DOI: https://doi.org/10.36094/sc.v91.2025.A_New_Perimidine_Based_Chemosensor.Roy.517

ARTICLE

Sci. and Cult. 91 (9–10) : 517-523 (2025)

A NEW PERIMIDINE BASED CHEMOSENSOR FOR DETECTION OF Cu^{2+} IONS IN AQUEOUS MEDIA AND EVALUATION OF ITS ANTIOXIDANT PROPERTY

DEBASISH ROY[1], ARIJIT CHAKRABORTY[2] AND SRABASTI CHAKRABORTY[3]*

In the present study, we have reported a unique perimidine derivative as a colorimetric sensor for the detection of aqueous Cu^{2+} ion, the third most abundant transition metal ion in human bodies. The chemosensor 2PP can be used as a highly selective and sensitive, naked-eye sensor for the detection of aqueous Cu^{2+} ion as it enables analysis of aqueous Cu^{2+} ion with a detection limit of 8.9 x 10^{-8} M, which is even below than that of the WHO acceptable limit (31.5 μM). The stoichiometry of the Cu^{2+}: 2PP complex as calculated from Job's plot measurements is 1:1. The binding constant (K_b) of 2PP for Cu^{2+} ion was determined by linear fitting of absorption titration curve. The high value of binding constant (0.69 x 10^4 M^{-1}) indicates the strong interaction between 2PP and Cu^{2+} ion. Additionally, the antioxidant activity of 2PP has also been evaluated in the study.

Introduction

A chemical sensor can be defined as a compact, portable device that provides real-time data by detecting specific compounds or ions in complex mixtures. Chemical sensors can be categorized into electrochemical, optical, mass sensitive and heat sensitive, according to the types of transducers[1] In comparison with the laborious and expensive traditional detection methods, fluorescent sensor molecules offer useful information about ions which could be chelated in cellular systems, because we can study the concentration or distribution of the particular metal ion in real time[2], for example fluorescence imaging of that metal ion has become a widely and frequently used technique. Up till now, significant advances have been made in the design of fluorescence probes for metal ions[3]. Most of them, however, have disadvantages such as insufficient selectivity or sensitivity, or interference problems from other transition metal ions, especially those, which appear in the same group of the periodic table and show similar properties. To this end, the design and synthesis of novel heterocycle based organochemosensor for metal ions have sufficient relevance and importance today. To develop a simple, facile and reliable metal ion sensor with better sensitivity, selectivity and solubility, we have tried to devise a sensor with functionalized oxygen and nitrogen-based heterocycle.

It has been an interesting job for the present-day chemists for the development of chemosensors for environmentally and biologically important cations and anions[4,5]. Among the many cations and anions, special attention has been given towards those having significant clinical importance[6-9]. Among the metal cations, Cu^{2+} demands its importance as it is the third largest abundant transition metal ion in human body. It is used by many proteins in electron transportation as cofactor, catalyses redox reactions in cells, acts as antioxidant, helping in

[1] Jonepur High School (H.S.), Kanchrapara, North 24 Parganas, West Bengal - 743145, India

[2] Department of Chemistry, Acharya B. N. Seal College, Cooch Behar, West Bengal-736101, India

[3] Department of Chemistry. Behala College, Parnasree, Behala, Kolkata - 700060, India

e-mail: srabasti123@gmail.com

reducing free radicals, as well as a pro-oxidant[10-16]. But over exposure of copper causes toxicity due to its catalytic activity manifested by generation of reactive oxygen species (ROS). Generation of ROS damages nucleic acid, proteins, lipids which in turn lead to various neurodegenerative and other diseases. Diseases like Alzheimer's disease, Wilson disease, Menkes disease, Indian childhood cirrhosis and prions disease[17-19] owe their occurrence due to cellular toxicity of copper. Due to extensive use of copper in daily lives Environment Protenction Agency (EPA) has limited its amount to 1.3 ppm which is roughly 20 μM in drinking water[20]. Hence its detection and estimation are of utmost significance.

Previously, the quantification of the Cu^{2+} was done via a number of analytical methods including atomic absorption/emission spectroscopy (AAS/AES), electrochemical methods, inductively-coupled plasma mass spectrometry (ICPMS) and X-Ray fluorescence spectroscopy (XRF)[21-24]. Nevertheless, these techniques of quantification of the Cu^{2+} involve the use of sophisticated instrumentation, time consuming sample pretreatment and separation methods, thus making these inconvenient. In current period, all these techniques have been mitigated by new colorimetric as well as spectrophotometric methods. The techniques depend upon UV-VIS absorption and fluorescence measurements of fluorophore moieties which can act as high performance chemosensors for metal ions detection[25-28]. These have drawn much attention due to several advantages such as high sensitivity, selectivity and comfortable operation in identifying the metal ions.

The present study is focused on synthesis of novel perimidines and testing their chemosensing on transition metal cations and also anions. To this end, we have synthesized a compound 2PP, and also carried out the studies on its activity as a Cu^{2+} ion chemosensor.

Experimental

General Information: Every solvent and reagent used for spectrophotometric study were of analytical grade. The salts employed as transition as well as alkali or alkaline earth metal ions source were $AgNO_3$, $Ba(NO_3)_2$, $Ca(NO_3)_2.4H_2O$, $Cd(NO_3)_2$, $Co(NO_3)_2.6H_2O$, $Cr(NO_3)_3$, $Cu(NO_3)_2.3H_2O$, $(NH_4)_2Fe(SO_4)_2.6H_2O$, $HgNO_3$, $MnSO_4.H_2O$, $NaNO_3$, $Ni(NO_3)_2.6H_2O$, $Pb(NO_3)_2$ and $ZnSO_4.7H_2O$. ^1H NMR and ^{13}C NMR studies were accomplished on a Bruker DPX-300 (300 and 75 MHz, respectively) spectrometer, and chemical shifts were filed in ppm. FT-IR data were collected from Perkin Elmer Spectrum 2 machine. Melting point measurements were done on a LabX India digital melting point device. UV-VIS spectra were run and collected at optimum temperature engaging a Perkin Elmer UV Lambda 25 spectrometer.

Synthesis of 2-(2,3-dihydro-1H-perimidin-2-yl) phenol (2PP): Salicylaldehyde (0.41 ml, 3.9 mmol) and 1,8-diaminonaphthalene (1 g, 6.32 mmol) were added to 15 mL of ethanol, and the reaction mixture was refluxed for 4 hours. A rosy brown solid product separated out from the reaction medium, and the product was filtered and washed with chilled ethanol, distilled water and dichloromethane, successively for 2 times to obtain the pure product. The product was recrystallised from ethanol. Yield: 0.9 g (84 %). M.P.: 190 °C.

^1H NMR (DMSO- d6, 400 MHz): δ 9.73 (1H, s, -O*H* proton), 7.48 (1H, dd, *J* = 8, *H*-Ar), 7.21-7.14 (3H, m, *H*-Ar), 7.0 (2H, d, *J* = 8, *H*-Ar), 6.9 (1H, d, *J* = 8, *H*-Ar), 6.87 (1H, t, *J* = 16, *H*-Ar), 6.59 (2H, br s, N*H* protons), 6.54 (2H, s, *J* = 8, *H*-Ar), 5.67 (1H, s, *H*-Ar). ^{13}C NMR (DMSO- d6, 100 MHz): δ 155.8, 143.7, 134.8, 129.5, 128.8, 127.5, 127.2, 119.3, 115.8, 115.7, 112.8, 104.9, 61.16. IR spectrum (cm^{-1}, KBr pellet): 3360 & 3323.44 (NH str.), 3600-2900 (broad OH str.), 3044 (CH str.), 1600 (C=C str.), 1492, 1413, 1381, 1414, 1248, 817, 766.

UV-Vis Spectral Titration of 2PP with Cu^{2+}: 10 mM solution of 2PP in acetonitrile and 10 mM solution of Cu^{2+} in water were taken as stock solutions. 10 μL of 2PP (10 mM) was diluted to 2 mL acetonitrile in a cuvette, and the UV-Vis spectrum was recorded. Thereafter, 1 μL of Cu^{2+} solution (10 mM) was poured into the same cuvette; after blending these homogeneously, UV-Vis absorption spectrum was recorded at room temperature. This process of collation of Cu^{2+} solution (10 mM) was recurred up to saturation.

Job Plot Measurements of 2PP with Cu^{2+} ion[29]: A solution (10 mM) of 2PP in acetonitrile and a Cu^{2+} (10 mM) solution in water were taken as stock solutions. Thereafter, 1, 2, 3, 4, 5, 6, 7, 8 and 9 mL from the stock solution of 2PP were taken successively and shifted to different vials. 9, 8, 7, 6, 5, 4, 3, 2 and 1 mL of the Cu^{2+} solution (10 mM) was added respectively, to the foresaid vials so that the mole fraction of 2PP in each vial end in 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9, respectively. Each vial was diluted with acetonitrile to make a total volume of 2 mL. After blending the solution of vials for a few seconds to form 2PP-Cu^{2+} complex, UV-vis spectra were recorded at room temperature. Similar step was taken for blank sample with solution of 2PP only.

Fluorescence Emission Spectroscopic Titration of 2PP with Cations: 5 mM solution of 2PP in acetonitrile

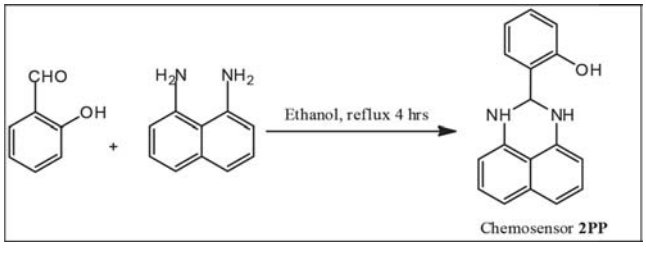
and 5 mM aqueous solution of Cu^{2+} were arranged as stock solutions. 5 μL of 5 mM solution of the 2PP was diluted to 2 mL acetonitrile, and 1 μL of 5 mM aqueous Cu^{2+} solution was then conferred at each time into the diluted 2PP solution. After mixing it homogeneously, emission spectra were taken by exciting the 2PP at 326 nm. This procedure was conducted up to saturation at room temperature.

***Evaluation of Antioxidant Activity by DPPH Assay*[30]: Step 1:** 0.01(M) methanolic solution of 2PP, DPPH (1,1-diphenyl-2-picrylhydrazyl) and L-ascorbic acid were arranged. Then, the 0.01(M) methanolic solution of 2PP were systematically diluted to 1/2, 1/4, 1/8, 1/16, 1/32.

Step 2: To the seven sample vials 10 μL of 0.01(M) DPPH was mixed up. Amongst these, to the six sample vials 10 μL of sequentially diluted solutions of 2PP arranged in step 1 was mixed. To one rest sample vial 10 μL of distilled methanol was added, but the seventh vial was operable as blank. Thereafter, these seven sample vial solutions were diluted to 2 mL. After blending homogenously, these seven solutions were granted to react for 30 minutes at room temperature under dark. UV-Vis spectra of all the seven solutions were conducted at room temperature and the absorbance of these taken at 517 nm.

Results and Discussion

Synthesis and Characterisation: The chemosensor 2PP was synthesised by coupling of salicylaldehyde with 1, 8-diaminonaphthalene in absolute ethanol under refluxing condition following literature procedure with some modification (Scheme 1); 84% yield of 2PP was obtained by this modification Compound 2PP was characterised by ^{1}H NMR, ^{13}C NMR, FT-IR (Fig. S1–S3).



Scheme 1 Synthesis of 2-(2,3-dihydro-1 *H*-perimidin-2-yl)phenol (2PP).

Visual and Spectral Changes of Chemosensor 2PP towards Cations: Due to the poor solubility of compound 2PP in water, the colorimetric sensing abilities of 2PP were investigated in acetonitrile upon the treatment with various kinds of aqueous cations such as Ag^{+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Hg^{+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+} and Zn^{2+}. To examine the spectral changes of 2PP toward cations, a solution of 2PP was treated with above mentioned metal ions (Fig. 1). Upon the collation of aqueous Cu^{2+} to the solution of 2PP (25 mM), λ_{max} 326 nm, molar extinction co-efficient = 1.48×10^{4} $M^{-1}cm^{-1}$ in acetonitrile), a change of colorless to yellow color along with an absorption change with isosbestic points at 327 nm and 343 nm were observed; whereas other cations did not reveal any visual change as well as spectral change. Consequently, appearance of two new absorption peaks at 403 nm and 473 nm imply the creation of a stable complex with Cu^{2+} ion (Fig. 1). Thus, compound 2PP is undoubtedly a potent candidate for a "naked-eye" chemosensor of Cu^{2+} ion.

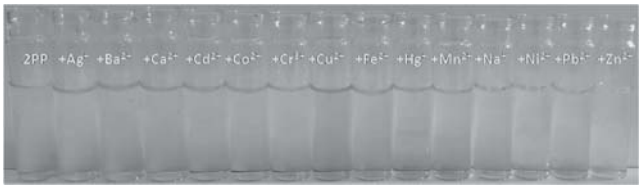


Fig. 1 (a) The color changes of 2PP (50 mM) upon addition of various aqueous cations (1 equiv.) in MeCN medium.

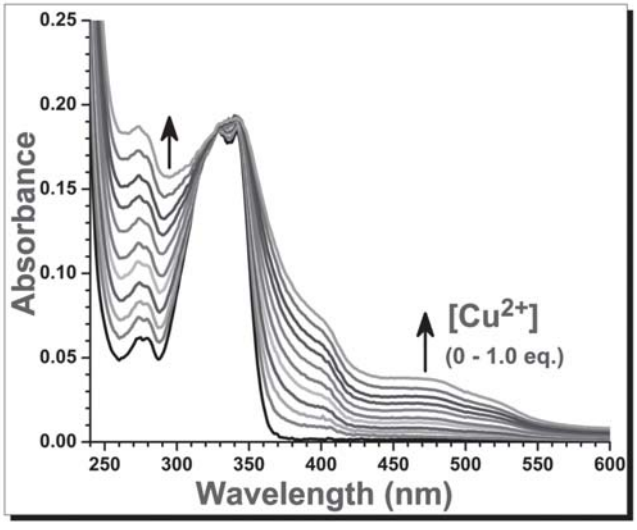


Fig. 1 (b)Absorption changes of 2PP (25 mM) upon the addition of aqueous Cu^{2+} (1.0 equiv.) in MeCN medium at room temperature.

Determination of Binding Stoichiometry: Job's method of continuous variation[30] was employed to estimate the stoichiometry of complexation of Cu^{2+} with compound 2PP using absorbance spectroscopy. The difference in absorbance (ΔA) between the absorbance of free ligand and absorbance after cationic interaction was plotted against the increasing mole fraction of Cu^{2+}. Sum of the concentrations of ligand and cation was maintained at 50 mM with varying their mole fractions from 0 to 1. The corresponding absorbances were taken at 351 nm for studies with chemosensor 2PP. The break point in the resulting plot corresponds to the mole fraction of Cu^{2+} in

the Cu^{2+}: 2PP complex. Therefore, the stoichiometry is obtained in terms of Cu^{2+}: ligand [x_{cation} : (1-x_{cation})] where x_{cation} is the mole fraction of cation calculated as the ratio of cations to the total molar concentration of receptors and cations.

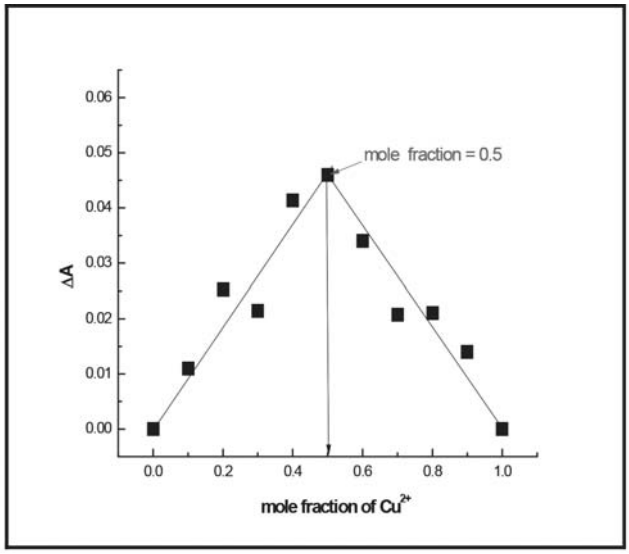


Fig. 2 Job's plot for binding of Cu^{2+} ion with 2PP.

Job's method of continuous variation was employed to determine the stoichiometry of complex formation between Cu^{2+} and compound 2PP using absorption profile. In our study, the inflection point was found to be at x_{Cu}^{2+} = 0.5. From this inflection point stoichiometry of the Cu^{2+}:2PP complex as calculated is 1:1 (Fig. 2).

Determination of Binding Constant: The spectrophotometric titration data were used to construct Benesi-Hildebrand[31] plot to determine binding constant from the following equation

$$\frac{\Delta A_{max}}{\Delta A} = 1 + \frac{1}{K[C]} \quad (1)$$

A_0, A_x and A_α are the absorbances of compound 2PP in the absence of Cu^{2+}, at an intermediate anion concentration and at a concentration of complete saturation with Cu^{2+} respectively. K represents the binding constant of the complexation between compounds 2PP and Cu^{2+}, [C] is the concentration of the variant (here Cu^{2+}). Plot was made with "A_{max}/"A as a function of 1/[C]. The binding constants can be obtained from the slope.

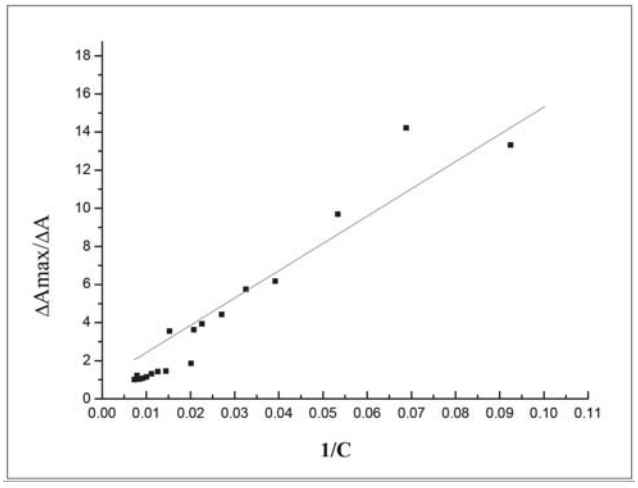


Fig. 3: Benesi–Hildebrand plots for evaluation of binding constant of 2PP for Cu^{2+} ion.

The binding constant (K) was determined by linear fitting of absorption titration curve (Fig. 3). The binding constant value (0.69 x 10^4 M^{-1}) indicates the good interaction between 2PP and Cu^{2+} ion.

Fluorescence Studies of 2PP: For the establishment of fluorescent characteristics of 2PP toward aqueous Cu^{2+}, the emission variations were also examined. Upon stepwise composition of an aqueous Cu^{2+} into the solution of 2PP in acetonitrile medium, an astonishing fluorescent quenching was observed with quenching efficiency = 98% (Fig.4).

For the sake of collisional quenching the startling decrement in intensity was portrayed by the famous Stern-Volmer equation (eqn.2) [32]

$$\frac{I_0}{I} = 1 + K_{S-V}\left[Cu^{2+}\right] \quad (2)$$

In the above equation, I_0 and I are the emission

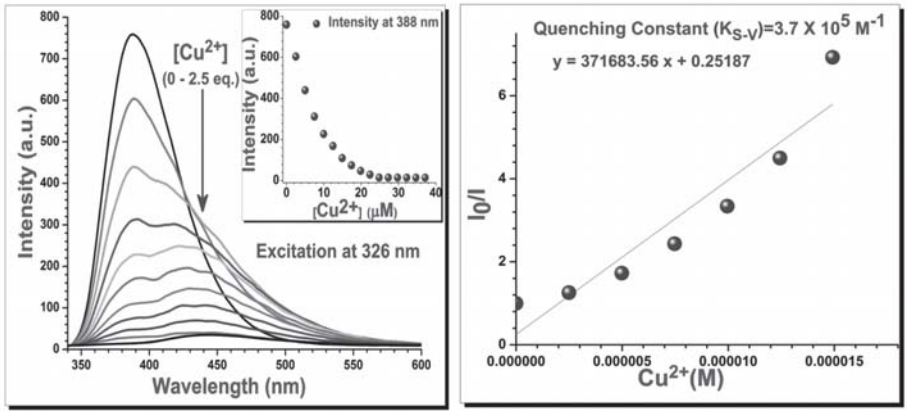


Fig. 4 (a) Fluorescent emission spectra of 2PP (12.5 μM) upon merging of aqueous Cu^{2+} (upto 2.5 equiv) in MeCN medium. Inset: Emission intensity changes of 2PP at 388 nm as a function of several aqueous Cu^{2+} concentrations. (b) Stern-Volmer plot to account the quenching constant of 2PP for Cu^{2+} ion.

intensities, respectively, in the non-appearance of and at the interim of the interaction of metal ions; [Cu^{2+}] is the concentration of cation; K_{S-V} is the Stern-Volmer quenching constant having value 3.7 x 10^5 M^{-1} for Cu^{2+}.

Solvatochromism[33]: Fluorescence emission spectra of 2PP in different solvents of several polarities are shown in Fig. 5. With increase in the polarity of the solvent, 2PP became red shifted at higher excited state. The solvent dependent fluorescence emission spectra have been shown in the Fig. 5. A vivid and measurable change in emission maxima is recorded from higher to lower wavelength with decremental polarity of the solvent. The rise of wavelength can be ascribed by the information that a polar excited state of 2PP is more stabilised by a polar solvent.

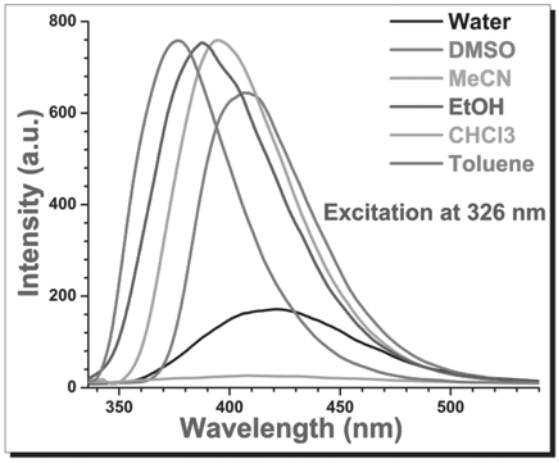


Fig. 5 Fluorescence emission spectra of 2PP in a series of solvents of various polarities.

Antioxidant Activity: This examination is based on the efficacy of the scavenging property of 2PP towards 1,1-diphenyl-2-picrylhydrazyl (DPPH). An odd electron on N in DPPH is attenuated by acquiring hydrogen atom from antioxidant 2PP to the corresponding hydrazine. Since DPPH can engage hydrogen radical or an electron for the acquirement of stability, 2PP is immutably oxidized. Owing to an odd electron, DPPH exhibits an absorbance maxima at 517 nm, and its solution appears deep violet, however, the absorption peak annihilates when the electron pairs off. The 50 μM methanolic solution of DPPH has intense colour, and at this concentration, the Lambert-Beer's law is followed over the workable range of absorbance. The equation for calculation of free radical scavenging ratio is:

$$\frac{Abs_0 - Abs_i}{Abs_0} \times 100 = 1\% \qquad (3)$$

here I% *is percentage of scavenging, Abs_0 and Abs_i are* the absorption values being showed by DPPH solution without appearance of antioxidant 2PP, and the absorbance of DPPH solution in the appearance of antioxidant 2PP, respectively. The IC_{50} is the concentration value of the antioxidant requisite for scavenging 50% DPPH and can be computed from the inhibition curve applying eqn. 3. From this inhibition diagram (Fig. 6) it can be finely decided that 2PP has more than 3.8 times greater antioxidant strength than the famous antioxidant L-ascorbic acid.

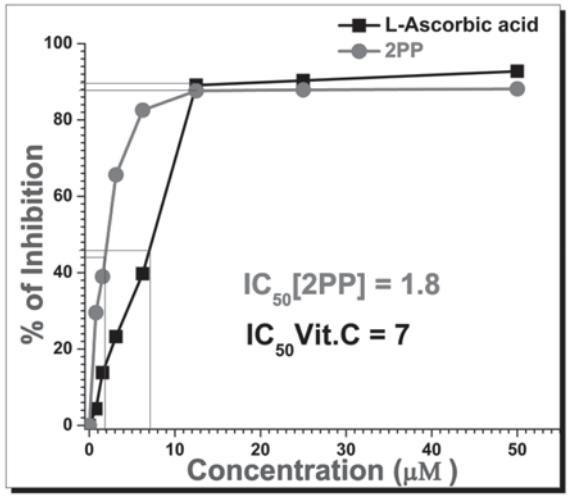


Fig. 6 Inhibition diagram of DPPH in the appearance of the chemosensor 2PP in incremental concentrations.

Determination of Detection Limits[34]: Employing fluorescence emission titration examination of 2PP in acetonitrile with aqueous Cu^{2+} (Fig.4a), the limit of detection (LOD) or detection limit was predicted applying the equation: LOD = 3σ/m, where σ the standard deviation of the blank solution, and m is the slope of the intensity vs. [Cu^{2+}] calibration curve.

The detection limits of 2PP toward aqueous Cu^{2+} ion is 6.2 × 10^{-9} M.

Life Time Measurement: We surveyed the outcomes of aqueous Cu^{2+} on the fluorescence decay attitude of 2PP (Fig. 7). The mean fluorescence lifetime of 2PP in acetonitrile was 1.604 ns where a superior mean

Table 1. Time resolved fluorescence decay parameters of 2PP in aqueous buffer in the presence of Cu^{2+} ion

Solution	τ_1 (ns)	a_1	τ_2 (ns)	a_2	τ_{avg} (ns)	χ^2
[2PP]	1.604161	-	-	-	1.604161	1.142444
[2PP] + Cu^{2+}	1.63259	0.693307808	2.684181	0.306692192	1.955104749	1.129367

fluorescence lifetime 1.955 ns was recorded in the presence of Cu^{2+} ion using eqn. 4.

$$\tau_{avg} = \tau_1 a_1 + \tau_2 a_2 \quad (4)$$

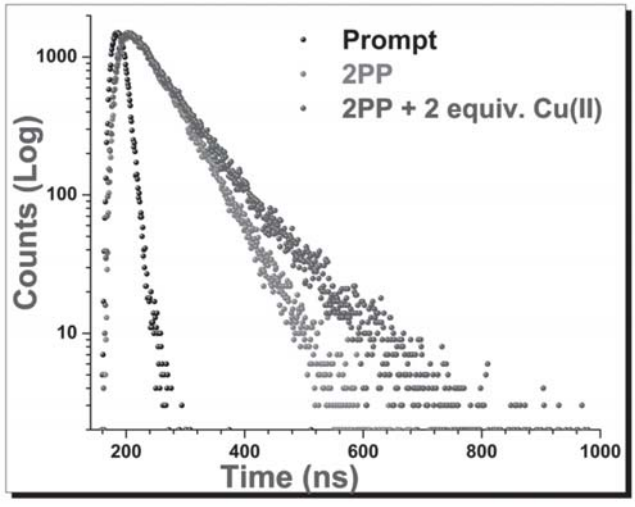


Fig. 7: The time resolved fluorescence decay curves of 2PP in the absence and presence of Cu^{2+} ions.

Conclusion

In this study, we have synthesized a novel perimidine based colorimetric chemosensor, derived from salicylaldehyde and 1,8-diaminonaphthalene, designed for the selective and sensitive detection of aqueous Cu^{2+} ions. Copper, being the third most abundant transition metal ion in the human body, plays a critical role in various biological processes, making its detection in aqueous media highly relevant. The developed chemosensor, referred to as 2PP, facilitates naked-eye detection of Cu^{2+} ions in aqueous environments and demonstrates a remarkable detection limit of 6.2 x 10^{-9} M. This value is significantly below the World Health Organization (WHO) permissible limit for copper in drinking water, which is 3.15 x 10^{-7} M, highlighting the chemosensor's practical utility for trace-level monitoring.

Spectroscopic studies revealed that the Cu^{2+} -2PP complex forms in a 1:1 stoichiometric ratio, as determined through Job's plot analysis. The strength of the interaction between 2PP and Cu^{2+} ions was quantified by calculating the binding constant (K) from the absorption titration data, yielding a high value of 0.69×10^4 M^{-1}. This strong binding affinity underscores the robustness and specificity of the sensor for Cu^{2+} ion detection.

Beyond its sensing capabilities, the perimidine-based chemosensor exhibited significant antioxidant activity. Comparative studies revealed that 2PP possesses an antioxidant strength exceeding that of the well-known antioxidant L-ascorbic acid by more than 3.8-fold. This dual function not only enhances the sensor's utility but also suggests its potential applications in fields requiring both metal ion detection and antioxidant activity, such as biomedical diagnostics and environmental monitoring.

Acknowledgement

The authors gratefully acknowledge the help and support extended by Dr. Rina Ghosh, former Professor, Department of Chemistry, Jadavpur University towards the present study. ❒

References

1. WHO, *World Heal. Organ. Website*, 1–16 (2023). at <https:/ 1. W. Wen, Introductory Chapter: What is Chemical Sensor? Progresses in Chemical Sensor, Intech Open, **2016**, https://doi.org/10.5772/64626
2. S. Yang, W. Zhang, Y. Xue, J. Zhang, *Chemosensors* **2023**, *11*(4), 226-258. DOI:10.3390/chemosensors11040226
3. W. Peveler, M. Yazdani, V. Rotello, *ACS Sens.* **2016**, *1*(11), 1282-1285. https://doi.org/10.1021/acssensors.6b00564
4. D. Wu, A.C. Sedgwick, T. Gunnlaugsson, E.U. Akkaya, J. Yoon, T.D. James, *Chem. Soc. Rev.* **2017**, *46*, 7105-7123. https://doi.org/10.1039/C7CS00240H.
5. A. Pundi, C.J. Chang, *Journal of Environmental Chemical Engineering* **2023**, *11*(5), 110346. https://doi.org/10.1016/j.jece.2023.110346
6. A. Rastergar, M. Soleimani, *Postgraduate Medical Journal* **2001**, *77*(914), 759-764. https://doi.org/10.1136/pmj.77.914.759
7. W.J. Fawcett, E.J. Haxby, D.A. Male, *Br. J. Anaesth.* **1999**, *83*, 302-320. https://doi.org/10.1093/bja/83.2.302.
8. D.A. Bushinsky, R.D. Monk, *Lancet.* **1998**, *352*(9124), 306-311. https://doi.org/10.1016/s0140-6736(97)12331-5.
9. J. Anastassopoulou, T. Theophanides, The Role of Metal Ions in Biological Systems and Medicine. In: Kessissoglou, D.P. (eds) *Bioinorganic Chemistry*. NATO ASI Series, **1995**, vol 459. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-0255-1_17
10. F. Richard., T. Dennis, *Curr Biol* **2008**, *21*(21), 877-883. https://doi.org/10.1016/j.cub.2011.09.040
11. M. Syed, A. Meshari, *Am. J. Anal. Chem.* **2018**, 09(01), 15-26. https://doi.org/10.4236/ajac.2018.91002
12. D.C. Crans, K. Kostenkova, *Commun. Chem.* **2020**, *3*, 104-107. https://doi.org/10.1038/s42004-020-00341-w
13. R.A. Festa, D.J. Thiele, *Curr Biol.* **2011**, *21*(21), 877-883. https://doi.org/10.1016/j.cub.2011.09.040
14. T. Tsang, C.I. Davis, D.C. Brady, *Curr Biol.* **2021,** 31(9), 421-427. https://doi.org/ 10.1016/j.cub.2021.03.054
15. O.A. Milach, I.V. Mel'sitova, I.L. Yurkova. *Russ. J. Gen. Chem.* **2020**, *90*, 987–993. https://doi.org/10.1134/S1070363220060080
16. J. Chen., Y. Jiang., H. Shi, *et al. Pflugers. Arch – Eur. J. Physiol.* **2020**, *472*, 1415–1429. https://doi.org/10.1007/s00424-020-02412-2

17. L.M. Gaetke., C.K. Chow, *Toxicology* **2003**, *189*(1-2), 147-163. https://doi.org/10.1016/S0300-483X(03)00159-8

18. D. Strausak, J.F.B. Mercer, H.H Dieter, W. Stremmel, G. Multhaup, *Brain Research Bulletin* **2001**, *55*(2), 175-185. https://doi.org/10.1016/S0361-9230(01)00454-3

19. I.C.J. Coenen, R.H.J. Houwen, Chapter 44 - Indian Childhood Cirrhosis and Other Disorders of Copper Handling, Editor(s): Nanda Kerkar, Eve A. Roberts, Clinical and Translational Perspectives on WILSON DISEASE, Academic Press **2019**, Pages 449-453. ISBN 9780128105320, https://doi.org/10.1016/B978-0-12-810532-0.00044-6

20. Lead and Copper Rule Compliance Help for Public Water Systems-EPA https://www.epa.gov/dwreginfo/lead-and-coppeer-rule-compliance-help-primacy agencies/, 2017

21. A. Tong, Y. Akama, S. Tanaka, Pre-concentration of copper, cobalt and nickel with 3-methyl-1-phenyl-4-stearoyl-5-pyrazolone loaded on silica gel. Analyst **1990**, *115*, 947–949. https://doi.org/10.1039/AN9901500947

22. G.P.C. Rao, K. Seshaiah, Y.K. Rao, M.C. Wang, *J. Arg. Food. Chem.* **2006**, *54*, 2868-2872. https://doi.org/10.1021/JF0600049

23. L. Pujol, D. Evrard, K. Groenen-Serrano, M. Freyssinier, A. Ruffien-Cizsak, P. Gros, *Front. Chem.* **2014**, *2*, 19. https://doi.org/10.3389/fchem.2014.00019

24. R. Sitko, B. Zawisz, Quantification in X-Ray Fluorescence Spectrometry. chapter 8, X-Ray Spectroscopy, Editor: Shatendra K. Sharma InTech **2012**. http://dx.doi.org/10.5772/29367

25. F. Rahmana, A. Alib, S. K. Khalild, R. Guoa, P. Zhanga, H. Wanga, Z. T. Lia, D. W. Zhanga, *Talanta* **2017**, *164*(1), 307-313. https://doi.org/10.1016/j.talanta.2016.10.085.

26. H. Xu, X. Wang, C. Zhang, Y. Wu, Z. Liu, *Inorg. Chem. Commu.* **2013**, *34*, 8-11. http://dx.doi.org/10.1016/j.inoche.2013.04.041

27. J. Panchompoo, A. Leigh, M. Baker, M. Wallace, R. Compton, *The Analyst*. **2012**, *137*, 2054-2062. https://doi.org/10.1039/c2an16261j

28. Z. Kowser, U. Rayhan, T. Akther, C. Redshaw, T. Yamato, *Mater. Chem. Front.* **2021**, *5*, 2173-2200 https://doi.org/10.1039/D0QM01008A

29. P. Job, *Ann. Chim. Appl.* **1928**, *9*, 113-203.

30. S. B. Kedare, R. P. Singh, *J. Food Sci. Technol.* **2011**, 48(4), 412-422. https://doi.org/10.1007/s13197-011-0251-1

31. H. A. Benesi, J. H. Hildebrand, *J. Am. Chem. Soc.* **1949**, *71*, 2703-2707. https://doi.org/10.1021/JA01176A030

32. M. H. Gehlen, *J. Photochem. Photobio. C: Photochemistry Reviews* **2020**, *42*, 100338. https://doi.org/10.1016/j.jphotochemrev.2019.100338

33. M. Homocianu, *Microchemical Journal* **2024**, *198*, 110166. https://doi.org/10.1016/j.microc.2024.110166

34. A. Shrivastava, *Chronicles of Young Scientists.* **2011,** *2*, 21-25. https://doi.org/10.4103/2229-5186.79345