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A Cu(II) complex of an imidazolium-based ionic liquid: synthesis, X-ray structure and application in the selective electrochemical sensing of guanine†

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An imidazolium-based ionic liquid containing a carboxylic acid group was synthesized and complexed with Cu(II). The resulting complex **R1** was fully characterized using various techniques, including IR spectroscopy and X-ray crystallography. Binding studies of the complex **R1** were performed with anions and biomolecules using cyclic voltammetry, which showed no change in its voltammogram upon the addition of various anions and most biomolecules. However, a shift in the reduction peak from +0.20 to −0.15 was observed upon the addition of guanine. This selective determination of guanine by **R1** was extended by using **R1** as an electrochemical sensor for guanine in various voltammetric techniques, including cyclic voltammetry, LSV and DPV. The proposed sensor showed excellent reproducibility and high selectivity and sensitivity towards guanine, with a linear range of 0–20 μM and a detection limit of 45 nM.

Introduction

The specific detection of biomolecules such as amino acids, nucleic bases, and DNA has numerous applications in medical diagnostics.[1,2] The separation and determination of the concentrations of individual nucleic acids and their ratios in DNA is a challenging task.[3,4] It can be achieved by the use of novel artificial receptors based on organic supra-molecular hosts[5–8] or metal complexes.[9–14] Many artificial receptors based on urea/thio-urea, benzimidazole and calixarene have been reported.[15–19] Also, various detection techniques have been developed for sensing biomolecules, such as spectrophotometry, fluorescence spectrometry, and electrochemical methods.[20–23] These techniques have been well explored in the detection of metal ions and inorganic anions;[24] still, biomolecule detection is a challenging field in this research arena. These analyte molecules have diverse shapes and sizes, so the respective receptors need a lot of engineered structures for compatibility with their binding sites. This problem can be addressed by using a metal complex in tandem with hydrogen bond donor sites for the organic receptor. Due to their surface smoothness, metal ions could not bind selectively with a particular guest. The cation could offer electrostatic interaction to facilitate the binding of analytes in aqueous medium, while hydrogen bonds could provide the binding sites required to ensure sensor selectivity.[25]

In the last decade, many research articles have been reported on imidazole and benzimidazole-based receptors that act as sensors for anions and cations.[26,27] Kim *et al.* have reported imidazolium cation-based receptors for the detection of anions and biomolecules.[28] Recently, our group has reported the benzimidazole-based cobalt(III) complex for selective detection of iodine using an electrochemical method.[29] Inspired by these results, we decided to explore an imidazolium ion liquid-based copper complex for such an electrochemical sensor activity. Imidazolium ion liquids show some interesting properties due to the availability of sites for hydrogen bonding. They are believed to be able to interact with anions and biomolecules through hydrogen bonding. In this regard, an imidazolium ionic liquid-containing carboxylic acid group was synthesized by substitution reaction of bromoacetic acid with *N*-methylimidazole. Upon complexation with copper metal, it forms a cavity large enough to interact with anions or biomolecules through electrostatic interactions with the metal ion as well as through hydrogen bonding with the imidazolium cation. To the best of our knowledge, there has been no report on a complex of imidazolium ionic liquid-based electrochemical sensor for the detection of biomolecules; thus this system presents an improvement over the existing sensors in the literature.

Result and discussion

Syntheses

Ligand **1** was synthesized by nucleophilic substitution reaction between *N*-methyl imidazolium and bromoacetic acid. The product was characterised using ^{1}H and ^{13}C-NMR. Ligand **1**

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was complexed with copper perchlorate in methanol–water (50 : 50) and fully characterised using single-crystal X-ray crystallography.

X-ray structure determination

The X-ray diffraction data for compound **R1** were collected on a Bruker X8 APEX II KAPPA CCD diffractometer at 100 K using graphite-monochromatized Mo-Kα radiation (λ = 0.71073 Å). The crystals were positioned at 50 mm from the CCD, and the diffraction spots were measured using a counting time of 10 s. Data reduction and multi-scan absorption were carried out using the APEX II program suite (Bruker, 2007). The structures were solved by direct methods with the SIR97 program[30] and refined using full-matrix least squares with SHELXL-97.[31] Anisotropic thermal parameters were used for all non-H atoms. The hydrogen atoms of C–H groups had isotropic parameters equivalent to 1.2 times those of the atom to which they were attached. All other calculations were performed using the programs WinGX[32] and PARST.[33] The molecular diagrams were drawn with DIAMOND.[34] Final *R*-values, together with selected refinement details, are given in Table S1.†

Structure description. The compound **R1** crystallizes in an orthorhombic crystal system with *Pnnm* space group. The complex consists of one cationic moiety $[Cu_2C_{24}H_{36}N_8O_{10}]^{4+}$, four anionic moieties (ClO_4^-) and four water molecules of crystallization. The anionic moieties and water molecules are highly disordered, and both are present at three (0.50, 0.25, 0.25) positions each. **R1** is a binuclear molecule possessing a paddle wheel conformation similar to copper acetate. Each copper atom is six-coordinated in an octahedral fashion. The valency around the copper center is satisfied by four equatorial oxygen atoms belonging to four carboxyl groups (Table S1†) with $Cu–O_{eq}$ distance that ranges from 1.966(2) to 1.968(2) Å. The O atoms at the apical positions belong to coordinated water molecules with a $Cu–O_{water}$ bond length of 2.163(5) Å. This elongation in bond length is due to the Jahn Teller distortion observed in copper(II) complexes. The Cu–Cu distance is small [2.642(1) Å]; hence it is also considered as a bond. The ORTEP diagram, along with the atom numbering scheme, is shown in Fig. 1 (the anionic moieties and water molecules are removed for clarity). In the further packing description of the structure, the water molecules and anion were not considered, due to their disorder. When the packing of the molecules is viewed down the *c* axis, the complex cations are arranged in the form of two different types of alternate layers, as shown in Fig. 2. Here, only C–H···O interactions were observed between complex cations.

Binding study with anions and biomolecules

The interaction of **R1** with tetrabutylammonium salts of various anions (F^-, Cl^-, Br^-, I^-, NO_2^-, CN^-, CH_3COO^-, HSO_4^- and $H_2PO_4^-$) was investigated by cyclic voltammetry (CV) in DMSO–H_2O (50 : 50) using tetrabutylammonium perchlorate (0.1 M) as the supporting electrolyte. The CV profile of **R1** showed a reduction peak at 0.20 V (Fig. 3). Upon addition of various anions to the **R1** solution, no change in the electrochemical signal was observed. Similar results were perceived on the addition of biomolecules (adenine, guanine, cytosine, thymine, uracil, ADP, ATP, NAD, NADH, NADP and glycine) (Fig. 3), except in the case of guanine, in which the reduction potential was changed from +0.20 V to −0.15 V. To verify its interaction with guanine, titration was performed with cyclic voltammetry and linear sweep voltammetry (Fig. 4 and 5).

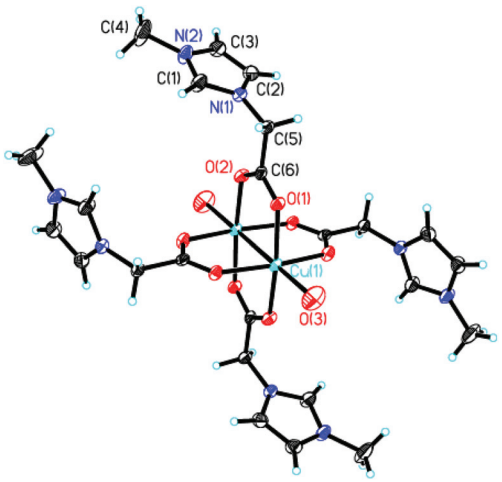


Fig. 1 ORTEP diagram of compound **R1** with 40% probability thermal ellipsoids and the atom-numbering scheme. Disordered water molecules and anions have been removed for clarity.

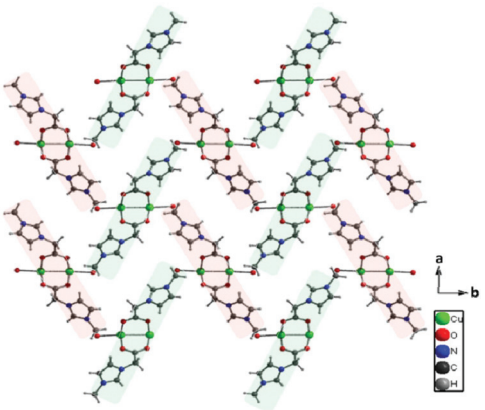


Fig. 2 Packing diagram of **R1** down the *c* axis, showing the arrangement of complex cations in the lattice.

The shift in the reduction peak from +0.20 V to −0.15 V can be purely attributed to the interaction of guanine with **R1** and the formation of a new complex that is altogether different from both parent species and has its own electrochemical profile (Fig. S-4 in the ESI†). To check the ability of **R1** as a sensor for guanine, titration of **R1** (10 μM) was performed by successive addition of 10 μL of guanine (1 mM) using various voltammetric techniques, including cyclic voltammetry, differential pulse voltammetry and linear sweep voltammetry.

During titration, it was observed that the shift in the peak is gradual in the case of cyclic voltammetry and linear sweep voltammetry (Fig. 5), while in the case of DPV a ratiometric

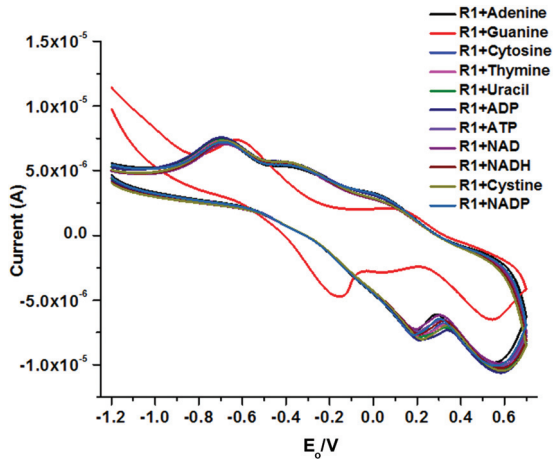


Fig. 3 Changes in the CV profile of **R1** (10 μM) upon addition of various biomolecules (20 μM) in DMSO–H_2O (50 : 50).

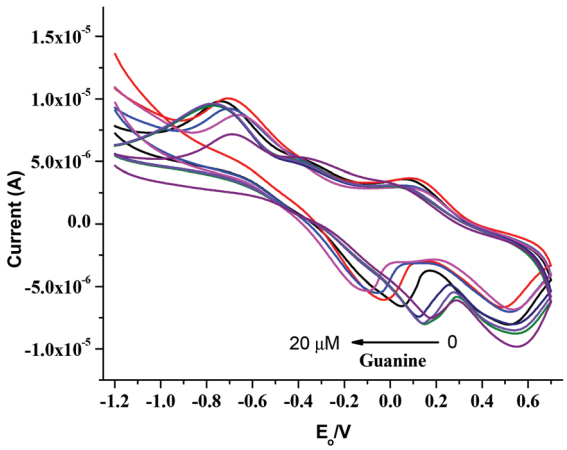


Fig. 4 Changes in the CV profile of **R1** (10 μM) upon continuous addition of guanine (0–20 μM) in DMSO–H_2O (50 : 50).

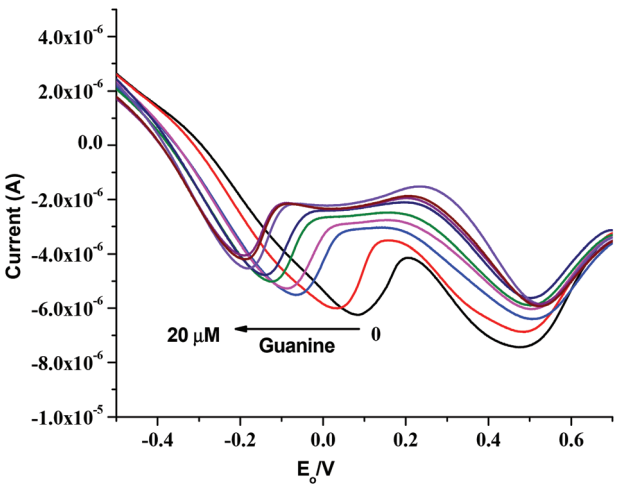


Fig. 5 Changes in the LSV profile of **R1** (10 μM) upon continuous addition of guanine (0–20 μM) in DMSO–H_2O (50 : 50).

variation in peak was observed; *i.e.*, the peak at −0.248 V increased gradually with the successive addition of guanine, while the peak at around 0.0 V decreased (Fig. 6), clearly indicating the gradual interaction of guanine with **R1** and the gradual formation of a new complex upon their interaction.

Differential pulse voltammetry can detect even closely spaced species having a difference of 0.01 V and can improve the detection limit up to a thousand times as compared to cyclic voltammetry. Although the results obtained by all the techniques were quite similar to each other, differential pulse voltammetry had the edge, with a better detection limit of 45 nM as compared to using cyclic voltammetry. A linear response was obtained between $I_{-0.23}/I_0$ and the concentration of guanine (Fig. 7). The limit of detection was determined using 3σ method:

$$\mathrm{DL} = \frac{3 \times \mathrm{S.D.}}{m}$$

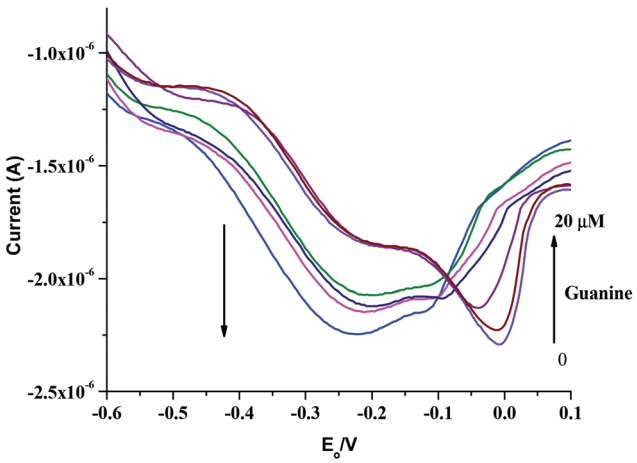


Fig. 6 Changes in the DPV profile of complex **1** (10 μM) upon continuous addition of guanine (0–20 μM) in DMSO–H_2O (50 : 50).

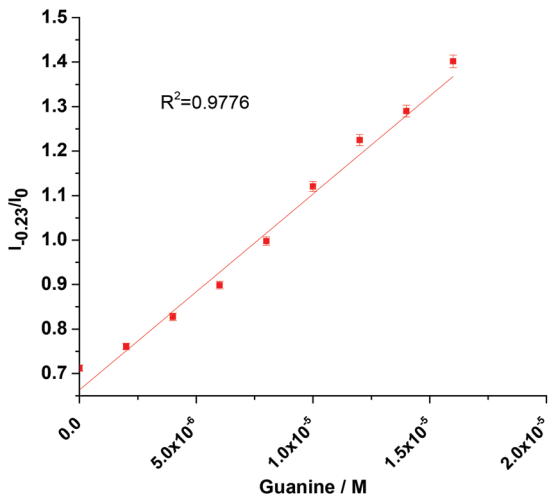


Fig. 7 Linear regression graph between concentration of guanine and $I_{-0.23}/I_0$ obtained by differential pulse voltammetry titration.

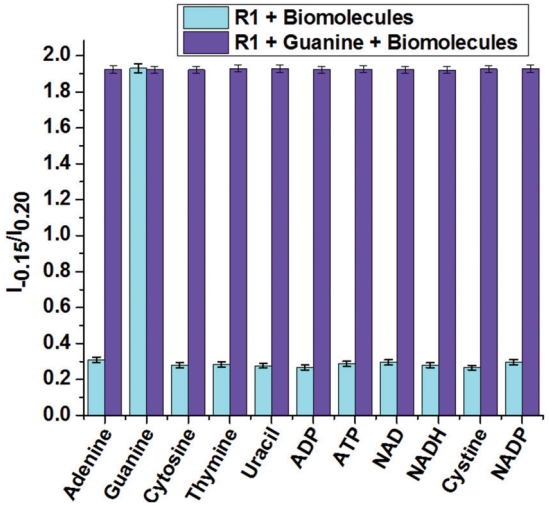


Fig. 8 Change in reduction potential of **R1** in CV profile upon the addition of guanine in the presence of various interfering species.

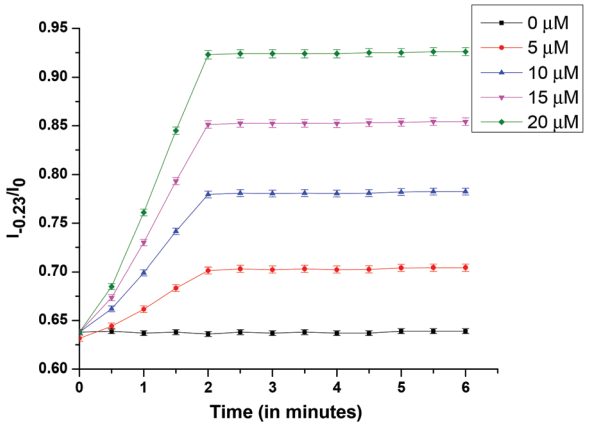


Fig. 9 Plot of current ratio of complex **R1** and guanine at different concentrations of guanine (0, 5, 10, 15 and 20 μM) as a function of time (minutes).

where S.D. is the standard deviation of the blank signal and *m* is the slope of the calibration curve.

To further extend the usage of the proposed sensor, competitive binding experiments were performed to estimate guanine in the presence of adenine, guanine, cytosine, thymine, uracil, ADP, ATP, NAD, NADH, NADP and glycine (Fig. 8). No significant change in electrochemical profile of the guanine-**R1** complex was observed in the presence of any other biomolecules. Hence, the proposed sensor can be used to determine guanine with high sensitivity and selectivity, *i.e.*, without any interference from any of the competitive biomolecules.

Interference studies were also carried out using some derivatives of guanine, *i.e.*, 7-methylguanine, 9-ethylguanine, 1-methylguanine and 6-*O*-methylguanine, in the presence of guanine (Fig. S8†). No change in the cyclic voltammogram of the guanine-**R1** complex was observed. Hence, none of the derivatives of guanine interfere in its determination. This can be attributed to the fact that alkylation of guanine at various positions does not allow hydrogen bonding and electrostatic interaction to take effect. Therefore, no interaction with **R1** is observed.

To check the effect of ionic concentration on **R1** response, the CV profile of **R1** was recorded with successive additions of perchlorate salt (0–200 equiv.) under the same concentration of **R1** and the same solvent system. It was observed that even 200 equivalents of perchlorate salt had no effect on the CV response of **R1**. The response of **R1** with guanine was also studied as a function of time by monitoring the changes in reduction peak in DPV with different concentrations of guanine (0, 5, 10, 15 and 20 μM) added to complex **R1** solutions of fixed concentration (10 μM), and DPV profiles were recorded after small time intervals (Fig. 9). The results showed that guanine interacts with complex **R1** within the first 2 minutes, and after this no change in the reduction potential is observed with increased time, even up to 6 minutes. The results of the proposed sensors were compared with various sensors recently reported in the literature. It was found that most of the reported sensors used either a modified electrode or some hybrid material as the working electrode; however, in our proposed sensor a better detection limit was achieved using the bare platinum electrode (Table S4†).

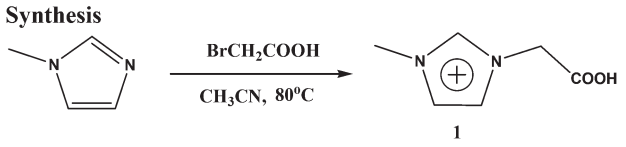
Conclusion

In the work reported here, a Cu(II) complex (**R1**) of synthesized imidazolium ionic liquid containing a COOH group was characterized by FT-IR and single-crystal X-ray crystallography. Further, **R1** was studied as an electrochemical sensor for guanine using various voltammetric techniques, including CV, DPV, *etc.* The studies revealed a great deal of selectivity and sensitivity in the determination of guanine, with a detection limit of 45 nM and a dynamic range of 0–20 μM.

Experimental section

General information and materials

All chemicals were purchased from Aldrich Co. and used as received without further purification. ^{1}H NMR spectra were recorded using a Jeol instrument, which was operated at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR. IR spectra of solid-state compounds were recorded using a Bruker Tensor 27 spectrometer with KBr discs. Mass spectra were recorded using a Waters Micromass Q-T of Micro. The pH measurements were carried out on ME/962P instrument. The redox properties were evaluated using a Potentiostat-Galvanostat BASI EPSILON, with a three-electrode system (Pt disk was used as the working electrode, a platinum wire was used as the counter electrode, and Ag/AgCl was used as the reference electrode), and tetrabutylammonium perchlorate was used as the



Scheme 1 Synthesis of imidazolium ionic liquid.

supporting electrolyte. The X-ray diffraction data for KPK 102 were collected on a Bruker X8 APEX II KAPPA CCD diffractometer at 100 K using graphite-monochromatized Mo-Kα radiation (λ = 0.71073 Å).

Synthesis procedure

Synthesis of ionic liquid. 1-Methyl imidazole (8.4 gm, 10 mmol) and bromoacetic acid (13.8 g, 10 mmol) were refluxed at 80 °C in dry acetonitrile (Scheme 1). White oil was separated out; thereafter, the solvent was evaporated on a rotary evaporator. The resulting colorless oil constituted a pure bromide salt of imidazolium ionic liquid with the following properties: ^{1}H NMR (400 MHz, DMSO, ppm) δ: 3.90 (s, 3H, CH_3), 5.12 (s, 2H, CH_2), 7.57–7.64 (d, 2H, ArH), 9.41 (s, 1H, ArH), ^{13}C NMR (100 MHz, D_2O, ppm) δ: 36.39, 50.07, 123.66, 124.13, 137.89, 168.58.

Synthesis of Cu(II) complex of imidazolium ionic liquid. Copper(II) perchlorate (10 mmol, 3.7 mg) was dissolved in methanol–water (50 : 50) (15 mL), followed by the addition of a methanolic solution (10 mL) of **1** (20 mmol, 2 mg), and was stirred for 30 min. The solution was left to allow slow evaporation. Green crystals of $[Cu_2C_{24}N_8O_{10}H_{36}](ClO_4)_4$, suitable for X-ray structure determination, were deposited after 12 hours. FT-IR results showed the following: ν_{max}: 3453 (COOH), 3111 (=CH), 3092 (=CH), 3066 (=CH), 3042 (CH_3), 1916, 1843, 1658 (C=O), 1605, 1573, 1489, 1384, 1237 (C–O), 1220, 1152, 867, 755.

Recognition studies. The recognition studies were performed at 25 ± 1 °C, and the solutions were shaken for a sufficient time before recording the voltammogram. The binding ability of **R1** (10 μM) in DMSO–water was determined by adding 20 μM of a tetrabutylammonium salt of the anion and the various biomolecules to a 5 mL solution of **R1** taken in volumetric flasks. The volumetric flasks were allowed to stand for 30 minutes before the voltammograms were recorded. To evaluate any possible interference due to different biomolecules for the estimation of guanine, solutions were prepared containing **R1** (10 μM) both with and without other interfering biomolecules (20 μM). The effect of ionic strength was explored by recording the voltammogram at different concentrations of tetrabutylammonium perchlorate (0–200 equivalent).

Acknowledgements

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