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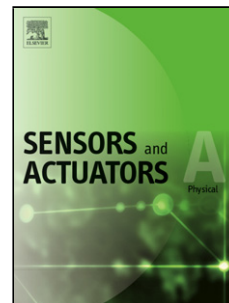
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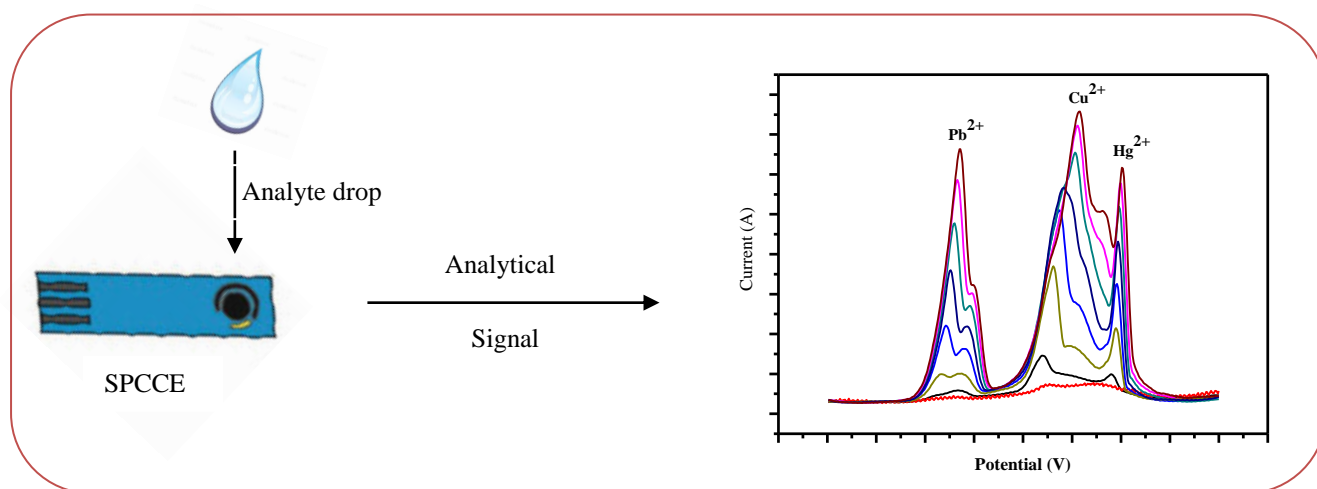
Calixarene bulk modified screen-printed electrodes (SPCCEs) as a one-shot disposable sensor for the simultaneous detection of lead(II), copper(II) and mercury(II) ions: Application to Environmental Samples

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Graphical Abstract



Highlights

- The proposed sensor is cost effective, portable and one-shot disposable.
- Easy fabrication
- Good reproducibility
- Very small sample volume (μL level) required
- Low detection limits at nanomolar / $\mu\text{g L}^{-1}$ level
- Applied to a range of different sample matrices

Abstract

Calixarene bulk modified screen-printed electrodes (SPCCEs) have been designed, fabricated and utilized as one-shot disposable electrochemical sensors towards the simultaneous measurement of toxic metal ions lead(II), copper(II) and mercury(II) within environmental samples. These SPCCEs have been fabricated upon polyester strips, which have been modified over a range of different calixarene compositions. These bespoke sensors have been utilized as disposable electrochemical sensors and quantified in model aqueous solution of 0.1 M HCl giving rise to the simultaneous sensing of lead(II), copper(II) and mercury(II) using cyclic voltammetry and differential pulse anodic stripping voltammetry techniques. These SPCCEs have additionally been applied for the sensing of lead(II), copper(II) and mercury(II) within industrial and environmental samples such as industrial effluents and wastewater samples, furthermore the presented method has been subjected to a comprehensive interference study and further validated with an atomic absorption spectrometric method.

Keywords: Calixarene modified screen-printed electrodes; toxic metal ions; electrochemical

sensor, stripping voltammetry, environmental samples.

1. Introduction

On site detection and quantification of heavy metal ions (at trace level) is a very challenging task and can require sophisticated instrumentation/devices [1]. On spot quantification of toxic metal ions within environmental samples is an essential and mandatory protocol within the industry and water treatment processes. Use of sophisticated instruments or devices for on-the-spot analysis may not be viable in the field due to various constraints. Hence over recent years, one-shot analysis using disposable sensors (*i.e. screen-printed electrodes (SPEs)*) have gained a significant focus within the field of electroanalysis [2, 3]. Metal ions such as lead, mercury and copper are highly toxic to many organs of both humans and animals causing disorders to the nerves, immunity, kidneys, reproductive and gastrointestinal systems [4]. Even trace level concentrations of these metal ions can cause a negative impact, not only on environment, but also to the ecosystem [5]. Over the recent decade, these ions have been noticeably diffusing into the environment from geochemical mechanisms as well as from industrial activities such as electroplating, batteries and paint industries [6]. The prescribed threshold limit values (TLV) of lead, copper and mercury ions in drinking water are 10, 3 and 1 $\mu\text{g/L}$ respectively according to the World Health Organization [7]. Traditional methods for the measurement of these metal ions include spectrophotometry and electrochemical techniques. Spectrophotometric methods include atomic absorption spectrometry (AAS), inductively coupled plasma-atomic emission spectrometry (ICP-AES), neutron activation analysis (NAA), X-ray fluorescence spectrometry (XRF), and more [8-11]. However, these techniques are expensive and require prolonged sample preparation procedures. Electrochemical techniques such as cyclic voltammetry (CV) and anodic stripping voltammetry (ASV) are the most suitable methods

for trace metal analysis due to their high sensitivity, good selectivity, low analysis time, portable instrumentation, easy operation and low maintenance cost [12]. Such features have made them more beneficial over spectroscopic methods for field analysis.

Initially, mercury film based electrodes were extensively employed for stripping analysis [13]. However, the toxicity of mercury has made this methodology undesirable for sensing applications. A number of alternatives have been reported, followed by various carbon based electrode materials with a great emphasis to increase the sensitivity and selectivity toward particular analytes by modifying the surface with selective functionalities having potential affinity towards the target metal ions [14,15]. Various forms of carbon materials have been reported as a substrate material for metal ion detection, such as glassy carbon, graphene, carbon nanotubes, carbon paste, carbon nanospheres, carbon paper, carbon fibers and screen-printed electrodes (SPEs) [16-18]. In recent years, screen-printing has gained much attention in the field of electroanalysis for the fabrication of sensors and biosensors [19-21]. Several reports have appeared recently on the application of various forms of sensing tools for trace metal quantification such as functional nucleic acids, carbon nanotubes and DNA-based biosensors [22-24]. SPEs usually include a three electrode configuration (working, counter and reference electrodes) printed on the same strip and also offer an attractive features such as broad potential range, easy fabrication, low cost and low sample volume (μL level) [25]. Moreover, SPEs have the advantage of being one-shot disposable sensor strips and furthermore require no polishing or smoothening of the electrode surface, as is often the case for more conventional solid electrodes [12]. The sensitivity and the selectivity can be enhanced *via* chemical modification of these carbon based SPEs with suitable functional motifs.

Calixarenes have received a great deal of interest over recent years due to their unique cavity-size, shape and its structure. The functionalized calixarenes have become useful metal

ion receptors due to their selective functional moieties that have been anchored on lower and upper rims of these molecules. The applications of functionalized calixarenes in the field of electrochemistry have been appraised and a number of successful applications have been reported in fabrication of ion-selective electrodes [26-28]. Due to this, in electroanalysis a number of calixarene molecules have been reported for the quantification of metal ions such as lead and copper [30-33]. Until now, very little focus has been made upon calixarenes as substrate materials within the fabrication of SPEs for the measurement of target analytes [32, 34, 35]. Hart *et al.* have used the drop-coating method to modify the carbon SPEs with calixarene molecules, which showed good analytical performance for the quantitative study of lead(II) and cadmium(II) individually within real-water samples [33,34]. Whereas, Sheng *et al.* reported the use of calixarene moiety for the simultaneous detection of lead and cadmium ions at trace level *via* carbon paste electrodes (CPEs). This method is complex and tedious, where calixarene, graphite powder and paraffin oil have been subjected to hand mixing. Finally, a portion of the modified carbon paste was packed into the tip of a piston glass tube electrode (3 mm in dia.), then utilized for the electrochemical study [35].

Herein we report a simple protocol for the simultaneous measurement of lead(II), copper(II) and mercury(II) ions using SPCCEs as an electrochemical sensor. The proposed sensor has been successfully applied to real environmental sample matrices using cyclic voltammetry (CV) and differential pulse anodic stripping voltammetry (DPASV) techniques. The present protocol has been aimed at utilizing calixarene moiety upon the polyester strips as a one-shot disposable electrochemical sensor as in the form of screen printed calixarene carbon electrode (SPCCE) in the simultaneous measurement of lead(II), copper(II) and mercury(II) that require no polishing and smoothening.

2. Experimental

2.1 Chemicals and reagents

All chemicals used were of analytical grade and were obtained from Sigma-Aldrich. 25, 26, 27, 28-tetrahydroxy-calixarene was synthesized according to the reported literature [29]. Acetic acid, sodium acetate and sodium hydroxide were purchased from SD Fine Chemicals, Mumbai. All buffer solutions in the pH range 2 – 9 were prepared using ultra-pure double distilled water from millipore water purifier with a resistivity of not less than $18.2 \text{ M}\Omega \text{ cm}^{-1}$ at 25 °C. Stock solutions of lead, copper and mercuric ionic solutions were prepared by dissolving the corresponding salts with appropriate mass in deionised water. Working standards were prepared by diluting the stock solutions on the day of use. The screen-printed electrodes (SPEs) utilized consist of a graphite working electrode, a graphite counter electrode and an Ag/AgCl reference electrode. The SPEs, which have a 3 mm diameter working electrode, were fabricated in-house (at Manchester Metropolitan University, UK) with appropriate stencil designs using a microDEK 1760RS screen-printing machine (DEK, Weymouth, UK). Such SPE design has been reported previously [36-38]. For the case of each fabricated electrode, first a graphite ink formulation (Product Code: C2000802P2; Gwent Electronic Materials Ltd, UK), which is utilized for the efficient connection of all three electrodes and as the electrode material for both the working and counter electrodes, was screen-printed onto a polyester (Autostat, 250 μm thickness) flexible film. After curing the screen-printed graphite layer in a fan oven at 60 °C for 30 minutes, a Ag/AgCl reference electrode was included by screen-printing Ag/AgCl paste (Product Code: C2030812P3; Gwent Electronic Materials Ltd, UK) onto the polyester substrates, which was

subsequently cured once more in a fan oven at 60 °C for 30 minutes. Finally, a dielectric paste (Product Code: D2070423D5; Gwent Electronic Materials Ltd, UK) was screen-printed onto the polyester substrate to cover the connections and define the active electrode areas, including that of the working electrode. After curing at 60 °C for 30 minutes, the SPEs were ready to be used. The calixarene moieties were incorporated into the bulk of the SPEs on the basis of the weight percent of M_P to M_I , where M_P the mass of particulate and M_I the mass of the ink formulation used in the printing process, *i.e.* $\% = (M_P/M_I) \times 100$. The weight percent of M_P to M_I varied from 0-10 % which resulted four separate inks that could subsequently be individually screen-printed on top of the working SPE electrode creating calixarene bulk modified screen-printed electrodes (SPCCEs).

2.2 Apparatus

All electrochemical measurements were carried out using electrochemical work station [CH Instruments, Texas, USA, model: CHI 619B] at room temperature (25 ± 2 °C) in an electrochemical cell of volume 10 mL with a standard three electrode configuration. The SPCCEs acted as the working electrode. All pH measurements were carried out using pH meter (Control Dynamics, Mumbai, India model: APX 175). Infrared measurements were recorded using FTIR Spectrometer (Bruker, model: 8400S) in the range 400-4000 cm^{-1} with a resolution of 4 cm^{-1} . Scanning electron microscopy images were recorded using JEOL (JSM-840A) scanning electron microscope. ^1H NMR spectra were recorded using 400-MHz NMR Spectrometer (Bruker) using dimethylsulfoxide (DMSO- d_6) as solvent and tetramethylsilane (TMS) as an internal standard. The mass spectral data was recorded using GCMS (Shimadzu, Japan, model: QP 2010S). Atomic absorption spectrometric (AAS) measurements were carried out using Shimadzu, Japan, model: AA-6200 instrument.

2.3 Sample preparation

The industrial effluents and sewage water samples were collected and stored in polyethylene containers. Initially the pH values are measured and they were found to be in the range 6.0 to 9.0. All these liquid samples were filtered to remove any suspended colloidal matter and they were treated with activated charcoal to remove the color and turbidity. Then they are filtered using Whatman filter paper and diluted to known volume. Known aliquots of sample solutions were taken and the required pH was adjusted by using suitable buffer solutions. However, suitable dilutions were carried out to bring the analyte concentration well within the working range of DPASV and AAS techniques.

2.4 Analytical procedure

Electrochemical measurements of lead(II), copper(II) and mercury(II) ions were carried out using DPASV in the potential range -1.0 to 0.6 V with an amplitude of 0.01 V and pulse width of 0.05 s. The analyses were performed after the removal of oxygen *via* bubbling N₂ for 15 min. unless otherwise stated. Known amounts of the analytes were taken in an electrochemical cell of 10-mL- volume capacity. The SPCCE was immersed into the cell containing 0.1 M HCl followed by medium exchange phenomena, buffer solution and target metal ions which were stirred for 3 min. to pre-concentrate the metal ions at open circuit. Then, the pre-concentrated metal ions were reduced at a reduction potential of -1.1 V and subsequently stripped off from the electrode surface into the bulk of the electrolytic solution by sweeping the potential in the positive direction after 20 s of equilibration time. The application study for real sample matrices was carried out utilizing a very tiny volume of

analyte solution (15 – 25 μL). The stripping analysis was adapted in the real sample analysis under optimized conditions.

3. Results and discussion

3.1. Characterization

The synthesized calixarene molecule was characterized *via* spectroscopic tools such as Fourier Transform Infrared Spectroscopy (FTIR), Gas Chromatography Mass Spectrometry (GC-MS) and ^1H Nuclear Magnetic Resonance (^1H NMR) Spectrometry to ascertain the functional groups as well as its molecular mass. Scanning electron microscopy was used to record the surface morphology of SPCCEs before and after metal ion accumulation.

3.2. FTIR Study

The infrared spectrum of calixarene was recorded in the wavelength range 4000-400 cm^{-1} to confirm the presence of functional groups in the synthesized molecule. A weak stretching frequency was observed at 3166 cm^{-1} due to the vibration of OH groups of cyclic tetramer. The bands at 1449, 1391 cm^{-1} might be due to the -COH bending vibrations (ESI: Fig. 1).

3.3. GC-MS Study

The molecular mass of calixarene was determined by GC-MS study. A molecular ion peak at m/z 648 was observed corresponding to the molecular weight of the synthesized compound. The peak observed at m/z 592 is due to the loss of one *tert*-butyl group from one of the upper rim of the compound (ESI Fig. 2).

3.4. ^1H NMR Study

^1H -NMR was recorded with TMS as the internal standard and $\text{DMSO-}d_6$ as the

solvent. A broad singlet observed at 8.69 ppm corresponds to the 4 phenolic –OH groups of the calixarene moiety. Methylene protons resonated at 3.80 ppm as a singlet whereas the singlet observed at 1.09 ppm corresponds to the methyl group of the tert-butyl moieties. All other aromatic protons were resonated as a singlet at 6.95 ppm (ESI Fig. 3).

3.5. Scanning Electron Microscopy Study

The surface morphology of the calixarene bulk modified screen-printed electrodes (SPCCEs) has been examined by scanning electron microscopy study. In this study, SEM images were recorded by dipping the screen-printed electrodes in an electrolyte solution containing metal ion mixture after their accumulation for a period of five minutes at open circuit potential. Similarly, SEM images were recorded for bare SPEs before the accumulation of metal ions also. ESI Fig. 4 depicts the SEM images of the SPEs before metal ion accumulation (A) and after metal ion pre-concentration (B). The metal ion accumulated screen-printed electrode has shown a distinguished surface morphological difference when compared to the bare counterpart. The metal ion accumulated surface has particles that are agglomerated non-uniformly on the surface of the screen-printed electrode. Hence, these studies reveal that during the pre-concentration step, these metal ions have become accumulated on the surface of SPCCEs.

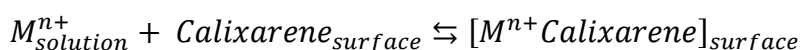
3.6. Electrochemical behavior of the SPCCEs

The electrochemical behavior of modified screen-printed electrodes was initially examined by using cyclic voltammetry. The cyclic voltammetric investigation of SPCCEs was carried out in presence of supporting electrolyte and buffer solution along with and without metal ions (Fig. 1(a-c)). CV depicts well-defined anodic peak currents for lead(II), copper(II) and mercury(II) ions pertaining to their respective potentials. The observed anodic peak currents were significant in presence of lead(II), copper(II) and mercury(II) metal ions (Fig. 1(c)) in comparison with their absence (Fig. 1(b)). The enhanced peak currents observed

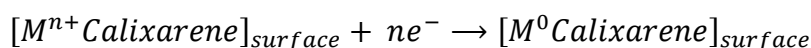
in the present investigation is due to the modified screen-printed electrode [33]. However, bare SPEs did not show its signature in presence of metal ions (Fig. 1(a)). Further, we have performed the experiment using bare and modified SPEs in presence of buffer solution (ESI Fig. 5) in order to understand the analytical behaviour. The observed results reveal that, larger background current on modified SPEs was observed in comparison with bare SPEs. This behavior might be due to the presence of higher concentration of protons at the electrode surface being bound by the oxygen functional groups of the polymeric calixarene. The increased current may be due to the commencement of proton reduction at the negative potential at which the scan is initiated [32]. However, a broad cathodic signal at -0.17 V could be due to the reduction of extraneous ions or the surface unevenness of the SPEs. The SPCCEs have shown an enhanced signal response upon its interface which might be attributed to the fact that the chelation/complexation phenomenon of the calixarene moiety in presence of metal ions [31]. The signal enhancement in the present study might be due to complexation process between the impregnated calixarene molecules in SPEs and metal ions in the electrolyte solution [33]. The binding and complexation behaviour between modifier molecule and the target metal ions are schematically shown in Scheme 1. The mechanism of pre-concentration and its subsequent stripping steps are given below [31].

Scheme 1

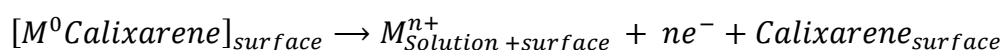
1. Accumulation by complex formation



2. Reduction of complexed metal ion (at -1.1 V)



3. Stripping (oxidation)



Cyclic voltammetric curves (in absence and in presence of metal ions) were recorded over a potential range of -1.2 to 0.6 V at a scan rate 50 mV/s. These results demonstrate that

the SPCCEs indeed accumulate metal ions from the solution and greatly enhance the signal response [31]. To achieve the maximum efficiency using SPCCEs as disposable electrochemical sensors in the quantification of lead(II), copper(II) and mercury(II) ions under aqueous medium, all the reaction variables like calixarene loading, medium pH, pre-concentration time and reduction potentials were optimized.

Insert Figure 1

3.7. Optimization Study

The analyte has been pre-concentrated on chemically modified electrode surface (SPCCE) at open circuit from the electrolyte solution for a period of 3 minutes and its measurements were carried out by a medium exchange phenomenon. This process minimizes the background current and reduces the noise level during signal peak current measurement [33]. The pre-concentrated metal ions were reduced at -1.1 V and stripped off from the electrode interface into the bulk of the electrolytic solution by reversing the sweep potential. The optimization study was carried out in presence of 3 μ M concentration of each ion in the solution throughout the experiment.

3.7.1. Effect of calixarene loading on the stripping current

The electrochemical studies were carried out using four different electrode compositions. The modifier/ligand percentage was chosen as 5 – 20%, with the best performance being obtained with that of 15%, so this was further employed in all subsequent experiments along with the presence of supporting electrolyte and target analytes. The modifier molecule percentage could lead to obtain higher metal ion accumulation at open circuit on the electrode interface which intern results in an increased sensitivity within the electro-analysis process and it has shown in Fig. 2.

Insert Figure 2

3.7.2. Effect of pH

The effect of medium pH on the differential pulse anodic stripping voltammetric response was investigated over the range of 2.0 to 9.0 (Fig. 3). Stripping peak current significantly increased as the pH increased from 2.0 to 4.0 and reached its maximum value at pH 4.0 and thereafter decreased slightly from 4.0 to 7.0. The observed results are in agreement with the literature [35]. The decrease in the signal current at higher pH values might be due to the hydrolysis of metal ions. Hence, pH 4.0 was used as an optimum pH condition in all further studies which was maintained by adding buffer solution.

Insert Figure 3

3.7.3. Effect of accumulation potential

The effect of accumulation potential on the anodic stripping peak currents of metal ions was investigated over the potential range of -0.1 to -1.4 V (Fig. 4). The stripping peak currents of lead(II), copper(II) and mercury(II) reached the maximum value at a reduction potential of -1.1 V. The peak current increases for copper(II) and mercury(II) from -0.1 V to -0.4 V, whereas for lead(II) the current decreases up to a potential of -1.0 V. However, the anodic peak currents reached maximum for all the metal ions at -1.1 V. Hence it is attributed to the fact that progressively more ions get reduced at this particular potential, then the decrease of peak currents are due to the reduction of extraneous ions which might be present in the electrolytic solution or from the material. Therefore, the accumulation potential of -1.1 V was applied in all further studies.

Insert Figure 4

3.7.4. Effect of accumulation time

The effect of accumulation time on the stripping peak currents of metal ions was studied from 1 – 10 min. (Fig. 5). The peak current increases upon an increase of the accumulation time up to 3 min. Such response is due to a progressive increase of ions

accumulating on the electrode interface. The peak current gradually decreases in the case of mercury and remains almost constant with other metal ions when the accumulation time exceeds 3 min. This could be attributed to a surface saturation process; therefore an accumulation time of 3 min. was chosen for all further investigations.

Insert Figure 5

The stripping voltammograms for different concentrations of metal ions at the SPCCEs are shown in Fig. 6(a). The analytical performance of the proposed method for metal ion determination was examined under optimized conditions. The calibration plots were constructed using varied concentrations in the range 100 – 2400 $\mu\text{g/L}$ for lead, copper and mercury with correlation coefficients of 0.999, 0.985 and 0.984 respectively (Fig. 6(b)). The limits of detection (LOD) ($\delta\sigma$) were found to be 38, 40 and 48 $\mu\text{g/L}$ respectively based on the peak current values of five blank measurements. The calibration equations obtained are as follows: Pb^{2+} : $I_{\text{pa}} (\mu\text{A}) = 0.0568 (\mu\text{A}/\mu\text{g/L}) C - 0.4311(\mu\text{A})$, $R^2= 0.999$, Cu^{2+} : $I_{\text{pa}} (\mu\text{A}) = 0.0645 (\mu\text{A}/\mu\text{g/L}) C + 20.80(\mu\text{A})$, $R^2= 0.985$ and Hg^{2+} : $I_{\text{pa}}(\mu\text{A}) = 0.04585 (\mu\text{A}/\mu\text{g/L}) C + 19.7872 (\mu\text{A})$, $R^2= 0.984$. The linearity and detection limits obtained in these metal ions quantification is very good. The attainment of low detection limits in this method could be due to the presence of functional groups such as $-\text{OH}$ on the lower rim of the indicator moiety. However, in calixarene molecule the hydroxyl groups will be in more number and also the presence of lone pair of electrons on the oxygen atom makes these molecules to complex with the target analyte metal ions. The performance of the proposed sensor has been compared with some of the reported sensors in table 2[31,33]. The multiple peaks appeared might be due to the leaching of the modifier molecule or due to non complexed analyte trapped on the surface of modified SPCCEs, whereas intercepts was differed in case of copper(II) and mercury(II) in comparison with lead(II) indicating kinetic limitation. The residual current composed of different components like Faradaic current from the electrochemical reaction, charging current, variable currents from electrochemical reactions of the electrode surface and trace currents from electroactive impurities present in the

electrolyte solution. The explanation for the shoulder peaks are in agreement with literature [31, 39].

Insert Figure 6

3.8. Interference Study

In order to check the applicability of the proposed protocol to real sample analysis detailed interference study was carried out. The possible cationic and anionic species were added into the electrolytic solution containing the target analytes and their impact on the anodic peak currents was studied. Most of the cations and anions did not interfere in the simultaneous measurement of Pb^{2+} , Cu^{2+} and Hg^{2+} ions, under optimized reaction conditions. The peak currents tolerate the following interferents: 1000 fold - Cl^- , F^- , SO_4^{2-} , K^+ , Mn^{2+} , Li^+ , Ag^+ ; 100 fold for Co^{2+} , $\text{C}_2\text{O}_4^{2-}$ and CO_3^{2-} , 20 fold for As^{3+} , Cr^{6+} , Tl^+ and In^{3+} and F^- ; 50 fold for Ni^{2+} , Fe^{2+} and Zn^{2+} will alter the anodic peak current. However, cadmium ions seriously interfere with simultaneous detection of lead(II) copper(II) and mercury(II) even at 100 micro moles. In order to overcome this interferent, we have masked cadmium ions with 2-octylamino pyridine prior to the electrochemical measurements [40]. The tolerance limit for the above mentioned ions were tabulated in ESI Table 1.

3.9. Repeatability and Reproducibility

The relative standard deviation (RSD) of 7 measurements of 1500 $\mu\text{g/L}$ of Pb^{2+} , Cu^{2+} and Hg^{2+} at SPCCEs were 4.4%, 3.8% and 4.9% respectively. The stripping voltammograms utilizing one single electrode were measured over a period of 15 days and the maximum deviation obtained were 5.1%, 5.5% and 4.7% respectively for these metal ions. These results suggest that the proposed interface showed excellent repeatability and longevity. Hence, the fabricated electrode *i.e.*, SPCCEs have been used for the simultaneous measurement of target ions in real samples.

3.10. Application Study

In order to evaluate the proposed analytical method using SPCCEs, it has been

successfully applied to quantify lead(II), copper(II) and mercury(II) ions present in industrial effluents and sewage water samples by following the recommended procedure. The stripping peak currents were measured and the concentrations were correlated through standard calibration plots (Fig. 6b). The results obtained by the proposed method are compared with the results obtained by the atomic absorption spectroscopy method. The results obtained by the proposed protocol are in agreement with the standard method. Known aliquots of standards were spiked with real samples and the total analyte content were measured. The recovery of spiked samples were within 98 ± 5 % (Table 1). The results obtained by the developed method are in good agreement with the AAS method (Table 1) and these results are compared with some of the literature reports (Table 2).

Insert table 1

Insert table 2

4. Conclusions

The proposed screen-printed calixarene carbon electrodes (SPCCEs) are simple to design and fabrication with different indicator loadings. These electrodes have been used as disposable one shot electrochemical sensors in the simultaneous measurement of lead(II), copper(II) and mercury(II) ions at trace levels. The modified SPCCEs have been successfully applied to real sample matrices and validated with the results of AAS method. The present protocol provides good sensitivity with wide working range and requires a lower volume (15-25 μ L) of the analyte solution for quantification. These SPEs could be used as an alternative device in the measurement of these toxic metal ions at trace level with suitable automation in industrial locations.

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Figures, schemes and its caption

Fig.1: Overlaid cyclic voltammograms of screen printed electrode (b) modified SPE in absence of metal ions, (a) bare SPE and (c) modified SPE in presence of 1 mM each of lead(II), copper(II) and mercury(II) ions in an acetate buffer and 0.1M HCl, scan rate 50 mV/s.

Fig.2: Effect of modifier composition

Fig.3: Effect of pH

Fig.4: Effect of accumulation potential

Fig.5: Effect of pre-concentration time

Fig.6: (a) Overlaid anodic stripping voltammograms of lead(II), copper(II) and mercury(II) with increasing concentration range from 100 – 2400 $\mu\text{g/L}$. Conditions: accumulation potential - 1.1 V, deposition time 180 s, potential window -1.0 to 0.6 V.

Fig.6: (b) Calibration plots for Pb^{2+} , Cu^{2+} and Hg^{2+} over the concentration range 100 – 2400 $\mu\text{g/L}$.

Scheme 1: Schematic representation of binding and complexation mode of metal ions with modifier molecule (calixarene) present on the surface of SPCCE .

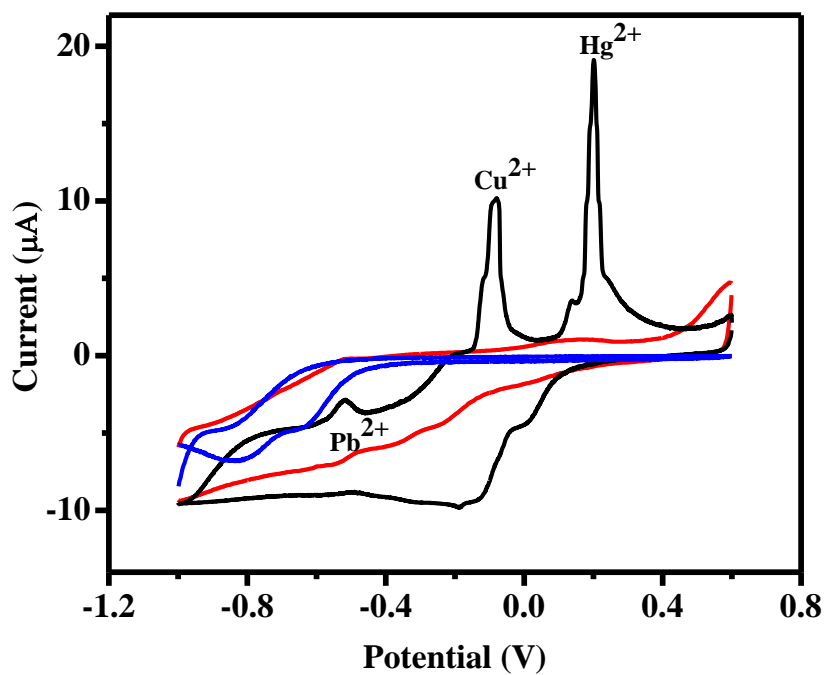


Fig.1

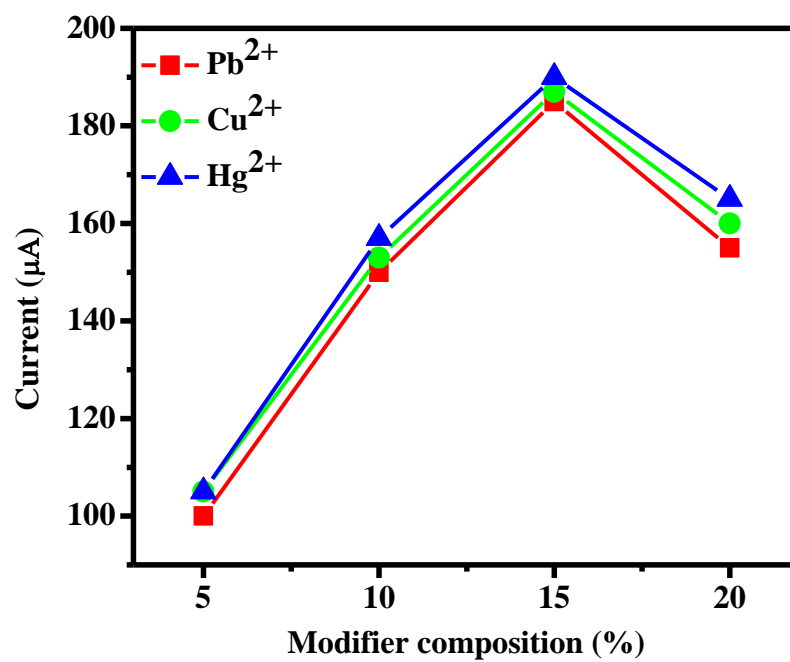


Fig.2

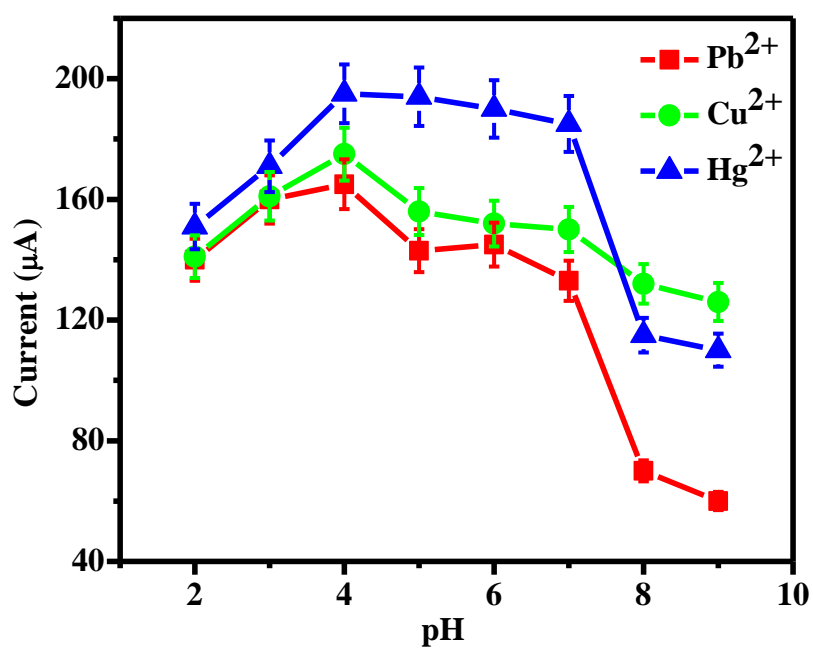


Fig.3

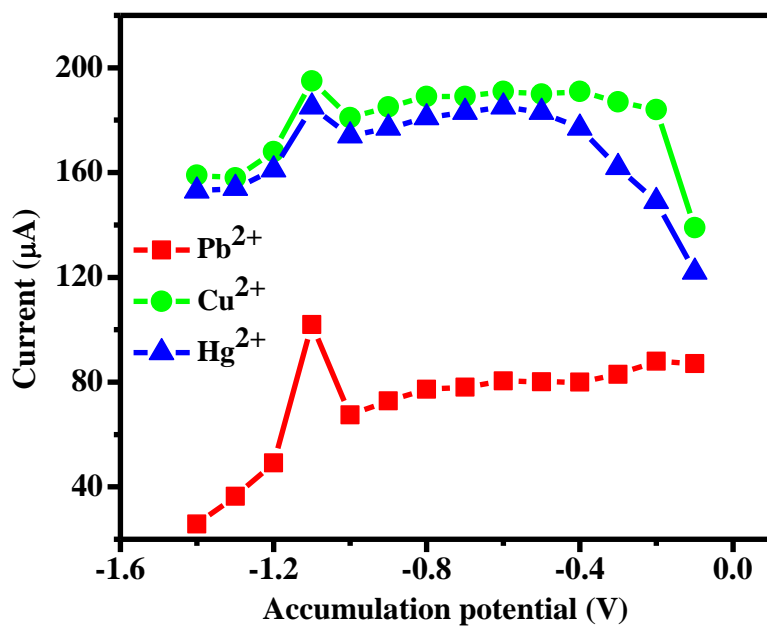


Fig.4

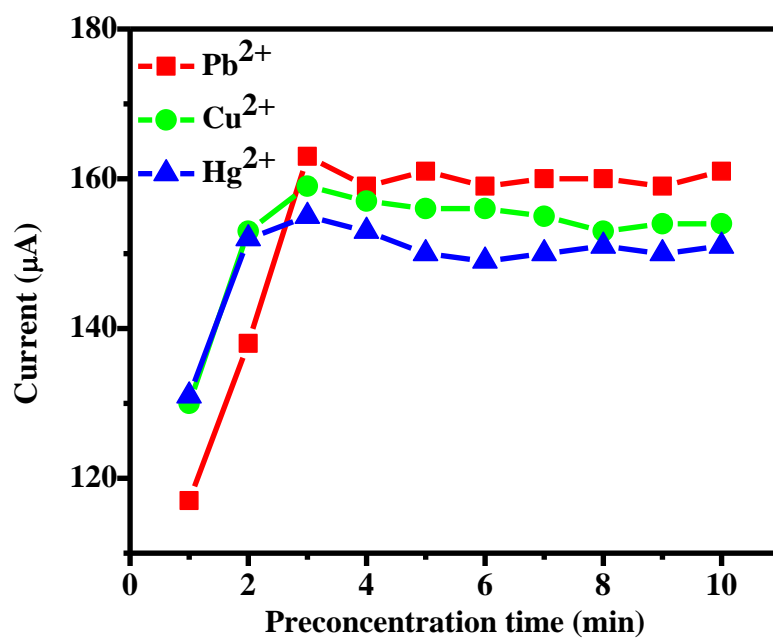


Fig.5

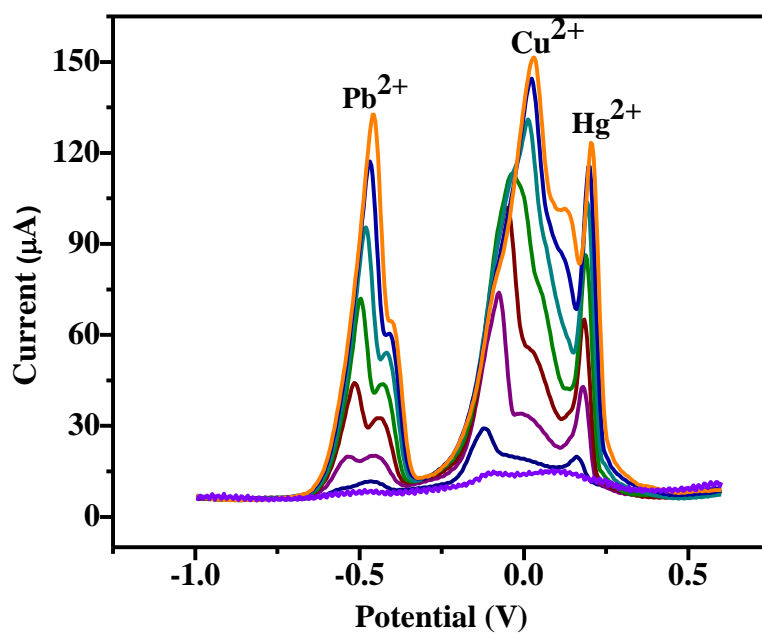


Fig.6 (a)

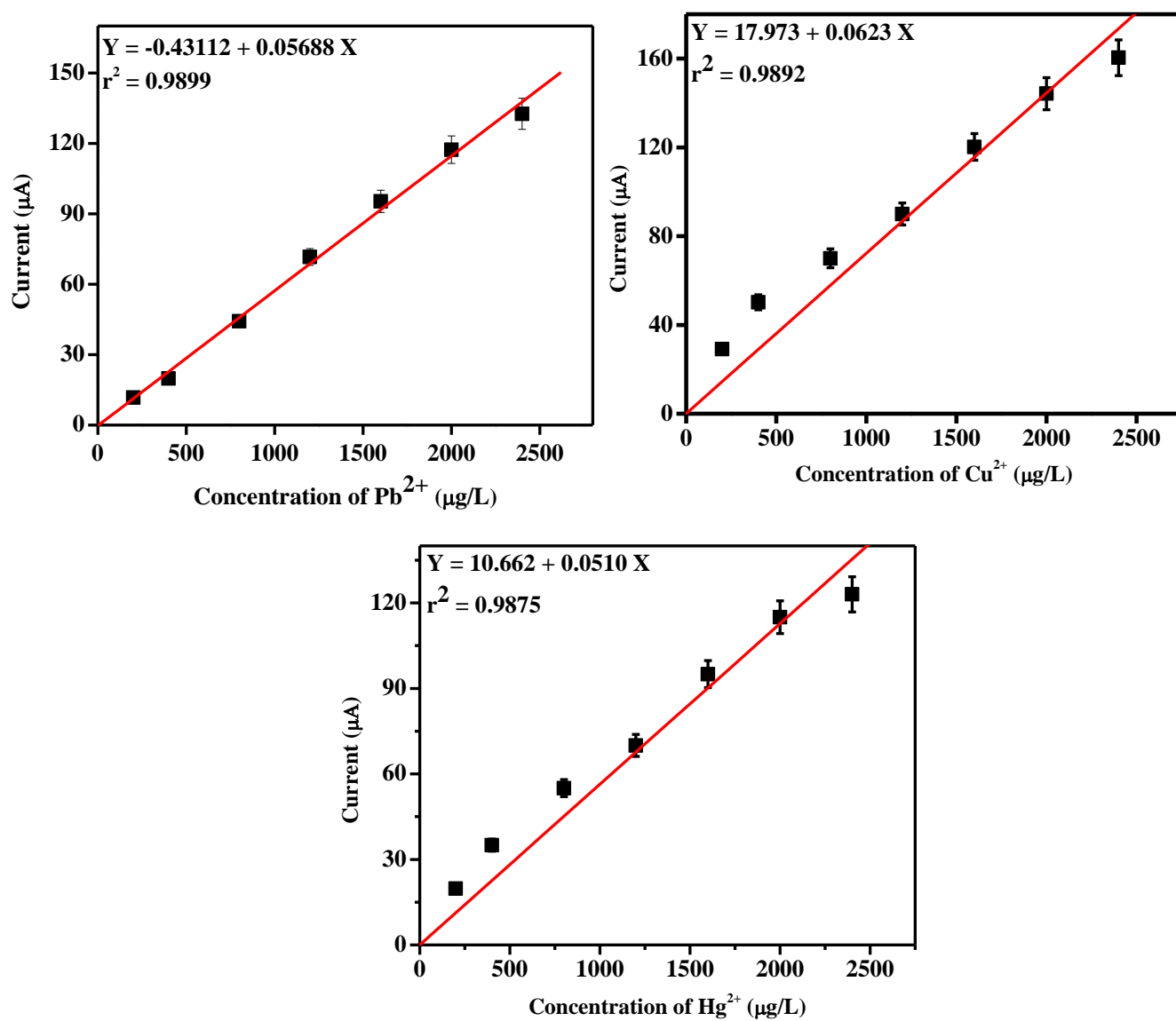
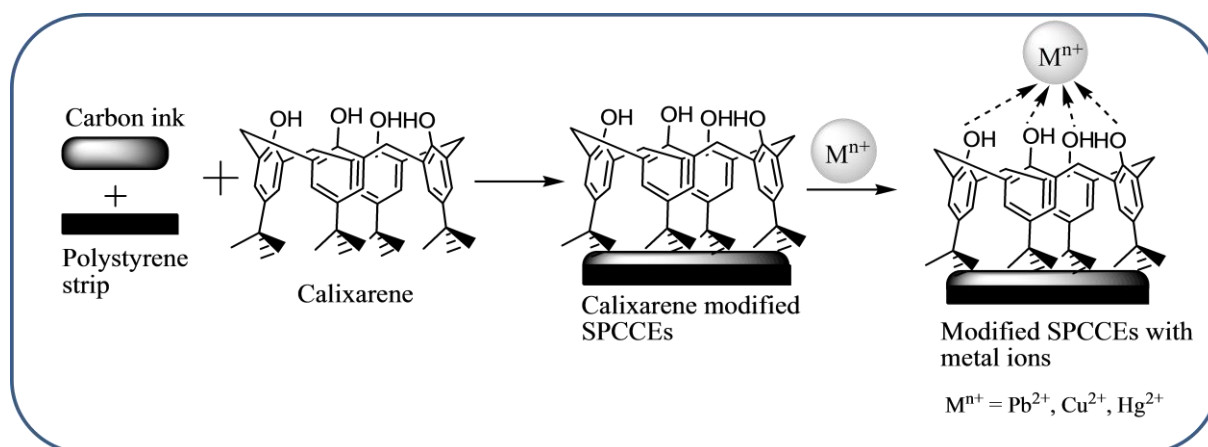


Fig.6 (b)



Scheme 1

Table 1 Application Study

Sample	Originally found ($\mu\text{g/L}$)			Added ($\mu\text{g/L}$)			Total ($\mu\text{g/L}$)						Recovery (%)					
	Pb^{2+}	Cu^{2+}	Hg^{2+}	Pb^{2+}	Cu^{2+}	Hg^{2+}	Proposed protocol			AAS protocol			Proposed protocol			AAS protocol		
							Pb^{2+}	Cu^{2+}	Hg^{2+}	Pb^{2+}	Cu^{2+}	Hg^{2+}	Pb^{2+}	Cu^{2+}	Hg^{2+}	Pb^{2+}	Cu^{2+}	Hg^{2+}
Industrial effluents	35	29	ND	50	50	50	84.5	78.3	49.2	84	78.5	49.5	99.4	99.1	98.4	98.8	99.3	99.0
Waste* water	27	ND	20	50	50	50	76.5	49.7	69.8	76.7	49.9	69.4	99.3	99.4	99.7	99.6	99.8	99.1
Waste* water	22	ND	29	50	50	50	71.8	49.1	78.6	71.5	49.5	78.7	99.7	98.2	99.4	99.3	99.4	99.0
Battery effluents	26	ND	ND	50	50	50	75.7	49.9	49.3	75.8	49.8	49.1	99.6	99.8	98.6	99.7	99.6	98.2

ND – Not Detected

* - The samples were obtained from different open sewer lines within the city limits in the vicinity of industries.

Table 2 Comparison of proposed sensor with certain reported methods

Electrode	Metal ions	Deposition time (s)	Linear range ($\mu\text{g/L}$)	Limit of Detection LOD($\mu\text{g/L}$)	Ref.
SnO ₂ /reduced graphene oxide - nanocomposite	Cd ²⁺ Pb ²⁺ Cu ²⁺ Hg ²⁺	120	0 - 1.3	0.1 0.2 0.2 0.2	[41]
BiO-SPE	Pb ²⁺ Cd ²⁺	300	20 - 100 20 - 100	2.3 1.5	[42]
Sb-SPCE	Pb ²⁺ Cd ²⁺ Cu ²⁺	120	11 - 63 11 - 72 5 - 100	5 3.4 1.6	[43]
Au-SPCE	Hg ²⁺	360	1 - 100	4.9	[44]
SPGEs	Pb ²⁺ Cu ²⁺ Hg ²⁺	240	5 - 300 5 - 300 6 - 300	1.2 1.0 1.7	[45]
SPE-Au-film	Pb ²⁺ Hg ²⁺	120	2 - 16	1.5 0.5	[46]
SAMMS-CPE	Pb ²⁺ Hg ²⁺	120	10 - 1500 20 - 1600	0.5 3.0	[47]
Ac-Phos SAMMS	Pb ²⁺ Cu ²⁺ Cd ²⁺	120	10 - 200	10 10 10	[48]

Thiolated CAL-SPEs	Pb ²⁺	600	5 - 100	5	[33]
Novel calix[4]arene modified GCE	Hg ²⁺	360	0.05 - 50	0.015	[49]
Langmuir–Blodgett film p-allylcalix[4]arene modified GCE	Hg ²⁺	210	0.07 - 40	0.002	[50]
Langmuir–Blodgett film p-tert-butylthiacalix[4]arene modified GCE	Hg ²⁺	90	0.005 - 0.15	0.002	[51]
TCA-MWCNT-modified glassy carbon electrode	Pb ²⁺	900	0.0002 - 0.001	0.0004	[52]
Benzothiozole CAL/GCE	Hg ²⁺	360	25- 300	5	[53]
SPCCEs	Pb ²⁺ Cu ²⁺ Hg ²⁺	180	100 - 2400	38 40 48	Proposed work

BIO-SPE = Bismuth oxide – screen-printed electrode; Sb-SPCE = Antimony film screen-printed electrode;

Au-SPCE = Gold screen-printed electrode; SPGEs = Screen-printed gold electrodes;

SPE-Au-film = Screen-printed electrodes modified with gold films;

SAMMS-CPE = Carbon paste electrode modified with thiol self-assembled monolayer on mesoporous silica;

Ac-Phos SAMMS = Carbon paste electrode modified with carbamoylphosphonic acid self-assembled monolayer on mesoporous silica; Thiolated CAL-SPEs = Tetra-tert-butyl-tetrakis-(2-mercaptoethoxy)-calix[4]arene; GCE = Glassy carbon electrode; TCA-MWCNT = Thiacalixarene multiwalled carbon nanotube; CAL-SPEs = Calixarene screen-printed electrodes; CAL/GCE = Calixarene glassy carbon electrode; SPCCEs = Screen-printed calixarene carbon electrodes.