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Electrochemical analysis of copper ion using a Gly–Gly–His tripeptide modified poly(3-thiopheneacetic acid) biosensor

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ABSTRACT

A novel biosensor harnessing a conducting polymer functionalized with a copper ion specific peptide proved to be highly effective for electrochemical analysis of copper ions. The developed sensor comprised a transducer based on a conducting polymer (poly(3-thiopheneacetic acid)) electrode and a probe (tripeptide, Gly–Gly–His) selectively cognitive of copper ions. For functionalization of the electrode, the carboxylic group of the polymer was covalently coupled with the amine group of the tripeptide, and its structural features were confirmed by X-ray photoelectron spectroscopy (XPS) and attenuated total reflection infrared (ATR-IR) spectroscopy. The peptide modified polythiophene biosensor was used for the electrochemical analysis of various trace metal ions by square wave voltammetry. The electrode was found to be highly sensitive and selective to Cu^{2+} in the range of 0.02–20 μM with almost no cross binding to other metal ions such as Ni^{2+} and Pb^{2+}. Furthermore, the developed sensor exhibited a high stability and reproducibility despite the repeated use of the sensor electrode and probe. With the advent of more diverse affinity bioprobes specific towards a broad range of analytes, the demonstrated strategy harnessing peptide modified polythiophene biosensor is likely to provide an excellent platform for the selective determination of trace amount of analytes whose detection is otherwise cumbersome.

1. Introduction

Environmental contamination by trace metals is a serious problem not only for ecosystems, but also for human health, as heavy metals accumulate at higher levels within the food chain (Pascal et al., 2007). This concern has resulted in an ever-increasing demand for reliable means of detecting metal contaminants in environmental matrices.

Current methods used for metal ion detection include liquid- or gas-phase chromatography, solid-phase extraction, atomic absorption spectroscopy, inductively coupled plasma mass spectrometry, and inductively coupled plasma optical emission spectroscopy. However, many of these techniques are inconvenient, expensive, and time-consuming (Chapman et al., 2007). In contrast, electrochemical methods afford high sensitivity and sufficient accuracy without the need for expensive and specialized equipment. Electrochemical stripping analysis has been recognized as one of the most sensitive methods for the detection of trace metals. This strategy involves capturing metal ions using solid electrodes and then stripping the electrodeposited metal. Stripping methods that use chemically-modified electrodes exhibit higher selectivity, due to the ability of the modifier to capture specific metal ions of interest (Strutwolf and Williams, 2005; Achterberg and Braungardt, 1999).

Recently, interest in the electro-analytical application of conducting polymer (CP) modified electrodes has surged. Electroactive polymeric films are easier to generate on electrode surfaces than monolayers and for this reason have become widely popular. Compared with metal electrodes modified with an appropriate thiol or disulfide self-assembled monolayer, the chemical and physical properties of CP modified electrodes (e.g. adhesion to the electrode surface, surface roughness, and conductivity) can be greatly enhanced through formation of polymers on an electrode surface (Willicut and McCarley, 1995; Dahlgren et al., 2000). Moreover, the increased number of active sites on the CP film produces enhanced electrochemical processes at its surface compared to a monolayer-modified electrode (Boopathi et al., 2004).

The incorporation of a functional ligand as a dopant and modifier ion into the CP has been reported to facilitate capture of metal ions (Imisides et al., 1991; Rahman et al., 2003; Wallace and Lin, 1988; Migdalski et al., 1999). Wallace et al. used modified CP electrodes for the determination of Ag^{+}, Hg^{2+} and Cr^{5+} (O'Riordan and Wallace, 1986; Teasdale et al., 1989). A polypyrrole film electrode doped with 2,6-pyridinedicarboxylic acid or ethylenediaminetetraacetic acid (EDTA) has been employed for the determination of Ag^{+} (Wallace and Lin, 1988). The use of CP-modified electrodes based on

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electropolymerization of the different anionic-complexing ligands (e.g. bathophenanthroline, dihydroxyanthraquinone derivatives, bathocuproine sulfonate, and sulfosalicylic acid) into polypyrrole film has also been used for electrochemical analysis of Cu^{2+} and Fe^{2+} (Imisides et al., 1991; Shiu et al., 1994). In this case, the metal ion sensitivity of CP film cannot be regenerated due to gradual loss of ligands from the polypyrrole film. These polymeric electrodes are generally less stable than those with active groups covalently attached to the polymeric skeleton. Because of this, the sensing membranes may lose reliable responsivity after repeated exposures to corrosive or fouling solutions.

Heitzmann et al., 2007 prepared modified polypyrrole-coated electrodes through electropolymerization of *N*,*N*′-ethylene bis[*N*-[(3-(pyrrole-1-yl)propyl) carbamoyl) methyl]-glycine], which exhibits suitable affinity towards Cu^{2+}, Cd^{2+} and Pb^{2+}. A conducting polymer modified electrode functionalized by EDTA was fabricated by electropolymerization of 3′,4′-diamino-terthiophene monomer on a glassy carbon electrode (GCE), followed by the reaction with EDTA in the presence of catalyst. These modified electrodes show good affinity to various metal ions such as Pb^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Hg^{2+}; however, they do not have high selectivity for any specific metal ions (Rahman et al., 2003).

Conducting polymer grafted with specific functional groups can exhibit unique physicochemical properties departing from those of the original polymer. Functional materials used for modification of electrode appear particularly attractive for trace metal analysis. The carboxylic group is one of the most frequently employed reactive residues for incorporation of functional ligands onto the polymer backbone. Coupling ligand or specific biological species including peptides, enzymes, antibiotics, DNA, and even whole cells can be grafted via the carboxylic group into the polymers, which are subsequently electropolymerized on the electrode for the construction of the conducting polymer based biosensors (Shiu et al., 1994; Lee et al., 2002).

The use of peptides for the development of electrochemical metal ion sensors offers a number of attractive benefits. Numerous examples of highly selective metal-binding peptide motifs are available from the protein literature (Yang et al., 2003). Peptides represent the simplest biological recognition elements for binding metals. As a consequence, the development of peptide-based electrochemical biosensors for detecting heavy metals is an area of major interest (Chow et al., 2005a, 2005b). Arrigan and Bihan, 1999 have reported the monolayer formation of L-cysteine and its Cu^{2+} electrochemistry, Liu et al., 1999 introduced the application of cysteine monolayers for electrochemical determination of Cu^{2+} at a sub-ppb level. Chow et al. also reported Cd^{2+} sensors with the use of biologically active peptide His–Ser–Gln–Lys–Val–Phe attached to a self-assembled 3-mercaptopropionic acid (MPA) monolayer on a gold electrode (Chow et al., 2005a), and Cu^{2+} sensors based on Gly–Gly–His tripeptide attached onto a MPA self-assembled monolayer modified gold electrode (Chow et al., 2005b) were also reported. However, no conducting polymer electrodes modified with a peptide have yet been reported.

Of current interest is to identify peptide moieties specifically cognitive of copper ions for their selective measurement at low concentration with minimal interference from other metal ions. As exemplified by numerous metal-binding peptides and proteins found in nature, peptides are likely to suit this purpose by providing ideal recognition interfaces between transducer (i.e. CP) and analyte (i.e. copper ion). Since peptides are small ligands with multidentate binding sites, they are conducive to exhibiting a strong affinity to metal ions and allowing the redox active centre to remain in close proximity to the transducer for maximum signal output (Yang et al., 2003; Chow et al., 2005b). In this paper, we introduce an electrochemical biosensor consisting of a Gly–Gly–His tripeptide covalently attached to a poly(3-thiopheneacetic acid) (PTAA) film on a gold electrode. We also describe the characterization of each modification step in the formation of the Gly–Gly–His modified PTAA electrode by X-ray photoelectron spectroscopy (XPS) and ATR-IR spectroscopy. In our study, the Gly–Gly–His modified PTAA electrode showed greater affinity towards Cu^{2+} ions, compared to the other ions studied, and is nearly insensitive to Pb^{2+} and Ni^{2+} ions. The electrode was found to be highly selective for Cu^{2+} ions in the range of 0.02–20 μM.

2. Experiment

2.1. Materials

We purchased 1-ethyl-3-(3-dimethylamino propyl)-carbodiimide hydrochloride (EDC), 3-thiopheneacetic acid (TAA, 98%), copper (II) chloride hydrate ($CuCl_2$), lithium perchlorate ($LiClO_4$), chlorobenzene (99%), and acetonitrile (ACN) from Sigma–Aldrich. *N*-hydroxysuccinimide (NHS) and tetrabutylammonium perchlorate (TBAP) were purchased from Fluka. MES hydrate (Sigma–Aldrich), ammonium acetate (Fluka), sodium hydroxide (Dukson Pure Chemical Co. Ltd.), and sodium chloride (Junsei Chemical Co. Ltd.) were used for buffer solutions. All chemicals were of reagent grade and used without further purification.

All solutions were freshly prepared using distilled water. Buffer solutions were 50 mM ammonium acetate (pH 7.0) and 0.1 M MES (pH 6.8), and pH was adjusted using either NaOH or HCl solutions. Stock solution of copper ions (5 mM) was prepared in ammonium acetate buffer and diluted to give various concentrations of copper ions.

2.2. Electrochemical polymerization of PTAA

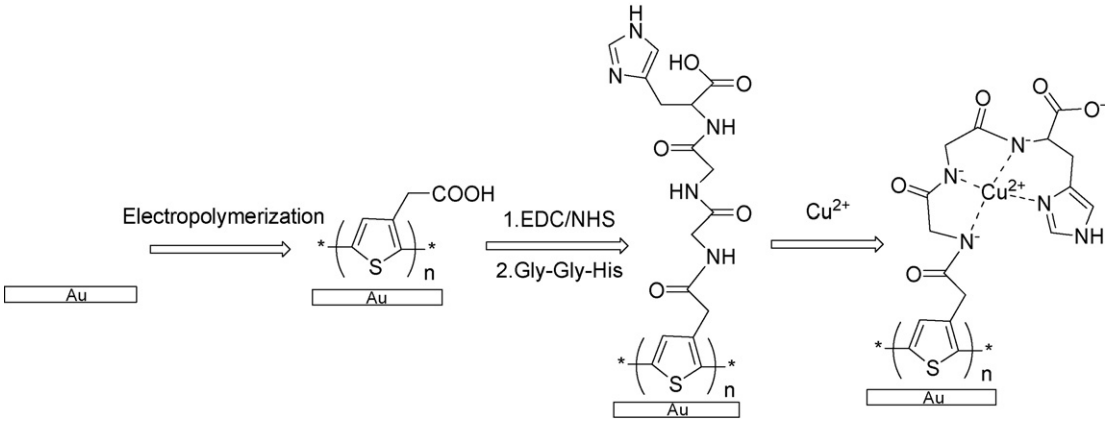
The optimum polymerization of PTAA was conducted according to a previously reported method (Liaw et al., 1999). During the electropolymerization, 0.2 M TAA in chlorobenzene containing 0.03 M TBAP was used to prepare a film of PTAA. The solution was degassed with nitrogen for 15 min to prevent oxidation of the monomer prior to polymerization. The polymer film was electro-polymerized onto a gold working electrode surface positioned parallel to a gold counter electrode of the same dimensions, held 2 mm away. A constant current density of 0.5 mA/cm^2 was applied between the gold electrodes for 15 min. The PTAA film on the electrode was immersed in fresh chlorobenzene to eliminate the residual monomer and dried with nitrogen.

2.3. Preparation of PTAA electrode modified with Gly–Gly–His

After polymerization, the carboxyl terminal groups of PTAA were activated by immersing the electrode in a solution of 50 mM EDC/50 mM NHS in 100 mM MES (pH 6.8) for 3 h. After a thorough rinse with 25 mM MES buffer, the modified polymer electrode was reacted overnight with Gly–Gly–His (3 mg/mL) in MES buffer to form the Gly–Gly–His modified PTAA electrode (Scheme 1). The modified electrode was rinsed thoroughly with MES buffer and stored at room temperature in 50 mM ammonium acetate buffer (pH 7.0) prior to use (Chow et al., 2005b).

2.4. Metal ion sensing

The surface area of modified electrode was 1.0 cm^2. The Gly–Gly–His modified PTAA electrode was immersed in 20 mL of 50 mM aqueous ammonium acetate buffer solution (pH 7.0) at various concentrations of copper ions for 10 min (Chow et al., 2005b; Bi and Yang, 2007) and washed with copper-free ammonium acetate buffer. The bound metal ions on the Gly–Gly–His modified PTAA electrode were reduced at −1.2 V for 40 s, and the electrochemical



Scheme 1. A schematic of the Gly–Gly–His modified PTAA electrode synthesis and its putative complexation with Cu^{2+}.

response was measured by square wave voltammetry (SWV) using a frequency of 50 Hz, an amplitude of 20 mA, and a step voltage of 5 mV. After each SWV measurement, the electrode was rinsed with ammonium acetate buffer, placed in 0.1 M EDTA solution for regeneration, rinsed carefully with acetate buffer, reduced and measured by SWV in a blank solution of ammonium acetate buffer to ensure complete stripping of all ions.

All electrochemical analysis was performed in a conventional three-electrode cell comprising the modified electrode as the working electrode, a platinum plate as the counter electrode, and a Ag/AgCl in saturated KCl solution as the reference electrode.

2.5. Characterization

UV–visible spectra were obtained by spectrophotometry (Labsphere RSA-HP-8453). ATR-IR spectra were obtained by infrared spectrophotometry (Bruker, Tensor 27) with 4 cm^{-1} resolution and 100 scans, using an ATR platform by pressing the solid electrodes onto the diamond crystal. XPS measurements were carried out using a VG Scientific ESCA 2000 spectrometer with an Mg-Kα X-ray source operating at a power of 170 W (13 mA and 13 kV). Cyclic voltammetry of the electrode was carried out in 0.1 M $LiClO_4$/ACN by potential scanning between −0.3 and 1.4 V (vs. Ag/AgCl KCl saturated) at a sweep rate of 10 mV/s. All electrochemical measurements were performed using a VSP potentiostat (Princeton Applied Research, USA).

3. Results and discussion

3.1. Electropolymerization and characterization of PTAA

In order to obtain a UV–vis spectrum of PTAA, ITO glasses were used as a working and a counter electrode. Prior to the polymerization, the ITO glass was cleaned in an ultrasonic bath for 20 min each in acetone, ethanol, and distilled water. The background spectra were recorded with blank ITO glass, the *in-situ* UV–vis spectra of PTAA during polymerization were measured in a standard quartz cuvette, with results recorded every minute. As shown in Fig. 1, *in-situ* UV–vis spectra measurement during electropolymerization of PTAA, the absorption band in the range of 320–350 nm is due to π–π* transition of the PTAA oligomer (Thuwachaowsoan et al., 2007; Wang et al., 2004; Kim et al., 1999). The bands appearing at λ_{max} of 604 nm and 952 nm correspond to the localized polaron state and bipolarons (Demanze et al., 1996). The overall absorbance increases with polymerization time; the bipolaronic state also amplifies, attributable to the increase in the conducting form of PTAA.

The cyclic voltammogram of the PTAA is depicted in the supplemental information Fig. S1. A slight anodic peak is observed during the sweep upto 1.4 V, and a cathodic peak is observed close to 1.04 V. No apparent peak during the anodic sweep confirmed the presence of intact carboxyl group on thiophene ring which is essential for conjugation of the tripeptide probe (Bartlett and Dawson, 1994).

The SEM images of the PTAA and Gly–Gly–His modified PTAA films are shown in the supporting information Fig. S2. The unmodified PTAA film (Fig. S2a) shows the dispersed microparticles on the surface, which is similar to the morphology of polythiophene, poly(3-methylthiophene), and poly(3-bromothiophene) surfaces (Hara et al., 2000). In contrast, the surface morphology of the Gly–Gly–His modified PTAA film (Fig. S2b) shows a smooth and uniform coating on Au surface, indicating the surface smoothness of the Gly–Gly–His modified PTAA film as compared with that of the PTAA film. These pores are likely to form during the tripeptide modification step due to the dissolution of PTAA microparticles.

3.2. Modification of PTAA electrode with Gly–Gly–His

3.2.1. ATR-IR spectroscopy

The ATR-IR spectrum of a PTAA film is shown in Fig. 2A. A very strong carbonyl band is observed at 1704 cm^{-1}, corresponding to the H-bonded acid oligomer in the polymer. The bands of moderate intensity between 1200 and 1600 cm^{-1} corresponded to aromatic and ring–ring stretches. The α-C–H and β-C–H out-of-plane

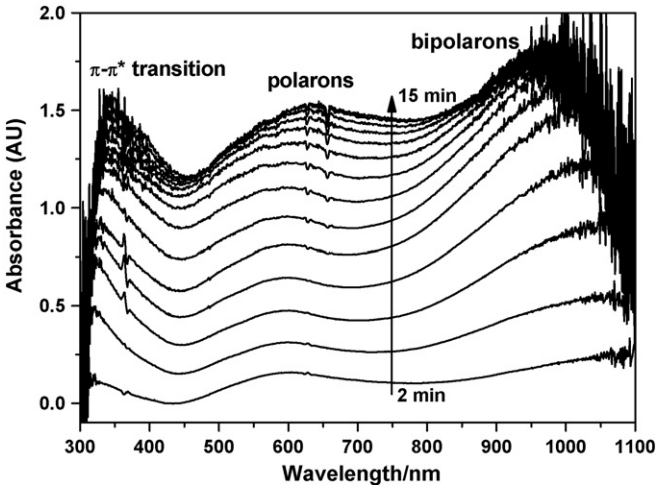


Fig. 1. UV–vis spectra of PTAA as a function of polymerization time.

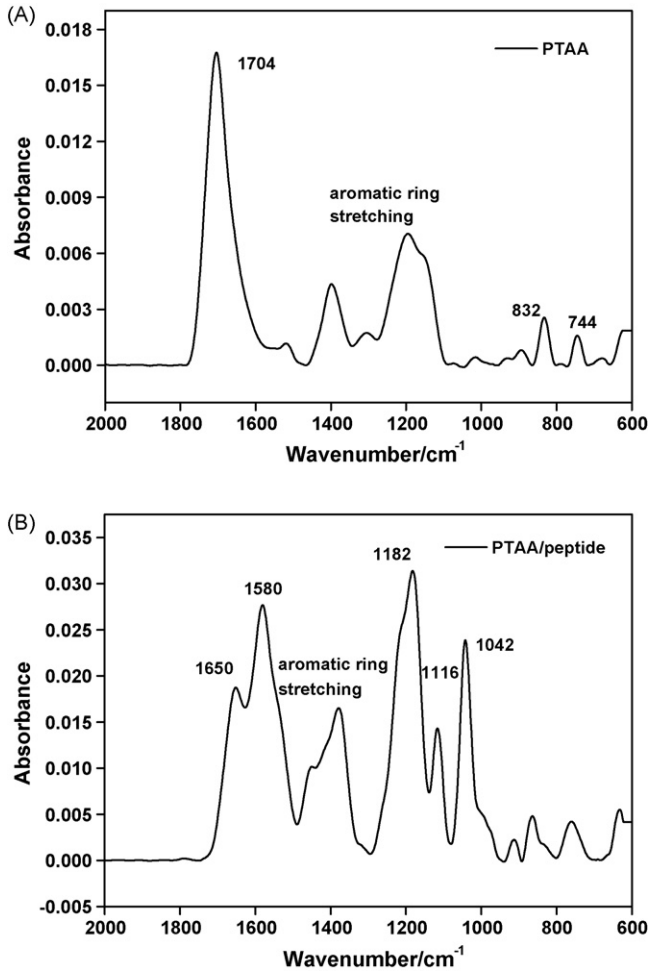


Fig. 2. ATR-IR spectra for the PTAA electrode (A) and Gly–Gly–His modified PTAA electrode (B).

bending of the thiophene ring in PTAA were observed at 744 and 832 cm^{-1}, respectively (Thuwachaowsoan et al., 2007; Bartlett and Dawson, 1994). In the spectrum obtained after modification of the tripeptide Gly–Gly–His on the PTAA film, as shown in Fig. 2B, the peak at 1650 cm^{-1} comes from the non-coordinated carboxylates (C=O) present in amide bonds, and the peak at 1580 cm^{-1} is primarily due to the secondary amide bond (N–H bending vibration strongly coupled to C–N stretching). Vibration absorption for C–O stretching is observed at 1182 and 1116 cm^{-1}. The band at 1042 cm^{-1} is attributed to a C–N stretching vibration. The absorption for aromatic and ring–ring stretching are observed between 1200 and 1600 cm^{-1}.

3.2.2. X-ray photoelectron spectroscopy

The XP survey spectrum for the formation of the PTAA film on gold showed a peak centered at 289.2 eV in the C_{1s} high resolution spectrum (Fig. 3A), characteristic of the carboxyl group of TAA. This is in good agreement with the peak position (i.e. 288.9 eV) reported by Cooper and co-workers (Chow et al., 2005b; Jiang et al., 1997; Liu et al., 2002). The peak at 285.0 eV can be attributed to the CH_2 groups of the PTAA film. As shown in the C_{1s} spectra in Fig. 3B, aromatic carbons at 284.9 eV are contributed by polythiophene and the photoelectrons emitted at 289.2 eV accounts for the presence of carbon atoms in the carboxylic acids. It is noted that three new Gaussian functions were generated by photoelectrons emitted from the carbon atoms of Gly–Gly–His in the C_{1s} core level spectrum recorded on the tripeptide modified PTAA/Au electrode.

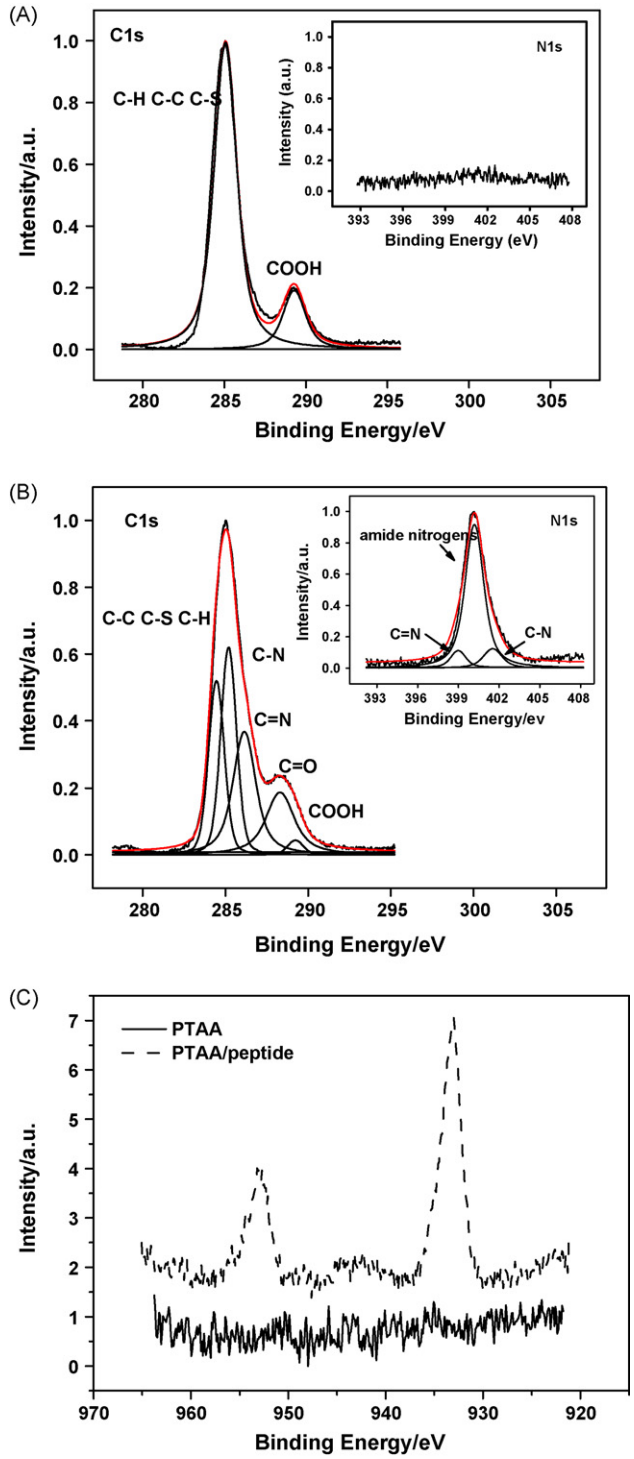


Fig. 3. XPS spectra (C_{1s} and N_{1s} (inset)) for PTAA electrode surface (A) and Gly–Gly–His modified PTAA electrode (B). (C) XPS spectra (Cu_{2p}) for PTAA electrode (solid line) and Gly–Gly–His modified PTAA electrode (dash line) following their incubation in Cu^{2+} solution.

Information regarding the successful attachment of Gly–Gly–His to PTAA can be obtained from the N_{1s} high resolution spectrum (Fig. 3B, inset). The nitrogen envelope can be fitted with three peaks. The first, at 398.9 eV, is attributed to the C=N nitrogen of the imidazole side chain of histidine, while the highest binding energy component (401.5 eV) originates from the C–N nitrogen of the imidazole ring (Abe and Watanabe, 2004). The peak at 400.1 eV is assigned to the amide nitrogens. The peak area ratio for these

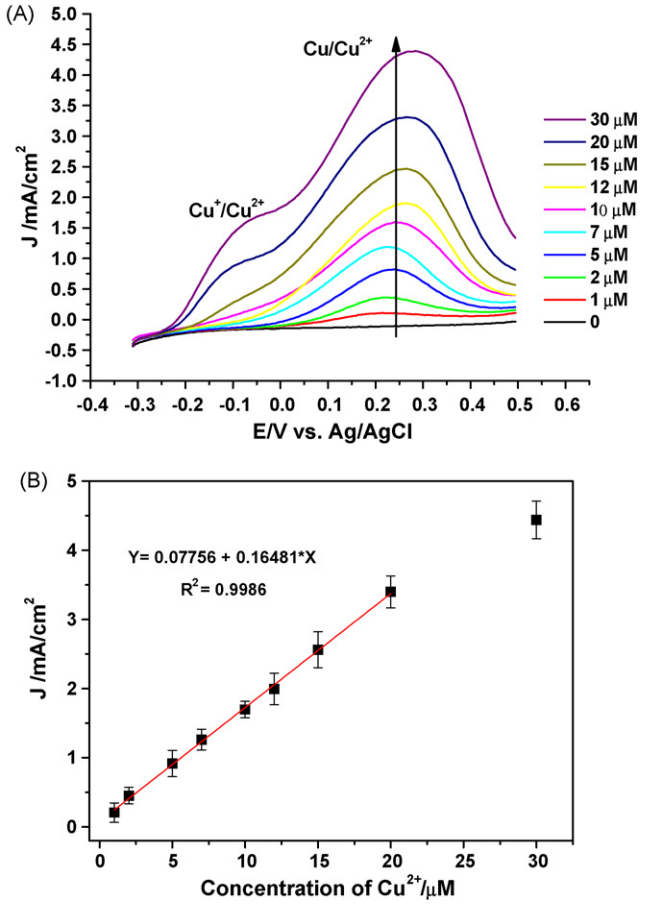


Fig. 4. (A) SWV curves for dissolution of Cu^{2+}captured by Gly–Gly–His modified PTAA electrode in the range of 1–30 μM Cu^{2+}, (B) SWV peak current for Gly–Gly–His modified PTAA electrode.

three components, from lowest to highest binding energy, is in good agreement with the predicted structure of a PTAA surface functionalized with Gly–Gly–His through an amide bond.

Our research goal is to investigate the formation of a copper complex with the immobilized Gly–Gly–His modified PTAA electrode. Fig. 3C shows the XPS spectra of Cu_{2p} for PTAA and Gly–Gly–His modified PTAA electrode following their immersion in buffer solutions containing copper ions for 10 min. $Cu_{2p3/2}$ peak at 933.0 eV and $Cu_{2p1/2}$ peak at 952.9 eV confirm the complexation of copper ions by the tripeptide modified electrode (Bi and Yang, 2007), indicating that the surface-exposed Gly–Gly–His could effectively capture copper ions.

3.3. *Electrochemical performance of Gly–Gly–His modified PTAA electrode*

SWV, more sensitive than cyclic voltammetry (CV), was conducted with the Gly–Gly–His modified PTAA electrode for detection of trace amount of copper ions. Fig. 4A and Fig. S3a (See supplemental information) show the SWV peaks originating from Gly–Gly–His modified PTAA electrodes. Following capture step, the modified electrode was transferred into a metal ion-free buffer (50 mM ammonium acetate, 50 mM NaCl, pH 7.0) for detection of Cu^{2+} where the captured metal ions on the electrode surface were reduced before recording the stripping current using SWV (Heitzmann et al., 2007; Yang et al., 2003; Bi and Yang, 2007).

For analytical purpose, SWV is used due to the lower detection limits afforded by square wave techniques. As shown in Fig. 4A and Fig. S3a (See supplemental information), SWV reveals a distinctive peak at 230 mV, associated with oxidization of Cu/Cu^{2+} (Heitzmann et al., 2007; Chow et al., 2005b) on a Gly–Gly–His modified PTAA electrode. However, a minor peak emerges at −75 mV when the solution concentration of Cu^{2+} was greater than approximately 15 μM (Fig. 4A) due to the partial reduction of entrapped Cu^{2+} to Cu^{+} (Yang et al., 2003; Liu et al., 1999). This is probably because the reduced Cu^{+} could have remained complexed to Gly–Gly–His rather than being dissolved in the solution (Chow et al., 2005b). For the Gly–Gly–His peptide immobilized onto PTAA film, the binding constant for Cu^{2+} was found to be $(4.4 \pm 0.3) \times 10^{8}\ M^{-1}$ in our experimental condition. Considering the loss in configuration freedom associated with the immobilization of the peptide, and the loss of carboxyl group either during electrochemical polymerization or modification step, it is expected that binding affinity of Cu^{2+} to immobilized or free peptide should be different. This is confirmed by the previous results where a binding constant of $(8.1 \pm 0.4) \times 10^{10}\ M^{-1}$ was reported for Cu^{2+} binding to the Gly–Gly–His immobilized on a MPA monolayer modified Au surface, which is nearly half of the previously reported affinity constant of $1.3 \times 10^{11}\ M^{-1}$ (Yang et al., 2003) for Cu^{2+} binding to free Gly–Gly–His in solution. The electrochemical response of the PTAA films during the oxidative scan was observed at 750 mV, much higher than that of the copper oxidation potential, as shown in supplemental information Fig. S1. This illustrates that the oxidation of the polymer does not interrupt detection of the pure Cu oxidation. The current from SWV is proportional to the amount of copper ions bound to the Gly–Gly–His modified PTAA electrode surface, the calibration curve (Fig. 4B) shows a linear increase in current density with the Cu^{2+} concentration from 1 to 20 μM (R^2 = 0.9986), illustrating a high sensitivity of the Gly–Gly–His modified PTAA electrode to Cu^{2+}. The modified electrode shows a Cu^{2+} concentration dependent behavior even for a sub-micromolar range (See supplemental information Fig. S3b).

Gly–Gly–His modified PTAA electrodes can be stored in buffer solution for reserved Cu^{2+} sensing. After each measurement, the regeneration of metal-free electrodes was performed by soaking in 100 mM EDTA solution for 10 min. From Fig. 5, it is clear that almost all the Cu^{2+} were stripped from the electrode by EDTA-mediated regeneration process. As a result, the SWV responses of the regenerated Gly–Gly–His modified PTAA electrodes are indistinguishable from those of fresh electrodes at two Cu^{2+} concentrations (i.e. 15 and 1 μM), there were only 1–2% decrease in peak currents compared to use the fresh prepared tripeptide modified electrode, indicating that the regenerated electrode can be used for Cu^{2+} detection in a reproducible manner. Each electrode was confirmed to be used for determination of copper ions without significant loss of sensitivity for several weeks. The loss of performance over time is attributed to gradual degradation or solubilization of modified PTAA film.

A sensor which relies on the extent of metal ion binding to the chelating ligand will be dependent on the solution pH. Higher pH conditions promote the deprotonation of amide nitrogens of Gly–Gly–His, rendering the formation of tetradentate Cu^{2+} complex with the deprotonated nitrogens. The larger the ratio of charge squared to the radius of the metal ion, the greater the ease of deprotonation (Chow et al., 2005b). Once the pH is raised above a certain level (e.g. pH > 7.53 for Cu^{2+} (Liu et al., 2006)), the amount of Cu^{2+} ions decreases with the formation of hydroxo complexes. In the present study, the pH effect on the capture of 1 μM Cu^{2+} by Gly–Gly–His modified PTAA electrode was investigated between pH 4 and 9. The optimum pH condition according to the maximum response using the tripeptide electrodes was obtained when the solution was buffered at pH 7, which is in a good agreement with the results reported elsewhere (Chow et al., 2005b). At this optimum pH, the electrode was highly selective to Cu^{2+} with minimal

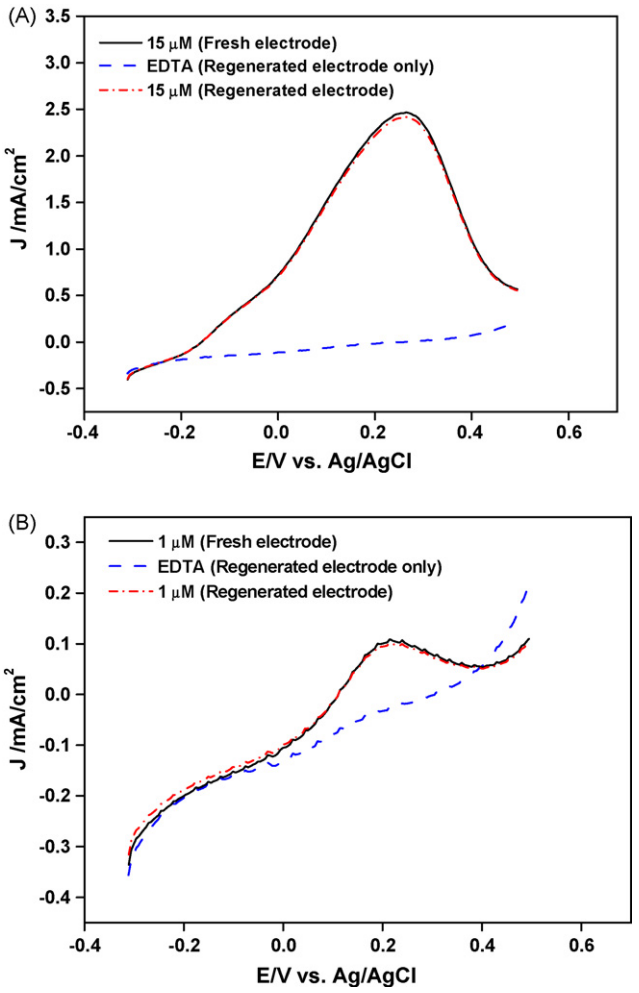


Fig. 5. SWV curves for the Gly–Gly–His modified PTAA electrodes which immersed in different solutions for their regeneration: (A) 15 μM Cu^{2+}, (B) 1 μM Cu^{2+}.

cross reactivity exhibited to the other metal ions tested (e.g. Pb^{2+} and Ni^{2+}).

The selectivity of the Gly–Gly–His modified PTAA electrode was investigated in the presence of common metal ion interferences, such as Ni^{2+} and Pb^{2+}. Ni^{2+} is the most concerning interfering species because it not only has a similar ionic radius but also a similar reduction potential as Cu^{2+} (Yang et al., 2003). Ni^{2+} can also form square planar 4N complexes with peptides, such as Gly–Gly–His, in solution (Kozlowski et al., 1999; Yang et al., 2003). When the peptide modified electrode was immersed in 70 μM Ni^{2+} solution, there was only a faint SWV peak current the magnitude of which is comparable to that from a significantly lower Cu^{2+} concentration (i.e. 1 μM) (See supplemental information Fig. S4). In the case of Pb^{2+}, there is no clear response even at 70 μM Pb^{2+}. This indicates that the Gly–Gly–His modified PTAA electrode has a preferentially higher affinity towards Cu^{2+} and is nearly insensitive to Pb^{2+} and Ni^{2+}.

Peptides with optimal amino acid sequences are considered as attractive molecular receptors for metal ion recognition (Bi et al., 2008). Gly–Gly–His tripeptide used in this study has well been known for its selective affinity to Cu^{2+} despite the presence of other divalent metal ions. In addition, its small size is ideal to locating the peptide probe in close proximity to the transducer, thereby allowing the tripeptide modified PTAA sensor to detect Cu^{2+} in a selective and reproducible manner at a sub-micromolar range.

4. Conclusions

The formation of PTAA was confirmed through UV–visible spectroscopy and cyclic voltammetry. The modification of PTAA with Gly–Gly–His was characterized by ATR-IR and XPS. The Gly–Gly–His modified PTAA electrodes were used for Cu^{2+} detection by square wave voltammetry. The results demonstrate that Gly–Gly–His modified PTAA electrodes are highly sensitive to Cu^{2+} in the range of 0.02–20 μM. The Gly–Gly–His modified PTAA electrode showed high affinity towards Cu^{2+} ions and is nearly insensitive to Pb^{2+} and Ni^{2+} ions. The Gly–Gly–His modified PTAA electrodes are also very stabile and easily reused, with regeneration through a simple wash in EDTA solution.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2009.05.035.

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