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Research paper

Nanomolar detection of lead using electrochemical methods based on a novel phthalocyanine



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Keywords: Cobalt phthalocyanine Lead sensor Heavy metals Cyclic voltammetry (CV) Amperometry	4-4-[(Bromo-phenylimino)-methyl]-2-methoxy-phenoxy-phthalocyanine (CoTBrIMPPc) complex was synthe- sized and characterized by different spectroscopic as well as analytical techniques for the first time. The imi- nephthalocyanine derivative displayed redox peaks ascribed to cobalt (Co^{+2}/Co^{+1}) metal and phthalocyanine ligand (Pc^{-2}/Pc^{-3}). The (CoTBrIMPPc) complex was immobilized by drop coating method on the glassy carbon surface and the modified electrode was utilised for detecting lead (Pb) at nanomolar concentration level by voltammetry and amperometric methods. The cyclic voltammetric method showed linearity in the 100–1000 nmol L ⁻¹ range with the limit of detection (LOD) 37 nmol L ⁻¹ . The addition of lead species did not show the considerable amperometric current response under normal amperometric conditions and hence ex- periments were performed by the modified methodology. Amperometry technique showed linearity in the 500 to 3000 nmol L ⁻¹ range, LOD value of 180 nmol L ⁻¹ . The current response vs. Lead (Pb) concentration in graph by amperometric method exhibited correlation coefficient of 0.9991 and sensitivity of 0.0035 μ A nM ⁻¹ . The fab- ricated iminephthalocyanine based sensor is stable, reproducible and free of interference from other metal ion impurities in the electrolyte. The designed sensor has been employed for practical application.

1. Introduction

Lead is a highly poisonous metal among the various heavy metals, both to humans and the environment [1,2]. A common source for lead is mining; available in mineral galena (PbS) and trace quantity of lead is also associated with zinc, silver and copper ores. Besides that, Pb finds potential application in lead ion batteries, projectiles, solder, plumbing, gasoline fuels as an antiknock agent, anti-corrosion agent, paint (black printing ink), coloring agent in ceramic glazes, radiation shielding, and electrode in the process of electrolysis, etc.

Lead is considered as a source of neurotoxin and has adverse affects on health. In particular, it affect the normal growth of the nervous system and brain causing permanent illness in adults with higher risk of kidney damage and varied blood pressure [3,4]. Lead is known to be a toxicant that harms multiple body organs including gastrointestinal, hematological, cardiovascular, neurological and renal organs. Lead stores in the teeth and bones, where it accumulates and concentrates with time and the stored lead in bone is slowly released into the blood system during the period of pregnancy in woman and responsible for the source of lead contact to the growing baby in womb. The lead in

blood results in abdominal pain, constipation, tiredness, headache, irritation, and memory loss behaviour, tingling in the body of hands or feet, tiredness, etc [5]. The lead exposure accounts for 1,43,000 deaths on average every year and it accounts for 0.6% of the global diseases [6]. The allowed limit of consumption of lead is $10 \,\mu\text{g/dL}$ as per WHO standards. Further, the Environmental Protection Agency (EPA) has estimated around 20% lead exposure incidents are due to the usage of lead contaminated drinking water [4].

Hence, it has become a priority to detect and monitor the amount of lead in environmental samples. A simple, sensitive and selective method for the detection of lead at nanomolar concentration is essential at the moment for environmental monitoring of lead. The different analytical techniques like chromatography, spectrophotometry, fluorogenic probing, chemiluminescence, electrochemical sensing are being used for the sensing of lead. Among these, electrochemical methods show great promise due to their advantages such as easy operation, cheaper cost, quick response and high sensitivity. But, it is very essential to find suitable catalyst material which is stable, decreases the overpotential and increases the sensitivity for lead detection [7]. In the last few years, high-performance electrochemical sensors have been

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developed based on cyclic voltammetry and amperometry as they are known to be simple, easy, reliable, reproducible and needs low cost. In recent years modified electrodes have been widely used owing to their favorable signal to noise ratio. Various modifiers including polymers, carbon nanotubes (CNTs), Graphene Oxide (GO), metal phthalocyanines and biological molecules such as DNA and enzymes have been employed to modify the electrode surface for the detection of heavy metals [8-11]. Tebello Nyokong et al. reports the simultaneous detection of mercury (II), Lead (II), copper (II) and cadmium (II) ions with low symmetric metallophthalocyanine via click chemistry [12]. 3-D ZnO@graphene nanocomposite was used to detect Pb by mixing with Bi at the surface of the modified electrode for improved sensitivity and generation of intense peaks [13]. Further, an electrochemical sensor fabricated by GO modified with N-doped quantum dots (QDs) has been used for adsorption of heavy metal ions due to availability of active sites in the sensor surface [14]. Bi based modified electrodes have been frequently used for the detection of lead ions [15]. However, in order to use Bi based modified electrodes, Bi (III) needs to be preconcentrated at the electrode to form an alloy with the targeted analyte for which an in situ electro-deposition or an electric discharge method or exfoliation method must be carried out [15]. Hence, there is a dire need of a more easy method and material for the direct detection of lead ions.

N4 macrocyclic molecules are known to be very good electrochemical catalysts for sensing and these molecules represent inexpensive alternatives to platinum and precious metals. More importantly Cobalt phthalocyanines (CoPcs) have received significant attention because of inherent redox behavior of cobalt and substituted functional groups. It is well known that the properties of the Pcs are dependent on metal center and functional groups at the peripheral sites. In turn, CoPcs are highly stable, conjugated with enhanced electron delocalization. Moreover, CoPcs have been shown to act as effective electrocatalysts towards a wide range of redox systems. Electrode modified with this compound has shown great promise for the electrocatalytic determination of many important compounds like catalytic oxygen reduction reaction and to decrease the effective oxidation or reduction potentials of various reactions. The redox transformation of Co^{II}/Co^{III} and Co^{III}/Co^{II} is very facile compared to other metals in the macrocyclic system and as such cobalt metal is very good electro-active metal [16]. Further, the imine group represents a class of biologically reactive intermediate and has been the focus of intensive toxicological research. The bis-imines are biologically important due to the presence of > C=N moiety. Compound containing an azomethine group (-CH= N-) are known as schiff bases and these Schiff bases form complexes with various metal ions which have shown significant biological activities [17]. In addition, imine groups enhances the electrocatalytic activity. Hence, we expected that the synthesis of cobalt phthalocyanine with imine peripheral group will lead to the biocompatible molecule which can be incorporated in the biosensor for the lead detection.

2. Materials and methods

2.1. Materials

The chemicals for the *CoTBrIMPPc* synthesis as well as electrochemical characterization were obtained from Sigma Aldrich or SD Fine chemicals, India, and all the analytical solutions were diluted and prepared from distilled water.

2.2. Preparation of 4-{4-[-bromo-phenylimino)-methyl]-2-methoxy-phenoxy}-phthalocyanine (CoTBrIMPPc)

2.2.1. Preparation of 4-[(4-bromo-phenylimino)-methyl]-2-methoxy-phenol (i)

A mixture of 4-bromoaniline (2.0 g, 0.0116 mol) and vaniline (1.76 g, 0.0116 mol) with 10 mL methanol as solvent and triethylamine (4–5 drops) as catalyst and refluxed for about 8 h to yield light yellow

colored product. It was recrystallized from ethanol to form 4-[(4-bromo-phenylimino)-methyl]-2-methoxy-phenol (i).

Mol. Wt.: 306. Melting point: 140 °C. Yield: 70%, Anal. For: $C_{14}H_{12}BrNO_2$: Calc. C, 54.92; H, 3.95; N, 4.58; O, 10.45; Br, 26.10. Found: C, 55.23; H, 4.08; N, 4.22. FTIR (cm⁻¹): 1626 (-C=C str.), 2932, 2866 (C–H str.) and 3383 (–OH str). ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.780 (s, 1H), 2.73 (s, 1H), 3.37 (d, 1H), 3.824 (s, 1H), 7.237 (m, 4H), 7.79 (d, 2H), 7.57 (d, 2H), 7.60 (t, 1H), 7.954 (d, 2H), 7.39 (s, 1H), 7.341 (s, 1H), 8.086 (s, 1H), 8.66 (s, 1H). Mass Spectroscopy: M: 306, M⁺¹: 307.

2.2.2. Preparation of 4-{4-[-bromo-phenylimino]-methyl]-2-methoxyphenoxy}-phthalonitrile) ligand (ii)

The precursor i (1.0 g, 0.00326 mol) and 4-nitrophthalonitrile (0.5657 g, 0.00326 mol) were taken in 10 mL DMF solvent and slowly added potassium carbonate (2.24 g, 0.01304 mol) with stirring in nitrogen atmosphere. Further, the mixture was stirred for 72 h to yield compound ii [18]. The solid product was washed with water and dried in vacuum. The ligand ii was recrystallized with ethanol to obtain pure imine phthalonitrile ligand (ii).

Mol. Wt.: 433. Melting point: 122 °C. Yield: 74%. Anal. For $C_{22}H_{14}N_3O_2Br$: Calc. C, 61.13; H, 3.26; N, 9.72; O, 7.40; Br, 18.48, Found: C, 61.58; H, 3.54; N, 9.36.FTIR (cm⁻¹): 1622 (C=C str.), 2928, 2856 (C-H str.), and 2233 (-CN str.). ¹H NMR:(400 MHz, DMSO- d_6): δ 1.49 (s, 2H), 3.358 (s, 1H), 7.197 (d, J = 4.00 Hz, 1H), 7.218 (d, 1H), 7.239 (d, 1H), 7.26 (dd, H), 7.34 (d, 2H), 7.535 (d, 2H), 7.54 (d, 2H), 7.924 (d, 2H), 8.174 (dd,1H), 8.675 (s, 2H). Mass Spectroscopy: M⁺²: 435.

2.2.3. Preparation of 4-{4-[-bromo-phenylimino)-methyl]-2-methoxyphenoxy}-phthalocyanine (CoTBrIMPPc) (iii)

Compound ii (1.0 g, 0.00231 mol) and $CoCl_2$ (0.137 g, 0.00057 mol) were taken in 10 mL of 1-pentanol and added small amount of DBU as catalyst. The reaction was progressed by refluxing the mixture for 24 h at 140 °C to form dark green colored product which is then washed with ethanol and hexane to yield pure compound iii.

Mol. Wt.: 1414. Yield: 78%. Anal. For $C_{88}H_{56}N_{12}O_8Br_4$ Co: Calc. C, 59.11; H, 3.16; N, 9.40; O, 7.16; Br, 17.88; Co, 3.30. Found: C, 59.55; H, 3.42; N, 9.21; Co, 3.66. FTIR (cm⁻¹):715, 844, 981, 1106, 1119 (skeletal vibrations of phthalocyanine) and 2860–2920 (Ar-CH stretching and aliphatic –CH stretching). UV–Visible (nm): B- band (325 nm), Q- band (667 nm).

2.3. Modification of GCE

The GCE was cleaned by polishing to mirror-like surface utilizing alumina (0.05 μ , Baikolox, Japan) over the polishing pad prior to modification. Then the electrode was sonicated sequentially in Millipore water and ethanol 2 times in each solvent to wash-off the trapped alumina particles on the surface and then dried. Suspension of phthalocyanine was prepared by dispersing 0.1 mM CoTBrIMPPc in 1 mL of isopropyl alcohol, sonicated and 5 μ L of suspension was drop-coated on a clean bare GCE and dried under vacuum to obtain GCE/CoTBrIMPPc electrode.

3. Characterization

The elemental analyzer Vario EL III CHNS, Germany was employed for the analysis of different elements in the complex. The cobalt content was determined by gravimetric procedure [19]. The Perkin-Elmer spectrophotometer model lambda 35 was used to record the UV–Vis spectrum in the 280–950 nm range with 0.1 mM of CoTBrIMPPc in DMSO. FTIR spectral analysis was carried out on Perkin-Elmer FT-IR spectrometer by employing KBr sampling technique. The thermogram of the CoTBrIMPPc was recorded over a STA6000 instrument in the 30–800 °C temperature with 10 °C min⁻¹ heating rate in air



Scheme 1. Schematic route for the preparation of cobalt phthalocyanine macrocyclic complex (CoTBrIMPPc).

atmosphere (30 mL min⁻¹). XRD profile of the complex was recorded on D8 Advance X-ray diffraction machine from Bruker. The ¹H NMR spectra (D₆-DMSO as solvent) of the ligands were performed using Bruker AMX-400. The electrochemical analyser (CHI6005E) with cyclic voltammogram and chronoamperometry techniques in a three-electrode setup with GCE as working, Pt foil counter and Ag/AgCl reference was employed for electrochemical investigation. The measurements were carried out in inert atmosphere of nitrogen by purging and stirring the electrochemical cell with N₂ gas for 20 min prior to the experiment. The voltammetric analysis of the complex was carried out with 0.1 mM CoTBrIMPPc in DMSO and 0.1 M TBAP. Further, 0.1 mM K₄[Fe(CN)₆] in buffer (phosphate buffer) was used for studying the charge transfer characteristics of the GCE/ CoTBrIMPPc.

4. Results and discussion

The synthetic scheme for the preparation of imine linkage cobalt phthalocyanine (CoTBrIMPPc) is presented in Scheme 1. The electrophilic carbon of aldehyde and ketone are the target for nucleophilic attack from the amine group. This reaction yields a product in which the C=O is replaced by C=N as shown in Scheme 1. This kind of compound formed is well-known by the name *imine* or Schiff base.

The mechanism for imine complex formation involves two steps. Firstly, the nitrogen in amine behaves like a nucleophile and attacks the carbonyl carbon. This is closely related to hemiacetal and hemiketal formation as shown in Scheme ES. 1a. According to the mechanism involved in the formation of acetal and ketal, the next step of the reaction includes the attack from a second amine group to yield a product with carbon binding to the both amine groups i.e. nitrogen version of the ketal. The nitrogen atom gets deprotonated, and electrons of the N–H bond slowly pushes the oxygen atom from the carbon forming imine, C=N bond (an imine) and water molecule is removed as shown in Scheme ES. 1b.

The synthesized *Pc* complex is obtained with good yield and possess bluish colour. The CoTBrIMPPc complex is amorphous powder-like material and dissolves only in DMF, DMSO, DMA and concentrated sulphuric acid without any degradation. The experimental elemental analytical data of CoTBrIMPPc displayed good agreement with theoretical data referring that the compounds synthesized are highly pure.

4.1. Characterization of the ligands and complex

4.1.1. Absorption studies

The synthesized cobalt phthalocyanine complex UV–Vis absorption spectrum was measured in DMSO solvent in 300–800 nm wavelength range. The CoTBrIMPPc solution was uniform, clear and the electronic absorption spectrum of CoTBrIMPPc exhibited characteristic absorption peaks for phthalocyanine as shown in Fig. S1. The absorption spectrum of the complex exhibited a strong and sharp absorption peak at 667 nm in Q-band region and is ascribed to π - π^* transition [20]. This absorption peak in the visible region is responsible for characteristic blue color of the phthalocyanine complex. The peak observed at 325 nm is due to the absorption in B-band region and can be ascribed to n- π^* and π - π^* transitions. The small shoulder-like peak appeared around 610 nm indicates the vibronic behavior of the complex and the presence of dimeric and oligomeric forms of phthalocyanine in solution [21].

4.1.2. IR spectroscopy

Fourier transform infrared spectra for the synthesized precursor, ligand and complex were performed in 4000 to 500 cm⁻¹ region and presented as shown in Fig. 1. The IR spectra of precursor (i), ligand (ii) and phthalocyanine complex iii exhibited a band at 1620 cm^{-1} corresponding to the aromatic C=C stretching and the peak at 2850-2930 cm⁻¹ is attributed to the -CH stretching of aromatic rings. The compound i exhibited broad peak at 3500 cm⁻¹ for -OH group. Whereas the phthalonitrile ligand ii showed a new peak at 2233 cm^{-1} with the disappearance of -OH peak at 3500 cm⁻¹. New peak observed at 2233 cm⁻¹ may be ascribed to the -CN group and this confirms the successful preparation of ligand ii from i. The spectrum of phthalocyanine complex iii, displayed characteristic bands for phthalocyanine macrocycle skeletal vibrations at 715, 844, 980, 1105, 1119 cm⁻¹ [22]. The-CN peak disappearance in CoTBrIMPPc and the appearance of phthalocyanine skeletal vibration peaks confirm the formation of phthalocyanine complex from ligand ii [23].

4.1.3. NMR spectra

The NMR spectrum of precursor (i) in DMSO- d_6 was recorded and is presented in Fig. S2a. The position of the peaks obtained for (i) are



Fig. 1. FTIR spectra of i) precursor, ii) phthalonitrile ligand and iii) phthalocyanine complex.

presented in synthesis sub-section. Peak at 8.66 ppm is due to proton of imine linkage and this confirms the imine bond formation. Further, the peak for the proton of phenolic –OH was noticed at 3.824 ppm.

Further, the ¹H NMR spectrum for 4-{4-[-bromo-phenylimino)-methyl]-2-methoxy-phenoxy}-phthalonitrile) ligand was recorded using DMSO- d_6 , Fig. S2b. The observed ¹HNMR peaks, splitting and their assignment to different protons of ligand are presented in the synthetic section. Further, the spectrum did not display the peak for –OH at 3.824 ppm and the –OH peak disappearance confirms the formation of phthalonitrile ligand from the precursor. The aromatic protons were observed in the chemical shift range 7 to 8.5 ppm.

4.1.4. Mass spectral characterization

The mass spectrum of 4-[(4-bromo-phenylimino)-methyl]-2methoxy-phenol precursor is shown in Fig. S3a. The theoretical molecular mass of the precursor compound is 306 and the peak at m/z = 306, 307 in the mass spectrum correspond to the molecular ion M and M⁺¹. The mass spectrum confirms the formation of (i) successfully.

The phthalonitrile ligand (ii) mass spectrum is presented in Fig. S3b. Theoretical molecular mass of the compound is 433 and the peak at m/z = 435 appeared in the spectrum due to molecular ion, M⁺². Hence, the formation of phthalonitrile ligand (ii) was confirmed by mass spectrum.

4.1.5. TGA analysis

TGA data was collected to study the thermal behaviour and decomposition nature of imine linkage cobalt 4-{4-[-bromo-phenylimino)methyl]-2-methoxy-phenoxy}-phthalocyanine (CoTBrIMPPc) complex shown in Fig. S4. The small weight loss in the weight of CoTBrIMPPc, noticed below 120 °C is due to the removal of moisture and volatile components in the complex. The thermogram reveals that the complex formed is stable upto 380 °C and the degradation starts at 385 °C in the air atmosphere. The gradual weight loss noticed in 380–500 °C temperature range is attributed to thermal degradation of the phthalocyanine inner structure in the elevated oxidizing atmosphere. The stable degradation product formed from the phthalocyanine complex was equivalent to the theoretical mass of CoO. The TGA data along as well as elemental composition data confirms the pure nature of the *CoT*-*BrIMPPc* [20].

4.1.6. Powder X-ray diffraction

Powder X-ray diffraction pattern for the synthesized novel CoTBrIMPPc complex was recorded in 2θ angle range $10-70^{\circ}$. The imine linkage phthalocyanine showed highly diffused and noisy pattern indicating that the compound is highly amorphous in nature and the diffraction pattern is as shown in the Fig. S5.

4.2. Electrochemical studies

4.2.1. Electrochemical behaviour of CoTBrIMPPc

Cyclic voltammograms (CVs) were performed in DMSO solution with 0.1 mM CoTBrIMPPc and 0.1 M TBAP as supporting electrolyte. The CV was recorded in the window of -1.1 to 0.9 V in nitrogen at mosphere at 50 mV/s. The voltammetric cycle showed that the *CoTBrIMPPc* complex exhibits two pairs of redox peaks revealing that the phthalocyanine molecule is electro-active [24]. The redox peaks that were observed at \sim 0.35 and 0.45 V with reduction and oxidation peaks difference (Δ E) of 0.1 V corresponds to the metal (Co²⁺/Co⁺¹) and another redox peak appeared at -0.5 and -0.3 V with a separation of the reduction and oxidation peaks (Δ E) of -0.2 V is due to the phthalocyanine macrocycle (Pc⁻²/Pc⁻¹) [9,25] as illustrated in the Fig. 2a.

The CoTBrIMPPc modified GCE was analyzed by cyclic voltammetry in dimethyl formamide (DMF) with 0.1 M tetrabutyl ammonium perchlorate (TBAP) in nitrogen (N_2) atmosphere. The CV of the modified electrode displayed all the redox peaks which are in well agreement with the redox peaks for *CoTBrIMPPc* in solution. The stability of the GCE/CoTBrIMPPc electrode was evaluated in the experimental condition by repeated potential cycling for 50 cycles in 1.0 to -1.0 V and Fig. 2b shows no-leaching effect of the *Pc* layer from the GCE.

4.2.2. Charge transfer behavior

The ferri/ferrocyanide redox couple provides the information regarding the charge transfer behavior of the CoTBrIMPPc complex which is on the GCE. Voltammograms for the 0.1 mM $[Fe(CN)_4]^{3-/4-}$ in PBS (phosphate buffer solution) pH 7.0 on the pristine and GCE/ CoTBrIMPPc is as in Fig. S6. The peak currents observed for the [Fe $(CN)_4]^{3-/4}$ decreased on the GCE/CoTBrIMPPc in comparison to the bare electrode. The GCE/CoTBrIMPPc film displayed slight hindrance for the transfer of charge compared to pristine GCE. The GCE/CoT-BrIMPPc film is slightly resistive in nature for the charge transfer that occurs between the electrode and electrolyte. The minimizing of peak current and blocking behavior of the coated film on GCE may be accounted for the thick CoTBrIMPPc film on the electrode which restricts the facile charge transfer [26].

4.2.3. Electrochemical impedance studies

The electrochemical impedance spectroscopy was employed to analyze the modified electrode to obtain information about charge transfer characteristics and interfacial resistance. The Nyquist plot observed for bare and GCE/CoTBrIMPPc electrode in phosphate buffer at amplitude of 5 mV is as shown in Fig. S7. The Nyquist plot has been fitted theoretically with an appropriate equivalent circuit as R(Q(RW))and is shown in the inset of Fig. S7. R₁ is electrolytic resistance, R₂ is charge transfer resistance (Ret), Q is the constant phase element and W is Warburg element. The equivalent circuit parameters of the equivalent circuit are presented in Table S1. The impedance spectra demonstrated that the R_{et} of the modified electrode varied slightly compared to bare electrode. The change in R_{et} value for the GCE/CoTBrIMPPc electrode in comparison to bare GCE confirms the coating of phthalocyanine on GCE [27].

4.2.4. Electrocatalytic sensing of lead by cyclic voltammetry

The cyclic voltammograms in Fig. 3a represents the electrocatalytic detection of lead at pristine GCE and GCE/CoTBrIMPPc electrode in PBS with 200 nM of lead. The catalytic peak appeared at -0.6 V for lead is due to the electrocatalytic reduction of lead at the modified electrode. The literature reports indicate that the reduction of lead occurs at -0.55 V in 1 M HCl at the substituted cobalt phthalocyanine [12] and -0.66 V in pH = 4.5 buffer solution at the substituted cobalt phthalocyanine [28]. The observed reduction potential values are in agreement with the literature values. The peak current for the reduction of lead at bare GCE was very minimal and reduction peak appeared at higher negative potential compared to that of GCE/CoTBrIMPPc. The redox peak noticed at -0.6 V for the GCE/CoTBrIMPPc may be ascribed for the electrocatalytic reduction of lead at -0.6 V. The appearance of the lead reduction peak at less overpotential with increased peak current on the GCE/CoTBrIMPPc electrode reveals that the GCE/ CoTBrIMPPc electrode could be a potential sensor for the detection and sensing of lead. Hence, voltammetric curves were carried out for varying concentrations of lead at GCE/CoTBrIMPPc and the peak current (i_p) for the Pb reduction increased as the Pb concentration increased as in Fig. 3b. Inset of figure Fig. 3b, depicts in vs. Pb concentration plot at the GCE/CoTBrIMPPc electrode displayed a linear behaviour in the 100-1000 nM concentration with LOD value of 30 nM (S/N = 3) in 0.1 M PBS at of 50 mV/s. The reduction i_p vs. concentration of Pb exhibited linear characteristics, Y = 0.0011x + 1.7829 with R^2 = 0.9996 and sensitivity of 0.0011 μA $nM^{-1}.$ Regression value (R^2) reveals a good linear dependency between the dependent variable (i_p) and independent variable (concentration of lead).



Fig. 2. Cyclic voltammograms for a) 0.1 mM CoTBrIMPPc and b) i) bare and ii) GCE/CoTBrIMPPc modified electrode in 10 mL DMSO containing 0.1 M TBAP supporting electrolyte in N_2 atmosphere.

4.2.5. Effect of scan rate

Fig. S8 presents voltammograms for 200 nM lead in 0.1 M PBS at different scan rates of 10–100 mV/s on GCE/CoTBrIMPPc electrode. The voltammograms clearly demonstrated an increasing peak current (i_p) when the scan rate (ν was increased and the inset in Fig. S8 presents the i_p vs. $\nu^{1/2}$ plot. The peak current exhibits a linear dependence on the $\nu^{1/2}$. This linear behavior infers that the CoTBrIMPPc modified electrode involves diffusion controlled mass transfer phenomena between the *Pc* thin film modified electrode/electrolyte interface.

The diffusion coefficient for the catalytic reaction of lead at the GCE/CoTBrIMPPc was calculated using the following equation (Eq. (1)) [29].

$$Ip = 2.69 \times 10^5 n^{3/2} C D^{1/2}$$
(1)

where, peak current response (Ip), number of electrons (n) take part in the redox process of lead having value n = 2, bulk concentration (C) of lead (200 nM). Diffusion coefficient (D_o) was 3.9×10^{-5} cm²s⁻¹. Larger value of D_o infers the rapid diffusion of the Pb species at the electrode-electrolyte interface. The value of D_o obtained for the lead reduction is appreciably higher when compared to the results documented for other electrodes [30].

4.2.6. Electrocatalytic sensing of lead by amperometry

The voltammetric measurements encouraged us to develop sensitive amperometric sensor for lead using GCE/CoTBrIMPPc electrode. Normal amperometric procedure could not be employed for the detection of lead as it did not show appreciable amperometric current response for successive addition of lead. Hence, a modified methodology has been employed where each addition of lead has been



Fig. 4. Amperometric i-t response for different concentration of lead on GCE/CoTBrIMPPc modified electrode in 0.1 M phosphate buffer pH 7.0 under stirring condition. Inset shows the plot of stabilised current response vs. concentration of lead.

monitored separately for the amperometric current response. Amperometric curve was recorded at an applied voltage of -0.6 V in phosphate buffer solution with continuous stirring to maintain the homogeneity in the solution. The Fig. 4, presents the step-wise current response on the successive incremental addition of 500 nM lead to 0.1 M PBS. On each addition of 500 nM lead to the electrolyte solution, the amperometric current increased linearly. The step-wise



Fig. 3. CV profile in phosphate buffer pH 7.0 for a) i) bare GCE without analyte, ii) bare GCE with analyte (200 nM lead), iii) GCE/CoTBrIMPPc without analyte and iv) GCE/CoTBrIMPPc with analyte (200 nM lead). b) electrocatalytic reduction of lead with different concentration (100–1000 nM) on CoTBrIMPPc electrode. Inset b: Plot of peak current at -0.6 V vs. lead concentration.

Table 1

Comparison of the analytical parameters observed for the detection of lead with the literature reports.

Electrode material	Method	Linear range (µM)	LOD (µM)	Sensitivity (μ A nM ⁻¹)	Reference
^a GCE/HDME ^b GCE/Pt wire ^c GCE/Ch- <i>N</i> -Gr ^d GCE/GSH-AgNPs ^e GCE/CoTBrImPPc	CV CV CV CV CV	20–1000 30–3000 10–1000 0.1–10000 0.1–1	3.55 2.7 0.06 0.049 0.03	13.01 50 0.0035	[31] [32] [33] [34] This work

^a HDME-Hanging dropping mercury electrode.

^b GCE/Pt wire – Platinum wire electrode.

^c GE/Ch-N-Gr-Glassy electrode N graphene.

^d GCE/GSH-AgNPs-glutathione stabilized silver nanoparticles.

^e CoTBrImPPc-4-4-[(Bromo-phenylimino)-methyl]-2-methoxy-phenoxy-phthalocyanine.

enhancement in the amperometric current for the successive and repetitive addition of lead demonstrates that the lead undergoes sensitive and quantitative reduction at GCE/CoTBrIMPPc electrode.

The inset in Fig. 4 exhibits the current response vs. Pb concentration plot at the GCE/CoTBrIMPPc. The straight line response was obtained in 500 to 3000 nM concentration range of lead with the LOD 180 nM and sensitivity of $0.0035 \,\mu A \, n M^{-1}$. The analytical parameters for detection of lead at GCE/CoTBrIMPPc was compared with other electrodes in literature and some of the values are better for some important parameters achieved by GCE/CoTBrIMPPc and the comparison data of our work with the literature values are shown in Table 1 [31–34].

In order to evaluate the fabricated sensor for selectivity and specificity, the interference from co-existing molecules was studied with various metal ions. The interference study involved amperometric measurement of lead in presence of interfering common ions such as 3 µM nickel sulphate, 3 µM zinc sulphate, 3 µM sodium chloride, 3 µM potassium chloride, along with 500 nM lead. Fig. S9 shows the amperometric response for lead and other interfering ions. The experiment was performed by recording the amperometric current with the successive incremental injection of lead and 3000 nM of different interfering molecules at a regular interval of 200 s with an applied voltage of -0.6 V at GCE/CoTBrIMPPc under stirring condition. The plot clearly demonstrates an increasing of the current on addition of lead but, the addition of co-existing species shows negligible or non-measurable current response. Despite the presence of excess interfering species, the GCE/CoTBrIMPPc demonstrated very good selectivity for the detection of lead. Hence, GCE/CoTBrIMPPc electrode provides highly selective and sensitive signal towards the lead detection even when the interfering molecules are present in higher concentration.

4.2.7. Real sample analysis at GCE/CoTBrIMPPc modified electrode

The commercial 1.0 g solder sample [1.0 g of lead solder (Recorn Classic, Delhi) contains 0.4 g of lead] was dissolved in 10 mL of 1 M HCl and diluted to obtain the applicable concentration range of the modified electrode. This commercial solder sample solution was used for the

real sample measurements and the results are reported in the Table 2. Different concentration of solder sample solution was added to 10 mL of PBS and lead was detected on GCE/CoTBrIMPPc electrode at -0.6 V by amperometry. The amount of lead detected by amperometry was agreeing with that of the theoretical value as given in Table 2. Further, the standard addition method was employed to understand the interference from the matrix substances in the real sample. To the 1000 nM solder solution in electrolyte, a standard lead solution was added. The amperometric response was used to find the recovery of lead and the results are documented in Table 2. The % recovery values of the lead are satisfactory at the GCE/CoTBrIMPPc and indicates that the impurity or matrix of the solder do not interfere in the detection of lead at GCE/ CoTBrIMPPc. Further, the Pb content in blood and urine samples was assessed at the GCE/CoTBrIMPPc. The blood sample was collected from the person working in soldering industry from a nearby hospital and was stored in a clean vial at 2 °C before use. 1 mL of blood sample was mixed with 10 mL PBS. The blood sample did not show any lead content by amperometric method in the detectable range of the GCE/CoT-BrIMPPc sensor. Therefore, the standard addition method was employed to detect lead in blood sample and known amount of lead was spiked into the blood sample. The amperometric method was used to detect lead and the recovery values were between 100 and 102% for different amounts of lead added to blood sample. In the same manner, the urine sample was also analysed for lead content by standard addition method and the recovery values varied between 100 and 101%, Table 2. The results illustrated that the GCE/CoTBrIMPPc electrode presents the feasibility and practical applicability of lead detection in biological samples which are comparable with the literature data [35].

4.2.8. Reproducibility, repeatability and stability of GCE/CoTBrIMPPc

The GCE/CoTBrIMPPc electrode was prepared in 4 sets to evaluate the reproducibility. The results from the fabricated four sets of sensor showed RSD value of less than 4% which indicates the acceptable reproducibility. The sensor repeatability parameter was investigated by taking five repeated amperometric measurements for 500 nM lead using the CoTBrIMPPc electrode. The designed sensor exhibited overwhelming repeatability with RSD of 2% for the detection of lead. The amperometric response was collected for freshly prepared and electrode stored in the desiccator for 10 days to monitor the stability of the modified electrode. The current response for the stored electrode was in well agreement and satisfactorily matching with the freshly prepared electrode response.

Further, to understand the type of interaction between lead and MPc during the lead detection, absorption experiments were carried out and shown in Fig. S10. It was noticed that the addition of an aqueous lead solution to CoTBrIMPPc in DMSO displayed small shift in both B and Q band towards lower wavelength with splitting of B-band which may be due to formation of adduct of CoPc with lead or aggregation [16].

5. Conclusions

N4 macrocycle containing imine linkage at the periphery was

Table	2
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Analytical data obtained for the commercial solder sample

Sample	Theoretical value (nM)	Detected	Spiking (nM)	Detected $\pm \text{RSD}^{\$}$ (%)	Recovery (%)
Solder Sample	500	503	100	606 ± 0.4157	103
•	1000	1004	200	1206 ± 0.6403	101
	1500	1505	400	1906 ± 0.3404	100.25
Blood Sample	_	-	500	510 ± 0.5024	102
-	_	-	1000	1015 ± 0.1054	101.5
	_	-	1500	1518 ± 0.2046	101.2
Urine Sample	_	-	500	504 ± 0.1804	100.8
•	_	-	1000	1012 ± 0.4404	101.2
	_	-	1500	1504 ± 0.2098	100.26

synthesised first time with good yield. Various analytical and physicochemical characterization like elemental composition, UV–Vis, IR spectra, XRD, TGA, NMR and mass spectroscopy have been performed to confirm the purity. The cyclic voltammetry reveals the redox-active nature of iminephthalocyanine. The synthesized CoTBrIMPPc was coated on GCE and investigated for the Pb detection by voltammetry and amperometry. The fabricated electrode showed improved analytical parameters with respect to low LOD, high sensitivity, stability and reproducibility. The GCE/CoTBrIMPPc electrode was highly selective for Pb detection and did not respond to the co-existing molecules.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ica.2020.119564.

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