Intracellular detection of toxic hypochlorite anion by using a nontoxic rhodamine based sensor

Dibyendu Sain\textsuperscript{a}, Shyamaprosad Goswami*\textsuperscript{a} and Chitrangada Das Mukhopadhyay\textsuperscript{b}

\textsuperscript{a}Department of Chemistry, \textsuperscript{b}Department of Centre for Healthcare Science & Technology, Indian Institute of Engineering Science and Technology, Shibpur, Howrah-711 103, West Bengal, India

\textit{E-mail} : spgoswamical@yahoo.com

\textit{Manuscript received 21 April 2017, accepted 08 May 2017}

Abstract: A simple bio-compatible 2,4-dinitrobenzene and rhodamine coupled probe was designed and synthesized for the detection of highly toxic hypochlorite ion with high selectivity and sensitivity. Practical applicability of the sensor was established through simple and fast naked-eye detection of ClO\textsuperscript{−}. Absorption and emission spectra proved the detection ability of the sensor towards ClO\textsuperscript{−}. The non toxic behaviour of the sensor in living cells increased its practical applicability and it was effectively applied in intracellular detection of toxic ClO\textsuperscript{−} in living cells.

Keywords: Hypochlorite sensor, Rhodamine hydrazide, naked-eye, intracellular detection, DFT computation.

Introduction

Hypochlorite anion (OCl\textsuperscript{−}) is commonly used in daily life as a bleaching agent, disinfectant for drinking water, cooling-water treatment and cyanide treatment in the millimolar to micromolar concentration range\textsuperscript{1}. However, high level of hypochlorite accumulation causes a lot of human diseases, such as cardiovascular diseases, atherosclerosis, lung injury, neuron degeneration, kidney disease, arthritis, and cancer\textsuperscript{2,3}. Therefore, the detection and precise determination of HOCl in the biological systems is the new challenge for the researchers. Among different sensitive and selecte methods, fluorescent probes are superior in this respect due to their high sensitivity and fine resolution capability\textsuperscript{4}. Moreover, colorimetric chemodosimeters are widely used for the detection of target analytes quantitatively by the naked-eye as well as \textit{in vivo} cell imaging technique\textsuperscript{5}. Previously, some useful fluorescent sensors have been reported for the detection of HOCl\textsuperscript{6,7}. But, most of the reported probes for hypochlorite suffer from poor selectivity and sensitivity, very high response time, and practically unsuitable for biological application\textsuperscript{8}. Therefore, the construction of a novel fluorescent chemodosimeters for the easy and accurate detection of hypochlorite in the living cells pays great attention.

Rhodamine based sensors are widely used for biological application due to their well-established photophysical properties such as longer excitation and emission wavelengths, high fluorescence quantum yield etc.\textsuperscript{9}. The spirolactam ring closed form of rhodamine moiety is normally non-fluorescent and by the influence of the guests (mostly metal ions) ring-opening state exists to show strong fluorescent character\textsuperscript{10}. Till now, there are only few reports of using rhodamine moiety as an effective structural scaffold for designing fluorescent chemodosimeters of hypochlorite\textsuperscript{11}.

Herein, a rhodamine based sensor coupled with 2,4-dinitrofluorobenzene (S1) was designed and synthesised (Scheme 1). The molecule was characterised by NMR and Mass spectroscopy.
Results and discussion

The sensing ability of the sensor (S1) was studied with various anions like F–, Cl–, Br–, I–, CN–, AcO–, HSO4–, H2PO4–, ClO– in 40% ethanol in water media. S1 displayed visual colour change from colourless to pink instantly after the addition of 1.0 equivalent of ClO– (Fig. 1). In contrast, other anions did not exhibit any colour change with S1. UV-Vis absorbance spectrum showed the peaks at 319 nm for only S1 \((1.4 \times 10^{-5} \text{M})\) but after gradual addition of ClO–, a new peak was generated at colour region (559 nm) with increasing peak intensity (Fig. 2). The bathochromic shift \((\lambda_{\text{max}} 319 \text{ to } 559 \text{ nm})\) in UV-Vis electronic spectra strongly supports the interaction of S1 with ClO–. This new peak was the indication of spirolactam ring opening form of rhodamine part by the reaction with hypochlorite. No such spectral change was observed in the presence of other anions.

The non-fluorescent solution of S1 turned to red fluorescent in the presence of ClO– under long wave-length UV light (Fig. 3). The solution of S1 remained unchanged with the other anions. The visual fluorescence change could be easily observed from the emission spectra of S1 \((1.7 \times 10^{-6} \text{M})\) in 40% ethanol in
Sain et al.: Intracellular detection of toxic hypochlorite anion by using a nontoxic rhodamine based sensor.

Water. The emission peak of S1 was visualized at 439 nm ($\lambda_{\text{exc}}$ 350 nm) but after addition of ClO$^-$, a new peak at 584 nm was generated and its intensity increased with the gradual addition of ClO$^-$ up to 1 equivalent (Fig. 4). No such spectral change was observed for other anions. Visually detectable red fluorescence of S1 in the presence of ClO$^-$ only along with the generation of a new emission peak at 584 nm ($\lambda_{\text{exc}}$ 350 nm) with ClO$^-$ also concluded that S1 is a selective and sensitive fluorosensor of ClO$^-$. 

**Probable mechanism for sensing of hypochlorite**

Based on the previously reported mechanism$^{12}$, we supposed that HClO promoted chlorination reaction took place followed by the opening of the spirolactam ring in S1. Here, the hydrazide moiety of S1 was oxidized by ClO$^-$ to form a diimide intermediate (Scheme 2), which experienced further hydrolysis to produce the strong chromogenic and fluorogenic rhodamine B. The rate of hydrolysis was dependent on solvent because water is highly responsible for the hydrolysis of the diimide intermediate.

**Cytotoxicity test**

Cytotoxicity test of S1 was performed to know whether the compound was toxic or not. The toxicity level and practical applicability of the molecule was carried out using MTT assay (Fig. 5). About 91% of these cells remained viable even upon addition of 30

---

**Fig. 4.** Fluorescence emission spectra of S1 with 1.0 equivalent ClO$^-$ in 40% ethanol in water.

**Scheme 2.** Reaction of S1 with ClO$^-$. 

---

675
μM concentration of S1 for 24 h which suggested that the toxicity level of S1 up to 30 μM was low enough and it could be easily applied for any biological activity including cell imaging study.

**Fluorescence cell-imaging study**

The long wavelength fluorescence emission property of the S1 was applied for intracellular hypochlorite detection in living RAW 264.7 cells, which proves the superiority of the sensor. To identify ClO\(^-\) in living cell, at first 5 μM of S1 was incubated into the cells (5% EtOH in H\(_2\)O) in Dulbecco’s modified Eagle’s medium (DMEM) for half an hour at 37 ºC. After half an hour, 5 μM solution of ClO\(^-\) was incorporated with the cell and incubated for again half an hour. Then fluorescence images were captured after processing the cells in the proper way. S1 containing cells did not show any fluorescent image in red channel of fluorescence microscope but the cells incubated with both S1 and ClO\(^-\) showed a bright red fluorescent in the red channel (Fig. 6). This observation suggested that S1 could be used as a real time useful molecule for detection of ClO\(^-\) in living cells.

**Quantum chemical DFT calculation**

Quantum chemical DFT calculation was performed using Gaussian 09 program with the help of GaussianView 5.0 visualization program\(^{13}\) to investigate the structural aspects of S1. The structures were optimized theoretically using B3LYP/6-311G+(d,p) basis set. The energy optimized structure of S1 suggested that 2,4-dinitrobenzene moiety was almost parallel to the xanthene part of the rhodamine moiety to maintain the stability of the system (Fig. 7a). The optimization energy value of S1 (−2095.6415 a.u.) suggested its very high stability. Molecular electrostatic potential (MEP) diagram displayed two different colour regions, where red colour signified the electro negative regions and blue colour was the indication of positive regions (Fig. 7b). Electron rich red regions were mainly distributed around the most electronegative centre of the molecule such as N and O atoms. These regions were responsible for easy deprotonation of the NH group by the influence of ClO\(^-\).

The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) diagram of S1 along with their spatial distributions were
Sain et al. : Intracellular detection of toxic hypochlorite anion by using a nontoxic rhodamine based sensor

displayed in Fig. 8. From the diagram of S1, it was cleared that the xanthene moiety contained HOMO orbitals whereas 2,4-dinitrobenzene moiety contained LUMO orbitals (Fig. 8). The orbital diagrams suggested the probable energy transfer from HOMO to LUMO i.e. from xanthene part to 2,4-dinitrobenzene part of S1.

Conclusion

As a conclusion, a simple bio-compatible 2,4-dinitrobenzene and rhodamine coupled probe was developed for the detection of highly toxic hypochlorite ion with high selectivity and sensitivity. Practical applicability of the sensor was established through simple and fast naked-eye detection of ClO⁻. The detection of ClO⁻ was carried out using UV-Vis and fluorescence spectral experiments. The non toxic behaviour of the sensor in living cells increased the practical applicability of S1 and it was effectively applied in intracellular detection of toxic ClO⁻ in living cells. Quantum chemical DFT calculation displayed the structural aspects of S1. So, this sensor might be useful in future for in vivo detection of ClO⁻ infected cells in the human body.

Experimental

All reagents (AR grade) for synthesis were purchased from Sigma-Aldrich chemicals commercially and used without further purification. Solvents were dried following standard procedures. ¹H NMR spectra
were recorded on a Bruker AV300 instrument using CDCl3 with TMS as an internal standard. ESI-MS measurements were carried out using a microTOF-Q II 10337 mass spectrometer instrument. UV-Vis spectra and fluorescence spectra were recorded using JASCO UV spectrometer (1.0 cm quartz cell) and Perkin-Elmer LS 55 fluorescence spectrometer, respectively. Fluorescence images of living cells were captured using epifluorescence microscopy (Carl Zeiss).

**Synthesis of compound 1**:
Rhodamine B (1 g, 2.88 mmol) in dry ethanol and excess of hydrazine (2 ml) was taken in a rb flask and the reaction mixture was refluxed for 12 h. A light pink precipitate was obtained. The precipitate was filtered and washed with ethanol for several times to obtained pure compound 1.

**Synthesis of S1**:
Compound 1 was taken in dry DMF and 2,4-dinitrofluorobenzene was added to it. The mixture was stirred at room temperature for 12 h. The mixture was then poured into ice water to get precipitate. The precipitate was then filtered and dried followed by purification through column chromatography to obtain pure S1.

**Characteristic experimental data of S1**:

1H NMR (300 MHz, CDCl3): δ 8.87 (1H1, s), 7.96 (1H2, d, J=7.5 Hz), 7.75 (1H3, d, J=6.9 Hz), 7.63–7.52 (2H4, m), 7.27 (2H5, d, J=7.5 Hz), 6.59 (2H6, d, J=9.3 Hz), 6.40 (1H7, s), 6.24–6.22 (4H8, m), 3.19 (8H9, s), 1.03 (12H10, s); 13C NMR (125 MHz, CDCl3): δ 165.6, 154.1, 149.0, 148.5, 148.4, 137.9, 133.7, 130.9, 129.6, 128.8, 128.2, 124.4, 123.5, 122.4, 116.4, 107.9, 97.4, 67.4, 44.1, 12.0; ESI-MS (ES+) (m/z): 623.41 (M+1).

**Procedure for cytotoxicity assay**
The cytotoxic effects of the sensor S1 and S1+ NaOCl were determined by an MTT assay following the manufacturer’s instructions (MTT 2003, Sigma-Aldrich, St. Louis, MO, USA). HCT cells were cultured into 96-well plates (approximately 104 cells per well) for 24 h. On the following day, media were removed and various concentrations of S1 and S1+ NaOCl (10, 25, 30, 50, 75, and 100 μM) made in DMEM were added to the cells and incubated for 24 h. Solvent control samples (cells treated with DMSO in DMEM), no cells and cells in DMEM without any treatment were also included in the study. Following incubation, the growth media were removed, and fresh DMEM containing MTT solution was added. The plate was incubated for 3–4 h at 37 ºC. Subsequently, the supernatant was removed, the insoluble colored formazan product was solubilized in DMSO, and its absorbance was measured in a microtiter plate reader (Perkin-Elmer) at 570 nm. The assay was performed in triplicate for each concentration of sensor S1 and S1+NaOCl. The OD value of wells containing only DMEM medium was subtracted from all readings to remove background influence. Data analysis and the calculation of standard deviation were performed with Microsoft Excel 2007 (Microsoft Corporation).

**Fluorescence cell-imaging procedure**
Frozen human colorectal carcinoma cell line HCT 116 (ATCC: CCL-247) was obtained from the American Type Culture Collection (Rockville, MD) and maintained in Dulbecco’s modified Eagle’s medium (DMEM, Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum (Invitrogen), penicillin (100 μg/mL), and streptomycin (100 μg/mL). The RAW 264.7 macrophages were obtained from NCCS, Pune, India, and maintained in DMEM containing 10% (v/v) fetal calf serum and antibiotics in a CO2 incubator. Cells were initially propagated in 25 cm² tissue culture flask in an atmosphere of 5% CO2 and 95% air at 37 ºC humidified air until 70–80% confluency.

For fluorescence imaging studies, RAW cells, 7.5×10³ cells in 150 μL of media were seeded on sterile 12 mm diameter poly-L-lysine-coated coverslip and kept in a sterile 35 mm covered Petri dish and incubated at 37 ºC in a CO2 incubator for 24–30 h. Next day cells were washed three times with HEPES (pH 7.4) and fixed using 4% paraformaldehyde in HEPES (pH 7.4) for 10 min at rt washed with HEPES followed by permeabilization using 0.1% saponin for 10 min. Then the cells were incubated with 10 μM of...
the sensor (S1) dissolved in 100 µL of DMEM at 37 °C for 1 h in a CO₂ incubator and observed under epifluorescence microscope (Carl Zeiss). The cells were again washed thrice with HEPES (pH 7.4) to remove any excess sensor and incubated in DMEM containing NaOCl (20 µM) followed by washing with HEPES (pH 7.4) three times to remove excess free NaOCl outside the cells. Again, images were taken using epifluorescence microscope. Before fluorescence imaging, all the solutions were aspirated, and the samples were mounted on slides in a mounting medium and stored in dark before microscopic images were acquired.

Acknowledgement

Dibyendu Sain acknowledges Science and Engineering Research Board (SERB), Department of Science & Technology, Government of India for awarding National Post-doctoral fellowship (File No. PDF/2015/000882).

References


Electrochemical sensor for simultaneous determination of dextromethorphan hydrobromide and paracetamol at carbon paste electrode modified with synthesized indium tin oxide nanoparticles and ionic liquid

Mal Phebe Kingsley, Pratima Ashok Sathe and Ashwini K. Srivastava*
Department of Chemistry, University of Mumbai, Vidyanagari, Santacruz (E), Mumbai-400 098, India

E-mail : aksrivastava@chem.mu.ac.in, akschbu@yahoo.com Fax : 91-22-26528547

Abstract: Indium tin oxide nanoparticles were synthesized by solution route using cetyltrimethyl ammonium bromide as surfactant and used as a modifier of the electrode. An electrochemical study for simultaneous quantification of dextromethorphan hydrobromide (DXM) and paracetamol (PA) by adsorptive stripping differential pulse voltammetry at a carbon paste electrode modified with the composite of indium tin oxide nanoparticles and 1-butyl-3-methylimidazolium chloride ionic liquid (ITO-IL-CME) is described. Phosphate buffer (0.05 M) at pH 7 was found to be the ideal supporting electrolyte for the electrochemical study. The voltammetric results indicate that ITO-IL-CME remarkably enhances the electrocatalytic activity towards the oxidation of DXM and PA in neutral solution. The peak current increased linearly with the concentration for DXM in the range from 9.70×10⁻⁷ M to 6.20×10⁻⁴ M with a correlation coefficient of 0.989 and for PA with the concentration in the range from 3.62×10⁻⁸ M to 7.18×10⁻⁴ M with a correlation coefficient of 0.995. The detection limits for simultaneous determination of DXM and PA were 4.66×10⁻⁸ M and 8.40×10⁻⁹ M for DXM and PA respectively.

The proposed procedure was successfully applied for determination of the drugs in pharmaceutical formulations, human serum and urine sample with good apparent recoveries without interference from matrix. The nanocomposite material modified carbon paste electrode has several advantages such as providing improved voltammetric behavior, long time stability, good selectivity, high sensitivity and excellent reproducibility.

Keywords: Dextromethorphan hydrobromide, paracetamol, 1-butyl-3-methylimidazolium chloride, indium tin oxide nanoparticles, modified carbon paste electrode, adsorptive stripping differential pulse voltammetry.

1. Introduction

Dextromethorphan hydrobromide (DXM, (+)-3-methoxy-17-methyl-(9α, 13α, 14α)-morphinan hydrobromide) is an over the counter nonnarcotic antitussive drug. It acts through depression of the medullary centers of the brain to decrease the involuntary urge to cough. It affects the signals in the brain that trigger cough reflex. The drug is beneficial when administered in recommended doses. However, DXM produces a range of toxicities when consumed in high doses and can cause liver damage, heart attack, stroke, and death. Though DXM is a very effective safe-to-use medicament, abuse of the drug replicates alcohol like effect such as hallucination, paranoia, euphoria and suicidal tendency. Number of adolescents in the United States and Europe intoxicate themselves with megadoses of DXM viz. 5 to 10 times the dose recommended for control of annoying nonproductive coughs. Over dosage of DXM lacks the ability to metabolize the drug normally, leading to rapid acute toxicity with profound psychological and physiological effects. Also, interaction between DXM and other substances (e.g. alcohol, acetaminophen, 3,4-methylenedioxy methamphetamine and other over the counter cough medicines) produces a synergistic effect that can be very dangerous. Several analytical methods have been employed for the determination of the DXM, like high performance liquid chromatography, micellar liquid chromatography, RP-HPLC, first-derivative spectrophotometry, capillary gas chromatography and voltammetric techniques. With respect to the use
of voltammetric techniques, very few articles were reported for the quantification of DXM with rare usage of modified electrodes.

Paracetamol or N-acetyl-p-aminophenol (PA) is the most widely antipyretic, analgesic drug. It relieves pain and fever. Generally, limited use of paracetamol is safe and has no harmful side effects. However, overdose or long-term use of it may cause adverse effects on health because of the accumulation of toxic metabolites, which can lead to severe and sometimes fatal hepatotoxicity and nephrotoxicity. PA is also combined with other active ingredients in prescription medicines that treat allergy, cough, colds, flu, and sleeplessness. Varieties of methods have been used for the determination of PA in pharmaceutical preparations and human plasma like reverse phase high performance liquid chromatography, RP-UPLC, spectrophotometry, flow-injection analysis and voltammetric techniques. PA is present in some pharmaceutical preparations containing DXM in order to achieve synergistic effect as a combinational drug, though with different mechanisms of action. The presence of analgesic and cough suppressant in pharmaceutical formulations work together in the brain to decrease pain and reduce the cough reflex.

Ionic liquids (IL) are salts those are liquid at or above room temperature. They possess several advantages (e.g. high intrinsic conductivity, wide electrochemical windows, low volatility, high thermal stability and good solvating ability). Higher currents (both faradaic and capacitive) are often observed at ionic liquid-carbon paste composite electrode (IL-CME) compared to traditional bare carbon paste electrode due to its larger electro-active surface area promoting increase in electron transfer at the electrode surface.

Voltammetric methods of analysis have been receiving considerable attention since past few decades due to their simplicity, efficiency and low cost. Modification of carbon paste working electrode surface imparts high sensitivity and selectivity to the analyte response. Use of nanoparticles as chemically modified electrodes for electroanalysis of drugs, has enhanced the scope and analytical applicability of the technique in the field of drug analysis.

In view of medical and pharmacological importance, as well as increasing trend of DXM abuse with potential increase in toxicity of combined drug formulations of DXM and PA on over dosage, there is a compelling need to develop reliable, selective and sensitive analytical method for simultaneous quantification of DXM and PA in pharmaceutical products and in human body fluids. However, to the best of our knowledge, only few reports on voltammetric method have been reported for the simultaneous determination of DXM and PA compound. Therefore, the objective of the present study is to develop an electrochemical sensor for the simultaneous determination of DXM and PA, to understand the electrochemical reaction mechanisms and to determine their diffusion coefficients in solution. Further, it aims to determine simultaneously the level of concentration of DXM and PA in pharmaceutical and clinical preparations in presence of preservatives and excipients with the developed sensor.

2. Material and methods

2.1. Chemicals

All chemicals were of analytical grade and were used without further purification. Graphite powder (amorphous graphite size, < 50 μm) was purchased from S.D. Fine Chemicals and mineral oil was from Fluka. Indium chloride (InCl₃·4H₂O), tin chloride pentahydrate (SnCl₄·5H₂O), ammonium hydroxide, ethanol, cetyltrimethylammonium bromide (CTAB), DXM, PA and 1-butyl-3-methylimidazolium chloride ionic liquid (IL) were purchased from Sigma Aldrich. Britton-Robinson buffer (BR) solution 0.04 M was used to investigate pH dependence of DXM and PA. Other buffer solutions used were phosphate (K₂HPO₄/KH₂PO₄-Phos), tris(hydroxymethyl)ammonium bromide (Tris) and 4-(2-hydroxy ethyl)-1-piperazine ethane sulphonic acid (HEPES). Double distilled water was used for the preparation of aqueous solutions. Tablets/syrup/lozenges of DXM and tablets/syrup of PA available from local pharmacies were subjected to analysis.

2.2. Instrumentation

Voltammetry measurements and electrochemical impedance spectroscopy (EIS) study have been per-
formed on Eco Chemie, Electrochemical Work Station, model Autolab PGSTAT 30 using GPES software version 4.9005 and Frequency Response Analyser, software version 2.0 respectively. A three electrode system employing an Ag/AgCl (3 M KCl) and platinum electrode were used as reference and counter electrodes respectively. Bare carbon paste electrode (CPE)/synthesized indium tin oxide nanoparticles modified carbon paste electrode (ITO)/1-butyl-3-methylimidazolium chloride ionic liquid modified carbon paste electrode (IL-CME)/synthesized indium tin oxide nanoparticles with 1-butyl-3-methylimidazolium chloride ionic liquid composite modified carbon paste electrode (ITO-IL-CME) were used as working electrodes. The EIS studies were performed in the frequency range from $10^{-1}$ to $10^5$ Hz at open circuit potential. The pH measurements were performed using an ELICO LI 120 pH meter. Conductivity measurement was carried out using four point probe instrument, Scientific Equipment, Roorkee. The energy dispersive spectroscopy (EDAX) and scanning electron microscopy (SEM) measurements were recorded using FEI Quanta-250 model, Transmission electron microscopy (TEM) images were registered by FEI TF 30 model, Powder X-ray diffraction (XRD, Rigaku, D/Max-RA,Cu Kα) and Perkin-Elmer FTIR spectrophotometer version 10.03.07 in wave range of 4000–400 cm$^{-1}$ were used for characterization of synthesized indium tin oxide nanoparticles.

### 2.3. Synthesis of indium tin oxide nanoparticles

A mixture of 1.105 g of InCl$_3$·4H$_2$O and 0.21 g of SnCl$_4$·5H$_2$O was dissolved in water and 20 mL ethanol was added followed by adding 0.05 g of CTAB surfactant and drop by drop addition of ammonia solution under constant stirring at ambient condition. The whole mixture was stirred for 1 h on magnetic stirrer. It was then transferred to an autoclave and heated at 150 °C for 5 h. The product was centrifuged and washed with de-ionized water and further by ethanol. Pale blue coloured nanocrystalline indium tin oxide was obtained after the dried product was subjected to heat treatment at 400 °C for 3 h in muffle furnace.

### 2.4. Preparation of CPE, ITO-CME, IL-CME and ITO-IL-CME

CPE was prepared by mixing graphite with mineral oil at composition 70 : 30 (w/w) using a motor and pestle and was homogenized for 30 min. ITO-CME, IL-CME and ITO-ILCME were prepared by mixing graphite powder, indium tin oxide nanoparticles and/or 1-butyl-3-methylimidazolium chloride, mineral oil with various weight ratios. The pastes were then packed into a Teflon micro tip (diameter 0.5 mm) and a copper wire was inserted into the paste to establish an electrical contact. A new surface was regenerated by pressing out an excess of paste out of the tip and polishing it against zero grade butter paper.

### 2.5. Voltammetry procedure – Determination of DXM and PA

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) studies were carried out with appropriate quantity of the DXM and/or PA with pH 7 phosphate buffer (Phos). The solution was then transferred into an electrochemical cell and the measurements were carried out at 25 ± 0.2 °C. N$_2$ gas purging was not required as oxygen did not interfere in the measurements. The cyclic voltammetric experiments were carried out by sweeping the potential between +0.65 to +1.25 V for DXM and between +0.15 to 0.75 V. For simultaneous recording of DPVs for DXM and PA, the potential range were within +0.20 to +1.1 V.

### 2.6. Sample treatment for scanning electron microscope (SEM) and transmission electron microscope (TEM) analysis

Morphology studies of ITO were carried out by SEM and TEM. Samples of ITO for TEM measurements were prepared by placing drops of the solution (sample in alcohol) on coated Cu grids and subsequently drying in air. For SEM analysis, ITO nanoparticles were sonicated in toluene, allowed to dry and then employed for analysis.

### 2.7. Sample preparation – Standard and real sample

The pharmaceutical preparations were Alkox-Dx (Alkem Laboratories Ltd.) and Ascoril-D (Glenmark...
Pharmaceuticals Ltd.) cough syrups each containing 10 mg of dextromethorphan hydrobromide per 5 mL, TusQ-D lozenges (Blue Cross Laboratories Ltd.) containing 5 mg of dextromethorphan hydro-bromide, P-250 (Apex Laboratories Ltd.) containing 250 mg of paracetamol, Pyrigesic (East India Pharmaceutical Works Ltd.) containing 500 mg paracetamol and Alex-P+ (Glenmark Pharmaceuticals Ltd.) containing 5 mg of dextromethorphan hydrobromide and 170 mg of paracetamol per 5 mL.

Five tablets/lozenges were accurately weighed and finely powdered with a mortar and pestle. To a corresponding weight of the real sample water was added, filtered through a Qualigens (615) filter paper into a 100 mL calibrated flask. The residue was washed several times with water and the solution was diluted to the mark. Appropriate volume of this solution was diluted to 25 mL with phosphate buffer (pH 7.0) and then transferred to an electrolytic cell for the determination of DXM and/or PA.

To 1 mL of blood serum/urine/syrup, supporting electrolyte was added and made up to 10 mL and analyzed. Standard addition method was employed for quantification of DXM and PA as individual/combinations in pharmaceutical preparations, blood serum and urine samples.

3. Results and discussion

3.1. Characterization of indium tin oxide nanoparticles

3.1.1. X-Ray diffraction studies (XRD) of synthesized indium tin oxide nanoparticles

The XRD of indium tin oxide is given in Fig. 1. All diffractions can be indexed as cubic crystal structure of indium oxide. Indium oxide nanoparticles exhibits the reflection from the (222) planes as the most predominant peak in the X-ray diffraction pattern. Tin atoms are doped into the indium oxide lattice since no evidence of characteristic peaks of Sn, SnO or SnO2 were indicated in the spectra. The peaks in the diffraction spectrum are found to be sharp indicating highly crystalline nature of the nanoparticles. No characteristic peaks of impurities were detected.

Table 1 shows that all the peaks can be perfectly indexed to crystalline indium oxide, not only in their peak positions, but also in their relative intensities.

Using Debye-Scherrer's formula, the crystallite size, $D_{hkl}$ is given by

$$D_{hkl} = \frac{K\lambda}{\beta \cos\theta}$$

where the Scherrer’s constant $K$ is taken to be 0.9, ‘$\lambda$’ is the wavelength of X-ray (1.5406 Å), ‘$\beta$’ is the full

![Fig. 1. XRD pattern of indium tin oxide nanoparticles.](image-url)
width at half maximum of the peak, ‘θ’, the Bragg angle and ‘D’ is the particle diameter size. The mean particle diameter of ITO nanoparticles was found to be 30 nm.

3.1.2. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) of synthesized indium tin oxide nanoparticles

The morphology and micro-structure of ITO were investigated by SEM and TEM. Fig. 2(A) is the SEM image of synthesized indium tin oxide nanoparticles, which is observed to be non-agglomerated with uniform morphology and of cubic shape. The chemical constituent of particles was analyzed by EDAX (Fig. 2(B)). The In Lα1 (3.286 keV) and Sn Lα1 (3.443 keV) peaks show the presence of indium and tin elements with the atomic molar percent determined for indium being 91 % and for tin 9 % in the lattice of the ITO nanoparticles.

The TEM image of synthesized indium tin oxide nanoparticles is given in Fig. 3.

<table>
<thead>
<tr>
<th>Table 1. XRD data for indium tin oxide nanoparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td>XRD data-d (nm)</td>
</tr>
<tr>
<td>Theoretical values-d (nm)</td>
</tr>
<tr>
<td>Crystalline plane (hkl)</td>
</tr>
</tbody>
</table>

Fig. 2. SEM image of (A) ITO nanoparticles and (B) Energy-dispersive X-ray spectrum for ITO nanoparticles.
The TEM examination of synthesized ITO indicates that the morphology is homogeneous showing a cubic structure.

3.1.3. FTIR spectral analysis of synthesized indium tin oxide nanoparticles

The peaks at 476, 557, 593 and 849 cm\(^{-1}\) in Fig. 4 show the characteristic bands of crystalline In\(_2\)O\(_3\). The observed bands at 476 and 557 cm\(^{-1}\) are attributed to the stretching vibrations of In-O whereas bands at 593 and 849 cm\(^{-1}\) are characteristic of In-O bending vibrations. The vibrations extending from 2328 and 1293 cm\(^{-1}\) indicate the presence of hydrogen bonds involved in O-H oscillations arising from adsorbed water\(^{26}\).

3.1.4. Conductivity measurements of synthesized indium tin oxide nanoparticles

Electrical conductivity measurement of synthesized indium tin oxide nanoparticles was performed using the DC four-point probe technique\(^{27}\) on synthesized ITO pellets (1.7 cm diameter, 0.42 mm thickness). Three pellets were prepared by pressing about 100 mg of synthesized ITO with an IR pressing tool. Sheet resistance ‘\(R_s\)’ across the pellets was determined to be \(2.41 \times 10^{-2}\) \(\Omega\)/sq, the value of the average electrical conductivity ‘\(\sigma\)’ calculated from \(R_s\) obtained was 98.79 S cm\(^{-1}\).

### 3.2. Effect of pH

The effect of pH on peak current and peak potential for DXM (1.0×10\(^{-5}\) \(M\)) and PA (2.5×10\(^{-6}\) \(M\)) are shown in Fig. 5(A) and (B) respectively in BR buffer from pH 2 to 10 at CPE using differential pulse voltammetry.

It is observed that the peak current of DXM increases till pH 7.0 followed by a decrease with further increase in pH. Similarly for PA, the maximum peak current is obtained at pH 7.0. Therefore, pH 7 was selected for the determination of DXM and PA in their mixture. The peak potentials for the oxidation of DXM and PA is shifted negatively with increasing pH, indicating that protons take part in the electrochemical reaction processes for both the molecules. A slope of \(-0.052\) V/pH and \(-0.050\) V/pH for DXM and PA were observed respectively, suggesting that same number of electrons and protons were involved for their electrooxidation. The effects of several supporting electrolytes (0.1 \(M\)) viz. HEPES, phosphate (KH\(_2\)PO\(_4\)+ K\(_2\)HPO\(_4\)), Tris and BR buffer at pH 7 for DXM (1.0×10\(^{-5}\) \(M\)) and PA (2.5×10\(^{-6}\) \(M\)) on peak current were tested as shown in Fig. 6.

Of these, phosphate buffer (Phos) gave the best response in terms of peak height and the peak shape. Optimization of buffer concentration was carried out by varying phosphate concentration in the range from 0.01 \(M\) to 0.2 \(M\), the best peak response was observed for 0.05 \(M\) of Phos (pH 7) and hence was used for further studies.

### 3.3. Effect of surface modification and optimization of the amount of the modifier

The cyclic voltammograms for DXM (1.0×10\(^{-5}\) \(M\)) and PA (2.5×10\(^{-6}\) \(M\)) in 0.05 \(M\) of Phos (pH 7) at
CPE, ITO-CME, IL-CME and ITO-IL-CME is shown in Fig. 7(A) and (B). It is seen that DXM and PA show irreversible peaks. It was evident from the figures that the modification of the CPE with indium tin oxide and ionic liquid enlarged the peak current considerably by about 2.5 and 3 times for DXM and PA respectively, indicating that the surface property of the modified electrode has been significantly changed due to synergistic effect of ITO with IL.

The influence on the amount of ITO nanoparticles and IL added to CPE was studied for a solution containing DXM (1.0 × 10^{-5} M) and PA (2.5 × 10^{-6} M) in pH 7 by DPV. Table 2 shows the weight ratios for the various modified electrode. The peak current increased with increase in the percentage weight of nanoparticles till the ratio of composition (w/w) for graphite : ITO nanoparticles : IL : mineral oil was 45 : 20 : 05 : 30.

The DPV peaks are well-resolved for PA and DXM at ITO-IL-CME with the peak potentials at about 0.391 V and 0.872 V respectively. The separation of peak potentials is 0.481 V for PA-DXM which is large enough to determine PA and DXM simultaneously.

The surface areas for CPE, IL-CME, ITO-CME and ITO-IL-CME having same bore size using 6 mM mixture of K_{3}Fe(CN)_{6} and K_{4}Fe(CN)_{6} system in 0.1 N KNO_{3} by cyclic voltammetric technique was calculated from Randles-Sevcik equation\textsuperscript{28}. It was found to be 0.00958, 0.0175, 0.0345 and 0.0847 cm\textsuperscript{2} respectively.

### 3.4. Cyclic voltammetry

The effect of scan rate to investigate electrode reaction mechanism is studied and are shown in Fig. 8(A) and (B) for DXM (1.0 × 10^{-5} M) and PA (2.5 × 10^{-6} M) at ITO-IL-CME in 0.05 M phosphate (pH 7).
respectively. The scan rates were from 50 to 900 mV s⁻¹.

The peak current varied linearly with increase in the scan rate for DXM (Fig. 9(A)) and PA (Fig. 10(A)) with regression equation

\[ I_p = 4.216 + 0.035 \nu \]
\[ [R^2 = 0.984; \ I_p : \mu A, \ \nu : (mV/s)] \] (2)

\[ I_p = 3.442 + 0.033 \nu \]
\[ [R^2 = 0.995; \ I_p : \mu A, \ \nu : (mV/s)] \] (3)

respectively, validating a typical adsorption-controlled process. A plot of log \( I_p \) against log \( \nu \) for 1.0×10⁻⁵ \( M \) DXM (Fig. 9(B)) and for 2.5×10⁻⁶ \( M \) PA (Fig. 10(B)) was linear with a slope of 0.666 and 0.941 indicating that the electrode process is adsorption controlled.

Also, the peak potential shifted to more positive values with the increase of scan rate, which confirmed the irreversibility of the oxidation process for both the molecules (Figs. 9(C) and 10(C)).

For an irreversible reaction the number of electrons (\( n \)) involved in the oxidation reaction was calcu-
lated from the following equation:

$$E_p - E_{p/2} = 47.7/n\alpha \text{ [mV at 25 ºC]}$$  (4)

$E_p - E_{p/2}$ value for DXM and PA was found to be 75 mV and 55 mV respectively. On substitution in the above equation and assuming $\alpha$ as 0.5, 'n' was calculated to be 1.2 for DXM i.e. approximately one proton is transferred and 1.7 for PA i.e. approximately two proton is transferred in the oxidation process. Since the oxidation occurred by transfer of the same number of electrons and protons for both the molecules, the oxidation of DXM is an one electron one proton process and for PA it is two electron two proton process at the ITO-IL-CME electrode.

Scheme 1(a) and (b) show the probable electrochemical reaction process for the oxidation of DXM and PA at the ITO-IL-CME.

In order to calculate the electron transfer rate constant the following Laviron’s equation was used

$$E_p = E^0 + \frac{RT}{\alpha n F} \ln \left( \frac{RTk^0}{\alpha n F} \right) + \frac{RT}{\alpha n F} \ln v$$  (5)

where, $k^0$ is the electron transfer rate constant, $\alpha$ is the electron transfer coefficient, $v$ is the scan rate, $n$ is the electron transfer number and $E^0$ is the formal po-
of $k_0$ were determined to be 330.0 s$^{-1}$ and 380.3 s$^{-1}$ for DXM and PA, respectively. Large values obtained for electron transfer rate constant indicate high ability of promoting electrons between the molecules and the ITO-IL-CME electrode surface.

3.5. Electrochemical impedance spectroscopy (EIS)

Electrochemical impedance spectroscopy (EIS) is a simple and effective way to measure the charge transfer resistance ($R_{ct}$) of the electrochemical reactions. The EIS measurements were performed for DXM ($1.0 \times 10^{-5} \text{ M}$) and PA ($2.5 \times 10^{-6} \text{ M}$), in a phosphate buffer solution pH 7 at CPE, IL-CME, ITO-CME and ITO-IL-CME electrode surfaces.
Kingsley et al.: Electrochemical sensor for simultaneous determination of dextromethorphan etc.

It can be seen from Fig. 11(A) and (B) that each spectrum exhibited a semicircular and a linear portion.

The semicircle corresponds to the charge transfer process at the electrode at a high frequency range, whereas the linear portion in the spectra is due to the diffusion process in the low frequency range. The diameter of the semicircle represents the magnitude of $R_{ct}$ at the electrode surface. The measured EIS data were fitted with an equivalent circuit known as the Randles equivalent circuit, consisting of ohmic resistance ($R_s$) of the electrolyte solution, the double layer capacitance ($C_{dl}$), electron transfer resistance ($R_{ct}$) and the Warburg impedance ($Z_w$) resulting from the diffusion of ions from the bulk of the electrolyte to the interface. The values of $R_{ct}$ at CPE, IL-CME, ITO-CME and ITO-IL-CME were 518.9 $\Omega$, 299.3 $\Omega$, 238.5 $\Omega$ and 208.4 $\Omega$, for DXM and 317.0 $\Omega$, 295.6 $\Omega$, 286.5 $\Omega$ and 235.1 $\Omega$, for PA respectively. By comparing the data obtained, the $R_{ct}$ for CPE is much higher than at ITO-IL-CME for both the molecules implying that the charge transfer process is relatively fast at ITO-IL-CME compared to CPE.

3.6. Chronocoulometry (CC)

Chronocoulometry is a controlled-potential technique in which the measurement of charge as a function of time is observed. Application of this technique helps to study of electrode kinetics by, determining the diffusion coefficient of the analyte in solution and by determining the surface coverage area of the electrode the extent of the adsorption of the analyte on the electrode can be ascertained. Double-potential step chronocoulometry permits an “in situ” double layer correction even in the presence of extensive adsorption. The above technique was applied individually for $1.0 \times 10^{-5}$ M DXM and $2.5 \times 10^{-6}$ M PA in 0.05 M phosphate (pH 7).

From following equation:

$$Q_t = \frac{(2nFD^{1/2}CA t^{1/2})}{\pi^{1/2} + nFA \Gamma^0 + Q_{dl}}$$

the Anson plot of total charge ($Q_t$) in coloumb vs the square root of time ($t^{1/2}$) in seconds should give a linear relationship. $Q_{dl}$ is the double layer charge in Coulombs, $n$ is the number of electrons, $F$ is the Faradays constant (96485 C), $C$ is the concentration in mol/cm$^3$, $A$ is the area of the electrode in cm$^2$, $\Gamma^0$ is surface coverage in mol cm$^{-2}$ and $D$ is the diffusion coefficient in cm$^2$ s$^{-1}$. The diffusion coefficient and surface coverage were estimated from the slope and intercept of the Ansons plot at CPE, IL-CME, ITO-CME and ITO-IL-CME as given in Table 3.
Table 3. Chronocoulometry of 1.0×10⁻⁵ M DXM and 2.5×10⁻⁶ M PA in 0.05 M Phos (pH 7)

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Electrode</th>
<th>Slope $Q_{ads}$ (µC s⁻¹/²)</th>
<th>Intercept $Q_{ads}$ (µC)</th>
<th>Surface coverage (10⁻¹⁰ mol cm⁻²)</th>
<th>Diffusion coefficient (cm² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXM</td>
<td>CPE</td>
<td>0.367±2.14</td>
<td>0.528±1.79</td>
<td>5.71</td>
<td>1.23×10⁻³</td>
</tr>
<tr>
<td></td>
<td>IL</td>
<td>0.945±3.48</td>
<td>1.280±0.92</td>
<td>7.59</td>
<td>2.45×10⁻³</td>
</tr>
<tr>
<td></td>
<td>ITO</td>
<td>2.510±3.79</td>
<td>3.260±2.13</td>
<td>9.78</td>
<td>4.45×10⁻³</td>
</tr>
<tr>
<td></td>
<td>ITO-IL-CME</td>
<td>7.830±1.81</td>
<td>8.990±4.07</td>
<td>10.99</td>
<td>7.19×10⁻³</td>
</tr>
<tr>
<td>PA</td>
<td>CPE</td>
<td>0.113±3.55</td>
<td>7.050±3.77</td>
<td>38.1</td>
<td>4.68×10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>IL</td>
<td>0.245±2.19</td>
<td>16.10±2.84</td>
<td>47.6</td>
<td>6.58×10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>ITO</td>
<td>0.574±1.03</td>
<td>42.40±1.62</td>
<td>63.7</td>
<td>9.32×10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>ITO-IL-CME</td>
<td>1.53±3.13</td>
<td>160.9±2.20</td>
<td>98.4</td>
<td>10.92×10⁻⁴</td>
</tr>
</tbody>
</table>

The values of $D$ and $\Gamma^0$ are found to increase from CPE to ITO-IL-CME indicating that maximum accumulation of DXM or PA on ITO-IL-CME from among all the electrodes studied.

3.7. Adsorptive stripping differential pulse voltammetry (AdSDPV)

3.7.1. Effect of accumulation parameters – Accumulation potential and accumulation time

AdSDPV was employed to study the influence of differential pulse anodic stripping peak current for 1.0×10⁻⁵ M DXM and 2.5×10⁻⁶ M PA on the accumulation potential ($E_{acc}$) and accumulation time ($t_{acc}$) at ITO-IL-CME in 0.05 M Phos buffer solution (pH 7). $E_{acc}$ was determined by employing a potential widow from −1.0 V to 0.7 V. The peak current remained almost constant when the potential was positive from 0 V to 0.7 V for both the molecules. The peak current gradually increased as $E_{acc}$ became negative and reached its maximum at −0.3 V and −0.50 V for DXM and PA respectively (Fig. 12(A)).

When potential shifts still further in the negative direction, there is drop in peak current with increase in the background current. Thus the optimum accumulation potential of −0.50 V was used for subsequent sensitive simultaneous determination of DXM and PA.

At the same accumulation potential, the effect of accumulation time on the efficiency of drugs onto the...
Kingsley et al.: Electrochemical sensor for simultaneous determination of dextromethorphan etc.

working electrode surface was evaluated over a time range from 0.0 s to 150 s (Fig. 12(B)). Peak current increased very rapidly with increasing accumulation time, which induced rapid adsorption of DXM and PA on the surface of the modified electrode. A steady enhancement in the peak current was observed over the range from 0.0 s to 90 s for DXM and from 0.0 s to 110 s for PA and thereafter the peak intensity gradually scales off at longer accumulation time. This indicates that, further increase of accumulation time did not increase the response of DXM and PA on the electrode surface due to a complete coverage of electrode surface. Therefore, the accumulation time was fixed at 110 s with an optimal stirring rate of 1200 rpm for the analysis.

3.7.2. Effect of potential sweep parameters

To improve the sensitivity of adsorptive stripping differential pulse voltammetry for the simultaneous determination of 1.0×10⁻⁵ M DXM and 2.5×10⁻⁶ M PA at ITO-IL-CME, the effect of pulse amplitude, scan rate and pulse width on peak current were investigated. It was found that the peak currents increased significantly with increasing pulse amplitude from 10 to 50 mV and then almost remained constant from 60 mV to 100 mV. The pulse amplitude chosen for practical purpose was 50 mV. Faster scan rates resulted in higher peak currents but background currents also increased. Taking peak current and background current into consideration the scan rate chosen was 50 mV s⁻¹. The optimum pulse width was 0.05 s.

3.8. Determination of DXM and PA by adsorptive stripping differential pulse voltammetry (AdSDPV)

Based on the optimal operating conditions (Table 4), AdSDPV measurements were undertaken in solutions containing different concentrations of DXM and PA individually and simultaneously.

Fig. 13(A) and (B) are the voltammograms of DXM and PA respectively carried out individually.

For simultaneous determination, two separate sets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AdSDPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7</td>
</tr>
<tr>
<td>Supporting electrolyte</td>
<td>0.05 M Phosphate buffer</td>
</tr>
<tr>
<td>Accumulation potential</td>
<td>−0.5 V</td>
</tr>
<tr>
<td>Accumulation time</td>
<td>90 s</td>
</tr>
<tr>
<td>Stirring rate</td>
<td>1200 rpm</td>
</tr>
<tr>
<td>Pulse amplitude</td>
<td>50 mV</td>
</tr>
<tr>
<td>Pulse width</td>
<td>0.05 s</td>
</tr>
<tr>
<td>Scan rate</td>
<td>50 mV s⁻¹</td>
</tr>
</tbody>
</table>

Table 4. Optimal parameters for determination of DXM and PA simultaneously by AdSDPV technique

Fig. 13. AdSDPV curves obtained at ITO-IL-CME for (A) DXM at different concentrations (a) 0.6, (b) 0.8, (c) 6.0, (d) 8.0, (e) 60.0, (f) 80.0, (g) 150, (h) 300, (i) 500, (j) 700 μM. (B) PA at different concentrations (a) 0.4, (b) 0.8, (c) 2.0, (d) 8.0, (e) 40.0, (f) 60.0, (g) 80.0, (h) 200.0, (i) 400.0, (j) 600.0 μM; scan rate 50 mV s⁻¹ in 0.05 M Phos (pH 7.0); pulse amplitude 50 mV. Inset of (A) and (B) shows the linearity graph of DXM and PA respectively.
of experiments were carried out. In the first instance, concentration of DXM was increased in the presence of fixed concentrations of PA and the voltammograms were recorded (Fig. 14(A)) and vice versa as given in Fig. 14(B). For the second instance, both DXM and PA were determined by simultaneously increasing their concentrations as given in Fig. 14(C). The linear working range (LWR), empirical limits of detection (LOD) (S/N = 3), linear regression equation (LRE) and correlation coefficient ($r$) were determined (Table 5).

The anodic peak current corresponding to the oxidation of DXM and PA correlates linearly with its concentration in the entire range of investigation.

From Table 5 it is observed that the LWR and LOD determined for DXM and PA simultaneously are in good agreement with when both were individually determined.

Table 6 shows a comparison of the proposed sensor with the reported electrochemical sensors for the determination of DXM and PA individually and simultaneously.

3.9. Validation and interference studies

The precision of the developed method was investigated with respect to both repeatability (single electrode), reproducibility (multiple electrodes), rugged-
ness and interferents with standard solution of $1.0 \times 10^{-4} \text{ M} \text{ DXM}$ and $1.0 \times 10^{-5} \text{ M} \text{ PA}$ in $0.05 \text{ M} \text{ Phosphate buffer (pH 7)}$ was assessed by continuous electrooxidative determination of the standard solution with the same ITO-IL-CME electrode for 10 analyses. The anodic current decreased by 1.05% and 1.32% with the RSD value of 1.75% and 2.19% for DXM and PA respectively after completing 10 scans, which showed that the electrode responds with good repeatability. Reproducibility was investigated by determination of three replicate samples containing standard solution of DXM and PA on five consecutive days using multiple electrodes where the mean concentrations were found to be $1.09 \times 10^{-4} \text{ M}$ and $0.983 \times 10^{-5} \text{ M}$ with associated RSD values of 1.19% and 3.67% respectively. The ruggedness of the method was assessed by comparison of the intra- and inter-day assay results for DXM and PA that had been performed by two analysts. The RSD values for intra- and inter-day assays of analyses performed in the same laboratory by two different analysts did not exceed 3.5%, thereby indicating the ruggedness of the method. The influence of peak current on DXM and PA in presence of interferents that are commonly present in biological media and 4-aminophenol (metabolite of paracetamol) were investigated. Also, DXM and PA are formulated in different dosage forms in combinations with drugs like aspirin, caffeine, phenylephrine, ascorbic acid and cetirizine dihydrochloride, therefore the extent of their interference along with DXM and PA were tested with the developed AdSDPV method. Under optimized experimental conditions, no effect on the determination of DXM and PA was observed up to 250-fold of Na+, K+, Mg2+, Ca2+, Cl−, SO42−, 200-fold of glucose and ascorbic acid, 150-fold of glucose and uric acid, 20-fold of citric acid, 100-fold of aspirin and caffeine,
60-fold of phenylepherine, 50-fold of cetirizine dihydrochloride and 70-fold of 4-aminophenol, indicating that the present modified electrode was highly selective towards the determination of DXM and PA.

### 3.10. Application to real matrices

Commercial pharmaceutical samples (tablets) containing DXM and/or PA present in tablets were analyzed in order to evaluate the practical applicability of

---

**Table 6. Comparison of the performances of electrochemical sensors for DXM and PA**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Modified electrode</th>
<th>Detection limit (M)</th>
<th>Linear range(M)</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextromethorphan hydrobromide</td>
<td>Carbon nanotubes-carbon microparticle-ionic liquid composite</td>
<td>8.81×10⁻⁶</td>
<td>2.50×10⁻⁴ to 3.30×10⁻³</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Reduced graphene oxide modified screen printed electrode after electromembrane extraction</td>
<td>1.50×10⁻⁶</td>
<td>5.0×10⁻⁶ to 1.50×10⁻³</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Hydrophilic carbon nanoparticulates at the surface of carbon paste electrode</td>
<td>2.90×10⁻⁶</td>
<td>8.0×10⁻⁶ to 8.0×10⁻⁴</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>ITO-IL-CME</td>
<td>2.61×10⁻⁸</td>
<td>8.0×10⁻⁷ to 8.0×10⁻⁴</td>
<td>This work</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>Boron doped diamond electrode modified with Nafion and lead films</td>
<td>1.70×10⁻⁷</td>
<td>5.0×10⁻⁷ to 2.0×10⁻⁴</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Nafion/TiO₂ – Graphene modified glassy carbon electrode</td>
<td>2.10×10⁻⁷</td>
<td>4.0×10⁻⁶ to 2.0×10⁻⁵</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Hydrophilic carbon nanoparticulates at the surface of carbon paste electrode</td>
<td>1.50×10⁻⁸</td>
<td>1.0×10⁻⁷ to 1.0×10⁻³</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>ITO-IL-CME</td>
<td>2.20×10⁻⁹</td>
<td>4.0×10⁻⁸ to 6.0×10⁻⁴</td>
<td>This work</td>
</tr>
</tbody>
</table>

**Table 7. Assay of DXM and/or PA in pharmaceutical preparations (n = 5)**

<table>
<thead>
<tr>
<th>Tablet/Syrup</th>
<th>Amount of drug in the sample (mg)</th>
<th>Amount of drug obtained in the proposed method (mg)±RSD</th>
<th>Tablet/Syrup</th>
<th>Amount of drug in the sample (mg)</th>
<th>Amount of drug obtained in the proposed method (mg)±RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alex-P⁺</td>
<td>5.0</td>
<td>4.8±2.13</td>
<td>Alex-P⁺</td>
<td>170.0</td>
<td>172.1±1.17</td>
</tr>
<tr>
<td>Tus Q-D</td>
<td>5.0</td>
<td>5.1±2.15</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Pyrigesic</td>
<td>500.0</td>
<td>498.4±2.94</td>
</tr>
</tbody>
</table>

---
the developed method (Table 7). Recovery experiments on the spiked samples (tablets, blood and urine) were also carried out to evaluate matrix effects (Table 8). Standard solution additions yielded good average recoveries which ranged between 97.5% and 102.9% for DXM and 97.6% and 103.2% for PA indicating high reproducibility and reliability of the modified electrode.

4. Conclusion

The present work demonstrates a simple and sensitive electrochemical method for the simultaneous determination of dextromethorphan hydrobromide and paracetamol at carbon paste electrode modified with indium tin oxide and 1-buty-3-methylimidazolium chloride ionic liquid. Cyclic voltammetry and electrochemical impedance spectroscopy study results confirmed the synergism of ITO nanoparticles and IL with unique catalytic activity at the electrode surface. Anodic stripping voltammetric technique was used for simultaneous determination of DXM and PA at ITO-IL-CME electrode and also was used for the first time. On comparison with unmodified carbon paste electrode and with some of the previously reported electrodes the ITO-IL-CME significantly enhanced the sensitivity, selectivity and reliability for individual and simultaneous determination of DXM and PA with low detection limits and large linear working range. The utilization of the developed sensor is an attempt to perform as a good alternative economically and environmentally friendly for simultaneous determination of DXM and PA, in quality control of pharmaceutical formulations, testing of blood and urine samples in terms of easy handling, low cost and stability.

<table>
<thead>
<tr>
<th>Std drug</th>
<th>Drug conc.</th>
<th>Apparent recovery (%)</th>
<th>Std drug</th>
<th>Drug conc.</th>
<th>Apparent recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkox-Dx</td>
<td>10–6 M</td>
<td>3.54 ±RSD</td>
<td></td>
<td>1.95</td>
<td>5.65 ±2.14</td>
</tr>
<tr>
<td></td>
<td>5.66</td>
<td>8.97 ±1.33</td>
<td></td>
<td>10.84</td>
<td>14.17 ±1.75</td>
</tr>
<tr>
<td>Ascoril-D</td>
<td>1.67</td>
<td>10.10 ±1.48</td>
<td></td>
<td>3.75</td>
<td>12.17 ±3.15</td>
</tr>
<tr>
<td></td>
<td>23.31</td>
<td>31.55 ±2.66</td>
<td></td>
<td>2.40</td>
<td>3.59 ±1.24</td>
</tr>
<tr>
<td>P-250</td>
<td>–</td>
<td>–</td>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Blood serum</td>
<td>7.41</td>
<td>7.43 ±1.39</td>
<td></td>
<td>1.18</td>
<td>1.21 ±1.24</td>
</tr>
<tr>
<td></td>
<td>10.70</td>
<td>10.8 ±2.16</td>
<td></td>
<td>2.31</td>
<td>2.35 ±2.93</td>
</tr>
<tr>
<td></td>
<td>13.8</td>
<td>13.7 ±2.94</td>
<td></td>
<td>3.40</td>
<td>3.51 ±1.68</td>
</tr>
<tr>
<td></td>
<td>21.9</td>
<td>21.7 ±1.52</td>
<td></td>
<td>4.44</td>
<td>4.43 ±3.35</td>
</tr>
<tr>
<td>Urine</td>
<td>7.41</td>
<td>7.50 ±2.44</td>
<td></td>
<td>3.31</td>
<td>3.35 ±2.93</td>
</tr>
<tr>
<td></td>
<td>16.70</td>
<td>16.40 ±2.86</td>
<td></td>
<td>6.74</td>
<td>6.58 ±3.14</td>
</tr>
<tr>
<td></td>
<td>26.50</td>
<td>26.30 ±1.13</td>
<td></td>
<td>11.90</td>
<td>11.72 ±1.87</td>
</tr>
</tbody>
</table>
Acknowledgement

One of the authors (MPK) is thankful to the University Grants Commission, New Delhi, India for providing financial assistance under Faculty Improvement Programme for this work. We are also grateful to Professor Sabir H. Mashraqui, University of Mumbai, for his valuable inputs.

References

A novel imidazolyl appended quinoline-hydrazide Schiff base fluorescent chemosensor for precise identification of Zn$^{II}$

Rakesh Purkait and Chittaranjan Sinha*

Department of Chemistry, Jadavpur University, Kolkata-700 032, India

E-mail : c_r_sinha@yahoo.com

Abstract : (E)-N'-((1H-imidazol-2-yl)methylene)quinoline-2-carbohydrazide (H$_2$L) is synthesized by the reaction of acid hydrazide and carbaldehyde. The probe has been characterized by spectroscopic data (FT-IR, UV-Vis, $^1$H NMR) and is weakly emissive. The H$_2$L selectively binds Zn$^{2+}$ and upon irradiation at 331 nm in presence of large number of cations shows high intense emission ($\lambda_{em}$, 575 nm) and serves as a “turn-on” fluorescence chemosensor. The limit of detection (LOD) for Zn$^{2+}$ is 0.36 $\mu$M. Formation of the 1 : 1 metal-to-ligand complex has been ascertained by Mass spectra and Job’s plot.

Keywords : Quinoline-carbohydrazide, Zn$^{2+}$-sensor, LOD 0.36 $\mu$M, 1 : 1 complex, spectral characterization.

Introduction

Elements are essential for the origination, growth and reproduction of living bodies. About 98% of the body mass of man is made up of nine nonmetallic elements$^1$. Transition metals present in trace and some are in ultratrace level in human body$^2$. Elements such as iron, zinc, copper etc. are essential components of enzymes and facilitate their conversion to specific end products. Zinc, the second-most abundant (transition) metal following iron in the human body, is an omnipotent metal and the average body content is 2–3 g in an adult$^3$. The standard daily requirement of zinc is 15–20 mg/day. Zinc plays an important role in cell proliferation, immunological and psychological functions and in metabolic activity. Plasma zinc levels are decreased in pregnancy, acute myocardial infarction, infections, and malignancies. It is essential for normal spermatogenesis and maturation, functioning of neurotransmitters, development of thymus, epithelialization, taste sensation, and secretion of pancreas and gastric enzymes. The excess “free zinc” may induce pathological diseases such as Alzheimer’s and Parkinson’s disease$^4$. Moreover, excess of zinc in the environment may reduce soil microbial activity, which has phytotoxic effects$^5$–$^7$. Thus detection of Zn$^{2+}$ is pressing important for monitoring human health. Exploration of selective and sensitive chemosensor for detection of ions in solution has been of considerable attention with biological and environmental interest$^8$–$^{14}$. In the last two decades, significant number of fluorescent probes have been designed and some have been used successfully in sensing of zinc at ultratrace level. Most of them have been developed based on quinoline$^{15}$, bipyridyl$^{16}$, coumarine$^{17}$, pyrazoline$^{18}$, tripyrrins$^{19}$, BINOL$^{20}$, fluorescein$^{21}$, rhodamine$^{22}$, fluorophores. Quinoline based fluorophore$^{15,23}$ is associated with imine (C=N), amide (-CONH-), sulfonyl derivatives etc.; however, quinoline-hydrazide has not been used for investigating sensor activity. In this work, we have designed and synthesized a hydrazide based imidazole derivative, (E)-N'-(1H-imidazol-2-yl)methylene)quinoline-2-carbohydrazide for the selective and sensitive detection of Zn$^{2+}$ in presence of other commonly available metal ions. The DFT computation of optimized geometry of H$_2$L and the complex has been used to explain the electronic spectral properties.

Experimental

Materials and methods

Quinaldic acid and imidazole-2-carbaldehyde were purchased from Sigma-Aldrich and quinoline-2-
carbohydrazide was synthesized following the published procedure\textsuperscript{24}. All other organic chemicals and inorganic salts were obtained from commercial suppliers Merck and used without further purification. Aqueous solutions were prepared using Milli-Q water (Millipore). Elemental analyses were performed using a Perkin-Elmer 2400 Series-II CHN analyzer, Perkin-Elmer, USA elemental analyzer. UV-Vis spectra were recorded on Perkin-Elmer Lambda 25 spectrophotometer and fluorescence spectra were obtained using a Perkin-Elmer spectrofluorimeter model LS55, FT-IR spectra (KBr disk, 4000–400 cm\textsuperscript{−1}) from a Perkin-Elmer LX-1FTIR spectrophotometer. NMR spectra were obtained on a Bruker (AC) 300 MHz FT-NMR spectrometer using TMS as an internal standard. ESI mass spectra were recorded from a Water HRMS model XEVO-G2QTOF#YCA351 spectrometer. All of the measurements were conducted at room temperature. The fluorescence quantum yield was determined using fluorescein as reference with a known quantum yield, $\phi_R = 0.79$ in 0.1 M NaOH\textsuperscript{25}. The experimental sample and reference were excited at same wavelength, maintaining almost same absorbance and fluorescence were measured. Area of the fluorescence spectra were measured using the software available in the instrument and the quantum yield was calculated by following the formula

$$\frac{\phi_S}{\phi_R} = \left[ \frac{A_S}{A_R} \right] \times \left[ \frac{(\text{Abs})_R}{(\text{Abs})_S} \right] \times \left[ \frac{\eta_S^2}{\eta_R^2} \right]$$

where, $\eta_S$ and $\eta_R$ are the fluorescence quantum yield of the samples and reference; $A_S$ and $A_R$ are the respective areas under emission spectra of the sample and reference respectively. $(\text{Abs})_R$, $(\text{Abs})_S$ are the absorbance of sample and reference at the excitation wavelength and $\eta_S^2$, $\eta_R^2$ are the refractive index of the solvent used for the sample and the reference.

**Synthesis of probe, H$_2$L**

The condensation of quinoline-2-carbohydrazide (0.187 g, 1.0 mmol) and imidazole-2-carbaldehyde (0.097 g, 1.0 mmol) under stirring condition in dry MeOH (15 ml) for 5 h at room temperature yields a grey precipitate. It was filtered off and washed several times with MeOH and dried in open air. Yield : 88%, m.p. >200 °C (Scheme 1). Microanalytical data : C$_{20}$H$_{13}$N$_3$O$_3$ Calcd. (Found) : C, 63.39 (63.35); H, 4.18 (4.19); N, 26.40 (26.37)%; $^1$H NMR (300 MHz, DMSO-$d_6$) : 15.29 (1H, s, imidazole-NH), 12.31 (1H, s, NH), 8.63 (1H, s, imine-H), 8.25–7.99 (2H, m), 7.99–7.85 (2H, m), 7.72 (1H, d, 18Hz), 7.51 (1H, t, 18Hz), 7.26 (1H, d, 15Hz), 7.09 (1H, s) (ESI\textsuperscript{†}, Fig. S1); IR : 3240 cm\textsuperscript{−1} (hydrazide -NH), 1668 cm\textsuperscript{−1} (–C=O), 1539 cm\textsuperscript{−1} (azomethine, -C=N), (ESI\textsuperscript{†}, Fig. S2).

**Synthesis of [ZnL(H$_2$O)]**

To THF-MeOH (1 : 1, v/v, 10 ml) solution of H$_2$L (1 mmol, 0.265 g), MeOH solution (10 ml) of Zn(NO$_3$)$_2$.6H$_2$O (0.297 g, 1 mmol) was added and refluxed for 3 h to yield a red precipitate. It was filtered off and washed several times with MeOH and dried in open air. Microanalytical data : Calcd. (%) C, 48.37; H, 3.48; N, 20.14; $^1$H NMR (300 MHz, DMSO-$d_6$) : 8.85 (1H), 8.75 (1H), 8.57 (1H), 8.41 (1H), 8.17 (1H), 7.95 (1H), 7.80 (1H), 7.42 (1H), 7.28 (1H), 7.09 (1H) (ESI\textsuperscript{†}, Fig. S3); IR : 3434 cm\textsuperscript{−1} (v(H$_2$O)), 1529 cm\textsuperscript{−1} (azomethine, -C=N), (ESI\textsuperscript{†}, Fig. S4).

**General method for UV-Vis and fluorescence studies**

The probe, H$_2$L (1.32 mg, 0.001 mmol) was dissolved in THF (5 ml) and 100 μL of H$_2$L solution diluted using 2 ml THF-MeOH (v/v : 1) containing HEPES buffer (pH 7.2) to make the solution with total volume 2.1 ml. Zn(NO$_3$)$_2$.6H$_2$O (2.97 mg, 0.001 mmol) was dissolved in water (10 ml). The Zn$^{2+}$ so-
Purkait et al.: A novel imidazolyl appended quinoline-hydrazide Schiff base fluorescent etc.

Solution (100 µL) were transferred to H₂L solution prepared above. This procedure for sample solution preparation also maintained for other cations. After mixing spectra were recorded at room temperature. For fluorescence study excitation wavelength used was 331 nm (excitation slit = 10.0 and emission slit = 10.0).

**Theoretical computation**

H₂L and [ZnL(H₂O)] were optimized to generate the structures by DFT/B3LYP method using Gaussian 09 software. 6-31G basis set was used for C, H, N, O, and LanL2DZ basis set was used as effective potential (ECP) set for Zn. To ensure the optimized geometries represent the local minima, vibrational frequency calculations were performed, and these only yielded positive eigen values. Theoretical UV-Vis spectra were calculated by time-dependent DFT/B3LYP method in methanol using conductor-like polarizable continuum model (CPCM). GAUSSSUM was used to calculate the fractional contributions of various groups to each molecular orbital.

**Results and discussion**

**Synthesis and formulation**

The condensation of quinoline-2-carbohydrazide and imidazole-2-carbaldehyde synthesised (E)-N’-((1H-imidazol-2-yl)methylene)quinoline-2-carbohydrazide (H₂L) in good yield (88%). It has been characterized by spectroscopic data (FTIR, Mass, NMR; ESI†). Molecular ion peak, (H₂L+H)+ 265.03 (Mwt., 265.27) supports the molecular identity. The broad band at 3240 cm⁻¹ refers to ν(hydrazide-NH) and strong stretches at 1668 cm⁻¹ and 1609 cm⁻¹ are assigned to ν(C=O) and ν(C=N) respectively. The 1H NMR spectrum of H₂L (300 MHz, DMSO-d₆) demonstrates singlet at 15.29 ppm corresponds to δ(imidazole-NH); hydrazine-NH appears at 12.31 ppm; imine-H (CH=N) appears at δ 8.63 ppm; and aromatic protons appear at 7.0–8.3 ppm. The reaction of H₂L with Zn(NO₃)₂.6H₂O in methanol has isolated mononuclear zinc complex, [ZnL.6H₂O]. The complex has shown a broad peak at 3434 cm⁻¹ corresponds to ν(H₂O) and ν(C=N) appears at δ 8.63 ppm; and aromatic protons appear at 7.0–8.3 ppm. The reaction of H₂L with Zn(NO₃)₂.6H₂O in methanol has isolated mononuclear zinc complex, [ZnL.6H₂O]. The complex has shown a broad peak at 3434 cm⁻¹ corresponds to ν(H₂O) and ν(C=N) at 1592 cm⁻¹ which is shifted to lower energy compared to H₂L. Mass spectrum shows molecular ion peak at 368.05 which may be due to [ZnL(H₂O)+Na]+. The absence of δ(hydrazide-NH) and δ(imidazolyl-NH) support ionization of probe and its binding with ZnII during synthesis. All other protons quantitatively appear in the spectrum. Thermal treatment eliminates coordinated H₂O at 112 ºC which is supported by elimination of broad stretch at 3434 cm⁻¹ in the infrared spectrum. Thus, the structure proposal of probe and the complex (Scheme 1) are established.

**UV-Vis spectroscopic studies**

The interaction of H₂L with Zn²⁺ has been examined by spectrophotometric titration of H₂L with incremental addition of Zn²⁺ in HEPES buffer (10 mM, pH 7.2) at 25 ºC in the same solvent, and has shown absorption enhancement at 383 nm and decrement at 332 nm, with the isosbestic point at 355 nm (Fig. 1) which suggests that the reaction is clean and straightforward. The red-shifting of the bands of H₂L upon Zn²⁺ addition is attributed to expulsion of intramolecular charge transfer (ICT) through the chelation. The change of absorbance is linear until the molar ratio [Zn²⁺] : [H₂L] reaches 1 : 1, and no longer changes with increase in [Zn²⁺]. It suggests that the stoichiometry between H₂L and Zn²⁺ is 1 : 1. To establish the binding stoichiometry of H₂L and Zn²⁺

![Fig. 1. Change in absorption spectrum of H₂L (50 µM) upon gradual addition of Zn²⁺ ions (5 µM each) in THF-MeOH (v/v 1 : 1) (pH 7.2).](image-url)
the Job’s plot has been generated by plotting absorbance against different mole fractions of Zn$^{2+}$ while volume of solution has remained fixed (Fig. 2) and the molar fraction maxima has been obtained at 0.5 mole fraction, which indeed supports 1 : 1 complex formation of H$_2$L and Zn$^{2+}$.

Fluorescence sensing for Zn$^{2+}$

Upon excitation the probe (H$_2$L) at 331 nm, a weak emission is observed at 500 nm with fluorescence quantum yield ($\phi_{HL}$) 0.0021. On addition of Zn$^{2+}$ the emission band is red shifted to 575 nm. The fluorescence emission of H$_2$L with other cations (Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, Fe$^{2+}$, Al$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Pd$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Cu$^{2+}$, Ba$^{2+}$, Pb$^{2+}$ and Al$^{3+}$) in THF-MeOH (v/v 1 : 1) (pH 7.2) is insignificant. Thus, the probe is selectively showing “turn-on” emission to Zn$^{2+}$ under the identical experimental condition (Fig. 3)$^{31,32}$. On incremental addition of Zn$^{2+}$ to the solution of H$_2$L the fluorescence intensity increases and becomes saturated when reached at 1 : 1 molar ratio which results enhancement of quantum yield to 0.0819 (39-fold increment compared to ligand). The emission intensity of the mixture does not change on excess addition of Zn$^{2+}$ (Fig. 4). The augmentation in fluorescence intensity for H$_2$L+Zn$^{2+}$ may arise from the elimination of photoinduced electron transfer (PET) in free H$_2$L and chelation enhancement effect (CHEF) through the co-ordination of imidazoly]-N, azomethine-N and hydrazide-O to metal ion (Scheme 1). To get further insight about the complexation reaction the fluorimetric titration has been done and $[(F_{\text{max}} - F_0)/(F - F_0)]$ vs $1/[\text{Zn}^{2+}]$ has been plotted following Benesi-Hildebrand equation (Fig. 5) and has determined the binding constant $[K_d: 7.6 \times 10^4]$. The limit of detection (LOD) of Zn$^{2+}$ has been calculated 0.36 $\mu$M following the 3$\sigma$ method (ESI, Fig. S5). The fluorescence...
cence enhancement of $\text{H}_2\text{L}-\text{Zn}^{2+}$ complex has been examined in presence of other metal ions (Fig. 6).

Quinoline substituted fluorogenic motives\textsuperscript{15,23} have been used in large number in the identification of different cations and anions; and the detection of $\text{Zn}^{2+}$ appears in highest account. Some of the literature reports (Table 1) are selected considering structural similarity of present chemosensor; but quinoline-hydrazide is first time reported herewith.

Effect of pH variation on fluorescence intensity of $\text{H}_2\text{L}$ and $\text{H}_2\text{L}-\text{Zn}^{2+}$ complex has been studied; it has observed that there is no significant fluorescence emission of $\text{H}_2\text{L}$ at the pH range 2 to 12 and in presence of $\text{Zn}^{2+}$ the ligand emits in the pH range between 3.0 to 11 (Fig. 7). At high acidic medium (pH 2) ligand may be protonated or hydrolyse Schiff base while in the basic medium (pH 12) may precipitate $\text{Zn(OH)}_2$ and thus, inhibits the complexation. This indicates that $\text{H}_2\text{L}$ is useful for detection of $\text{Zn}^{2+}$ in the biological pH that is at much lower concentration than that of WHO recommended value (76 $\mu$M) in drinking water\textsuperscript{33}.

Lifetime data were obtained upon excitation at 450 nm, and the fluorescence decay curve was deconvoluted with respect to the lamp profile. The observed fluorescence decay fits nicely with the bi-exponential decay profile for both $\text{H}_2\text{L}$ and complex (Fig. 8), which is supported by goodness of fit ($\chi^2$) data in the regression analyses. The average lifetime value of $[\text{H}_2\text{L}-\text{Zn}^{2+}]$ (0.5 ns) is longer than that of $\text{H}_2\text{L}$ (0.038 ns). The metal-ligand orbital mixing in $[\text{H}_2\text{L}-\text{Zn}^{2+}]$ may be the reason for the longer lifetime of the excited state.

**Density functional theory calculation**

Geometry optimization of $\text{H}_2\text{L}$ and $[\text{ZnL(H}_2\text{O)}]$ has been performed using DFT calculation with B3LYP method. The DFT optimized structure of the complex is a distorted tetrahedral where $\text{H}_2\text{L}$ acts as O,N,N chelator to $\text{Zn}^{2+}$. The calculated $\text{Zn-N}$ (imine), $\text{Zn-O}$ (amide carbonyl) and $\text{Zn-N}$ (imidazolium-N) distances are 2.04, 2.08 and 2.02 Å respectively and have been comparable with similar structure\textsuperscript{31,32}. Upon coordination of metal ion with the $\text{H}_2\text{L}$, the energy of HOMO increased relative to those of free $\text{H}_2\text{L}$ while LUMO decreases its energy relative to those of free $\text{H}_2\text{L}$. The decrease in the LUMO level is more significant indicating that the LUMO was more stabilized than the
### Table 1. Structure of quinolinyl fluorophore, LOD of Zn$^{2+}$ (and Reference)

<table>
<thead>
<tr>
<th>Structure</th>
<th>LOD (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure 23b" /></td>
<td>0.066 µM</td>
</tr>
<tr>
<td><img src="image" alt="Structure 23c" /></td>
<td>3.2 µM</td>
</tr>
<tr>
<td><img src="image" alt="Structure 23d" /></td>
<td>33.6 x 10$^{-3}$ µM</td>
</tr>
<tr>
<td><img src="image" alt="Structure 23e" /></td>
<td>5.81 µM</td>
</tr>
<tr>
<td><img src="image" alt="Structure 23f" /></td>
<td>0.01 µM</td>
</tr>
<tr>
<td><img src="image" alt="Structure 23g" /></td>
<td>7.1 µM</td>
</tr>
<tr>
<td><img src="image" alt="Structure 23h" /></td>
<td>4.48 µM</td>
</tr>
<tr>
<td><img src="image" alt="Structure 23i" /></td>
<td>0.256 µM</td>
</tr>
<tr>
<td><img src="image" alt="Structure 15a" /></td>
<td>0.12 µM</td>
</tr>
<tr>
<td><img src="image" alt="Structure Present work" /></td>
<td>0.36 µM</td>
</tr>
</tbody>
</table>

---

**Fig. 7.** Effect of pH on fluorescence intensity of receptor H$_2$L and H$_2$L-Zn$^{2+}$ complex.

**Fig. 8.** Decay profile of H$_2$L and [H$_2$L-Zn$^{2+}$] complex.
Purkait et al. : A novel imidazolyl appended quinoline-hydrazide Schiff base fluorescent etc.

HOMO. The HOMO-LUMO gap in H₂L (3.55 eV) has been decreased in [ZnL(H₂O)] (3.31 eV) which supports the red shift of absorption band from 332 nm to 483 nm in UV-Vis spectra (Fig. 9).

Conclusion

Quinoline-hydrazide (H₂L) has been successfully used as “turn-on” fluorescence chemosensor to Zn²⁺ ion in presence of large number of other metal ions upon irradiation at 331 nm and shows high intense emission (λ_em, 575 nm). The limit of detection (LOD) for Zn²⁺ is 0.36 mM which is far below the WHO recommended limit (76 μM). The 1 : 1 metal-to-ligand complex has been ascertained by Mass spectra and Job’s plot.

Acknowledgement

Financial support from the Council of Scientific and Industrial Research (CSIR, Sanction no. 01(2894)/09/EMR-II), New Delhi, India is gratefully acknowledged. One of the authors (RP) is thankful to Department of Science and Technology (DST), Govt. of India for providing DST-INSPIRE research fellowship.

References

1. I. Kienlen, Ann Anesthesiol Fr., 1977, 18, 1019.
Triphenylamine-based oxidized bis(indolyl)methane as fluorogenic and chromogenic chemosensor for anions

Kumaresh Ghosh* and Indrajit Saha

a Department of Chemistry, University of Kalyani, Kalyani-741 235, Nadia, West Bengal, India
b Department of Chemistry, R. K. Mahavidyalaya, Kailashahar, Unakoti-741 235, Tripura, India

E-mail: ghosh_k2003@yahoo.co.in

Manuscript received 21 April 2017, accepted 08 May 2017

Abstract: Triphenylamine coupled oxidised bis(indolyl)methane 1 has been designed and synthesized for optical sensing of anions in organic (CH₃CN) and semi aqueous (CH₃CN/H₂O, 4 : 1, v/v) medium. The sensory system exhibited ratiometric change in emission upon addition of fluoride anion in CH₃CN. Further, the chemosensor 1 displayed selective coloration from yellow to pink in presence of F⁻ anion in CH₃CN while in aqueous acetonitrile sharp colour change from yellow to pink was observed upon addition of HSO₄⁻ only. The differential selectivity of 1 in different medium is attributed to the different proton transfer signaling modes.

Keywords: Bis(indolyl)methane, fluoride sensing, hydrogen sulphate sensing, ratiometric sensing, triphenylamine.

Introduction

The significant role that anions play in biological, environmental and medicinal science has inspired supramolecular chemists to design and synthesize numerous receptors for the recognition and sensing of anionic substrates. Owing to their diverse geometry, low charge to radii ratios, narrow pH window and high solvation energy, design of anion receptor that operate with high sensitivity and selectivity towards desired analytes is particularly difficult. Charge-charge interactions, hydrogen bonding interactions, coordination bonds and hydrophobic interactions are the key tools that have been widely utilized for the recognition and sensing of anionic species. As a result, numerous reports that take the advantages of metal ions, pyridinium, imidazolium/benzimidazolium, guanidinium, polyammonium, urea/thiourea, amide, pyrrole and calixpyrrole functionalities have been documented in literature. In this regard, synthetic receptors that signal the presence of anions through change in luminescence as well as colour of the solution have drawn significant attention in recent years.

Among the various anions, fluoride has received special attention due to its potential toxicity in various living systems. Scientific challenges along with societal issues associated with the smallest anion fluoride have made the recognition and sensing of this anion by synthetic hosts is of special interest. For example, low concentration of fluoride in human diet has beneficial health effects, particularly in dental care. However, excess consumption of fluoride has toxic side effects, related to various human pathologies such as neurological dysfunction, osteosarcoma etc. Although significant progress has been made in the field of fluoride recognition and sensing, there are only a few easy-to-use chemosensors that operate with high sensitivity owing to its high charge density and high hydration energy.

The oxidised bis(indolyl)methane is a well established chromophoric unit that recognizes anions in both organic and aqueous organic solvents by exhibiting color change of the solution. It provides an acidic hy-
hydrogen bond donor moiety and a basic hydrogen-bond acceptor moiety for anion binding. Shao and co-workers first reported that the oxidised bis(indolyl)methane is an efficient chromogenic-sensing system for anion. This colorimetric anion sensor exhibited selective coloration either for $\text{F}^-$ in aprotic solvent or for $\text{HSO}_4^-$ in water containing medium based on different proton transfer signaling modes. The same group modified the bis(indolyl)methane system to tris(indolyl)methene, a new kind of unexplored chromogenic molecules. These molecules can act as excellent chemosensors for $\text{F}^-$ by two stages of proton transfer along with stepwise drastic colour changes, and could also distinguish $\text{AcO}^-$ and $\text{H}_2\text{PO}_4^-$ through spectral behaviours. Kim et al. also designed and synthesized dual colorimetric sensing system based on this bis-(indolyl)methane skeleton.

Results and discussion

The synthesis of the designed compound was achieved according to the Scheme 1. It starts with 4-(diphenylamino)benzaldehyde which was synthesized using literature procedure. The condensation reaction of with indole in CH$_3$OH afforded the compound in 90% yields. The subsequent oxidation of with DDQ in CH$_3$CN gave the desired compound in 37% yields. All the compounds were characterized using $^1\text{H}$ NMR, $^{13}\text{C}$ NMR, FTIR, and Mass spectroscopic tools.

The anion binding properties of were investigated using fluorescence, UV-Vis, and $^1\text{H}$ NMR spectroscopic techniques. The receptor exhibited a well defined fluorescence band with maxima at 365 nm and 512 nm upon excitation at 300 nm in CH$_3$CN. The bands at 365 nm and 512 nm were assigned to triphenylamine and bis(3-indolyl)methane moieties, respectively. Upon complexation with the anions, the emission intensities of both the bands were perturbed differently depending upon the anionic species (Fig. 1). While the emission intensity at 365 nm was found to be enhanced greatly upon complexation of $\text{H}_2\text{PO}_4^-$,
Ghosh et al.: Triphenylamine-based oxidized bis(indolyl)methane as fluorogenic and chromogenic etc.

Fig. 1. Change in emission of receptor 1 \((c = 3.55 \times 10^{-5} M)\) upon addition of 15 equivalent amounts of putative anions in CH\(_3\)CN.

The peak at \(\sim 512\) nm was changed merely (Fig. 2). Among the halides, only F\(^-\) ion interacted with 1 significantly. The change in emission of 1 with the increase in concentration of F\(^-\) ion is different from the other anions examined in the present study (Fig. 1).

Fig. 2. Change in emission of receptor 1 \((c = 3.55 \times 10^{-5} M)\) upon addition of H\(_2\)PO\(_4^-\) in CH\(_3\)CN.

Fig. 3 demonstrates that the titration of 1 with F\(^-\) follows a ratiometric change in emission with an isosbestic point at 468 nm. With the increase in concentration of F\(^-\), while the intensity of the peak at 365 nm was increased, the emission at 512 nm was decreased gradually. Such characteristic spectral change was observed only with F\(^-\) and is thus diagnostic to differentiate F\(^-\) fluorimetrically from other anions taken in the study. It is worthy to note that ratiometric fluorescent probes have the important feature in that they permit signal rationing increase the dynamic range and provide bait-in correction for environmental effects. Other anions such as Cl\(^-\), Br\(^-\), I\(^-\), NO\(_3^-\), CH\(_3\)COO\(^-\), ClO\(_4^-\) and HSO\(_4^-\) interacted very weakly in CH\(_3\)CN showing no significant change in emission of 1.

In aqueous CH\(_3\)CN (CH\(_3\)CN : H\(_2\)O, 4 : 1, v/v), the receptor 1 showed only one emission band at 372 nm upon excitation at 300 nm. However, no significant change in emission intensity of this band was observed upon addition of various anions. Fig. 4, in this aspect, represents the change in emission of 1 upon addition of 15 equivalent amounts of anions in aqueous CH\(_3\)CN.

The ground state interaction of 1 with the anions was investigated through UV-Vis spectroscopic technique in both CH\(_3\)CN and aqueous CH\(_3\)CN (CH\(_3\)CN :
H2O, 4 : 1, v/v) solvents. The receptor 1 showed two absorption bands at 284 nm and 436 nm along with a weak shouldering at around 550 nm in CH3CN. The strong absorption band at 436 nm, responsible for yellow colour of the solution, was assigned as the ICT absorption band of the conjugated bis(indole) skeleton. The less strong shoulder peak at 550 nm is related to its intermolecular hydrogen bond interaction, which disappeared upon addition of polar protic solvent such as H2O and CH3OH.

Fig. 5 collectively represents the change in absorption of 1 upon addition of various anions. Except fluoride, other anions did not show any red or blue shift in the absorption spectra. Fig. 6 illustrates the change in absorption of 1 with increase in concentration of F− in CH3CN. As the concentration of F− ion increases, absorbance at 436 nm decreases with a blue shift of 35 nm and a new band at 520 nm appeared showing an isosbestic point at 480 nm. Three other isosbestic points were noticed at 246 nm, 310 nm and 366 nm. In the event the colour of the solution changes from yellow to pink and this is attributed to the formation of deprotonated receptor (Fig. 7A). Initially, F− ion forms hydrogen bonding complex and at high concentration, by virtue of its basic nature, it deprotonates the receptor. The new band at 520 nm was related to the formation of deprotonated receptor. Such deprotonation was related to the acidity of the H-bond donor sites and the particular stability of [HF2]− hydrogen bond complex.

On the other hand, in aqueous CH3CN (CH3CN : H2O, 4 : 1, v/v), the compound 1 behaved differently towards the same anions. In this solvent composition,
no characteristic change in UV-Vis spectra of 1, upon titration with various anions, was observed except for HSO$_4^-$ and CH$_3$COO$^-$.  

Fig. 8 represents the change in absorbance with increase in concentration of F$^-$, CH$_3$COO$^-$ and HSO$_4^-$. During complexation of AcO$^-$, the band at 455 nm moved to 478 nm with the appearance of a weak shoulder peak at 540 nm (Fig. 8b). In the case of HSO$_4^-$, the band at 455 nm underwent red shift showing new absorption band at 528 nm, which was gradually intensified upon complexation (Fig. 8c). This resulted in a visual color change from yellow to pink (Fig. 7B). Since the HSO$_4^-$ anion is a relatively strong acid (p$K_a$ = 1.99 in aqueous solution), the HSO$_4^-$-induced change is mainly the consequence of a simple protonation of receptor 1. The band that develops at 500 nm pertains to the protonated receptor [H$_2$1]$^+$, which was confirmed by the protonation of receptor 1 by using perchloric/acetic acid in CH$_3$CN/H$_2$O or in CH$_3$CN$^{13}$. The interaction of 1 with F$^-$ was also probed using $^1$H NMR experiments, carried out in DMSO-$d_6$ (solubility problems in CD$_3$CN in $^1$H NMR concentration range prevented the study in CD$_3$CN). The signals for NH-proton in 1 was difficult to identify. This may be either due to significant broadening or overlapping with the other signals appeared in the aromatic region. However, it was found that the signals for indole ring protons in 1 underwent upfield shift upon addition of 0–2 equivalent amounts of F$^-$ ion (Fig. 9). These observations reveal that F$^-$ first establishes H-bond interaction with the NH subunit of 1, while the second F$^-$ induces the deprotonation of the NH fragment, which brings electron density onto the $\pi$-conjugated framework with through-bond propagation, thus causing a shielding effect and inducing upfield shift. In the full deprotonated form, the polarization effect is no longer present since the anion does not remain in proximity to the receptor.
Conclusion

In conclusion, it has been shown that triphenylamine coupled oxidized bis(indolyl)methane 1 can report the selective recognition of anions through the change in photophysical properties of the triphenylamine unit. The compound works both in organic and aqueous organic solvents. The ratiometric change in emission, observed only with F⁻ in CH₃CN, is diagnostic to differentiate F⁻ anion fluorimetrically from other anions taken in the study. Also, the sensor exhibited selective coloration either for F⁻ in aprotic solvent and or for HSO₄⁻ in water containing medium based on different proton transfer signaling modes. The change in color of the receptor solution upon complexation of F⁻ and HSO₄⁻ is sharp and reproducible. Further work along this direction is underway in the laboratory.

Experimental

General: Reagents and solvents were purified using standard techniques. Solvents were dried over the appropriate drying agent following standard procedure. Solvents for spectroscopic measurements were of spectroscopic or HPLC grade. Infrared spectra were recorded on Perkin-Elmer L120-00A spectrophotometer. ¹H NMR spectra were recorded at 400 MHz using Bruker instrument. Mass spectra were recorded on API 2000 LCMS/MS instrument. Fluorescence measurements were done using Perkin-Elmer LS55 and UV-Vis absorption spectra were recorded at room temperature using Perkin-Elmer Lambda 25.
4-(Di(1H-indol-3-yl)methyl)-N,N-diphenylaniline 3

KHSO₄ (0.170 g, 1.25 mmol) was added to a mixture of indole (0.292 g, 2.5 mmol) and the aldehyde 2 (0.342 g, 1.25 mmol) in dry methanol (10 mL), and the reaction was stirred at room temperature for 4 h. Then water (10 mL) was added to quench the reaction, and the aqueous phase was extracted with CH₂Cl₂ (3×10 mL). The organic phase was dried with anhydrous Na₂SO₄, and was removed under reduced pressure to give the crude product. Repeated crystallization of the crude product using the mixture solvent (ethyl acetate : petroleum ether = 1 : 3, v/v) afforded the product 3 as white solid (0.445 g, yield 90%), m.p. 100 ºC (decomposed); ¹H NMR (400 MHz, CDCl₃) : δ 7.93 (2H, s), 7.43 (2H, d, J=8 Hz), 7.36 (2H, d, J=8 Hz), 7.23–7.15 (8H, m), 7.08–6.94 (10H, m), 6.72 (2H, s), 5.84 (1H, s); ¹³C NMR (100 MHz, CDCl₃) : 147.9, 145.7, 138.5, 136.7, 129.4, 129.1, 127.1, 125.6, 124.1, 123.9, 123.5, 122.4, 121.9, 119.9, 119.8, 119.2, 111.0; FTIR (KBr, cm⁻¹) : 3417, 3057, 3035, 1587, 1505, 1493.

General procedures of UV-Vis and fluorescence titration

Stock solutions of the hosts and guests were prepared in CH₃CN or CH₃CN-H₂O and 2.5 ml of the individual host solution was taken in the cuvette. The solution was irradiated at the excitation wavelength maintaining the excitation and emission slits. Upon addition of guest anions, the change in fluorescence emission of the host was noticed. The corresponding emission values during titration were noted. For UV-Vis titration the receptors were dissolved in dry UV mixture was further stirred for 4 h, gave a dark red precipitate, which was filtered, washed with CH₃CN, and recrystallized from ethanol to afford pure compound 1 (0.100 g, yield 37%), m.p. 140 ºC (decomposed); ¹H NMR (400 MHz, DMSO-d₆) : δ 7.96 (2H, s), 7.54 (2H, d, J=8 Hz), 7.41 (4H, t, J=8 Hz), 7.33 (2H, d, J=8 Hz), 7.22–7.15 (8H, m), 7.00 (4H, d, J=8 Hz), 6.85 (2H, d, J=8 Hz); ¹³C NMR (100 MHz, DMSO-d₆) : δ 151.8, 149.7, 146.2, 133.4, 131.9, 129.8, 129.3, 128.1, 125.4, 124.5, 124.3, 123.2, 122.2, 120.8, 119.9, 116.1 (one carbon in aromatic region is unresolved); FTIR (KBr, cm⁻¹) : 3392, 3063, 1608, 1588, 1556, 1529, 1503, 1489; MS (LCMS) C₃₅H₂₅N₃ requires 487.20; found 488.4 (M+H)⁺.
grade CH$_3$CN or CH$_3$CN-H$_2$O and 2.5 ml of the individual host solution was taken in the cuvette. Then, anions, dissolved in dry CH$_3$CN were individually added in different amounts to the receptor solution and the corresponding absorbance values during titration were noted.

Acknowledgement

KG thanks DST [755 (Sanc.)/ST/P/S & T/4G-3/ 2015-16/MRP/NERO/1083 dated 29.03.2015] is also gratefully acknowledged.

References


Chemosensors based on diazole derivatives

Manjira Mukherjee and Pabitra Chattopadhyay*

Department of Chemistry, The University of Burdwan, Golapbag, Burdwan-713 104, West Bengal, India

E-mail: pabitrac@ yahoo.com

Abstract: The heterocyclic diazole compounds and their derivatives not only exhibit a wide range of pharmacological activity but also can act as bioagent and revolve around its ability to bond to various guests as host. Hence it is useful in designing chemosensors. This review summarizes the most recent and relevant advances in this area.

Keywords: Diazole, bioagent, chemosensors.

Introduction

Azoles are five-membered heterocyclic compounds having a nitrogen atom and at least one other non-carbon atom (i.e. nitrogen, sulfur or oxygen) as part of the ring, diazoles refer to a special class of azoles as these are also five-membered aromatic heterocyclic compounds consisting of three carbon atoms and two nitrogen heteroatoms. Their names have been derived from the Hantzsch-Widman nomenclature (also called the extended Hantzsch-Widman system), where the numbering of the ring atoms in azoles starts with the heteroatom that is not part of a double bond, and then proceeds towards the other heteroatom. These compounds having two double bonds are aromatic in nature due to the participation of only one lone pair of electrons of each heteroatom in the ring as a part of the aromatic bonding in an azole. The two double bonds of the parent compound could be successively reduced to produce reduced analog with fewer like azolines and azolidines and upon reduction the names of azoles maintain the prefix e.g. pyrazoline, pyrazolidine.

Such heterocyclic units have many uses. They exhibit a wide range of pharmacological activity, ubiquitous in nature and play a critical role in many structures within the human body, notably histamine and histidine which contain imidazole unit. A special interest of researchers towards benzimidazole derivatives was stimulated by the fact that 5,6-dimethyl-1-(α-dribofuranosyl)benzimidazole is an basic part of the structure of vitamin B12, and benzimidazole is a structural unit of naturally occurring nucleotide, due to which it can easily interact with the biopolymers of living system, which is responsible for its numerous biological aspects. Diazoles like benzimidazole and pyrazole ring become an important pharmacophore in modern drug discovery. Moreover dazoles can be used as bioagent and its ability to bond to various guests as ligand make it useful in designing chemosensors for various ions. This review covers recent important development of diazole (here benzimidazole and pyrazole) as receptors for the recognition of various anions, cations and neutral molecules.

Imidazole/benzimidazole based compounds as chemosensor

Benzimidazole ring behaves as an excellent hydrogen bond donor moiety in synthetic anion receptor systems, and the acidity of the NH proton of the imidazole can be tuned by changing the electronic properties of the imidazole substituents. On the other hand, the presence of a donor pyridine-like nitrogen atom within the ring, capable of selectively binding cationic species also converts the imidazole derivatives into excellent metal ion sensors. Due to the emissive properties of the aromatic ring, benzimidazole has also been commonly used in molecular recognition of all cations, anions and neutral molecules. As a result, this
moiety has been used not only as a binding unit for cations and anions, as the imidazole derivatives do, but also as a fluorogenic antenna. Another fact with the imidazole ring is the two NH protons of different acidity caused by the electronic effect of the benzene ring. Here we cover important development of benzimidazole derivative receptors for the recognition of various anions, cations and neutral molecules. We have grouped the receptors to their ion sensing which include cations, anions, cations-anions both and neutral molecules which behaving as sensors for different ions.

Chemosensors for cations:

In the field of molecular recognition of cations Garcia and co-workers had mentioned the macrocyclic ligand, 1 (Table 1). The N and S atoms present in this system act as recognition sites while the two benzimidazole units act as fluorescent antenna for the detection of transition metal cations in water. A bathochromic shift in the UV-Vis bands upon addition of the corresponding cations is due to the metal coordination. Titration experiments carried out by using this technique were used to calculate the association constant of the different complexes, showing a higher affinity towards Cr$^{3+}$, followed by the Fe$^{2+}$ cation, while other metal cations as Hg$^{2+}$ or Cd$^{2+}$ have much smaller association constants. The selectivity found in this receptor has been attributed to the relative Lewis acidity of the cations that dominates the coordination capabilities of the receptor. The small selectivity in the process indicates that fluorescence quenching has a diffusive behaviour.

Fahrni’s group developed a Zn$^{2+}$-selective ratiometric biologically suitable probe (2) showed the cation-induced inhibition of excited-state intramolecular proton transfer (ESIPT) with a series of 2-(2’-benzenesulfonamidophenyl)benzimidazole derivatives in emission study. In the absence of Zn$^{2+}$ at neutral pH, the fluorophores undergo ESIPT to yield a highly Stokes’ shifted emission from the proton-transfer tautomer. Coordination of Zn$^{2+}$ inhibits the ESIPT process and yields a significant hypsochromic shift of the fluorescence emission maximum. Due to the modular architecture of the fluorophore, the Zn$^{2+}$ binding affinity can be readily tuned by implementing simple structural modifications. The synthesized probes are suitable to gauge free Zn$^{2+}$ concentrations in the micromolar to picomolar range under physiological conditions.

Guo and co-workers devised a fluorescent 2-(2’-pyridinyl)benzimidazole based Zn$^{2+}$ sensor (3). The sensor demonstrates a Zn$^{2+}$-specific emission shift and enhancement with a 1 : 1 binding ratio. Due to the Zn$^{2+}$-induced coplanation via reversion/rotation, the sensor behaves as a ratiometric sensor. The intracellular Zn$^{2+}$ imaging ability of the sensor has been tested in HeLa cells using a confocal microscope.

Rao and co-workers has reported the benzimidazole-substituted calix[4]arene (4) for the selective recognition of Hg$^{2+}$ versus a large number of divalent cations (Table 1) in MeOH, anhydrous CH$_3$CN and also in different aqueous mixtures of this organic solvent. In 50% aqueous solution, a 10-fold quenching in the fluorescence was observed only in the case of Hg$^{2+}$, whereas all other divalent metal cations tested exhibited almost no quenching in fluorescent intensity. However, when the amount of water decreases, the selectivity for this cation also decreases, due to the effect of a less competitive media. Binding of Hg$^{2+}$ has been attributed, by $^1$H NMR experiments, to the nitrogen atoms in the benzimidazole ring and the oxygen atoms of the amide bridge, as the theoretical calculations also predicted. The Hg$^{2+}$-complex formed has been isolated, characterized and studied on surface by TEM, SEM and AFM microscopies.

Minkin and co-workers synthesized a number of 1-(9-anthrylmethyl)-2-aryl-1H-benzimidazoles (5). Study on their luminescent properties and complexing ability showed that the sensitivity of these systems to H$^+$ and Hg$^{2+}$ ions is related to fluorescence quenching by the action of these cations and effectively selective towards H$^+$ and Hg$^{2+}$ ions.

Jang’s group synthesized a benzimidazole-based fluorescent receptor (6) bearing imine linkages with two sets of sp$^2$ nitrogens, and investigated its binding properties toward various metal ions. The receptor exhibited a shift in emission band upon binding with Fe$^{3+}$ ions, and no such significant response was noticed in other metal ions. The receptor shows a prop-
Singh and co-workers have synthesized a novel fluorescent receptor (7) based upon a benzimidazole moiety in a dipodal framework. The receptor exhibited a dual fluorescence emission which is quenched upon addition of Cu$^{2+}$ or Fe$^{3+}$. Interestingly, the receptor offers a ratiometric property and an ‘OR’ logic gate property to Cu$^{2+}$ and Fe$^{3+}$.

A different approach has been followed by Tang and Nandhakumar who designed a Rhodamine B colorimetric chemosensor (8) based on the chemical changes induced by Cu$^{II}$ anion (Table 1). UV-Vis experiments demonstrated that upon addition of Cu$^{II}$ the

<table>
<thead>
<tr>
<th>Table 1. Benzimidazole based chemosensors recognizing cations</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Chemical structures" /></td>
</tr>
</tbody>
</table>

![Chemical structures](image2)
Table-1 (contd.)
Table 1 (contd.)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>JICS-19</td>
<td><img src="image1" alt="Structure Image" /></td>
</tr>
<tr>
<td>(25)</td>
<td><img src="image2" alt="Structure Image" /></td>
</tr>
<tr>
<td>(26)</td>
<td><img src="image3" alt="Structure Image" /></td>
</tr>
<tr>
<td>(27)</td>
<td><img src="image4" alt="Structure Image" /></td>
</tr>
<tr>
<td>(28)</td>
<td><img src="image5" alt="Structure Image" /></td>
</tr>
<tr>
<td>(29)</td>
<td><img src="image6" alt="Structure Image" /></td>
</tr>
<tr>
<td>(30)</td>
<td><img src="image7" alt="Structure Image" /></td>
</tr>
<tr>
<td>(31)</td>
<td><img src="image8" alt="Structure Image" /></td>
</tr>
<tr>
<td>(32)</td>
<td><img src="image9" alt="Structure Image" /></td>
</tr>
<tr>
<td>(33)</td>
<td><img src="image10" alt="Structure Image" /></td>
</tr>
<tr>
<td>(34)</td>
<td><img src="image11" alt="Structure Image" /></td>
</tr>
<tr>
<td>(35)</td>
<td><img src="image12" alt="Structure Image" /></td>
</tr>
<tr>
<td>(36)</td>
<td><img src="image13" alt="Structure Image" /></td>
</tr>
<tr>
<td>(37)</td>
<td><img src="image14" alt="Structure Image" /></td>
</tr>
<tr>
<td>(38)</td>
<td><img src="image15" alt="Structure Image" /></td>
</tr>
<tr>
<td>(39)</td>
<td><img src="image16" alt="Structure Image" /></td>
</tr>
</tbody>
</table>
change of colour in the solution from colourless to pink with appearance of a low energy band. This colour change has been attributed to a ring-opening process of the spirolactam form of chemosensor (8). Addition of other metal cations does not produce such changes in the absorption spectrum and does not disturb the effect of Cu\textsuperscript{II} in competitiveness experiments. The reversibility of the process has confirmed by the addition of EDTA to the medium that cause the disappearance of colour, returning the solution to the initial colourless appearance.

A rhodamine-benzimidazole conjugate (9) was designed and synthesized by Li and co-workers which detect selectivity Fe\textsuperscript{3+} in a 1:1 stoichiometry over other metal ions in acetonitrile\textsuperscript{13}. A thousand time fluorescence intensity enhancement was observed at the maximum emission wavelength of 582 nm upon the addition of 10 equiv. of Fe\textsuperscript{3+} the detection limit was $1.5 \times 10^{-8}$ M.

Wang and co-workers synthesized a dehydroabietyl molecule (10) with a benzimidazole unit for the molecular recognition of metal cations\textsuperscript{14}. Receptor (10) decreases its fluorescent emission at $\lambda_{em} = 540$ nm upon addition of Cu\textsuperscript{II} whilst the rest of the cations do not modify or increase the fluorescent emission. Moreover, addition of up to 2 equiv. of Cu\textsuperscript{II} also modifies the absorption spectrum. The Job’s plot of the UV-Vis data yielded a 1:1 stoichiometry for its Cu\textsuperscript{II} complex.

Ghosh et al. reported a simple tripodal shaped chemosensor (11) for metal ions\textsuperscript{15}. In CH\textsubscript{3}CN containing 0.04\% DMSO, upon excitation at 370 nm, the chemosensor exhibited structured emission centred at 418 nm, which increased to a significant extent upon complexation of Cu\textsuperscript{II}. The other metal ions except Zn\textsuperscript{2+} and Hg\textsuperscript{2+} examined in this study did not exhibit any marked change in emission under a similar condition. Although Cu\textsuperscript{2+} ion showed strong interaction with (11), Zn\textsuperscript{2+} and Hg\textsuperscript{2+} ions exhibited moderate binding.

Jang’s group synthesized a novel benzimidazole-based, anthracene-coupled fluorescent receptor (12) capable of recognizing and estimating the concentrations of Fe\textsuperscript{3+} in semi-aqueous solution by ratiometric estimation\textsuperscript{16}. The sensor can be made highly selective for Fe\textsuperscript{3+} over other metal ions by changing the solvent composition.

Ghosh et al. reported a benzimidazole based chemosensor (13), derived from L-valine\textsuperscript{17}. The chemosensor effectively recognises Hg\textsuperscript{2+} in the open cleft in CH\textsubscript{3}CN containing 0.2\% DMSO by exhibiting significant enhancement in fluorescence emission. The steric isopropyl groups in (13) play the key role in the selectivity. The ensemble of (13).Hg\textsuperscript{2+} also showed the fluorescence sensing of L-cysteine, homocysteine, and glutathione over the other amino acids with no thiol group in aq. DMSO (DMSO : H\textsubscript{2}O, 4 : 1, v/v).

Luxami et al. reported a probe (14) selectively bind with Zn\textsuperscript{2+} ions at pH 7.0 (10 mM, HEPES) in CH\textsubscript{3}CN-H\textsubscript{2}O (4 : 1) and give a new emission band at 490 nm\textsuperscript{18}. These can estimate 20 nM Zn\textsuperscript{2+} ions as the lowest detection limit. The determination of Zn\textsuperscript{2+} ions by probe (14) is not interfered by the presence of other metal ions expects in the case of Cu\textsuperscript{2+} causes only the fluorescence quenching. In acetonitrile, the addition of different concentrations of Cu\textsuperscript{2+} (2 mM, 5 mM, 10
mM) and a fixed amount of F– (25 mM) to a solution of (14) (0.25 mM, CH₃CN) elaborates Cu²⁺ ion concentration dependent NOR, INH and AND Boolean operators with three distinct emission channels at 585, 515 and 400 nm, respectively.

Dessingou’s group showed the benzimidazole containing moiety (15) act as a selective “turn-on” fluorescence response toward Ag⁺. The selectivity of (15) has been explored in aqueous methanol, resulting in selective 8-fold switch-on fluorescence response toward Ag⁺ among 14 different transition, alkali, and alkaline earth metal ions studied. The complexation of Ag⁺ by (15) has been addressed by ESI-MS, ¹H NMR, and UV-Vis spectra. Microstructural features of (15) and its Ag⁺ complex have been measured by AFM and TEM. The morphological features of (15) alone and (15) in the presence of Ag⁺ differ dramatically both in shape and size, and the ion induces the formation of chains owing to its coordinating ability toward benzimidazole. Further, the in situ [Ag⁺.(15)] complex was titrated against 20 naturally occurring amino acids and found that this complex acts as a secondary recognition ensemble toward Cys, Asp, and Glu by switch-off fluorescence.

Singh et al. developed a new benzimidazole-based receptor (16) with potential functional groups for excited state proton transfer (ESPT) through ketoenol tautomerism. The enol form of the receptor selectively recognizes Zn²⁺, allowing it to be used as a ratiometric fluorescence sensor in DMSO/CH₃CN (1 : 9, v/v). The binding event triggers a blue-shifted band through the modulation of charge transfer transitions. The sensor is applicable for recognizing Zn²⁺ in the cytoplasm of Saccharomyces cerevisiae.

An imine-linked, benzimidazole-based sensor (17), reported by Jang et al., was used for chromogenic recognition of Mg²⁺ and fluorescent recognition of Cr³⁺. Addition of Mg²⁺ to a solution of (17) in CH₃CN-HEPES buffer (8 : 2, v/v, pH 7.0) led to a stepwise decrease in absorbance at 400 nm and an increase at 350 nm with a clear isosbestic point at 385 nm. Cr³⁺ binding with sensor (17) caused a change in the fluorescence spectra of (17) with quenching at 415 nm and enhancement at 475 nm. In the absence of Cr³⁺, the enol form of (17) was in equilibrium with its keto tautomer in the excited state. The modulation of the fluorescence spectrum of (17) with the addition of Cr³⁺ ions was due to the formation of a stable Cr³⁺ complex with the keto form of (17). In addition, (17) was applicable for staining the cytoplasm of microbial cells enriched with Cr³⁺.

Two Zn²⁺ ion selective fluorescent probes (18a) and (18b) based on a coumarin Schiff base were designed and synthesized by Guo et al. on the basis of C=N isomerization mechanism. Both the X-ray crystal structure of the zinc complex and the Job’s plots showed a 1 : 1 probe-Zn²⁺ ion identification with high selectivity and sensitivity. The probe (18a) was also used to image intracellular Zn²⁺ ions in MCF-7 cells with a good performance.

Interestingly, imidazo-benzocrown ether-based ionophores (19-21) bearing arylthienyl and bithienyl π-conjugated bridges have been evaluated as fluorimetric sensors for CuⅡ and PdⅡ ions (Table 1). Moreover, systems (20 and 21) have also proved to be efficient sensors for basic anions such as F–, due to changes in emission after deprotonation of the imidazole NH by the F⁻ ion.

Molina et al. reported a redox, chromogenic, and fluorescent chemosensor molecule (22) based on a deazapurine ring which could selectively detect aqueous Pb²⁺ in acetonitrile over other metal ions. Probe (22) showed a redox shift (E₁/₂ = 0.15 V of the Fe²⁺/Fe³⁺ redox couple) with a color change from the colorless to orange, and an emission change of 620-fold, with an unprecedented detection limit of 2.7 μg L⁻¹. The signal transduction occurs via a reversible CHEF with this inherent quenching metal ion.

A mercury non-sulfur and simple fluorescent chemosensor (23) based on the benzimidazole group and quinoline group as the fluorescence signal group has been designed and synthesized by Chen’s group. The receptor could instantly detect the Hg²⁺ cation over other cations by fluorescence spectroscopy in H₂O/DMSO (1 : 9, v/v) solution with specific selectivity and high sensitivity with the fluorescence color change.
of the solution from blue to colorless only after the addition of Hg$^{2+}$ in aqueous media. Moreover, further study demonstrates that the detection limit on the fluorescence response of the sensor to Hg$^{2+}$ is $9.56 \times 10^{-9}$ M. Test strips based on (23) were fabricated, which could act as convenient and efficient Hg$^{2+}$ test kits. Thus, the probe should have potential applications in an aqueous environment for the monitoring of mercury.

Zhang et al. reported a fluorescent sensor (24) based on benzimidazo[2,1-a]benz[de]isoquinoline-7-one which can be used as chemosensor for various metal ions. By the introduction of N,N-bis(pyridin-2-ylmethyl)-ethane-1,2-diamine as an electron-donating receptor, chemosensor (24) exhibited weak fluorescence due to fluorescence quenching by the PET (photoinduced electron transfer) process from the lone pair electrons on the nitrogen atom to the fluorophore. On the other hand, the receptor may have a strong binding ability toward metal ions due to its multiple coordinating groups. After binding with metal ions, a large chelation-enhanced fluorescence (CHEF) effect would be observed because the chelation abrogated the PET process giving it possible to be used as a fluorescent sensor in pure water.

Mashraqui et al. synthesized a chemosensor (25) in two steps from readily accessible 2,6-bis(2-benzimidazole)pyridine. Photophysical studies revealed that of the several metal ions examined, biologically and environmentally significant Zn$^{2+}$ exhibited highly selective emission wavelength shifts under the buffer condition. In contrast to Zn$^{2+}$, the coordinatively competing and toxic Cd$^{2+}$ elicited less remarkable optical responses as evidenced by its two order of magnitude lower stability constant compared to that of Zn$^{2+}$. Moreover, metal ions, viz. Li$^+$, Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Ba$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Hg$^{2+}$ and Pb$^{2+}$ exhibited insignificant optical perturbations even in concentrations far exceeding Zn$^{2+}$. Clearly, the probe has the attributes to selectively target Zn$^{2+}$ by ratiometric analysis under buffer conditions.

A rhodamine-benzimidazole based chemosensor (26) was designed and prepared by Li’s group for Fe$^{3+}$ via opening of the spiro-ring to give fluorescent and colored species. (26) exhibited high selectivity and excellent sensitivity in both absorbance and fluorescence detection of Fe$^{3+}$ in aqueous solution with comparatively wide pH range (5.8–7.4). The detection limit of this newly developed probe was shown to be up to 2.74 µM. The reversibility establishes the potential of both probes as chemosensors for Fe$^{3+}$ detection. Fluorescence imaging experiments of Fe$^{3+}$ in living MGC803 cells demonstrated its value of practical applications in biological systems. It also displayed enhanced fluorescence intensities and clear color changes upon recognition. Moreover, fast response (in 10 s) and neutral aqueous medium made it possible to be practical application for Fe$^{3+}$ ions. The probe could be applied in biological systems for the detection of Fe$^{3+}$ through confocal laser scanning microscopy experiments.

On the basis of fluorescence resonance energy transfer (FRET) from benzimidazole to a rhodamine moiety, another rhodamine-benzimidazole conjugate (27) ratiometric fluorescent probe has been designed and synthesized by Goswami et al. (27) selectively binds to Cu$^{2+}$, showing visually observable changes in absorption and emission behavior, and demonstrates an effective intracellular Cu$^{2+}$ imaging ability, allowing it to function as a cytoplasm marker.

Tang’s group reported a phenylbenzimidazole derivatized sensor (28) that behaves as a ratiometric fluorescent receptor for Zn$^{2+}$ in water has been described. In HEPES buffer at pH 7.4, sensor (28) displays a weak fluorescence emission band at 367 nm. Upon addition of Zn$^{2+}$, the emission intensity at 367 nm is decreased, concomitantly, a new emission band centered at 426 nm is developed, thus facilitates a ratiometric Zn$^{2+}$ sensing behavior. Sensor (28) binds Zn$^{2+}$ through a 1 : 1 binding stoichiometry with high selectivity over other metal cations. Sensor displays a linear response to Zn$^{2+}$ concentration from 0 to $6.0 \times 10^{-5}$ M, sensor (28) also exhibits high sensitivity to Zn$^{2+}$ with a detection limit of $3.31 \times 10^{-7}$ M.

Li and co-workers reported an optical and fluorometric pH sensor (29) by attaching pH sensitive group
benzimidazole at the 2-position of borondipyrromethene31. Due to the electron-donor effect, benzimidazole group caused obvious bathochromic shift in the absorption and fluorescence spectra. Upon protonation, the solution exhibited drastic blue-shifted absorption and enhanced fluorescence, and the color changed from yellow to green simultaneously. The sensing mechanism was elucidated by quantum chemistry calculation approach. Cell experiments were carried out to verify the compound could selectively locate in the acidic lysosome. These results indicated the benzimidazole-BODIPY could be used as an optical and “off-on” fluorescent pH indicator.

A highly sensitive and selective benzimidazole based colourimetric chemosensor (30) for the efficient detection of Ni2+ has been reported by Mondal et al.32. The synthesized chemosensor (30) is highly efficient in detecting Ni2+ over other metal ions that commonly coexist with Ni2+ in physiological and environmental samples. Probe (30) also shows distinct color change from orange yellow to blue visible under the “naked-eye” due to specific binding with Ni2+. This color change corresponds to a large red shift of the UV-Vis spectrum from 403 nm to 600 nm with a distinct isosbestic point at around 500 nm. The cation sensing property of the receptor (30) has been examined by UV-Vis spectroscopy. Electronic structure of its Ni2+ complex and sensing mechanism has been interpreted by DFT and TDDFT calculations.

Naphthalene benzimidazole conjugate (31) bearing a hydroxyl group was synthesized by Nandhakumar et al. Its binding properties towards various metal ions were examined and it showed a high selectivity and sensitivity towards Al3+ ions in CH3CN-H2O solution (1 : 1, v/v, HEPES 50 mM, pH 7.0)33. The sensor was designed in such a way that the naphthalene acts as fluorophore which is covalently attached to the benzimidazole scaffold which contains the heterocyclic nitrogen atoms. The recognition processes follows a photo induced electron transfer (PET) mechanism assisted with the restricted intramolecular C-C single bond rotation and are scarcely influenced by other coexisting metal ions. In addition, determination of Al3+ in a variety of sewage water samples was also determined. These authors synthesized a quinoline benzimidazole-conjugate (32) for the highly selective detection of ZnII both by colorimetry and fluorimetry34. Probe (32) senses Zn2+ over other cations as fluorescence “off-on” behaviour in HEPES-buffered CH3CN/H2O (1 : 1, v/v, pH 7.0) solution. A possible mechanism is proposed based on the inhibition of PET and intramolecular restricted torsional rotation through the C-C single bond between the quinoline benzimidazole-conjugate. This probe has been reported as a useful chemosensor to detect Zn2+ ions in much real sample analysis. Singh’s group synthesized a novel receptor and multianalyte fluorescent probe with benzimidazole moieties in a tripodal framework (33)35. The receptor displays rarely observed metal specific fluorescence enhancement at two different wavelengths. The receptor was investigated for the simultaneous analysis of Cu2+ and Fe3+ and successfully quantified the ions without interference over a wide concentration range.

Recently reported some benzimidazole derivatives having quinazoline moiety (34-42) (viz. Table 1) showed metal sensing property either by formation of chelating environment for incoming metal ions. Interestingly, in some cases this type of compounds ligate the metal ions selectively through solvent/metal ion assisted 1,5-σ tropic shift (34-42). The first example where reported a probe 2,6-bis(5,6-dihydrobenzo-[4,5]imidazo[1,2-c]quinazolin-6-yl)-4-methylphenol (34) as a selective and sensitive fluorescent probe for Zn2+ in a HEPES buffer (50 mM, DMSO : water = 1 : 9 (v/v), pH 7.2) at 25 ºC36, where formation of the zinc complex needs a [1,5] sigmatropic-type shift of the secondary N-H proton of the probe (34) prior to binding with Zn2+ to form a Schiff base structure evidenced by the solid state crystal structure. The selective enhancement of fluorescence intensity in presence of Zn2+ is due to the formation of dinuclear Zn2+ complex [Zn2(C35H25N6O)(OH)(NO3)2(H2O)] with increase of fluorescence quantum yield of the chemosensor by more than 12-fold in the presence of 2 equiv. of the zinc ion. Here, upon complexation, the
lone pair of electrons on the N atom of the organic moiety is no longer available for PET, leading to fluorescence enhancement. This probe is also potent to identify the distribution of Zn$^{2+}$ ions in the living cells (A375 and HT-29) using fluorescence microscopy. Similar type compound (35) have also reported as a highly selective ratiometric fluorescent sensor for Fe$^{2+}$ at pH 4.0–5.0 and Fe$^{3+}$ at pH 6.5–8.0 in acetonitrile-HEPES buffer (1/4) (v/v) medium. The probe is a “naked-eye” chemosensor for two states of iron and is efficient for detecting in vitro Fe$^{3+}$ ions in the biological organelles by developing a fluorescence images. According to the same group, probe (36) was rationally illustrated as a highly selective cell-permeable ratiometric fluorescent chemosensor for ReO$^V$ ion in acetonitrile : water = 9 : 1 (v/v) at 25 ºC. The fluorescence quantum yield of the chemosensor (36) was only 0.198 at 410 nm, and it increased more than 3-fold in the presence of 2 equiv. of the ReO$^V$ ion at 478 nm with almost no interferences of other metal ions and relevant anions. In another example a benzimidazole based fluorescent Cr$^{3+}$ receptor, (37) was reported in vitro, developing a good image of the biological organelles. Recently there is example of two structurally modified quinazoline derivatives (38 and 39) act as highly selective chemosensor for Al$^{3+}$ ions in DMSO-H$_2$O (1 : 9, v/v) over the other competitive metal ions. Both the probes show a red shifted fluorescence after addition of Al$^{3+}$ ions and later the further fluorescence enhancement is due to chelation enhanced fluorescence (CHEF) through inhibition of photoinduced electron transfer (PET). (38) detects Al$^{3+}$ ions as low as 9 nM whereas (39) can detect Al$^{3+}$ ions as low as 1.48 nM in 100 mM HEPES buffer (water/DMSO, 9/1, v/v) at biological pH within a very short responsive time (15–20 s). This two non cytotoxic probe can efficiently detect the intercellular distribution of Al$^{3+}$ ions in living cells under fluorescence microscope to exhibit its sensible applications in the biological systems. In this context Chellappa et al. reported a ratiometric chemosensor, 6-imidazo-2-yl-5,6-dihydrobenzo[4,5]imidazo[1,2-c]quinazoline (40) selective for Al$^{3+}$ ions as low as 5.3×10$^{-7}$ M in aqueous-DMSO. A highly fluorescent and efficient ratiometric “turn-on” probe for Hg$^{2+}$ and Al$^{3+}$ based on quinazoline (41) has been designed and synthesized by Pandey and co-authors. Limit of detection (LOD) and association constants revealed its superior binding affinity and sensitivity toward Hg$^{2+}$ over Al$^{3+}$. Paul et al. reported structurally characterised quinazoline functionalized benzimidazole-based fluorogenic chemosensor (42) as a highly selective colorimetric and fluorescence sensor for Cu$^{2+}$ ions in DMSO/HEPES buffer (1:4, v/v) medium. Again, this copper(II) complex acts as a metal based highly selective and sensitive chemosensor for S$^{2–}$ ions even in the presence of other commonly coexisting anions such in DMF/0.02 M HEPES (1 : 1, v/v, pH 7.4) medium due to the liberation of probe (42). Quantification analysis indicates that these receptors, (42) and its Cu$^{II}$ complex, can detect Cu$^{2+}$ ions and S$^{2–}$ ions upto 1.6×10$^{-9}$ M and 5.2×10$^{-6}$ M, respectively.

**Chemosensors for anions:**

The benzimidazole based organic moieties of judicious designs are also effective to detect the anions selectively. Jang and co-workers reported a compound comprise of three bezimidazole units connected by a tripodal 2,2',2''-trioxietylamine linker (43) which behaves as a good I$^–$ ion selective chemosensor in CH$_3$CN/water (9 : 1) buffered with HEPES at neutral pH based on quenching mechanism. The $^1$H NMR titration with iodide showed that this anion is fundamentally bound by the NH protons of the benzimidazole moiety.

The receptors (44 and 45), reported by Jang and co-workers, in which one or two 2-aminobenzimidazole groups are connected to an anthracene ring are significant in the field of molecular recognition of anions. Receptor (44) displayed strong fluorescence emission in CH$_3$CN, which is quenched with the addition of halide, AcO$^–$ and H$_2$PO$_4$$^–$ anions. The quenching mechanism has been attributed to a photoinduced electron transfer (PET) process. Compound (45), bearing two benzimidazole units, presents similar results and selectivities, though higher association constants were
found and a preference for AcO$^-$ over F$^-$ was also observed.

Jang’s group synthesized a fluorescent receptor (46) bearing benzimidazole moiety as recognition sites (viz. Table 2)$^{46}$. The recognition behaviour of the receptor towards various anions has been evaluated in CH$_3$CN. The receptor showed ratiometric fluorescent changes only with CH$_3$COO$^-$, and it showed no significant response to any of other anions such as Cl$^-$, Br$^-$, I$^-$, HSO$_4^-$, NO$_3^-$, C$_6$H$_5$COO$^-$ and H$_2$PO$_4^-$.

Lin and co-workers published a chromogenic “naked-eye” anions receptor (47) having a dinitrobenzimidazole unit connected to a phenol donating group in an aqueous media$^{47}$. The presence of the nitro groups increases the polarity of the molecule, making it more suitable for chromogenic detection and increases the acidity of the NH group of benzimidazole. Addition of F$^-$ to a solution of compound (47) induces a clear color change that is not observable with other halide anions. The fluoride ion binds strongly with (47) because of its highest electro-negativity and smallest size among all of the anions being investigated here. In the UV-Vis spectrum of the complex formed, a new charge-transfer band appears which is responsible for this colour change. The $^1$H NMR study carried out to investigate the coordination modes illustrates that the NH group of the benzimidazole and the phenolic OH are responsible for the F$^-$ ion coordination.

Within the concept of two-arms receptors, Jang and co-workers synthesized two isophthalamide receptors containing benzimidazole (48a) and nitrobenzimidazole (45b) units$^{48}$ and a 1,3-bisurea bearing two benzimidazole moieties (49)$^{49}$. Compound (45b) experiences a bathochromic shift of the UV-Vis bands with the addition of F$^-$ and AcO$^-$ anions in CH$_3$CN/DMSO (99 : 1) observable by “naked-eye”, while the rest of the anions tested do not cause any important variations. When the same titrations are carried out on the related receptor (48a), it exhibited no change in the absorption spectrum upon addition of any of the anions assayed. In the case of (49), after addition of 5 equiv. of the sodium salt of a large variety of anions, only the phosphate anion quenches the fluorescence of the receptor in a DMSO/water mixture while the other anions do not modify importantly the fluorescence emission. A PET quenching process has been addressed to explain the fluorescence changes. The $^1$H NMR titration of the receptor with phosphate exhibits an upfield shifting of the thioureia and benzimidazole NH protons involved in the recognition process that points to the presence of hydrogen bonds in the neat receptor that are stronger than in the complex. The popularity of the tweezer-like structures for anion recognition is again demonstrated with the preparation of two molecules bearing a phenanthroline, two imide and two benzimidazole moieties as receptor units$^{50}$. Lin’s group reported two novel and neutral benzimidazole derivatives bearing a 1,10-phenanthroline fluorophore (50) as anion receptors based on “turn-on” and “turn-off” fluorescence responses to various anions in DMSO solution. In the process of anions binding, there were two different fluorescent responses in presence of anions: a quenching of the fluorescence emission for F$^-$ and AcO$^-$ ions and an enhancement of the fluorescence emission for Cl$^-$, Br$^-$ and I$^-$ ions. Two different luminescent mechanisms of the receptors resulting from various anions were exploited to rationalize quenching and enhancement of the fluorescence emission: a photoinduced electronic transfer mechanism (PET) and the increase of the rigidity of the host molecules, respectively. In particular, chloride could be recognized selectively from the anions tested according to changes of fluorescence spectrum.

Tomapatanaget and co-workers synthesized anion receptors (51 and 52) fabricated from the imidazole unit and anthraquinone moieties. $^1$H NMR spectroscopy and UV-Vis titrations in DMSO-$d_6$ and DMSO, respectively, showed that both receptors underwent deprotonation at the NH moiety of the amide-anthraquinone unit in the presence of basic anions such as F$^-$ and AcO$^-$ $^{51}$. These phenomena gave a dramatic color change due to charge transfer transition corresponding to the shift of $\lambda_{\text{max}}$ from 371 nm to 489 nm. Redox chemistry of (51) and (52) in the presence of anions (F$^-$, Cl$^-$, AcO$^-$, BzO$^-$, and H$_2$PO$_4^-$) using cy-
cyclic voltammetry showed the different cyclic voltammogram (CV) responses upon addition of various anions. In the case of (51) with various anions, the CV changes are dependent on the basic strength of anions in order of F– > AcO–, BzO– > H₂PO₄– > Cl–, Br–. Interestingly, the CV responses of (52) with H₂PO₄– exhibited the most significant changes. Thus, (52) has an excellent electrochemical selectivity toward H₂PO₄– ion.

Ghosh et al. designed and synthesized an anthracene-appended benzimidazolium-based receptor (53). The receptor shows selective recognition of iodide in the excited state by exhibiting quenching of emission of anthracene52. In the ground state, receptor shows different selectivity and prefers to bind bromide with higher binding constant value as established from NMR titration experiment. Other anions in the study indicated weak or no interaction. Hydrogen bonding and charge-charge interactions are the forces responsible for strong binding. The interaction properties of the new receptor were evaluated by ¹H NMR, UV-Vis and fluorescence spectroscopic methods. The anthracene-linked bis(benzimidazole)diamide (54) has also been described as a simple receptor for the selective detection of metal ions and organic sulfonic acids53. Differences in the monomer/excimer ratio of fluorescent emission of the anthracene moieties were used to detect the presence of diverse organic acids in CHCl₃ and CH₂CN/water (4 : 1) solutions. The change in emission was considerable in the presence of methanesulfonic acid and p-toluenesulfonic acid while the addition of acetic, mandelic or trifluoacetic acids less perturbed the emission of receptor (54) due to the protonation of host by the most acidic sulfonic acids and the formation of different ion pairs. Changes in the absorption spectrum correlate well with the fluorescence results. Moreover, metal complexation was studied in CH₃CN and addition of increasing amounts of transition metal cations decreases both the monomer and the excimer emission, although the effect on the excimer band is more pronounced. Cu²⁺, Co²⁺ and Ni²⁺ ions cause the most marked changes in comparison to the other transition metals.

Table 2. Benzimidazole based chemosensors selective for anions

---

Table 2. Benzimidazole based chemosensors selective for anions

<table>
<thead>
<tr>
<th>Structure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(43)</td>
<td></td>
</tr>
<tr>
<td>(44)</td>
<td></td>
</tr>
<tr>
<td>(45)</td>
<td></td>
</tr>
<tr>
<td>(46)</td>
<td></td>
</tr>
<tr>
<td>(47)</td>
<td></td>
</tr>
<tr>
<td>(48a)</td>
<td>R=H</td>
</tr>
<tr>
<td>(48b)</td>
<td>R=NO₂</td>
</tr>
</tbody>
</table>
### Table-2 (contd.)

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>(49)</td>
<td><img src="image1" alt="Structure" /></td>
</tr>
<tr>
<td>(50a)</td>
<td><img src="image2" alt="Structure" /></td>
</tr>
<tr>
<td>(50b)</td>
<td><img src="image3" alt="Structure" /></td>
</tr>
<tr>
<td>(51)</td>
<td><img src="image4" alt="Structure" /></td>
</tr>
<tr>
<td>(52)</td>
<td><img src="image5" alt="Structure" /></td>
</tr>
<tr>
<td>(53)</td>
<td><img src="image6" alt="Structure" /></td>
</tr>
<tr>
<td>(54)</td>
<td><img src="image7" alt="Structure" /></td>
</tr>
<tr>
<td>(55)</td>
<td><img src="image8" alt="Structure" /></td>
</tr>
<tr>
<td>(56)</td>
<td><img src="image9" alt="Structure" /></td>
</tr>
<tr>
<td>(57)</td>
<td><img src="image10" alt="Structure" /></td>
</tr>
<tr>
<td>(58)</td>
<td><img src="image11" alt="Structure" /></td>
</tr>
<tr>
<td>(59)</td>
<td><img src="image12" alt="Structure" /></td>
</tr>
<tr>
<td>(60)</td>
<td><img src="image13" alt="Structure" /></td>
</tr>
</tbody>
</table>
Table-2 (contd.)
Ghosh et al. designed and synthesized a new anthracene-coupled benzimidazolium-based tripodal, tricationic fluorescent chemosensor (55). Receptor exhibits high degree of selectivity towards H$_2$PO$_4^-$ in CH$_3$CN through anion-induced quenching of emission along with the formation of a weak excimer complex in the excited states. Furthermore, receptor shows selective sensing of ATP over ADP and AMP by exhibiting an increase in emission in aqueous CH$_3$CN (CH$_3$CN : H$_2$O, 3 : 2, v/v). The electrostatic charge-charge interaction along with both conventional (NH···X; X=O, halides) and unconventional (C$^+$H···X; X=O, halides) hydrogen bonding between the host and the guest molecule synergistically interplays in the complexation. The anion-binding properties of receptor were understood by $^1$H NMR, UV-Vis and fluorescence spectroscopic methods.

Lee and co-workers synthesized a novel 1,3,5-substituted triethylbenzene derivative (57) with a 2-aminobenzimidazole moiety as a binding and signaling subunit. The sensor was tested in a buffered CH$_3$CN/H$_2$O (99 : 1, v/v) solution and found to be selective for iodide as demonstrated by the photophysical properties obtained through UV-Vis absorption and fluorescence spectroscopy analyses.

A simple CN$^-$ sensor (58) bearing a naphthol and an imine group was designed and synthesized by Zhang’s group, which showed both a colorimetric and a fluorescence “turn-on” response for cyanide ions in aqueous solution. The probe shows an immediate visible change in color from yellow to orange which then becomes colorless followed by a final change to pink, when cyanide is added; these color changes can be readily observed visually. The mechanism for the
cyanide ion sensing was established by using $^1$H NMR and FT-IR spectroscopy and mass spectrometry. Moreover, test strips based on the sensor were fabricated, which could act as a convenient and efficient CN$^-$/ test kit.

Zhang et al. reported a series of novel acridine derived bis-benzimidazolium macrocyclic fluorescent sensors (59 and 60)$^{58}$. X-Ray crystal structures demonstrated the self-assembly behavior of these cyclophanes in the solid state driven by hydrogen bond and $\pi$--$\pi$ interactions. Anion binding studies of these sensors revealed a significant effect of the macrocyclic size and rigidity for H$_2$PO$_4$$^-$ sensing via the obvious “turn-on” as well as bathochromic-shift in fluorescence emission. Different cavity size or rigidity of the sensors showed different bathochromic-shifts of 36 to 126 nm in fluorescence emission induced by H$_2$PO$_4$$^-$/, which resulted in significant color changes of fluorescence from blue to orange red, orange, green and blue-green respectively. The unique fluorescence response toward H$_2$PO$_4$$^-$ may be attributed to H$_2$PO$_4$$^-$-induced assembly of sensors forming the excimer between two acridine rings to a different extent.

A series of pyridine-coupled benzimidazolium-based receptors (61, 62 and 63) have been designed and synthesized by Ghosh et al. In the series, only receptor (61) is structurally appealing in the selective recognition of H$_2$PO$_4$$^-$ in CHCl$_3$ as well as in CH$_3$CN over a series of other anions.$^{59}$ The ratiometric change in emission with a triplet band at 420 nm is the distinctive feature of selective recognition of H$_2$PO$_4$$^-$ in CHCl$_3$. In CH$_3$CN, a “turn-on” response is selectively observed. Binding studies have been carried out using fluorescence, UV-Vis, $^1$H NMR and $^{31}$P NMR spectroscopic techniques. Experimental results have been correlated with the theoretical findings.

Molina et al. reported a benimidazole based receptor (64) which senses aqueous H$_2$PO$_4$$^-$/ and HP$_2$O$_{7}$$^{3^-}$/ anions through different channels in DMSO. A red-shift of the emission band with an important chelation enhanced fluorescence effect and a large cathodic shift of the ferrocene. The recognition event has also been studied by $^1$H NMR spectroscopy. It is worth mentioning that a series of ruthenium complexes containing bis-benzimidazole derivatives have been identified as able to target mitochondria and induce caspase-dependent apoptosis in cancer cells through superoxide overproduction$^{60}$. Following with the family of ruthenium complexes, Ye and co-workers prepared mononuclear complexes with 2,2-bisbenzimidazole and the corresponding 4,4$'$-bismethylated ligand$^{61}$. In a similar way as in the aforementioned complexes, compounds (65) and (66) experienced successive deprotonations in CH$_3$CN with F$^-$ and AcO$^-$ that were followed by UV-Vis, emission spectroscopy, $^1$H NMR and electrochemical methods. Additionally, a strong hydrogen bond coordination of the less basic anions is observed in emission spectroscopy and $^1$H NMR experiments. A theoretical paper published by Zhang and co-workers studies the coordination/deprotonation process in this sort of ruthenium bis(benzimidazol-2-yl)imidazole complexes$^{62}$. Computational results, obtained with (TD)-DFT methods in the Gaussian09 program, support a proton transfer process for the spectroscopic and electrochemical changes experienced in these compounds.

Liu and co-workers reported a fluorescence sensor (67) bearing NH and OH subunits displayed a highly selective and sensitive recognition property for fluoride over other anions$^{63}$. Fluoride-driven ESPT, poorly used in anion recognition and sensing, was suggested to be responsible for the fluorescence enhancement with a blue shift of 35 nm in the emission spectrum. Singh et al. reported a Co$^{3+}$ complex of similar type of benzimidazole-based probe (68) and evaluated as a sensor for I$^-$ and HSO$_4$$^-$ in aqueous media$^{64}$. The complex showed electrochemical changes with I$^-$ over other anions, whereas it had a ratiometric response towards HSO$_4$$^-$ ions in UV-Vis spectroscopic study even in the presence of other anions.

A simple, tailor made receptor (69) based on azo dye featuring with benzimidazole unit with hybrid
-OH and -NH binding sites was synthesized and characterized by Singh’s group. The addition of CN⁻, AcO⁻, F⁻, and H₂PO₄⁻ in acetonitrile solution of (69) resulted in a large bathochromic shift from 315 nm to 470 nm allowing “naked-eye” color change from colorless to orange⁶⁵. Anion sensing characteristics were determined using visual inspection, UV-Vis and ¹H NMR spectroscopy pointing toward the improved chromogenic ability after introduction of -N=N- group in the azo dye (69). The mechanism of anion binding with receptor (69) showed 1 : 2 (69/Anion) and the association constants were found in the order of AcO⁻ > CN⁻ > F⁻ > H₂PO₄⁻.

1,3-Bis(5,6-dimethyl-1H-benzo[d]imidazol-2-yl)benzene (70) a neutral tridentate ligand, is synthesized and employed as a chemosensor by Iyer et al. for the detection of different anions with highest affinity for fluoride⁶⁶. The detection ability of this ligand (70) is confirmed using UV-Vis spectroscopy, fluorescence spectroscopy and ¹H NMR techniques. Anion binding studies using ¹H NMR and fluorescence spectroscopy revealed that (70) exhibits high selectivity for fluoride over other anions.

A benzimidazole-based compound (71) built on the piperazine motif has been designed and synthesized by Ghosh et al.⁶⁷. The chemosensor (71) is highly selective and sensitive towards ATP over ADP and AMP in CH₃CN/H₂O (1 : 1, v/v, using 10 mM HEPES buffer, pH 6.4) as evidenced by the significant change in emission. Furthermore, sensor (71) is cell permeable and can detect the presence of ATP in Human cervical cancer cells (HeLa).

Samanta’s group synthesized a new dibenzimidazole diimine sensor (72) for selective detection of acetate ion⁶⁸. Significant “naked-eye” recognized color change of (72) solution from light yellow to pink upon addition of only acetate ion is accompanied with near infrared (NIR) emission exploiting excited state intramolecular proton transfer (ESIPT).

Sen Gupta et al. synthesized ESIPT based benzimidazole derivative and investigated their photophysical behaviour towards various anions⁶⁹. The probe (73) has been used for selective estimation of F⁻ ions as compared to other anions and signaled the binding event through formation of new absorption band at 360 nm and emission band at 420 nm. The probe (73) showed fluorescence behaviour towards fluoride ions through hydrogen bonding interactions and restricted the ESIPT emission at 540 nm from OH to nitrogen of benzimidazole moiety to release its enol emission at 420 nm.

In another work Sen Gupta et al. synthesized excited state intramolecular proton transfer based benzimidazole derivative having push-pull effect has been synthesized and investigated their photophysical behavior towards various anions⁷⁰. The probe (74) has been used for selective estimation of F⁻ and CN⁻ anions and signaled the binding event through formation of new absorption band at 465 nm. The probe (74) opens different emission channels at 425 nm in the presence of CN⁻ ions and two new emission bands at 435 nm and 365 nm in case of F⁻ ions. The probe (74) behaved as chemodosimeter for CN⁻ ions which have been proved by ¹H NMR and whereas fluoride caused hydrogen bonding interactions with probe (74) and restricted the ESIPT emission at 505 nm from OH to nitrogen of benzimidazole moiety to release its enol emission. The differential behaviour of F⁻ ions and CN⁻ has been confirmed through DFT calculations.

A sensitive fluorescent probe, 2,2'-bisbenzimidazole (75), for CN⁻ has been developed by Liu and co-workers. This structurally simple receptor displays great selectivity for the cyanide anion over other common inorganic anions in an aqueous environment⁷¹. In addition further study demonstrates the lower detection of the fluorescence response of the sensor to CN⁻ is in 10⁻⁹ mol/L range. Thus, the present probe should be applicable as a practical system for the monitoring of cyanide concentrations in aqueous samples.

In recent study two new 2-(2-aminophenyl)benzimidazole-based HSO₄⁻ ion selective receptors, (76) and (77) have synthesized⁷²a. Both receptors (76 and 77) behave as highly selective chemosensor for HSO₄⁻.
ions at biological pH in ethanol-water HEPES buffer (1/5) (v/v) medium over other anions. Theoretical and experimental studies showed that the emission efficiency of the receptors was tuned successfully through single point to ratiometric detection by employing the substituent effects. Using 3s method the LOD for HSO$_4^-$ ions were found to be very low. The structure property relationship of the bisulphate ensemble was established in another work reported by the same authors.$^{72b}$ Here quinazoline based receptor (78) selectively senses HSO$_4^-$ ions of nanomolar region in 0.1 M HEPES buffer (ethanol-water : 1/5, v/v) at biological pH over other competitive ions through the proton transfer followed by hydrogen bond formation and subsequent anion coordination.

**Benzimidazole based for both cations and anions:**

In the field of both recognition of cations and anions the Cu$^{2+}$ and cyanide successive recognition feature was examined by Tang and co-workers. A benzimidazole-based imine linked sensor (79) (viz. Table 3) exhibits highly selective and sensitive recognition properties to Cu$^{2+}$ in CH$_3$OH/H$_2$O (1/1, v/v, HEPES 10 mM, pH 7.0) solution with a 1 : 1 binding stoichiometry.$^{73}$ The *in situ* prepared Cu$^{2+}$ complex solution displays high selectivity to cyanide through Cu$^{2+}$ displacement approach and possesses excellent tolerance to other common interference anions. The detection limits of sensor (79) to Cu$^{2+}$ and Cu$^{2+}$ complex to cyanide were estimated to be $1.82\times10^{-8}$ M and $1.62\times10^{-6}$ M, respectively. Proof-of-concept experiment results demonstrate that sensor (82) has potential utilities for Cu$^{2+}$ and sulphide ion concentration evaluation in real water samples. This successive recognition features of sensor (82) makes it a potential utility for Cu$^{2+}$ and sulphide anion detection in water.

Das *et al.* synthesized and characterized a benzimidazole-based triprodal fluorescence derivative (81). Combining (81) with Cu$^{2+}$ shows excellent selectivity toward sulphide anions.$^{75}$ The binding stoichiometry of (81) with Cu$^{2+}$ is established by Jobs’ plot analysis and mass spectral evidence. The initial fluorescence of (81) is lost on complexation with Cu$^{2+}$ and is regained in presence of sulphide. The “off-on” behavior of (81) is studied by fluorescence, UV/Vis and mass spectroscopic methods.

To realize highly selective relay recognition of Cu$^{2+}$ and sulphide ions, a simple benzimidazole-based fluorescent chemosensor (82) was designed and synthesized by Tang’s group.$^{76}$ Sensor (82) displays rapid, highly selective and sensitive recognition to Cu$^{2+}$ in 100% water solution at pH 6.0. The *in situ* generated Cu$^{2+}$ complex solution exhibit a fast response and high selectivity toward sulphide anion via Cu$^{2+}$ displacement approach. The detection limits of sensor (82) to Cu$^{2+}$ and Cu$^{2+}$ complex to sulphide anion were estimated to be $3.5\times10^{-7}$ M and $1.35\times10^{-6}$ M, respectively. Proof-of-concept experiment results demonstrate that sensor (82) has potential utilities for Cu$^{2+}$ and sulphide ion concentration evaluation in real water samples. This successive recognition features of sensor (82) makes it a potential utility for Cu$^{2+}$ and sulphide anion detection in water.

The 2-(2’-aminophenyl)benzimidazole (2-APBI) derivatized fluorescent sensor (83) that also behaves relay recognition of Cu$^{2+}$ and S$^{2-}$ in water solution (pH 7.4) has been developed by the same authors.$^{77}$ Sensor (83) displays excited-state intramolecular proton transfer (ESIPT) featured two emission bands and performs highly selective and sensitive recognition to Cu$^{2+}$ through two emissions simultaneous quenching. The on-site formed Cu$^{2+}$ complex exhibits excellent selectivity to S$^{2-}$ with fluorescence “off-on” response via Cu$^{2+}$ displacement approach, which exerts ESIPT recovery. Thus, through modulation the ESIPT state of sensor (83), relay recognition of Cu$^{2+}$ and S$^{2-}$ in water has been achieved.
A fluorescent probe 6-(2,4-dihydroxyphenyl)-5,6-dihydrobenzoimidazo[1,2-c]quinazoline (84) was designed and synthesized by Wang’s group. Its fluorescence can be quenched upon the addition of Cu²⁺ in aqueous media. The binding constant for Cu²⁺ and (84) was determined to be 9.56×10³ M⁻¹ in DMSO/H₂O (1 : 1, v/v). The crystal structure of the Cu²⁺ complex, revealed that the Schiff base complex formed in the response system of probe (84) to Cu²⁺ via Cu²⁺-assisted C-N bond breakage of the quinazoline ring in (84) to form a Schiff base. Consequently, complex was used as a “turn-on” fluorescence chemosensor for direct detection of CN⁻. It showed high selectivity to CN⁻ over a number of anions in the aqueous media. CN⁻ replaced the probe in the complex to form [Cu(CN)₃]²⁻ and fluorescence recovered. The detection limit of CN⁻ was 4.0×10⁻⁶ M. The cell images showed that the complex could be used to detect intracellular CN⁻.

Benzimidazole-based derivative (85) has been designed and synthesized by Nandhakumar and co-author as a fluorescent probe highly selective to Cu²⁺ in HEPES-buffered CH₃OH/H₂O (1 : 1, v/v, pH 7.0) solution. The in situ formed Cu²⁺ complex solution displays high selectivity to CN⁻ through fluorescence relay enhancement. This Cu²⁺ and CN⁻ sequential recognition via fluorescence relay enhancement make probe (86) has a potential utility for Cu²⁺ and CN⁻ detection in aqueous media. In this case the detection limit of Cu²⁺ complex for CN⁻ was also evaluated and was calculated to be 1.74×10⁻⁶ M, which is smaller than the upper limit 1.9 µM for cyanide in drinking water defined by the World Health Organization, thus Cu complex probe has a potential utility in detection of cyanide in drinking water as well as cyanide polluted water. Subsequently, the reversibility of the cyanide recognition process was also evaluated. Upon further addition of excess Cu²⁺ to the Cu complex + CN⁻ solution, the fluorescence spectrum of the resulted solution is almost identical with that of Cu complex solution, which demonstrates that the cyanide recognition process is reversible.

In this field a water soluble non-fluorescent copper(II) complex (87) behaves as a highly selective and sensitive for HSO₄⁻ ions through the enhancement of fluorescence of the system based on intermolecular hydrogen bonding assisted chelation enhanced fluorescence (CHEF) process in “turn-on” style, which has been confirmed by systematic optical techniques and electrochemical studies. This mode of sensing pathway and binding of HSO₄⁻ ions with the receptor (87) has also been validated by optimizing the structures of Cu complex and HSO₄⁻ adduct with the help of theoretical calculations. This non-cytotoxic probe senses HSO₄⁻ ions as low as 3.18×10⁻⁷ M in water : DMSO (9 : 1, v/v) at biological pH (using 1 mM HEPES buffer) and it is also useful for the detection of intracellular HSO₄⁻ ions under a fluorescence microscope.
Table 3. Benzimidazole based chemosensors for both cations and anions

For neutral molecules:

Amongst various reports where compounds behave as sensor for neutral molecules an azo dye-coupled benzimidazole-based receptor (88) was synthesized and investigated as a receptor for metal ions in semi-aqueous medium by Singh et al.82. The receptor recognizes Cu²⁺ with high selectivity over other metal ions. The resultant complex was found to selectively bind oxalic acid via counter ion displacement.

L-Valine derived benzimidazole based bis-urea (89) has been designed and synthesized by Ghosh et al. The bis-urea (89) (viz. Table 4) is found to act as enantioselective chemosensor for tartrate. The sensor shows greater fluorescence response for L-tartrate in DMSO83. In comparison, less steric receptor (90) exhibits a marginal preference for D-tartrate over the L-tartrate in DMSO and validates the steric role of the substituents around the benzimidazole motif in (89) toward enantioselective recognition of tartrate.

Wang’s group designed and synthesized 1,3,5-
tri(1H-benzo[d]imidazol-2-yl)benzene derivatives, as a fluorescent chemosensor (91) (which has structural similarities with compound (57)) for the detection of nitroaromatic explosives, by simple N-hydrocarbylation. Among 16 obtained compounds, compound (91) has the best capability for detection of picric acid (PA), having good selectivity and high sensitivity. The detection of PA with (91) solution-coated paper strips at the picogram level is developed. A simple, portable, and low cost method is provided for detecting PA in solution and contact mode. Benzimidazole receptors have also been used for the recogni-
tion of neutral receptors as carboxylic acids, hydrogen bond donors, as urea or barbital, and O-methylated aminoacids. Neutral molecules are in general weakly bound and a better control of the complementarity between host and guest is then required.

Moran and co-workers showed a receptor (92) for carboxylic acids that connects two benzimidazole units through a xanthene molecule in a tweezer-like structure. The cavity created between the two benzimidazole arms effectively binds methanol, DMSO or acetone in chloroform and the structure of the complexes was determined by single X-ray crystal diffraction. When carboxylic acids were studied, it was observed that the acid group protonates one of the nitrogen atoms present in the benzimidazole moieties while the other remains neutral and participates in the coordination of the resulting anion. When the acid used was anthracene carboxylic acid, a strong decrease of the emission intensity was observed compared to the free acid through a PET mechanism responsible for the quenching of fluorescence. The single crystal structure for the complex shows how the benzimidazole on the amide arm protonates while this NH and the other three NH groups coordinate the carboxylate moiety.

Gale and co-workers prepared a simple tweezer-like receptor (93) connecting two benzimidazole units through a 2,6-pyridinedicarboxylic amide. Receptor (93) binds preferentially with barbital over the structurally related urea, thiourea or imidazolinone in DMSO/MeNO₂. In the crystal structure of the complex a good complementarity is observed that leads to the formation of four hydrogen bonds.

Other examples of the use of benzimidazole in molecular recognition are the two related macrocycles with the general structure (94) in which these heterocycles are connected through polyether alkyl chains. These compounds have been used for the recognition of O-methylated α-aminoacids in chloroform. In the study it has been observed how the length of the macrocyclic arms influences the selectivity due to the complementarity of hydrogen bonds and π-π stacking.

Das et al. developed a benzimidazole-derived ICT-based probe (95) for trace level determination of water. In presence of water, the “naked-eye” color of (95) changes from red to yellow, while it turns to green from red under UV light. Upon addition of water, (115) shows a ratiometric absorbance change in methanol.

Recently a newly designed rhodamine-benzimidazole hybrid molecule (96) has been developed as a FRET-based chemosensor for the selective detection of trace level water in both polar protic and aprotic organic solvents.

Pyrazole based compounds as chemosensor

Pyrazoles are important nitrogen containing 5-membered heterocyclic compounds with stronger fluorescence, have higher hole-transport efficiency and excellent emitting blueness property. Therefore, pyrazoline derivatives have widely been used as whitening or brightening reagents for synthetic fibers, fluorescent chemosensors for recognition of transition metal ions, hole-transport materials in the electrophotography and electroluminescence fields. Moreover, pyrazoline with membrane permeability, low toxicity, and high quantum yield render the fluorophore attractive for biological applications. However, a few pyrazoline derivatives are as effective “turn-on” fluorescent sensors in literature.

Li and co-authors have developed a chromogenic and fluorescent probe (97), (Table 5) based on an anion catalyzed intramolecular hydrogen transfer, which displayed drastic changes in UV-Vis absorption as well as fluorescence emission intensities showing selectively for F⁻ over other anions.

According to Li’s group two simple fluorescent anion receptors based on 1-phenyl-3-methylpyrazole-5-one-4-one-phenylhydrazone (98) and 1-phenyl-3-methylpyrazole-5-one-4-one p-nitrophenyl-hydrazone (99) with similar configuration exhibited different anion binding behaviors in DMSO solution. The experimental results indicate that (98) bind anions such as F⁻, AcO⁻, H₂PO₄⁻ ions in 2 : 1 host-guest complexation fashion, while (99) bind anions in 1 : 1 host-
guest complexation in the solution. Here, the anion binding ability of the receptors both (98 and 99) was evaluated through UV-Vis titrations. Upon the addition of fluoride ions in case of (98) a significant decrease in the absorption of 394 nm and appearance of two new absorption peaks at 306 nm and 431 nm with two isosbestic points at 346 nm and 410 nm. But, in the case of (99), out of two absorption bands at 407 nm and 537 nm, a significant decrease in the absorption band at 407 nm and an increase in the absorption of 537 nm upon addition of F{}^{-} ions accompanied by a “naked-eye” color changes from red to purple have been recorded. Here, (98 and 99) displayed a tautomeric equilibrium between the hydrazone form and the azophenol form. Where the hydrazone form dominated in the solution. Upon addition of the strong basic anions, the hydrazone form would be transferred to the azophenol form, which interacts with anionic analytes and is responsible for the spectral changes and “naked-eye” color changes, established also by the $^{1}$H NMR titration experiment. Upon excitation at 397 nm, (98) showed a weak emission at 415 nm and the visible fluorescent enhancement was observed with addition of the strong basic anions such as F{}^{-}. However, (99) exhibited a fluorescent emission band centered at 667 nm where the $\lambda_{ex}$ is 450 nm and such emission would be gradually quenched upon addition of fluoride ions. The possible explanation for this result might be that the receptors (98 and 99) employed two different signaling transduction mechanisms for anion binding. Upon complexation with anions, the configuration of (98) was rigidified, resulting in inhibiting vibrational and rotational relaxation modes of non-radiative decay, and thus the fluorescent enhancement of (99) takes place. In the case of (99), introduction of an electron-withdrawing substituent (-NO$_{2}$) would be a photoinduced electronic transfer (PET) from the pyrazole unit (a fluorophore) to the electron-withdrawing substituent (-NO$_{2}$) and as a result the fluorescent emission would be gradually quenched upon addition of fluoride ions. Merkoc et al. used N-alkylaminopyrazole ligands (100 and 101) in a proof-of-concept fluorescent sensor that can operate as a simple ‘paper test’ for rapid and highly sensitive mercury detection in water$^{95}$. A significant enhancement of fluorescence emission in aqueous media for the Hg$^{2+}$ detection at concentrations lower than 0.1 ppb is achieved. A linear range from 10 to 100 ppb [Hg$^{2+}$] was obtained in the paper-based system. Two respective contradictory “turn-on” and “turn-off” fluorescence mechanisms in the ‘paper test’ and water solution (pH 1) systems can be explained by the concept of PET, CHEF and H-bonded interaction.

Zhang’s group develop a pyrazoline-based fluorescent sensor (102) for biological Zn$^{2+}$ detection$^{96}$. The sensor shows good binding selectivity for Zn$^{2+}$ over competing metal with 40-fold fluorescence enhancement in response to Zn$^{2+}$ presumably due to the blocking of the photoinduced electron transfer (PET) process upon complexation with Zn$^{2+}$. The probe is cell-permeable and can be used to detect intracellular zinc ions in living neuron cells.

The synthesis, characterization, binding and deprotonation studies with anions for four 5-(1H-indol-3-yl)-pyrazolyl derivatives (103-106) have been described by Ahmad et al.$^{97}$. It is worthy to mention that sensor (103) shows a drastic change in absorption spectrum (ca. 335 nm) and colour (colourless to blue) upon addition of F{}^{-} in DMSO solution due to the deprotonation of indole -NH proton, as confirmed by $^{1}$H NMR titration. Sensor (105) recognizes F{}^{-} and CN{}^{-} ions by deprotonation mechanism with visible colour change of the solution in a similar manner to that of (103). However, in contrary to (103 and 105), sensor (104) binds with F{}^{-}, CN{}^{-}, H$_{2}$PO$_{4}${}^{-}, AcO{}^{-} and PhCOO{}^{-} ions exploiting hydrogen-bonding interaction with the shifting of absorption band to longer wavelength and subsequent colour change of the solution. Compound (106) recognizes F{}^{-} without any visual colour change and its binding is studied by $^{1}$H NMR titration to acquire the important information about the nature of binding between F{}^{-} and (106).

Zhao and co-authors present the preparation of a pyrazoline compound and the properties of its UV-Vis absorption and fluorescence emission$^{98}$. The structure of the compound was characterized by IR, $^{1}$H NMR
and HRMS. Moreover, this compound can be used to determine Hg$^{2+}$ ion with selectivity and sensitivity in the EtOH : H$_2$O = 9 : 1 (v/v) solution. This sensor forms a 1 : 1 complex with Hg$^{2+}$ and shows a fluorescent enhancement with good tolerance of other metal ions. The association constant $K_a$ measured for coordination of the sensor with Hg$^{2+}$ was $3.03 \times 10^4$ M$^{-1}$, and the detection limit of the sensor toward Hg$^{2+}$ was $3.85 \times 10^{-10}$ M. Detailed information on the interactions between (107) and Hg$^{2+}$ ion from $^1$H NMR spectroscopic studies suggested that the nitrogen atoms in the benzimidazole, thioamide and pyrazoline of the sensor (107) participated to the complex with Hg$^{2+}$. In addition, the fluorescent probe has practical application in cells imaging.

Wong and co-workers told about a luminescent cyclometalated iridium(III) complex-based chemosensor (108) bearing a zinc-specific receptor, tris(2-pyridylmethyl)amine, and the 3-phenyl-1H-pyrazole ligand showed a pronounced luminescence color change from blue to green upon addition of Zn$^{2+}$ ions to a solution of iridium(III) complex$^{99}$. It is attributed to the suppression of photoinduced electron transfer upon complexation of iridium(III) complex with Zn$^{2+}$ ions, which interaction has been established by UV-Vis absorption titration, emission titration, and $^1$H NMR titration. Selectivity of this iridium(III) complex (108) for Zn$^{2+}$ over other common metal ions and the application of this probe in visualizing intracellular Zn$^{2+}$ distribution in live zebrafish have been demonstrated.

### Table 5. Pyrazole based chemosensors recognising ions

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Ions Recognised</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(97)</td>
<td><img src="image" alt="" /></td>
<td>Hg$^{2+}$</td>
<td>Fluorescent enhancement</td>
</tr>
<tr>
<td>(98)</td>
<td><img src="image" alt="" /></td>
<td>Zn$^{2+}$</td>
<td>Color change from blue to green</td>
</tr>
<tr>
<td>(99)</td>
<td><img src="image" alt="" /></td>
<td>Zn$^{2+}$</td>
<td>Color change from blue to green</td>
</tr>
<tr>
<td>(100)</td>
<td><img src="image" alt="" /></td>
<td>Zn$^{2+}$</td>
<td>Color change from blue to green</td>
</tr>
<tr>
<td>(101)</td>
<td><img src="image" alt="" /></td>
<td>Zn$^{2+}$</td>
<td>Color change from blue to green</td>
</tr>
<tr>
<td>(102)</td>
<td><img src="image" alt="" /></td>
<td>Zn$^{2+}$</td>
<td>Color change from blue to green</td>
</tr>
<tr>
<td>(103)</td>
<td><img src="image" alt="" /></td>
<td>Zn$^{2+}$</td>
<td>Color change from blue to green</td>
</tr>
<tr>
<td>(104)</td>
<td><img src="image" alt="" /></td>
<td>Zn$^{2+}$</td>
<td>Color change from blue to green</td>
</tr>
<tr>
<td>(105)</td>
<td><img src="image" alt="" /></td>
<td>Hg$^{2+}$</td>
<td>Fluorescent enhancement</td>
</tr>
<tr>
<td>(106)</td>
<td><img src="image" alt="" /></td>
<td>Hg$^{2+}$</td>
<td>Fluorescent enhancement</td>
</tr>
<tr>
<td>(107)</td>
<td><img src="image" alt="" /></td>
<td>Hg$^{2+}$</td>
<td>Fluorescent enhancement</td>
</tr>
</tbody>
</table>
The design, synthesis, and photophysical properties of a new fluorene-based fluorescent chemosensor, 4-((E)-2-(2-(benzo[d]thiazol-2-yl)-9,9-diethyl-9H-fluoren-7-yl)vinyl)-N,N-bis((3,5-dimethyl-1H-pyrazol-1-yl)methyl)benzenamine (109), is described by Frazer and co-workers for the detection of Al$^{3+}$. Fluorescent probe (109) exhibited absorption at 382 nm and strong fluorescence emission at 542 nm (fluorescence quantum yield, $\Phi_F$, of 0.80). The capture of Al$^{3+}$ by the pyrazolyl aniline receptor resulted in nominal change in the linear absorption (372 nm) but a large hypsochromic shift of 161 nm in the fluorescence spectrum (542 to 433 nm, $\Phi_F=0.88$), from which Al$^{3+}$ was detected both ratiometrically and colorimetrically. The large hypsochromic shift could be attributed to a reduction in intermolecular charge transfer (ICT). This dramatic result found with Al$^{3+}$ and the probe may be a consequence of the size and polarizability of the Al$^{3+}$ ion. The Al$^{3+}$ is believed to interact strongly with the pyrazolyl nitrogens and, even though the resulting
molecular assemblies are unknown, it is believed to restrict the excited state vibrations of the chromophore, resulting in an increase in fluorescence properties. A single set of isobestic points was observed in both the absorption and emission spectra, suggesting a single equilibrium was reached in complexation. The addition of other metal ions, namely Mg$^{2+}$, Ca$^{2+}$, Mn$^{2+}$, Fe$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Hg$^{2+}$ and Pb$^{2+}$ produced only minimal changes in the optical properties of this probe. The emission band of this probe was also accessed by two-photon excitation in the near-IR, as two-photon absorption (2PA) is important for potential applications in two-photon fluorescence microscopy (2PFM) imaging. The 2PA cross section of the free fluorenyl ligand ($\sigma_{2PA}$) was 220 GM at 810 nm and 235 GM at 810 nm for the Al-ligand complex, practically useful properties for 2PFM. The probe exhibited a peak absorption at 382 nm and a gradually blue-shift upon the sequential addition of Al$^{3+}$. The emission spectra of the free ligand titrated with Al$^{3+}$ resulted in the appearance of a new fluorescence band at 432 nm, shifted from 532 nm for the pure ligand.

Two novel rhodamine-pyrazolone-based colorimetric “off-on” fluorescent chemosensors for Fe$^{3+}$ ions were designed and synthesized by Jadeja et al., using pyrazolone as the recognition moiety and rhodamine 6G as the signalling moiety. The photophysical properties and Fe$^{3+}$-binding properties of sensors (110) and (111) in acetonitrile-aqueous solution were also investigated. Both sensors successfully exhibit a remarkably “turn-on” response, toward Fe$^{3+}$, which was attributed to 1 : 2 complex formation between Fe$^{3+}$ and the two receptors. After the addition of metal ions, UV/Vis absorption spectra showed a distinct change and the appearance of a new spectral band with a maximum at 530 nm for Fe$^{3+}$ ions. The absorption band at 530 nm in the case of Fe$^{3+}$ is due to the formation of a delocalized xanthane moiety of rhodamine by selective Fe$^{3+}$-induced ring opening of spirolactam, which also explains the change in colour from colourless to pink in the presence of this Fe$^{3+}$ ion. Without metal ions, probe (110) and (111) show almost no fluorescence upon excitation at 530 nm, suggesting that receptors (110) and (111) exist in a ring-closed non-fluorescent spirolactam conformation.

Das et al. synthesized a new chemosensor (112) and its spectral response was screened with most alkali, alkaline earth, transition and lanthanide metal ions in THF-water (2 : 3, v/v, pH 7.2, 20 mM HEPES buffer) medium. This is found to bind four different transition metal ions such as Hg$^{2+}$, Cu$^{2+}$, Ag$^+$ and Ni$^{2+}$ in mixed aqueous solution at pH 7.2; while binding affinity towards the Hg$^{2+}$ is found to be an order of magnitude higher as compared to other three cations mentioned. The fluorescence quenching of these metal ions could be explained based on the spin-orbit coupling phenomena. Observed luminescence change for Hg$^{2+}$ was more appreciable than the other three metal ions for a comparable concentration of the respective metal ion. Chemosensor (112) has an anthracenyl unit as the signalling moiety, covalently coupled to an N-N donor receptor fragment. Fluorescence based emission read-out signal of the anthracenyl unit is governed by different photoinduced electron transfer (PET) processes in the absence and presence of the coordinated metal ion. Experimental studies revealed that pyridyl pyrazole, used as the receptor fragment for Hg$^{2+}$ showed a higher affinity for this ion in aqueous/organic media. Experimental results reveal that the (112) could be used for colorimetric detection of Hg$^{2+}$, present in the lower organism like Pseudomonas putida.

Yang’s group reported a new pyrazole-based fluorescent sensor, 5-amino-3-(5-phenyl-1H-pyrrol-2-yl)-1H-pyrazole-4-carboxamide (113), and studied for fluoride anion (F$^-$) detection in organic or water-containing solution. This compound displayed both changes in UV-Vis absorption and fluorescence emission spectra upon addition of F$^-$. With increasing of F$^-$, blue emission intensity increases drastically and reaches saturation with 607-fold enhancement at 424 nm. The results indicate that (113) has highly selectivity for fluoride detection over other anions, such as Cl$^-$, Br$^-$, I$^-$, HSO$_4^-$, H$_2$PO$_4^-$ and AcO$^-$ in DMSO or aqueous DMSO solutions. $^1$H NMR titration and other experiments confirm that the sensing process is mainly from
the deprotonation of the pyrazole-NH in (113). Because of the high charge density and small size, fluoride as strong base can deprotonate the compound (113) to afford deeply the heterocyclic conjugated anion which gives strong emission when excited at 365 nm.

Qin’s group reported an original Schiff base type fluorescent chemosensor 1-phenyl-3-methyl-5-hydroxypyrazole-4-carbaldehyde(benzoyl)hydrazone (114) for Cu$^{2+}$104. An obvious fluorescence quenching only for Cu$^{2+}$ demonstrates that ligand (114) exhibits high selectivity and efficient signaling behavior toward micromolar concentration of Cu$^{2+}$ compared with other metal ions. At the same time, the coordination form between ligand and Cu$^{2+}$ is elucidated via crystal structure. The paramagnetic nature of Cu$^{2+}$ and the Cu$^{2+}$-F interaction was the cause of fluorescence quenching.

A structurally characterized naphthelene-pyrazol conjugate (115) behaves as an Al$^{III}$ ion-selective chemosensor through internal charge transfer (ICT)-chelation-enhanced fluorescence (CHEF) processes in 100 mM HEPES buffer (water-DMSO 5:1, v/v) at biological pH with almost no interference of other competitive ions105. The probe can detect Al$^{3+}$ ions as low as 31.78 nM within a very short response time (15–20 s).

Conclusions

The important progress achieved in the development of diazole based receptors can be exemplified by the number of systems that have recently been described in this field. In most cases, such receptors are able to recognize either cations or anions. Although too many diazole derivatives have been devised as probes for the detection of several types of cationic/anionic/neural species with help of different photophysical processes, comparatively more imidazole/benzimidazole based compounds including their metal chelates have showed imperative photophysical properties in favour of developing the fluorescent chemosensors highly selective for the target species. Among the explored chemosensors for cations, several Zn$^{2+}$ sensors have been reported. Here, the benzimidazole based compound, 2,6-bis(5,6-dihydrobenzo[4,5]imidazo[1,2-c]quinazolin-6-yl)-4-methylphenol36 is the most superior one as it is highly sensitive and can detect Zn$^{2+}$ ions as low as 0.53 nM in almost aqueous medium at biological pH, and this probe is also efficient to detect the distribution of Zn$^{2+}$ ions in living cells like other diazole-Zn$^{2+}$ sensors106. Interestingly it is noteworthy to mention that the diazole derivative is capable to detect Al$^{3+}$ ions as low as 9.0 nM in green solvent system comparable with the lowest LOD available in the literature107. In case of anion detection, this derivatives are not good enough compared to the detection cations in terms of LOD, solvent systems. The diazole derivatives are also important to detect the neutral molecules like picric acid, tartarate, etc.; some toxic anions and also cations of different oxidation states. And, this review indicates that more imidazole/benzimidazole based compounds have been explored in developing the chemosensors for different species compared to the pyrazole based chemosensors. As a consequence of this background, we thus predict that this topic constitutes an area of supramolecular chemistry that is ripe for future growth.

Acknowledgement

Financial support from Council of Scientific and Industrial Research (CSIR), New Delhi, India is gratefully acknowledged.

References

Mukherjee et al. : Chemosensors based on diazole derivatives

107. S. Pal, B. Sen, M. Mukherjee, M. Patra, S. Lahiri (Ganguly) and P. Chattopadhyay, RSC Adv., 2015, 5, 72508.
A novel thiourea-based colorimetric chemosensor for sensitive and selective detection of sulphate ion in semi-aqueous medium

Aman Bhasin\textsuperscript{a}, Simanpreet Kaur\textsuperscript{b}, Mayank\textsuperscript{c}, Narinder Singh\textsuperscript{c} and Navneet Kaur\textsuperscript{a,}\textsuperscript{a}

\textsuperscript{a}Department of Chemistry, Punjab University, Chandigarh-160 014, India
E-mail: navneetkaur@pu.ac.in

\textsuperscript{b}Centre for Nanoscience and Nanotechnology (UIEAST), Punjab University, Chandigarh-160 014, India

\textsuperscript{c}Department of Chemistry, Indian Institute of Technology, Ropar (IIT Ropar), Rupnagar-140 001, Punjab, India
E-mail: nsingh@iitrpr.ac.in

\textit{Manuscript received 21 April 2017, accepted 08 May 2017}

Abstract: Within the pool of naturally available anions, the sulphate anion has gained a certain level of notoriety by playing a critical role in contaminating the animal food chain, apart from disturbing marine life via paralysis of certain ecological cycles. Under such circumstances, it becomes imperative to regulate the influx of this potentially hazardous anion into the ecosystem. An important section of the remediation strategies in this area would involve an efficient sensing and quantification of this toxic anion, particularly in the natural water systems. In continuation of our efforts to exploit the unique photophysical properties of ligand-tagged nanoparticles to achieve selective ion-recognition potential, this report highlights the chemical synthesis and chemosensing efficacy of a new and selective sulphate-recognising organic receptor OR in aqueous medium. Upon decoration of OR onto ZnO nanoparticles, the nanohybrid OR@ZnO demonstrated a rapid response and selectivity towards the hydrogen sulphate anion, as marked by a significant alteration in the absorbance and fluorescence profile of OR@ZnO in aqueous system with a significantly low limit of detection (100 nM).

Keywords: ZnO, surface decoration, sulphate, thiourea, fluorescence, sensor.

Introduction

Inorganic sulphates are naturally available in several minerals, including barite (BaSO\textsubscript{4}), epsomite (MgSO\textsubscript{4}·7H\textsubscript{2}O) and gypsum (CaSO\textsubscript{4}·2H\textsubscript{2}O)	extsuperscript{1}. Amongst the several non-transition metal sulphates, sodium, potassium and magnesium sulphates are all highly soluble in water whereas many heavy metal sulphates (like barium sulfate) are less soluble. In addition to a plethora of industrial applications, inorganic sulphates also harbor potential biological relevance at physiological concentrations\textsuperscript{2}. For instance, the sulphate moiety constitutes an integral part of several polysaccharides (found in animal tissues) like heparin sulphate, which upon binding to a variety of protein ligands, regulates biological processes like cell signaling, angiogenesis and regulation of enzyme activity\textsuperscript{3}. In the recent decades and as a consequence of the industrial revolution, a meteoric rise in the concentration of the inorganic sulphate ion in natural aquatic systems has been broadly attributed to acid rain, non-regulated use of sulphate-containing fertilizers and oxidation of pyrite deposits in the subsoil\textsuperscript{4}. A serious consequence of an overload of this otherwise harmless sulphate ion is the aberrant increase in phosphorus levels in the aquatic system, leading to an uncontrolled growth of certain algae and lower oxygen levels in the water systems. Such a phenomenon, known as eutrophication, poses a serious ecological hazard to marine life\textsuperscript{5}. In addition, another phytotoxic effect of sulphate-overdose has been increasingly realised in the form of an excess generation and accumulation of methyl mercury in the deep waters\textsuperscript{6–8}. Therefore, in due recognition of the ubiquitous nature
of the sulphate anion and a unique sulphatemethyl mercury relationship, it is imperative to recognise and quantify sulphate concentrations in the natural water bodies in order to promote potential remediation strategies for specifically limiting the production and accumulation of MeHg in the sediment pore-water.

In an attempt to do so, there has been a growing interest in developing highly selective chemical receptors to facilitate a facile and selective recognition and quantification of the sulphate ion in water systems. In contrast to a broad spectrum of metal ion-sensors, several reports in the past have harboured a certain bias towards positively-charged/metal-containing chemical receptors as a favourite class for anion detection in aqueous medium. Interestingly, certain natural anion-binding systems exhibit high affinity and selectivity for certain substrates under physiological conditions, apparently upon shielding of the latter from neighbouring solvent molecules via recognition into a cleft or cavity of the folded polypeptide chains. For example, sulphate binding proteins (SBP) responsible for anion transport in bacteria bind sulphate exceptionally well in water via hydrogen bonding. However, some critical factors that may impede the binding of sulphate anion to neutral receptors in aqueous systems include the former’s relatively high hydration energy, thereby making the anion strongly hydrophilic and difficult to detect in aqueous phase. Such challenges encountered in the detection of sulphate-anion in aqueous systems have been often cited in scientific literature. For example, despite a high selectivity for sulphate anion in organic solvent medium, Shao et al. demonstrated a limited environmental applicability of their novel indole-based chemosensor in aqueous medium. Molina et al. have also postulated a similar trend by demonstrating a measurable sulphate-recognizing potential of a ferrocene-incorporated chemoreceptor in acidic medium, whose sensitivity, however, diminishes drastically in a neutral medium. In the above and certain other related studies, the sulphate-sensing ability of potential chemoreceptors has been proposed to heavily rely on protonation of the host receptors, followed by a subsequent anion coordination within the receptor cleft via H-bonding and electrostatic interactions. Therefore, a rational incorporation of specific sulphate-binding chemical moieties into a suitable neutral host would be ideal for selective recognition of a sulphate anion in aqueous medium, thereby circumventing the need for protonation of the receptor ligand, prior to sulphate detection.

In this regard, several urea/thiourea-based host ligands are amongst the most successful group of neutral chemical receptors for recognising sulphate ion in a highly competitive aqueous medium. This may be attributed to a facile hydrogen-bonding interaction between the acidic H-bond donor moiety (NH of host ligand) and sulphate anion (guest), thereby ensuring a sensitive chemical affinity between the two species. Upon an evaluation of previously reported sulphate-sensing chemosensors, a major concern regarding their environmental relevance lies in their efficacy in physiological medium and their limit of sensitivity.

In due recognition of the same and our long-standing interest in finely tunable ion-recognition properties of biocompatible ZnO nanoparticles, we have exploited this unique “amine-sulphate chemistry” in the chemical synthesis of a novel nanohybrid. The subsequent modulatory effects of this nanosensor, resulting in a sensitive and selective recognition of a physiologically relevant sulphate-ion, has been discussed henceforth.

Results and discussion

Synthesis and characterization of organic receptor (OR): The rationale of this investigation is to obtain the selectivity of a chemical receptor towards a specific anionic guest; which is being governed through multiple interactions between host and guest in a complementary fashion. The work is themed on the fact that synthetic receptors with flexible arms possess a shape complementary to tetrahedral anions because the geometry and orientation of the host molecule favour the formation of stable host-guest complexes. This concept has been corroborated with the
Bhasin et al.: A novel thiourea-based colorimetric chemosensor for sensitive and selective detection etc.

excellent work of Wu et al., which highlighted the exceptional sulphate-binding properties of a novel tripodal hexaurea through the encapsulation of the tetrahedral anion with six urea groups involving multiple hydrogen bonds. The reported chemical sensors involve multiple steps and tedious purification techniques for the synthesis of such functional organic receptors. However, the organic receptor projected in our work OR was synthesized by a relatively shorter and facile protocol. The OR was furnished as a yellow solid in quantitative yield (86% overall) through the nucleophilic addition of a commercially available derivative of isothiocyanate to a secondary amine (Fig. 1A). The latter, in turn, was easily synthesized by sodium-borohydride-mediated reduction of a Schiff base which was synthesized by a one-step condensation reaction between commercially available salicylaldehyde and n-propyl amine in water-free methanol. Purity of the title ligand L was confirmed by its detailed physico-chemical characterization using IR, 1H/13C NMR and electrospray ionisation (ESI) mass spectroscopy in addition to elemental analysis. In accordance with the results obtained from the IR spectrum, an observation of a distinct stretching frequency band at 1621 cm\(^{-1}\), which is characteristic of an imine-linked (-CH=N) functional group, provided evidence of the formation of the final product L. As per the result obtained from NMR spectroscopy, the peak corresponding to the iminic functionality (-CH=N) at 7.27 ppm and at 165.9 ppm in the 1H NMR and 13C NMR, respectively, confirmed the complete consumption of the reactant, 2-hydroxy benzaldehyde. In addition, an upfield shift in the NMR signal (3.7 ppm) corresponding to the CH\(_2\)-N protons of L, relative to a rather deshielded environment around its precursor proton in benzaldehyde (10.64 ppm) can be related to the lower electronegativity of a nitrogen atom (as in L).

In order to identify the mass spectrum of the receptor ligand L on the basis of its mass/charge (m/z) ratio, a solution of the same was subjected to ionisation via electrospray technique. The appearance of an intense molecular ion peak (at 346.1) confirmed the presence of a protonated adduct of OR. A close difference in the observed and calculated percentage of the individual elements in OR further confirmed its purity in bulk.

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) : \(\delta\) 9.93 (1H, br, -OH), 9.72 (1H, s, -NH), 8.1 (2H, d, Ar-H), 7.6 (2H, d, Ar-H), 7.13 (2H, m, -CH\(_2\)), 6.86 (1H, d, Ar-H), 6.81 (1H, t, Ar-H), 4.9 (2H, s, Ar-CH\(_2\)), 3.7 (2H, t, -N-CH\(_2\)) and 0.86 (3H, t, -CH\(_3\)); \(^{13}\)C NMR (400 MHz, DMSO-\(d_6\)) : \(\delta\) 178.34, 154.90, 152.28, 149.82, 147.80, 142.22, 128.35, 123.75, 123.25, 119.12, 115.08, 39.70, 39.28, 39.08 and 11.01; ESI-MS m/z = 346.1 [M+H]\(^+\); CHN analysis Calcd. for

![Fig. 1](image-url)
Surface decoration of ZnO with organic receptor (OR) and characterization of OR@ZnO

ZnO is a wide band gap semiconductor, possessing high exciton binding energy with a stable Wurtzite structure. It has attracted exhaustive efforts into an elucidation of its unique applications in antireflection coatings, solar cells, diode lasers and antibacterial properties\(^{26}\). Nowadays, ZnO nanoparticles, in conjugation with a plethora of organic and bio-chemical ligands are being popularly utilized as a potential ion-sensing candidate due to their immense selectivity and sensitivity. A significant biocompatibility and highly adjustable photophysical properties in the presence of the ion-of-interest renders ZnO nanoparticles as a desirable analytical probe\(^{27}\). In an attempt to explore a potential ion-sensing capability of a new thiourea-based aromatic derivative OR the new organic receptor was further decorated onto ZnO nanoaggregates in order to achieve a stable analytical sensor system in aqueous medium\(^{28,29}\). Details of the procedure for synthesis of OR-linked ZnO nanoparticles include mixing an alcoholic solution of Zn(ClO\(_4\))\(_2\)-6H\(_2\)O (595 mg, 2.0 mmol) and an alcoholic solution of NaOH (120 mg, 3.0 mmol). A white product was separated out and the product was washed and dried at 150 °C. Compound OR (804 mg, 3 mmol) was mixed with freshly prepared ZnO nanoparticles (100 mg) under constant stirring at 25 °C in dry CHCl\(_3\) and the solution was refluxed for 15 h. The progress of the reaction was monitored with IR and UV-Vis absorption spectroscopy. Upon completion of reaction, the final product was isolated by centrifugation and washed successively with ethanol and water. The procured product OR@ZnO was dried for 24 h at 50 °C. The size, morphology, chemical composition and spectroscopic features of the receptor-capped Zn nanoparticles were elucidated by XRD, SEM, EDAX, solid state UV-Vis and fluorescence studies, respectively. The particle size distribution of the receptor-decorated ZnO nanoparticles was estimated by dynamic light scattering (DLS) studies using a probe based Metrohm microtrac Ultra nanotrac particle size analyzer. The SEM image depicts that modified ZnO nanoparticles form supramolecular self-assembled morphology with growth limited in particular directions and thereby generating tripod morphology (Fig. 2A). The EDAX analysis of OR@ZnO points towards the simultaneous presence of zinc, oxygen and carbon, thereby distinctively confirming a coating of the organic receptor OR onto the surface of ZnO (Fig. 2B). The thickness of OR-capped ZnO nanoparticles (OR@ZnO) was characterized by dynamic light scattering (DLS), which estimates the hydrodynamic diameter of OR@ZnO to be 560 nm (Fig. 2C). Herein, variation in the particle size measured by SEM (200 nm, in this case) and DLS was observed. This can be attributed to the fact that SEM only estimates the size of the particle core neglecting the surrounding solvent layer. In contrast, DLS studies measures the size of the solvent-adhering particles under the influence of brownian motion, thereby making it a more reliable technique in comprehending and optimizing the nanoparticles’ performance in analytical and biological assays. In contrast, SEM estimates the size of the particles without the hydration layer and is a more reliable technique to measure the size of coated nanoparticles\(^{26}\). The solid state UV-Vis absorption spectra of pure ZnO exhibit a peak at 360 nm corresponding to the exciton state of ZnO (Fig. 2D, Supporting Information)\(^{29}\). The \(\lambda_{\text{max}}\) of the absorption spectra of OR@ZnO was blue-shifted as compared to that of pure ZnO (Fig. 2D). The solid-state photoluminescence spectrum of OR@ZnO, which was recorded at an excitation wavelength of 325 nm, displayed an intense peak at 330 nm and 456 nm (as of pure ZnO) (Fig. 2E); however, the intensity of a characteristic green band at 570 nm (corresponding to pure ZnO) quenched in case of OR@ZnO. According to Dijken’s postulate, the visible emission band can be attributed to the formation of a recombination center (Vo*)

containing one electron (Vo*)\textsuperscript{27}. This recombination of a shallow trapped electron with a deeply trapped hole in a Vo** center is responsible for visible emission, thereby explaining the role of multiple surface defects in pure ZnO as a probable cause leading to intense emissions in the visible region. Such defects at the grain boundaries of ZnO may enable a probable conjugation with -OH and -CH=N groups of OR, thereby resulting in a decrease in interfacial defects in OR-capped ZnO and a consequent lowering in green region emission (Fig. 2E). Finally, X-ray powder diffraction pattern of the prepared OR@ZnO nanopowder, calcined at 500 °C was also recorded (Fig. 2F), depicting scattering angles (2\theta) of 31.79, 34.40, 36.25, 47.56, 56.56, 62.82 and 67.91, corresponding to reflections from 100, 002, 101, 102, 110, 103 and 112 planes of the crystal, respectively. All diffraction peaks are in good agreement with literature reports, which can be indexed as the hexagonal Wurtzite structure of ZnO with lattice constants of \(a = 3.253\) Å and \(c = 5.207\) Å. No other peak (except assigned for ZnO) was visualized, thereby confirming the purity of the sample. In accordance with Debye-Scherrer’s equation \(D = 0.89\lambda/\beta\cos\theta\), where \(\lambda\) is the wavelength of incident X-ray (0.15418 nm), \(\beta\) is the full width at half maximum (FWHM) of diffraction peak and \(\theta\) is the diffraction angle, the average crystalline size of the ZnO nanocrystals was estimated to be 30.55 nm.

**Anion recognition properties of OR@ZnO**: The title ligand OR belongs to a class of thiourea-based derivatives (Fig. 1A). As discussed earlier, such type of compounds possess multiple NH (as hydrogen bond donor) functionalities, therefore being able to demonstrate ion-sensing potential receptors by facilitating anion coordination through multiple hydrogen bonding\textsuperscript{13,14}. However, due to the rather high hydrophili-
city of biologically relevant anions, a chemical recognition and quantification of such ionic species has posed a major challenge in aqueous phase\textsuperscript{12}. This, in turn, has been attributed to the water-mediated disruption of H-bonding and/or electrostatic interactions between the ion of interest and host species\textsuperscript{30}. In order to explore a probable chemical affinity of OR@ZnO towards a pool of tetrabutyl ammonium salts of physiologically relevant anions (F\textsuperscript{−}, Cl\textsuperscript{−}, Br\textsuperscript{−}, I\textsuperscript{−}, CN\textsuperscript{−}, CH\textsubscript{3}COO\textsuperscript{−}, HSO\textsubscript{4}\textsuperscript{−}, PO\textsubscript{4}\textsubscript{3}\textsuperscript{−}, NO\textsubscript{3}\textsuperscript{−} and ClO\textsubscript{4}\textsuperscript{−}), its photophysical properties were inspected by UV-Vis and fluorescence spectroscopy in HEPES buffered acetonitrile/H\textsubscript{2}O (98 : 2) system. Accordingly, due to the presence of an arene-conjugated thiocarbonyl moiety, the native absorption spectrum of OR@ZnO exhibited an intense absorption band (at 450 nm) in the near UV region (Fig. 3A). This characteristic peak is primarily attributed to $\pi-\pi^*$ transitions resulting from an electron dipole movement, commonly observed in aromatic carbonyl compounds\textsuperscript{31}. Further, upon an addition of small aliquots of a solution of each tetrabutyl ammonium anion salt (5 equivalents) to a solution of OR@ZnO, the absorption profile of OR@ZnO experienced a distinct hypochromic shift and a slight bathochromic shift in the receptor’s charge transfer band (460 nm) only in the vicinity of hydrogen sulphate anion. This could apparently be attributed to certain alterations in the electronic levels (HOMO/LUMO) of OR@ZnO, upon coordination to the electron-rich hydrogen sulphate anion\textsuperscript{32}. In addition, the absorption spectrum of native OR@ZnO displayed an uphill slope from 380–465 nm, in contrast to a downhill slope observed in the same region in the vicinity of hydrogen sulphate ion. A similar trend was, however, not observed in the presence of other anions which indicated towards a certain physico-chemical sensitivity of OR@ZnO towards the hydrogen sulphate anion.

On the other hand, OR@ZnO nanoaggregates did not experience any marked alterations in its UV-Vis absorption spectrum in the presence of any of the select metal ions, thereby pointing towards an inherent inability of the metal cations in altering the electronic environment of the chemical sensor in its ground state. In order to corroborate the selective binding of OR@ZnO with HSO\textsubscript{4}\textsuperscript{−} in aqueous medium, titration studies were carried out. Accordingly, upon a successive addition of HSO\textsubscript{4}\textsuperscript{−} (0–80 \textmu M) to a solution of the chemoreceptor OR@ZnO in aqueous medium, a distinct drop in the intensity of its native absorption peak at 455 nm was observed with a concomitant formation of a new peak at 360 nm (Fig. 3B). In consequence, a clear isobestic point was observed at 390 nm, indicating the formation of a well-defined OR@ZnO-HSO\textsubscript{4}\textsuperscript{−} scaffold (Fig. 3B). The titration experiment indicated a considerable linearity (98\%) within a concentration range of 0 \textmu M to 50 \textmu M (3σ method)\textsuperscript{33}. Upon an introspection of the library of potentially novel HSO\textsubscript{4}\textsuperscript{−}-sensing chemosensors proposed in the past, the ultra-sensitivity of OR@ZnO matches closely to one of our recently reported and highly sensitive HSO\textsubscript{4}\textsuperscript{−}-detecting chemosensor\textsuperscript{10,33}. Such a significantly low detection limit is encouraging enough to introduce this newly synthesized receptor into the elite class of environmentally relevant and highly selective HSO\textsubscript{4}\textsuperscript{−}-sensing chemosensors. To summarize, this titration experiment confirmed a selective and sensitive affinity of OR@ZnO towards HSO\textsubscript{4}\textsuperscript{−} ion in aqueous medium. In order to further validate the on-field applicability and selective chemical affinity of OR@ZnO towards HSO\textsubscript{4}\textsuperscript{−} in a competitive aqueous medium, an interference experiment was carried out by subjecting a solution of OR@ZnO to HSO\textsubscript{4}\textsuperscript{−} and a pool of select interfering anions (F\textsuperscript{−}, Cl\textsuperscript{−}, Br\textsuperscript{−}, I\textsuperscript{−}, CN\textsuperscript{−}, CH\textsubscript{3}COO\textsuperscript{−}, PO\textsubscript{4}\textsubscript{3}\textsuperscript{−}, NO\textsubscript{3}\textsuperscript{−} and ClO\textsubscript{4}\textsuperscript{−}), simultaneously. In accordance with the results obtained, there was an insignificant variation in the absorbance profile of HSO\textsubscript{4}\textsuperscript{−}-tagged OR@ZnO in the presence of the competing anions (Fig. 3D). Therefore, it could be deduced that OR@ZnO selectively coordinates with HSO\textsubscript{4}\textsuperscript{−} irrespective of the presence of other competing anions.

The physico-chemical stability of ligand-capped
nanoparticles are influenced by their local environment. Presence of electrolyte ions in the immediate vicinity may influence the charge on the nanoparticle surface (basically in first order by the Debye-Hückel effect), thereby resulting in electrochemical instability and consequent agglomeration. A similar degradative effect on the aggregation state of nanoparticles may also be exerted by a pH-mediated protonation/deprotonation of acidic groups on surface of ligand-coated nanoparticles. As a consequence, such environmental factors serve as critical parameters in governing the stability and ion-sensing relevance of fluorophore-labelled NPs under water. In order to identify an appropriate pH range in which nanoaggregates can selectively probe HSO₄⁻, acid/base titrations were carried out. The absorbance of the title ligand-coated ZnO nanoaggregates OR@ZnO was observed to vary negligibly within a pH range of 2.7 to 11.2 (Fig. 4A). To conclude, a change in pH over a broad range apparently exerted a minimal effect on the physico-chemical stability of the nanoaggregates of R1. The effect of ionic strength on the ion-sensing potential of the nanoaggregates OR@ZnO was investigated by comparing the absorbance spectrum of native OR@ZnO.
(0.5 μM) with the absorbance spectrum obtained upon addition of 0–100 equiv. of the tetrabutyl ammonium (TBA) salt of perchlorate to OR@ZnO (Fig. 4B)\(^{33}\). In accordance with the procured data, there was a negligible variation in the absorbance curves, thereby indicating that even an excessive addition of salts yields a marginal effect on the ion-sensing ability of the nanoaggregates OR@ZnO. In order to investigate the response time of OR@ZnO towards HSO\(_4^-\), the absorbance spectrum of the same was recorded in the absence and presence of different concentrations of HSO\(_4^-\) (15, 30, 50 μM) and analyzed as a function of time. In accordance with the data obtained (Fig. 4C), the absorbance of OR@ZnO corresponding to the select concentrations of HSO\(_4^-\) did not vary after 35 seconds of start of the experiment. In other words, the response period of OR@ZnO towards HSO\(_4^-\) was found to be less than 35 s.

**Proposed mechanistic pathway for selective binding of receptor N1 to sulphate anion**

There is ample scientific evidence which confirms the critical reliance of certain biological sulphate-ion transporters on hydrogen bonding for the selective binding and transport of this hydrophilic anion across the cellular membrane\(^{11}\). For instance, an elucidation of the X-ray structure of the sulphate-binding site in a specific sulphate-binding protein reveals the encapsulation of the anion in a peptide pocket via H-bonding with the N-H and O-H groups of the protein. In addition, ion-sensing studies and structural data of the sulphate-engulfed single crystals of a plethora of syn-

---

![Fig. 4.](image-url) (A) Effect of pH on stability of OR@ZnO nanoaggregates in aqueous medium; (B) Salt perturbation studies of ion-sensing OR@ZnO nanoaggregates in aqueous medium upon addition of (Bu4N)**+ClO4**– (0–100 equiv.); (C) Time-dependent response of OR@ZnO towards varying concentrations of HSO\(_4^-\) (15, 30, 50 μM).
thetic urea-based receptors further validate the significant role of H-bonding interactions in the sulphate-recognizing ability of chemical receptors with a common amide/thioamide containing structural motif10,11. In due consideration of the same, a probable mode of sensing of sulphate ion by the title thiourea-based ligand 1B, via a potential H-bonding interaction with the acidic H-bond donor moiety (NH and OH) of the receptor ligand has been postulated.

Conclusion

A diverse library of potential chemical sensors against the physiologically relevant sulphate anion have been proposed in the past34–37. However, a limited water solubility and relatively low sensitivity continue to restrict their applicability in the immediate environment. In due consideration of the same, we propose a new thiourea-based ligand OR as a potentially novel sulphate ion-sensing ligand. The new thiourea-based receptor OR was quantitatively synthesized by the nucleophilic addition of a secondary amine to a phenyl isothiocyanate derivative under ambient reaction conditions. Upon capping onto environmentally compatible ZnO nanoparticles, the novel nanohybrid OR@ZnO demonstrated a distinct chemical sensitivity and selectivity towards sulphate anion over other biologically relevant ions in aqueous medium, with a significantly low detection limit of 100 nM. This potentially novel sulphate sensor may find industrial applicability in the global fight against sulphate-induced water pollution.

Acknowledgement

AB and NK are grateful to CSIR (Project No. 0(0216)/14/EMR-II) for the financial support in pursuing this research work.

References

23. C. Jia, B. Wu, S. Li, X. Huang, Q. Zhao, Q. S.
A new highly selective and ratiometric chromogenic sensor for Cu$^{2+}$ detection

Puspendu Roy, Sangita Das, Krishnendu Aich, Saswati Gharami, Lakshman Patra and Tapan Kumar Mondal*

Department of Chemistry, Jadavpur University, Kolkata-700 032, India

E-mail: tkmondal@chemistry.jdvu.ac.in

Abstract: A new azo-phenol based chemosensor has been designed, synthesized and its structure is confirmed through different spectroscopic techniques. The synthesized chemosensor (HL) has shown a marked colorimetric response from light yellow to purple upon complexation with Cu$^{2+}$ which is easily detectable through naked eyes, makes the sensor efficient one to detect Cu$^{2+}$ and remain nonresponsive in presence of other competitive metal ions. The 1:1 complex formation of HL with Cu$^{2+}$ is determined from Job’s plot analysis and mass spectroscopic studies. Association constant ($K_a$, $8.94 \times 10^4$ M$^{-1}$) of HL-Cu$^{2+}$ complex suggests HL has high affinity towards Cu$^{2+}$.

Keywords: Azo dye, chemosensor, colorimetric detection, ratiometric, Cu$^{2+}$ sensor, DFT calculations.

1. Introduction

Cu$^{2+}$ is the third most abundant essential trace element after Fe$^{3+}$ and Zn$^{2+}$ in human body and plays a crucial role in many important physiological processes. Copper is being used as catalyst in a variety of biological processes and it is the cause of its requirement in every living organism. Oxygen transportation, hormone maturation, and signal transduction etc. are the important biochemical activities of Cu$^{2+}$. For the formation of hemoglobin, red blood cells and bones of the human system, copper is treated as an essential element. Besides its usefulness, copper ion at excessive concentration is very toxic. Due to its adverse effect on living beings, Cu$^{2+}$ is also treated as a notable metal pollutant. Enormous use of Cu$^{2+}$ in our daily life makes it easily accumulate in environment which exposes to human body through contamination of food and water. The U.S. Environmental Protection Agency (EPA) has set limits for copper in drinking water and food to 1.3 ppm. Many severe neurodegenerative diseases, such as Alzheimer’s and Wilson’s diseases, amyotrophic lateral sclerosis, Menke’s syndrome and hematological manifestations are the effect of disorder in Cu$^{2+}$ metabolism. Thus, it is important to monitor the concentration of copper ion in environmental sample.

In this regard, an optical sensor for Cu$^{2+}$ ion, which leads a prominent change in color is in high demand. Colorimetric sensors have been studied actively over the recent decades due to their simple, inexpensive, and rapid implementation. Especially, colorimetric sensors are quite promising because the change of color can easily be noticed by the naked-eye. Thus, the colorimetric sensor requires much less labor and need no equipments. Therefore, the development of new chemosensor for Cu$^{2+}$ detection, especially those which exhibit selective ratiometric Cu$^{2+}$ detection is in high demand which not only contributes to the accurate measurement of the intensities of the two absorbance peaks, but also results in a huge ratiometric value.

Herein, we have reported the synthesis and photophysical characterizations of ONS donor thioether containing azo phenol based derivative showing a prominent ratiometric sensing towards Cu$^{2+}$. Experimental studies reveal that the sensor exhibits a remarkable
affinity for Cu$^{2+}$ in acetonitrile media which is associated with a color change from light yellow to pink due to the enhancement of ICT (internal charge transfer) effect.

2. Experimental

2.1. Materials and methods

CuCl$_2$.H$_2$O was purchased from Arrora Matthey, Kolkata, India. 2-(Ethylthio)benzenamine was synthesized following the published procedure$^{17}$. All other chemicals and solvents were of reagent grade and were used without further purification.

Microanalyses (C, H, N) were performed using a Perkin-Elmer CHN-2400 elemental analyzer. HRMS mass spectra were obtained on a Waters (Xevo G2 Q-TOF) mass spectrometer. The electronic spectra were obtained on a Lambda 750 Perkin-Elmer spectrophotometer in acetonitrile solution. IR spectra were recorded on RX-1 Perkin-Elmer spectrophotometer in the spectral range 4000–400 cm$^{-1}$ with the samples in the form of KBr pellets. $^1$H NMR spectra were recorded in CDCl$_3$ on a Bruker (AC) 300 MHz FT-NMR spectrometer in the presence of TMS as internal standard.

2.2. Synthesis

2.2.1. Synthesis of 4-chloro-2-((2-(ethylthio)phenyl)diazenyl)phenol (HL)

A solution of 2-(ethylthio)benzeneamine (3.06 g, 0.02 mol) in 1 : 1 HCl (10 mL) was cooled in an ice bath. An ice cold solution of NaNO$_2$ (2.0 g in 10 mL water) was added under stirring. This diazotized solution was added to an ice cold solution of Na$_2$CO$_3$ (6 g in 25 mL) and 4-chlorophenol (2.56 g, 0.02 mol) with vigorous stirring. An orange-red precipitate was observed. The precipitate was filtered and washed with cold water, then dried over CaCl$_2$. The product was purified further by column chromatography using silica gel (60–120 mesh) and an orange-red band was eluted by 30% (v/v) ethyl acetate-petroleum ether mixture. Yield 3.7 g (72%). The receptor HL has been characterized by elemental and mass spectral analysis along with several other spectroscopic techniques (IR, UV-Vis, NMR etc.).

Scheme 1. Synthesis of chemosensor HL : (i) Na/dry MeOH/ethyl iodide, (ii) NaNO$_2$/HCl/4-chlorophenol in Na$_2$CO$_3$ (0-5 °C).

Anal. Calcd. for C$_{14}$H$_{13}$ClN$_2$OS (HL) : C, 65.34; H, 5.09; N, 10.89%. Found : C, 65.13; H, 5.01; N, 10.77%; IR data (KBr, cm$^{-1}$) : 3434 $\nu$(O-H), 1421 $\nu$(N=N); $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ (ppm) : 12.67 (1H, s), 7.95 (1H, s), 7.85 (1H, d, $J$=8.0 Hz), 7.28–7.45 (4H, m), 7.02 (1H, d, $J$=8.8 Hz), 3.04 (2H, q, $J$=7.2 Hz), 1.40 (3H, t, $J$=7.3). HRMS m/z, 280.19 (Calcd. for C$_{14}$H$_{13}$N$_2$OS+Na$^+$ : 280.321).

2.3. Synthesis of the HL-Cu$^{2+}$ complex

CuCl$_2$.H$_2$O was added in acetonitrile light yellow colored solution of HL and the stirring was continued for 4 h. The solvent was then removed under reduced pressure in rotary evaporator. The residue was washed by cold water followed by n-hexane and dried in a CaCl$_2$ desiccator. Yield was, 0.093 g, 78%. Anal. Calcd. for C$_{14}$H$_{13}$Cl$_2$N$_2$OS : Calcd. (%) : C, 43.03; H, 3.10; N, 7.17. Found (%) : C, 43.04; H, 3.09; N, 7.21. HRMS m/z, 413.523.

Scheme 2. Synthesis of HL-Cu$^{2+}$ complex.

2.4. General method for UV-Vis titration

2.4.1. UV-Vis method

Stock solution of the receptor HL (10 μM) was prepared in CH$_3$CN at 25 °C. The aqueous solution of the guest cations using their chloride salts in the order
Roy et al. A new highly selective and ratiometric chromogenic sensor for Cu$^{2+}$ detection

of $10^{-4} \text{M}$ were prepared. Solutions of various concentrations containing host and increasing concentrations of cations were prepared separately. The changes in UV-Vis spectra of receptor (10 $\mu\text{M}$) upon gradual addition of metal ion solutions were recorded.

2.4.2. Job’s plot by absorbance method

For Job’s plot experiment, a series of solutions containing HL (10 $\mu\text{M}$) and Cu$^{2+}$ (10 $\mu\text{M}$) were prepared in such a manner that the sum of the total metal ion and HL volume remained constant (4 ml) in MeCN : H$_2$O (1 : 5, v/v) medium. Job’s plots were drawn by plotting $\Delta$A versus mole fraction of Cu$^{2+}$ ($\Delta$A = change of intensity of the absorption spectrum at 515 nm).

3. Results and discussion

3.3. Cation sensing studies of HL

3.3.1. UV-Vis study

Absorption study of the receptor upon gradual addition of increasing concentrations of Cu$^{2+}$ (0–2.0 equiv.) is shown in Fig. 1. The probe shows two prominent absorbance peaks at 323 nm and 410 nm in absence of any guest analytes. After addition of Cu$^{2+}$ a significant changes in the absorbance profile was noticed. Upon gradual addition of Cu$^{2+}$ to the solution of HL (10 $\mu\text{M}$) in MeCN : H$_2$O (1 : 5, v/v) medium the absorption band at 410 nm gradually decreases and a new band appears at 515 nm which gradually increases after incremental addition of Cu$^{2+}$ with a prominent isobestic point at 466 nm. The intensity of the new band (at 515 nm) increases appreciably with progressive addition of Cu$^{2+}$ (up to 2 equiv.) (Fig. 1).

This photophysical changes leads to a prominent color change of the solution from light yellow to pink. This large red shift (105 nm) of the absorption spectra of HL is attributed due to the enhancement of ICT mechanism after complexation with guest Cu$^{2+}$ ion. The absorbance at 515 nm exhibits a good linearity with added Cu$^{2+}$ concentration with a very good R$^2$ value (Fig. 2). The detection limit of the sensor for Cu$^{2+}$ is determined from the absorption spectral change, upon addition of Cu$^{2+}$ to be $6.26 \times 10^{-8}$ M, using the equation DL = K Sb1/S, where K = 3, Sb1 is the standard deviation of the blank solution, and S is the slope of the calibration curve. Upon incremental addition of Cu$^{2+}$ solution, a complete reduction in the intensity at 410 nm band accompanied by a ratiometric increase in intensity at 515 nm was observed which achieved saturation after the addition of a ~ 1.0 equivalent (10 $\mu\text{M}$) solution of Cu$^{2+}$ ions (Fig. 1). The ratio of two absorption intensities ($A_{410}/A_{515}$) also maintained a good linear relationship with [Cu$^{2+}$] (Fig. 3).

![Fig. 1. Changes in UV-Vis spectra of HL upon gradual addition of Cu$^{2+}$ in MeCN : H$_2$O (1 : 5, v/v).](image)

![Fig. 2. The linear response curve of HL at 515 nm depending on the Cu$^{2+}$ concentration.](image)

The selectivity and interference are the two very important parameters to determine the novelty of any receptor. Now the sensitivity and selectivity of the probe HL towards Cu$^{2+}$ were examined by employing different guest analytes. In addition of other guest ions (except Cu$^{2+}$) no noticeable change was observed in the absorbance profile (Fig. 4). Upon addition of Cu$^{2+}$, the absorbance of the sensor at 515 nm was
increased by 27-fold. Competition study with different metal ions (30 μM) in the solution of HL (10 μM) and Cu²⁺ (20 μM) suggesting that there is no significant interferences of other metal ions (Cd²⁺, Co²⁺, Cr³⁺, Fe³⁺, Hg²⁺, K⁺, Mg²⁺, Na⁺, Mn²⁺, Ni²⁺, Al³⁺, Pb²⁺, In³⁺ and Zn²⁺) for the detection of Cu²⁺ (Fig. 5). This phenomenon indicates that the sensor can be employed conveniently for Cu²⁺ detection by simple visual inspection. So the selectivity of HL towards Cu²⁺ is thus well proven.

To understand the interaction of HL and Cu²⁺ in solution Job’s plot by absorbance method was performed. Stock solution of same concentration of HL and Cu²⁺ was prepared in the order of 10 μM in MeCN : H₂O (1 : 5, v/v) at 25 °C. The UV-Vis spectrum in each case with different host-guest ratio but equal in volume was recorded. Job’s plots were drawn by plotting ∆I.Xhost vs Xhost (∆I = change of intensity of the absorbance spectrum at 515 nm during titration and Xhost is the mole fraction of the host in each case, respectively). From the Job’s plot titration it can be concluded that the stoichiometry between HL and Cu²⁺ is 1 : 1.

The stoichiometry of the probe in the presence of Cu²⁺ is found to be 1 : 1, which is supported by the Job’s plot (Fig. 6) and confirmed by HRMS experiment. The HRMS spectrum of the HL-Cu²⁺ complex gives a signal at m/z 413.5, which may be for [Cu(L)Cl+Na⁺] species. Further to understand the stability of the HL-Cu²⁺ complex association constant calculated according to the Benesi-Hildebrand equation. Kₐ was calculated following the equation stated below:

\[
\frac{1}{(A - A₀)} = \frac{1}{Kₐ(Aₜₐₜₐₜ - A₀)} \left[ M^{X⁺} \right] n \]

\[
+ \frac{1}{Aₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜₐₜ}_{758}
Roy et al.: A new highly selective and ratiometric chromogenic sensor for Cu$^{2+}$ detection

Fig. 6. Job’s plot diagram of receptor for Cu$^{2+}$ (where $X_h$ is the mole fraction of the host and $\Delta I$ indicates the change of absorbance intensity at 515 nm).

to be $8.94 \times 10^4$ M$^{-1}$ (Fig. 7). Thus the experiments strongly suggest that there is a strong association between the radii of Cu$^{2+}$ and the cavity space in the probe HL, which ultimately gives rise to strong interaction between Cu$^{2+}$.

Fig. 7. Benesi-Hildebrand plot from absorption titration data of receptor HL (10 $\mu$M) with Cu$^{2+}$.

For extending the scope of experiment of synthesized probe HL, we have studied the sensing at different pH medium. The absorption intensity at 515 nm of the receptor is almost unaffected at different pH condition but in presence of Cu$^{2+}$ ion there is large change of absorption intensity between pH 8–11. Receptor can form stable complex with Cu$^{2+}$ between pH 5–8. At very low pH (<3), there is a tendency to combine with proton, hence become less effective in detection of Cu$^{2+}$. So it is clear that our synthesized receptor can sense Cu$^{2+}$ ion between pH 5–8.

Fig. 8. Absorbance response of HL and HL-Cu$^{2+}$ at 515 nm (10 $\mu$M) as a function of pH.

4. Conclusion

A new azo-phenol based chemosensor has been successfully synthesized and characterized by several spectroscopic techniques. HL showed a marked colorimetric response from light yellow to purple in presence of Cu$^{2+}$ which is easily detectable through naked eyes. Moreover, the sensor showed a selective color change in presence of Cu$^{2+}$ and remain non responsive in presence of other competitive metal ions. The 1 : 1 host-guest complex formation of HL with Cu$^{2+}$ is determined from Job’s plot analysis and mass spectroscopic studies. Association constant ($K_a$, $8.94 \times 10^4$ M$^{-1}$) of HL-Cu$^{2+}$ complex suggests HL has high affinity towards Cu$^{2+}$.

Acknowledgement

Financial supports received from the Council of Scientific and Industrial Research, New Delhi, India (No. 01(2831)/15/EMR-II) and the Department of Science and Technology, New Delhi, India (No. SB/EMEQ-242/2013) are gratefully acknowledged. P. Roy, S. Gharami and L. Patra are thankful to UGC, New Delhi for their fellowships.
References

Orbital and molecular design of new naphthyl-salen type transition metal complexes toward DSSC dyes

Marin Yamaguchi\textsuperscript{a}, Yuki Tsunoda\textsuperscript{a}, Shinnosuke Tanaka\textsuperscript{a}, Tomoyuki Haraguchi\textsuperscript{a}, Mutsumi Sugiyama\textsuperscript{b}, Shabana Noor\textsuperscript{c} and Takashiro Akitsu\textsuperscript{*a}

\textsuperscript{a}Department of Chemistry, Faculty of Science, Tokyo University of Science, Shinjuku-ku, Tokyo, Japan
\textsuperscript{b}Department of Electrical Engineering, Faculty of Science and Technology, Tokyo University of Science, Chiba, Japan
\textsuperscript{c}Department of Chemistry, Aligarh Muslim University, Aligarh-202 002, Uttar Pradesh, India

\textit{E-mail}: akitsu@rs.kagu.tus.ac.jp

\textbf{Abstract :} Dye sensitized solar cells (DSSC) emerged as a new class of low cost energy conversion devices. In this study, we investigated a class of transition metal complexes [ML(CH\textsubscript{3}OH)]; \(M = \text{Mn(1), Fe(2), Co(3), Ni(4), Cu(5) and Zn(6)}\) and \(H_2L\) is naphthyl-salen Schiff base ligand. The COOH group of the complexes leads to better ability for adsorption of dyes. These complexes were characterized employing elemental analysis, powder X-ray analysis, IR, UV-Vis-NIR electronic spectroscopy and so on. Light absorption of the all the complexes shows that \(\pi\)-conjugated naphthyl ring makes these complexes efficient as dyes in DSSC. However, spatial distribution of excited orbitals associated with absorption properties (UV-Vis absorption spectra) calculated by TD-DFT method revealed one of the important factors about orbitals and molecular design of metal complexes for DSSC dyes.

\textbf{Keywords :} Schiff base complexes, orbital design, TD-DFT calculation, UV-Vis spectra, dye sensitized solar cell (DSSC).

\textbf{Introduction}

In the year 1991, Grätzel and O'Regan firstly reported on a low cost organic material type solar cell, namely dye sensitized solar cells (DSSC) based on TiO\textsubscript{2} materials. Thus, DSSC is one of the organic type solar cells, producing electrical energy by light harvesting of dye molecules. An electron is excited by the photoexcitation of the dye and transferred to conduction band of TiO\textsubscript{2} which transfers to positive electrode and finally returns to ground state dye molecule via oxidation-reduction reaction of electrolyte. At present, DSSC are the most extensively investigated area owing to their applications as low cost photovoltaic devices especially suited for building and automobiles integrated PV and portable or indoor light harvesting applications. Many researches have been performed on DSSC using different type of dyes\textsuperscript{1a}.

\textbf{For example, Ru\textsuperscript{2+} polypyridyl complexes, [Ru(dcbpyH\textsubscript{2})\textsubscript{2}(NCS)\textsubscript{2}], [Ru(4,4'-dicarboxy-2,2'_{-}bipyridine)\textsubscript{2}(NCS)\textsubscript{2}]} (commonly called "N3")\textsuperscript{1b} had been widely used as dye component and shows higher conversion efficiency because of long excitation lifetime; however, the metal is rare and expensive\textsuperscript{2–7}.

In addition, reports on transition metal complexes have demonstrated that organic ligands play important role in enhancing the luminescent color and intensity. Especially, narrowing HOMO-LUMO gap makes expansion of absorption band toward NIR region\textsuperscript{8–10}. Although some organic molecules and complexes have been developed as dyes in DSSCs, design and synthesis of organic ligands and their transition metal complexes for application on DSSCs is still a great challenge\textsuperscript{11–17}. In the design of complexes, Schiff base ligands have been widely used as ligands due to their
convenient synthesis.

Herein we have focused on designing of dyes based on salen type Schiff base complexes. We also consider the effect of substituents on the absorption conversion performance theoretically. Our prediction shows that the first row transition metal complexes may be effectively exploited as dyes. In the present article, we present the synthesis and characterization of the new transition metal complexes \[\text{ML(CH}_3\text{OH)}; \text{M = Mn(1), Fe(2), Co(3), Ni(4), Cu(5) and Zn(6)}\] and \[\text{H}_2\text{L}\] is naphthyl-salen Schiff base ligand and discusses the effect of central metal on absorption characteristic along with computational calculations. The complexes 1-6 were employed to prepare DSSC devices, which could compensate for the absorption of N3 in the high-energy band region of the visible spectrum. Thus, performance of DSSCs based on complexes as sensitizers is investigated in detail.

**Experimental**

**Chemicals and solvents:**

All starting materials such as 1-vinyl-naphthalen-2-ol and 2,3-diamino-succinic acid and metal salts M(OAc)_2·xH_2O were obtained from commercial sources. The solvents like methanol and DMF, DMSO were reagent grade and used as supplied.

**Instrumentation:**

Elemental analyses (C, H, N) were carried out with a Perkin-Elmer 2400II CHNS/O analyzer at the Tokyo University of Science. FT-IR spectra were recorded as KBr pellets on a JASCO FT-IR 4200 plus spectrophotometer in the range 4000–400 cm\(^{-1}\) at 298 K. UV-Vis-NIR absorption spectra and diffuse reflectance electronic spectra were measured on a JASCO V-570 UV-Vis-NIR spectrophotometer. Cyclic voltammetry (CV) was performed on a AIOS/DY2323 with a traditional three electrodes. The working electrode and auxiliary electrode was Pt disc. Ag/AgCl and Pt wire electrode, respectively. Magnetic studies were performed using a Quantum design MPMS-XL5QUID magnetometer. The X-ray diffraction patterns were collected at 298 K on Rigaku Smart lab (CuKα radiation) at the University of Tokyo. Powder crystal structure analysis was carried out with a Rigaku PDXL2, commercially available program package.

**Computational calculations:**

All calculation was performed by the Gaussian 09W software Revision A.02 (Gaussian, Inc.)\(^{18}\). Calculated UV-Vis spectra was gained by TD-DFT with B3LYP functional. Basis sets were Lan12DZ on central metal ion to consider the effect core potential and 6-31(d) on other atoms.

**Synthesis:**

Naphthyl-salen Schiff base ligand (H_2L) and 5 were synthesized according to the reported method by us\(^{19}\). To methanol solution of 1 mmol H_2L ligand, 1 mmol M(OAc)_2·xH_2O (M = Mn, Fe, Co, Ni, Cu and Zn) was added and dissolved. The reaction mixture was stirred for 2 h, subsequently filtered and collected at room temperature.

1 : Yield 17.9%; IR (KBr, cm\(^{-1}\)) : 457w, 508w, 565w, 657w, 725w, 753w, 831w, 979w, 1038w, 1097w, 1191w, 1250w, 1293w, 1340w, 1362w, 1385m, 1456w, 1478w, 1542s, 1599s, 1618s (C=N), 1727w, 2950w, 3075w, 3253w; Anal. Calcd. for C_{27}H_{22}MnN_2O_7 : C, 52.96; H, 3.62; N, 4.57. Found : C, 51.95; H, 3.32; N, 4.79%.

2 : Yield 75.0%; IR (KBr, cm\(^{-1}\)) : 501w, 566w, 618w, 654w, 751w, 831w, 863w, 990w, 1040m, 1028s, 1128s, 1192m, 1212m, 1243m, 1295w, 1342m, 1364m, 1384m, 1423w, 1453w, 1540m, 1579s, 1600s, 1617s (C=N), 1728w, 2962w, 3066w, 3200w; Anal. Calcd. for C_{27}H_{22}FeN_2O_7 : C, 51.96; H, 3.62; N, 4.57. Found : C, 51.95; H, 3.32; N, 4.79%.

3 : Yield 61.4%; IR (KBr, cm\(^{-1}\)) : 506w, 571w, 655w, 751m, 829w, 982w, 1045w, 1106w, 1188w, 1251w, 1295w, 1340m, 1361m, 1389m, 1435w, 1452w, 1510w, 1541m, 1590s, 1619s (C=N), 1731w, 2954w, 3076w, 3252w; Anal. Calcd. for C_{27}H_{22}CoN_2O_7 : C, 52.28; H, 3.57; N, 4.52. Found : C, 51.98; H, 3.48; N, 4.84%.

4 : Yield 25.0%; IR (KBr, cm\(^{-1}\)) : 505w, 567w, 659w, 753w, 829w, 977w, 1037w, 1099w, 1188w, 1251w, 1295w, 1340m, 1361m, 1389m, 1435w, 1452w, 1510w, 1541m, 1590s, 1619s (C=N), 1731w, 2954w, 3076w; Anal. Calcd. for C_{27}H_{22}NiN_2O_7 : C, 52.28; H, 3.57; N, 4.52. Found : C, 51.98; H, 3.48; N, 4.48%.

5 : Yield 25.0%; IR (KBr, cm\(^{-1}\)) : 506w, 571w, 655w, 751m, 829w, 982w, 1045w, 1106w, 1189m, 1260w, 1294m, 1364m, 1391m, 1485w, 1542s, 1586s, 1603s, 1618s (C=N), 1721w, 2941w, 3074w; Anal. Calcd. for C_{27}H_{22}NiN_2O_7 : C, 52.32; H, 3.58; N, 4.52. Found : C, 51.98; H, 3.48; N, 4.05%.
Yamaguchi et al.: Orbital and molecular design of new naphthyl-salen type transition metal complexes etc.

6: Yield 50.0%; IR (KBr, cm⁻¹): 505w, 575w, 723w, 752w, 835w, 974w, 1032w, 1101w, 1199m, 1282m, 1371m, 1419m, 1483w, 1518w, 1546m, 1575s, 1627s (C=N), 1731w, 2938w, 3062w, 3208w; Anal. Calcd. for C₂₇H₂₂ZnN₂O₇: C, 51.21; H, 3.50; N, 4.42. Found: C, 51.86; H, 3.45; N, 4.78%.

DSSC fabrication:
Negative electrodes were prepared by adding 1.5 g of TiO₂ (P25) to 1.5 g polyethylene glycol (PEG) and 15% (v/v) of acetic acid. The mixture was stirred for 1 h, resulting in a paste. The paste was extended into 1 cm² of Indium Tin Oxide (ITO) glass 2.5 cm² by squeegee method. Then the ITO was heated up to 450 °C for 30 min. Finally, the glass was immersed in dye (1-4, 6) solutions (5 × 10⁻⁴ M) for 24 h. Electrolyte was composed of mixture of 0.127 g I₂, 0.127 g of dimethyl-3-propylimidazolium iodide (DMPImI), 0.134 g of LiI and 0.405 g of tertbutylpyridine (TBP) in 10 mL MeCN. 2–3 drops of the resulting solution were added before measurement.

We prepared three types of positive electrode (ITO glass). One ITO glass was coated with graphite using 10B pencil. Another was ITO glass with rough surface, on which graphite was pasted using 10B pencil. The other ITO glass was exposed to burning candle and covered with graphite. We employed the type 3 positive electrode for measurements (Fig. 1).

Results and discussion
Synthesis:
The Schiff base ligand was synthesized employing the condensation reaction of 1-vinyl-naphthalen-2-ol and 2,3-diamino-succinic acid in the ratio 2:1. The reaction of the preformed Schiff base with metal acetate, M(OAc)₂·xH₂O (M = Mn(1), Fe(2), Co(3), Ni(4) and Zn(6)) afforded polycrystalline solid compounds (Scheme 1). 2,3-Diamino-succinic acid and Cu(5) complex was previously synthesized and characterized thoroughly and reported. The stoichiometry of the rest of the complexes 1-4 and 6 obtained was established through elemental analysis and FT-IR which was confirmed by structural characterization.

IR spectral studies:
FT-IR spectra of the complexes have provided conclusion for the mode of coordination from the dianionic moiety (nap)²⁻ to the metal ions. The positions of bands are summarized in Experimental section. The spectra of all the complexes contain a band in 1617–1627 cm⁻¹ range is the characteristic of ν(C=N) iminic bond. The spectra also contained bands in 1721–1731 cm⁻¹ range, represent the characteristic stretching frequency for free uncoordinated -COOH of the unionized car-

![Scheme 1. Preparation and chemical structures of 1-6.](image-url)
Boxylic functions of ligand. The appearance of medium intensity band in low frequency region characteristic of M–N ($\sim 570$ cm$^{-1}$) and the bands M–O ($\sim 505$ cm$^{-1}$) stretching vibrations were also present in the spectra of the complexes.

**X-Ray crystallographic studies**

The results of the X-ray analysis on crystals of the complexes 1-4 and 6 with refinement parameters are summarized in Table 1. As shown in Fig. 2, the structures of the independent molecules in the unit cells are presented for respective complexes (significant atoms are labeled). The selected bond lengths are summarized in Table 2. The selected bond angles for the complexes (1-3) and (4 and 6) are compiled in Table 3 and Table 4 respectively. All the complexes are within common range for the related ones$^{20}$. The metal ions

![Crystal structures of complexes](image)

**Fig. 2.** Crystal structures of complexes (a) [MnL(CH$_3$OH)](1), (b) [FeL(CH$_3$OH)](2), (c) [CoL(CH$_3$OH)](3), (d) [NiL(CH$_3$OH)](4), (e) [CuL(CH$_3$OH)](5) and (f) [ZnL(CH$_3$OH)](6).
Table 1. Crystal data and structural refinement for 1-4 and 6

<table>
<thead>
<tr>
<th></th>
<th>CCDC</th>
<th>Empirical formula</th>
<th>Formula weight</th>
<th>Crystal system</th>
<th>Space group</th>
<th>a (Å)</th>
<th>b (Å)</th>
<th>c (Å)</th>
<th>(\alpha) (°)</th>
<th>(\beta) (°)</th>
<th>(\gamma) (°)</th>
<th>V (Å³)</th>
<th>Z</th>
<th>Rwp (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1540630</td>
<td>(C_{27}H_{22}MnN_2O_7)</td>
<td>541.41</td>
<td>Triclinic</td>
<td>P-1</td>
<td>11.21(12)</td>
<td>14.10(2)</td>
<td>10.779(7)</td>
<td>92.46(11)</td>
<td>111.79(10)</td>
<td>93.28(15)</td>
<td>1550(3)</td>
<td>2</td>
<td>2.86</td>
</tr>
<tr>
<td>2</td>
<td>1540629</td>
<td>(C_{27}H_{22}FeN_2O_7)</td>
<td>542.32</td>
<td>Monoclinic</td>
<td>P2</td>
<td>15.47(6)</td>
<td>12.87(2)</td>
<td>16.177(4)</td>
<td>95.43(11)</td>
<td>106.02(2)</td>
<td>99.04(18)</td>
<td>1921.16(6)</td>
<td>2</td>
<td>3.97</td>
</tr>
<tr>
<td>3</td>
<td>1540628</td>
<td>(C_{27}H_{22}CoN_2O_7)</td>
<td>513.37</td>
<td>Monoclinic</td>
<td>P2</td>
<td>9.63(11)</td>
<td>110.11(2)</td>
<td>7.740(11)</td>
<td>95.43(11)</td>
<td>106.02(2)</td>
<td>99.04(18)</td>
<td>1332.86(6)</td>
<td>2</td>
<td>1.92</td>
</tr>
<tr>
<td>4</td>
<td>1540631</td>
<td>(C_{27}H_{22}NiN_2O_7)</td>
<td>545.17</td>
<td>Triclinic</td>
<td>P-1</td>
<td>15.47(6)</td>
<td>12.87(2)</td>
<td>16.177(4)</td>
<td>107.62(7)</td>
<td>99.48(7)</td>
<td>107.62(7)</td>
<td>1118(2)</td>
<td>2</td>
<td>5.66</td>
</tr>
<tr>
<td>6</td>
<td>1540627</td>
<td>(C_{27}H_{22}ZnN_2O_7)</td>
<td>551.89</td>
<td>Triclinic</td>
<td>P-1</td>
<td>12.568(19)</td>
<td>12.87(2)</td>
<td>16.177(4)</td>
<td>109.83(18)</td>
<td>102.46(11)</td>
<td>1118(2)</td>
<td>2096(6)</td>
<td>2</td>
<td>3.90</td>
</tr>
</tbody>
</table>

Table 2. Selected bond lengths (Å) for 1-4 and 6

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn–O1</td>
<td>1.933(2)</td>
<td>1.949(3)</td>
<td>1.896(5)</td>
<td>1.874(4)</td>
<td>2.312(3)</td>
</tr>
<tr>
<td>Mn–O2</td>
<td>1.987(2)</td>
<td>1.960(5)</td>
<td>1.892(6)</td>
<td>2.064(3)</td>
<td>1.879(3)</td>
</tr>
<tr>
<td>Mn–O3</td>
<td>2.087(2)</td>
<td>2.102(4)</td>
<td>2.124(3)</td>
<td>1.850(15)</td>
<td>2.125(3)</td>
</tr>
<tr>
<td>Mn–N1</td>
<td>2.144(3)</td>
<td>2.131(6)</td>
<td>1.910(7)</td>
<td>1.944(3)</td>
<td>2.321(3)</td>
</tr>
<tr>
<td>Mn–N2</td>
<td>2.130(14)</td>
<td>2.149(3)</td>
<td>1.9305(5)</td>
<td>2.142(3)</td>
<td>2.268(3)</td>
</tr>
</tbody>
</table>

Table 3. Selected bond angles (°) for 1-3

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1–Mn–O3</td>
<td>101.76(8)</td>
<td>102.533(4)</td>
<td>92.085(17)</td>
</tr>
<tr>
<td>O2–Mn–O3</td>
<td>97.76(10)</td>
<td>96.518(16)</td>
<td>93.355(8)</td>
</tr>
<tr>
<td>O1–Mn–O2</td>
<td>97.76(10)</td>
<td>108.420(4)</td>
<td>89.902(7)</td>
</tr>
<tr>
<td>O1–Mn–N1</td>
<td>80.99(17)</td>
<td>83.554(5)</td>
<td>91.488(7)</td>
</tr>
<tr>
<td>O1–Mn–N2</td>
<td>147.30(6)</td>
<td>147.158(9)</td>
<td>164.861(4)</td>
</tr>
<tr>
<td>O2–Mn–N1</td>
<td>162.23(2)</td>
<td>161.950(5)</td>
<td>173.835(2)</td>
</tr>
<tr>
<td>O2–Mn–N2</td>
<td>83.73(10)</td>
<td>84.626(9)</td>
<td>91.471(5)</td>
</tr>
<tr>
<td>O3–Mn–N1</td>
<td>92.54(11)</td>
<td>93.845(16)</td>
<td>92.596(7)</td>
</tr>
<tr>
<td>O3–Mn–N2</td>
<td>106.23(8)</td>
<td>105.795(5)</td>
<td>102.880(16)</td>
</tr>
<tr>
<td>N1–Mn–N2</td>
<td>79.49(11)</td>
<td>78.341(6)</td>
<td>85.632(5)</td>
</tr>
</tbody>
</table>

occupy the inner chamber of the Schiff base ligand and exhibits penta-coordinated geometry whereby the dianonic (nap)²⁻ binds in tetradentate fashion via two oxygen (O1 and O2) and two nitrogen (N1 and N2) of the Schiff base ligand which are approximately coplanar. The tetragonality index, \(\tau = (\beta - \alpha)/60\), where \(\alpha\) and \(\beta\) are given by opposing angles in the coordination polyhedron. The values of \(\tau\) are zero and unity, for perfect square pyramidal and trigonal bipyramidal respectively. The values of \(\tau\) for complexes are found to be 0.248(1), 0.246(2), 0.149(3), 0.184(4) and 0.164(6). According to these values, the coordination geometry around the metal ions is best described as distorted square-based pyramidal with one methanol at axial position.

Magnetic properties:
Magnetic susceptibility measurements for powder
samples (1-4) were carried out by the SQUID based magnetometer in the temperature 5–300 K. The magnetic susceptibilities are shown as a function of temperature in Fig. 3. The magnetic data were fitted by employing Curie-Weiss law, $\chi = C/(T+\theta)$. Magnetic moments were obtained from the relation $\mu = 2.828(\chi T)^{1/2}$ (B.M.). The number of unpaired electrons ($n$) was predicted from $\mu$ by the relation $\mu = \{n(n+2)\}^{1/2}$. The observed effective magnetic moment along with number of unpaired electrons are shown in Table 5. The $n$ values are in agreement with expected d-electron configurations of the coordination geometries determined.

### Table 4. Selected bond angles (º) for 4 and 6.

<table>
<thead>
<tr>
<th></th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1–Ni–O3</td>
<td>101.23(9)</td>
<td>O1–Zn–O3</td>
</tr>
<tr>
<td>O2–Ni–O3</td>
<td>99.35(8)</td>
<td>O2–Zn–O3</td>
</tr>
<tr>
<td>O1–Ni–O2</td>
<td>95.50(11)</td>
<td>O1–Zn–O2</td>
</tr>
<tr>
<td>O1–Ni–N1</td>
<td>87.98(11)</td>
<td>O1–Zn–N1</td>
</tr>
<tr>
<td>O1–Ni–N2</td>
<td>156.43(5)</td>
<td>O1–Zn–N2</td>
</tr>
<tr>
<td>O2–Ni–N1</td>
<td>167.51(2)</td>
<td>O2–Zn–N1</td>
</tr>
<tr>
<td>O2–Ni–N2</td>
<td>87.88(12)</td>
<td>O2–Zn–N2</td>
</tr>
<tr>
<td>O3–Ni–N1</td>
<td>91.70(8)</td>
<td>O3–Zn–N1</td>
</tr>
<tr>
<td>O3–Ni–N2</td>
<td>101.21(9)</td>
<td>O3–Zn–N2</td>
</tr>
</tbody>
</table>

### Table 5. Effective moment ($\mu$) for the paramagnetic complexes (1-4)

<table>
<thead>
<tr>
<th>Complexes</th>
<th>$\mu$ (B.M.)</th>
<th>No. of unpaired electrons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.03</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>4.64</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>4.05</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>2.91</td>
<td>2</td>
</tr>
</tbody>
</table>

UV-Vis-NIR electronic spectra and TD-DFT calculations:

The electronic spectra of the $3 \times 10^{-5}$ M DMF solutions of the complexes are shown in Fig. 4(a). The intense high energy absorption bands appear at ~270 nm (Band 1) and a weak band at ~400 nm (Band 2) in all complexes are due to $\pi-\pi^*$. The complex 4 shows highest absorption coefficient whereas in complex 6, band 2 is broad and prominent as compared to other complexes.
complexes. From these observations, it may be concluded that structure of the ligand affects the absorption bands. Comparing with the absorption spectra of Ru$^{2+}$ polypyridyl complexes, [Ru(dcbpyH$_2$)$_2$(NCS)$_2$], N3, apparently all the complexes could better compensate for the absorption of N3 in the low wavelength region of the visible spectrum. Therefore, we apply complexes (1-6) as dyes to apply in the DSSC devices. The high molar extinction coefficient indicates that they possess a better light-harvesting ability in this low wavelength region compared with N3. Meanwhile the ligand has carboxylic groups, which could adsorb on the TiO$_2$ surface.

The solid state adsorption spectra were also obtained to gain insight for the d-d transitions in the complexes 1-6 shown in Fig. 4(b). It has been found that absorption band at ~700 nm appears in all complexes is due to d-d transitions.

The electronic transition in UV spectra of complexes 1-6 have been carried out employing TD-DFT (B3LYP/6-31+G(d,p)) method and provided in Fig. 5. The molecular orbitals (MOs) HOMO and LUMO were provided in Fig. 6. To support the DFT geometry optimizations, the calculated geometries were compared with the experimental spectra data. A comparison of the theoretical data for the complexes allows us to support the assignment.
to assess the extent of spectra and to correctly describe the electronic spectra.

The broadening of the peak in Fig. 5 is generated by two peaks in the absorption spectra at ~300 nm and 400 nm. Band 1 and 2 arise due to $(\pi-\pi^*)$ charge transfer of the naphthyl ring. It is clear from Fig. 5 that these two bands are comparable, but complex 6 show the broadest absorption band among all molecules.

It may also be concluded from Fig. 6 that the complex 4 shows longest wavelength. It is plausible to
conclude that visual appearance of electron transition by band 2 that causes power generation are shown in Fig. 6. It may also be concluded that it is only the complexes 4 that can be used as dye as this follow the trend for DSSC power generation effective. In contrast to 4, other complexes are not suitable for this purpose due to unsuitable orbital distribution of excited states (far from -COOH groups).

<table>
<thead>
<tr>
<th>Complexes</th>
<th>( E_{p}^{a} ) (V vs Ag/AgCl)</th>
<th>( E_{p}^{c} ) (V vs Ag/AgCl)</th>
<th>( E_{p}^{a} ) (V vs NHE)</th>
<th>( E_{p}^{c} ) (V vs NHE)</th>
<th>HOMO (eV)</th>
<th>LUMO (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1.170</td>
<td>0.307</td>
<td>-0.970</td>
<td>0.507</td>
<td>-4.95</td>
<td>-3.47</td>
</tr>
<tr>
<td>2</td>
<td>-1.150</td>
<td>0.476</td>
<td>-0.950</td>
<td>0.676</td>
<td>-5.12</td>
<td>-3.49</td>
</tr>
<tr>
<td>3</td>
<td>-1.170</td>
<td>0.103</td>
<td>-0.970</td>
<td>0.303</td>
<td>-4.74</td>
<td>-3.47</td>
</tr>
<tr>
<td>4</td>
<td>-1.120</td>
<td>0.187</td>
<td>-0.920</td>
<td>0.387</td>
<td>-4.83</td>
<td>-3.52</td>
</tr>
<tr>
<td>5</td>
<td>-1.300</td>
<td>0.313</td>
<td>-1.100</td>
<td>0.513</td>
<td>-4.95</td>
<td>-3.34</td>
</tr>
<tr>
<td>6</td>
<td>-1.130</td>
<td>0.545</td>
<td>-0.93</td>
<td>0.745</td>
<td>-5.19</td>
<td>-3.51</td>
</tr>
</tbody>
</table>

\( E_{p}^{a} \) and \( E_{p}^{c} \) are the onset oxidation potentials in cyclic voltamograms (CV), these sensitizers have low lying highest occupied molecular orbital (HOMO) levels from -4.74 to -5.19 eV. All HOMO are lower than the redox potential of \( \Gamma/\Gamma^{3-} \) (−4.85 eV vs vacuum), indicating the favorable regeneration. The lowest unoccupied molecular orbital (LUMO) energy of the complexes are -3.47(1), -3.49(2), -3.47(3), -3.52(4), -3.34(5) and -3.51(6) eV, respectively. All calculated

### Electrochemical properties:

We investigated the UV-Vis absorption spectra of complexes 1-6. The HOMO-LUMO energy levels are crucial in selecting sensitizers for DSSCs, which were determined by cyclic voltammetry in a 0.1 \( M \) tetrabutylammonium perchlorate (TBAP). The electrochemical parameters are compiled in Table 6. As estimated from the onset oxidation potentials in the

### Characterization parameters for DSSC:

The solar to electricity conversion efficiency (\( \eta \)) of the solar cell is calculated by the photocurrent density measured at short-circuit (\( J_{sc} \)), the open-circuit photovoltage (\( V_{oc} \)), the fill factor of the cell (FF) and the
intensity of the incident light \( (P_{\text{inc}}) \) by the relation,

\[
\eta = \frac{FF(V_{\text{oc}}J_{\text{sc}})}{P_{\text{inc}}} \\
FF = \frac{I_{\text{mp}}V_{\text{mp}}}{J_{\text{sc}}V_{\text{oc}}}
\]

where, \( I_{\text{mp}} \) is the maximum power point current, \( V_{\text{mp}} \) is the maximum point voltage.

**Photovoltaic properties of DSSCs:**

The complexes (1-6) were assembled into sensitized DSSCs devices to study the influence of the electron-donating group and coordination on the DSSCs performance was tested under irradiation of 100 mW cm\(^{-2}\) by ADCMT 6241 DC voltage/current source/monitor. The data of \( J_{\text{sc}}, V_{\text{oc}} \) and FF for these devices are shown in Fig. 7 and presented in Table 7. The higher value of \( V_{\text{oc}} \) corresponds to higher harvest of the light energy. The efficiencies are found in the order \( 4 > 6 > 1 > 5 > 3 > 2 \). Complex (4) and (6) show higher \( V_{\text{oc}} \) and \( J_{\text{sc}} \), which indicates the higher efficiency as compared to the other complexes. The strongest light harvesting ability of the complex 6 is also supported by the appearance of band 2 in calculated electronic spectra. However, band 2 of 4 indicating the highest \( V_{\text{oc}} \) value is ascribed to electron transfer towards -COOH group.

![Fig. 7. The J-V plots for complexes 1-6.](image)
The metal order of efficiency is Ni > Zn > Mn > Cu > Co > Fe as listed in Table 6. Efficiencies of DSSC with 4 and 5 showed an order of magnetite larger value than other cells, this is due to comparatively higher $V_{oc}$ and $J_{sc}$.

The enhanced $J_{sc}$ values will be discussed in conjunction with the incident photon-to-current electron conversion efficiency (IPCE) spectra shown in Fig. 8 and the parameters are provided in Table 6 to know the degree of energy conversion from light harvesting toward electricity at each light wavelength. The complex (4) show the highest value (7%) around 350 nm corresponds to band 2 in calculated UV-Vis spectra. This transfer results in high value of IPCE. Complex 6 show the efficiency (η) next to 4 and also exhibit the high IPCE value too. It may be concluded that complexes 4 and 6 can be exploited as dyes in DSSC cells and increase the conversion efficiency of the cell.

**Conclusion**

The newly synthesized naphthyl-salen type mononuclear complexes of Mn, Fe, Co, Ni, Zn have been characterized using elemental analysis and FT-IR. The structures are ascertained through X-ray diffraction on crystal analysis. The metal ions in all the complexes were pentacoordinate, square pyramidal geometry. The UV-Vis-NIR, TD-DFT calculation, magnetic properties were also measured and all the five complexes along with the Cu complex previously synthesized and characterized by us. Similar to previous systems, UV-Vis-NIR electronic spectra of these complexes were also measured and inference of electron transfer of each absorption band is estimated by TD-DFT calculations. The complexes (1-6) were assembled into sensitized DSSCs devices to study the influence of the electron-donating group and coordination in the DSSC. The Ni-nap and Zn-nap show comparatively stronger absorption, high $V_{oc}$, $J_{sc}$ and show suitable HOMO-LUMO band of dye for electron cycle in cell as compared to other complexes. Thus, these two complexes may be exploited as dye in DSSC.

CCDC 1540627-1540631 contain the supplementary crystallographic data. These data can be obtained free of charge via [http://www.ccdc.cam.ac.uk/conts/retrieving.html], or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: (+44) 1223-336-033; or E-mail: deposit@ccdc.cam.ac.uk.

**Table 7. The parameters of DSSC performance for 1-6**

<table>
<thead>
<tr>
<th>Dye</th>
<th>$V_{oc}$ (V)</th>
<th>$J_{sc}$ (mA cm$^{-2}$)</th>
<th>FF</th>
<th>η (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N$_3$</td>
<td>0.6803</td>
<td>6.0247</td>
<td>0.26</td>
<td>1.0824</td>
</tr>
<tr>
<td>Mn-nap</td>
<td>0.0861</td>
<td>0.0084</td>
<td>0.28</td>
<td>2.05×10$^{-4}$</td>
</tr>
<tr>
<td>Fe-nap</td>
<td>0.396</td>
<td>0.0050</td>
<td>0.26</td>
<td>0.52×10$^{-4}$</td>
</tr>
<tr>
<td>Co-nap</td>
<td>0.425</td>
<td>0.0103</td>
<td>0.26</td>
<td>1.15×10$^{-4}$</td>
</tr>
<tr>
<td>Ni-nap</td>
<td>0.1668</td>
<td>0.0305</td>
<td>0.29</td>
<td>1.48×10$^{-4}$</td>
</tr>
<tr>
<td>Cu-nap</td>
<td>0.0304</td>
<td>0.0264</td>
<td>0.22</td>
<td>1.75×10$^{-4}$</td>
</tr>
<tr>
<td>Zn-nap</td>
<td>0.0739</td>
<td>0.0660</td>
<td>0.26</td>
<td>1.26×10$^{-4}$</td>
</tr>
</tbody>
</table>

**Fig. 8.** IPCE spectra for fabricated DSSC with complexes 1-6.
Acknowledgement

This XRD work was conducted at Advanced Characterization Nanotechnology Platform of the University of Tokyo (Professor Kazuhiro Fukawa), supported by "Nanotechnology Platform" of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

References


13. B. Kilic, S. Turkdogan and A. Astam, Sci. Re-

ports, 2016, 6, 27052.


Peanut proteins in selective sensing of Bi\textsuperscript{III} at trace concentrations

Sumanta Kumar Ghatak\textsuperscript{a}, Dipanwita Majumdar\textsuperscript{b}, Achintya Singha\textsuperscript{b} and Kamalika Sen*\textsuperscript{a}

\textsuperscript{a}Department of Chemistry, University of Calcutta, 92, Acharya Prafulla Chandra Road, Kolkata-700 009, India
\textsuperscript{b}Department of Physics, Bose Institute, 93/1, Acharya Prafulla Chandra Road, Kolkata-700 009, India

Abstract: Bismuth is an environmentally significant element due to its usefulness in different aspects of life especially in semiconductors, cosmetics and pharmaceutical industries. It is less soluble in aqueous medium so it is poorly absorbed in human cells, however it plays a crucial role in various diseases mainly gastrointestinal disorders and neurological disorders. In this report selective recognition of trace concentration of bismuth is observed with peanut proteins conarachin II, conarachin I and arachin, which are available from cheap source and their extraction is cost effective too. For avoiding bismuth precipitation, we have optimized the pH of the solutions and they were subjected to spectrophotometric analysis. Absorption, fluorescence, circular dichroism and Raman spectra support the interaction of bismuth with peanut proteins.

Keywords: Peanut, bismuth, sensing, absorbance, fluorescence, circular dichroism, Raman spectra.

Introduction

Designing new organic molecules for the sensing of biologically important and environmentally hazardous metal ions at trace concentration levels is an exciting research in recent years\textsuperscript{1,2}. Some metals are of environmental concern as they cause various health hazards including carcinogenicity while some others have useful applications. Among transition-metal ions, mercury is the most dangerous cation for the environment. Being highly reactive to biological molecules, it enters the living cells causing prenatal brain damage, serious cognitive disorders and minamata diseases\textsuperscript{3-6}. Lead is known to be a larger problem for children than it is for adults at higher concentrations. Brain damage and nervous disorders are the most common outcomes of high level lead exposure\textsuperscript{7}. Studies also indicate that lead accumulation inhibits plant growth due to denaturation of proteinaceous enzymes that, control cellular processes\textsuperscript{8}. Antimony and its compounds are however milder in causing acute human health effects, with the exception of antimony potassium tartrate, a prodrug used to treat leishmaniasis. They rather have medical applications and are used in antiprotozoan drugs\textsuperscript{9,10}. Thallium (Tl\textsuperscript{+}) is known to cause isomorphous replacements with potassium in biological systems and activate some enzymes as a consequence. Sometimes this poses a threat as the thallium ion may bind more strongly than potassium to a site and then fail to bind additional sites as required for the biological activity. Thallium actively concentrates in mitochondria of the cell and thereby interferes in oxidative phosphorylation\textsuperscript{11}. \textsuperscript{201}Tl radio-isotopes are however potential candidates for heart imaging\textsuperscript{12}. Bismuth is present at trace concentration (8 \textmu g/kg) in earth’s crust. It is used in our daily lives in semiconductors, cosmetic products, medicines (for treatment of syphilis, peptic ulcers and dermatological disorders), alloys, catalyst in the chemical industry, metallurgical additives, and preparation and recycling of uranium in nuclear fuels\textsuperscript{13}. \textsuperscript{212}Bi and \textsuperscript{213}Bi isotopes have been used for cancer treatment\textsuperscript{14,15}. However bismuth causes several toxic effects in human body such as nephropathy, osteoarthropathy, hepatitis and neuropathology when present at high concentrations\textsuperscript{16}. 

773
Spectroscopic techniques e.g. atomic absorption spectrometry (AAS)\textsuperscript{17}, hydride generation-atomic fluorescence spectrometry (HG-AFS)\textsuperscript{18}, inductively coupled plasma atomic emission spectrometry (ICP-AES)\textsuperscript{19}, inductively coupled plasma mass spectrometry (ICP-MS)\textsuperscript{20} and electrochemical methods\textsuperscript{21} have been used for detecting bismuth in several samples at trace concentrations. These methods however need costly instrumentation and rigorous sample preparation techniques.

To the best of our knowledge reports on metal ion sensing using natural bioactive polymers like plant proteins are still lacking in the literature. Peanuts represent cheap and widely available source of plant proteins growing all over the world. In this report we have developed a spectrophotometric method of bismuth sensing using the peanut protein fractions conarachin II, conarachin I and arachin selectively out of other heavy metals like Hg\textsuperscript{II}, Pb\textsuperscript{II}, Sb\textsuperscript{III} and Tl\textsuperscript{III} in a very convenient and cost effective way.

**Experimental**

**Materials**: Sodium citrate and citric acid were obtained from Fischer Scientific for buffer preparation. Bi(NO\textsubscript{3})\textsubscript{3}, Tl(NO\textsubscript{3})\textsubscript{3}, Pb(NO\textsubscript{3})\textsubscript{2}, HgCl\textsubscript{2}, K\textsubscript{2}Sb\textsubscript{2}(C\textsubscript{4}H\textsubscript{2}O\textsubscript{6})\textsubscript{2} salts were taken from Merck. All the solutions were prepared in triple distilled water. Peanuts were purchased from the local market. For the determination of protein concentration, the components of Bradford reagent i.e. CH\textsubscript{3}CH\textsubscript{2}OH, H\textsubscript{3}PO\textsubscript{4}, NaOH, CH\textsubscript{3}OH were obtained from Merck. Coomassie brilliant blue G was obtained from HIMEDIA and all the other chemicals in this experiment were of analytical grade.

**Apparatus**: The pH of the solutions was maintained using the pH/ion meter (S 220-K) of Mettler. The absorption spectra were measured using Agilent 8453 diode array spectrophotometer and fluorescence spectra were obtained on a Perkin-Elmer LS-55 spectrofluorimeter equipped with a quartz cell with a 1.0 cm optical path length. The excitation and emission slits of the monochromator were adjusted to 5 nm. RAM HR, Jobin Yvon spectrometer and a Peltier-cooled charge-coupled-device (CCD) detector was used for the determination of the Raman vibrational frequencies of the experimental samples. The far-UV CD spectra were obtained using JASCO J-815 spectropolarimeter.

**Methods**

The extraction, purification and characterization of the peanut protein fractions conarachin II, conarachin I and arachin were done using the methods described in the earlier report\textsuperscript{22}. The obtained protein fractions were characterized using the absorption spectra and SDS-PAGE pattern. The maximum absorption intensity of conarachin II at 278 nm, conarachin I at 265 nm and arachin at 280 nm, agrees with the literature data. The SDS-PAGE pattern of conarachin II, and arachin show molecular weight bands at 39800, 33100, 26900, 24000, 21900, 18600, 15800 Da and 72400, 60300, 39800, 33100, 26900, 21900 Da and conarachin I shows a single band at 18600 Da. The concentration of the proteins was measured using Bradford reagents\textsuperscript{23}. The concentration of conarachin II, conarachin I and arachin were 0.9, 0.8 and 0.76 mg/mL respectively.

**Absorption and fluorescence spectra**:

Absorption and fluorescence study were used to determine the selectivity and sensitivity of peanut proteins in the presence of various heavy metal ions, such as Bi\textsuperscript{III}, Tl\textsuperscript{III}, Pb\textsuperscript{II}, Hg\textsuperscript{II} and Sb\textsuperscript{III}. The salt solutions (1 mM each) of Bi(NO\textsubscript{3})\textsubscript{3}, Tl(NO\textsubscript{3})\textsubscript{3}, Pb(NO\textsubscript{3})\textsubscript{2}, HgCl\textsubscript{2}, K\textsubscript{2}Sb\textsubscript{2}(C\textsubscript{4}H\textsubscript{2}O\textsubscript{6})\textsubscript{2} were prepared with triple distilled water. For preparation of Bi(NO\textsubscript{3})\textsubscript{3} solution a weighed amount of the salt was first dissolved in a drop of HNO\textsubscript{3} and then diluted with water to required volume. This was done to avoid precipitation of Bi as its oxynitrate directly in water medium. To identify the possible complexation, 0.3 mL each of these metal ion solutions was mixed with 0.2 mL of protein solutions and volume was made up to 1.5 mL with 0.1 M sodium citrate-citric acid buffer of pH 3. To optimize the Bi-protein complexation, 1 mM bismuth(III) solution was added to the protein solution in different stoichiometric ratios. The solutions were subjected to absorption and fluorescence spectral studies. The experi-
Ghatak et al.: Peanut proteins in selective sensing of Bi^{III} at trace concentrations

ment was also studied at the pHs 5.6, 6.7 and 7.9 of 0.1 mM phosphate buffer medium. But due to the metal hydroxide precipitation, the experiment was done at pH 3 of 0.1 mM citrate buffer medium.

Circular dichroism (CD) spectra:

To obtain CD spectra, 1 mM Bi^{III} solution was added to the 50 µL of protein solution in a quartz cylindrical cuvette of 0.1 cm path-length at 25 °C in different stoichiometric ratios. The final spectra have been taken after three consecutive scans and base line correction.

Raman spectra:

The Raman spectra of the different peanut protein fractions were compared with the protein-Bi^{III} complexes. 20 µL of different peanut proteins and the solutions of protein-Bi^{III} complexes were taken on glass plate coated with Al foil. The solutions were dried in vacuum desiccator overnight. Then the samples were then taken for Raman spectroscopy.

Results and discussion

Absorption spectra: Conarachin II, conarachin I and arachin, show the absorption maxima at 278, 265 and 280 nm respectively. The absorption data (Fig. 1) suggest the selective interaction of Bi^{III} ions with all the three proteins. With increasing Bi concentrations in the protein solutions a gradual increase in the absorbance of the three peanut proteins were observed. From the absorbance data we have calculated the binding constant and free energy changes upon complexation using Benesi-Hildebrand equation[22].

\[
\frac{1}{A - A_0} = \frac{1}{A_1 - A_0} + \frac{1}{(A_1 - A_0)K[M]}
\]

\[Y = A + BX\]

where \(A\) is the absorbance of the experimental solution containing metal and protein. \(A_0\) is the absorbance of the protein only. \(A_1\) is the absorbance when protein is completely bound with the metal. \([M]\) is the metal ion concentration. \(K\) is the binding/association constant.

Eq. (1) can be represented as eq. (2) which is a

Fig. 1. Plot of absorbance of conarachin II, conarachin I, arachin with different metal ions at pH 3 in citrate buffer (0.1 M) medium.

Fig. 2. Benesi-Hildebrand plot of conarachin II, conarachin I and arachin-Bi^{III} complexes at pH 3.
straight line having intercept \(1/(A_1 - A_0)\) and slope \(1/(A_1 - A_0)K\). From the Benesi-Hildebrand (BH) plot (Fig. 2), we have calculated the binding constant values. Corresponding free energy change (\(\Delta G\)) values were calculated at a temperature (\(T\)) of 298 K using the equation

\[\Delta G = -RT \ln K\]  

(3)

Binding constant, free energy change and stoichiometric ratio (obtained from the BH plot) of the three peanut protein-bismuth complexes are tabulated in Table 1.

The binding constants of the conarachin II, conarachin I and arachin-Bi complexes are, \(2.4737 \times 10^3\) M\(^{-1}\), \(3.7614 \times 10^3\) M\(^{-1}\) and \(0.92623 \times 10^3\) M\(^{-1}\) respectively. The \(\Delta G\) values upon complexation of conarachin II, conarachin I and arachin with Bi are \(-19.488\) kJ/mol, \(-20.533\) kJ/mol and \(-17.038\) kJ/mol respectively. The greater value of binding constant and more negative value of free energy change indicate the highest interaction of conarachin I with Bi\(^{III}\) amongst the three proteins.

**Fluorescence spectra**: The emission maxima for conarachin II, conarachin I and arachin, were observed at 365 nm, 425 nm and 340 nm respectively. Fluorescence spectra also support the selective interaction of Bi\(^{III}\) with the three peanut proteins as compared to other heavy metals like Tl\(^{III}\), Pb\(^{II}\), Hg\(^{II}\) and Sb\(^{III}\). Figs. 3, 4 and 5 reflect this selective behavior of conarachin II, conarachin I and arachin respectively. With increasing bismuth concentration emission intensity decreases which, indicates the interaction of Bi\(^{III}\) with three proteins. The plot (Fig. 6) \(F_0/F\) versus [Bi\(^{III}\)] shows a curved nature which suggests that the fluorescence quenching is both static and dynamic type indicating both ground state and excited state complexations of the proteins with Bi\(^{III}\).

**CD spectra**: The far UV CD spectra (Figs. 7, 8 and 9) of the three peanut proteins showed two minima at 208 and 222 nm which indicates \(\alpha\) helical structures of conarachin II, conarachin I and arachin. With in-

---

**Table 1. Some physical parameters of Bi\(^{III}\) complexes of the protein fractions**

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding constant (M(^{-1}))</th>
<th>Free energy change (kJ/mol)</th>
<th>Stoichiometric ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conarachin II-Bi(^{III})</td>
<td>(2.4737 \times 10^3)</td>
<td>(-19.488)</td>
<td>1 : 1</td>
</tr>
<tr>
<td>Conarachin I-Bi(^{III})</td>
<td>(3.7614 \times 10^3)</td>
<td>(-20.533)</td>
<td>1 : 1</td>
</tr>
<tr>
<td>Arachin-Bi(^{III})</td>
<td>(0.92623 \times 10^3)</td>
<td>(-17.038)</td>
<td>1 : 1</td>
</tr>
</tbody>
</table>
creasing bismuth concentration, the gradual decrease of ellipticity indicates the lower value of $\alpha$ helical content in the protein molecules which leads to the formation of $\beta$ sheet structures in all the three proteins. The change in the circular dichroism spectra of the three proteins upon interaction with bismuth clearly indicates the modification of the secondary structure of the proteins.

**Raman study**: Upon interaction with Bi, a large number of vibrational frequencies were observed (Fig. 10 and Table 2) to appear or disappear for the three proteins conarachin II, conarachin I and arachin. The vibrational frequency near 1000 cm$^{-1}$ indicates the presence...
Table 2. Details of the Raman spectral data

<table>
<thead>
<tr>
<th>Raman modes (cm⁻¹)</th>
<th>Conarachin II</th>
<th>Conarachin II-Bi&lt;sup&gt;III&lt;/sup&gt;</th>
<th>Conarachin I</th>
<th>Conarachin I-Bi&lt;sup&gt;III&lt;/sup&gt;</th>
<th>Arachin</th>
<th>Arachin-Bi&lt;sup&gt;III&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-C-C out of plane bending</td>
<td>–</td>
<td>380</td>
<td>400</td>
<td>–</td>
<td>390</td>
<td>–</td>
</tr>
<tr>
<td>C-C-C out of plane skeletal deformation</td>
<td>457</td>
<td>455</td>
<td>455</td>
<td>–</td>
<td>475</td>
<td>–</td>
</tr>
<tr>
<td>C-C-C out of plane bending</td>
<td>466</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>475</td>
</tr>
<tr>
<td>C-C-C inplane skeletal deformation</td>
<td>–</td>
<td>520</td>
<td>538</td>
<td>530</td>
<td>557</td>
<td>570</td>
</tr>
<tr>
<td>N-H₂ wagging</td>
<td>615</td>
<td>620</td>
<td>625</td>
<td>620</td>
<td>619</td>
<td>–</td>
</tr>
<tr>
<td>Tyr</td>
<td>636</td>
<td>630</td>
<td>–</td>
<td>–</td>
<td>822</td>
<td>829</td>
</tr>
<tr>
<td>Trp</td>
<td>–</td>
<td>900</td>
<td>884</td>
<td>897</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C-H out of plane bending</td>
<td>–</td>
<td>945</td>
<td>–</td>
<td>939</td>
<td>920</td>
<td>930</td>
</tr>
<tr>
<td>C-N-H₂ stretching</td>
<td>976</td>
<td>989</td>
<td>975</td>
<td>–</td>
<td>990</td>
<td>–</td>
</tr>
<tr>
<td>Phe</td>
<td>995</td>
<td>1000</td>
<td>1000</td>
<td>–</td>
<td>1000</td>
<td>–</td>
</tr>
<tr>
<td>C-C, C-N and C-O stretching mode</td>
<td>1075</td>
<td>1075</td>
<td>1091</td>
<td>1050</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C-C stretching mode</td>
<td>1130</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tyr, Phe</td>
<td>1197</td>
<td>–</td>
<td>–</td>
<td>1190</td>
<td>1171</td>
<td>–</td>
</tr>
<tr>
<td>Amide III</td>
<td>–</td>
<td>1260</td>
<td>1264</td>
<td>1260</td>
<td>1260</td>
<td>–</td>
</tr>
<tr>
<td>CH₃ deformation</td>
<td>–</td>
<td>1427</td>
<td>–</td>
<td>1425</td>
<td>1451</td>
<td>–</td>
</tr>
<tr>
<td>Phe</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1617</td>
<td>1607</td>
<td>–</td>
</tr>
<tr>
<td>Trp</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1630</td>
<td>–</td>
</tr>
<tr>
<td>Amide 1, β-sheet</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1666</td>
<td>–</td>
</tr>
</tbody>
</table>
ence of phenylalanine in the three proteins. C-C-C out of plane bending and C-N-H2 stretching were shifted for conarachin II, but were disappeared for conarachin I and arachin. Shifting of the peak positions near 630 cm–1 and 830 cm –1 for tyrosine and 900 cm –1 for tryptophan24–27 in the three proteins indicates the interaction of bismuth with peanut proteins. Raman spectra also suggest that the interaction of the three peanut proteins with bismuth is due to the presence of tyrosine, tryptophan and phenylalanine in the side chain of the protein molecules. This observation is also in agreement with individual interactions of BiIII with the three aromatic amino acids, tyrosine, tryptophan and phenylalanine28.

Conclusion

Peanut proteins were subjected to interaction with different heavy metal ions and the solutions were analyzed using different spectral techniques. Absorption, fluorescence, circular dichroism and Raman spectra indicate the preferential interaction of peanut proteins with bismuth amongst the heavy metals mercury, thallium, lead, bismuth and antimony. The results reveal the stoichiometric ratio of peanut protein-BiIII complex is 1 : 1 for all the three cases. The greater value of binding constant and free energy change supports the highest interaction of conarachin I with bismuth. The Raman spectra also indicate the presence of three amino acids tyrosine, tryptophan and phenylalanine in the side chain of the three peanut proteins. The modification in the secondary structure of conarachin II, conarachin I and arachin obtained from the circular dichroism spectra are due to the interaction of bismuth with three amino acids tyrosine, tryptophan and phenylalanine within the protein molecules. The results strongly support a selective sensing of BiIII amongst a series of heavy metals at their trace concentrations.

Acknowledgement


References


An efficient pyrimidine-based colorimetric chemosensor for naked-eye recognition of copper in aqueous medium

Anamika Dhara\textsuperscript{a}, Nikhil Guchhait\textsuperscript{b,*} and Subash Chandra Bhattacharya\textsuperscript{a,*}

\textsuperscript{a}Department of Chemistry, Jadavpur University, Kolkata-700 032, India
\textsuperscript{b}Department of Chemistry, University of Calcutta, 92, Acharya Prafulla Chandra Road, Kolkata-700 009, India

E-mail: nguchhait@yahoo.com

Manuscript received 19 April 2017, accepted 08 May 2017

Abstract: A pyrimidine-based receptor 1-(9\textit{H}-fluoren-9-ylidene)-2-(4,6-dimethylpyrimidin-2-yl)hydrazine (HPF) was designed for naked-eye detection of Cu\textsuperscript{2+} with high selectivity in the presence of other competitive metal ions. In aqueous solution, upon addition of Cu\textsuperscript{2+} to HPF a color change from yellow to wine-red due to complex formation. The detection limit (5.03×10\textsuperscript{-8} M) of HPF for Cu\textsuperscript{2+} ions is much lower than that recommended by WHO in drinking water (31.5 \textmu M). The sensor shows consistent performance in a pH value range of 4 to 11. Moreover, test strips based on receptor HPF were fabricated, which could act as a handy and efficient Cu\textsuperscript{2+} test for “in-the-field” measurement of Cu\textsuperscript{2+}.

Keywords: Colorimetric chemosensor, hydrazino pyrimidine, Cu\textsuperscript{2+} recognition, DFT study, test kits.

1. Introduction

The metal copper plays a significant role in the areas of biological, environmental, and chemical systems\textsuperscript{1}. It is the third most abundant metal after iron and zinc in the human body and it plays an important role in a number of biological processes, including iron absorption, haemopoiesis, various enzyme-catalyzed and redox reactions\textsuperscript{2\textendash}5. Thus, daily ingestion of copper is indispensable for our good health\textsuperscript{6}. However, copper is extremely toxic to some organisms such as many bacteria and viruses. Owing to its toxicity to bacteria, elevated concentrations of copper impeded self-purification capability of the sea or rivers and destroy the biological reprocessing systems in water. On the other hand, unregulated overloading of copper can induce severe neurological diseases, including Alzheimer’s, Parkinson’s, Menkes’, and Wilson’s diseases\textsuperscript{7}. The World Health Organization (WHO) has set the safe limit of copper in drinking water at 2 ppm (31.5 \textmu M)\textsuperscript{8}. Accordingly, facile techniques, enabling professionals to monitor the concentration of copper ions in environmental water samples and visualize its subcellular distribution in physiological processes, are of considerable significance for environment protection and human health.

For effortlessness, convenience and low cost, the fabrication of small molecular chemosensors into colorimetric test kits is highly challenging\textsuperscript{9}. Even though some colorimetric/fluorescent chemosensors have been developed for the detection of Cu\textsuperscript{2+} so far, the sensing methods for fast detection of Cu\textsuperscript{2+} in aqueous solution, especially using colorimetric sensors without resorting to instruments, are relatively rare. Current approaches for environmental and clinical samples rely on costly, time-consuming methods like atomic absorption/emission spectroscopy\textsuperscript{10} or inductively coupled plasma mass spectrometry\textsuperscript{11} which are not very convenient and handy for “in-field” detection. Moreover, in most cases the sensing experiments are carried out using metal solutions in water and organic solvent.
mixture\textsuperscript{12} and hence are of greater academic interest than practical. Therefore, to develop simple-to-use and naked-eye diagnostic tool for selective detection of copper in aqueous medium is a demanding and attractive topic. Keeping in mind, we have chosen to design a fluorenone-appended pyrimidine based scaffold HPF as a receptor. Because of chemosensors based on pyrimidine are being widely studied in recent years owing to their great variety of biological activity ranging from antimalarial, antibacterial, antitumoral, antiviral activities, etc.\textsuperscript{13,14} which have often been associated to their chelating capability with trace metal ions. The higher (\(\pi\)) acidity of pyrimidine and presence of more than one hetero atom in the same ring take part in an important role in its coordination chemistry\textsuperscript{15–17}. On the other hand, fluorenone family compounds are base materials for the production of dyes and optical brightening agents. Fluorenones have been used extensively as catalyst precursors for electro-catalytic oxidation\textsuperscript{18}, inhibition of DNA tumor viruses\textsuperscript{19}, light-emitting materials\textsuperscript{20}, etc., but have rarely been used as chemical sensors. Fluorenone has been investigated as an attractive element in organic solar cells and display devices. This has led us to design and synthesize a colorimetric sensor containing a fluorenone framework that is particularly suitable for environmental and biological applications.

In the current paper, we report the synthesis, characterization, and sensing properties of HPF as a selective colorimetric receptor for detection of Cu\textsuperscript{2+}. The receptor HPF displayed a highly selective and sensitive (detection limit 5.03 \times 10^{-8} \textit{M}) colorimetric recognition toward Cu\textsuperscript{2+} by a change in the color from yellow to wine red in an aqueous solution with the formation ligand metal complex. Moreover, HPF can also be used as a practical, visible colorimetric detection kit for Cu\textsuperscript{2+}. So far our knowledge is concerned, this is one of the rare examples of the pyrimidine derivative employed for the detection of Cu\textsuperscript{2+}.

2. Experimental

2.1. Materials

9H-Fluoren-9-one was purchased from Aldrich. 2-Hydrazinyl-4,6-dimethyl pyrimidine was previously prepared in the laboratory\textsuperscript{21}. Other commercially available chemicals and solvents were used and purified by standard procedure. Perchlorate salts of some cations such as Na\textsuperscript{+}, K\textsuperscript{+}, Mg\textsuperscript{2+}, Li\textsuperscript{+}, Ag\textsuperscript{+}, Al\textsuperscript{3+}, Pb\textsuperscript{2+}, Cr\textsuperscript{3+}, Mn\textsuperscript{2+}, Fe\textsuperscript{2+}, Fe\textsuperscript{3+}, Co\textsuperscript{2+}, Ni\textsuperscript{2+}, Cu\textsuperscript{2+}, Zn\textsuperscript{2+}, Cd\textsuperscript{2+} and Hg\textsuperscript{2+} were purchased from Merck.

2.2. Apparatus

Elemental analyses (carbon, hydrogen and nitrogen) were performed with a Perkin-Elmer CHN analyzer 2400. Melting points were determined using a Buchi 530 melting apparatus. NMR spectra were recorded on a Bruker spectrometer at 500 (\textit{1H NMR}) MHz and 300 (\textit{13C NMR}) in DMSO-\textit{d}_6. Chemical shifts (\(\delta\) values) were reported in ppm down field from internal Me\textsubscript{4}Si. The electronic spectra were recorded in MeOH-water solution on a Hitachi model U-3501 spectrophotometer. IR spectra (KBr pellet, 400–4000 cm\textsuperscript{-1}) were recorded on a Perkin-Elmer model 883 infrared spectrophotometer.

2.3. Synthesis

2.3.1. Synthesis of reference compound REF

9H-Fluoren-9-one (200 mg, 1.109 mmol) was added to a methanol solution (5 mL) of 1-phenylhydrazine (0.109 mL, 1.109 mmol). The above mixture was refluxed at 80 °C for 2 h, then the resulting mixture was cooled to ambient temperature. Crude product was obtained and filtered off, then washed with methanol several times and dried under vacuum. The final compound REF was obtained as yellow crystalline solid (Scheme 1). Yield: 0.147 g, 48.5%; \textit{1H NMR} (300 MHz, DMSO-\textit{d}_6): 10.77 (1H, s), 8.24 (1H, d, \(J=7.65\) Hz), 7.94 (1H, d, \(J=7.45\) Hz), 7.87 (1H, d, \(J=7.40\) Hz), 7.81 (1H, d, \(J=7.45\) Hz), 7.45 (1H, d, \(J=0.75\) Hz, 6.8 Hz), 7.44–7.39 (2H, m), 7.37 (1H, d, \(J=0.80\) Hz), 6.82 (1H, s), 2.40 (6H, s); \textit{13C NMR} (300 MHz,
Dhara et al.: An efficient pyrimidine-based colorimetric chemosensor for naked-eye recognition etc.

DMSO-$d_6$ $\delta$ (ppm): 31.14, 114.58, 120.91, 121.58, 126.26, 128.47, 129.79, 138.23, 140.42, 145.75; Anal. Calcd. for C$_{19}$H$_{14}$N$_2$: C, 84.43; H, 5.23; N, 10.48%. Found: C, 84.42; H, 5.22; N, 10.36%.

2.3.2. Synthesis of receptor HPF

The compound 3 was synthesized following a literature method$^{21}$. To a solution of 9H-fluoren-9-one (1 g, 5.55 mmol) in 10 mL of methanol, then previously prepared 1-(4,6-dimethylpyrimidin-2-yl)hydrazine (0.77 g, 5.55 mmol) was added to the mixture and cooled in ice water. Then glacial acetic acid (5 drops) was added to the mixture drop by drop with continuous stirring. Finally the total reaction mixture was refluxed at 90 ºC for 12 h. The reaction mixture was cooled to room temperature. The yellow colored crystalline compound was separated out (Scheme 1). The solution was filtered and the compound was collected and dried in vacuum desiccators. Bright yellow solid of HPF was obtained. Yield: 0.72 g, 43.37%; $^1$H NMR (500 MHz, DMSO-$d_6$): 10.77 (1H, s), 8.24 (1H, d, $J$=7.65 Hz), 7.94 (1H, d, $J$=7.45 Hz), 7.87 (1H, d, $J$=7.40 Hz), 7.81 (1H, d, $J$=7.45 Hz), 7.45 (1H, t, $J$=0.75 Hz, 6.8 Hz), 7.44–7.39 (2H, m), 7.37 (1H, d, $J$=0.80 Hz), 6.82 (1H, s), 2.40 (6H, s); $^{13}$C NMR (300 MHz, DMSO-$d_6$) $\delta$ (ppm): 23.87, 31.06, 40.75, 113.75, 120.47, 120.84, 121.73, 127.17, 128.38, 129.62, 129.89, 130.69, 137.59, 139.09, 141.05, 146.03, 160.69, 168.10; IR (KBr): 1527, 1437, 1336, 1183, 1122, 1085, 1029, 953 cm$^{-1}$. Anal. Calcd. for C$_{19}$H$_{16}$N$_4$: C, 76.10; H, 5.42; N, 18.66%. Found: C, 75.98; H, 5.37; N, 18.65%.

2.4. General procedure for UV-Vis titration

Stock solution of the receptor, HPF was prepared in 10 mM HEPES buffer-CH$_3$OH (99 : 1, v/v) at room temperature in the concentration range of 10$^{-5}$ mol/L. 2 mL of the receptor (HPF) solution was taken in the cuvette. Stock solutions of guests in the concentration range of 10$^{-5}$ M, were prepared in the same solvents and were individually added in different amounts to the receptor solution. The same stock solution of receptor and guests were used to perform the UV-Vis at 25 ºC.

2.5. Job plot measurements

Copper perchlorate and HPF were dissolved in 10 mM HEPES buffer-CH$_3$OH (99 : 1, v/v) to make the concentrations 20 µM. Volumes of 5.0, 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5 and 0 mL of the solution...
of HPF were taken and transferred to vials. Volumes of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mL of the copper solutions were added to separate solutions of HPF. Each vial had a total volume of 5 mL. After shaking the vials for a few seconds, UV-Vis spectra were taken at room temperature.

2.6. Association/binding constant

The binding constant\textsuperscript{22} of the \([\text{Cu}^{2+} \cdot \text{HPF}]\) complex was determined using the Benesi-Hildebrand (B-H) plot.

\[
\frac{1}{(A - A_0)} = \frac{1}{K (A_{\text{max}} - A_0) C} + \frac{1}{(A_{\text{max}} - A_0)} \tag{1}
\]

\(A_0\) is the absorbance of HPF at absorbance maxima (\(\lambda = 428\) nm), \(A\) is the observed absorbance at that particular wavelength in the presence of a certain concentration of the metal ion (C), \(A_{\text{max}}\) is the maximum absorbance value that was obtained at \(\lambda = 428\) nm during titration with varying \([C]\), \(K\) is the association constant (M\(^{-1}\)) and was determined from the slope of the linear plot, and \([C]\) is the concentration of the Cu\(^{2+}\) ion added during titration studies. The goodness of the linear fit of the B-H plot of \(1/(A - A_0)\) vs \(1/([\text{Cu}^{2+}]\) for 1 : 1 complex formation confirms the binding stoichiometry between HPF and Cu\(^{2+}\).

2.7. Detection limit

The detection limit\textsuperscript{23} (DL) of HPF for Cu\(^{2+}\) was determined using the following equation:

\[
\text{DL} = K^* \frac{S_b}{S} \tag{2}
\]

where \(K^* = 2\) or 3 (we take 3 in this case), \(S_b\) is the standard deviation of the blank solution and \(S\) is the slope of the calibration curve.

2.8. Crystallographic measurements

Measurements were done on a Bruker SMART APEX II CCD area detector equipped with graphite monochromated Mo K\(_\alpha\) radiation (\(k = 0.71073\) Å) source in \(\omega\) scan mode at 296 K. The structure of the receptor HPF were solved using the SHELXS-97 package of programs and refined by the full-matrix least square technique based on \(F^2\) in SHELXL-97\textsuperscript{24}. All non-hydrogen atoms were refined anisotropically. Positions of hydrogen atoms attached to carbon atoms were fixed at their ideal position.

3. Results and discussion

The compound REF (reference compound) was synthesized by refluxing 1-phenylhydrazine and 9H-fluoren-9-one in methanol and characterized by \(^1\)H NMR, \(^{13}\)C NMR and CHN elemental analysis (Figs. S1-S2, in Supplementary material). The compound 3 was synthesized following a literature method and characterized by \(^1\)H NMR spectra, Mass data, and FT-IR spectra\textsuperscript{21}. We have synthesized pyrimidine-based receptor HPF (Scheme 1) by condensing hydrazino pyrimidine (3) and 9-fluorenone in methanol. The structure HPF was established by \(^1\)H NMR, \(^{13}\)C NMR, FT-IR, CHN elemental analysis and Mass spectrometry techniques (Figs. S3-S6 in Supplementary material) and also X-ray crystal structure analysis (Fig. 1). We obtained single crystal for receptor HPF by slow evaporation of DMF/ether (1 : 1 v/v) over a period of one week and crystallized in a monoclinic \(P2_1/c\) space group (Tables S1 and S2 in Supplementary material). The crystal structure of HPF (CCDC 1050465) is shown in Fig. 1. The C-N and N-N distances for HPF are in the range of 1.30–1.38 Å. Two adjacent HPF recep-
Dhara et al.: An efficient pyrimidine-based colorimetric chemosensor for naked-eye recognition etc.

3.1. Visual sensing of Cu$^{2+}$

Visual color change of receptor HPF and reference compound REF (20 μM) were investigated in an aqueous medium [10 mM HEPES buffer-CH$_3$OH (99 : 1, v/v) at room temperature]. Upon addition of Cu$^{2+}$ ion to HPF, a detectable naked-eye color change was observed from yellow to wine red (Fig. 3). Simultaneously, no naked-eye color change was observed upon addition of Cu$^{2+}$ to the REF (inset of Fig. 7). Complexation does not occur between REF and Cu$^{2+}$ due to the absence of pyrimidine ‘N’ in REF. Hence it can be said that pyrimidine ‘N’ is responsible for complexation. Other ions did not exhibit any detectable color change.

The sensing abilities of HPF (20 μM) toward various cations (Na$^+$, K$^+$, Li$^+$, Mg$^{2+}$, Ag$^+$, Al$^{3+}$, Pb$^{2+}$, Cr$^{3+}$, Mn$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Cd$^{2+}$ and Hg$^{2+}$) were investigated by UV-Vis spectroscopy in an aqueous medium [10 mM HEPES buffer-CH$_3$OH (99 : 1, v/v) at room temperature]. Upon the addition of 1.2 equiv. of each cation, only Cu$^{2+}$ induced distinct spectral change is observed, while the aforementioned other metal ions did not show colour change or slight change in the absorption spectra relative to the free receptor HPF (Fig. 4). For comparison, their optical densities at 428 nm upon addition of the above mentioned cations to HPF solution are charted in Fig. 5.

The absorption spectrum of receptor HPF (20 μM) exhibits strong absorption bands at 360 nm, which is attributed $\pi\rightarrow\pi^*$ transition of fluorenone unit of HPF. Upon concomitant addition of Cu$^{2+}$ to receptor HPF, a new red shifted absorption band centered at 428 nm appears with increasing intensity which is due to com-
plexation of pyrimidine of HPF with Cu$^{II}$ (Fig. 6). Meanwhile, the original absorption band centered at 360 nm decreases gradually, generating an isosbestic points at 389 nm, which indicates the formation of a new complex between HPF and Cu$^{2+}$. As shown in the inset of Fig. 6, a nearly linear dependence of the absorbance at 428 nm band as a function of Cu$^{2+}$ concentration from 0 to 1.2 equiv. was observed.

Again, the absorption behaviour of REF (20 μM) exhibits initially two absorption bands at 388 and 244 nm. The compound REF neither observed any UV-Vis absorption spectral change nor naked-eye detection of color upon addition of Cu$^{2+}$ (Fig. 7). From above two experiments we have concluded the indis-
Dhara et al.: An efficient pyrimidine-based colorimetric chemosensor for naked-eye recognition etc.

Pensable roles of the pyrimidine ‘N’ within receptor HPF and Cu\(^{2+}\) for complexation and naked-eye colour change.

We have studied the better selectivity of HPF as a colorimetric receptor for the recognition of Cu\(^{2+}\) in the presence of various competing metal ions. For competition studies, receptor HPF (20 \(\mu\)M) was treated with 1.2 equiv. of Cu\(^{2+}\) in the presence of 1.2 equiv. of other metal ions, as indicated in Fig. 8. There was no interference in the detection of Cu\(^{2+}\) from various competitive cations. Thus, HPF could be used as a selective colorimetric sensor for Cu\(^{2+}\) in the presence of most of the competing metal ions.

For further determination the stoichiometry between HPF and Cu\(^{2+}\), Job’s plot\(^{25}\) analyses were also used. When molar fraction of Cu\(^{2+}\) was 0.5, the absorbance at 428 nm reaches to maximum (Fig. 9), demonstrating the formation of 1 : 1 complex between HPF and Cu\(^{2+}\). For further information on the coordination mode of the HPF-Cu\(^{2+}\) complex, we performed \(^1\)H NMR titration of HPF with Cu\(^{2+}\), but it was not doing well owing to the paramagnetic nature of Cu\(^{2+}\) ions. Based on the Job’s plot study, we propose the structure of the HPF-Cu\(^{2+}\) complex as shown in Scheme 2.

Based on UV-Vis titration, the association constant\(^{22}\) (\(K\)) of HPF with Cu\(^{2+}\) ion was calculated using the Benesi-Hildebrand equation (Fig. S7 in Supplementary material). The \(K\) value turned out to be 4.69\(\times\)10\(^3\) M\(^{-1}\). The detection limit\(^{23}\) of receptor HPF for the analysis of Cu\(^{2+}\) ions was calculated to be 5.03\(\times\)10\(^{-8}\) M (Fig. S8 in Supplementary material). The detection limit of HPF for Cu\(^{2+}\) was much lower than that recommended by the US EPA in drinking water (1–4 ppm). Therefore, HPF could be a good indicator for the detection of copper ions in the drinking water.

We investigated the effect of pH on the absorption response of receptor HPF to the Cu\(^{2+}\) ion in same media with pH values ranging from 2 to 12 (Fig. 10). The color of the Cu\(^{2+}\)-HPF complex remained in the
wine red region between pH 4 and 11, while its color changed to the original yellow at pH 2, 3 and 12. These results indicate that Cu^{2+} could be clearly detected by the naked-eye or UV-Vis absorption measurements using HPF over the wide pH range of 4.0–11.0. The color change of the Cu^{2+}-HPF complex from wine red to yellow at very low pH (2 and 3) and high pH (>11) might be due to demetallation of the complex, thus regenerating the receptor HPF with its yellow color.

To ensure the practical application of receptor HPF, test strips were ready by immersing filter papers into a methanol solution of HPF (20 μM) and then drying in air. These test strips were applied for sensing different Cu^{2+} concentrations (0, 10 μM, 30 μM), exhibiting colorimetric changes differentiable by bare eyes (Fig. 11). Development of such test strips is helpful as instantaneous qualitative information without resorting to the instrumental analysis.

To better understand the nature of the coordination of Cu^{2+} with HPF, energy-optimized structures of HPF and HPF-Cu^{2+} (Fig. 12a) were obtained on Density Functional Theory (DFT) calculations at the B3LYP level using 6-311G** basis set for simple receptor (HPF) and LANL2DZ basis set for metal complex using the Gaussian 09 program. The spatial distributions and orbital energies of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of HPF and the corresponding Cu^{2+} complex were also generated using this calculations (Fig. 12b). The HOMO is spread over the whole molecule, whereas LUMO is distributed on the -C=N bond and pyrimidine N in HPF, and the imine N and pyrimidine N are favorably coordinated with Cu^{2+}. In HPF-Cu^{2+} complex, the π electrons of HOMO are mainly located on the C=N and the LUMO is mostly spread over the pyrimidine ring. The energy gaps between the HOMO and LUMO of the probe and corresponding Cu^{2+} complex were found to be 2.094 eV and 0.184 eV, respectively (Table S3 in Supplementary material). This calculated results exhibit the red shifting absorption band compared to the free ligand which corroborates with the observed absorption spectral band position. Calculation also predicts better stabilized of HPF-Cu^{2+} complex than the bare HPF ligand.

4. Conclusions

In summary, we have developed a pyrimidine based sensor HPF, which could detect Cu^{2+} in aqueous solution with specific selectivity and high sensitivity in a very short time. Our method is simple, rapid, cost-effective and could find potential application as a 'na-
Dhara et al.: An efficient pyrimidine-based colorimetric chemosensor for naked-eye recognition etc.

In particular, there was no interference response for detection of Cu$^{2+}$ in the presence of competing metal ions. The detection limit of Cu$^{2+}$ was found to be $5.03 \times 10^{-8} \text{ M}$. On the other hand, the decrease in calculated HOMO-LUMO band gap energy evidences red shifted absorption band of the complex than the bare molecule which corroborates well with experimental findings and calculation also predicts stabilization of complex of receptor HPF with Cu$^{2+}$. Moreover, test strips based on sensor HPF were fabricated, which could serve as a practical colorimetric sensor for “in-field” measurement of Cu$^{2+}$ and does not require any labor-intensive synthetic protocols. We anticipate that this report of a colorimetric pyrimidine-based sensor for Cu$^{2+}$ ion could prompt the development of more sensitive and cost-effective methods for Cu$^{2+}$ ion detection from the scientific community.

**Supporting Information**

Supporting Information is available in the Website: indianchemicalsociety.com.

**Acknowledgement**

AD acknowledges the financial support provided by University Grants Commission, India (Award Letter No. F.4-2/2006(BSR)/CH/14-15/0163, dated May, 2015) through the Dr. D. S. Kothari Post Doctoral Fellowship (DSKPDF).
References


A thienyl-pyridine-based Hantzsch ester fluorescent probe for the selective detection of nitric oxide and its bio-imaging applications

Syed Samim Ali, Ajoy Kumar Pramanik, Sandip Kumar Samanta, Uday Narayan Guria and Ajit Kumar Mahapatra

Department of Chemistry, Indian Institute of Engineering Science and Technology, Shibpur, Howrah-711 103, West Bengal, India

E-mail: akmahapatra@chem.iiests.ac.in

Abstract: A new Hantzsch ester fluorescent probe (TPC) containing thienyl-pyridine group appended to the dihydropyridine ring was synthesized and characterized and successfully applied in the fluorescent sensing nitric oxide (NO) in aqueous solution. The fluorescence of probe TPC shows extremely strong blue fluorescent, while its fluorescence was greatly switched off upon the addition of NO solution and showed high selectivity and sensitivity to NO. The limit of the detection was calculated to be ~0.4 μM. A reaction mechanism for dihydropyridine with NO is proposed in this study. The probe shows good stability over a broad pH range (pH > 4). The structure of the TPC probe has been established by single-crystal XRD. DFT and TDDFT calculations were done to demonstrate the electronic properties of the probe and the corresponding aromatic product. Moreover, the utility of the TPC probe in detecting NO in live cells has also been demonstrated using Raw 264.7 cells as monitored by fluorescence imaging.

Keywords: Nitric oxide, single crystal X-ray, DFT, live cell imaging.

Introduction

Nitric oxide (NO) is one of the smallest gaseous molecules which exists in the atmosphere. It is a highly reactive and multifunctional free radical, and was primarily accepted as an air pollutants known as nitrogen oxides (NOx)\(^1\). NO is produced from the reaction of \(\text{N}_2\) and \(\text{O}_2\) during high-temperature combustion processes in car engines, power plants or industrial processes and naturally by lightning during a thunderstorm. High NO\(_x\) levels in the atmosphere can cause serious diseases such as carcinogenesis and asthma\(^2\). NO is also a ubiquitous bioactive signaling molecule\(^3\). Recently, researchers have discovered that NO also serves as an indicator for many biological processes in the human immune system, nervous system, epithelium system and its pivotal role in cardiovascular system\(^4\). Several analytical methods for NO detection have been developed such as electrophoresis, electron paramagnetic resonance (EPR) or GC-mass spectroscopies, chemiluminescence and electrochemical methods\(^5\). In comparison to these protocols, colorimetric or fluorescence-based techniques present a large number of advantages, such as simple detection in situ or at site without any sample pre-treatment or use of low-cost, widely available equipment. Thus, the construction of small fluorescent sensors suitable for specific NO detection in living systems has received great attention. A number of fluorescent probes have been reported to date\(^6\). The most common approach for NO detection involves the use of \(o\)-diamino aromatics under aerobic conditions. These molecules react with \(\text{N}_2\text{O}_3\) (formed from NO and \(\text{O}_2\)), to yield fluorescent triazole derivatives\(^7\). Another family of probes, in this case reacting directly with NO, is based on transition-metal complexes\(^8\). An increasing number of other strategies have been described in the literature\(^9\).

A widely adopted approach involves a two-component “fluorophore-modulator” strategy for designing reaction-based fluorogenic NO probes. The reaction between \(o\)-phenylenediaminophenyl and NO is the most
well known and widely used modulating mechanism of fluorogenic NO probes. It is believed that an aromatic vicinal diamine can efficiently quench a fluorophore subunit through the photoinduced electron transfer (PET) mechanism. The reaction of an aromatic vicinal diamine with NO in the presence of oxygen leads to the formation of triazole that blocks the nonradiative PET relaxation pathway and restores fluorescence emission of the fluorophore. However, this developed strategy still has its limitations as follows: (i) Self-oxidation-sensitive electron-abundant o-phenylenediamine groups makes it easily react with other reactive oxygen/nitrogen species, lighting up its fluorescence and interfering with the sensing process of NO; (ii) oxidized species rather than NO itself are demanded to react with the vicinal aromatic diamines. To solve those problems, our group recently reported a highly selective fluorogenic probe for NO sensing based on the 4-substituted Hantzsch 1,4-dihydropyridines (DHP). According to the former work, DHP is not only able to quantitatively react with NO to give the corresponding pyridine, but also exhibits a good selectivity for NO against other interfering reactive oxidative species (ROS), a reaction that is independent of oxygen.

Taking the above information into consideration, we report herein the rational design and experimental validation of a highly selective fluorogenic probe for NO sensing based on 6-(2-thienyl)-2-pyridinecarboxaldehyde (1) as shown in Scheme 1. The structure of TPC was confirmed by 1H NMR and LCMS spectra. The molecular structure of TPC has been further established on the basis of single-crystal XRD data. Subsequently, we examined their reactivity toward NO through UV-Vis and fluorescence spectra in HEPES buffers (15 mM, pH 7.4, containing 40% DMSO) at 25 ºC. Isoamyl nitrite was used as the source of NO, which is commercially available with a half life of 30 min.

**Scheme 1. Reagents and conditions** : *Ethylacetoacetate, NH3 (I), reflux for 8 h.

**Crystal structure of TPC**

The fine and X-ray quality single crystal of pale-yellow colored TPC was grown by slow evaporation of the solvent from a solution in MeOH-CH3CN (1 : 1) at room temperature with ambient condition over a week. The probe crystallizes as monoclinic system with space group P21 and CCDC entry no. 1547182. A summary of the crystallographic data and structure refinement parameters of the probe TPC are given in Experimental section. The X-ray bond lengths and angles well reproduced with the standard parameters.

The unit cell structure of TPC exhibits a regular twinned aggregation of the same species joined together in some definite mutual orientation. Twinning may occur when a unit cell has higher symmetry than implied by the space group of the crystal structure. The molecular structure constitutes of two moieties i.e. thienyl-pyridine moiety and Hantzsch ester moiety. Now, two planes between the two thienyl-pyridine moieties of the twin are almost parallel to each other having angle of 177.11º and distance of 7.457 Å. Similarly, planes between the two Hantzsch ester moieties of the twin are almost parallel having angle of 177.58º and distance of 2.551 Å.

The crystal structure of TPC is stabilized by intermolecular hydrogen bonding which forms a 1-dimen-
Fig. 1. ORTEP structure of TPC, showing 35% thermal ellipsoids probability level for non-H atoms with atom numbering scheme.

Fig. 2. The crystal packing diagram of TPC shows 1-D supramolecular network involving face-to-face H···bonding viewed along the b-axis (H atoms not involved are omitted in for clarity).

Spectroscopic properties and response to TPC

The absorption spectra of free probe TPC and the colorimetric sensing ability with NO in HEPES buffer (15 mM, pH 7.4, containing 40% DMSO) was monitored at 25 °C and exhibited different bands from 200 to 400 nm. These spectra are typical of an aromatic compound. Fig. 3 shows that the UV-Vis absorption spectra of a mixture of probe TPC with different concentrations of NO in aqueous-DMSO solution. When increasing the concentration of NO, a new absorption band at ~338 nm was gradually enhanced, while the intensity of other two bands at ~265 and ~305 nm were decreased correspondingly. The color of the so-

Colour
aromatic and the aromatic species. The spectra here are very similar to those of the probe we reported previously. On the account of the quantitatively NO-induced conversion of Hantzsch ester into the pyridine form, the change of the fluorescence herein inspired us to utilize the probe TPC as effective NO fluorescent probe.

To check the fluorescence response of probe TPC to NO, the fluorescence spectra of TPC and its titration experiments upon the addition of various equivalents of NO in HEPES buffer (50 mM, pH 7.4, containing 40% DMSO) was monitored at 25 ºC. The probe TPC exhibits strong fluorescence emission at ~ 439 nm owing to the inhibition of photoelectron transfer (PET) process from the thienyl-pyridine aromatic part to dihydropyridine moiety due to geometrical constrained (orthogonal arrangement), when excited at ~ 338 nm in HEPES buffer. Titration of probe TPC with NO results in the decrease of the fluorescence intensity as a function of the added NO concentration (Fig. 4) and the intensity saturates at ≥ 3 equiv.

The switch off fluorescence emission is expected to the photoelectron transfer (PET) upon the aromatization of the Hantzsch ester, which caused the attain-
Samim Ali et al.: A thienyl-pyridine-based Hantzsch ester fluorescent probe for the selective detection of NO.

In addition, a linear relationship between the fluorescent intensity and the concentrations of NO was constructed. Meanwhile, the calculated lowest limit detection (LOD, 3σ/slope) was determined to be about 0.40 μM, which is similar with the values in literatures\textsuperscript{13}, suggesting that probe TPC is a highly sensitive agent for detecting NO.

In order to check whether probe TPC is sensitive to only NO or even to the other reactive oxygen species (ROS), fluorescence titrations were carried out in the same medium with different ROS, viz. NO, HNO, OH\textsuperscript*-, NO\textsubscript{3}–, H\textsubscript{2}O\textsubscript{2}, NO\textsubscript{2}–, ClO– and ONOO– no significant fluorescence change was found in the presence of these ROS (Fig. 5a).

Moreover, the interfering experiment was conducted by adding 10 equiv. of the above mentioned reactive species to the solutions of TPC (Fig. 5b) in the presence of 3.0 equiv. of NO. Noticing that the added interfering species were largely excessive, it demonstrated that TPC can serve as a highly selective fluorescence probe for NO.

Under UV light, the solution of probe TPC is intense blue fluorescent, whereas in the presence of NO

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{scheme2.png}
\caption{Proposed reaction mechanism between TPC and NO.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig5.png}
\caption{(a) Fluorescence spectral changes of TPC (4×10^{-5}) in the presence of 10 equiv. of different analytes. (b) Competitive selectivity of TPC (4×10^{-5}) toward NO (3 equiv.) in the presence of other analytes (10 equiv.).}
\end{figure}
non-fluorescent was observed (Fig. 6) and no such fluorescent color change was observed in case of the other ROS. Thus, NO can easily be differentiated by its fluorescent color change from the other ROS.

Next, we studied the influence of pH on the fluorescence of probe TPC during NO titration. The titrations carried out at different pH of the medium exhibited no variation in the fluorescence intensity in the pH range 2.0–10.0. These measured results proved that probe TPC was a potential fluorometric candidate for monitoring NO in the environment and living cells.

Considering the importance of the responsive time to the analyte for a reactive probe, the time dependent fluorescence spectra were investigated after addition of 3.0 equiv. of NO to the solution of probe TPC (Fig. 7). After addition of NO into the solution of probe TPC, the fluorescence emission decreased rapidly, and it became stable in 30 min.

**Computational studies**

Here NO transformed a nonaromatic species dihydropyridine (TPC) derivative to its corresponding aromatic pyridine (TPC-Ar) moiety, which makes a dramatic electronic and structural changes occurs in this chemical transformation.

To gain insights into the nature of this fluorescence switching-off mechanism based on NO-induced aromatization of the Hantzsch ester, computational stud-
ties based on density functional theory (DFT) and time-dependent DFT (TDDFT) were performed at the B3LYP/6-31G(d,p) level of the Guassian 09 program.

Results from calculation revealed that the \( E_{\text{LUMO}} \) level of the dihydropyridine Hantzsch ester (Fig. 9) was about \(-1.899\) eV, higher than that of the \( E_{\text{LUMO}} \) level \((-2.483\) eV) of the thienyl pyridine moiety. Upon NO-induced aromatization of the Hantzsch ester, the \( E_{\text{LUMO}} \) level \((-3.17\) eV) of the pyridine was below that of the \( E_{\text{LUMO}} \) level \((-2.483\) eV) of the thienyl-pyridine moiety, which drive PET process and therefore turn off fluorescence of the probe.

The TDDFT calculation revealed the absorption bands in the region of \( \lambda_{\text{max}} \), 250–450 nm. The vertical transitions were found to have a good agreement with the experimentally found data.

TDDFT calculations showed absorption band at \( \sim 357 \) and \( \sim 339 \) nm belonging to the HOMO\(\rightarrow\)LUMO (84.31\%) \((f = 0.1302)\) and HOMO\(\rightarrow\)LUMO+5 (74.41\%) \((f = 0.034)\) energy states respectively for probe TPC.

**Bioimaging in living cells**

We then assessed the potential utility of TPC for the specific imaging of NO in living cells. For this purpose, the cytotoxicity of probe TPC toward Raw 264.7 cells were evaluated by using a standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

The results in Fig. 10e showed that compound TPC displayed low cytotoxicity in the adoptive concentration ranges, indicating the high feasibility in biologi-
cal measurement. Probe TPC was then applied for detecting endogenously generated NO by incubating Raw 264.7 cells in the presence or absence of bacterial lipopolysaccharide (LPS)\textsuperscript{14}. Raw 264.7 cells were incubated in PBS buffer (pH 7.4) containing 15 \(\mu\text{M}\) of the probe TPC for 20 min at 37 \(^\circ\text{C}\), followed by washing the cells with the same buffer to remove the excess of the probes. At this stage, the fluorescence microscopy image of Raw 264.7 cells displayed intense blue fluorescence. However, upon the addition of isoamyl nitrite (source of NO) into the cells via incubation with LPS solution for 20 min at 37 \(^\circ\text{C}\), the cells exhibited non-fluorescent (Fig. 10d). These results demonstrate that the probe TPC is capable of detecting NO in living cells.

**Conclusion**

We have synthesized and characterized thienyl-pyridine-based Hantzsch ester, TPC fluorescent probe for detecting nitric oxide (NO) in HEPES buffer medium. The selectivity and sensitivity was demonstrated on the basis of fluorescence, absorption, LC-mass spectrometry, and visual fluorescent color changes. Fluorescence titrations showed the probe is sensitively and linear-dependent to the NO concentration and can be detect up to \(-0.4 \mu\text{M}\). The probe displayed a high specificity for NO when compared with other N-, O-sensitive species. NO-induced aromatization of the Hantzsch ester is responsible for the fluorescence change mechanism in the sensing process. The structure of TPC has been established by single-crystal XRD. Theoretical calculations were performed to understand the photo-physical behaviors for probe TPC and its aromatized compound, and the obtained results also supported the sensing mechanism. The probe TPC demonstrated good photophysical stability in the pH range of 4.0–9.0 and low cytotoxicity. The probe was successfully applied in the field of cell imaging for detecting NO in the cell. Strong fluorescent images were observed when Raw cells were incubated with the probe TPC alone. However, non-fluorescence was observed in Raw cells in the presence of NO. Further work to improve the sensitivity and put into practical applications is in progress.

**Experimental**

**Chemicals, method and measurements** : UV grade dimethyl sulfoxide (DMSO) was purchased from spectrochem. Isoamyl nitrite was purchased from Sigma-Aldrich Pvt. Ltd. (India). Other chemicals were obtained from commercial suppliers and were used without further purification. Mass spectra was carried out using a Waters QTOF Micro YA 263 mass spectrometer. \(^1\)H spectra was recorded on a Brucker 400 MHz instrument. For NMR spectra, DMSO-\(d_6\) and CDCl\(_3\) were used as solvent using TMS as an internal standard. Chemical shifts are expressed in \(\delta\) ppm units and 1H–1H and 1H–C coupling constants in Hz. UV spectra were recorded on a JASCO V-530 spectrophotometer. Fluorescence spectra were recorded on a Perkin-Elmer Model LS 55 spectrophotometer.

**Cell culture procedure** : Raw 264.7 cell lines were prepared from continuous culture in Dulbecco’s modified Eagle’s medium (DMEM, Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum (Invitrogen), penicillin (100 \(\mu\text{g/mL}\)), and streptomycin (100 \(\mu\text{g/mL}\)). The Raw 264.7 were acquired from the American Type Culture Collection (Rockville, MD) and maintained in DMEM containing 10% (v/v) fetal bovine serum and antibiotics in a CO\(_2\) incubator. At first cells were initially cultivated in 75 cm\(^2\) polystyrene, filter-capped tissue culture flask in an atmosphere of 5% CO\(_2\) and 95% air at 37 \(^\circ\text{C}\) in CO\(_2\) incubator. When the cells almost reached the logarithmic phase, the cell density was adjusted to 1.0 \times 10\(^5\) per/well in culture media. Then the cells were used to inoculate in a glass bottom dish, with 1.0 mL (1.0 \times 10\(^4\) cells) of cell suspension in each dish. Culture medium was removed just after cell adhesion. The cell layer was rinsed thrice with phosphate buffered saline (PBS), and then used according to the experimental need.

**Confocal imaging procedure** : For confocal imaging studies Raw 264.7 cell, 1 \times 10\(^4\) cells in 1000 \(\mu\text{L}\) of medium, were seeded on sterile 35 mm covered glass bottom, Petrisids, culture dish (ibidi GmbH, Germany), and incubated at 37 \(^\circ\text{C}\) in a CO\(_2\) incubator for 10 h. Then cells were washed with 500 \(\mu\text{L}\) DMEM and then incubation with (8 \(\mu\text{M}\)) isoamyl nitrite dissolved in 500 \(\mu\text{L}\) DMEM at 37 \(^\circ\text{C}\) for 1 h in a CO\(_2\) incubator and analyzed under an Olympus IX81 microscope.
equipped with a FV1000 confocal system using 1003 oil immersion Plan Apo (N.A. 1.45) objectives. All images obtained through section scanning were analyzed by Olympus Fluoview (version 3.1a; Tokyo, Japan) with excitation at 338 nm monochromatic laser beam, and emission spectra were integrated over the range 400–650 nm (single channel). The cells were again washed thrice with phosphate buffered saline PBS (pH 7.4) to remove any free isoamyl nitrite and incubated in PBS containing probe TPC to a final concentrations of 4.0×10⁻⁶ M, incubated for 10 min followed by washing with PBS three times to remove excess probe outside the cells and images were captured. The confocal microscope settings for all images, such as scan speed and transmission density were held constant for comparing the relative intensity of intracellular fluorescence.

**General computational method** : Geometries have been optimized using the B3LYP/6-31G (d,p) level of theory. The geometries are verified as proper minima by frequency calculations. Time-dependent density functional theory (TDDFT) calculation has also been performed at the same level of theory. All calculations have been carried out using Gaussian 09 program.

**Determination of the detection limit** : The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectra of TPC were measured, and the standard deviation of blank measurements was acquired. To obtain information about the slope, the fluorescence intensity at I₀/I was plotted as a concentration of NO. Then, the detection limit was calculated using the following equation : Detection limit = 3σ/k. In which σ is the standard deviation of 10 blank measurements, k is the slope between the fluorescence intensity versus NO concentration.

**General procedure for preparation of solution for fluorescence titration** : A stock solution of the probe TPC (4×10⁻⁵ M) was prepared in DMSO/H₂O (4 : 6 v/v). All experiments were carried out in DMSO/H₂O solution (4 : 6 v/v, 15 mM HEPES buffer, pH = 7.4). Titration experiment was carried out by adding (4×10⁻⁵ M) solution TPC in a quartz optical cell of 1 cm optical path length, and the analyte stock solutions (4×10⁻⁴ M) were added into the quartz optical cell slowly by using a micropipet.

**Synthesis of TPC** : 6-(2-Thienyl)-2-pyridinecarboxaldehyde (1) (0.3 g, 1.58 mmol), ethyl acetooacetate (1.1 g, 9.2 mmol) and ammonia solution (0.2 g, 9.2 mmol) were dissolved in 10 ml ethanol and refluxed for 10 h. After cooling to room temperature, the reaction mixture was concentrated in vacuo to get crude product which was purified by column chromatography using ethyl acetate in hexane (1 : 9) and finally get pure compound TPC. Yellow solid, (260 mg, 44%), m.p. 190–195 ºC, MS (LCMS) : (m/z, %) : 413.2 [(TPC +H⁺), 100 %]; Calculated for C₁₂H₂₄N₂O₄S : 412.15.

**X-Ray crystal structure determination**

The X-ray quality single crystal of TPC was grown by slow diffusion in MeOH-CH₂CN (1 : 1) under ambient condition for a week. Block shaped pale-yellow crystal of dimension 0.18×0.14×0.09 mm. Unit cell parameters were determined from least-squares method with the data in the range of −9 < h < 9, −22 ≤ k ≤ 22, −23 ≤ l ≤ 23 and angle varies 1.197 < θ < 28.85º. Other unit cell parameters are a = 7.3479(6), b = 16.3371(13), c = 17.2146(12) (Å) and α = γ = 90 (°), β = 98.693(4). Out of total reflections 68151 unique data 6125 (I > 2σ(I)) were used for structure determination.
Acknowledgement

The authors would like to acknowledge DAE-BRNS [Project No. 36(1)/14/12/2016-BRNS/36012] for financial support. SSA and AKP thanks to the UGC-MANF and SERB-NPDF, New Delhi for the financial support.

References

Toward dithia-aza based malachite green probe : Colorimetric chemosensor for Hg$^{2+}$ ions

Samaresh Ghosh* and Rajkumar Manna

Department of Chemistry, Bankura Sammilani College, Kenduadihi, Bankura-722 102, West Bengal, India

E-mail : gsamaresh@yahoo.com

Manuscript received 07 June 2017, accepted 12 June 2017

Abstract : A new probe 1 based on a malachite green derivative bearing two dithia-aza subunits was prepared, and studied as colorimetric chemosensor for the sensitive detection of Hg$^{2+}$ ions over other representative metal ions.

Keywords : Hg-sensor, dithia-aza, malachite green, naked-eye.

Introduction

In recent years, there has been growing interest in the development of chemosensors for the sensitive detection of mercuric (Hg$^{2+}$) ion because of its toxicity causing serious problems for public health and ecology$^{1-5}$. Although a number of molecular probes for the detection of Hg$^{2+}$ ions have been developed, colorimetric chemosensors for the selective detection of Hg$^{2+}$ are quite attractive because of their excellent sensitivity, rapid response, and ability to do the so-called “naked-eye” detection in a straightforward and inexpensive manner$^{6-14}$. Thus, developing simple and practical colorimetric chemosensors for Hg$^{2+}$ ions is still a challenge. However, dithia-aza binding motif is currently used$^{15,16}$ in the design of chemosensors owing to its ability to coordinate specific metal ions through N and S-donors. Furthermore, the malachite green entity is one of the most attractive chromogenic units due to its well characterized UV-Vis absorption and easy synthesis. To continue our interest in chemosensor development for transition and heavy metal ion monitoring$^{17}$, we report herein a simple malachite green (MG) derivative 1 as colorimetric chemosensor bearing dithia-aza chelating units for the rapid detection of Hg$^{2+}$ ions in aqueous media. Malachite green (MG) 2 was taken as reference compound. However, to the best of our knowledge, there is no report on the design of MG-derivatives for “naked-eye” detection of Hg$^{2+}$ ions.

Results and discussion

N-Phenyl-5,11-dithia-8-azapentadecane 1a was synthesized according to the Scheme 1. 1a was then condensed with benzaldehyde to give 1 in good yield. Titled molecule 1 was characterized by FT-IR, UV-Vis, NMR, MS, and elemental analyses. The UV-Vis absorption spectrum of 1 in THF-water (4 : 1, v/v) solution ([I] = ~4.9×10$^{-5}$ M) exhibited three absorption bands at 307, 435 and 625 nm (weak) ($\varepsilon$ = 1.01×10$^4$, 6.42×10$^2$, and 1.42×10$^3$ M$^{-1}$ cm$^{-1}$, respectively) typical of MG subunit. Absorption titra-
tion experiments were carried out by using the set of representative metal ions, such as Ni2+, Cu2+, Zn2+ (all as sulphates), Pb2+, Mg2+, Mn2+ (all as perchlorates) and Hg2+ (as nitrate) to evaluate the metal ion binding selectivity and sensitivity of 1.

Upon interaction with this set of metal ions, only Hg2+ induced a progressive and sizable increase in absorption band at 626 nm. Fig. 1 gives detailed UV-Vis spectral changes of 1 upon titration with Hg2+ ions. Remarkable feature pertaining to this molecular probe is the change of color from light yellow to greenish blue (Fig. 2) which can be used for a “naked-eye” detection of Hg2+ ions in aqueous solution.

No significant color/absorption changes were observed upon interaction with other potentially competing metal ions mentioned (Figs. 2 and 3) demonstrating its unique selectivity and sensitivity toward Hg2+

**Scheme 1.** (i) p-TsCl, pyridine, 0–5 °C (80%); (ii) (a) Thiourea/EtOH, reflux, (b) NaHCO3/H2O, 80–90 °C; (iii) (a) dry THF/NaH, reflux, (b) n-BuBr, rt, 65%; (vi) (a) PhCHO, ZnCl2 (anh.), THF (dry), reflux, (b) PbO2, conc. HCl, water, rt, 70%.

**Fig. 1.** UV-Vis spectral changes of 1 (4.9×10⁻⁵ M) upon the incremental addition of 14 equivalents of Hg2+ ion in THF-water (4 : 1, v/v).

**Fig. 2.** Color changes of 1 (4.9×10⁻⁵ M) upon the addition of tested metal ions in THF-water (4 : 1, v/v).

**Fig. 3.** Plot of absorption intensity at 625 nm as a function of metal ion concentration.

The stoichiometry of the complex (1.Hg2+) in the interaction process was determined from the titration curve (Fig. 4). The break of the titration curve at Hg2+/[1] = 2 indicates the formation of 1 : 2 complexes (sensor 1 : Hg2+ ion).
The signaling mechanism might be attributed to the Hg$^{2+}$ chelation induced perturbation of the π-clouds of non-planar propeller-like twisted structure (A) toward the corresponding more conjugated quinoid-like cationic forms (B and C) as illustrated in Scheme 2. Both nitrogen and sulfur atoms of dithia-aza subunit participate in Hg$^{2+}$-ion binding cooperatively.

In order to look into the thiophilic speciality in the chelation induced absorption effect in 1, UV-Vis titration experiments of well known reference compound 2 lacking S-donors were carried out. We noticed that adding Hg$^{2+}$ to the solution of 2 induced no change in color/absorption features. This observation revealed that the presence of two proximal S-donors is a prerequisite for promotion of M$^{2+}$-N interaction induced signaling mechanism.

The complexation induced signaling phenomenon of 1 with, for instance, Hg$^{2+}$ ions was further evidenced by LCMS and $^1$H NMR measurements. The $^1$H NMR spectrum of 1 in the presence of Hg(NO$_3$)$_2$ was broadened to some extent and resulted in downfield shifting of the MG-protons, especially in the dithia-aza moiety. These shifts suggest that dithia-aza moieties are involved in the coordination with Hg$^{2+}$ ions. LCMS measurement provided additional evidence for the formation of 1 : 2 stoichiometry of the complex 1-Hg$^{2+}$.

**Conclusion**

In summary, dithia-aza based malachite green chemosensor 1 has been synthesized for the first time. A distinct color change was observed when 1 was treated with Hg$^{2+}$ ions. This is presumably attributed to the perturbation of the π-system upon complexation of Hg$^{2+}$ ions at the binding domains. Such impressive color change as well as the high reproducibility and the large persistence (days) of the coloration of probe 1 permit a “naked-eye” detection of Hg$^{2+}$ ions in aqueous media. Work in this direction is currently under progress in our laboratory.

**Experimental**

Synthesis of 1a: A solution of aniline (0.5 g, 5.38 mmol), 2-chloroethanol (4.3 g, 53.76 mmol), and calcium carbonate (2.5 g, 25.0 mmol) in 50 ml water was refluxed for 18 h, and the filtrate was extracted with dichloromethane. The organic phase was dried over anhyd. Na$_2$SO$_4$ and filtered. The solvent was removed in vacuo to get the crude product, which was purified by column chromatography on silica gel eluating with petroleum ether gradually increasing to 1 : 1 petroleum ether/EtOAc to obtain pure product of N-phenylated diethanolamine (70%). Conversion of N-phenylated diethanolamine to the corresponding dithiol was achieved by following the reported procedure$^{15}$. 

![Graph](image_url)  
**Fig. 4.** Plot of absorption change of 1 at 625 nm as a function of [Hg$^{2+}$]/[1].

![Scheme](image_url)  
**Scheme 2.** Suggested co-ordination induced sensing mechanism of 1 for Hg$^{2+}$ ions.
Under nitrogen atmosphere, to a solution of the corresponding dithiol (0.154 g, 0.723 mmol) in dry THF was added NaH (0.0696 g, 2.90 mmol) and refluxed for 2 h. Then n-butylbromide (0.397 g, 2.90 mmol) was added to the reaction mixture and stirred overnight at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The residue was redissolved in EtOAc, washed with brine solution, dried over anhyd. Na$_2$SO$_4$ and filtered. The solvent was removed in vacuo to get the crude product, which was purified by column chromatography (silica gel 60–120, petroleum ether/EtOAc 1 : 50) to obtain pure product 1 (65%). FT-IR (KBr) $\nu_{\text{max}}$: 2957, 2929, 2862, 1591, 1505, 1458, 1343, 1277, 1181, 1143, 743, 686 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 7.23 (2H, t, $J=7$Hz), 6.70 (1H, t, $J=7$Hz), 6.66 (2H, d, $J=8$Hz), 3.52 (4H, t, $J=7.5$Hz), 2.71 (4H, t, $J=7.5$Hz), 2.57 (4H, t, $J=7.5$Hz), 1.62–1.56 (4H, m), 1.46–1.38 (4H, m), 0.92 (6H, t, $J=7.5$Hz). Anal. Calcd. for C$_{18}$H$_{31}$NS$_2$: C, 66.40; H, 9.60; N, 4.28. Found : C, 66.38; H, 9.59; N, 4.28.

Synthesis of 1: Compound 1 (0.02 g, 0.061 mmol) was dissolved in minimum volume of dry THF. Benzaldehyde (0.0016 g, 0.0153 mmol) was then added into the solution of 1 and the resulting solution was refluxed with anhyd. ZnCl$_2$ for 24 h. After solvent evaporation, the residue was treated with water, PbO$_2$ and few drops of conc. HCl. The reaction mixture was stirred at room temperature overnight. Then the mixture was treated with water and extracted with CH$_2$Cl$_2$. The organic phase was dried over anhyd. Na$_2$SO$_4$ and filtered. The crude product obtained after removal of solvent was purified by preparative thin layer chromatography using 6% EtOAc/petroleum ether to yield 1 (70%) as a green semisolid. FT-IR (KBr) $\nu_{\text{max}}$: 2930, 2859, 1726, 1596, 1510, 1453, 1348, 1281, 1167, 1119, 1024, 891, 814, 691, 614 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 7.28 (4H, t, $J=10$Hz, ArH), 7.12–7.06 (5H, m, ArH), 6.96–6.90 (1H, t, $J=7$Hz), 6.70 (1H, t, $J=7$Hz), 5.54 (2H, d, $J=10$Hz, ArH), 3.94–3.91 (4H, m), 3.55–3.51 (4H, m), 3.07–3.05 (4H, m), 2.90–2.80 (4H, m), 2.73–2.71 (6H, m), 2.62–2.60 (2H, m), 1.73–1.70 (4H, m), 1.42–1.40 (4H, m), 1.29–1.21 (8H, m), 0.94–0.86 (12H, m), 13C (CDCl$_3$, 125 MHz) $\delta$: 141.5, 130.3, 129.7, 129.5, 129.3, 128.9, 127.8, 120.4, 116.6, 116.0, 110.4, 81.0, 70.0, 52.3, 51.8, 51.5, 44.7, 41.1, 32.1, 29.7, 26.7, 14.1, 13.7. Anal. Calcd. for C$_{43}$H$_{65}$N$_2$S$_4$Cl: C, 66.75; H, 8.47; N, 3.60; m/z (ES$^+$) 772.4 (M$^+$), 737.7 (M–Cl$^+$).

Acknowledgement

Funding from Department of Science and Technology (SR/FTP/CS-88/2007), Government of India, is gratefully acknowledged.

References

Fluorescent sensing of $\text{H}_2\text{PO}_4^-$ by *in situ* prepared Cu$^{2+}$ complex in aqueous medium via displacement approach

Soma Mukherjee*, Palash Mal and Shrabani Talukder

Department of Environmental Science, University of Kalyani, Kalyani-741 235, Nadia, West Bengal, India

*E-mail :* somam580@gmail.com, sommukh445@yahoo.co.in

Abstract : The *in situ* prepared Cu$^{2+}$ chelate of luminescent ligand, pyridine-2-carboxaldehyde-benzoic acid hydrazone (1) was investigated for fluorescent sensing of $\text{H}_2\text{PO}_4^-$ in aqueous medium via restoration of the fluorescence intensity of the receptor through Cu$^{2+}$ displacement approach. The sensing behavior of this ligand towards Cu$^{2+}$ via fluorescence quenching has already been reported in our previous work. The present study depicts *in situ* prepared Cu$^{2+}$ chelate as a turn-on fluorescent chemosensor of $\text{H}_2\text{PO}_4^-$ detecting in the micro molar range. The Cu$^{2+}$ chelate possesses excellent tolerance to other interfering anions Cl$^-$, Br$^-$, I$^-$, OAc$^-$, NO$_3^-$, SO$_4^{2-}$.

**Keywords :** Luminescent, hydrazone, fluorescent chemosensor, anion recognition, $\text{H}_2\text{PO}_4^-$.  

Introduction

The development of anion selective sensors has received considerable attention in recent years as anions play a potential role in several environmental processes. Especially, chemosensors based on the anion induced luminescence changes are attractive due to the simplicity, selectivity and high sensitivity$^{1-3}$. Among the significant anions, phosphate and its derivatives act as essential ingredients in biological systems and are responsible for different biological and environmental functions$^{4-12}$. Therefore, a number of phosphate recognition methods have been investigated$^{13-18}$. At physiological pH phosphate exists as both $\text{H}_2\text{PO}_4^-$ and $\text{HPO}_4^{2-}$ and the selective detection of dihydrogen phosphate ($\text{H}_2\text{PO}_4^-$) gets more attraction to the researchers in phosphate recognition. Due to the very high free energy of hydration ($\Delta G^0 = -465$ mol$^{-1}$), it is difficult to design a chemosensor for $\text{H}_2\text{PO}_4^-$ sensing in water$^{20}$. The receptors having the ability of detection of $\text{H}_2\text{PO}_4^-$ in an aqueous medium at neutral pH is highly desirable and of special significance due to their potential applications in biological sciences$^{21,22}$. A number of chemosensors are developed that rely on hydrogen bonding via the amide$^{23}$, urea and thiourea subunits$^{24}$. However, a serious disadvantage of such receptors is that they are not able to sense anions in aqueous solvents because the aqueous medium strongly competes for hydrogen bonding sites$^{25}$. To overcome this problem, sensory systems were developed in which an electrostatic affinity for transition metal ions facilitates a strong interaction$^{26}$. In the context of metal complexes as anion sensors, the paradigm has shifted toward the metal ion displacement approach where, a metal ion quenches the fluorescent intensity of the receptor and the treatment of such a metal complex with an anion displaces the metal ion from the coordination sphere of the original organic receptor, restoring the fluorescence intensity of the receptor$^{27-29}$. However, a lot of works have already been reported and most of the receptors are developed using tedious synthetic protocols and sometimes shows poor compatibility in water$^{30-36}$.

Earlier the selective response of the receptor 1 (pyridine-2-carboxaldehyde-benzoic acid hydrazone) towards Cu$^{2+}$ was investigated and reported$^{37}$. In continuation to the previous work herein we have explored the sensing behavior of the *in situ* prepared Cu$^{2+}$ chelate, I-Cu$^{2+}$, towards $\text{H}_2\text{PO}_4^-$ by turn-on mechanism via Cu$^{2+}$ displacement approach.
Table 1. Some reported receptors used for $\text{H}_2\text{PO}_4^-$ sensing via metal displacement approach

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Structure</th>
<th>Metal displaced</th>
<th>Quantum yield</th>
<th>Limit of detection of receptor-metal chelate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><img src="image1" alt="Structure" /></td>
<td>Zn$^{2+}$</td>
<td>-</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>2.</td>
<td><img src="image2" alt="Structure" /></td>
<td>Zn$^{2+}$</td>
<td>-</td>
<td>-</td>
<td>31</td>
</tr>
<tr>
<td>3.</td>
<td><img src="image3" alt="Structure" /></td>
<td>Cu$^{2+}$</td>
<td>-</td>
<td>1.41 $\mu$M</td>
<td>32</td>
</tr>
<tr>
<td>4.</td>
<td><img src="image4" alt="Structure" /></td>
<td>Zn$^{2+}$</td>
<td>$2.0 \times 10^{-3}$</td>
<td>-</td>
<td>33</td>
</tr>
</tbody>
</table>
**Experimental**

**Reagents :**  
All starting materials and solvents were purchased from Sigma Aldrich and Merck chemical company and used without further purification unless otherwise stated.

**Physical measurements :**  
Spectroscopic studies were carried out with a Hitachi-F7000 Luminescence Spectrometer at ambient temperature in aqueous medium (CH$_3$CN/H$_2$O; 1 : 4, v/v) at fixed concentration of 1 ($1.0 \times 10^{-5}$ M). Titration experiments were carried out in 10-mm quartz cuvettes at 25 °C. Copper(II) salt and anions as tetrabutylammonium salts (1 μM; except for NO$_3^-$ and SO$_4^{2-}$) in aqueous medium (CH$_3$CN/H$_2$O; 1 : 4, v/v) were added to the host solution and used for the titration experiment. 1-Cu$^{2+}$ solution for H$_2$PO$_4^-$ detection was prepared by addition of 1.0 equiv. of Cu$^{2+}$ to compound 1 solution in aqueous medium (CH$_3$CN/H$_2$O; 1 : 4, v/v). Mass spectra were recorded using Xevo G2-SQT.

**Synthesis and characterization of receptors :**  
The receptor (1) was synthesized following the reported method$^{37}$. The characterization data and the single crystal X-ray structure of the receptor 1 were already reported in our previous work$^{37}$.

**Results and discussion**

**Spectral studies :**  
The binding stoichiometry of the receptor 1 with Cu$^{2+}$ ion was found to be 1 : 1 as reported in our

---

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Reagent</th>
<th>Stoichiometry</th>
<th>Concentration</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.</td>
<td></td>
<td>Fe$^{3+}$</td>
<td>0.89</td>
<td>$1.0 \times 10^{-9}$ mol/L</td>
<td>34</td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td>Cu$^{2+}$</td>
<td>–</td>
<td>–</td>
<td>35</td>
</tr>
<tr>
<td>7.</td>
<td></td>
<td>Fe$^{3+}$</td>
<td>–</td>
<td>$1.69 \times 10^{-8}$ mol/L</td>
<td>36</td>
</tr>
<tr>
<td>8.</td>
<td></td>
<td>Cu$^{2+}$</td>
<td>0.0167</td>
<td>$2.53 \times 10^{-6}$ M</td>
<td>37</td>
</tr>
</tbody>
</table>

*and the present work*
The positive ion mass spectrum of 1 upon addition of Cu$^{2+}$ indicated that a peak at $m/z = 401.10$ which was assignable to $[1 + Cu^{2+} + Br^- + H_2O]$ (Calcd. 401.65). Thus, the 1 : 1 stoichiometry of the 1-Cu$^{2+}$ chelate was also supported by the ESI-mass spectrometry analysis.

The sensing of 1 towards Cu$^{2+}$ was reported in our earlier study. The 1-Cu$^{2+}$ chelate exhibits strong absorption band in the visible region ($\lambda_{max} = 435$ nm) in aqueous medium. The emission peak of 1 ($\lambda_{em} = 405$ nm), undergoes quenching upon complexation with Cu$^{2+}$. In the present work, the in situ prepared Cu$^{2+}$ chelate was investigated as a turn-on fluorescent chemosensor for H$_2$PO$_4^-$ ions in aqueous medium. The successive addition of H$_2$PO$_4^-$ (0–100 $\mu$M) to the in situ prepared solution of 1-Cu$^{2+}$ (10 $\mu$M) resulted in a step-wise enhancement of emission intensity at 405 nm and gradually restored to the original emission state of 1. The proposed binding mode of receptor 1 involving Cu$^{2+}$ and H$_2$PO$_4^-$ ions is illustrated in Scheme 1. Fluorescence-ON receptor 1 selectively binds to Cu$^{2+}$ to form a Fluorescence-OFF 1-Cu$^{2+}$ chelate. This is accompanied by the loss of a proton as revealed from mass spectroscopic studies. The 1-Cu$^{2+}$ chelate selectively interacts with H$_2$PO$_4^-$ thereby resulted in the restoration of the Fluorescence-ON emission spectra of receptor 1.

The detection limit of the 1-Cu$^{2+}$ chelate as a fluorescent sensor for the analysis of H$_2$PO$_4^-$ was determined from a plot of emission intensity as a function of concentration of the added anion. Based on the emission titration curve it was found that the fluorescence enhancement factor $(I - I_0)/I_0$ was proportional to the concentration of H$_2$PO$_4^-$ ions (Fig. 2). The linear range of fluorescence response of 1-Cu$^{2+}$ for H$_2$PO$_4^-$ ions was 0–10 $\mu$M ($R^2 = 0.9818$) and detection limit for H$_2$PO$_4^-$ ions was calculated as $2.53 \times 10^{-6}$ M following the reported method.

The response of 1-Cu$^{2+}$ (10 $\mu$M) with selected anions (H$_2$PO$_4^-$, Cl$^-$, Br$^-$, I$^-$, OAc$^-$, NO$_3^-$, SO$_4^{2-}$) was examined through spectrofluorimetric study in aqueous medium (CH$_3$CN/H$_2$O; 1:4, v/v) at 298 K. The intensity of 1-Cu$^{2+}$ solution containing other competitive anions (Cl$^-$, Br$^-$, I$^-$, OAc$^-$, NO$_3^-$, SO$_4^{2-}$) and H$_2$PO$_4^-$ ions is shown in Fig. 3, indicating negligible interference of other anions for selective sensing of H$_2$PO$_4^-$ via Cu$^{2+}$ displacement approach.

A plot of $II_0$ (where, $I$ and $I_0$ represent the fluorescence intensity at the given anion concentration and in absence of anion, respectively) for selected anions is shown in Fig. 4. The selectivity of 1-Cu$^{2+}$ for H$_2$PO$_4^-$ was also confirmed from Job’s method of continuous variation at the emission maximum ($\lambda_{em} = 405$ nm).

The detection limit of 1-Cu$^{2+}$ for H$_2$PO$_4^-$ was determined from the emission titration curve and was found to be $2.53 \times 10^{-6}$ M.

**Scheme 1.** Proposed mechanism of fluorescence sensing of H$_2$PO$_4^-$ by 1-Cu$^{2+}$.
Mukherjee et al. : Fluorescent sensing of $\text{H}_2\text{PO}_4^-$ by *in situ* prepared $\text{Cu}^{2+}$ complex in aqueous *etc.*

Fig. 2. (a) Plot of the fluorescence enhancement factor $(I - I_0)/I_0$ at 405 nm versus the concentration of $\text{H}_2\text{PO}_4^-$ ions added. (b) Representation of linear range.

Fig. 3. (a) Changes in the fluorescence spectra ($\lambda_{\text{ex}} = 290$ nm) of 1-$\text{Cu}^{2+}$ (10 $\mu$M) upon addition of tetrabutylammonium salts of anions (100 $\mu$M) in aqueous medium (CH$_3$CN/H$_2$O; 1 : 4, v/v). (b) Changes in the emission intensity of 1-$\text{Cu}^{2+}$ (10 $\mu$M) on addition of $\text{H}_2\text{PO}_4^-$ (100 $\mu$M) over selected anions (100 $\mu$M) in aqueous medium (CH$_3$CN/H$_2$O; 1 : 4, v/v) at 405 nm at 298 K. The blue bars represent the emission intensity of 1-$\text{Cu}^{2+}$ in the presence of selected anions; the red bars represent the emission intensity of the above solution on addition of $\text{H}_2\text{PO}_4^-$. 

Fig. 4. Emission intensity ratios $(I/I_0)$ for 1-$\text{Cu}^{2+}$ (10 $\mu$M) at 405 nm in the presence of selected anions in aqueous medium (CH$_3$CN/H$_2$O; 1 : 4, v/v) at 298 K.

Fluorescence intensity of various metal ions and that of free metal respectively) at 405 nm (Fig. 4), shows a ratio =1 for most of the metal ions with the notable enhancement in emission intensity (> 8-fold) for $\text{H}_2\text{PO}_4^-$ ions.

**Conclusion**

The *in situ* prepared 1-$\text{Cu}^{2+}$ chelate was investigated as a fluorescent sensor for $\text{H}_2\text{PO}_4^-$ by the $\text{Cu}^{2+}$ displacement approach in aqueous medium. No significant interference was observed in presence of selected anions. The sensor 1-$\text{Cu}^{2+}$ was highly sensitive and selective for $\text{H}_2\text{PO}_4^-$ with detection limit in the micro molar range.
Acknowledgement

Financial supports received from DST-PURSE and DST-FIST, New Delhi, India, are gratefully acknowledged for financial support and instrumental facilities. We are also grateful to the University of Kalyani for providing infrastructural facilities.

References

A novel compartmental Schiff base ligand as a fluorescence chemosensor: Microscopic to macroscopic detection of Zn$^{II}$ acetate

Bhaskar Biswas

Department of Chemistry, Raghunathpur College, Purulia-723 133, West Bengal, India

E-mail: mr.bbiswas@rediffmail.com, icbbiswas@gmail.com

Abstract: A novel compartmental Schiff base ligand, \((L = N,N'-\text{bis(salicyaldehyde)-1,3-diaminopropan-2-ol)}\) serves as a fluorescence chemosensor for the detection of zinc(II) acetate salt. The fluorescent probe \((L)\) undergoes a remarkable fluorescence enhancement upon binding to zinc acetate in pure acetonitrile \((\text{MeCN})\). Interestingly, addition of other 3d-metal acetate salts to the probe, exhibit usual fluorescence quenching phenomenon in MeCN solution. The fluorescent probe \((L)\) can selectively detect Zn$^{II}$ acetate salt in a concentration range of \(1.0 \times 10^{-6}\) to \(1.0 \times 10^{-4}\) mol/L. Further, trinuclear Zn$^{II}$-Schiff base complex has been characterized by single crystal X-ray diffraction study supports the selectivity and binding ability of \(L\) with Zn$^{2+}$ ion.

Keywords: Molecular recognition, zinc(II), Schiff base, X-ray structure, fluorescence probe.

Introduction

The creation of organic molecular species having the ability to sense analytes selectively, especially cations, are of biologically and environmentally important and have been emerged as a significant goal in the field of chemical sensors in recent years\(^1\)–\(^3\). Chemical fluoro-sensors which are based on ion-induced fluorescence changes have created huge interests to chemists in terms of sensitivity, selectivity, response time, simplicity, high degree of specificity and low detection limit\(^1\)–\(^4\). Fluorescent sensors remain an indispensable tool for visualizing or monitoring metal ions in real-time and real-space at a molecular level without any special instrumentation, and are applicable in many fields such as medical diagnostics, environmental control, living cells, and electronics\(^1\)–\(^4\),\(^7\). Zinc is a very important bio-essential element and plays significant roles in the living system effecting, DNA synthesis, microtubule polymerization, gene expression, apoptosis, immune system function, and the activity of different metallo-enzymes such as carbonic anhydrase and matrix metalloproteinase\(^8\). Moreover, zinc ion is also a contributory factor in neurological disorders such as epilepsy and Alzheimer’s diseases\(^9\).

Over the past few decades, many fluorescent chemosensors of different class for Zn$^{2+}$ have been developed, using quinoline\(^10\), anthracene\(^11\) and coumarin\(^12\) as fluorophores. However, some of them require complicated syntheses and are insoluble in aqueous medium. Further, various fluorescence-based zinc probes have been recently developed – some of these are fluorescent adducts of Zn-chelating peptides\(^13\)–\(^15\) and proteins\(^16\), while others are dye adducts of Zn-chelating macrocyclic compounds\(^17\)–\(^19\). Although some of these fluorescent probes can be utilized to detect zinc ions in environmental or biological samples, they have serious disadvantages like insufficient selectivity or sensitivity. Schiff bases (SBs) have the potential to form stable complexes of varied nuclearities with transition metal ions and act as ion carriers. The feature of SBs gives geometric and cavity control of host-guest complexation and modulation of its lipophilicity, and produces remarkable selectivity, sensitivity and stability for a specific ion. Thus, in this present report a novel
compartmental Schiff base ligand, (L = N,N’-bis(salicyaldehydene)-1,3-diaminopropan-2-ol), Scheme 1, is used as an ionophore with N and O donor atoms for construction of a ZnII chemosensor. Further, a trinuclear ZnII-Schiff base complex is characterized in the form of single crystals in MeCN medium with an external addition of little amount of DMF, and this phenomenon supports and consolidates the selectivity and efficient binding ability of L with Zn2+ ion in a 2 : 3 binding manner.

Results and discussion

Syntheses and formulation of the Schiff base (L)
The Schiff base ligand, L, N,N’-bis(salicyaldehydene)-1,3-diaminopropan-2-ol was synthesized by condensing 1,3-diaminopropan-2-ol with 2-salissaldehyde in 1 : 2 molar ratio in dry ethanol following a reported procedure20. The formulation was confirmed by elemental analysis, IR, UV-Vis, 1H NMR, and ESI-MS spectral analysis. The schematic presentation of synthesis is given below:

photophysical and solution properties of L

The photophysical study and the structural integrity in solution state for the Schiff base ligand (L) has been examined by proton NMR, UV-Vis spectroscopy, fluorescence study and ESI mass spectral studies. The ligand is soluble in common organic solvents like methanol, acetonitrile, dichloromethane etc. The 1H NMR spectral data for this Schiff base compound is provided in Experimental section and accounts in favour of the formulation of L. The UV-Vis spectrum for L shows high intensity transitions in the range of 200 to 500 nm (Fig. 1). The Schiff base solution shows the characteristic absorption bands at 316, 363 and 414 nm in acetonitrile solvent (10–4 M; Fig. 1). The highly intense band at 316 nm is assigned to π-π* transition of the C=N chromophore, whereas the band with low intensity at 363 nm is due to n-π* transition and a broad band ~414 nm is appeared for intraligand charge transfer21 (Fig. 1). The fluorescence spectral measurement for receptor, L in the absence of any divalent metal ions was carried out in acetonitrile using 10–4 M at room temperature and the spectrum is also given in Fig. 1. The ligand, excited at 363 nm, have shown emission band around 424 nm. The lower fluorescence intensity of L may be attributed for losing of co-planarity.

In order to probe the solution stability of L, ESI-MS spectrum in acetonitrile solution at room temperature is taken. It consolidates the solution stability of L which exhibits the molecular ion peak as base peak at m/z 299.17 in acetonitrile medium. ESI-MS (MeCN) m/z 299.17 [L+H+] (Calcd. 299.13).

Fluorogenic ZnII-acetate recognition

To establish the practical applications, the fluorescence response behavior of the ligand was examined upon addition of 3d divalent metal acetate in ace-tonitrile medium. Fig. 2 shows the fluorescence intensity of L in the presence of different metal acetates. Only Zn-salts resulted in a pronounced fluorescence enhancement, whereas other transition metal salts including Cu2+, Ni2+, Co2+, and Mn2+ did not change fluorescence (Fig. 3) even at concentrations 10-fold higher than the corresponding Zn2+ ion concentration as there is a probability of an electron and energy transfer be-
Biswa: A novel compartmental Schiff base ligand as a fluorescence chemosensor etc.

Fig. 1. Left: UV-Vis spectrum of $\text{L}$ in MeCN; Right: Fluorescence spectrum of $\text{L}$ in MeCN ($\lambda_{ex} = 363$ nm).

Fig. 2. Fluorescence titration of $\text{L}$ ($1 \times 10^{-6} \text{ M}$) in MeCN medium upon addition of different divalent late 3d acetate solution of $1 \times 10^{-6} \text{ M}$ concentration ($\Lambda_{ex} = 363$ nm). Inset: Visual fluorescence changes of sensor $\text{L}$ in the presence of zinc acetate. The photo was taken under a hand held UV (365 nm) lamp.

Fig. 3. 3D bar diagram of fluorescence intensity of $\text{L}$ ($1 \times 10^{-6} \text{ M}$) in MeCN with different 3d acetate solution.

between the metal ion and ligands or quenching of fluorescence intensity of a ligand by transition metal ions during complexation is a rather common phenomenon which is explained by processes such as magnetic perturbation, redox-activity, electronic energy transfer, etc. When the experiment was carried out with ubiquitous intracellular metal ions such as $\text{K}^+$, $\text{Na}^+$ and $\text{Ca}^{2+}$, which exist at very high concentrations inside the cell, no significant fluorescence was observed, even at concentrations that were 100-fold higher than $\text{Zn}^{2+}$ ion concentration. However, treatment of various zinc(II) salts like chloride, nitrate, sulphate, bisulphate, phosphate with $\text{L}$ exhibit similar kind of enhancement of fluorescence intensity. Enhancement of fluorescence through complexation is,
however, of much interest as it opens up the window for photochemical applications of the metal complexes\textsuperscript{24,25}. Factors like a simple binding of ligand to the d\textsuperscript{10} metal ions\textsuperscript{25}, an increased rigidity in structure of the complexes\textsuperscript{26}, a restriction in the photoinduced electron transfer (PET)\textsuperscript{24,27}, etc. are assigned to the increase in the photoluminescence. For this case, the enhancement of fluorescence intensity may be accounted by considering the imposed rigidity through complexation and decrement of the non-radiative decay of the excited-state with increasing radiative decay.

The photophysical responses of the Schiff base ligands are modulated when the fluorophore and the guest binding units are covalently integrated, resulting in a possible excited-state electron transfer phenomenon between the two. This can be termed as a Zn\textsuperscript{2+} ion selective chelation enhanced fluorescence. The experiment shows that there is a significant increase in fluorescence intensity for L upon addition of zinc(II) acetate salt. The emission peak intensity of L at 424 nm upon successive addition of zinc acetate concomitantly increases (Fig. 4) whether excited at 363 nm. Other salts of zinc(II) ion can enhance the fluorescence intensity of the L but the extent of enhancement is significantly small compared to the acetate salt. At least 2–3 fold more enhancement of fluorescence intensity is observed for the acetate salt compared to other zinc(II) salts. Thus, it is concluded that the Zn\textsuperscript{2+} ion selectively in a concentration range from 10\textsuperscript{-6} (M) to 10\textsuperscript{-4} (M) in acetonitrile medium using L as a fluorescence chemosensor.

**Isolation of zinc-L complex in macroscopic scale**

In order to evaluate the coordination nature of L to a Zn\textsuperscript{2+} ion, Zn\textsuperscript{2+}.L complex is isolated and crystallized. Simple mixing of zinc acetate and L, instantly produced crystalline colourless compounds. But successful isolation of single crystals, external addition of little amount of DMF is very much essential. Different ratios of zinc acetate and L are used (10\textsuperscript{-2} M each) to isolate monomeric or dimeric or trimeric or polymeric Zn-L complex, but obtained beautiful colourless crystals of rectangular shape when 3 : 2 Zn-acetate and L molar proportion is used. In this synthesis, acetate ions have contributed as bridging units to bind adjacent zinc ions together. The coordination geometry of trinuclear zinc(II) complex, [Zn\textsubscript{3}(L\textsubscript{2})(OAc)\textsubscript{2}]\textsubscript{2}DMF was determined by mainly single crystal X-ray diffraction study along with different spectroscopic and analytical techniques.

**Description of crystal structure**

The X-ray structural determination of [Zn\textsubscript{3}(L\textsubscript{2})(OAc)\textsubscript{2}]-2DMF reveals a trinuclear neutral zinc(II) complex with the space group P\textbar. The compound has been found to have a trinuclear molecule with a central Zn\textsuperscript{II} ion lying on a center of inversion. An ORTEP view\textsuperscript{28} of µ-acetato-µ-phenoxy-trizinc(II) complex of [Zn\textsubscript{3}(L\textsubscript{2})(OAc)\textsubscript{2}]-2DMF with an atom labelling scheme is shown in Fig. 5. The crystallographic parameter for the compound is given in Table 1. The trinuclear complex is built up of two mononuclear Zn-L moieties linked through bridging acetate and µ\textsuperscript{2}-phenolato groups to the central Zn atom. The coordination geometry around the terminal Zn centers (Zn1 and Zn1\textsuperscript{1}) may be regarded as square pyramidal geom-

---

**Fig. 4.** Fluorescence titration of L (1×10\textsuperscript{-6} M) in MeCN medium upon successive addition of Zn-acetate solution of 1×10\textsuperscript{-6} M concentration (Excitation wavelength, 316 nm or 363 nm).
metrical where the equatorial plane of a terminal Zn1/Zn1i atom is formed by phenoxy-bridged oxygen (O2/O2i), phenoxy oxygen (O1/O1i), imine nitrogen (N3/N3i), imine nitrogen (N4/N4i) and the axial positions are occupied by acetato bridged oxygen (O3/O3i). However, the coordination geometry of the central zinc ion (Zn2) may also be best described as distorted octahedral geometry, formed by six oxygen atoms from the same Schiff base ligands and bridging acetate ions which coordinate the terminal zinc ions. Thus four phenoxyoxygen (O1/O1i, O2/O2i) from two Schiff base ligands and two acetate ions bridge the two terminal zinc ions (Zn1 and Zn1i) and the central zinc ion (Zn2). The four equatorial positions for terminal zinc ions (Zn1 and Zn1i) are occupied by two phenoxy oxygen atoms (O2/O2i, O1/O1i) and two imine nitrogen atoms (N3/N3i, N4/N4i) where as the central zinc ion is surrounded by four phenoxy-bridged oxygen atom (O1, O1i, O2, O2i) from the two Schiff base ligands. Axial positions for all three zinc ions are coordinated by acetate bridged oxygen atom (O3, O4, O4i, O3i). The three zinc ions are in a perfect linear arrangement (∠Zn1-Zn2-Zn1i = 180.0º). The distances between the central zinc ion (Zn2) and the two terminal zinc ions (Zn1/Zn1i) are 3.061(1) Å.

### Conclusion
Herein, I report a novel compartmental Schiff base ligand as a fluorescence chemosensor for the detection of zinc(II) acetate salt. The fluorescent probe (L) undergoes enhancement of fluorescence emission intensity upon binding to zinc acetate or any zincII salts in MeCN while addition of other 3d-metal acetate salts to the probe, exhibit usual fluorescence quenching phenomenon in MeCN solution. The fluorescent probe (L) can selectively detect ZnII acetate salt in a concentration range of 1.0×10−6 to 1.0×10−4 mol/L. Further, isolation of trinuclear zinc-Schiff base complex, [Zn3(L)2(OAc)2]·2DMF in the form of single crystals.
in MeCN medium with an external addition of DMF supports and consolidates the selectivity and binding ability of L with Zn$^{2+}$ ion in 2 : 3 ratio. Thus this compartmental Schiff base, L can effectively and selectively recognize Zn$^{2+}$ ion in a mixture of other bivalent 3d ions and behaves an efficient fluorescence chemosensor from micro to macro scale of concentration.

**Experimental**

*Materials and methods:*

High purity salisaldehyde (E. Merck, India), 1,3-diaminopropan-2-ol (Lancaster, UK), methylene blue (Aldrich, UK), zinc(II) acetate dihydrate (E. Merck, India), copper(II) acetate dihydrate (E. Merck, India), nickel(II) acetate (E. Merck, India), cobalt(II) acetate dihydrate (E. Merck, India), manganese(II) acetate dihydrate (E. Merck, India) and iron(II) acetate dihydrate (Sigma-Aldrich, USA) were purchased from respective concerns and used as received. All the other reagents and solvents are of analytical grade (A.R. grade) and were purchased from commercial sources and used as received.

*Physical measurements:*

Infrared spectrum (KBr) was recorded with a FTIR-8400S Shimadzu spectrophotometer in the range 400–3600 cm$^{-1}$. $^1$H NMR spectrum in DMSO-$d_6$ was obtained on a Bruker Avance 300 MHz spectrometer at 25 °C and was recorded at 299.948 MHz. Chemical shifts are reported with reference to SiMe$_4$. Ground state absorption was measured with a JASCO V-730 UV-Vis spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer. Elemental analyses were performed on a Perkin-Elmer 2400 CHN microanalyser.

*Preparation of L and zinc(II) complex:*

Salisaldehyde (0.244 g, 2 mmol) was heated under reflux with 1,3-diaminopropan-2-ol (0.089 g, 1 mmol) in 30 ml dehydrated alcohol. After 10 h the reaction solution was evaporated under reduced pressure to yield a gummy mass, which was dried under vacuum and stored over CaCl$_2$ for subsequent use. Yield : 0.278 g (82.8%). Anal. Calcd. for C$_{17}$H$_{18}$N$_2$O$_3$ (L) : C, 68.48; H, 6.08; N, 9.39. Found : C, 68.40; H, 6.02; N, 9.35; $^1$H NMR (CDCl$_3$) $\delta$ : 3.68 (2H, dd, $J$=12.4, 6.8 Hz), 3.84 (2H, dd, $J$=12.4, 4.0 Hz), 4.23–4.25 (1H, m), 6.88 (2H, t, $J$=7.2 Hz), 6.96 (2H, d, $J$=8.4 Hz), 7.25 (2H, dd, $J$=7.6, 1.6 Hz), 7.32 (2H, td, $J$=8.8, 1.6 Hz), 8.36 (2H, s) ppm; $^{13}$C NMR $\delta$ : 62.9, 70.2, 117.0, 118.5, 118.6, 131.5, 132.5, 161.1, 167.3 ppm; IR (KBr, cm$^{-1}$) : 3412 ($\mu$OH), UV-Vis ($\lambda_{max}$, nm) : ~316, 363, 424 nm.

An acetonitrile solution of Zn(OAc)$_2$·2H$_2$O (0.657 g, 3 mmol) (10 ml) was added dropwise to a solution of L (0.596 g, 2 mmol) in the same solvent (15 ml). The yellow solution of the ligand immediately became colourless with quick precipitation of white crystalline compound. It was filtered and recrystallized from acetonitrile-dimethyl formamide (DMF) solvent mixture. The solvent mixture produced colourless crystals. Yield : 0.453 g (69% based on metal salt). Anal. Calcd. for C$_{38}$H$_{38}$N$_4$O$_{10}$Zn$_3$ (1) : C, 50.32; H, 4.22; N, 6.18. Found : C, 50.40; H, 4.27; N, 6.27%; IR (KBr, cm$^{-1}$) : 3436 ($\nu$OH), 1634, 1605 ($\mu$C=N), 1497, 1460, 1420 ($\mu$OAc), 1276 ($\nu$PhO) ; UV-Vis ($\lambda_{max}$, nm) : 266, 361.

*X-Ray diffraction study:*

Single crystal X-ray diffraction data were collected using a Rigaku XtaLABmini diffractometer equipped with Merury CCD detector. The data were collected with graphite monochromated Mo-K$\alpha$ radiation ($\lambda$ = 0.71073 Å) at 295(2) K using $\omega$ scans. The data were reduced using Crystal Clear suite and the space group determination was done using Olex2. The structure was resolved by direct method and refined by full-matrix least-squares procedures using the SHELXL-97 software package using Olex2 suite.$^{28,29}$

Zn$^{2+}$ sensing experiments:

The spectral analyses display an excellent chemosensory response both in absorption and fluorescence of
the Schiff base ligand upon addition of trace amounts of zinc acetate in acetonitrile solution. The selectivity of receptor for Zn$^{2+}$ ion over other 3d cations was studied in detail.

**Acknowledgement**

Financial support from the Science and Engineering Research Board (SB/FT/CS-088 dated 21/5/2014), New Delhi, India is gratefully acknowledged by BB. The author is greatly indebted to Dr. Tapas Majumdar, Department of Chemistry, University of Kalyani, Nadia, West Bengal, India for his kind co-operation throughout the fluorescence measurements. Thanks to Dr. Arabinda Mallick, Department of Chemistry, Kashipur Michael Madhusudan Mahavidyalaya, Purulia, West Bengal for valuable discussions. Furthermore, BB sincerely acknowledges Dr. Anshuman Roy Choudhury of IISER, Mohali for collecting X-ray diffraction data for the complex.

**References**

29. (a) CrystalClear 2.0, Rigaku Corporation, Tokyo, Japan; (b) G. M. Sheldrick, *Acta Cryst.*, 2008, A64, 112.
A rhodamine-based fluorescent sensor for rapid detection of Hg$^{2+}$ exhibiting aggregation induced enhancement of emission (AIEE) in aqueous surfactant medium

Dipankar Das$^{a}$, Rahul Bhowmick$^{a}$, Atul Katarkar$^{b}$, Keya Chaudhuri$^{b}$ and Mahammad Ali$^{a, *}$

$^a$Department of Chemistry, Jadavpur University, Jadavpur, Kolkata-700 032, India

E-mail: m.ali2062@yahoo.com Fax: 91-33-24146223

$^b$Department of Molecular & Human Genetics Division, CSIR-Indian Institute of Chemical Biology, 4, Raja S. C. Mallick Road, Kolkata-700 032, India

Manuscript received 20 June 2017, accepted 25 June 2017

Abstract: An easily synthesizable rhodamine-based chemosensor, L$_2$, selectively recognizes Hg$^{2+}$ and Al$^{3+}$ ions in the presence of all biologically relevant and toxic heavy metal ions. Very low detection limit (47 nM for Hg$^{2+}$) along with cell permeability and negligible cytotoxicity provides a good opportunity towards cell imaging of Hg$^{2+}$. SEM studies reveal rod-like microstructure for L$_2$ in water, which changes to a porous microstructure in the presence of Hg$^{2+}$. It was interesting to note that the presence of SDS solubilized the otherwise insoluble probe in pure aqueous medium. In case of Al$^{3+}$ it was astonishingly observed a fluorescence quenching on increasing the SDS concentration, while a ~33-fold enhancement of FI of [L$_2$-Hg$^{2+}$] complex was observed compared to that in the absence of SDS, making the probe selective towards Hg$^{2+}$ over Al$^{3+}$ in the aqueous SDS medium. In SDS/water system, there is a steep rise in FI, reaches a maximum at ~7 mM of SDS and then fluorescence intensity decreases gradually with the increase in [SDS] up to 28 mM. These observations clearly signify the SDS-assisted formation of polymer aggregates of the complex on the surface of monolayer of SDS formed in pre-micellar concentrations with higher FI, which is converted to the monomer being trapped inside the micellar cavity beyond the critical micellar concentration (cmc) with comparatively lower FI, indicating an interesting AIEE phenomenon. This proposition is further supported by the dependence of fluorescence anisotropy ($r$) on [SDS].

Keywords: Rhodamine-based turn-on Hg$^{2+}$ sensor, aggregation induced enhancement of emission (AIEE), fluorescence anisotropy ($r$), microstructure formation, live cell imaging.

Introduction

The fabrication of appealing supramolecular assemblies are achieved through bottom-up approach by an elegant use of noncovalent interactions like electrostatic, hydrophobic, van der Waals, hydrogen bonding etc.\(^1\)-\(^4\). Out of these, ionic self-assembly by various combinations between peptides, polyelectrolytes, surfactants and extended rigid organic scaffolds has attracted considerable attention of the researchers due to its application towards the fabrication of optical materials and advanced nano-devices. Another interesting feature of these supramolecular assemblies is their different behaviour in solution and solid state exhibiting either aggregation induced emission enhancement (AIEE) or aggregation caused quenching (ACQ)\(^5\)-\(^9\). Till date, the AIEE mechanism has been observed in silole derivative\(^10\), 1,1,2,2-tetraphenylethene (TPE)\(^11\)-\(^13\), 1-cyano-trans-1,2-bis-(4-methylphenyl)ethylene (CN-MBE) etc.\(^14\),\(^15\).

The amphiphilic nature of surfactants readily produces various supramolecular aggregates like micelles and vesicles in aqueous solution\(^16\),\(^17\). It could be used as a coupling unit to induce structural changes by ionic self-assembly. Recently surfactants have been suitably exploited to generate AIEE\(^18\)-\(^20\).

Heavy metals like mercury, lead, cadmium and
semimetal arsenic are widely distributed, extensively used, and highly toxic, and pose the greatest environmental threat; as soils and sediments are the ultimate sink for them. The water-soluble Hg$^{2+}$ ion is highly toxic and can damage the brain, nervous system, kidneys, and endocrine system. Again, due to very high thiophilicity Hg$^{2+}$ can deactivate many thiol-containing enzymes, thereby stopping or altering the metabolic processes. Over the past decade, increasing attention has been paid to the development of efficient chromo- and fluorogenic sensors for Hg$^{2+}$ ions for real-time monitoring of environmental, biological and industrial samples.

Here, a rhodamine-based probe with potential NO$_3$ donor atoms have been synthesized and used successfully for the selective and rapid recognition of toxic Hg$^{2+}$ ion (Scheme 1) exhibiting chromo- and fluorogenic metal-induced OFF-ON responses through the opening of the spirolactam ring.

In addition, although the current probe is poorly soluble in 100% aqueous medium, the presence of SDS makes it soluble in this medium, thereby making it useful for monitoring Hg$^{2+}$ ion in the purely aqueous medium in the presence of SDS with enhanced sensitivity and selectivity, even in the presence of Al$^{3+}$ which otherwise gives fluorescence response with the probe in the absence of SDS.

**Experimental**

*Materials and reagents:*

All solvents used for the synthetic purposes were of reagent grade (Merck). For spectroscopic (UV/Vis and fluorescence) studies double-distilled water and HPLC-grade MeCN were used. Rhodamine 6G hydrochloride, ethylene diamine, methyl acrylate and perchlorate salts of Na$^+$, K$^+$, Ca$^{2+}$, Ni$^{2+}$, Zn$^{2+}$, Pb$^{2+}$, Cd$^{2+}$, Fe$^{2+}$, Co$^{2+}$, Hg$^{2+}$ and Cu$^{2+}$ were purchased from Sigma-Aldrich and used as received. Sodium salts of anions like SO$_4^{2-}$, NO$_3^-$, PO$_4^{3-}$, S$^{2-}$, Cl$^-$, F$^-$, Br$^-$, I$^-$, OAc$^-$, H$_2$AsO$_4^-$ and N$_3^-$ were of reagent grade and used as received.

Steady-state fluorescence studies were carried out with a PTI (QM-40) spectrofluorimeter. UV/Vis absorption spectra were recorded with an Agilent 8453 diode array spectrophotometer. Bruker spectrometers of 300 and 500 MHz were used for $^1$H and $^{13}$C NMR.

![Scheme 1. Synthetic steps leading the formation of $[L_2-Hg^{2+}]$ complex.](image-url)
Das et al.: A rhodamine-based fluorescent sensor for rapid detection of Hg\textsuperscript{2+} exhibiting aggregation etc.

Studies. The ESI-MS\textsuperscript{+} spectra were recorded on a Waters XEVO G2QToF (Micro Y263) mass spectrometer.

**Synthesis of rhodamine 6G hydrazide (L\textsubscript{1})**:
It was synthesized by the literature method\textsuperscript{36}.

**Synthesis of rhodamine 6G probe (L\textsubscript{2})**:
To a suspension of 30 mL ice-cooled (0 °C) methanolic solution of L\textsubscript{1} (0.456 g, 1.0 mM), a 4 mL methanol solution of methylacrylate (0.36 mL, 10 mmol) was added dropwise over a period of 30 min. The reaction mixture was allowed to warm slowly to room temperature at which it was stirred for 3 days. The final product was obtained after evaporation of methylacrylate and methanol under vacuum as white crystals (yield 0.55 g, 88%) with m.p. 134–136 °C, \( R_f = 0.66 \) in a toluene/ethanol = 2 : 1 solvent system.

FT-IR (KBr) cm\textsuperscript{-1} : 3324 (-NH); 2942, 2896 (-CH); 1723 (-C=O); 1670 (-COOMe); 1622, 1518 and 1450 (-ArCH).

\[ ^1\text{H} \text{NMR (DMSO-}d_6, 500 \text{MHz)}, \delta \text{ ppm} : 7.75-7.77 (1H, t, -Ar-H), 7.48-7.53 (2H, m, Ph-H), 6.98-7.00 (1H, t, -Ar-H), 6.25 (2H, s, Ph-H), 6.03 (2H, s, -Ar-H), 5.0 (2H, t, ArNHCH\textsubscript{2}), 3.33 (6H, s, -COOCH\textsubscript{3}), 3.09-3.17 (4H, m, ArNHCH\textsubscript{2}), 2.89-2.92 (2H, t, -CH\textsubscript{2}NCOAr), 2.42-2.44 (4H, t, -CH\textsubscript{2}-CH\textsubscript{2}COOMe), 2.11-2.14 (4H, t, -CH\textsubscript{2}-COOMe), 2.01-2.05 (2H, t, -CH\textsubscript{2}N), 1.88 (6H, s, -ArCH\textsubscript{3}), 1.18-1.20 (6H, t, -CH\textsubscript{2}CH\textsubscript{3}); \text{ES}^+ \text{MS} = 629.3890 [L\textsubscript{2}+H\textsuperscript{+}]\].

**Methods of characterization**:
A scanning electron microscope (SEM) (ZEOL, JSM 8360) operating at an accelerating voltage of 5 kV was used for the study of morphologies of the free probe (L\textsubscript{2}) in aqueous medium and also in the presence of Hg\textsuperscript{2+} (L\textsubscript{2}-Hg\textsuperscript{2+}). Before SEM, the samples were vacuum dried and then gold coated to minimize the sample charging.

Fluorescence anisotropies (\( r \)), defined by eq. (1), were measured on a PTI QM-40 spectrofluorometer.

\[ r = \left( I_{VV} - G \cdot I_{VH} \right) / \left( I_{VV} + 2G \cdot I_{VH} \right) \]  \hspace{1cm} (1)

where, \( I_{VV} \) and \( I_{VH} \) indicate the emission intensities with the excitation polarizer oriented vertically and emission polarizer oriented vertically and horizontally, respectively, and corresponding \( G \) factor is calculated as in eq. (2)\textsuperscript{19},

\[ G = I_{HV}/I_{HH} \]  \hspace{1cm} (2)

where, \( I_{HV} \) and \( I_{HH} \) refer to the intensities corresponding to the vertical and horizontal positions of the emission polarizer, with the excitation polarizer being horizontal.

**Cell culture and cell cytotoxicity assay**:
HepG2 (Human hepatocellular liver carcinoma) cell lines (NCCS, Pune, India) grown in DMEM was supplemented with 10% FBS and antibiotics (penicillin \( \approx \) 100 \( \mu \)g/ml; streptomycin \( \approx \) 50 \( \mu \)g/ml). Conditions for the culture of cells are: 37 °C in 95% air, 5% CO\textsubscript{2} incubator. To test the cytotoxicity of L\textsubscript{2}, the 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay was performed following the procedure described previously\textsuperscript{36-39}.

**Cell imaging studies**:
Cell imaging studies were performed by using the protocol as described previously\textsuperscript{36-39}.

**Results and discussion**
A simple reaction between L\textsubscript{1} and methyl acrylate in methanol leads to the formation of L\textsubscript{2} in quantitative yield (Scheme 1) which was thoroughly characterized by \(^1\text{H} \text{NMR (Fig. S1)}, ^{13}\text{C} \text{NMR (Fig. S2)}, \text{ESI-MS}^+ \text{ (Fig. S3)} \) and IR studies (Fig. S4a).

**Steady-state absorption and emission studies**:
The UV-Vis titrations reveals that on gradual addition of Hg\textsuperscript{2+} to a 50 \( \mu \)M solution of L\textsubscript{2} there occurs a gradual growing of two peaks at 350 nm and 528 nm (Fig. S5) clearly manifesting the chelation induced opening of the spirolactam ring of the probe. The probable coordination mode of L\textsubscript{2} towards Hg\textsuperscript{2+} is demonstrated in Scheme 1. When absorbances were plotted against [Hg\textsuperscript{2+}] it gives a non-linear curve of decreasing slope (Fig. S5). Eq. (3)\textsuperscript{19} was employed to solve such dependence with \( a \) and \( b \) as the absorbance in the absence and presence of excess metal ions, \( c (= K_f) \) is the apparent formation constant and \( n \) is the stoichio-
metry of the reaction. The evaluated apparent association constant $K_f$ is $(3.08 \pm 0.53) \times 10^3$ M$^{-1}$ with $n = 1.0$.

$$y = \frac{a + bx^n}{1 + cx^n}$$ (3)

Job’s method also gives 1 : 1 (Fig. S6) complexation between $L_2$ and Hg$^{2+}$ which was further supported by mass spectrometric analysis ($m/z = 440.2579$) [Hg(L$_2$)(MeOH)(H$_2$O)]$^{2+}$ (see Fig. S3a in the Supporting Information).

The fluorescence titration was carried out by gradual addition of Hg$^{2+}$ (0–130 µM) to a fixed concentration of $L_2$ (20 µM) in MeCN/water (1 : 1, v/v, HEPES buffer, pH 7.2) which yielded ~126-fold enhancement in fluorescence intensity at 558 nm on excitation at 502 nm (Fig. 1). The titration data were again solved by employing eq. (3) under the condition $1 \gg c \times x^n$ with $n = 1$ prevailing a linear form. A linear least-square fitting of data gives the apparent association constant $K_f = (1.01 \pm 0.01) \times 10^4$ M$^{-1}$ (see Fig. 1 inset). Al$^{3+}$ also induces an opening of spirolactam ring of the probe leading to enhancement of fluorescence intensity. So, analogously the fluorescence titration data for $L_2$-Al$^{3+}$ complexation was solved and the apparent formation constant was calculated to be $(1.45 \pm 0.02) \times 10^4$ M$^{-1}$ (Fig. 2 inset).

**Selectivity of the probe:**

The probe was found to be sensitive towards Hg$^{2+}$, but interfered by the presence of Al$^{3+}$. However, in the presence of SDS in aqueous medium the fluorescence of $L_2$-Al$^{3+}$ was completely quenched, but not the $L_2$-Hg$^{2+}$ complex, instead there was an increase in FI (vide infra). In case of $L_2$-Al$^{3+}$ complexation the quenching of fluorescence intensity may arise due to abstraction of Al$^{3+}$ from the [$L_2$-Al$^{3+}$] complex by SDS arising out of strong hard-hard interaction between sulfonic-O and Al$^{3+}$ ion; which is absent for $L_2$-Hg$^{2+}$ complex. Again, the detection of Hg$^{2+}$ was not perturbed by 5 equivalents of metal ions like Na$^+$, K$^+$, Ca$^{2+}$, Mg$^{2+}$, Fe$^{3+}$, Co$^{2+}$, Cu$^{2+}$, Cr$^{3+}$, Mn$^{2+}$, Fe$^{2+}$, Ni$^{2+}$, Zn$^{2+}$, Cd$^{2+}$ and Pb$^{2+}$ (Fig. 3) under the identical reaction conditions. Also, the introduction of 5 equivalents of anions like SO$_4^{2-}$, NO$_3^-$, PO$_4^{3-}$, S$^{2-}$, CN$^-$, Cl$^-$, F$^-$, Br$^-$, I$^-$, OAc$^-$, H$_2$AsO$_4^-$ and N$_3^-$ into the solution of $L_2$ (Fig. S7) did not show any appreciable fluorescence change. However, I$^-$ has a strong affinity towards Hg$^{2+}$. As a result I$^-$ abstracts Hg$^{2+}$ ion from the [$L_2$-Hg$^{2+}$] complex resulting the disappearance of emission band at 558 nm through the re-establishment of the spirolactam ring (Fig. S8). The quantum yield ($\phi$) of the [$L_2$-Hg$^{2+}$] complex and ligand were determined to be 0.8609 and
Das et al.: A rhodamine-based fluorescent sensor for rapid detection of Hg$^{2+}$ exhibiting aggregation etc.

The limits of detection (LOD) of Hg$^{2+}$ and Al$^{3+}$ were found to be as low as 47 and 73 nM, respectively (Fig. S9) as delineated by 3σ method. The increased quantum yield ($\phi$) and lifetime ($\tau$) of [L$_2$-Hg$^{2+}$] over the free ligand L$_2$ clearly indicate the enhanced stability of the formed complex in the excited state.

pH-stability of the probe was checked over a wide range of pH (2–12). There is no obvious fluorescence emission of L$_2$ in the range of pH 4–12, establishing the fact that the spirolactam form of L$_2$ is stable over this wide pH range (Fig. S10). However, the presence Hg$^{2+}$ ion induces the opening of spirolactam ring at pH $\geq$ 7.0 resulting a fluorescence enhancement and hence seems to be compatible for biological applications under physiological conditions.

IR studies showed a characteristic amidic “C=O” stretching frequency of the rhodamine moiety at 1723 cm$^{-1}$ which is shifted to a lower wave number (1657 cm$^{-1}$) in the presence of 1.2 equivalent of Hg$^{2+}$ (Fig. S4b). Thus a strong binding of L$_2$ to the Hg$^{2+}$ ion and the cleavage of N-C bond in spirolactam ring is apparent. The $^1$H NMR spectra showed the ring proton “b” (Fig. 4 and Fig. S1) of the rhodamine moiety is shifted downfield in the presence of 1.2 equivalents of Hg$^{2+}$ ions. The “f” proton of -NH$^+$ group vanishes as this group possesses a positive charge due to ring opening upon binding with Hg$^{2+}$ ion. The “a” proton also shows a down-field shift. The down-field shift of “a” and “b” protons in the presence of Hg$^{2+}$ arises mainly due to decrease in electron density on opening of the spirolactam ring. The signal pattern of the other aromatic protons in [L$_2$-Hg$^{2+}$] also indicates the involvement of the receptor unit of L$_2$ in the binding to Hg$^{2+}$.

**Time resolved fluorescence studies:**

The fluorescence decay behaviour of the L$_2$ and [L$_2$-Hg$^{2+}$] were studied in aqueous medium (Fig. S11) both in the absence and presence of SDS. The bi-exponential decay of L$_2$ resulted life times of 1.53 ns ($\tau_1$) and 6.11 ns ($\tau_2$). But in the presence of SDS it prevails a mono-exponential decay with $\tau = 3.63$ ns. In the presence of Hg$^{2+}$ the decay processes are mono-exponential both in the absence and presence of SDS with respective $\tau$ values of 4.47 and 5.44 ns (Fig. S11). Bi-exponential decay of free ligand may arise due to $\pi\cdots\pi$ stacking interactions between the probe molecules. The enhanced life time of L$_2$ and [L$_2$-Hg$^{2+}$] complex in the presence of SDS may arise due to enhanced stability of the probe and its complex in the

---

Fig. 3. (a) Histogram of the fluorescence responses of different metal ions (100 mM) towards L$_2$ (20 mM) in 1 : 1 v/v MeCN/water in HEPES buffer at pH 7.2 with $\lambda_{ex} = 502$ nm, $\lambda_{em} = 558$ nm. (b) Fluorescence responses of different cations (100 mM) towards L$_2$ (20 mM) in 1 : 1 v/v MeCN/water in HEPES buffer at pH 7.2.

Fig. 4. $^1$H NMR spectra of (a) L$_2$ and (b) L$_2$ in presence of 1.2 equivalent of Hg$^{2+}$. Both spectra were recorded on a Bruker 500 MHz spectrometer in DMSO-$d_6$ (For atom numbering, please see Fig. S1).
excited state in the presence of SDS. Thus this observation clearly indicates the fact that SDS imposes more restriction on the movement of the probe in microheterogeneous environments through the formation polymeric aggregates.

Steady-state fluorescence studies in aqueous SDS:

In purely aqueous solution the L₂⁻Hg²⁺ complex is weakly fluorescent, however, in the presence of SDS enhanced fluorescence was observed. Thus, steady state fluorescence studies were also carried out in the presence of SDS in two separate experiments. In one case, the SDS concentration was kept fixed at 7 mM and [Hg²⁺] was varied in the range 0–40 μM giving a non-linear curve of decreasing slope which was solved by adopting eq. (3) (Fig. 5b) and evaluated apparent formation constant $K_f = (1.00±0.02) \times 10^5$ M⁻¹ was found to be an order of magnitude higher than that obtained in the absence of SDS. This enhanced stability constant value may be due to the restricted movement of the doubly positively charged L₂⁻Hg²⁺ complex, which were held fixed in position by the strong electrostatic interaction with the negatively charged sulphonic acid head groups of SDS in the form of layer structure. This causes the formation of aggregates of L₂⁻Hg²⁺ complex through strong cooperative π⋯π interactions among the complexes held sidewise (Scheme 2).

In another experiment both [L₂⁻] and [Hg²⁺] was kept fixed at 20 and 150 μM, respectively and [SDS] was varied between 0–28 mM. A plot of FI vs [SDS] showed a gradual increase in FI with the increase in [SDS], reaches a maximum at ~7 mM and then gradually decreases with the increase in [SDS] (Fig. 6b). The fluorescence maximum at [SDS] ~7 mM clearly points out a critical micellar concentration (CMC) of SDS as ~7 mM under the experimental conditions. The decrease in FI with [SDS] beyond 7 mM may be attributed to a change in polymeric aggregates of the complex to a monomer arising due to the formation of spherical micelle on increasing the [SDS] (Scheme 2) in which the complex is trapped. The increase in FI with [SDS] beyond 7 mM may be explained by considering the fact that sulphonic acid group abstract Al³⁺ ion from [L₂⁻-Al³⁺] complex by strong electrostatic interaction. So in aqueous SDS the probe becomes more selective towards Hg²⁺. The fluorescence quenching experiment by iodide ion in the presence of [SDS] = 7 mM was carried out to verify such a proposition and was evaluated to be $K_{SV} = (7.23±4.3) \times 10^6$ indicating an easy accessibility of the [L₂⁻-Hg²⁺] complex located on the laminar surface of the SDS to I⁻ ion to form HgI₂.

Fig. 5. (a) Fluorescence titration of L₂⁻ (20 μM) by Hg²⁺ (0–40 μM) in presence of [SDS] = 7 mM with $\lambda_{ex} = 502$ nm. (b) Plot of fluorescence intensity as a function of [SDS].

Das et al.: A rhodamine-based fluorescent sensor for rapid detection of Hg$^{2+}$ exhibiting aggregation etc.

Determinant of steady-state fluorescence anisotropy:

Steady-state fluorescence anisotropy is usually taken as a measure of the extent of restriction imposed by the micro heterogeneous environments on the dynamic properties of the probe. An increase in rigidity of the fluorophore results in an increase in the fluorescence anisotropy$^{19}$. We have monitored the fluorescence anisotropy as a function of SDS concentration at a fixed concentration of L$_2$ and Hg$^{2+}$ (20 and 150 μM, respectively) at 558 nm which showed a marked increase in anisotropy on increasing SDS concentration up to 3.5 mM, then gradually decreases with SDS concentration reaches a plateau at ~5 mM and maintains steady value up to 12 mM. In the range 1–3.5 mM concentration, the SDS arranges them in a layered fashion. Now, the doubly charged [L$_2$-Hg$^{2+}$] complexes are held firmly by the strong electrostatic interactions between negatively charged sulfonic acid head groups and doubly positively charged complexes; which are again held together by strong $\pi \cdots \pi$ interactions thereby restricting their free movement. As a result, there occurs a sharp increase in anisotropy in the [SDS] ~1–3.5 mM. Further increase in the SDS concentration, a phase transition occurs through the formation of micelle. The slight drop in $r$ values with [SDS] beyond 3.5 mM may be rationalized by considering the formation of a monomer of [L$_2$-Hg$^{2+}$] complex which is again trapped inside the cavity of the micelle. The higher values of $r$ in case of polymeric aggregates arise due to cooperative interactions among the [L$_2$-Hg$^{2+}$] complexes which is absent in monomer trapped inside the cavity of the micelle. The variation of fluorescence anisotropy ($r$) as a function of SDS concentration is presented in Fig. 7.

**Fig. 6.** (a) Fluorescence titration of L$_2$ (20 μM) by [SDS] in presence of [Hg$^{2+}$] (130 μM) with $\lambda_{ex} = 502$ nm; (b) plot of fluorescence intensity as a function of [SDS]; (c) fluorescence titration of L$_2$ (20 μM) by [SDS] in presence of [Al$^{3+}$] (130 μM) with $\lambda_{ex} = 502$ nm; (d) plot of fluorescence intensity as a function of [SDS].

**Fig. 7.** Plot of fluorescence anisotropy ($r$) as a function of [SDS] in purely aqueous medium at 25 °C and [L$_2$] = [Hg$^{2+}$] = 20 μM, $\lambda_{ex} = 502$ nm, $\lambda_{em} = 558$ nm.

**SEM study:**

The SEM micrographs of L$_2$ (0.50 mM) prevals rod-like microstructures which interestingly changes to porous like architecture in presence of Hg$^{2+}$ (0.50 mM) (Fig. 8). In case of pure ligand in water the
structures are similar to the hexagonal prisms that arises due to the presence of two different polar ends (xanthine moiety and carboxylic ester moiety) favoring the stacking of L₂ one over another. However, in presence of Hg²⁺ these stacking interactions are disrupted leading to the formation of porous microstructures centering Hg²⁺ with the ligands at the periphery.

**Cell imaging applications:**

Hg²⁺ capturing capability of L₂ was assessed by performing the fluorescence imaging of L₂ with Hg²⁺ into the live HepG2 cells (Fig. 9). The cytotoxicity effects of L₂ determined by MMT assay indicate no significant cell cytotoxicity for HepG2 cells up to 60 µM (<30% cytotoxicity) of L₂ (Fig. S13). Interestingly, up to 10 µM of L₂ there was more than 90% of cell viability and fluorescence imaging were carried out at 1 µM, 5 µM and 10 µM of L₂. Significantly, an excellent red intracellular cytoplasmic fluorescence was observed inside the live HepG2 cells pre-incubated with 10 µM of Hg²⁺ followed by washing with 1X PBS and subsequent incubation with 1 µM, 5 µM and 10 µM of L₂. Interestingly, we observed that L₂ has excellent Hg²⁺ capturing capability even at low concentration likely at 1 µM and 5 µM at cytoplasmic level of Hg²⁺ ions (Fig. 9). Moreover, the concentration dependent binding of the L₂ with Hg²⁺ ions was observed (Fig. 9). Parallel staining of cells were carried out with DAPI and superimposed with the correspondingly treated cells with Hg²⁺ (10 µM) followed by L₂ (10, 1, 5, 10 µM) to show the cytoplasmic staining of L₂ with HepG2 cells.

**Fig. 8.** SEM images of microstructures conditions: (i) L₂ 0.5 mM and (ii) L₂+Hg²⁺ (0.5 mM each) in aqueous medium.

**Fig. 9.** Cell imaging study of Hg²⁺ ions with L₂. The fluorescence images of HepG2 cells were captured (40X and 100X) after incubated with 10 µM of L₂ for 30 min at 37 °C, also in pre-incubated 10 µM of Hg²⁺ for 3 h at 37 °C followed by washing twice with 1X PBS and, subsequent incubation with 1 µM, 5 µM and 10 µM of ligand L₂ for 30 min at 37 °C. The imaging studies showed the strong red fluorescence when L₂ binds with cytoplasmic Hg²⁺ ions. The merge images show the cytoplasmic Hg²⁺-L₂ fluorescence.
Das et al.: A rhodamine-based fluorescent sensor for rapid detection of Hg$^{2+}$ exhibiting aggregation etc.

Conclusions

In summary, we present herein a rhodamine-based chemosensor with potential NO$_3$ donor atoms for the selective and rapid recognition of toxic Hg$^{2+}$ ions. The binding stoichiometry of the sensor with Hg$^{2+}$ was established by the combined Job’s and HRMS ($m/z$) methods. All biologically relevant as well as toxic heavy metal ions did not interfere with the detection of Hg$^{2+}$ ion. The detection limit of Hg$^{2+}$ calculated by $3\sigma$ method gives a value of 1.52 nM. Its exhibits live cell imaging application of Hg$^{2+}$ with no or negligible cytotoxicity. SEM studies reveal a rod-like microstructure for L$_2$ which changes to a porous microstructure in presence of Hg$^{2+}$ (0.50 mM). The presence of SDS causes enhanced quantum yield ($\theta$), life time ($\tau$), and stability constant ($K_f$) by an order of magnitude compared to those in the absence of SDS. Again, the FI of [L$_2$-Hg$^{2+}$] complex is enhanced by 33-fold in the presence of 7 mM SDS to that in the absence of SDS. In SDS/water system, there is a steep rise in FI with the increase in [SDS], reaches a maximum at ~7 mM and then FI decreases gradually with the increase in [SDS] up to 28 mM, indicating the formation of polymeric aggregates of [L$_2$-Hg$^{2+}$] complex on layers of the SDS at pre-micellar concentrations with higher FI values – a phenomenon reminiscent with the aggregation induced emission enhancement (AIEE). However, it turns into monomer and trapped inside the cavity of the micelle beyond CMC with comparatively lower FI. This proposition is further supported by the dependence of fluorescence anisotropy ($r$) with [SDS].

Acknowledgement

Financial supports from DST, New Delhi (Ref. SR/ S1/IC-20/2012) and UGC CAS-II are gratefully acknowledged.

References


