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ON–OFF Fluorescent Imidazole Derivative for Sensitive and Selective Detection of Copper(II) Ions

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Abstract—A novel multichromophoric hybrid compound, 2-[1-(4-*tert*-butylphenyl)-4,5-diphenyl-1*H*-imidazol-2-yl]-6-(pyren-1-yl)quinoline (TDIPQ) has been synthesized as an ON-OFF fluorescent chemosensor for copper(II) ions. Colorless TDIPQ in acetonitrile–water (2:1, v/v) selectively turns yellow along with fluorescence quenching upon addition of copper(II) ions. The fluorescence quenching is directly proportional to the concentration of copper(II) ions. The interaction between TDIPQ and copper(II) was investigated with the aid of UV-Vis, fluorescence, ^{1}H NMR, and MALDI mass spectral techniques. The stoichiometry of the TDIPQ–Cu complex was determined to be 2:1 by Job's Plot. Under similar experimental conditions, other competitive metal ions had negligible or no interference in the detection ability of TDIPQ. The detection and quantification limits of TDIPQ were estimated at 2×10^{-6} M and 6.2×10^{-6} M. respectively. This method showed an excellent precision of 0.98 ± 0.011 and recovery characteristic of 99.09±1.4%. It is applicable for the quantification of copper(II) in various samples such as drinking water, lab waste water, and soil. A mixture of TDIPQ with the BZA-Co-BZMA polymer can be cast as a film on a glass slide to be used as a sensor device to indicate the presence of copper. Polymer-coated TDIPQ chemosensing property was analyzed by SEM imaging.

Keywords: imidazole–quinoline–pyrene conjugate, chemosensor, copper(II)-selective, polymer, fluorescence quenching.

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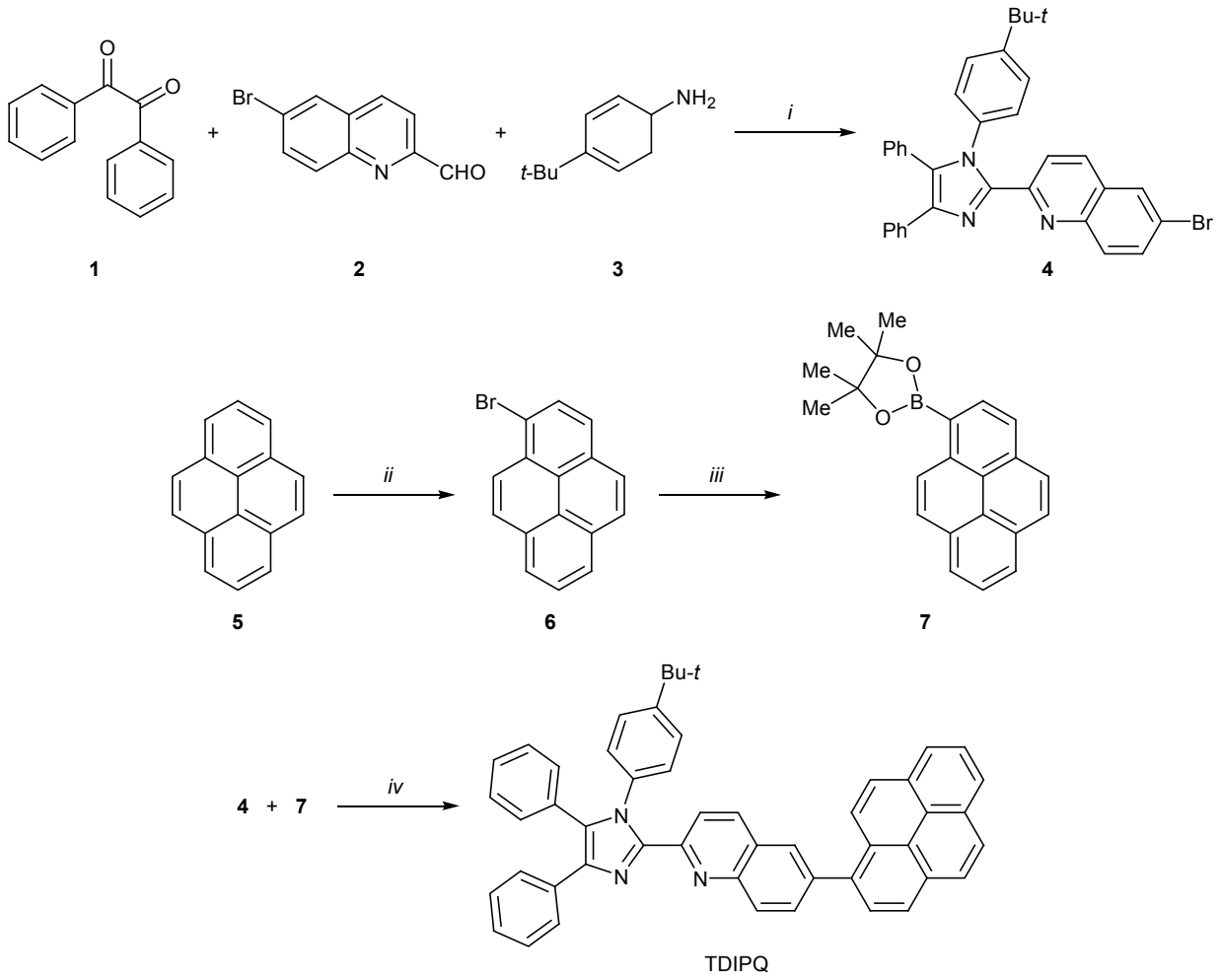
For many years researchers are keenly looking into coordination compounds of metal salts and organic ligands due to their potential application in homogeneous catalysis, bioinorganic applications, chemosensing, etc. In recent years, the research has been focused on developing selective and sensitive chemosensors. There is a strong necessity for the development of simple, cost-effective, selective, and sensitive analytical methods for the detection and determination of transition metals due to increased demand for detection of heavy metal ions [1]. Among transition metals, copper has a vast utility in biotic and abiotic processes. Copper is an essential trace element in all living forms due to its involvement in several biochemical processes. Copper-containing proteins act as redox catalysts in biological processes where copper shifts between the cuprous (Cu^{1+}) and cupric (Cu^{2+}) forms, though the majority of copper exists in the Cu^{2+} form in the body [2]. Even though copper is an essential trace element, it exhibits toxicity or causes illness when ingested in higher doses. Excess concentration of copper can cause both Indian childhood cirrhosis (ICC) [3] and non-Indian childhood cirrhosis (NICC) [4], as well as Alzheimer's [5] or Parkinson's disease [6]. According to the World Health Organization (WHO), the maximum limit of copper in drinking water is 2 mg/L (30 mM) [7]. In aqueous solution Cu^{2+} is more stable than Cu^{1+} since the energy required to remove one electron from Cu^{1+} to Cu^{2+} is compensated by high hydration energy of Cu^{2+} ion [8]. Copper detection is an important aspect in forensic science due to copper sulfate poisoning; in India it is commonly consumed to commit suicidal; however, accidental poisonings are also reported [9]. Current techniques used to detect copper in toxicological and environmental samples include atomic absorption spectrometry [10], inductively coupled plasma mass spectroscopy (ICP-MS) [11], inductively coupled plasma atomic emission spectrometry (ICP-AES) [12], and voltammetry [13]. Fluorescence-based techniques have gained much attention

due to their high sensitivity, selectivity, and speed and low maintenance cost. In recent years, a number of fluorescent probes for the detection of copper in various biological and environmental samples have been developed [14–19]. Imidazole-derived compounds have engrossed the attention of researchers due to their coordination properties, photoluminescence, and pH sensing [20]. The electronic nature of imidazole as N donor can be tuned by substitution of the active NH proton [21]. Polymers provide a good matrix to cast chemosensor molecules to obtain a device for the detection of copper ions in field samples. Dispersion polymerization consists of polymerization of monomers that are moderately soluble in aqueous medium. Benzyl acrylate and methyl methacrylate monomers were chosen for this purpose due to their high refractive indices (~1.53) and low cost.

During recent years, our research group has focused on the design of new fluorimetric and colorimetric chemosensors for the recognition of various cations and anions [22–26]. Based upon this experience and previously discussed considerations, in the present work we have designed and synthesized a multichromophoric hybrid TDIPQ molecule (Scheme 1), as an ON–OFF fluorescence chemosensor for selective detection of copper(II) ions in acetonitrile–water (2:1, v/v). A TDIPQ solution exhibited a color change to yellow, fluorescence quenching, and clear isosbestic points in the UV-Vis absorption spectrum upon addition of Cu^{2+} ions. The TDIPQ–Cu^{2+} complex showed a new red-shifted peak at λ ~382 nm in the UV-Vis spectra. The MALDI mass spectrum along with Job's plot indicated 2 : 1 stoichiometric ratio for the TDIPQ complex with copper(II). It is thermodynamically

Scheme 1.



Reagents and conditions: *i*: NH_4Ac, AcOH, reflux overnight; *ii*: HBr, H_2O_2, MeOH–Et_2O (1:1), 0–5°C to room temp.; *iii*: *n*-BuLi, THF, 4,4,5,5-tetramethyl-2(propan-2-yloxy)-1,3,2-dioxaborolane, –78°C to room temp.; *iv*: $Pd(PPh_3)_4$, toluene, Na_2CO_3, H_2O, EtOH, reflux.

favored for a metal ion to form a chelate complex with polydentate and bidentate ligands rather than a complex with monodentate coordination mode due to the entropy factor [27–29]. The ^{1}H NMR, IR, and MALDI mass spectrum of the 2TDIPQ–Cu^{2+} complex predicted the site of copper(II) binding to TDIPQ. Finally, the behavior of a polymer-mixed colorless dye is described with the observation of yellow colored complex as a sensing response.

2-[1-(4-*tert*-Butylphenyl)-4,5-diphenyl-1*H*-imidazol-2-yl]-6-(pyren-1-yl)quinoline (TDIPQ) was synthesized as shown in Scheme 1 and was characterized by spectral analyses. The TDIPQ molecule contains a pyrene fragment as signaling moiety and quinoline-linked imidazole as receptor moieties. The presence of pyrene and quinoline moieties improves the quantum yield up to ~0.7. The imidazole N^3 atom and nitrogen atom of the quinoline ring appear at 1,4-position with respect to each other, which makes the ligand more favorable for the interaction with Cu^{2+} ion. The *tert*-butyl group improves the solubility of the ligand and prevents intermolecular interactions through steric hindrance. TDIPQ alone (10 μM) and in presence of various metal ions (260 μM) like Na^+, Mg^{2+}, K^+, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Ba^{2+}, and Hg^{2+} is colorless in acetonitrile–water (2:1, v/v), but it selectively turns to yellow along with fluorescence quenching in presence of Cu^{2+} ions. Selective color change of TDIPQ for Cu^{2+} ion enables naked-eye detection of copper.

Spectroscopic studies. The UV-Vis and fluorescence spectrophotometric data were used to ascertain the interaction between copper(II) ions and TDIPQ in solution. TDIPQ in MeCN–H_2O (2:1, v/v) was

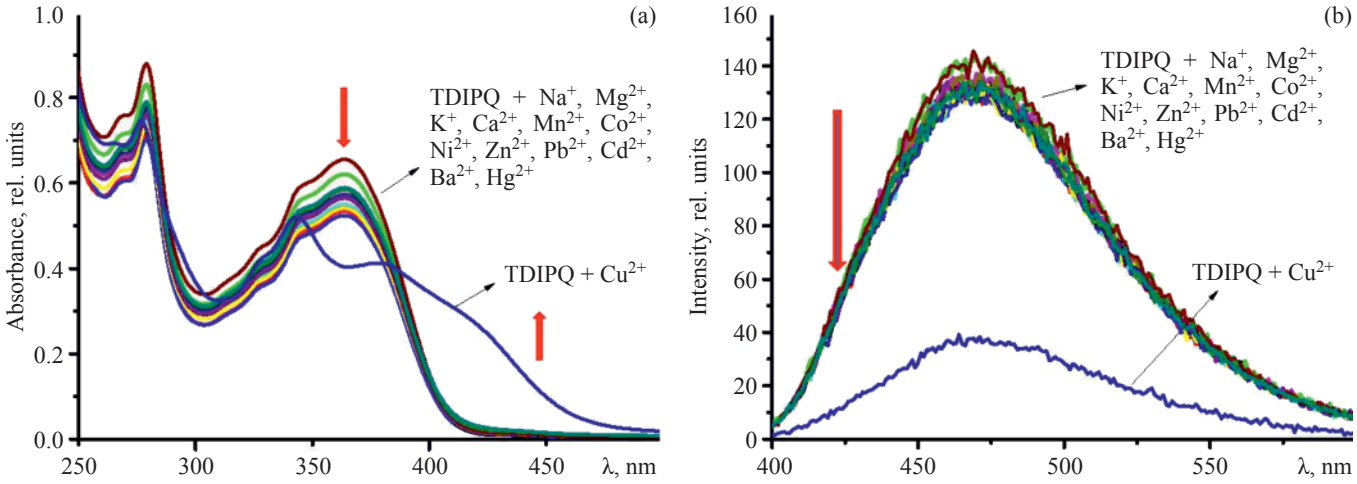


Fig. 1. Metal ion (260 μM) induced variations in the (a) absorption and (b) fluorescence spectra of TDIPQ (10 μM); excitation wavelength λ 364 nm.

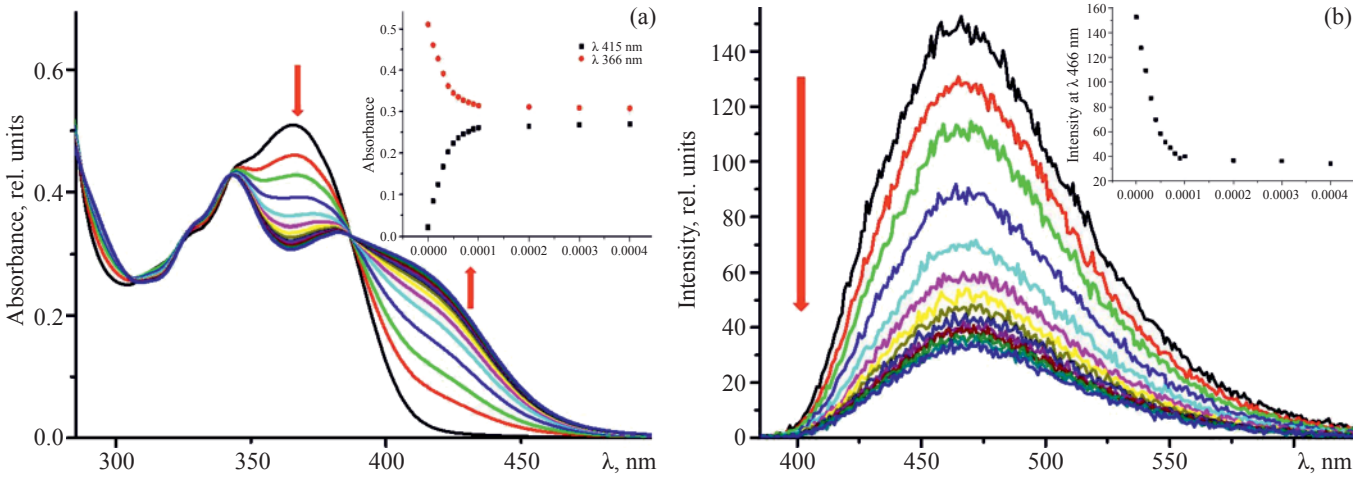


Fig. 2. Copper(II) concentration-dependent variations in the (a) absorption and (b) fluorescence spectra of TDIPQ.

Table 1. Detection of copper(II) using various chemosensors[a]

Signaling moiety	Solvent	LOD (μM)	Stoichiometry	NMR data	MS data	Color change	Remarks	Reference
Naphthalene	DMSO/H_2O (1:1,v/v)	0.65	1:1	NA	NA	NA	On/off	[14]
Coumarin	MeCN/HEPES (4/6, v/v)	5.80	1:1	NA	Present	Green to yellow	On/off	[15]
Quinazolinone	MeCN/H_2O (9:1, v/v)	37.5	1:1	Present	NA	Red to yellow	Colorimetric detection	[19]
Coumarin	DMSO/H_2O (1:1,v/v)	NA	1:1	NA	Present	Light yellow to red	On/off	[31]
Quinoline	DMSO/H_2O (2:1,v/v)	0.53	1:1	Present	Present	Colorless to pink	Colorimetric detection	[32]
Calix[4]arene	CH_2Cl_2/MeCN (1000:1, v/v)	NA	2:1	NA	Present	NA	On/off	[33]
Rhodamine B	Tris–HCl/MeCN (1:1, v/v)	0.06	1:1	Present	Present	Colorless to yellow	Off/on	[34]
Benzimidazole	DMSO/H_2O (7:3, v/v)	9.76	1:1	Present	Present	NA	On/off	[35]
Quinoline	MeCN/HEPES (9:1, v/v)	1.86	1:1	NA	NA	Yellow to pink	On/off	[36]
Pyrene	MeCN/H_2O (2:1,v/v)	2	2:1	Present	Present	Colorless to yellow	On/off	This work

[a] NA stands for not applicable.

screened against various metal ions. The electronic absorption spectrum of TDIPQ showed a negligible effect in the presence of various metal ions, and a similar trend was reflected in the fluorescence characteristics of TDIPQ. The absorption maximum of TDIPQ was red shifted in the presence of Cu^{2+} ions (Fig. 1a), and fluorescence quenching of TDIPQ was observed (Fig. 1b). These selective changes in the absorption and emission characteristics of TDIPQ indicated its ability to detect Cu^{2+} ions. TDIPQ exhibits an absorption maximum at λ 364 nm (ε = 5.25×10^4 L mol^{-1} cm^{-1}). Upon incremental addition of Cu^{2+} (260 μM), the absorption maximum of TDIPQ shifted red to λ 382 nm with an isosbestic point at λ 386 nm. This shift in the absorption wavelength was saturated when the concentration of Cu^{2+} reached 240 μM (Fig. 2a). Under similar conditions, TDIPQ (10 μM) showed an emission maximum at λ 467 nm upon excitation at λ 364 nm with a fluorescence quantum yield of 0.69. TDIPQ selectively responded to the addition of Cu^{2+} ions by quenching its emission (fluorescence quantum yield 0.01), and the emission quenching was saturated at 240 μM of Cu^{2+} ions (Fig. 2b).

The limit of detection (LOD) and limit of quantification (LOQ) of TDIPQ for Cu^{2+} were calculated using the Cu^{2+} ion concentration-dependent variation in the fluorescence intensity of TDIPQ. The minimum detectable limit was found to be 2×10^{-6} M. The LOD values of various chemosensors for Cu^{2+} ions [25, 26, 30–36], including TDIPQ, are compared in Table 1. The LOD of the TDIPQ is well in range to detect maximum limit of copper in drinking water that is 3×10^{-5} M (2 mg/L; according to the WHO). The limit of quantification was found to be 6.2×10^{-6} M. The LOD was calculated as per the reported procedures

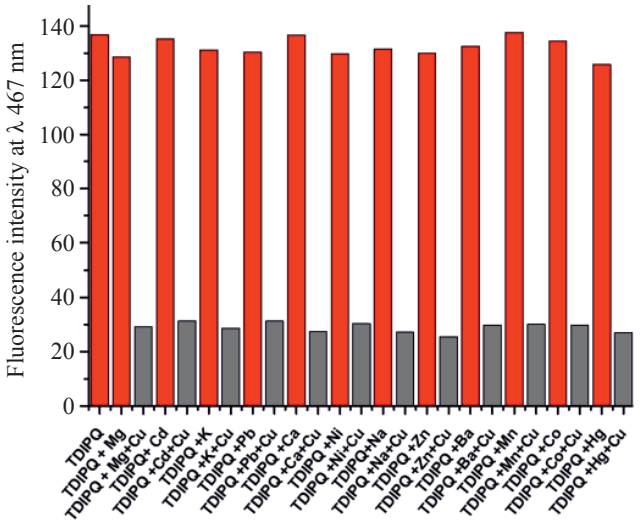


Fig. 3. Metal-ion selectivity of TDIPQ (10 μM) in MeCN–H_2O (2:1). Red bars represent the fluorescence emission intensity of solutions containing TDIPQ (10 μM) and 20 equiv of the corresponding cation; gray bars show the fluorescence intensity after addition of 20 equiv of Cu^{2+} to the solution containing TDIPQ (10 μM) and the cation (200 μM).

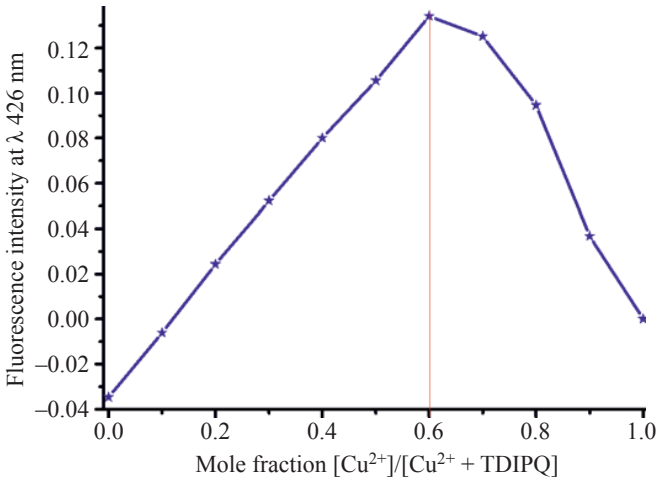


Fig. 4. Job's plot indicating the 2:1 stoichiometry for the complex TDIPQ–Cu^{2+}.

using LOD = 3σ/*S*, where σ is the standard deviation (0.075) and *S* is the slope of the linearity curve [37], and the LOQ was determined as 3×LOD. The LOD and LOQ values indicated the high sensitivity of TDIPQ toward Cu^{2+} ions. Next, precision, accuracy, and recovery of Cu^{2+} added were estimated for the present method, and the results indicated that TDIPQ is an efficient fluorescent chemosensor for both qualitative and quantitative analysis of Cu^{2+} ions.

Competition study. A chemosensor should be selective for the target metal ion but should have minimal response toward coexistent metal ions normally present in various matrices. To understand the ability of TDIPQ (MeCN:H_2O (2:1 v/v)) to selectively respond to Cu^{2+} ions (200 μM) in the presence of other metal ions (200 μM; Na^+, Mg^{2+}, K^+, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Ba^{2+}, and Hg^{2+}), competition experiment was conducted, and the resultant emission maximum at λ 467 nm confirmed that TDIPQ can recognize Cu^{2+} ions with its characteristic fluorescence quenching even under coexistence with other metal ions (Fig. 3).

Job's plot. A binding assay was carried out using Job's plot. The absorbance at λ 426 nm was plotted against the mole fraction of TDIPQ to get the stoichiometric ratio. For TDIPQ and Cu^{2+} ion, the maximum of Job's plot was observed at a 0.6 mole fraction of the ligand (Fig 4), which strongly suggests 2:1 complex formation. The 2:1 stoichiometry of the complex was confirmed by the MALDI mass spectrum which showed a peak at *m*/*z* 1422.86 corresponding to 2TDIPQ·Cu species. This is a direct evidence of complex formation showing changes in the absorption and emission spectra of TDIPQ in the presence of copper(II) ions.

^{1}H NMR study. To complement spectrophotometric studies and further explicate the coordination mode of TDIPQ with the metal ion, we performed ^{1}H NMR analysis of the complex formation by adding Cu^{2+} (0.6 mM) to a solution of TDIPQ. The ^{1}H NMR spectrum of the free receptor has doublets at δ 8.38 and 7.56 ppm corresponding to the quinoline protons. Upon addition of Cu^{2+} ion, these signals shift to δ 8.36 and 7.57 ppm, respectively, with a decrease in their intensity, indicating complex formation (Fig. 5). These findings suggest coordination of copper(II) to the quinoline and imidazole nitrogen atoms.

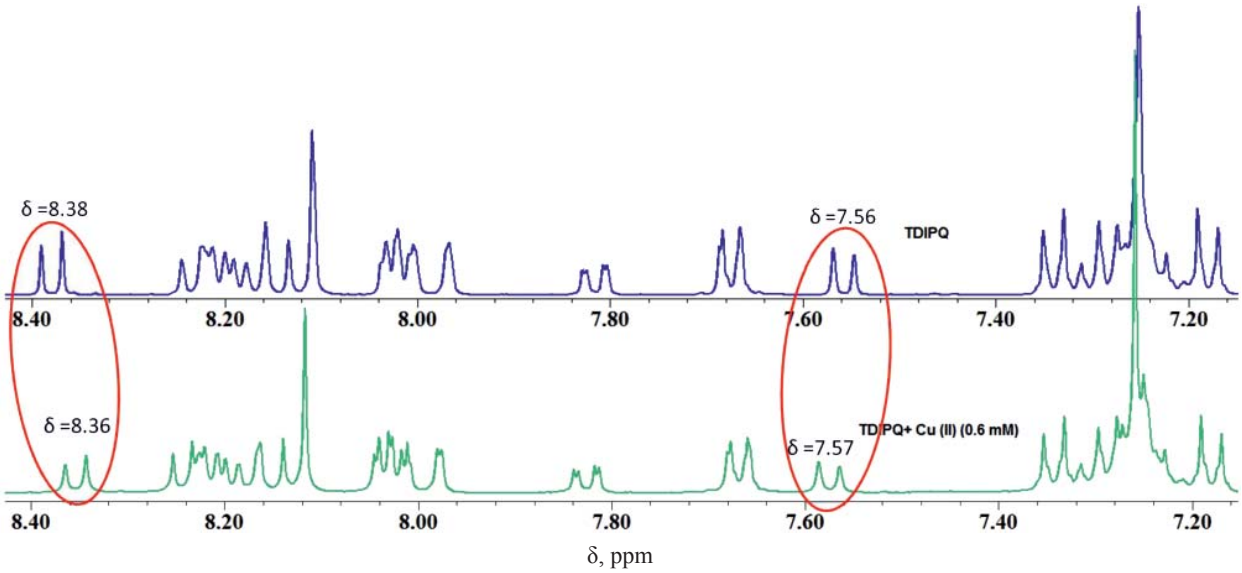
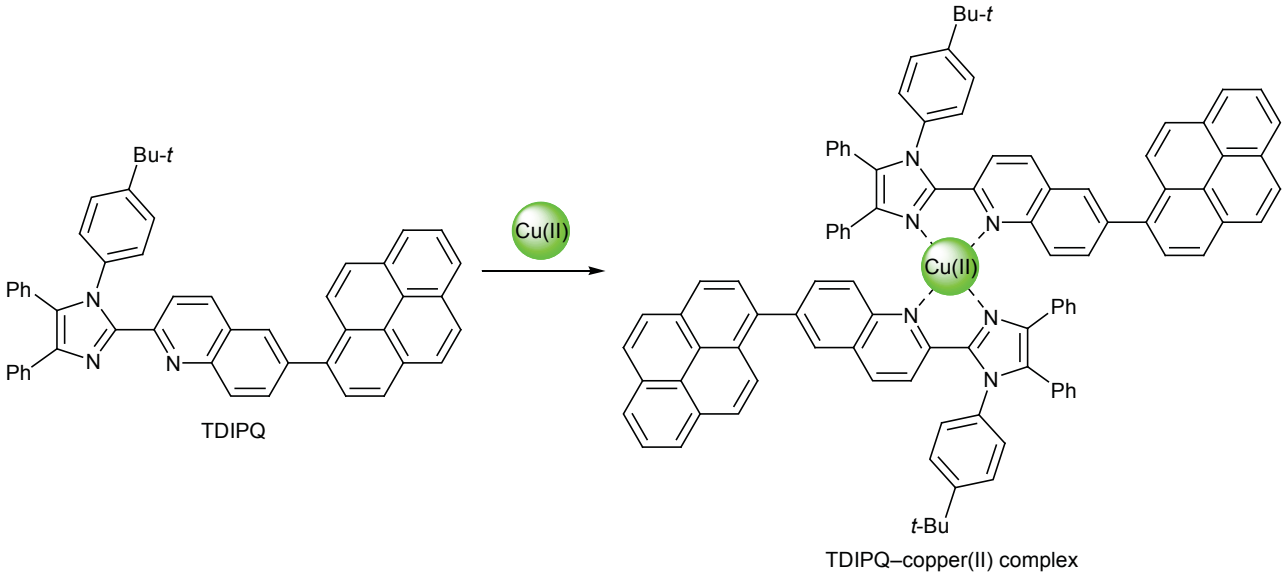


Fig. 5. Variations in the ^{1}H NMR spectra of TDIPQ upon addition of 0.6 mM of Cu^{2+} ions.

Scheme 2.



Plausible mechanism of Cu^{2+} sensing. Conclusions from the preceding discussions put forward a binding mode of TDIPQ with Cu^{2+} ion as shown in Scheme 2. The interaction involves nitrogen atoms of the quinoline and imidazole rings of two TDIPQ molecules and one Cu^{2+} ion, as is well evident from the MALDI spectrum and Job's plot. The interaction between TDIPQ and Cu^{2+} ions brings about a change in color to yellow and fluorescence quenching of TDIPQ.

Analytical applications. To understand the practical utility of TDIPQ for detection of Cu^{2+} ions in environmental samples such as drinking water, soil, and lab wastewater, experiments have been conducted by spiking the samples with $CuCl_2$. The samples were then analyzed for Cu^{2+} ions using the proposed method. Control experiments were performed using ICP-OES to correlate the results and to estimate the reliability of the proposed method. The detailed procedure for sample preparation is provided in Experimental. The results obtained by spectrophotometry employing TDIPQ as an indicator and ICP-OES were in agreement, indicating that TDIPQ is suitable to detect Cu^{2+} ions present in samples (Table 2). Moreover, advantages like high sensitivity, good selectivity, speed, low cost, and simple sample preparation procedure associated with spectrophotometer make this method more preferable that conventional ICP-OES method.

Instant sensing of Cu^{2+} ion on a polymer (BZA-Co-BZMA) film containing TDIPQ. To make the method more feasible and readily available, polymer-based thin films were cast on a glass slide doped with TDIPQ. The polymer (BZA-Co-BZMA) and TDIPQ were taken into a round-bottom flask and dissolved in chloroform. After addition of copper(II) to the polymer (BZA-Co-BZMA)–TDIPQ mixture, the color of the solution changed to yellow and was retained even after solidification of the mixture. On exposure to UV light, the (BZA-Co-BZMA)–TDIPQ film showed blue fluorescence, whereas the (BZA-Co-BZMA)–TDIPQ–Cu^{2+} film showed fluorescence quenching (Fig. 6).

The morphology of the (BZA-Co-BZMA)–TDIPQ and (BZA-Co-BZMA)–TDIPQ–Cu^{2+} films was inves-

Table 2. Estimation of Cu^{2+} concentration (μg/mL) in various samples using TDIPQ and ICP-OES methods

Sample	[Cu^{2+}] added	[Cu^{2+}] found using TDIPQ[a]	[Cu^{2+}] found by ICP-OES
Drinking water	0.13	0.13	0.13
	0.32	0.32	0.32
Laboratory wastewater	0.13	0.12	0.13
	0.32	0.32	0.31
Soil	0.13	0.10	0.12
	0.32	0.31	0.32

[a] Mean value of three experiments.

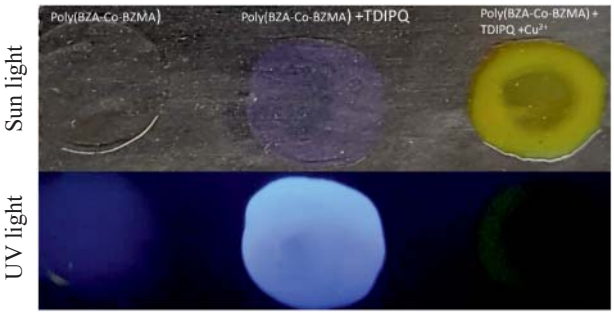


Fig. 6. Films of polymer (BZA-Co-BZMA), (BZA-Co-BZMA)–TDIPQ, and (BZA-Co-BZMA)–TDIPQ–Cu^{2+} under sunlight and UV light.

tigated by scanning electron microscopy. The SEM images (Fig. 7) revealed tightly packed spherically shaped arrangement of the (BZA-Co-BZMA)–TDIPQ film. The size of the spheres varied from ~0.5 μm to ~1 μm. In the presence of copper, the size of the spheres increased to ~6.6 μm, and the spheres were surrounded by satellite ovals. The difference in the size and morphology of the films may be due to the formation of copper complex with two molecules of TDIPQ.

The above (BZA-Co-BZMA)–TDIPQ mixture was used to cast a film on a glass slide. After solidification of the polymer on the glass slide, it was used as a device to indicate the presence of copper ion in solution. When a drop of a 1×10^{-5} M Cu^{2+} solution was applied onto the coated glass slide, it showed a color change to yellow along with fluorescence quenching (Fig. 8).

In summary, we have developed a method for qualitative detection and quantification of copper(II) ions in various samples by using a novel multichromophoric hybrid molecule, TDIPQ. TDIPQ selectively responds to Cu^{2+} ion through color change to yellow along with fluorescence quenching, and the detection ability of TDIPQ for Cu^{2+} ion remains unaffected even in the presence of common coexisting metal ions. The method is characterized by limit of detection and limit of quantification values of 2×10^{-6} and 6.2×10^{-6} M, respectively. The stoichiometry of the complex formed between TDIPQ and Cu^{2+} was determined to be 2:1 using Job's plot. A polymer–TDIPQ mixture cast on a glass slide can be used to detect copper ion in solution. Practical application of the proposed method, simple synthesis of TDIPQ, uncomplicated operation procedures, and cost effectiveness demonstrate the potential utility of TDIPQ for monitoring copper in various samples.

EXPERIMENTAL

All chemicals used were obtained from Aldrich, Alfa Aesar, SD Fine Chem Ltd., and Merck. All reactions were carried out under nitrogen atmosphere, and the synthesized compounds were purified by silica gel column chromatography. Redistilled organic solvents were used. Metal salt stock solutions were prepared in Millipore water. The ^{1}H and ^{13}C NMR spectra were recorded on 400- and 500-MHz Bruker UltraShield Plus spectrometers using tetramethylsilane as internal standard and $CDCl_3$ as solvent. The UV-Vis absorption spectra were measured on an Agilent Cary 5000 UV-Vis-NIR spectrophotometer at room temperature using 10-mm quartz cells. Fluorescence analysis was done at room temperature on a Cary Eclipse fluorescence spectrophotometer with a slit width of 2.5 nm (10-mm quartz cell). The mass spectra (positive electrospray ionization) were recorded on a Thermo Scientific mass

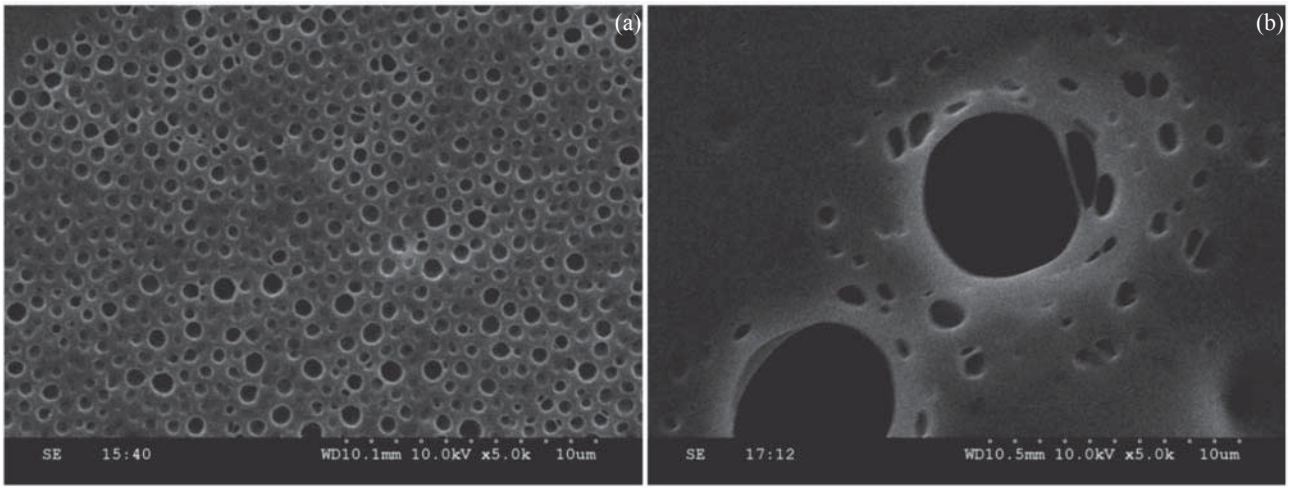
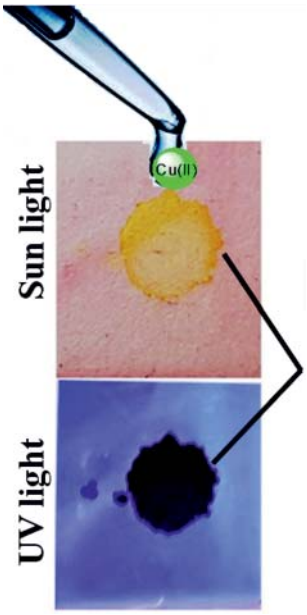


Fig. 7. SEM images of (a) poly(BZA-Co-BZMA) and (b) poly(BZA-Co-BZMA)–TDIPQ.

spectrometer (Waltham, MA, USA). The high-resolution mass spectra were obtained with an orbitrap mass analyzer. The IR spectra were recorded on a Bruker Alpha FTIR spectrophotometer. A Thermo Fisher Scientific UK iCAP 6500 DUO inductively coupled plasma–optical emission spectrophotometer (ICP-OES) was used to analyze copper in real/simulated samples. Scanning electron microscopy (SEM) images were obtained using a Hitachi S-3000N scanning electron microscope instrument. A stock solution of TDIPQ with a concentration of 1×10^{-2} M was prepared using HPLC-grade chloroform. Metal ion stock solutions (1×10^{-2} M) were prepared from the corresponding metal chlorides using Millipore water as a solvent. A working solution of TDIPQ (1×10^{-5} M) was prepared by adding 3 μL of the stock solution to HPLC-grade acetonitrile–water (2:1, v/v), followed by adjusting the volume to 3 mL. All experiments were carried out in acetonitrile–water (2:1, v/v).

6-Bromo-2-[1-(4-*tert*-butylphenyl)-4,5-diphenyl-1*H*-imidazol-2-yl]quinoline (4). Benzil (1.00 g, 4.75 mmol), ammonium acetate (3.66 g, 47.50 mmol), 6-bromoquinoline-2-carbaldehyde (1.12 g, 4.75 mmol), and 4-*tert*-butylaniline (663 mg, 7.12 mmol) were mixed in glacial acetic acid under stirring, and the mixture was refluxed for 12 h under nitrogen. The mixture was cooled, and the precipitate was filtered off and washed with water (5 × 10 mL). The filtrate was dissolved in ethyl acetate, dried over Na_2SO_4, and purified on a silica gel column using hexane–ethyl acetate (10:2, v/v) as eluent. Yield 60%, white solid. ^{1}H NMR spectrum (400 MHz), δ, ppm: 8.30 d (1H, J = 8.7 Hz), 8.00 d (1H, J = 8.7 Hz), 7.87 d (1H, J = 2.1 Hz), 7.65–7.61 m (2H), 7.58–7.55 m (1H), 7.30–7.18 m (11H), 7.10–7.07 m (2H), 1.31 s (9H). ^{13}C NMR spectrum (125 MHz), δ_C, ppm; 151.01, 149.57, 145.61, 144.92, 138.87, 135.53, 134.70, 134.33, 133.06, 132.59, 131.08, 131.03, 130.27, 129.38, 128.25, 128.15, 128.09, 128.04, 127.39, 126.72, 125.13, 121.84, 120.17, 34.64, 31.34. Mass spectrum: m/z 560.15161 $[M + H]^+$.

1-Bromopyrene (6). 1-Bromopyrene was prepared according to the procedure described in [30]. A solution of pyrene (10 g, 49.5 mmol) in methanol–diethyl ether (60 mL, 1:1, v/v) was cooled to 5–10°C, and a mixture of 48% aqueous HBr (9.2 mL, 54.4 mmol) and 30% aqueous hydrogen peroxide (5.6 mL, 49.5 mmol) was added over a period of 15 min with stirring. The mixture was stirred at room temperature for 12 h, and the progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was diluted with water (50 mL) and extracted with methylene chloride (3 × 100 mL). The combined extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure, and the residue was subjected to column chromatography using hexane as eluent. Yield 90%, mp 81–83°C. ^{1}H NMR spectrum (300 MHz), δ, ppm: 8.43 d (1H, J = 9.8 Hz), 8.25–8.13 m (4H), 8.11–7.98 m (4H).



Spot formed after addition of a solution containing Cu^{2+} ions on a polymer–TDIPQ film

Fig. 8. Polymer (BZA-Co-BZMA)–TDIPQ film indicating the presence of copper as a yellow sport under sunlight and fluorescence quenching under UV light.

4,4,5,5-Tetramethyl-2-(pyren-1-yl)-1,3,2-dioxaborolane (7). A solution of compound **6** (6.0 g, 21.4 mmol) in anhydrous THF (100 mL) was cooled to –78°C, and a 2.0 M solution of *n*-BuLi in hexane (11.7 mL, 23.5 mmol) was slowly added under nitrogen atmosphere. The mixture was stirred for 1 h, 4,4,5,5-tetramethyl-2-(propan-2-yloxy)-1,3,2-dioxaborolane (6.4 g, 34.2 mmol) was added, and the mixture was stirred at room temperature for 3 h. After completion of the reaction (TLC), water (3 × 10 mL) was added, and the mixture was extracted with methylene chloride (3 × 60 mL). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure, and the residue was subjected to column chromatography on silica gel (60–120 mesh) using hexane–ethyl acetate (98 : 2) as eluent. Yield 70%. ^{1}H NMR spectrum (300 MHz), δ, ppm: 9.07 d (1H, J = 9.15 Hz), 8.53 d (1H, J = 7.62 Hz), 8.23–8.10 m (5H), 8.08–8.05 m (1H), 8.02–7.98 m (1H), 1.49 s (12H). ^{13}C NMR spectrum (75 MHz), δ_C, ppm: 136.37, 133.81, 133.38, 131.02, 130.69, 128.47, 127.95,

127.73, 127.41, 125.62, 125.28, 125.13, 124.53, 124.31, 124.02, 83.82, 25.00. Mass spectrum: *m*/*z* 328.1749 $[M + H]^+$ (^{10}B).

2-[1-(4-*tert*-Butylphenyl)-4,5-diphenyl-1*H*-imidazol-2-yl]-6-(pyren-1-yl)quinoline (TDIPQ). Compounds **4** (1 g, 1.99 mmol) and **7** (0.784 g, 2.390 mmol) and $Pd(PPh_3)_4$ (5 mol %) were added to toluene (40 mL) while stirring. A solution of sodium carbonate (2.11 g, 19.92 mmol) in a mixture of water (16 mL) and ethanol (8 mL) was added with stirring, and the mixture was refluxed at 110°C overnight. The mixture was then cooled to room temperature, 50 mL of water was added, and the mixture was extracted with 100 mL of chloroform. The extract was evaporated, and the residue was purified by silica gel column chromatography using hexane–ethyl acetate (95:5, v/v) as eluent. Yield 60%. IR spectrum, ν, cm^{-1}: 3041 ($C–H_{arom}$), 1596 (C=C), 1474, 1413, 1377 (C–N). ^1H NMR spectrum (400 MHz), δ, ppm: 8.38 d (1H, *J* = 8.5 Hz), 8.26–8.10 m (7H), 8.04–7.99 m (3H), 7.97 s (1H), 7.84–7.80 m (1H), 7.70–7.65 m (2H), 7.56 d (1H, *J* = 8.5 Hz), 7.36–7.16 m (12H), 1.35 s (9H). ^{13}C NMR spectrum (100 MHz), δ_C, ppm: 151.01, 149.46, 146.38, 145.35, 139.40, 138.81, 136.81, 135.87, 135.71, 134.47, 132.98, 132.22, 131.45, 131.17, 130.91, 130.79, 130.44, 129.28, 128.67, 128.55, 128.26, 128.24, 128.17, 128.01, 127.75, 127.70, 127.61, 127.46, 127.38, 127.08, 126.69, 126.08, 125.26, 125.20, 124.96, 124.66, 124.83, 121.54, 34.68, 31.38. Mass spectrum: *m*/*z* 680.30569 $[M + H]^+$.

Job's plot. A binding assay was carried out by constructing Job's plot [38] using a series of TDIPQ–Cu^{2+} solutions (10 μM in total). Solutions were prepared by mixing different ligand/metal concentration ratios and diluting to a constant volume so that the total concentration of the mixture remained constant, followed by measuring their emission profile. The intensity of the emission maximum was plotted against mole fraction of TDIPQ to get the stoichiometric ratio.

^1H NMR study of the complex formation of TDIPQ with copper(II). A 0.6 mM solution of Cu^{2+} was added to a solution of TDIPQ (12.3 mM) in $CDCl_3$, and the resulting solution was subjected to ^1H NMR analysis.

Precision and recovery studies. The precision of the proposed method was experimentally determined by estimating the corresponding spectrophotometric responses for five repeated analysis of a known quantity of the Cu^{2+} ion from working solution. The resulting five concentration values were plotted to get precision values. Recovery studies were also carried out to illustrate the accuracy of the method. All experiments were performed in triplicate and the mean value for each concentration was plotted to get recovery values.

Quantum yield (Φ) determination. The fluorescence quantum yield was determined by integrating the area under the curve of the sample and fluorescence standard using the equation

$$\Phi = \Phi_R(ID_R n^2)/(I_R D n_R^2),$$

where Φ is the quantum yield, *I* is the integrated intensity, *n* is the refractive index, *D* is the optical density, and the subscript *R* refers to the standard (diphenylanthracene in cyclohexane) [39]. The fluorescence quantum yields of TDIPQ were calculated in the presence and in the absence of Cu^{2+} ions.

Analytical applications. The proposed method was employed to determine Cu^{2+} ions in drinking water, soil, and lab wastewater. The samples were spiked with $CuCl_2$ and filtered to remove solid impurities. The amount of copper present in all samples was analyzed by ICP-OES and by the proposed method. Copper(II) chloride, 6.7 mg, was added to 5 mL of a sample to get a concentration of 1×10^{-2} M. Soil sample spiked with 6.7 mg of $CuCl_2$ was dissolved in Millipore deionized water (5 mL), and the solution filtered to remove insoluble impurities. A portion from all samples was diluted to concentrations of 2×10^{-5} M and 5×10^{-5} M for the ICP-OES analysis and the proposed method, respectively. Available polymer (BZA-Co-BZMA) along with TDIPQ was used to cast a device as a spot test for Cu^{2+} ions. For this purpose, 150 mg of BZA-Co-BZMA was dissolved in 5 mL of chloroform, and 340 μg of TDIPQ was added. The mixture was stirred at room temperature for 30 min and transferred to a round-bottom flask, 5 mL of chloroform was added, a solution containing Cu^{2+} ions (1×10^{-5} M) was slowly added, and the mixture was stirred at room temperature for 30 min.

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