



# Diazonium functionalized exfoliated graphitic carbon as a binderless and covalently modified electrochemical interface for mercury sensing



Raghu G. Kempegowda, Pandurangappa Malingappa\*

Department of Chemistry, Central College Campus, Bangalore University, Bangalore 560 001, India

## ARTICLE INFO

### Article history:

Received 27 March 2013

Received in revised form 28 May 2013

Accepted 7 June 2013

Available online 19 June 2013

### Keywords:

Exfoliated graphite

Mercury

Anodic stripping voltammetry

Mercaptobenzothiozole

Industrial effluent analysis

## ABSTRACT

A sensitive and selective electrochemical sensing platform for mercury quantification at picomolar level has been described. The interface was constructed using exfoliated graphitic carbon covalently functionalized with mercaptobenzothiozole (MBT) as a selective indicator species in mercury determination. It was characterized using Fourier transform infrared spectroscopy (FTIR), cyclic voltammetry (CV) techniques and subsequently used in the fabrication of surface renewable binderless bulk modified robust pellet electrode. The fabricated electrode was used in the measurement of mercury in alkaline medium using differential pulse anodic stripping voltammetry (DPASV). The developed interface showed linearity in the concentration range 1–20 pM with a detection limit of 1 pM. The analytical utility of the proposed interface has been validated by determining the mercury levels from various water and industrial effluent samples with least interference from commonly encountered cations and anions that are generally present in industrial effluents. The storage stability of the proposed interface has been studied over a period of 6 months and the results were found to be highly reproducible with a relative standard deviation of  $\pm 6\%$ .

© 2013 Elsevier B.V. All rights reserved.

## 1. Introduction

Designing and construction of an electrochemical interface with selective functional moieties which are having potential affinity toward a specific metal ion is of a great interest in the development of chemically modified electrodes (CMEs) in the area of electroanalysis [1]. Mercury ( $\text{Hg}^{2+}$ ) is one of the highly toxic, volatile element and widely distributed in the environment. Exposure of this element even at trace level may pose negative impact on both mankind and aquatic systems [2]. As a result of this, the world health organization (WHO) has prescribed maximum threshold limit value (TLV) of 2 ppb in drinking water [3]. Hence, there is an increasing need for the development of analytical protocols with high sensitivity which determine the mercury well within the prescribed limits.

Several interfaces have been reported in the literature in the electrochemical measurement of mercury including thin film based electrodes which can be easily fabricated by simple drop casting methodology [4]. The storage stability of these interfaces has been found to be very poor and sometimes they may require recoating of the modifier during routine and continuous analysis [5]. Metal nanoparticle modified electrodes have been extensively used in

recent years in the determination of mercury due to their high sensitivity as well as better detection limits [6]. The possibility of oxide formation and the loss of few particles during surface renewing process restricts their utility [7]. The surface renewability of the interface is essentially required for precise analytical measurements. In this direction, several bulk modified electrodes like carbon paste electrodes (CPE) were used due to the possibility of introducing selective functionalities [8]. These electrodes are beneficial from the point of surface renewability but associated with the leaching of modifier from the electrode body into the bulk of the electrolytic solution during prolonged electrochemical measurements [9]. The presence of binder imparts an additional barrier for electron transfer and reduces the electrode kinetics at the interface [10]. Surface renewability coupled with long term storage stability is essentially required for long term and continuous analytical applications. Therefore, it is desirable to develop a robust, stable and easily renewable electrode using covalently derivatized carbon substrate material in order to prevent the leaching of modifier molecules, to improve the electrode kinetics and also to provide long term storage stability to the fabricated electrode.

Mercaptobenzothiozole (MBT) is a well-known analytical reagent which has been used in the determination of mercury [11]. It consists of exocyclic dissociable thiol group as well as endocyclic nitrogen and sulfur atoms in two distinctive environments. Application of MBT as a modifier molecule, carbon epoxy composite and

\* Corresponding author. Tel.: +91 80 22961352; fax: +91 80 22961331.

E-mail address: [mpurangachem@gmail.com](mailto:mpurangachem@gmail.com) (P. Malingappa).

polymer film modified electrodes have been designed and used in the determination of mercury [12,13]. The major limitation of the polymer modified interface is the slow diffusion across the films and also the swelling of modifier during prolonged electrochemical measurement [14]. Surface ion imprinted electropolymerized interfaces have also been reported but the analytical measurements have to be carried out in the solutions having the pH 5 or less than 5 due to the alkaline solubility of the MBT molecule [11]. The interaction of MBT molecule with mercury is expected to be through thiol and either N or S atoms. In acidic condition, it is in protonated form (HMBT) whereas in neutral and alkaline medium it is in deprotonated form ( $\text{MBT}^-$ ) [15]. Hence in acidic medium, there is no effective interaction between mercury and MBT molecule. As a result, it is essential to perform the analysis in alkaline medium in order to get maximum interaction, but due to alkaline solubility of the modifier molecule it is not possible to use it in basic medium. Hence its broad use in alkaline medium as a sensing platform is not possible. As a result of this, it is desirable to develop a sensing platform containing MBT molecule as a modifier molecule which can perform better in basic medium.

Exfoliated graphite (EG) is an expanded form of graphite whose surface area is 100 times greater than its unexfoliated form [16]. Due to its increased surface area, more number of modifier molecules will get covalently anchored on the EG which results in high sensitivity and selectivity toward particular analyte. It has a better homogeneity on the surface than any other forms of graphite. Based on these properties, it has been widely used for its chemical modification in recent years especially in sensing and bio-sensing applications [17,18].

In the present investigation, all these limitations have been overcome by anchoring the indicator (MBT) molecule covalently through the reduction of its corresponding diazonium salt at the surface of exfoliated graphite and subsequently used in the fabrication of binderless bulk modified electrochemical interface for the determination of mercury at picomolar level. Due to the covalent attachment of the modifier molecule, leaching and alkaline solubility of the MBT does not arise and working in basic medium provides an efficient route for the effective interaction of mercury and modifier molecule than in acidic medium which in turn results in the enhanced analytical signal (sensitivity) even at trace level concentration. The mechanism behind the formation of robust pellet electrode is the interlocking of graphitic layers under high pressure and the absence of binder during electrode fabrication may facilitates its electrode kinetics as well as long term storage stability over a period of several months. Since the interface is covalently bulk modified, its surface can be easily renewed for repetitive analytical measurements.

## 2. Experimental

### 2.1. Chemicals and reagents

All reagents used were Analar grade (AR) and used without any purification. Graphite (size  $>20\ \mu\text{m}$ ), amino mercaptobenzothiozole ( $\text{NH}_2\text{-MBT}$ ) and potassium bromide were obtained from Sigma–Aldrich. Acetic acid, sodium acetate and sodium hydroxide were purchased from SD fine Chemicals, Mumbai, India. Solutions of known pH in the range pH 1–12 were prepared using deionised water from Milli-Q water purifier (Millipore, USA) with a resistivity of not less than  $18.2\ \text{M}\Omega\ \text{cm}^{-1}$  at  $25^\circ\text{C}$ . Stock solutions of 1 mM of mercury was prepared using AR grade mercuric bromide in Teflon volumetric flasks and stored in refrigerator. Working standards were prepared by diluting appropriate aliquots of stock solution on the day of use. Double distilled water was used in all reagent preparations.

### 2.2. Apparatus

All electrochemical measurements were carried out using electrochemical analyzer [CH Instruments, model 619B, Texas, USA] at room temperature in an electrochemical cell of volume 10 mL with a standard three-electrode configuration. Binderless bulk modified pellet electrode (dia. = 5 mm) acted as the working electrode, A Pt (purity 99.99%) wire as the counter electrode and Ag/AgCl (3 M KCl) as a reference electrode (CH Instruments, Texas, USA). All the solutions were degassed using ultra pure nitrogen gas for 15 min before electrochemical measurements. All pH measurements were carried out using Control Dynamics pH meter (model APX 175). Infrared measurements were recorded using FTIR Shimadzu Scanning spectrometer (model 8400S) in the range  $1000\text{--}4000\ \text{cm}^{-1}$  with a resolution of  $4\ \text{cm}^{-1}$ .

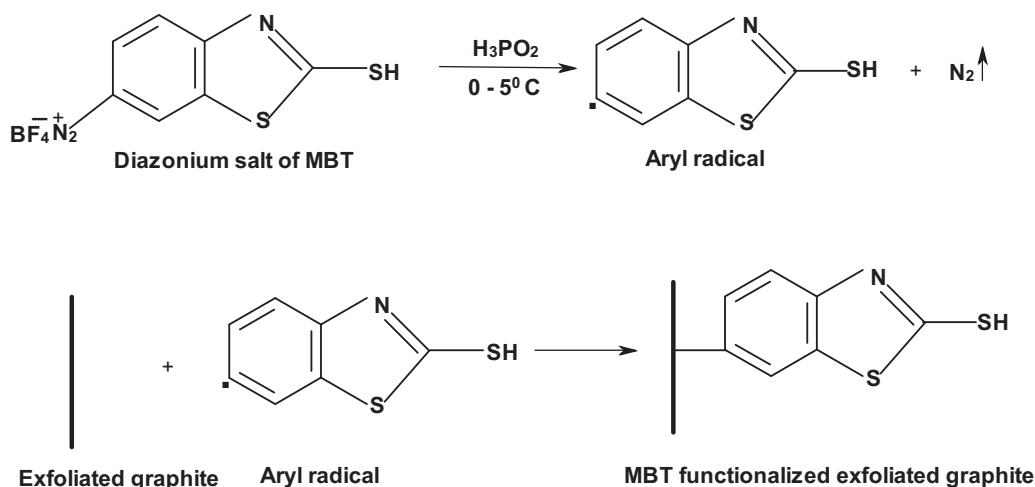
### 2.3. Exfoliation of graphitic carbon and its covalent derivatization through diazonium salt reduction

Graphitic carbon was intercalated by immersing 10 g of native graphite powder in 100 mL mixture of conc.  $\text{H}_2\text{SO}_4\text{--HNO}_3$  in 3:1 (v/v) ratio for 24 h under ambient conditions. The volume of the material has been found to be increased approximately twice due to intercalation of bisulfate ions. The material was washed well with distilled water to remove the excess of acid and air dried. Exfoliation was subsequently carried out by introducing the intercalated graphite in a furnace preheated at  $800^\circ\text{C}$  for 2–3 min. The resulting puffy carbon material was then gradually cooled to room temperature and stored in an air tight container [9].

The bulk modification of exfoliated graphite with mercaptobenzothiozole was carried out according to the procedure described below [19]. One gram of exfoliated graphitic carbon (EG) was mixed with 10 mL of a solution containing 30 mM of corresponding diazonium salt (synthesized according to the literature [20]) to which 30 mL of hypophosphorous acid was added slowly. The reaction contents were mixed well and allowed them to stand at  $5^\circ\text{C}$  for 30 min with occasional stirring at regular time intervals (once in every 5 min). Then the solution was filtered by water suction and washed thoroughly with distilled water in order to remove the excess of the acid and finally with acetonitrile to remove unreacted diazonium salt from the reaction mixture. The functionalized EG was then air dried by placing it inside a fume hood for a period of 12 h and finally stored in an air tight container. The schematic representation of covalent bulk modification of exfoliated graphite (EG) with mercaptobenzothiozole (MBT) as indicator species is shown in Scheme 1.

### 2.4. Fabrication of pellet electrode

Electrodes were fabricated using unmodified and MBT modified exfoliated graphitic carbon. About 200 mg of exfoliated graphite was compressed into pellet form at a pressure of  $8\ \text{ton cm}^{-2}$  for about 8 h without using any binder. The resistivity of the pellets were measured using multimeter and it was found to be  $1\text{--}2\ \Omega$  for unmodified and  $2\text{--}3\ \Omega$  for modified electrode. In each case, the compressed pellet was cut into small pieces of 5 mm diameter and mounted onto the one end of the Teflon tube with an external diameter of 8 mm and internal diameter of 5 mm. A copper wire was used for electrical contact and silver paste serves as the conducting medium between the pellet and the copper wire. The other end of the Teflon tube was sealed with araldite. Then the surface of the electrode was polished with 600- and 1500-grit emery sheets followed by 4/0, 5/0 and 6/0 emery polishing papers to obtain a smooth surface before its use. Roughness on the electrode surface was generated by scratching the electrode surface against emery sheets in unidirection for the reproducible results.



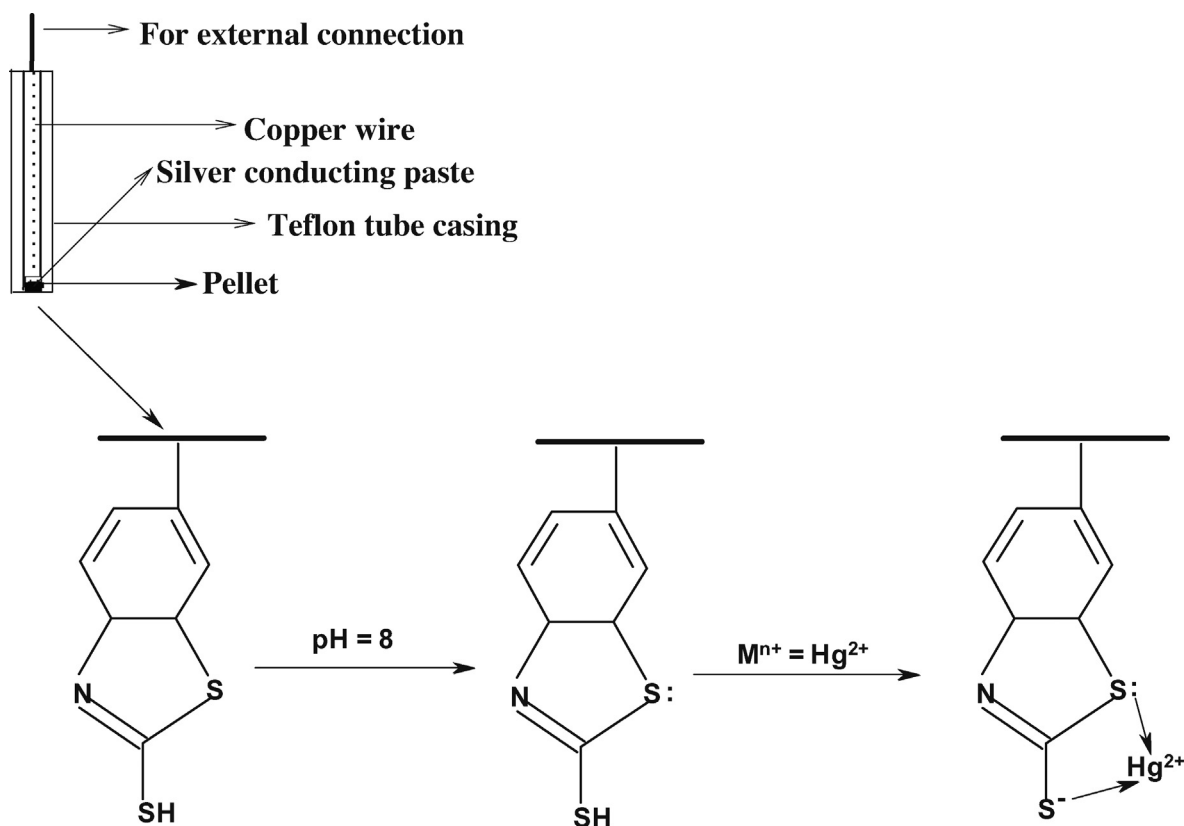
**Scheme 1.** Schematic representation of covalent bulk modification of exfoliated graphite (EG) with mercaptobenzothiozole (MBT).

The schematic representation of fabrication of electrode and the plausible mechanism of its interaction with mercuric ions has been shown in Scheme 2.

### 2.5. Analytical procedure

The determination of mercury was carried out using medium exchange differential pulse anodic stripping voltammetry (DPASV) in the potential range from  $-0.2$  to  $0.4$  V with an amplitude of  $0.01$  V and pulse width of  $0.05$  s. Known aliquots of mercury was taken in an electrochemical cell of  $10$  mL volume containing buffer solution of pH 8 which is fitted with a tab controlled magnetic stirrer. The fabricated pellet electrode was immersed into the pH solution

containing mercury and continuously stirred for 2 min to preconcentrate the metal ions at the interface at open circuit potential through specific interaction of mercury with the functional groups of the modifier molecule. Then the electrode was taken out from the preconcentration cell and washed thoroughly with distilled water in order to remove the loosely bounded mercury ions at the interface. Then the electrode was kept in another electrochemical cell containing supporting electrolyte of  $1$  M HCl and a reduction potential of  $-0.4$  V was applied for 20 s to reduce all the preconcentrated mercury ions into its atomic state and subsequently stripped off from the electrode surface into the bulk of the solution by scanning the potential in the positive direction after 15 s of equilibration time.



**Scheme 2.** Schematic representation of electrode fabrication and plausible interaction of modifier molecule with mercury ion at the interface.

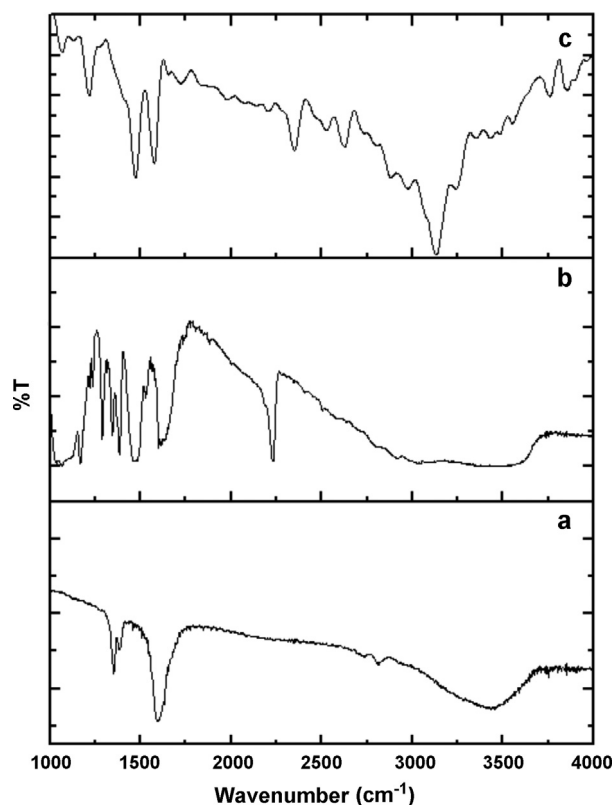


Fig. 1. FTIR spectra of (a) exfoliated graphite, (b) diazonium salt of MBT and (c) modified exfoliated graphite.

## 2.6. Sample preparation

The samples were collected from tap supply, treated industrial effluents, polluted lake, chrome plating industry and from textile industry. The collected samples were filtered through Whatman filter paper to remove any suspended colloidal matter present in the samples. Then known aliquots of filtered samples were added into electrochemical cell in which the electrolytic pH was maintained at 8 using phosphate buffer solution and analyzed by the procedure described as above.

## 3. Results and Discussion

### 3.1. Characterization of MBT modified exfoliated graphite

Exfoliated graphite was covalently anchored with mercapto-benzothiozole as a modifier molecule by the chemical reduction of its corresponding diazonium salt in presence of hyphosphorous acid as a chemical reducing agent. The reduction process results in the formation of dinitrogen and a highly reactive aryl radical which then subsequently gets anchored onto the exfoliated graphite forming a covalent bond between EG and MBT molecule [21]. The covalently modified EG was characterized by Fourier transform infrared spectroscopy (FTIR) and cyclic voltammetric (CV) techniques in order to decipher the functional groups present on the surface of EG. The FTIR spectra of unmodified exfoliated graphite, diazonium salt of MBT and modified exfoliated graphite were recorded and compared to confirm the nature of attachment of MBT molecule on the surface of EG (Fig. 1). The spectra were recorded by using thin pellet made by uniformly mixing of carbon (1 mg) with KBr (100 mg) using mortar and pestle. The transmission spectra of unmodified EG (Fig. 1a) has revealed the presence of C–C ( $1315\text{ cm}^{-1}$ ), C=C ( $1592\text{ cm}^{-1}$ ) and aromatic protons ( $2815\text{ cm}^{-1}$ ).

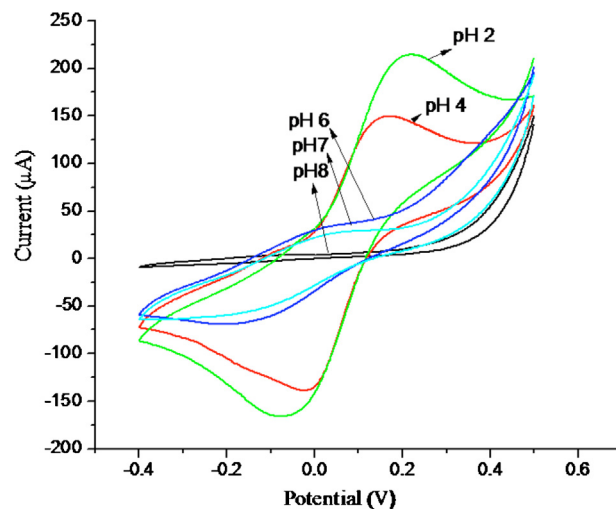
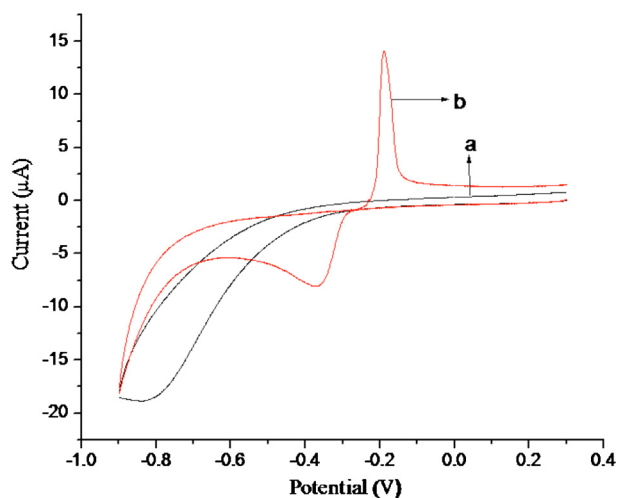


Fig. 2. Overlaid cyclic voltammograms of  $\text{K}_4[\text{Fe}(\text{CN})_6]$  (5 mM) redox system at MBT modified interface recorded in buffer solutions of varying pH (2–8). Scan rate: 50 mV/s.

The spectra of diazonium salt of MBT (Fig. 1b) showed a peak corresponding to the diazonium group ( $2230.7\text{ cm}^{-1}$ ). It has also showed peaks due to the presence of  $\text{C}=\text{S}$  ( $1215\text{ cm}^{-1}$ ), benzene ( $2349\text{ cm}^{-1}$ ) and  $\text{N}-\text{H}$  ( $3136\text{ cm}^{-1}$ ) functional groups. All these observations are in good agreement with the reported literature [22]. The most striking feature in case of the spectra of MBT modified EG is the absence of the stretching mode due to diazonium functionality (Fig. 1c). This indicates that, the diazonium group of the modifier molecule has undergone one electron reduction and subsequently gets covalently attached onto the EG during derivatization process.

The modified exfoliated graphite was electrochemically characterized by making use of blocking effect posed by MBT molecule toward the electrochemical behavior of a standard redox probe  $\text{K}_4[\text{Fe}(\text{CN})_6]$ . Cyclic voltammetry has been applied to characterize the modified interface in order to understand the nature of interfacial charge in the buffer solution of varying pH 2–8. Cyclic voltammograms of  $\text{K}_4[\text{Fe}(\text{CN})_6]$  (5 mM) in a buffer solution of different pH (2–8) were shown in the Fig. 2. It was observed that the standard redox probe showed well resolved redox peaks upto pH 4, above which the peaks starts decreasing and completely suppressed at pH 8. It may be due to the fact that, in acidic medium the MBT molecule is completely protonated (the thiol group is undissociated and the lone pair of electrons present on the N and S atoms are protonated) hence the interface is positively charged and therefore, there exist an electrostatic force of attraction with the negatively charged coordination sphere of the standard redox probe toward the interface. However in neutral and alkaline medium, the MBT molecule is deprotonated (the thiol group is dissociated into thiolate ion and the lone pair of electrons present on N and S atoms are free) hence the interface is negatively charged. Due to this, it repels the negatively charged coordination sphere of the standard redox probe, hence no redox reaction was observed. This type of behavior of the standard redox probe with respect to MBT modified interface is in agreement with the literature [23]. All these experimental observations clearly indicate that the nature of charge at the interface mainly depends on the protonation and deprotonation tendency of the MBT molecule which in turn depends on the pH value of the electrolytic solution. This study clearly demonstrates that covalently modified interface can effectively interact with the positively charged species (metal ions) in neutral or alkaline medium more effectively than in acidic medium. Therefore working in basic medium may result



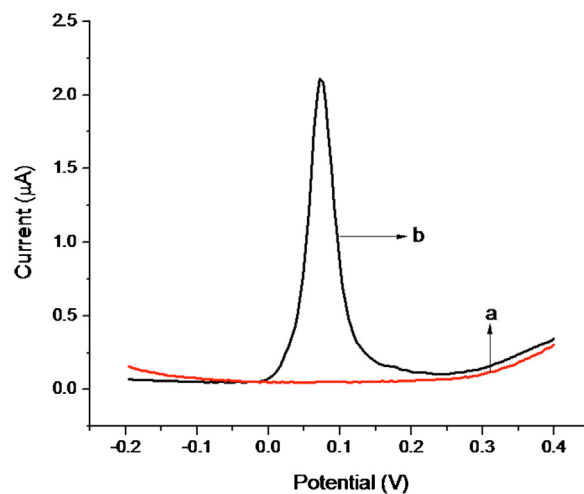
**Fig. 3.** Cyclic voltammograms recorded in HCl (1 M). Preconcentrated using phosphate buffer at pH 8 (a) in absence and (b) in presence of  $\text{Hg}^{2+}$  (10  $\mu\text{M}$ ) at MBT modified exfoliated graphite. Preconcentration time: 120 s.

with enhanced sensitivity as well as selectivity toward mercury metal ion under optimized conditions.

### 3.2. Electrochemical behavior of $\text{Hg}^{2+}$ at modified interface

The electrochemical response of MBT modified interface in presence of mercury was first examined using cyclic voltammetry in order to understand the primary information about the potential affinity of the modifier molecule toward mercuric ions in solution. The typical cyclic voltammetric response in presence and in absence of mercury at MBT modified interface in the potential window from 0.3 to  $-0.9\text{ V}$  is shown in Fig. 3. The modified interface did not show any peaks in the absence of mercury in the potential window used for present analysis. Hence, it can be applied for the quantification of analytes (metal ions) which are active in this potential region. In presence of metal ions, a voltammetric peak was observed during cathodic sweep at  $-0.38\text{ V}$  which can be ascribed to the two electron reduction process of  $\text{Hg}^{2+}$  to  $\text{Hg}^0$ , whereas in the reversal anodic sweep an intense peak at  $-0.18\text{ V}$  was observed which corresponds to the reoxidation (stripping) of  $\text{Hg}^0$  to  $\text{Hg}^{2+}$ . Such voltammetric response was not observed at unmodified interface (data not shown). These studies reveal that the observed response for mercury ion at modified interface is due to the presence of MBT modifier molecule and can be due to the effective interaction or complexing ability of the modifier molecule with mercury ion. At the proposed electrochemical interface, the modifier molecules contains thiol group, sulphur and nitrogen atoms as potential donor atoms and are known to show potential affinity toward the mercury ion due to soft–soft interaction according to HSAB concept [24]. Since the working pH is 8, the covalently modified MBT interface is in deprotonated form that is the exocyclic thiol group dissociated into thiolate ion and the lone pair of electrons present on sulphur and nitrogen atoms are set free [15]. Hence, the interaction of MBT molecule with mercury is expected to be through the thiolate ion and the lone pair of electrons present on sulphur atom than on the nitrogen because sulphur is more thiophilic and softer than the nitrogen. Therefore, covalently modified MBT interface can be used as a sensitive and selective sensing platform for the quantification of mercury at trace level concentration.

The oxidation peak (stripping) is sharper and more significant than the corresponding reduction peak, hence the oxidation peaks were systematically studied using differential pulse anodic



**Fig. 4.** Differential anodic stripping voltammograms recorded in HCl (1 M). Preconcentrated using phosphate buffer at pH 8 (a) in absence and (b) in presence of  $\text{Hg}^{2+}$  (14 pM) at modified exfoliated graphite. Preconcentration time: 120 s, reduction potential:  $-0.4\text{ V}$  and reduction time: 20 s.

stripping voltammetry (DPASV) in order to achieve the desired detection limits. The stripping voltammetric determination of mercury proceeds through the following three steps

- (i) Chemical preconcentration of mercury at MBT interface at open circuit potential for a given preselected time in a buffer solution of known pH containing known amount of  $\text{Hg}^{2+}$ .
- (ii) Reduction of chemically preconcentrated mercury at the interface at constant reduction potential for a preselected time and finally
- (iii) Stripping (oxidation) of reduced mercury into the bulk of the electrolytic solution.

A typical DPASV of  $\text{Hg}^{2+}$  (14 pM) is shown in Fig. 4. The MBT interface did not show any voltammetric signature in the absence of mercury ions where as in presence of mercury ions it showed a well-defined sharp oxidation peak at  $0.07\text{ V}$ . This indicates that the modified interface showed a potential affinity toward mercury and hence this kind of interface can be used as a suitable platform for the electrochemical measurement of mercury at very low concentration levels.

### 3.3. Optimization of the factors influencing the analytical measurements

In order to achieve the maximum efficiency for the developed interface toward the electrochemical measurement of mercuric ions in aqueous solution, the parameters like pH of buffer, nature of stripping media, preconcentration time, reduction potential and time, which controls the analytical signal intensity have been optimized.

The choice of buffer solution plays a crucial role in the preconcentration of metal ions from the bulk of the electrolytic solution onto the surface of modified electrode at the interface when the preconcentration is through specific chemical interaction between the metal ion and the functional groups of the modifier molecule [25]. Hence various buffer solutions like phosphate, ammonia and borate have been examined for the preconcentration of mercury. Among these, phosphate buffer has showed well-resolved and sharp peaks with enhanced peak currents. Hence, phosphate buffer was used as an optimum medium for the accumulation of mercury ions at MBT modified interface. Since the accumulation of mercury ions mainly depends on the protonation and



deprotonation behavior of the functional groups of the MBT molecule, the effect of pH of the phosphate buffer solution was studied in the pH range 3–9. The peak current increases from 3 to 8 and thereafter it decreased (ESI Fig. 1). The peak potential for the stripping of mercury remains unchanged as the stripping was carried out in the same buffer medium (ESI Fig. 1). This type of analysis in alkaline medium is not possible with existing reports because of the cleavage of the modifier molecule in alkaline medium from the electrode interface [11]. The increase of peak current could be attributed to the increased extent of dissociation of exocyclic thiol group and deprotonation of endocyclic sulphur or nitrogen atoms of the MBT molecule at neutral and basic pH conditions. In alkaline condition, the MBT molecule is in deprotonated form, i.e. the thiol group is in thiolate form and the lone pair of electrons present on the sulphur atom are free, hence thiolate and the lone pair of electrons present on the sulphur atom are involved in the chemical preconcentration of mercury at open circuit potential (Scheme 2).

The magnitude of stripping current depends on the choice of electrolyte used in the electrochemical study. Different supporting electrolytes like  $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_3$ ,  $\text{HCl}$  and  $\text{KCl}$  have been examined. The modified interface showed significantly higher stripping currents when  $\text{HCl}$  was used as a supporting electrolyte and it is attributed to the complexing ability of the  $\text{Cl}^-$  ions with  $\text{Hg}^{2+}$  hence, it has been used as an ideal supporting electrolyte throughout this study. Then the effect of supporting electrolyte concentration was examined using hydrochloric acid at different concentration ranges starting from 0.1 to 2.0 M. The stripping current increases from 0.1 to 1.0 M and beyond 1 M it did not affect the stripping response, hence 1 M  $\text{HCl}$  was used as a supporting electrolyte in all further studies. Then the effect of  $\text{H}^+$  and  $\text{Cl}^-$  ions were examined separately. The effect of  $\text{H}^+$  ion was studied by using the stripping medium containing 1 M  $\text{KCl}$  +  $x$  M  $\text{HNO}_3$ , where the  $x$  ranging from 0.1 to 2. Similarly the effect  $\text{Cl}^-$  ions were also studied by using the stripping medium containing 1 M  $\text{HNO}_3$  +  $x$  M  $\text{KCl}$ , where  $x$  ranges from 0.1 to 2. It has been found that the variation of  $\text{H}^+$  ion concentration has no effect on the stripping performance where as with increase of chloride ion concentration, the peak potentials have shifted toward more negative values up to 1 M there after it has no change which might be due to the complexing ability of  $\text{Cl}^-$  ions with oxidized metal ionic species.

Then we have studied the time required for the preconcentration of mercury ion (preconcentration time) from 40 to 240 s (ESI Fig. 2). The peak current for the stripping of mercury increases from 40 to 120 s and afterwards it decreased. The increase of peak current is due to the fact that longer the preconcentration time more and more analytes get accumulated at the electrode/solution interface. After 120 s, the peak decrease of peak current may be attributed to either surface saturation or the attainment of equilibrium between the metal ions at the interface and the ions in the bulk of the solution. Hence a preconcentration time of 120 s has been used as an optimized time in all future studies.

The effect of reduction potential on the anodic peak current of mercury was varied in the range  $-0.1$  to  $-0.6$  V (ESI Fig. 3), the peak current increased up to  $-0.4$  V thereafter it decreased. The increase of peak current is due to increase in extent of reduction of more and more mercury ions and the decrease in peak current may be due to the interference from extraneous process. Therefore a reduction potential of  $-0.4$  V was used as an optimum potential in all subsequent studies.

Similarly the reduction time which is the time required to reduce the metal ions into its atomic state. The effect of reduction time on the stripping current for mercury is studied in the range 10–40 s (ESI Fig. 4). The peak current increased from 10 to 20 s and thereafter it decreased. Therefore the reduction time of 20 s was fixed as an optimum time in all subsequent studies.

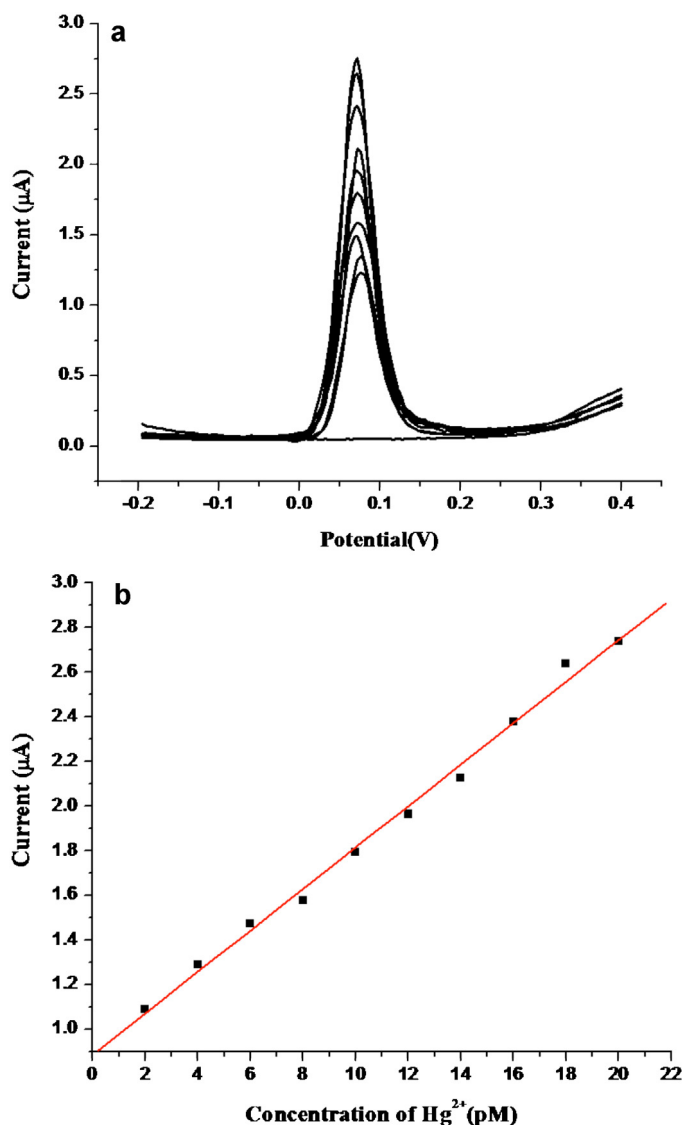


Fig. 5. (a) Overlaid differential pulse anodic stripping voltammograms with increasing additions of mercury ion under optimized conditions. (b) Calibration plot.

### 3.4. Calibration and detection limit

By using the optimized conditions described above, calibration plot was constructed by measuring the peak currents after successive additions of mercury ions (2 pM) into an electrochemical cell (10 mL) containing phosphate buffer (pH 8). The peak current for the stripping of mercury increased linearly with increase of mercury up to 20 pM (Fig. 5) and the detection limit ( $3\sigma$ ) was found to be 1 pM. The detection limit of the proposed method is well below the prescribed maximum threshold limit value of 2 ppb for drinking water recommended by world health organization [3]. The proposed electrode has showed an excellent stability, low detection limit, long term storage and reproducible analytical measurements. The analytical performance of the proposed sensor electrode has been compared with other existing electrodes (Table 1) used in the determination of mercury. It is evident from the table that the proposed binderless bulk modified electrode has a very low detection limit with low preconcentration time when compared to other electrodes. Hence the proposed sensor electrode could be used as an alternative tool to the existing sensors in industrial atmospheres for routine monitoring of toxic mercury present at ultra trace level.

**Table 1**  
Comparison of selected modified electrodes with the proposed interface.

Electrode	Method	Preconcentration time (s)	Limit of detection (LOD)	Reference
GC-MBT polymer	DPASV	300	40 nM	[13]
GC-MWCNTs	DPASV	300	20 nM	[26]
GC-MWCNTS	DHP SWASV	300	32 nM	[27]
GC-Au nanoparticles	DPASV	120	0.2 nM	[27]
CPE-S-nanoporous	DPASV	450	8 nM	[28]
GC-MWCNTs-Chi	LSASV	120	2.4 nM	[29]
EG-MBT	DPASV	120	1 pM	Present work

GC: glassy carbon; MWCNTs: multiwalled carbon nanotubes; DHP: dihexadecyl hydrogen phosphate; Au: gold; S: silica gel; Chi: chitosan; EG: exfoliated graphite; MBT: mercaptobenzothiozole.

**Table 2**  
Interference study.

Interfering ions	Tolerance limits ( $\mu\text{M}$ )
$\text{Cd}^{2+}$ , $\text{Ag}^+$ , $\text{Fe}^{2+}$ , $\text{Ni}^{2+}$ , $\text{Co}^{2+}$ , $\text{Cu}^{2+}$ , $\text{Zn}^{2+}$ , $\text{Pb}^{2+}$	250
$\text{Na}^+$ , $\text{Cs}^+$	520
$\text{Co}^{2+}$ , $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ , $\text{Ba}^{2+}$ , $\text{Be}^{2+}$ , $\text{Cr}^{3+}$ , $\text{Fe}^{3+}$ , $\text{As}^{3+}$	450
$\text{C}_2\text{O}_4^{2-}$ , $\text{CO}_3^{2-}$ , $\text{F}^-$ , $\text{I}^-$ , $\text{SO}_4^{2-}$ , $\text{SO}_3^{2-}$ , $\text{NO}_2^-$ , $\text{NO}_3^-$	380

### 3.5. Interference study

In order to check the suitability of the proposed sensor for real sample analysis the selectivity of the modifier molecule toward mercury in presence of other commonly encountered foreign ions were studied. The interference study has been carried out by the addition of different concentrations of cations or anions in the form of their respective salts into the electrochemical cell containing mercury (10 pM) during electrochemical investigation. The different interfering ions and their maximum concentration levels (tolerance limit) in the present study have been listed in Table 2. It is clear that the developed sensor has showed least interference from most of the common ions due to its specific and selective interaction of the modifier functionalities of the MBT modifier with mercury. It is very clear that the developed sensor can be successfully used in the determination of mercury from a variety of environmental matrices.

### 3.6. Surface renewability and storage stability of the modified interface

The surface renewability and reproducibility of the results toward a specified target analyte is very significant in order to apply it to real samples. The proposed sensor electrode is based on the covalent bulk modified material and it has been fabricated as a robust pellet electrode. Its surface can be easily renewed by simple mechanical polishing using 600- and 1500-grit emery sheets followed by 4/0, 5/0 and 6/0 emery polishing papers to obtain a smooth surface and the roughness on the electrode surface was generated by simply scratching the electrode surface against 400 grit emery sheets in unidirection. The electrochemical performance of the newly renewed surface with respect to mercury is essential

**Table 3**  
Determination of mercury from real sample matrices.

Sample	Originally present mercury (pM)	Added mercury (pM)	Total mercury (pM)	Recovery (%)
1. Tap water	ND	3	3.12	100.32
2. Industrial water	4.1	3	7	98.59
3. Lake water	15	3	17.9	99.44
4. Chrome plating industrial effluent <sup>a</sup>	12.8	3	15.7	99.36
5. Chrome plating industrial effluent <sup>b</sup>	11.2	3	14.3	100.70
6. Textile industrial effluent	16.4	3	19.2	98.96

ND: not detected.

<sup>a,b</sup>The samples were obtained on different days.

for precise analytical measurements. Hence, the surface renewability was checked by using mercury (10 pM) with 10 freshly renewed surfaces of the same electrode. The relative standard deviation in the peak current response has been found to be  $\pm 3.1\%$ . This effect revealed that the surface renewability of the electrode did not affect the electrochemical response which in turn confirms that the covalent bulk modification is uniform throughout the matrix, hence a single electrode of this type can be used and reused for several measurements with similar analytical performance. For long term analytical applications, the developed interface should be capable of producing identical analytical results. Therefore, the analytical performance of the electrode was checked over a period of 6 months using the same electrode under identical experimental conditions. In all these experiments, the magnitude of stripping current varied within a deviation of  $\pm 6\%$ . These experimental observations have revealed that the proposed electrode can be used for long time and continuous analyses with precise analytical measurements. This type of sensing electrode is required in industrial atmospheres where the measurement of target analyte is generally measured continuously at trace level concentration.

### 3.7. Application study

The analytical utility of the proposed sensor has been validated by applying it to measure mercury levels present in various real sample matrices like tap water, industrial effluents, lake water, waste water samples from chrome plating, electroplating and textile industries. The recovery study was carried out for all these samples by spiking with known concentrations of mercury and its recovery along with the originally present mercury in these samples (Table 3).

## 4. Conclusions

A simple and effective protocol for the covalent modification of exfoliated graphite in bulk quantities has been described for the development of sensitive and selective interface toward the electrochemical determination of mercury. The modified interface has showed very low detection limit of 1 pM with least interference from commonly present cations and anions. The analytical utility of the sensor has been validated by measuring the mercury levels

from various natural samples. The developed interface showed good storage stability over a period of several months and it can be used as an alternative tool to the existing sensing devices for mercury quantification.

## Acknowledgement

The authors acknowledge the financial support and award of Senior Research Fellowship (SRF) to Raghu GK by the Department of Science & Technology, New Delhi, India.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.snb.2013.06.028>.

## References

- [1] D.W.M. Arrigan, Tutorial review. Voltammetric determination of trace metals and organics after accumulation at modified electrodes, *Analyst* 119 (1994) 1953–1966.
- [2] S. Chilov, Determination of small amounts of mercury, *Talanta* 22 (1975) 205–232.
- [3] Guidelines for Drinking Water Quality, 4th ed., World Health Organization, 2011.
- [4] G.K. Raghu, M. Pandurangappa, Covalently modified multiwalled carbon nanotubes as a new voltammetric interface for the determination of mercury at picomolar level, *Electrochem. Commun.* 25 (2012) 83–86.
- [5] M.R. Khan, S.B. Khoo, Epoxy graphite tube bulk modified with 2-mercaptobenzothiazole as a robust electrode for the preconcentration and stripping analysis of Hg(II), *Anal. Chem.* 68 (1996) 3290–3294.
- [6] J. Gong, T. Zhou, D. Song, L. Zhang, X. Hu, Stripping voltammetric detection of mercury(II) based on a bimetallic Au–Pt inorganic–organic hybrid nanocomposite modified glassy carbon electrode, *Anal. Chem.* 82 (2010) 567–573.
- [7] N.Y. Stojko, K.Z. Brainina, C. Falleer, G. Henze, Stripping voltammetric determination of mercury at modified solid electrodes. I. Development of the modified, electrodes, *Anal. Chim. Acta* 371 (1998) 145–153.
- [8] I. Vancara, K. Vytras, K. Kalcher, A. Walcarious, J. Wang, Carbon paste electrodes in facts, numbers, and notes: a review on the occasion of the 50-years jubilee of carbon paste in electrochemistry and electroanalysis, *Electroanalysis* 21 (2009) 7–28.
- [9] P. Ramesh, S. Sampath, A binderless, bulk modified, renewable surface amperometric sensor for NADH and ethanol, *Anal. Chem.* 72 (2000) 3369–3373.
- [10] Y. Li, X. Liu, X. Zeng, Y. Liu, X. Liu, W. Wei, S. Luo, Simultaneous determination of ultra trace lead and cadmium at hydroxyapatite modified carbon ionic liquid electrode by square wave stripping voltammetry, *Sens. Actuat. B: Chem.* 139 (2009) 604–610.
- [11] X.C. Fu, X. Chen, Z. Guo, L.T. Kong, J. Wang, J.H. Liu, X.J. Huang, Three-dimensional gold micro/nanopore arrays containing 2-mercaptobenzothiazole molecular adaptors allow sensitive and selective stripping voltammetric determination of trace mercury(II), *Electrochim. Acta* 56 (2010) 463–469.
- [12] M.F.B. Sousa, R. Bertazzoli, Preconcentration and voltammetric determination of mercury(II) at a chemically modified glassy carbon electrode, *Anal. Chem.* 68 (1996) 1258–1261.
- [13] M.F.B. Sousa, E.J. Dallan, S.b. Yamaki, R. Bertazzoli, Preparation of polymer coated electrodes containing metal ions complexing sites by electropolymerization of 2-mercaptobenimidazole and 2-mercaptobenzothiazole on glassy carbon, *Electroanalysis* 9 (1997) 614–618.
- [14] W. Yantasee, Y. Lin, T.S. Zemamian, G.E. Fryxell, Voltammetric detection of lead(II) and mercury(II) using a carbon paste electrode modified with thiol self-assembled monolayer on mesoporous silica (SAMMS), *Analyst* 128 (2003) 467–472.
- [15] R. Woods, G.A. Hope, K. Watling, A SERS spectroelectrochemical investigation of the interaction of 2-mercaptobenzothiazole with copper, silver and gold surfaces, *J. Appl. Electrochem.* 30 (2000) 1209–1222.
- [16] P. Ramesh, S. Sampath, Electrochemical and spectroscopic characterization of quinone functionalized exfoliated graphite, *Analyst* 126 (2001) 1872–1877.
- [17] G.K. Ramesha, H.B. Vijay Kumara, S. Muralidhara, S. Sampath, Graphene and graphene oxide as effective adsorbents towards anionic and cationic dyes, *J. Colloid Interface Sci.* 361 (2011) 270–277.
- [18] M.P. Somashekarappa, S. Sampath, Sol–gel derived silicate–phthalocyanine functionalized exfoliated graphite electrodes, *Anal. Chim. Acta* 503 (2004) 195–201.
- [19] G.K. Raghu, S. Sampath, M. Pandurangappa, Chemically functionalized glassy carbon spheres: a new covalent bulk modified composite electrode for the simultaneous determination of lead and cadmium, *J. Solid State Electrochem.* 16 (2012) 1953–1963.
- [20] H. Yang, R.L. McCreery, Effects of surface monolayers on the electron-transfer kinetics and adsorption of methyl viologen and phenothiazine derivatives on glassy carbon electrodes, *Anal. Chem.* 71 (1999) 4081–4087.
- [21] M. Pandurangappa, N.S. Lawrence, R.G. Compton, Homogeneous chemical derivatization of carbon particles: a novel method for functionalizing carbon surfaces, *Analyst* 127 (2002) 1568–1571.
- [22] Y. Feng, S. Chen, H. Zhang, P. Li, L. Wang, W. Guo, Characterization of iron surface modified with 2-mercaptobenzothiazole self assembled monolayers, *Appl. Surf. Sci.* 253 (2006) 2812–2819.
- [23] Y.Y. Jun, K.S. Beng, electrochemical study of monolayers of heterocyclic thiols self assembled on polycrystalline gold electrode: the effect of solution pH on redox kinetics, *Electrochem. Commun.* 6 (2004) 87–90.
- [24] E. Yavuz, G. barin, B.F. Senkal, Thiol containing sulfonamide based polymeric sorbent for mercury extraction, *J. Appl. Polym. Sci.* 114 (2009) 1879–1883.
- [25] G.K. Raghu, M. Pandurangappa, A binderless covalently bulk modified electrochemical sensor: application to simultaneous determination of lead and cadmium at trace level, *Anal. Chim. Acta* 728 (2012) 9–17.
- [26] H.C. Yi, Anodic stripping voltammetric determination of mercury using multi-walled carbon nanotubes film coated glassy carbon electrode, *Anal. Bioanal. Chem.* 377 (2003) 770–774.
- [27] O. Abollino, A. Giacomino, M. Malandrino, G. Piscionieri, E. Mentasti, Determination of mercury by anodic stripping voltammetry with a gold nanoparticle-modified glassy carbon electrode, *Electroanalysis* 20 (2008) 75–83.
- [28] M. Javanbakht, H. Khoshafar, M.R. Ganjali, A. Badiei, P. Norouzi, A. Hasheminasab, Determination of nanomolar mercury(II) concentration by anodic-stripping voltammetry at a carbon paste electrode modified with functionalized nanoporous silica gel, *Curr. Anal. Chem.* 5 (2009) 35–41.
- [29] B.C. Janegitz, L. Carlo, S.F. Filho, L.H.M. Junior, R.P. Filho, O.F. Filho, Development of a carbon nanotubes paste electrode modified with crosslinked chitosan for cadmium(II) and mercury(II) determination, *J. Electroanal. Chem.* 660 (2011) 209–216.

## Biographies

**Raghu G. Kempegowda** received his MSc in Chemistry from Bangalore University, India in 2008. Since then he is pursuing doctoral work at Department of Chemistry, Bangalore University under the supervision of Dr. M. Pandurangappa. His area of research work is on the development of chemically modified electrochemical interfaces and their applications toward toxic metal ion sensing at trace level.

**Pandurangappa Malingappa** is an Associate Professor at the Department of Chemistry, Bangalore University. He was awarded his PhD degree from Indian Institute of Technology, Madras, Chennai (1996) in Department of Chemistry. After that he has worked as a Postdoctoral Fellow in Prof. Richard Compton Group at the University of Oxford, UK under Commonwealth Fellowship during 2001–2002 as well as 2008 under World Developing Scholarship awarded by the Royal Society of Chemistry, London. His research areas include electroanalysis and electrochemistry of chemically modified electrodes, spectroscopic characterization and their application in environmental sensing. Quantification of oxides of nitrogen/nitrite/nitrate, sulphur dioxide, hydrogen sulfide and heavy metal ions at trace level with its application to a variety of environmental matrices by spectrophotometry is another area in which he is working at present.