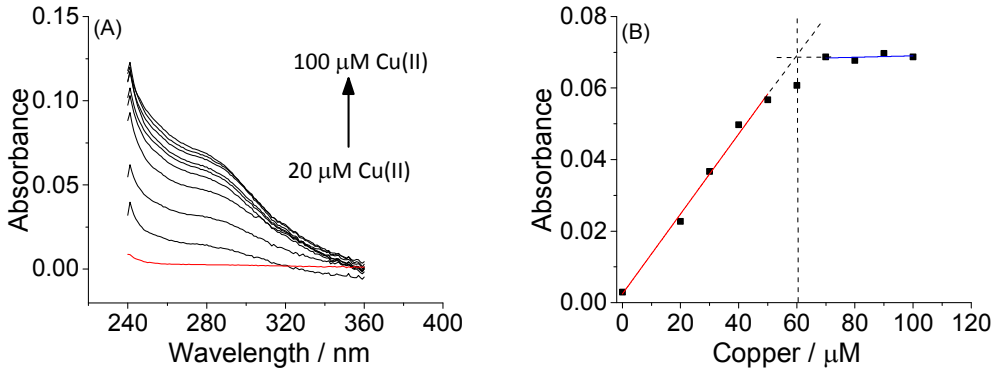
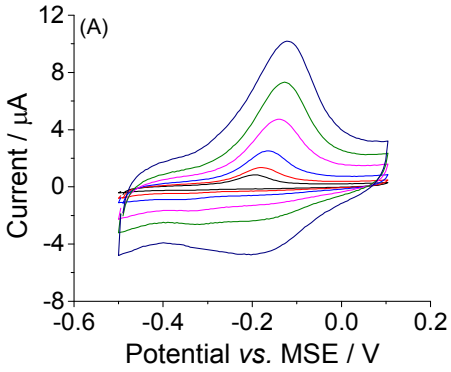
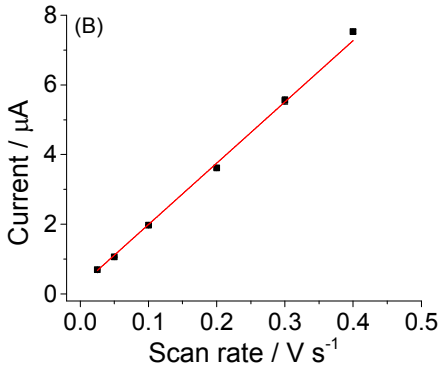


Fig. 2. (A) UV-Vis absorption spectra of 50 μM cysteine and various concentrations of copper(II) ion as indicated in the figure. The background absorption spectra of the nitrate ion has been subtracted for the figure. The absorption spectra of a solution that contained cysteine alone is indicated with a red solid line. (B) Plot of the absorbance at 280 nm obtained from the Figure 2A as a function of copper(II) concentration. The extrapolation of the best fit line between 0 μM and 50 μM (red line) and that from 70 μM to 100 μM (blue line) intercepts at cysteine concentration of ca. 60 μM, indicating the copper(II) to cysteine stoichiometric ratio is ca. 1:1. The analyte was contained in a quartz cuvette with light path length of 10 mm. All experiments were performed using ultra-pure water as a reference.

3.2 Cyclic voltammetry of the copper(II)-cysteine complex

Cyclic voltammograms were recorded in 0.1 M KNO_3/4.0 mM HNO_3 with 6.0 μM cysteine and 14.3 μM of copper(II) at scan rates between 0.025 V s^{-1} and 0.40 V s^{-1}. The resulting voltammograms are presented in Figure 3A in which the anodic peak potential for copper(II)-cysteine complex was found at ca. – 0.16 V *vs.* MSE. This value is significantly more negative than the direct oxidation of cysteine found in the Figure 1 (pink line). The anodic peak current increases linearly with the scan rate (Figure 3B); an expected voltammetric behaviour from a surface-bound species. The surface coverage of the complex compound is estimated as 2.1 (± 0.2) × 10^{-10} mol cm^{-2} from the integrated area under the oxidation peak. This value is independent of the scan rate as shown in Figure 3C. These observations again suggest that a copper(II)-cysteine complex compound is adsorbed onto the electrode and the electrochemical oxidation is a surface controlled process.[44]





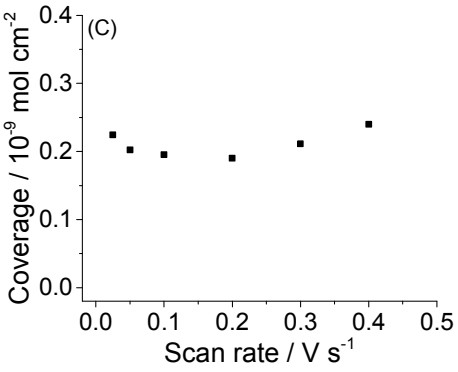


Fig. 3. (A) Cyclic voltammograms of a bare GCE in the 0.1 M KNO_3/4.0 mM HNO_3 electrolyte solution with 6.0 μM cysteine and 14.3 μM copper(II) at different scan rates. Black: 0.025 V s^{-1}; red: 0.050 V s^{-1}; blue: 0.10 V s^{-1}; pink: 0.20 V s^{-1}; green: 0.30 V s^{-1} and, dark blue: 0.40 V s^{-1}. (B) Anodic peak current as a function of the scan rate. (C) The surface coverage of cysteine as a function of the scan rate.

3.3 Determination of cysteine

The determination of cysteine in the 0.1 M KNO_3/4.0 mM HNO_3 solution was conducted using cyclic voltammetry at a scan rate of 0.05 V s^{-1} in the presence of 14.3 μM copper(II). The resulting voltammograms presented in Figure 4A show the oxidation of the copper(II)-cysteine complex near – 0.16 V *vs.* MSE. This potential is much lower than that required for the direct oxidation of cysteine. It is found that the anodic peak current gradually increases with respect to the concentration of cysteine up to ca. 10.0 μM (Figure 4B). With excess copper(II) present, the anodic peak current indicating the oxidation of the copper(II)-cysteine complex increases linearly with the concentration of the cysteine ligand, as the number of the redox active species in the solution increases.[45] The peak current becomes essentially constant once the cysteine concentration surpasses that of copper(II) ions. The intercept of the two extrapolated best-fit lines illustrated in Figure 4B is found at 11.4 μM, again suggesting a near 1:1 stoichiometric ratio as seen with the spectroscopic analysis.

A likely mechanism for the electrochemically assisted oxidative dimer formation of cysteine in the presence of copper (II) ion in acidic solution is as follows.

$$Cu^{II} + CySH \rightarrow Cu^{II}\text{–}CySH \quad (1)$$

$$2\,(Cu^{II}\text{–}CySH) \rightarrow 2Cu^{II} + CyS\text{–}SCy + 2H^{+} + 2e^{-} \quad (2)$$

where Cu^{II}–CySH indicates only the formal oxidation state of copper; literature suggests the possibility of Cu(I) formation partly as $Cu^{I} - CySH^{+\bullet}$.[46,47]

The calibration curve derived from Figure 4B reveals the following relationship (n = 3):

$$I_p = (0.18 \pm 0.006)[Cysteine] \qquad R^2 = 0.994 \qquad (3)$$

where I_p is the peak current in micro Amperes (μA) and the cysteine concentration is in micro molar (μM). The reproducibility of the anodic peak current is 2.1%. This was determined after triplicated collection of cyclic voltammograms measured for a solution containing 10.0 μM cysteine. The limit of detection (LOD) of 0.03 ± 0.001 μM was calculated using the equation: $LOD = \frac{3\sigma}{S}$ where σ and S indicate the standard addition and the sensitivity (= 0.18 μA/μM), respectively. This LOD is one of the lowest among the presently reported methods (Table 1). The dynamic linear range of this method is from 1.0 to 10.0 μM. As the concentration of free cysteine in human plasma is reported to be 3–17 μM[48–50], this range is suitable for the analysis to be carried out after a simple dilution. Note that the sample pre-treatment of pre-concentration is not necessary with this approach under practical applications. This is particularly important in order to reduce cost, time, and chemical waste during for example clinical analysis.

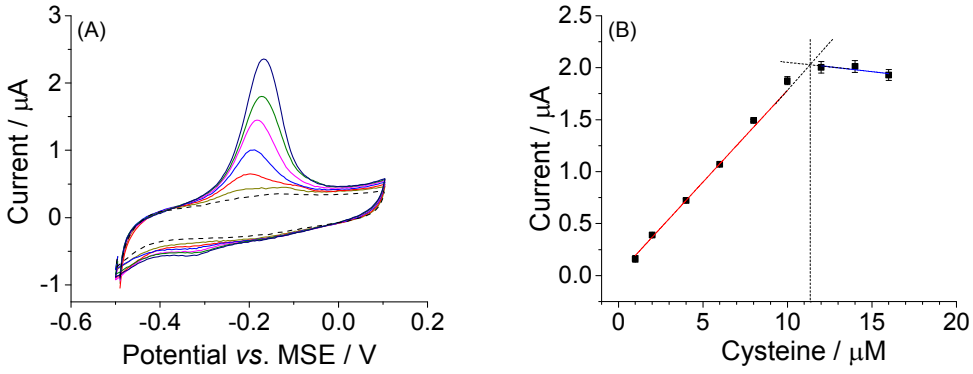


Fig. 4. (A) Cyclic voltammograms of a bare GCE in the 0.1 M KNO_3/4.0 mM HNO_3 electrolyte solution in presence of 14.3 μM of copper(II) and different concentrations of cysteine: No cysteine (black dash), 1.0 μM (dark yellow), 2.0 μM (red), 4.0 μM (blue), 6.0 μM (pink), 8.0 μM (green), and 10.0 μM (dark blue). All voltammograms were recorded at a scan rate of 0.05 V s^{-1}. (B) Plot of the peak currents obtained from the Figure 4A as a function of cysteine concentration. The calibration curve (red line, R^2=0.994) was obtained from the cysteine concentration range from 1.0 μM to 10.0 μM.

The limit of detection was 0.03 ± 0.001 μM. The blue line is the best-fit of data points obtained from cysteine concentration range from 12.5 μM to 16.0 μM. The extrapolation of the two lines intercepts at the cysteine of 11.4 μM, indicating that the stoichiometry is 1:1.

3.4 Determination of Cysteine in the presence of Glutathione

It is advantageous for a sensing technique to selectively determine the target chemical from other structurally similar molecules. The present method is selective towards free thiols that can form complex with copper(II) ion so that glutathione can potentially interfere with the cysteine determination in biological samples. Glutathione is one of the most abundant free thiol compounds and an antioxidant that is produced naturally in human body as a part of the methionine cycle from cysteine, glutamate, and glycine.[51,52] Because both glutathione and cysteine have the same thiol functional groups, their voltammetric response in neutral pH are similar.[18] Furthermore, the physiological concentration of glutathione in blood plasma and urine is highly comparable (1–8 μM)[7,48–50,53,54] with that of cysteine. Hence, it is particularly important for the proposed method to be able to accurately determine the cysteine concentration in the presence of glutathione. In the following, analyte solutions were prepared by mixing known amounts of cysteine and glutathione (5.0 μM each to give a total thiol concentration of 10.0 μM). Then the two concentrations are recovered via two separate standard addition experiments. First, an experiment is carried out at pH 4.0 to determine the total thiol concentration. Second, the glutathione concentration is determined at pH 7.0 as per our previous work.[18] The cysteine concentration is then derived by subtracting the amount of glutathione from the total thiol concentration.

In slightly acidic solution, it is found that voltammetric response of copper(II)-cysteine complex is much more stable than that at a neutral pH as implicitly demonstrated throughout this work. The voltammetric peak of the copper(II)-cysteine (Fig. 5 red line) appears at more negative (ca. – 0.19 V *vs.* MSE) potential than that of copper(II)-glutathione (ca. – 0.10 V

vs. MSE) (Figure 5 green line). When cyclic voltammetry was carried out in a solution that contained equi-molar concentrations (4.0 μM) of cysteine and glutathione with an excess of copper(II), a single anodic peak appears at ca. – 0.13 V *vs.* MSE (Figure 5, blue line). The integrated charge under the voltammetric peak of the combined solution is found roughly equal to the sum of that of cysteine and glutathione alone, consistent with the expectation that the voltammogram is the combined response from the two ligands.

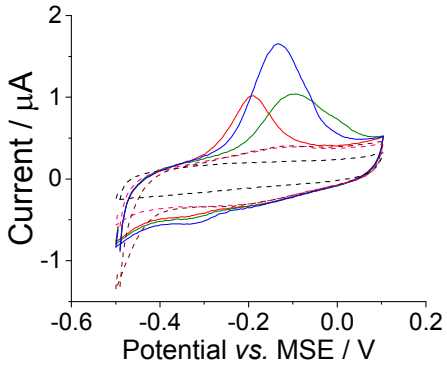


Fig. 5. Cyclic voltammograms of a bare GCE in the 0.1 M KNO_3/4.0 mM HNO_3 electrolyte solution with 14.3 μM copper(II) ion in presence of 4.0 μM cysteine (red), 4.0 μM glutathione (green), and a mixture of 4.0 μM cysteine and 10.0 μM glutathione (blue). The dashed black line is recorded in 0.1 M KNO_3 alone, the pink dashed line in 4 mM HNO_3 and 0.1 M KNO_3, and the brown dashed line is recorded in absence of the thiol compounds. All voltammograms were recorded at a scan rate of 0.05 V s^{-1}.

To determine the total thiol concentration, the test solution was prepared by mixing the analyte solution with a 0.1 M KNO_3/4.0 mM HNO_3 electrolyte solution that contained 44.3 μM of copper(II) ion. This test solution was then spiked with a standard cysteine solution to increase the concentration from 3.0 μM to 10.0 μM. Cyclic voltammograms were recorded at a bare GCE after placing the electrode in the test solution for analyte adsorption for 120 s. Stable voltammograms were obtained after cycling the potentials three times; thereafter the analytical measurements were carried out in triplicate.

Figure 6A shows characteristic cyclic voltammograms of a solution that contains copper complexes of cysteine and glutathione in acidic electrolyte media. The anodic peak

current increases as the amount of the cysteine standard present increases. 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 standard as shown in Figure 6B. The relation between the peak current and the cysteine concentration can be expressed as (n=3):

$$I_p = (0.19 \pm 0.003)[Cysteine] \qquad R^2 = 0.999 \qquad (4)$$

where I_p has the unit of μA and cysteine concentration is in μM. Extrapolating the best-fit line to the zero current, this analysis successfully finds 10.1 μM which is in good agreement with the original amounts of the total thiol (= 10.0 μM). In order to verify the consistency of the methodology experiment was repeated using an analyte solution that contained 10.0 μM cysteine and 5.0 μM glutathione. The result gave a concentration of 14.7 μM with a relative accuracy of 2%.

To determine the glutathione concentration in the analyte solution, a standard addition was conducted in neutral 0.1 KNO_3 solution which also contained 44.3 μM of copper(II) ion. At pH 7, the voltammetric response from the copper(II)-cysteine complex is unstable and becomes negligible after 20 min of equilibration time.[18] The test solution was spiked with a standard glutathione solution to increase the concentration from 3.0 μM to 10.0 μM. After each spike, the reaction mixture was equilibrated under N_2 atmosphere at 25 ^oC for 20 min. Then a bare GCE was immersed in the solution to adsorb analyte for 120 s prior to the electrochemical analysis. Stable voltammograms were obtained after the third cycle, and the scan was triplicated thereafter.

Figure 6B (circles) shows the results from the standard addition using the glutathione standard at neutral pH (voltammogram not shown). The lower peak current observed during this experiment than the previous standard addition at pH 4.0 is attributable to the difference

in surface coverage of the copper(II)-glutathione complex (1.5×10^{-10} mol cm^{-2}) in neutral pH as compared to that of copper(II)-cysteine (2.1×10^{-10} mol cm^{-2}) in acidic media. The relation between the peak current and the concentration of the glutathione standard can be expressed as (n=3):

$$I_p = (0.10 \pm 0.002)[Glutathione] \qquad R^2 = 0.995 \qquad (5)$$

where I_p has the unit of μA and glutathione concentration is in μM. Extrapolating the best-fit line to the zero current, this analysis gave 5.3 μM. Hence a cysteine concentration of 4.8 μM can be derived by subtracting the glutathione concentration (5.3 μM) from total thiol concentration (10.1 μM). The results show that the cysteine can be detected by unmodified GCE using adsorptive stripping voltammetry at low anodic potential and with high sensitivity. The ability to detect cysteine in the presence of glutathione makes the present approach applicable to physiological samples such as blood plasma and urine as well as commercial pharmaceutical products in which cysteine and glutathione are the predominant thiols.

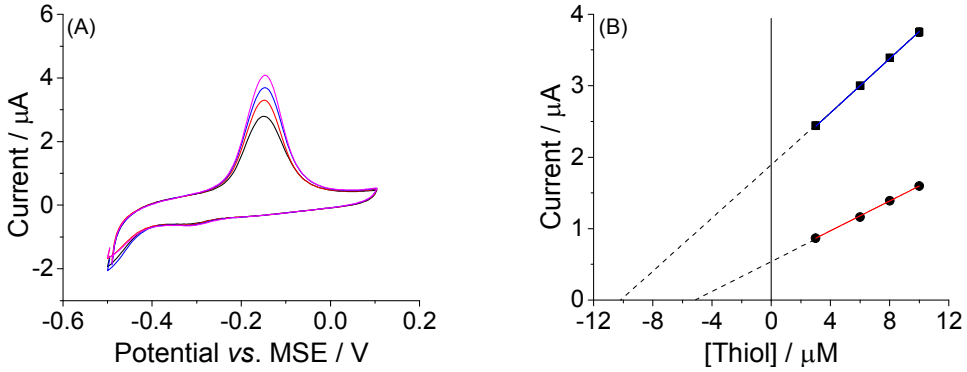


Fig. 6. (A) Cyclic voltammograms of a bare GCE recorded at 50 mV s^{-1} in the 0.1 M KNO_3/4.0 mM HNO_3 electrolyte solution containing 44.3 μM of copper(II) and various amount of cysteine standard: Black (3.0 μM); red (6.0 μM); blue (8.0 μM) and pink (10.0 μM) in presence of an analyte (5.0 μM cysteine + 5.0 μM glutathione). (B) Standard addition with glutathione standards (●) retrieving the glutathione concentration (5.3 μM) in the analyte. Standard addition with cysteine standards (■) showing the total thiol concentration (10.1 μM) in the analyte. The cysteine concentration is obtained subtracting glutathione concentration from the total thiol concentration (4.8 μM).

4. Conclusions

This work presents an electrochemical detection of cysteine at a bare GCE in an acidic electrolyte medium in the presence of a small amount of copper(II) ion. UV-visible spectra indicate the formation of a copper(II)-cysteine complex, which gives a characteristic voltammetric response for the oxidation of a surface bound species with the low potential anodic peak at ca. – 0.17 V *vs.* MSE. The voltammetric detection of cysteine using a bare GCE has a limit of detection of 0.03 μM that is one of the lowest values reported for voltammetric techniques for this reagent. Furthermore, with the dynamic linear range of 1.0 to 10.0 μM and the sensitivity of 0.18 ± 0.006 μA μM^{-1}, the present technique is highly applicable to the analysis of the physiological cysteine concentration with simple dilution as necessary. It is also demonstrated that the concentration of cysteine in the presence of glutathione can be determined using a two-step conditioned procedure. Such ability to determine the two physiologically significant thiols without significant sample preparation makes this approach highly suitable for possible clinical applications.

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