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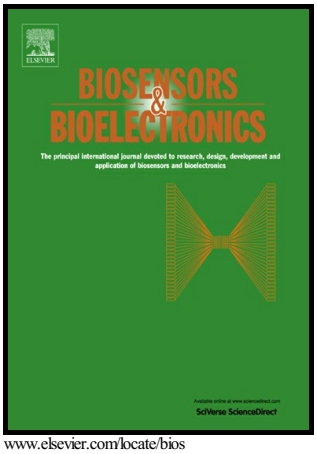

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A bioinspired ionic liquid tagged cobalt-salophen complex for nonenzymatic detection of glucose

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Abstract

The development of efficient and cost effective nonenzymatic biosensors with remarkable sensitivity, selectivity and stability for the detection of biomolecules, especially glucose is one of the major challenges in materials- and electrochemistry. Herein, we report the design and preparation of nonenzymatic biosensor based on an ionic liquid tagged cobalt-

[1] Authors contributed equally to this work.

salophen metal complex (Co-salophen-IL) immobilized on electrochemically reduced graphene oxide (ERGO) for the detection of glucose via an electrochemical oxidation. The bioinspired Co-salophen-IL complex has been synthesized and immobilized on ERGO, which was previously deposited on a screen printed carbon electrode (SPE) to form the Co-salophen-IL/ERGO/SPE nonenzymatic biosensor. The electrochemical behavior of this modified electrode was studied using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Notably, the Co-salophen-IL/ERGO/SPE biosensor exhibited excellent electrocatalytic activity towards glucose oxidation in 0.1 M NaOH, based on which an amperometric sensor has been developed. The modified electrode has shown prominent performance towards glucose detection over a wide linear range from 0.2 µM to 1.8 mM with a detection limit and sensitivity of 0.79 µM and 62 µA mM^{-1} respectively. The detection was carried out at 0.40 V and such a less working potential excludes the interference from the coexisting oxidizable analytes. The role of Co-salophen, IL and ERGO in the electrocatalytic activity has been systematically investigated. Furthermore, the biosensor demonstrated high stability with good reproducibility.

Keywords: nonenzymatic biosensor, bioinspired complex, salophen, ionic liquid, graphene oxide, glucose.

1. Introduction

Glucose represents an indispensable biomolecule and is omnipresent in most of the living organisms. It is essential for mankind since it is the primary source of energy for brain and also for the cells throughout the body. However, diabetes mellitus, which is one of the major health issues worldwide leads to imbalance in the blood glucose level (Wang et al., 2015; Galant et al.,

2015; Heller et al., 2010). Thus, sensitive and selective determination of glucose has gained enormous importance not only in healthcare, but also in food industry, environmental assessment (Wang, 2008) and renewable energy sector (Karuppiah et al., 2015). Numerous enzyme based biosensors (Chen et al., 2013; Turner, 2013) have been developed for the detection of glucose and few of them have been commercialized (Newman and Turner, 2005). Despite their noticeable applications, the major problems such as high cost of the enzymes, tedious enzyme immobilization procedures, requirement of additional redox mediator or oxygen, denaturation of the enzymes and insufficient long-term stability inspired researchers to look for cost effective and highly durable materials. Accordingly, enzyme free biosensors based on direct electrocatalytic oxidation of glucose (Tian et al., 2014; Cheng et al., 2016; Toghill and Compton, 2010) have recently garnered massive attention in sensor and biosensor design.

The performance of the nonenzymatic biosensor is very much dependent on the characteristics of the electrode materials and hence the choice of electrode materials is an important task (Parlak et al., 2013). Reduced graphene oxide (RGO) constitutes itself as a promising material for electrochemical applications owing to its well-recognized advantages such as high conductivity (Stankovich et al., 2007; Moon et al., 2010), large surface area (Li et al., 2010; Sharma et al., 2010), cost effectiveness and remarkable flexibility (He et al., 2011; Pei et al., 2010). In particular, electrochemically reduced graphene oxide (ERGO) has attracted more interest, because it is free from metallic impurities which is a preferred characteristic in electrochemical investigation and also it does not require any toxic reducing agents like hydrazine, $NaBH_4$ etc. (Haque et al., 2012; Bharadwaj et al., 2016). Hence, we preferred to utilize ERGO deposited on screen printed carbon electrode (SPE) as the electrode material in the present investigation (Cinti et al., 2015).

In general, the nonenzymatic biosensors for the determination of glucose have been prepared using Pt, Au, Cu and Ni metals (Zhu et al., 2015; Barsan et al., 2016), alloys (Su et al., 2016), metal oxides (Solanki et al., 2011) and transition metal complexes (Lee et al., 2015; Lee et al., 2014). Noteworthy, the performance of these sensors rely on the nature of metal centers, which play a prominent role in electrocatalytic reactions. With significant advancement in nanotechnology, variety of materials are being explored continuously as electrocatalysts for glucose oxidation (Talarico et al., 2015; Hwang and Gu, 2012; Mondal et al., 2016; Zamora-Galvez et al., 2016; Parlak et al., 2016) and undoubtedly, all these investigations are incrementally contributing to the design of nonenzymatic biosensors. However, the mechanism for glucose oxidation at these metal centers or on the nanostructures is uncertain and therefore there is no universally agreed mechanism for nonenzymatic glucose biosensing. On the other hand, enzymatic biosensors have established clear mechanism for their sensing behaviour. Though nonenzymatic biosensors possess significant advantages in terms of easy electrode fabrication, long term stability and cost effectiveness, they can replace the currently utilized enzymatic biosensors, only if they fulfill all the requisites of a viable biosensor with detailed mechanistic investigations. Hence, the demand for exploring new materials for electrocatalytic oxidation of glucose is rising deliberately.

The earth abundant transition metal (Fe, Co, Mn, Cu and Ni) complexes with bioinspired salen type ligands (Murphy et al., 2001; Shen et al., 2016; Goger et al., 2013; Diaz-urrutia et al., 2016) have been demonstrated as functional mimics of metalloproteins and utilized as catalysts for various redox reactions. The major advantage with this type of metal complexes is the conformational flexibility that can be tuned by suitable choice of ligands. This structural tunability can facilitate different geometries of the metal complex and consequently generate

desired active sites for carrying out the redox reactions (Carter et al., 2015; Yamamoto et al., 1996; Chang et al., 1997). Based on their potential advantages, attempts have been made to extend the efficacy of these complexes towards the electrocatalytic oxidation and electrochemical sensing of significant analytes (Wang et al., 2015; Martin et al., 2012), but are scarce. In spite of their huge proficiency, salen type metal complexes have been less investigated towards electrochemical sensing. Thus, there is substantial scope for designing new metal complexes comprising salen ligands and their derivatives to bridge the gap between the proficiency of these complexes and their electrochemical behaviour.

In this direction, the redox activity of these complexes has to be adequately improved to employ them in electrochemical sensing and biosensing. Ionic liquids (ILs) play a significant role in electrochemistry owing to their tunable physicochemical characteristics (Shiddiky and Torriero, 2011), wide potential window (Pauliukaite et al., 2011) and ionic conductivity (Kwak and Senthil, 2014). Thus, linking of IL to salen type ligand could dramatically improve the electrochemical behaviour of these complexes. In addition, the introduction of ionic moieties into the ligand would influence the coordinating ability of the ligand and in turn the electrocatalytic activity of the complex (Zhang et al., 2014; Bekhouche et al., 2010; Ambrose et al., 2013). Hence, it is unambiguous that tagging of IL into the metal salen complexes could significantly enhance its electrocatalytic activity through the conducting nature of IL and also by favouring a convenient catalytic environment.

In the present investigation, we have synthesized an ionic liquid tagged cobalt-salophen (Co-salophen-IL) complex (salophen-IL = N,N′-(o-phenylene)bis-[(3-ethyl-1H-benzimidazole-1-ium-1-yl)methylene hexafluorophosphato]salicylideneimine), and immobilized it on the ERGO deposited over the SPE to fabricate the Co-salophen-IL/ERGO/SPE modified electrode. The

efficacy of Co-salophen-IL/ERGO/SPE modified electrode towards nonenzymatic biosensing of glucose has been demonstrated. The modified electrode exhibited an excellent performance with respect to linear range, sensitivity, selectivity and stability for glucose detection. Furthermore, the developed nonenzymatic biosensor has been effectively utilized for the detection of glucose in real samples.

2. Experimental Section

2.1. Chemicals and Materials

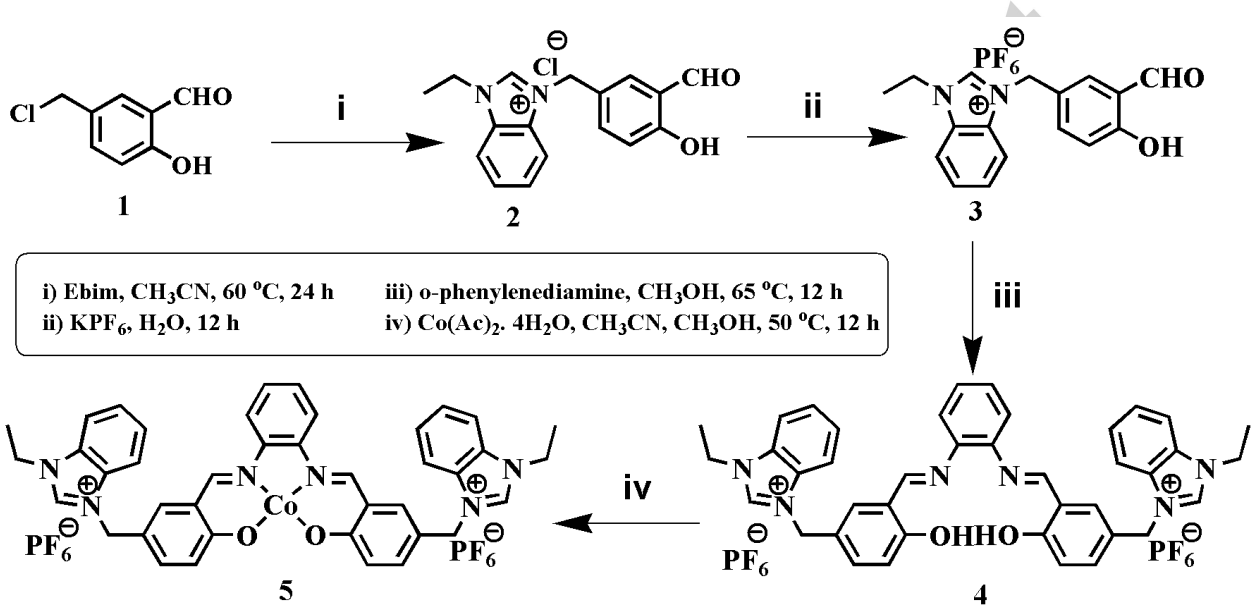
Salicylaldehyde, formaldehyde (37 wt % in H_2O), zinc chloride ($ZnCl_2$), benzimidazole, sodium hydride (60 % dispersion in mineral oil), bromoethane, potassium hexafluorophosphate, o-phenylenediamine, cobalt acetate [$Co(OAc)_2.4H_2O$], graphite powder, sodium hydroxide, $CDCl_3$, D_2O and DMSO-d_6 were purchased from Sigma-Aldrich. Potassium ferrocyanide, D-glucose and hydrochloric acid were obtained from Merck Chemicals. All solvents and reagents are of analytical grade and were used as received unless otherwise stated. All the solutions were prepared using Milli-Q water with electrical resistivity of 18.2 MΩ/cm at 25 °C. Screen printed carbon electrodes (SPE) with 0.196 cm^2 working geometric area were procured from Zensor R&D (Taichung, Taiwan).

2.2. Instrumentation

Electrochemical experiments were carried out using an electrochemical workstation (CHI 760E; CH Instruments, USA). A standard three electrode electrochemical cell was used consisting of a preanodized screen printed carbon electrode (SPE) (with or without modification) as the working electrode, Pt wire as counter electrode and Ag/AgCl (3 M KCl) electrode as reference electrode, to which all potentials in this paper are referred. All electrochemical

measurements were carried out with 0.1 M NaOH as the supporting electrolyte. Preanodization of SPE was conducted in 0.1 M H_2SO_4 with an applied potential of +2.0 V for 300 s under stirring conditions. Prior to each measurement, all the solutions were purged with N_2 for 15 min at room temperature.

2.3. Synthesis of N,N'-(o-phenylene)bis-[((3-ethyl-1H-benzimidazole-1-ium-1-yl)methylene hexafluorophosphato)salicylideneiminato]cobalt(II) **(*Co-salophen-IL*, 5)**



Scheme 1. Synthesis of salophen-IL ligand **(4)** and Co-salophen-IL complex **(5)**.

The Co-salophen-IL complex was synthesized in multiple steps as mentioned in Scheme 1. Procedures for the synthesis of compounds **1-4** are provided in the supplementary information. The synthesized ligand **(4)** (4.621 g, 5 mmol) was dissolved in a mixture of acetonitrile and methanol (9:1) and then added to a stirring solution of equimolar amount of $Co(OAc)_2.4H_2O$, (1.245 g, 5 mmol) in methanol (4 mL). The reaction mixture was refluxed at 50 °C under stirring for 12 h to complete the complex formation and the resulting reaction mixture was concentrated under vacuum. Then, diethyl ether was added slowly into the concentrated reaction mixture to

precipitate the complex. The precipitate was collected by filtration and washed with cold methanol and dried in air to afford a brown solid **(5)**. Yield: 3.156 g; 64 %. Melting point: 226 °C. IR (ATR) (ν/cm^{-1}): 2970 (C-H) (aliphatic), 1624 (imine C=N), 1525 (benzimidazole C=N), 1456 (C-C) (phenyl ring), 829 and 555 (P-F), 474 (Co-N), 424 (Co-O). UV-vis {λ_{max}, nm/ DMF}: 336, 394 (MLCT), 474(d-d). HRMS (EI): m/z calculated for $[M]^+$ $[C_{40}H_{36}CoF_{12}N_6O_2P_2]^+$ 691.6882, Found 691.6880.

2.4 Fabrication of Co-Salophen-IL/ERGO/SPE

Graphene oxide (GO) has been synthesized from graphite using Hummers and Offeman's method (Hummers and Offeman, 1958) with minor modification and the detailed procedure is presented in the supporting information (SI) and the TEM image of prepared GO is shown as Fig. S1A. Deposition of ERGO on SPE was carried out using the procedure reported earlier (Zhou et al., 2009), with slight modification. In brief, 5 µL of 0.1 mg/mL GO in DMF (dispersed and sonicated) was dropcasted on the pre-anodized SPE. After complete drying at room temperature, electrochemical reduction of GO was carried out by subjecting the electrode to potential cycling in the range from 0 to -1.6 V in PBS (pH 7.0) until constant current was achieved. The electrochemical reduction of GO to ERGO has been confirmed with the help of Raman spectroscopy through the increase in intensity ratio of D to G band (Fig. S1B). The obtained ERGO deposited SPE (ERGO/SPE) was dried and used for further modification. Co-salophen-IL was dissolved in methanol (2 mg/mL) and 5 µL of this solution was dropcasted on ERGO/SPE and allowed to dry in air to obtain Co-salophen-IL/ERGO/SPE.

3. Results and Discussion

3.1. Characterization of Co-salophen-IL

The stepwise synthesis of salophen-IL ligand followed by complexation with Co to form Co-salophen-IL has been monitored using ^{1}H and ^{13}C NMR, FTIR, UV-vis and Mass spectroscopy. ^{1}H and ^{13}C NMR spectra of 1-ethylbenzimidazole and compounds **1 to 3** and salophen-IL ligand are presented in supporting information as Fig. S2-S13. It could be observed that the recorded ^{1}H and ^{13}C NMR spectra matched well with that of the expected compounds and the respective protons and carbons for all the compounds are labelled in the figures.

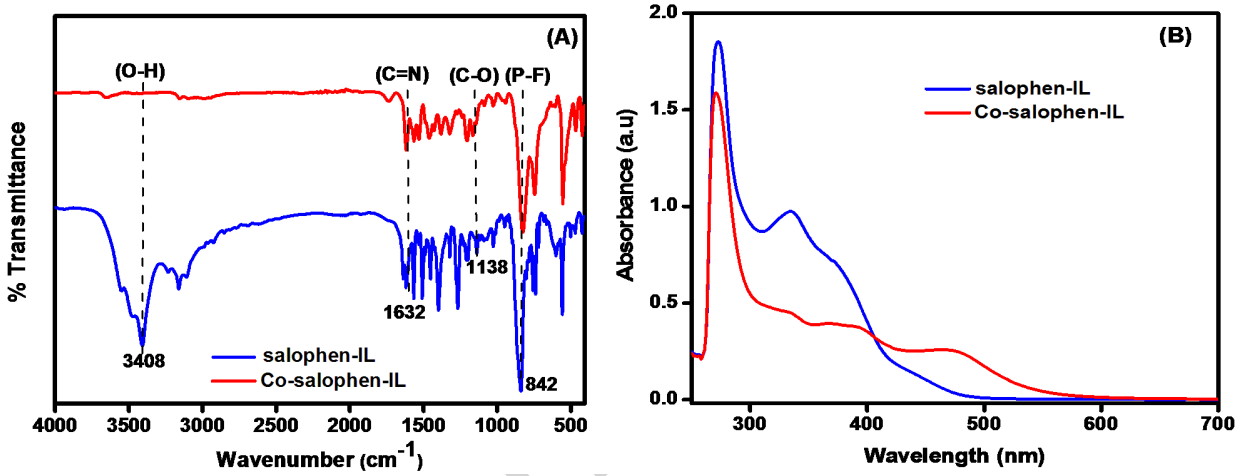


Fig. 1. (A) FTIR and (B) UV-vis spectra of salophen-IL (blue) and Co-salophen-IL (red).

FTIR spectra of salophen-IL and Co-salophen-IL complex are compared in Fig. 1A. The characteristic bands of the ligand have shifted along with a decrease in their intensities after complex formation and further the spectrum of Co-salophen-IL showed additional characteristic vibrational bands. Stretching vibration of the -OH group of free ligand ν(O-H) at 3408 cm^{-1} was not observed in the spectrum of the complex, suggesting deprotonation of the hydroxyl group and formation of a new M–O bond, which appeared as a strong band at 424 cm^{-1}. The imine bands ν(C=N) were observed at 1624 and 1637 cm^{-1} for complex and ligand respectively. The higher energy ν(C=N) metal –dependent band was found to be shifted by 13 cm^{-1} to the lower energy upon coordination of azomethine nitrogen to cobalt ion. A new weak band at 474 cm^{-1}

due to Co-N stretching appeared in the complex. The strong and sharp bands observed at 842 cm^{-1} in ligand and 560 cm^{-1} in complex are characteristics of the uncoordinated hexafluorophosphate anion (Souza et al., 2003). Quaternized-N-ethyl (C-N-$(CH_3)_3^+$) stretching at 954 cm^{-1} and alkylated N-methyl (CH_2-N) stretching at 1209 cm^{-1} indicate that both the ligand and complex are tagged with the IL (Fringeli and Gunthard, 1981).

UV-vis spectra of salophen-IL ligand and Co-salophen-IL complex were recorded in DMF and shown in Fig. 1B. Salophen-IL and Co-salophen-IL complex exhibited strong bands at 334 and 336 nm respectively which is assigned to the n→π* transition involving the molecular orbital of the C=N chromophore and the benzene ring. Co-salophen-IL complex showed an absorption band at 394 nm corresponding to metal to ligand (d→π*) charge transfer. A broad band observed at 474 nm in the case of Co-salophen-IL evidences d-d transition (arising from $d_z^2 \rightarrow d_{xy}$ transition) of Co(II) ion, which confirms the complex formation (Ortiz and Park, 2000).

The mass spectrum of salophen-IL ligand exhibited the molecular ion peak m/z at 633, corresponding to $[C_{40}H_{38}N_6O_2]^{2+}$. The molecular ion peak m/z at 691 in the mass spectrum of Co-salophen-IL complex confirms the composition of $[C_{40}H_{36}CoN_6O_2]^{2+}$. The fragmentations of the ligand and complex are indicated in Fig. S14 and S15 respectively. Thus, all the spectral data are consistent with the proposed structure of salophen-IL ligand and Co-salophen-IL complex (Scheme 1).

3.2. Electrochemical behaviour of Co-salophen, Co-salophen-IL complex and Co-salophen-IL/ERGO/SPE modified electrode

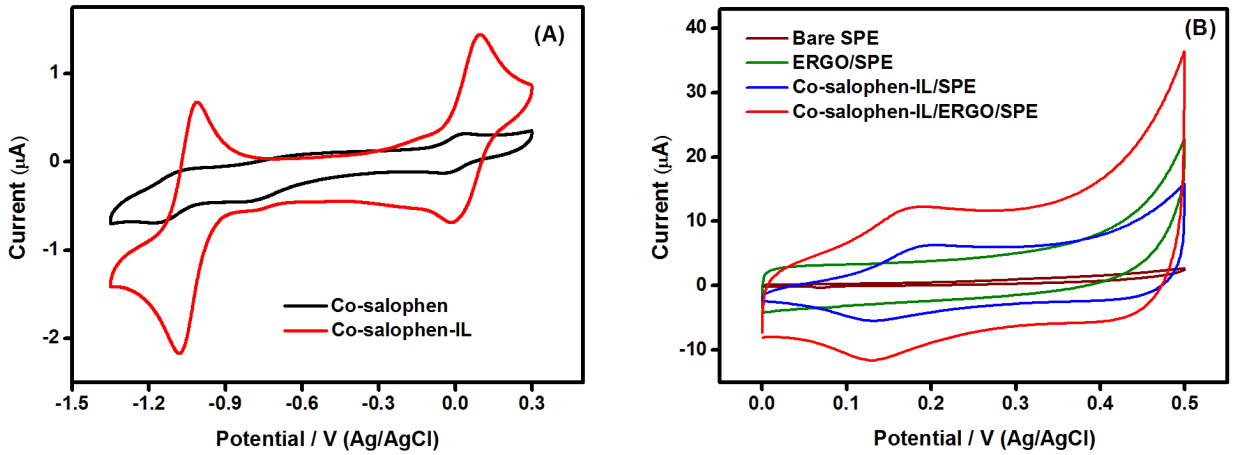


Fig. 2. (A) CVs of 1 mM solution of Co-salophen (black) and Co-salophen-IL (red) in DMSO containing 0.1 M TBAP at Pt electrode; scan rate: 50 mVs^{-1} (B) CVs of bare SPE (brown), ERGO/SPE (green), Co-salophen-IL/SPE (blue) and Co-salophen-IL/ERGO/SPE (red) in 0.1 M NaOH at a scan rate of 50 mVs^{-1} (C) Nyquist plots obtained for bare SPE (brown), ERGO/SPE (green), Co-salophen-IL/SPE (blue) and Co-salophen-IL/ERGO/SPE (red) in 0.1 M KCl containing 2 mM $[Fe(CN)_6]^{4-/3-}$.

We have synthesized an IL tagged Co-salophen, to improve its redox characteristics making it suitable for electrocatalytic oxidation of glucose. In order to probe the role of IL on the electrochemical behaviour of Co-salophen-IL, Co-salophen (without IL tagging) has been synthesized and the voltammograms of Co-salophen complex before and after IL tagging has been recorded in DMSO at 50 mVs^{-1} (Fig. 2A). Both the voltammograms of Co-salophen (before and after IL tagging) were found to display two sets of well-defined redox peaks and the peaks at high negative potentials are attributed to Co(II/I) redox couple and that at the positive potentials

are assigned to Co(II/III) redox couple. In the case of Co-salophen without IL, the anodic (E_{pa}) and cathodic (E_{pc}) peak potentials for Co(II/I) redox couple were found to be -1.065 and -1.167 V respectively with a ΔE_p of 102 mV. On IL tagging, the potentials corresponding to Co-salophen-IL have shifted to -1.010 and -1.079 V respectively with a decrease in ΔE_p of 69 mV. Similarly, E_{pa} and E_{pc} for Co(II/III) redox couple of Co-salophen without IL were found to be 0.045 and -0.055 V with a ΔE_p of 100 mV and that of Co-salophen-IL have shifted to 0.090 and -0.007 V with a ΔE_p of 97 mV. It is interesting to note that both the anodic and cathodic peak currents of Co-salophen-IL for both the redox peaks are very much higher than that of Co-salophen without IL. The lesser ΔE_p along with an enhanced current response observed with Co-salophen-IL when compared to that at Co-salophen (without IL), clearly reveals that IL tagging has significantly improved the redox behaviour of the complex. The observed results indicate that Co-salophen-IL possesses better redox behaviour than Co-salophen and thus making it suitable to be explored as an electocataytic material. Henceforth, we immobilized Co-salophen-IL on ERGO and carried out further investigations to demonstrate the electrocatalytic oxidation of glucose.

The electrochemical behaviour of the modified electrode was studied stepwise during the modification process in 0.1 M NaOH at a scan rate of 50 mVs^{-1} and the results are presented in Fig. 2B. As expected, bare SPE and ERGO deposited SPE did not show any redox peak. Co-salophen-IL/ERGO/SPE has shown a pair of well-defined redox peaks with E_{pa} and E_{pc} of 0.177 and 0.129 V respectively with ΔE_p = 48 mV, which corresponds to Co(II/III) redox couple. For comparison, Co-salophen-IL has been immobilized (without ERGO) directly on SPE to form Co-salophen-IL/SPE and the corresponding voltammogram exhibited a pair of redox peaks with E_{pa} and E_{pc} of 0.197 and 0.128 V respectively with ΔE_p = 69 mV. The higher peak currents and

lesser ΔE_p observed for Co-salophen-IL/ERGO/SPE than that of Co-salophen-IL/SPE showed that ERGO contributes significantly in improving the conductivity of the Co-salophen-IL/ERGO/SPE modified electrode.

The effect of scan rate on the electrochemical behaviour of Co-salophen-IL/ERGO/SPE (Fig. S16) and Co-salophen/IL/SPE (Fig. S17) was studied by recording the CV of the modified electrodes at different scan rates from 5 to100 mVs^{-1}. Both the anodic and cathodic peak currents were found to increase with increasing scan rate and plots of anodic and cathodic peak currents against the scan rates for both the modified electrodes were found to be linear (inset to Fig. S16 and S17) indicative of a surface confined redox process.

Electrochemical impedance spectroscopy is used to analyze the charge transport characteristics at the electrode/electrolyte interface based on the Nyquist plot, which is a significant tool in monitoring the electrode modification process. Fig. 2C depicts the plot of Z' (real component) versus Z" (imaginary component) recorded in 0.1 M KCl containing 2 mM $[Fe(CN)_6]^{4-/3-}$ at the formal potential of the redox couple. The variation in diameters of the semicircles corresponds to the change in charge-transfer resistance (R_{ct}) during the modification process. The Nyquist plot for bare SPE exhibited a well-defined semicircle with R_{ct} of 223 Ω at higher frequency showing the greater impedance at the interface. Upon electrodeposition of ERGO on SPE, the R_{ct} came down to 143 Ω and this decrease in R_{ct} is attributed to the conductive nature of ERGO (Ambrosi et al., 2011). In the meantime, immobilization of Co-salophen-IL on SPE also resulted in a reasonable decrease in R_{ct} (106 Ω) than that at the bare SPE owing to the conducting nature of IL tagged complex. Furthermore, when the same Co-salophen-IL was immobilized on ERGO deposited SPE, the R_{ct} was found to decrease drastically upto 49 Ω owing to the higher conductivity offered by ERGO and IL. These studies show that

Co-salophen-IL/ERGO/SPE modified electrode exhibit an enhanced conductivity making it competent enough for the electrocatalytic oxidation of glucose.

3.3. Electrocatalytic oxidation of glucose at the Co-salophen-IL/ERGO/SPE modified electrode

In order to examine the electrocatalytic oxidation of glucose, the voltammetric response of glucose was recorded at bare SPE, ERGO/SPE, Co-salophen-IL/SPE and Co-salophen-IL/ERGO/SPE in 0.1 M NaOH at a scan rate of 50 mVs^{-1} and the results are presented in Fig. 4A. As shown in the inset to Fig. 3A, bare SPE and ERGO/SPE did not show any significant response for glucose oxidation. Upon addition of 1 mM glucose, Co-salophen-IL/SPE modified electrode has shown significant increase in anodic current due to the electrocatalytic oxidation of glucose at the Co-salophen-IL. However, with the same concentration of glucose, the anodic current of Co-salophen-IL/ERGO/SPE modified electrode increased drastically and glucose oxidation was found to occur at similar potential as that of Co-salophen-IL/SPE modified electrode. It can be noticed that in both these cases, the anodic current starts increasing at the peak corresponding to Co(II/III) redox couple of Co-salophen-IL indicative of the mediated electrocatalytic activity of the complex (Fig. 3E). When the oxidation potential of Co-salophen-IL is reached, Co(II)-salophen-IL gets oxidized to Co(III)-salophen-IL. Upon addition of glucose to the test solution, the Co(III)-salophen-IL oxidizes glucose to glucanolactone and consequently gets reduced to Co(II)-salophen-IL. The electrochemical oxidation of Co(II)-salophen-IL and the chemical oxidation of glucose to glucanolactone by Co(III)-salophen-IL occur in a cycle, as long as glucose is available at the modified electrode surface and this results in the enhancement of anodic current for every addition of glucose. The excellent electrocatalytic oxidation obtained at the Co-salophen-IL/ERGO/SPE modified electrode is attributed to the combined synergistic effect of the catalytic sites generated by the conformational flexibility of Co-salophen-IL

complex and conductivity of the IL and ERGO. The electrocatalytic oxidation occurring at Co-salophen-IL/ERGO/SPE is explained in Fig. 3E.

3.4. Amperometric response towards determination of glucose

After successful demonstration of electrocatalytic oxidation of glucose at the Co-salophen-IL/ERGO/SPE modified electrode, we examined its efficacy towards amperometric sensing. It is well known that the amperometric sensing performance is highly dependent on the operating potential and hence we have tested the amperometric response of Co-salophen-IL/ERGO/SPE modified electrode towards glucose sensing at different applied potentials. As observed from the electrocatalytic studies in Fig. 3A, the current response for glucose oxidation starts increasing from ~0.20 V and it increases continuously with the increase in potential. Thus, we have examined the amperometric response of the modified electrode for glucose sensing in 0.1 M NaOH at different operating potentials of 0.30, 0.35 and 0.40 V and the results are depicted in Fig. S19. The sensitivity was found to increase with increase in the operating potential and maximum current response was observed at 0.40 V. Though the current response could increase further with increase in the operating potential beyond 0.40 V, interference from different analytes is highly possible beyond this potential and hence we have fixed 0.40 V as the optimum potential for amperometric sensing.

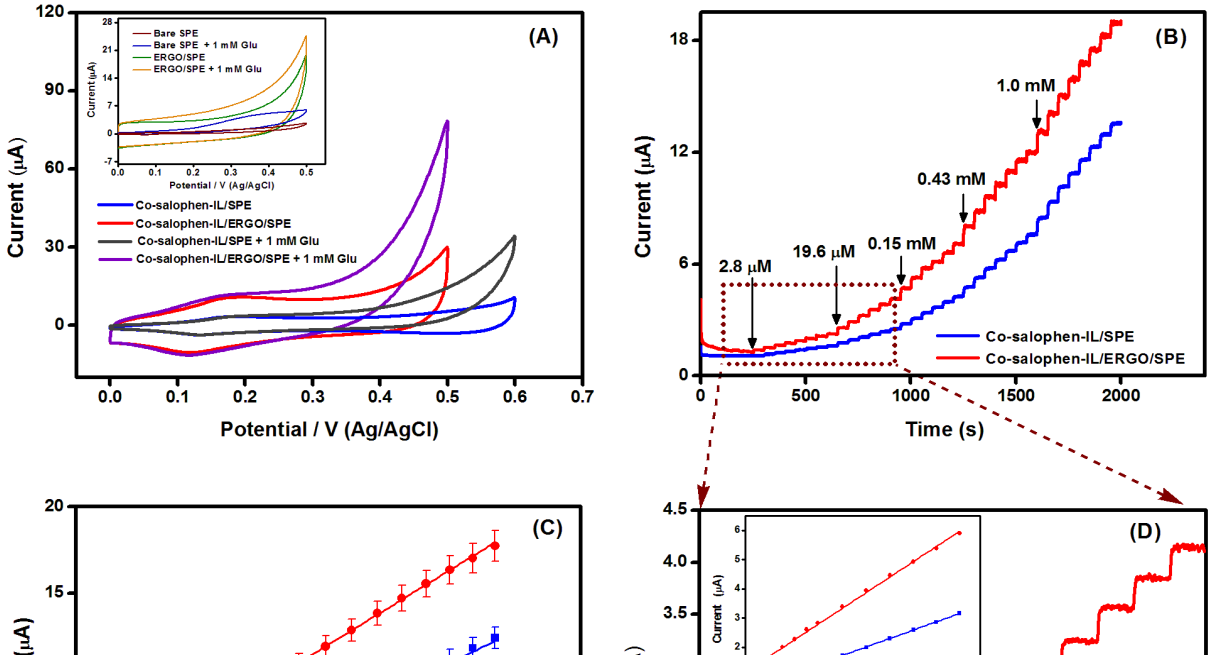


Fig. 3. (A) CVs of Co-salophen-IL/SPE in the absence (blue) and presence (grey) of 1 mM glucose, CVs of Co-salophen-IL/ERGO/SPE in the absence (red) and presence (violet) of 1 mM glucose in 0.1 M NaOH at 50 mVs^{-1} (B) Amperometric response of Co-salophen-IL/SPE (blue) and Co-salophen-IL/ERGO/SPE (red) on successive additions (interval 50 s) of different concentration of glucose into 0.1 M NaOH; Applied potential: 0.4 V (C) Calibration plots for the determination of glucose (D) Enlarged part on the amperometric response in B for low concentration of glucose and the corresponding calibration graph as inset. (E) Schematic representation of the electrocatalytic oxidation of glucose at Co-salophen-IL/ERGO/SPE.

Fig. 3B demonstrates the amperometric responses between the Co-salophen-IL/SPE and Co-salophen-IL/ERGO/SPE at an operating potential of 0.40 V towards incremental addition of different concentration of glucose into 0.1 M NaOH solution. Also, calibration plots were constructed by plotting the increase in the current response at the modified electrodes against the concentration of glucose added (Fig. 3C). As shown in Fig. 3B and 3D, Co-salophen-IL/ERGO/SPE shows relatively a better sensitivity **(**62 μA mM^{-1}**)** than Co-salophen-IL/SPE modified electrode (33 μA mM^{-1}) and the enhanced sensitivity is due to the highly conducting platform offered by ERGO. The Co-salophen-IL/ERGO/SPE exhibited an excellent response for glucose sensing over a wide linear range from 0.2 μM to 1.8 mM with a detection limit and sensitivity of 0.79 μM and 62 μA mM^{-1} respectively. The obtained parameters such as operating potential, linear range, detection limit and sensitivity are comparable with or better than the recently reported nonenzymatic glucose biosensors (Table 1).

Table 1. Comparison of the Performance of Various Nonenzymatic Glucose Sensors

Electrodes	Applied Potential (V)	Linear Range	Sensitivity	LOD (μM)	Ref.
3D porous Co_3O_4/GC	0.59	1–300 μM	471.5 μA mM^{-1} cm^{-2}	0.10	Han et al., 2015
Co_3O_4 NPs/GC	0.59	5 μM- 0.8 mM	520.7 μA mM^{-1} cm^{-2}	0.13	Hou et al., 2012
CoO/$Co(OH)_2$/MWCNTs/ GC	0.55	Up to 4.5 mM	162.8 μA mM^{-1} cm^{-2}	2.0	Yang et al., 2011

PSAC/CO_3O_4/GC	0.55	0.05 µM- 22 mM	34.23 µA mM^{-1} cm^{-2}	0.02	Madhu et al., 2015
CoO Nanorods/FTO	0.50	Up to3.5 mM	0.057 µA mM^{-1} cm^{-2}	0.06	Kung et al., 2011
CuO/r-GO/GC	0.50	0.50 µM - 3.75 mM	52.1 µA mM^{-1}	0.10	Zheng et al., 2016
CoO NPs/r-GO paste electrode	0.45	0.04 – 4 mM	1.21 µA mM^{-1} cm^{-2}	1.44	Heidari and Habibi, 2016
CoOOH nanosheets	0.40	30 µM - 0.7 mM	341 µA mM^{-1} cm^{-2}	30.9	Lee et al., 2012
PG/OPPy nanofiber Co-phthalocyanine	0.40	0.25–20 mM	5.695 µA mM^{-1} cm^{-2}	100	Ozcan et al., 2008
Co-Salophen-IL/SPE	0.40	4.8 µM to 1.8 mM	33 µA mM^{-1}	2.14	This Work
Co-Salophen-IL/ERGO/SPE	0.40	0.2 µM to 1.8 mM	62 µA mM^{-1}	0.79	This Work

3.5. Stability and reproducibility

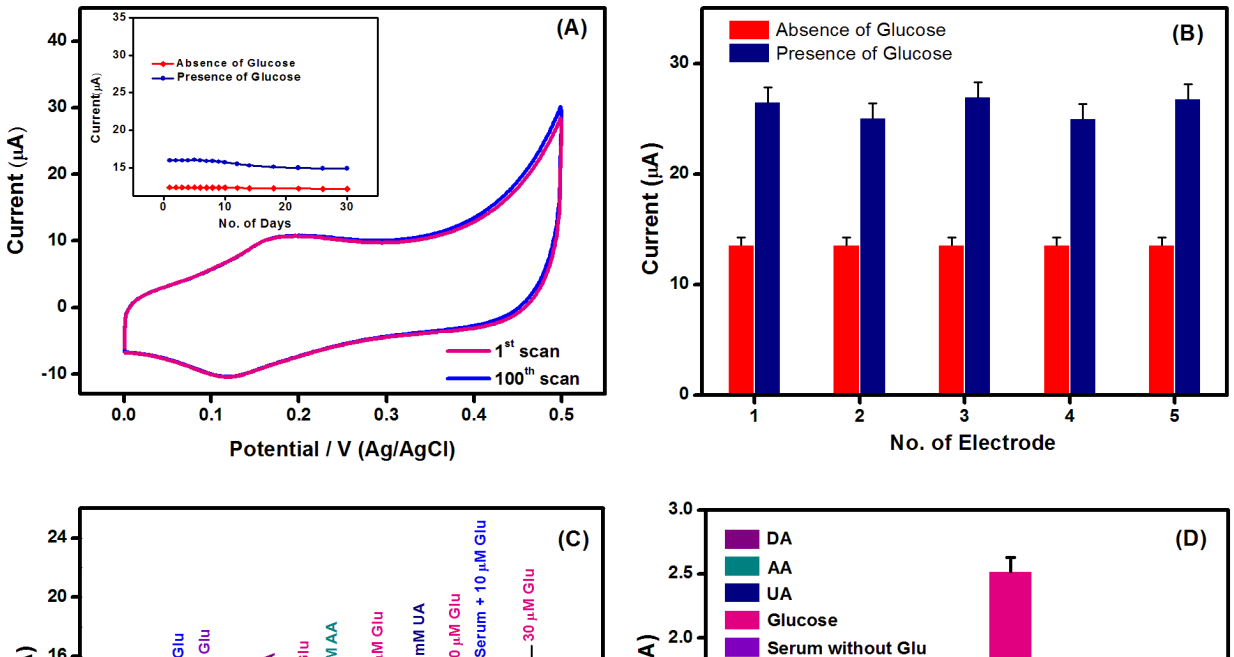


Fig. 4 (A) CV of Co-salophen-IL/ERGO/SPE in 0.1 M NaOH: (a) 1st and (b) 100th cycle at a scan rate of 50 mVs^{-1}; Inset: Plot of day-to-day current response of the biosensor (a) in the absence (red) in the presence 60 µM glucose (B) Demonstration of reproducibility of electrode fabrication and electrocatalytic oxidation (C) Influence of electroactive interferences (ascorbic acid, dopamine, uric acid and serum) on the response of 30 µM glucose (D) Current response columnar diagram of the tested analytes compared with glucose.

The operational stability of Co-salophen-IL/ERGO/SPE was verified by monitoring the voltammetric response of the modified electrode in the potential range of 0 to 0.5 V for 100 continuous cycles (Fig. 4A). The electrode exhibited remarkable stability without any significant change in the peak current or peak potential. The long-term stability of the sensor was tested in the absence and presence of glucose on alternative days for one month and shown as inset to Fig. 4A. The Co-salophen-IL/ERGO/SPE retained about 98 % of its original current in the absence of glucose and 93 % of its response towards oxidation of 60 µM glucose on the 30th day, which shows that the electrode possesses remarkable stability for glucose sensing.

Further, we have evaluated the reproducibility of Co-salophen-IL/ERGO/SPE by fabricating 5 different electrodes using similar procedure and comparing their electrocatalytic oxidation current response in presence of 1 mM glucose. Current responses measured with 5

different Co-salophen-IL/ERGO/SPE (Fig. 4B) exhibited a relative standard deviation (RSD) of 3.7 % revealing a very good electrode-to-electrode reproducibility. In the meantime, five measurements using same Co-salophen-IL/ERGO/SPE exhibited very good reproducibility with a relative standard deviation (RSD) of 3.3 %, confirming the reproducibility of our measurement. The observed results make Co-salophen-IL/ERGO/SPE as a highly promising sensor towards the nonenzymatic determination of glucose.

3.6. Interference studies

The selective oxidation of glucose is another key issue in determining the suitability of the biosensor. Therefore, the selectivity of Co-salophen-IL/ERGO/SPE for glucose biosensing was tested in presence of easily oxidisable interferents like ascorbic acid, uric acid and dopamine. It can be observed from the amperometric studies (Fig. 4C) and columnar diagram (Fig. 4D), that the addition of 0.3 mM ascorbic acid, 0.3 mM dopamine, 0.3 mM uric acid and glucose free serum sample show a slight or no increase in the current response. It is obvious that the concentration of glucose (between 4 and 7 mM) in human blood is at least 10 times higher than that of these interfering species and this slight increase in the current response can be considered as negligible. On the other hand, a drastic increase in current response is observed upon the addition of 30 µM glucose and serum sample containing 10 µM of glucose. This observation indicates that Co-salophen-IL/ERGO/SPE exhibits a good selectivity towards glucose sensing making it suitable for determination of glucose even in real samples. The selectivity is attributed to the less operating potential offered by the mediated electrocatalytic activity of Co-salophen-IL towards the oxidation of glucose. Though the exact reason for the selective electrocatalytic oxidation is not clear at present, the selectivity can be attributed to the

conformational flexibility and structural tunability offered by the IL tagged salophen ligand, which could have generated appropriate active sites to facilitate the oxidation of glucose.

3.7. Determination of glucose in real samples

In order to examine the utility of Co-salophen-IL/ERGO/SPE in real time analysis, glucose determination was carried out in real biological samples such as human blood serum and urine (Table S1, SI). The amperometric response of Co-salophen-IL/ERGO/SPE for the determination of glucose in real samples was recorded in 0.1 M NaOH at an operating potential of 0.40 V (Fig. S20). To the real samples obtained with certified concentration from the clinical laboratory, a known concentration of glucose was spiked and 1 mL of the spiked real sample was added to 9 mL of stirring 0.1 M NaOH at 100^{th} second and the amperometric responses for different samples were recorded. The concentration of glucose detected in human blood serum and urine samples using the prepared nonenzymatic sensor agrees well with the certified concentrations and the obtained recovery values are in the range from 92.1 % to 103 %. Thus, it can be concluded that Co-salophen-IL/ERGO/SPE modified electrode can efficiently determine glucose in real samples.

4. Conclusion

We have prepared a bioinspired ionic liquid tagged Co-salophen metal complex and successfully demonstrated its electrocatalytic activity towards oxidation of glucose for the first time. This durable nonenzymatic biosensor based on Co-salophen-IL/ERGO/SPE exhibited glucose sensing at a very less operating potential of 0.40 V with an excellent sensitivity and low detection limit of 62 μA mM^{-1} and 0.79 μM respectively. Notably, the modified electrode has shown high selectivity, good stability and reproducibility. The competent analytical

characteristics achieved by our biosensor is attributed to the combined synergistic effect of the electrocatalytic sites generated by the conformational flexibility of ionic liquid tagged Co-salophen complex and conductivity of the IL and ERGO. The present work opens an avenue for designing new variety of new ionic liquid tagged metal complexes by tuning the transition metal, ligand system and IL, which could find important applications not only in biosensors but also in many other electrochemical devices.

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Supplementary Information

Procedures for the synthesis of 1-ethylbenzimidazole, 5-chloromethyl-2-hydroxybenzaldehyde and GO, TEM image of GO, Raman spectra of GO and ERGO, ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectra and HRMS data of synthesized compounds, CVs recorded for the demonstration of effect of scan rate, CVs demonstrating the electrocatalytic oxidation of glucose along with the linear increase in peak current upon addition of glucose, and Amperometric sensing performance of the biosensor at different operating potentials

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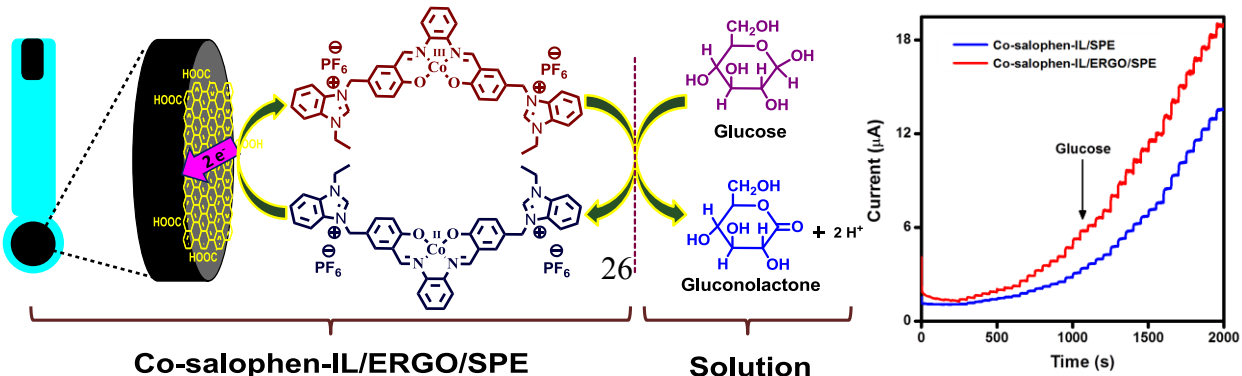
Graphical Abstract



Highlights

- A bioinspired ionic liquid tagged Co-salophen complex was synthesized
- Nonenzymatic biosensor for glucose was developed by immobilizing the complex on ERGO
- Glucose detection carried out at less potential of 0.4 V eliminates the interference
- Role of Co-salophen, IL and ERGO in the electrocatalytic activity was investigated