A regenerable screen-printed voltammetric Hg(II) ion sensor based on tris-thiourea organic chelating ligand grafted graphene nanomaterial

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An electrochemical Hg(II) ion sensor has been developed by using a miniaturized carbon paste screen-printed electrode (CSPE) modified with reduced graphene oxide (rGO) sheets and tris-thiourea (TTU) chelating ligand compound, i.e. N,N',N''-((nitrilotris(ethane-2,1-diyli))tris(azanediyl))tris(carbonothioyl))tribenz amide. In view of the strong cation-exchange characteristic and adsorption of aromatic TTU tridentate ligand on the graphene nanomaterial surface by non-covalent π-π stacking interaction, the differential pulse voltammetry (DPV) peak current response of the voltammetric sensor was linearly dependent on a broad Hg(II) ion concentration detection range from 0.1–0.00 mg L⁻¹ with a limit of detection (LOD) estimated at 0.02 mg L⁻¹ after accumulation for 10 min. The electrochemical sensor is reusable up to three consecutive Hg(II) ion assays by using 0.05 M acetate buffer (pH 8) as the sensor regeneration solution with a reversibility RSD value of 3.9%. The voltammetric sensor based on TTU derivative element and rGO nanosheets revealed satisfactory selectivity for Hg(II) ion over a large number of potential interfering ions, e.g. Ca(II), Co(II), Cu(II), Fe(II), Ni(II), Na(I) and Zn(II), and demonstrated reliable quantitative results as compared to the results obtained with inductively coupled plasma-mass spectrometer (ICP-MS) standard method for Hg (II) ion detection in river water samples.

1. Introduction

Mercury is a naturally occurring metal exists mainly in three forms i.e. metallic element, inorganic salt and organic compound [1]. Since the 1930s, mercury has been widely used as a preservative in a number of biological and pharmaceutical products, including many vaccines, nasal spray, drop points, antiseptic and ointment diaper rashes [2]. Mercury is very toxic and extremely bioaccumulative. Its presence in the water resources, such as river, ocean and lake where it is taken up by microorganisms can be concentrated in the fish or shellfish bodies in the form of methylmercury [CH3Hg(I)], and undergoing biomagnification to eventually confer toxicity in aquatic lives [3,4]. Consumption of contaminated seafoods and fish products is the major route of human exposure to mercury toxicity [5]. In the cement industry, mercury exposure among the workers could result in chronic asthma and bronchitis heart attacks. As such, the emission of mercury either by natural resources or anthropogenic activities can have a wide range of negative impacts on humans, plants and wild lands [6].

Conventional methods for detection of inorganic mercury and organic mercury compounds [e.g. ethylmercury [C2H5Hg(I) and CH3Hg(I)] in the blood and seafood samples have been using liquid chromatography-inductively coupled plasma-tandem mass spectrometry (LC-ICP-MS) with a rapid ultrasound-assisted extraction procedure [7,8]. Combination of diffusive gradient in a thin film probe and ion chromatography coupled to ICP-MS (IC-ICP-MS) has been deployed by Hong et al. [9] for in situ simultaneous quantification of CH3Hg(I) and mercury(II) (Hg(II)) ion in aquatic environments. By integrating a chip-based magnetic solid-phase microextraction system with micro high-performance liquid chromatography (microHPLC)-ICPMS, it can be used for speciation analysis of mercury [Hg(I) or CH3Hg(I)] in HepG2 cells to understand its cytotoxicity effect and cell protection mechanism [10]. Detection of Hg(II) ion concentration in water and wastewater can be done by using cold vapor atomic absorption spectrometry (CV-AAS) [11]. However, a major problem with coupled chromatographic and atomic spectroscopic procedures is achieving the necessary detection limits for target analyte either in waters or volatile forms,
whereby preconcentration of the targeted species followed by coupling of
effective derivatization is normally necessary prior to the detection step. Al-
though laboratory techniques are routinely used for the analysis of metal
ions as they are generally characterized as having high reliability, however
laboratory-based methods very often are costly, require skills to execute
and not to mention that they are time consuming. Thus, simple and portable
device for rapid analysis of heavy metal contaminants, which is comple-
mentary to the traditional laboratory techniques is greatly demanded.

For this reason, the development of sensing devices e.g. chemical sensor
or biosensor that will allow rapid and on-site analysis of inorganic mercury
has gained increasing interest among researchers. Potentiometric
sensor has been exploited for the determination of Hg(II) ion using tert-
butylcalix[4]arene-tetrakis(N,N-dimethylthioacetamide) as anion-
selective ionophore and tridodecylmethylammonium chloride as ion ex-
changer. An asymmetric membrane rotating ion-selective electrode config-
uration is required to improve the detection sensitivity, and dilution of the
water sample is needed to allow practical application of the potentiometric
sensor [12]. The Hg(II) ion sensor based on anodic stripping technique
(ASV) utilizing carbon nanoparticles-modified screen-printed electrode
(SPE) is described by Aragay et al. [13]. However, heating treatment at
40 °C is necessary to increase the edge-like planes of the modified electrode
for greater surface area available for metals deposition and electron transfer
enhancement, thereby an enhanced electroanalytical performance over de-
tection of Hg(II) can be achieved. A disposable bismuth SPE modified with
multi-walled carbon nanotubes (MWCNTs) was developed by Niu et al.
[14] for electrochemical stripping measurement of Hg(II) concentration
with square-wave anodic stripping voltammetry (SWASV). In order to min-
imize the cost in mass production of modified electrodes considering a large
number of samples is required for generating a high-quality analysis result.
Therefore, a cost-effective alternative to the disposable type of sensing ele-
trode would be a more economical substitution for it.

Among analytical methods, electrochemical methods have been shown
to have more advantages compared to other analytical methods due to their
portability, low cost, good selectivity and high sensitivity for electroanalyt-
cal determination of different types of compounds, such as drugs, food and
environmental pollutants [15]. With this, a wide-ranging effort has been
made to modify the electrode surface with various conductive
nanomaterials in order to increase the electron transfer rate for significant
enhancement of the resulting electrode sensitivity, which is the reason
why surface modification with nanomaterials is important to sensor/bio-
sensor performance. A novel analytical strategy for the detection of N-
hydroxysuccinimide water pollutant was achieved by carbon paste elec-
trode (CPE) amplified with tri-component nanohybrid composite of plat-
imum nanoparticle/polyoxometalate/two-dimensional hexagonal boron
nitrile nanosheets and 1-hexyl-3-methylimidazolium chloride as conduc-
tive mediators [16]. The application of palladium-nickel nanoparticles dec-
corated on functionalized-MWCNT was employed as a sensitive non-
enzymatic electrochemical glucose sensor [17]. A highly sensitive electro-
catalytic sensor was fabricated with a new nickel-based co-crystal complex
electrocatalyst incorporated with NIO dope Pt nanostructure hybrid as con-
ductive mediator on the CPE matrix for the determination of cysteamine
and serotonin in the drug and pharmaceutical serum samples [18]. Simple
modification of CPE electrode with novel modifiers composed of MWCNTs
and a series of noncyclic crown-type polyether ligands along with a room
temperature ionic liquid was employed for measuring thallium(I) in some
natural waters without interference from sample matrix using differential
pulse anodic stripping voltammetry (DPASV) method [19]. The hybrid
nanocomposite of MWCNTs and magnetic nanoparticles functionalized
with tannic acid and Au nanoparticles deposited on the glassy carbon elec-
trode (GCE) showed excellent electrochemical oxidation of 17α-
ethinylestradiol with electrochemical current increased by 28 times com-
pared to the conventional GCE [20]. Thiophene and p-phenylenediamine
were utilized as monomers to prepare low band gap copolymer and nano-
composites with core–shell construction by a simple in situ emulsion
polymerization. The electrical conductivity of the nanocomposites is con-
siderably higher than the copolymer which is dependent on the iron
content and doping degree [21]. A non-enzymatic amperometric hydrogen
peroxide (H2O2) sensor was fabricated by incorporating novel nanostruc-
tured orthorrhombic vanadium pentoxide into the CPE electrode, which
provides significant catalytic activities for H2O2 reduction [22]. Electro-
chemical aptasensor using gold nanoparticles (AuNPs) immobilized on
functional cupper magnetic nanoparticles and MWCNTs modified with
aptamer and 6-mercapto-1-hexanol [23], or immobilized on a conjugate be-
tween MWCNT and thiol-functionalized magnetic nanoparticles [24] that
were modified with an aptamer showed ultra-sensitivity towards label
free detection of bisphenol A (BPA) due to a synergistic augmentation on
the surface of the modified electrode. Direct electro-oxidation of BPA was
later achieved by utilizing novel nanocomposite based on MWCNT/thiol
functionalized magnetic nanoparticles as an immobilization platform and
AuNPs as an amplifying electrochemical signal [25].

Reduced graphene oxide (rGO) or known as graphene material possesses
superior mechanical, electrical and thermal properties by virtue of the highly
mobile π electrons that are located above and below the graphene sheet [26].
It is an effective absorbent towards any chemical or biological compound
having aromatic rings to strongly absorb at the graphene or graphene oxide
surface [27]. A composite film consisted of polycrystallite and rGO nanosheets
casted on the SPE with immobilized hemoglobin protein has been reported in
the electrocatalytic reduction of nitrite study by Raja Jamaluddin et al. [28].
In addition, graphene oxide sheets can be an ideal substrate for the study of
enzyme immobilization as they are enriched with oxygen-containing groups,
which makes them possible to immobilize enzymes without any surface
modification or any coupling reagents [29]. A CPE electrode modified with
 copper oxide decorated rGO, with 1-methyl-3-octylimidazolium tetrafluoro-
borate as a binder has been developed for high performance simultaneous
determination of cholesterol, ascorbic acid and uric acid [30]. An electro-
chemical As(III) ion sensor fabricated by casting graphene oxide/zinc based
metal-organic nanocomposite on a GCE electrode, followed by an electro-
chemically reduction of graphene oxide was performed by DPASV showed
excellent electrochemical performance, such as a wide linear range at part
per billion levels [31]. And, there are a lot more reports demonstrating the
adaptation of graphene nanomaterials for the applications of DNA detection
either as a label-free or amplification-free approach [32].

Thiourea derivative compound i.e. selenolthiol has been shown capable
capturing Hg(II) ion on mammalian by demonstrating enhanced inhibi-
tion activity as a result of detoxification mechanism of NADPH-reduced thioredoxin reduceant (TrxR) as an implication for treatment of mercury
poisoning [33]. A thiourea-based chemosensor was developed by Vonlanthen et al. [34] to be employed for fluorescence microscopy imaging of Hg(II)
ion concentration in living mammalian cells. Additionally, modi-
fication of label-free gold nanoparticle (AuNP) as a probe for colorometric
Fe(II) ion detection has been reported to occur in acidic thiourea mixture
as medium to catalyze the leaching of gold at room temperature [35].

In this study, we demonstrate the application of reduced graphene oxide
(rGO) as both superb conducting platform for efficient electron transfer and
immobilization platform for binding with tris-thiourea (TTU) chelating
agent on the miniaturized carbon paste screen-printed electrode (CSPE). In
view of the TTU aromatic species, which is rich in π orbitals, it can be
strongly adsorbed onto aromatic macromolecules such as graphene mate-
rials through π–π stacking interaction. Preliminary study by means of UV–
Vis spectrometry titration revealed the TTU-Hg(II) complex with tridentate
binding mechanism of NADPH-reduced thioredoxin (TrxR) as an implication for treatment of mercury poisoning [33]. A thiourea-based chemosensor was developed by Vonlanthen et al. [34] to be employed for fluorescence microscopy imaging of Hg(II) ion concentration in living mammalian cells. Additionally, modification of label-free gold nanoparticle (AuNP) as a probe for colorimetric Fe(II) ion detection has been reported to occur in acidic thiourea mixture as medium to catalyze the leaching of gold at room temperature [35].

2
2. Experimental

2.1. Instrumentation

The Fourier transform infrared (FTIR) spectrum of the tris-thioureia (TTU) ligand derivative was recorded in the wavenumber range of 4000–400 cm⁻¹ by using Perkin Elmer Spectrum GX spectrophotometer. ¹H and ¹³C nuclear magnetic resonance (NMR) experiments were performed on a Bruker Advance III 400 MHz instrument utilizing DMSO-d₆ as a solvent. Single-crystal X-ray diffraction (SXRD) investigations were performed on a Bruker D-QUEST diffractometer with graphite monochromated Mo and α radiation (0.71073 and 1.54178 Å). Preparation of homogenous mixture containing reduced-graphene oxide (rGO) and TTU ligand compound was conducted by using Elmasonic sonicator. The measurement of pH for 0.05 M acetic acid buffer was made with Metrohm pH meter. Field Emission Scanning Electron Microscopy (FESEM) was used to study the surface morphology and size distribution of rGO and immobilized TTU compound on the rGO-modified carbon paste screen printed electrode (CSPE). Differential pulse voltammetry (DPV) and cyclic voltammetry (CV) were conducted using Autolab PGSTAT electrochemical workstation (Netherlands). The three-electrode setup consists of a CSPE modified with rGO and TTU derivative element as the working electrode, a platinum counter electrode and a Ag/AgCl reference electrode. Inductively coupled plasma mass spectrometer (ICP-MS) was employed to validate the electrochemical Hg(II) ion in river water matrices.

2.2. Reagents and solutions

All the chemicals and reagents used in this study were of analytical grade and were used as received without any further purification. All solutions were prepared with deionized water of resistivity not less than 18.2 MΩ cm⁻¹. Graphite powder, sulfuric acid (H₂SO₄, 95–98%), potassium persulfate (K₂S₂O₈, 98%), phosphorus pentoxide (P₂O₅, 99%), potassium permanganate (KMnO₄, ≥ 99%), potassium hydroxide (KOH, ≥ 85%) and hydrazine monohydrate (NH₂NH₂·H₂O, 98%) were supplied by Sigma-Aldrich and were utilized in the synthesis of reduced graphene oxide (rGO). Benzoyl chloride (C₆H₅COCl, 99%), ammonium thiocyanate (NH₄SCN, ≥ 97.5%) and tris(2-ethylamino) ethanol (C₉H₂₁N₃O, ≥ 98%) were obtained from Aldrich and were used in the synthesis of TTU chelating compound. 0.05 M acetic acid buffer from pH 3 to pH 8 was prepared by mixing appropriate amount of 0.05 M glacial acetic acid (CH₃COOH, R & M Chemicals, 100%) with 0.05 M sodium acetate (CH₃COONa, Friendemann Sdhmidf Chemical, 99%). Mercury(II) acetate [Hg(CH₃COO)₂], ACS Reagent, ≥ 98%) was prepared in 0.05 M acetic acid buffer (pH 3). 0.01 M potassium ferricyanide (K₃[Fe(CN)₆]), ACS Reagent, > 99%) was prepared by dissolving 0.165 g K₃[Fe(CN)₆] in 50 mL of 0.05 M acetic acid buffer at pH 3.

2.3. Synthesis of rGO

rGO was synthesized according to the methods which have been described elsewhere [28]. In general, GO was prepared by using natural graphite powder through a modified Hummers and Offeman method [36]. Prior to the synthesis of GO procedure, a graphite oxidation procedure was first carried out according to the protocol recommended by Kovtyukhova et al. [37] in order to avert the formation of graphite-core/GO-shell particles in the final product as a result of incomplete oxidation reaction. The precursor of GO was then dispersed in deionized water followed by the addition of KOH solution as a strong base so as to afford a large negative charge through reactions with hydroxyl, epoxy and carboxylic acid functional groups on the GO sheets and that yielded an extensive coating of the sheets with negative charges and K⁺ ions [38]. 0.25 mL of 100 mmol hydrazine monohydrate was added to the KOH-treated GO to produce a homogeneous yellow-brown aqueous suspension GO. The as-obtained GO suspension was ultrasonicated and heated in an ice bath at 95 °C for 24 h to gradually produced the rGO as a black solid precipitate. The as-synthesized rGO was eventually washed with abundant deionized water followed by acetone, and dried in a vacuum desiccator.

2.4. Synthesis of TTU organic ligand

TTU chelating ligand was synthesized according to the method described by Misral et al. [39] with slight modifications. As Fig. 2 signifies, the TTU ionophore compound was prepared by mixing 1 mmol of benzoyl chloride (a) with 1 mmol of ammonium thiocyanate (b) in acetone under stirring for 15 min at 60 °C. The white precipitate of NH₄Cl (d) was filtered, and the yellowish solution of benzoyl isothiocyanate intermediate (c) was directly reacted with 0.3 mmol of tris(2-ethylamino) ethanol (e) in an ice bath under continuous stirring for about 5–6 h. The white TTU precipitate formed was washed several times with deionized water and dried in a vacuum desiccator.

2.5. Fabrication of electrochemical Hg(II) ion-selective sensor

The electrochemical Hg(II) ion sensor based on CSPE modified with rGO and TTU was prepared by first dispersing 1 mg of rGO in 200 µL of ethanol to form a stabilized colloidal rGO sheets suspension. Homogenous TTU solution with concentration of 15 mM, on the other hand, was prepared by dissolving 0.00653 g of TTU compound in 200 µL of ethanol followed by ultrasonication for 30 min. The CSPEs used were designed by Universiti Kebangsaan Malaysia and manufactured by Scrint Technology Pvt. Ltd., Malaysia with an electrode diameter of 4 mm and an active surface area estimated at 12.57 mm². The CSPEs were drop-coated with 15 µL or colloidal rGO sheets and left to dry at room temperature (25 °C) before dispensing 15 µL of TTU solution on the rGO electrode, and dried at ambient conditions. The electrochemical conductivity of the TTU-grafted rGO (TTU-rGO) electrode and its response with Hg(II) ion was measured by CV and DPV techniques versus Ag/AgCl reference electrode, and 10 mM K₃[Fe(CN)₆] electroactive probe in 0.05 M acetic acid buffer at pH 3 was used as supporting electrolyte.

2.6. Optimization of voltammetric Hg(II) ion-selective sensor

Optimization of electroanalytical performance of the TTU derivative-based Hg(II) ion sensor was conducted by means of DPV and K₃[Fe(CN)₆] was used as the redox probe. pH effect on the Hg(II) ion sensor was investigated by immersing six individual TTU-rGO electrodes into the respective
100.0 mg L\(^{-1}\) Hg(II) ion solutions at pH 3, pH 4, pH 5, pH 6, pH 7 and pH 8 (0.05 M acetate buffer) for 10 min before DPV peak current response was measured. Accumulation time of the sensor was performed by dipping the TTU-rGO electrode into 90 mg L\(^{-1}\) Hg(II) ion in 0.05 M acetate buffer at pH 3 for 1 min, 2 min, 5 min, 7 min, 10 min and 15 min before electrochemical response was recorded at DPV anodic peak current of 0.23 V. The electrochemical DPV response of the TTU-rGO based sensor towards determination of different levels of Hg(II) ion concentrations was carried out by using 0.1 mg L\(^{-1}\) Hg(II) ion solutions at pH 3. The life span of the electrochemical Hg(II) ion sensor was determined by measuring the voltammetric response of three chemically modified electrodes with 20.0 mg L\(^{-1}\) Hg(II) ion. The DPV measurement of the sensor was performed periodically within 38 days. The regeneration study was conducted by using 0.05 M acetate buffer (pH 8) as a regeneration agent. The TTU-rGO modified CSPE was immersed in 0.05 M acetate buffer at pH 8 for 10 min for removal of the Hg(II) ion after every Hg(II) ion measurement at 80 mg L\(^{-1}\) was done using the modified electrode. The selectivity study for Hg(II) ion sensor was tested using Ca(II), Co(II), Cu(II), Fe(II), Ni(II), Na(I) and Zn(I) ions during determination of Hg(II) ion at different molar ratios between Hg(II) ion and interfering ion i.e. 1:0:1, 1:0:1.0 and 1:0:10.0. All the electroanalytical experiments were performed in triplicate.

2.7. Voltammetric Hg(II) ion chemical sensor for real sample analysis

This study performed by using river water samples collected from two different points along Langat River, Selangor, Malaysia. Two sterilized polyethylene bottles were used for water sampling. The river water was filtered using Whatman filter paper No.1 to remove suspended particles. The river water pH was then adjusted to pH 3 by adding 0.05 M glacial acetic acid and 0.05 M sodium acetate. 80 mg L\(^{-1}\) Hg(II) ions were spiked into the river water sample, and examined by using the developed Hg(II) ion electrochemical sensor and validated with ICP-MS standard method.

3. Results and discussion

3.1. Characterization of TTU ligand compound and immobilization on the rGO nanomaterial

The as-prepared TTU organic ligand was obtained as white solid powder (yield: 2.07 g, 89%) with melting point of 257–269 °C. Chemical characterization of the TTU compound was conducted by using spectroscopy methods e.g. FTIR, \(^{1}H\) NMR, \(^{13}C\) NMR and single crystal X-ray diffraction analysis. The FTIR spectrum of the thiourea derivative (Fig. S1) shows strong absorption band at 3186 cm\(^{-1}\), which confirms the presence of NH functional group. The two most distinctive FTIR bands at 1660 cm\(^{-1}\) and 1527 cm\(^{-1}\) are associated with the carbonyl (C=O) and thiono (C=S) functional groups of the TTU ligand compound. \(^{1}H\) NMR analysis of TTU derivative showed characteristic peaks at \(\delta\) 10.93 ppm and \(\delta\) 8.94 ppm, which indicate the presence of thioamide (NHCS) and (NHCO) amide group (Fig. S2). This is also strongly supported by \(^{13}C\) NMR measurements (Fig. S3) that indicates chemical shift at \(\delta\) 207.07 ppm and \(\delta\) 179.93 ppm, which confirms the presence of CS and CO groups. The downfield chemical shifts of functional groups in \(^{1}H\) and \(^{13}C\) NMR are due to anisotropic and electronegative effect of oxygen and sulfur atom [40]. SXRD investigation revealed that the TTU ligand is in the crystalline form as two non-centrosymmetric molecules in one-unit cell (Fig. S4) having triclinic system with space group of P1. The crystallographic data of the as-synthesized TTU ligand derivative is tabulated in Table S1.

Chemical and physical characterization of rGO have been described in previous paper [28]. Fig. 3a shows the field emission scanning electron micrograph of the as-synthesized rGO that is ultrathin, flexible, sheet-like and transparent wrinkled-type [41] with average thickness of 2–4 nm. As TTU organic ligand is introduced on the rGO surface, the loosely stacked morphology of TTU chemical receptor-functionalized rGO sheets (Fig. 3b) is anticipated to enhance the Hg(II) ion diffusion and migration rate, and that the electron transfer rate at the TTU-rGO electrode surface can thus be enhanced [42]. The TTU is grafted on the rGO sheets by \(\pi-\pi\) interaction or non-covalent bond through molecular stacking between aromatic carbon in graphene and aromatic group in TTU ligand [43,44].

3.2. Chemical interaction between TTU ligand and Hg(II) ion

The chemical interaction between TTU chelating ligand and Hg(II) ion has been performed in un-immobilized state using DMSO as the solvent at three different molar concentration ratios of 1:1, 1:2 and 1:3 between TTU and Hg(II) ion. The colourless mixed TTU/Hg(II) ion solution dramatically turned into light yellow solution as increasing the molar concentration of Hg(II) ion from 0.001–0.003 M, whilst TTU concentration remained constant at 0.001 M of the mixed solutions (Fig. S5). This was attributed to the ionochromic behavior of the TTU ligand towards Hg(II) ion, i.e. mercury ionophore [45].

In addition, UV–Vis spectroscopy titration was also carried out to determine stoichiometry ratio of TTU-Hg(II) chelate complex by gradual addition of 150 \(\mu\)L of 0.01 M Hg(II) ion into 0.001 M TTU ligand until a slight sigmoidal curve is observed on the spectroscopic titration curve (inset of Fig. S6), which indicates a conformational change to the ligand is occurred. By drawing and extrapolating the standard lines in the molar-ratio curve, the TTU-Hg(II) complex establishes stoichiometry ratio of 1:3, which implies one TTU molecule binding to three Hg(II) ions via a lone pair on the respective oxygen and sulfur atoms of the TTU derivative donating to the Hg(II) ion through coordinate covalent bonds. A bathochromic shift of the absorption peak from 230 nm to 235 nm is observed along with the hyperchromic shift (Fig. S6), which indicates a conformational change to the ligand is occurred. By drawing and extrapolating the standard lines in the molar-ratio curve, the TTU-Hg(II) complex establishes stoichiometry ratio of 1:3, which implies one TTU molecule binding to three Hg(II) ions via a lone pair on the respective oxygen and sulfur atoms of the TTU derivative donating to the Hg(II) ion through coordinate covalent bonds. A bathochromic shift of the absorption peak from 230 nm to 235 nm is observed along with the hyperchromic shift (Fig. S6), which indicates a conformational change to the ligand is occurred.
mediator. The bare CSPE demonstrated rather lacking electronic conductivity (blue line) and that modification of the CSPE with graphene material is necessary to improve the electrochemical response of the electrode. The presence of the rGO nanosheets on the CSPE had shown to improve the heterogeneous electron transfer of the electrode [47] with both well-defined anodic peak current ($I_{pa}$) and cathodic peak current ($I_{pc}$) of K$_3$[Fe(CN)$_6$] redox indicator are discernible at 0.40 V and 0.08 V, respectively (red line). Further modification of the rGO electrode with TTU tridentate chelating ligand via non-covalent π-π stacking between benzene rings of TTU ionophore and hexagonal cells of graphene sheets depicted higher voltammetric response (green line). This was ascribed to the immobilized rGO sheets, which has increased the electrochemical active area on the CSPE and provided stable mobility of electron [27]. After pre-concentration of the TTU-rGO electrode in Hg(II) ion solution for 10 min, a remarkable increment in the electrochemical response was noticeable (purple line). This was due to the fact that the TTU multidentate ligand, which consisted of electron donor groups (Lewis base) bonded to the electron acceptor, i.e. the metal ion (Lewis acid), and when the electron from the donor transferred to the orbital d of the electron acceptor group, it produced a kind of internal oxidation/reduction process due to changes in oxidation states [48]. The electrochemical detection of Hg(II) ion using this chemically modified rGO electrode can thus be transduced into electrical signal based on the reversible redox reaction of the K$_3$[Fe(CN)$_6$] electron shuttling agent.

3.4. Electroanalytical performance of TTU ionophore-based Hg(II) ion sensor

The DPV peak current response of the Hg(II) ion chemical sensor as a function of pH is illustrated in Fig. S7 in the supplementary material. The electrochemical response of the Hg(II) ion sensor was noticed to decline steadily with increasing pH value of 0.05 M acetate buffer from pH 3 to pH 8, therefore the optimum pH for metal-chelation reaction was remained constant at pH 3 in the next electroanalytical optimization experiments. In acidic condition, i.e. pH 3, the optimum reaction medium can contribute to tautomeric effect of the immobilized TTU chelating ligand, and promoted deprotonation reaction of thioamide group to form complex assembly with Hg(II) ion as presented in Fig. S8 in the supplementary material [49]. At higher pH conditions, i.e. greater than pH 3 and towards alkaline medium, H$^+$ ions were depleted and Hg(II) ions were prone to exchange with exchangeable OH$^-$ ion to form mercury(II) hydroxide [Hg(OH)$_2$] as a result of ion exchange reaction [50]. In view of the metal-chelation reaction is pH-dependent, high pH would result in both thioamide and amide functional groups of TTU ligand no longer efficient to participate in metal binding at all.

The time dependent DPV response of the Hg(II) ion sensor showed gradual increase in the electrochemical voltammetric response from 1 min to 10 min pre-concentration time of TTU-rGO electrode in Hg(II) ion solution (Fig. S9). As the highest sensor response was obtained at 10 min of accumulation of the sensor electrode in Hg(II) ion solution at pH 3. This signifies maximum chelation reaction has attained at the electrode surface. However, after prolonged incubation of the electrochemical sensor in analyte solution above 10 min, it has reduced the efficiency of K$_3$[Fe(CN)$_6$] redox mediator transferring electron to the electrode surface and that a slight decrease in the sensor response was perceived.

The voltammetric pattern of the Hg(II) ion sensor is characterized by DPV $I_{pa}$ at 0.23 V. The DPV response of the TTU polydentate ligand-based sensor over electrochemical quantification of Hg(II) ion in the concentration range of 0.1 mg L$^{-1}$ to 180.0 mg L$^{-1}$ at 0.23 V and pH 3 is shown in Fig. 5a. The corresponding differential pulse voltammograms of the Hg(II) ion sensor response is exhibited in the inset of Fig. 5a. The electrochemical sensor response increased proportionally with increasing the Hg(II) ion...
concentration from 0.1–100.0 mg L\(^{-1}\) as metal-chelation reaction rate increased as increasing the analyte concentration. This allowed higher diffusion rate of K\(_3\)[Fe(CN)\(_6\)] redox probe to accelerate the redox reaction, and to facilitate the mediated electron transfer reaction at the electrode-electrolyte interface. The sensor response become saturated thereafter due to thioamide and amide functional groups of TTU chemical sensing phase were occupied by Hg(II) ion via dative covalent bonds. nd reproducibility relative standard deviation (RSD) of the sensor response was calculated at 4.9%. The limit of detection (LOD) of the voltammetric Hg(II) ion sensor calculated using the equation LOD = 3 \(s/m\), where \(s\) is the average standard deviation of blank and \(m\) is the slope of the calibration curve \([51]\) was estimated at 0.02 mg L\(^{-1}\) Hg(II) ion.

The lifetime performance of the TTU-rGO-modified CSPE electrode is illustrated in Fig. 5c. The electrochemical sensor was found to be tremendously stable throughout the course of the experiment towards determination of 20.0 mg L\(^{-1}\) Hg(II) ion, and the DPV peak current response of the sensor decreased slightly or not more than 95% of its initial response after 35 days of storage period. This was largely due to the strong adsorption of aromatic TTU sensing ionophore on the basal plane of the immobilized rGO nanosheets by \(\pi-\pi\) stacking on the CSPE, and thus preserving the electronic conductivity of graphene materials from physical or chemical structure interference. The electrochemical sensor showed high stability in the detection of inorganic Hg(II) ion right through the experimental period of 35 days with a RSD acquired at 1.2%.

Sensor repeatability was evaluated via four consecutive binding and regeneration cycles of the TTU-rGO electrode. As Fig. 5d indicates, 100% of the sensor response was remained after three consecutive binding and regeneration cycles of the sensor electrode. The use of 0.05 M acetate buffer at pH 8 as the regeneration solution has literally reacted with immobilized TTU-Hg(II) chelate complex by removing the bound Hg(II) ion from the TTU-rGO electrode, and Hg(OH)\(_2\) was precipitated out to regenerate the sensor electrode for next metal-chelation reaction by using 80 mg L\(^{-1}\) Hg(II) ion. The repeatability RSD of the sensor was calculated at 3.9%. However, when the TTU-rGO-based sensor was undergoing the fourth binding and regeneration cycle, the electrochemical sensor response dropped to

![Image](84x459 to 511x744)

**Table 1**
The percentage interference effect of various interfering ions e.g. Ca(II), Co(II), Cu(II), Fe(III), Ni(II), Na(I) and Zn(II) ions on the electrochemical Hg(II) ion sensor response at Hg(II):interfering ion molar ratios of 1.0:0.1, 1.0:1.0 and 1.0:10.0.

<table>
<thead>
<tr>
<th>Interfering ion</th>
<th>Interference level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Molar ratio of Hg(II):interfering ion</td>
</tr>
<tr>
<td>Ca(II)</td>
<td>0</td>
</tr>
<tr>
<td>Co(II)</td>
<td>0</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>0</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>0</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>0</td>
</tr>
<tr>
<td>Na(I)</td>
<td>0</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 2**
Validation of the electrochemical sensor based on TTU-rGO modified CSPE electrode with ICP-MS standard method for quantification of Hg(II) ion concentration in river water matrices.

<table>
<thead>
<tr>
<th>Method</th>
<th>Spiked Hg(II) ion concentration (mg L(^{-1}))</th>
<th>Sample A Found Hg(II) ion concentration (mg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTU-rGO modified CSPE</td>
<td>80</td>
<td>76.01 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>93.28 ± 0.07</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>80</td>
<td>79.34 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>99.60 ± 0.00</td>
</tr>
</tbody>
</table>
3.5. Selectivity of the Hg(II) ion electrochemical sensor

To establish specificity of the sensor in detecting Hg(II) ion, interference study was conducted against some metal ions e.g. Ca(II), Co(II), Cu(II), Fe (III), Ni(II), Na(I) and Zn(II) ions with the same electrochemical detection procedure implemented for Hg(II) ion. As shown in Table 1, among these potential interfering metal ions, Ca(II), Cu(II), Ni(II) and Zn(II) ions presented more than ±5% interference effect to the electrochemical quantita
tion of 80 mg L$^{-1}$ Hg(II) ion at Hg(II)interferent molar concentration ratio of 1.0:0.1. This is in accordance with the principle of Pearson soft and hard acidic-base (HSAB), whereby the immobilized TTU sensing ligand acted as soft base and that having most intense interaction with mild to soft Pearson acidic ions that includes metal ions, such as Ca(II), Cu(II), Na(I) and Zn(II) ions driven by simple electron transfer effect, and rendered competitive binding of the foreign metals ion with target Hg(II) ion for binding sites at the TTU ligand [46]. Nevertheless, according to the National Water Quality Standards for Malaysia [52], these metal ions are not exist in livestock drinking except Hg(II) ion at 0.004 mg L$^{-1}$ in class III water (i.e. polluted water). As such, the interference effect from those potential interfering ions can be neglected upon detection of Hg(II) with the developed electrochemical sensor based on TTU-rGO modified CSPE. Otherwise, ethylenediaminetetraacetic acid (EDTA), a widely applicable masking agent could be used to form complexes with great affinity for Cu(II) and Zn(II) ions in a simple binary mixture or a more complex mixture [53].

3.6. Application of the TTU derivative-based Hg(II) ion sensor for river water sample analysis

The accuracy of the proposed chemically modified sensor based on screen printed electrode for Hg(II) ion quantification has been validated with ICP-MS conventional method. The river water samples spiked with known amount of standard Hg(II) ion concentration at 80 mg L$^{-1}$ and 100 mg L$^{-1}$ were analysed by both methods. Based on the comparison results between conventional ICP-MS and electrochemical sensor tabulated in Table 2, both methods showed comparable results for Hg(II) ion determination in river water samples with a maximum total error of lesser than ±7% for Hg(II) ion concentration results obtained by voltammetric chemical sensor compared to ICP-MS. This indicates the Hg(II) ion electrochemical sensor based on TTU-rGO modified CSPE is in good agreement with the ICP-MS standard method for Hg(II) ion detection.

3.7. Performance comparison study between TTU-rGO-based sensor and other electrochemical sensors for inorganic Hg(II) ion detection

A performance comparison study on Hg(II) ion sensor developed in this study and previously reported Hg(II) ion electrochemical sensors based on different conducting materials and chemical chelating compound immobilized on the SPE electrode, such as sodium montmorillonite-modified CSPE [54], sumichellate Q10 chelating resin containing dithio
carbamate groups-modified CSPE [55], carbon black-gold nanoparticles (CBNP-AuNP) nanocomposite-modified CSPE [56], graphite nanoparticle-
modified SPE [13], multi-walled carbon nanotubes (MWCNTs)-modified bismuth SPE [14] and N220 nanostructured carbon black (CB) film-modified SPE [57] is outlined in Table 3. The developed Hg(II) ion chemical sensor demonstrated the potential for rapid, economical and convenient analysis of Hg(II) without the need of skilled personnel or burdensome equipment in the field. The polydentate TTU ionophore possesses multiple active sites that could increase the binding reaction rate with Hg(II) ion [58]. Direct grafting of TTU derivative onto the rGO electrode via π-π stacking interaction between aromatic rings could keep its intrinsic electronic properties remained intact in order to maintain the high electrochemical performance and led to faster electron transfer [26,59]. The TTU-rGO-modified CSPE can simply be regenerated in 0.05 M acetate buffer at pH 8, and be reused for screening of Hg(II) ion at room temperature.

4. Conclusions

The electrochemical Hg(II) ion sensor fabricated from miniaturized CSPE with electrode surface modified with rGO sheets and TTU chemical receptors by an easy drop casting procedure making this approach extendible to an automatable mass preparation of the modified CSPEs. The excellent electrical conductivity of the graphene material and good compatibility with aromatic thiourea compound rendered high electrochemical sensing performance of Hg(II) analysis with good stability of the sensor electrode. The polydentate TTU chemical sensing ionophore permitted multiple interaction with Hg(II) ion has widen the chemical sensor detection range to allow direct screening of inorganic mercury in water samples without additional dilution step is required, and holds wide application prospects in both environmental and industrial monitoring. The regenerable screen-printed voltammetric Hg(II) ion sensor is affordable and economical where it minimizing the environmental footprint of chemical materials as the electrode is reusable.

Author's comment

We wish for a fast peer review process for speedy journal publication.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jelechem.2020.114670.


