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NEW CHEMO-SENSORS BASED ON SIMPLE SPECTROSCOPIC METHODS

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Abstract
Molecular probes with interesting frameworks for detection of metal ions have been extensively studied in the past. Schiff bases however have proven to be more effective and efficient. In this study, two new Schiff bases NT1 and NT2 were synthesized from anthranilamide using salicylaldehyde and 5-bromosalicylaldehyde. The ligands were characterized using Electrospray Ionization Mass Spectrometry and Fourier Transform Infrared Spectroscopy. Results of analysis showed a yield of 65-70 % and melting points in the range of 188-190 and 195-197 for NT1 and NT2 respectively. Electrospray Ionization Mass Spectrometry confirmed the purity and exact mass of the ligands. Fourier Transform Infrared Spectroscopy revealed stretching frequencies for imine bands around 1607-1614 cm^{-1}, while hydroxyl and amide groups appeared in the range of 3409-3411 cm^{-1} and 3192-3288 cm^{-1}.The electro-activeness of the ligands was also studied and both ligands were investigated as potential chemo-sensors for copper metal ion using Ultraviolet spectroscopic technique. The chemo-sensing studies of the Schiff bases revealed a red-shift in the electronic spectra of the ligands on complexation with copper (II) metal ion. Peaks around 620-690nm were observed for the complexes with copper (II) metal ion. These can be attributed to d-d transition that occur within the complexes. Presence of peaks at low concentrations indicates the use of these probes as analytical tool.

Keywords: *Anthranilamide, chemo-sensors, copper (II), Schiff base, UV-spectroscopy*

Introduction

In the last few decades, the awareness regarding the significance and toxic effect of cations is well understood as well as the detection of heavy metal ions by scientist in the field of chemistry, biology and the environment (Zhang *et al.*, 2013, Kim *et al.*, 2012). The use of various heavy metal ions is banned by many international agencies, because of their toxic nature, non-biodegradable property and hence can accumulate in the environment and food chain (Witkowska *et al.*, 2021, De Vries *et al.*, 2007, WHO, 2008, Qasem *et al.*, 2021, Mitra *et al.*, 2022, Sall*et al.*, 2020, Piwowarska *et al.*, 2024). Today, scientists and chemists are trying to develop cation chemical sensor which can be used for analysis of environmental, biological and industrial samples.

Chemo-sensors are molecules specifically designed for the qualitative and quantitative monitoring of analytes. These chemo-sensors are widely used in biological and analytical chemistry, in medicine and environmental sciences (Li *et al.*, 2020, Singh *et al.*, 2020). Collection, concentration, or preparation of samples using complicated and expensive devices are no longer required for the analysis of cations, anions and even molecules due to facile analyte detection. The binding of an analyte causes substantial changes in physical properties of a system such as absorption and/or fluorescence spectra. The most common types of chemo-sensors consist of two components: a receptor and a signaling unit tethered via a spacer (Montalti, 2006, Kubo & Sakurai, 2000, Callan *et al.*, 2005, Li *et al.*, 2014, Fu *et al.*, 2013, Alfimov *et al.*, 2003, Huang *et al.*, 2013).

Metals are involved in many vital biological functions such as transmission, muscle contraction, cell activity, etc. Sensing of metal ions is gaining more attention by many scientists, including chemists, biologists, and environmentalists (Valeur & Leray, 2000, Lehn, 1995, Silva *et al.*, 1997, Atwood *et al.*, 1996). Metallic cations are extensively used in different fields and some of these regulate thousands of biological processes that support life. However, excess of these ions can cause one of the most serious environmental problems because they are non-biodegradable and can accumulate in food chain, which poses a severe threat to the environment and human health even at low concentrations (Fu & Wang, 2010, Sharma *et al.*, 2021). Metal ions like Pb^{2+}, Hg^{2+} and Cr^{3+} can cause various health problems including allergy, lung injury, anemia, kidney failure, neurotoxicity, genotoxicity, oxidative toxicity, steroidogenic toxicity, sperm toxicity, apoptotic toxicity and axillary toxicity (Fu *et al.*, 2010, Duraisamy & Inbaraj, 2020).

Schiff base has been employed as a strong absorbing and colorful chromophores in the design of chemo-sensors (Ejiah *et al.*, 2023). In recent years, Schiff base based fluorescent probes have been developed for the detection of various toxic analytes and imaging of various analytes in biological systems (Spichiger-Keller, 2008). Schiff bases have been explored successfully for the determination of diverse metal ions. The chemical interactions between Schiff bases and metal ions are critical for generating sensing signals (Stern, 2010).

Copper is essential to all living organisms as a trace dietary mineral. In humans, copper is found mainly in the liver, muscle, and bone (Padhan *et al.*, 2019), and it is necessary for the proper growth, development, and maintenance of bone, connective tissue, brain, heart, and many other body organs. It is involved in the formation of red blood cells, iron metabolism, the metabolism of cholesterol and glucose, and the synthesis and release of life-sustaining proteins and enzymes. These enzymes, in turn, produce cellular energy and regulate nerve transmission, blood clotting, and oxygen transport. Copper also stimulates the immune system to fight infections, to repair injured tissues, and to promote healing (Mcrae *et al.*, 2009).Copper deficiency is linked to symptoms including deficiencies in blood cells, bone and connective tissue abnormalities, and neurologic disorders (Wazir & Ghobrial, 2017, Myint *et al.*, 2018). Many methods for the detection of copper have provided valuable information for a better understanding of the complex handling of copper in cells (Gerdan *et al.*, 2022). A coumarin-salicylalidene Schiff base “on-off” probe has been reported. The probe exhibits fluorescence quenching caused by Cu^{2+} through a chelation-enhanced quenching process (Wang *et al.*, 2020).

In our search, we observed few reports on the synthesis of Schiff bases containing anthranilamide moiety, but none for its use as chemo-sensor. Therefore, it is worthwhile to carry out the synthesis and study the spectral properties of Schiff bases derived from the reaction of anthranilamide with 2-hydroxylsalicylaldehyde and 5-bromosalicylaldehyde, and to provide a baseline of structural data using spectroscopic techniques, and their chemo-sensor applications.

Materials and Methods

Materials

The chemicals and reagents used were purchased from Sigma-Aldrich chemical Co Ltd and used without further purification. They include methanol, salicylaldehyde, 5-bromosalicylaldehyde, anthranilamide, n-hexane and ethyl acetate.

The melting points of the synthesized compounds were determined using the Stuart melting point apparatus model. FT-IR spectra of the compounds were recorded on a PerkinElmer Spectrum Two FT-IR Spectrometer directly on small samples of the compounds in the range 400 to 4000 cm^{-1}. Electrospray Ionization (ESI) analyses were performed in positive ionization mode on a Micromass LCT Time of Flight Mass Spectrometer.

The electronic absorption spectra data were recorded on PGT80/T+ UV-visible spectrometer using $1x10^{-5}$ M dimethyl sulfoxide (DMSO) solution in a 1 cm quartz cell at room temperature. Electrochemical studies were performed using a platinum electrode as the auxiliary electrode, glassy carbon electrode (GCE) as the working electrode, and Ag/AgCl electrode as the reference electrode using Epsilon Ec. Vet. 213.77 Xp voltammetry machine. Electrochemical parameters: EpsilonEc.Vet.213.77Xp, Scan rate (mV s^{-1}): 100*mV*; number of segments: 2; quiet time (s): 2; scale: µA; supporting electrolyte, triethylammonium phosphate (TEAP); solvent, acetonitrile.

Synthesis of Schiff bases

*Synthesis of (E)-2-((2-hydroxybenzylidene) amino) benzamide (**NT1**)*

To a stirred methanolic solution of 40 mL of anthranilamide (10 mmol, 1.36 g), 40 mL methanolic solution of salicylaldehyde (14 mmol, 1.708 g) was added. 10 drops of phosphoric acid were added to reaction mixture. The reaction mixture was stirred for 48 hours at room temperature. The reaction was monitored by TLC using (1:2) n-hexane and ethyl acetate solvent system. The white product obtained was collected by filtration and recrystallized using methanol. This afforded a white crystalline compound **NT1**.

*Synthesis of (E)-2-((5-bromo-2-hydroxybenzylidene) amino) benzamide (**NT2**)*

To a stirred methanolic solution of 40 mL of anthranilamide (10 mmol, 1.36 g), 40 mL methanolic solution of 5-bromo salicylaldehyde (14 mmol, 2.813 g) was added. 10 drops of phosphoric acid were added to reaction mixture. The reaction mixture was stirred for 72 hours at room temperature. The reaction was monitored by TLC using (4:1) n-hexane and ethyl acetate solvent system. The white product obtained was collected by filtration and recrystallized using methanol. This afforded a white crystalline compound **NT2**

Electrochemical studies of NT1 and NT2 Schiff bases

Electrochemical study of **NT1** and **NT2** was carried out using cyclic voltammetry. A solution of triethylammonium phosphate (0.0231 g) was prepared in 100 ml acetonitrile. Then, 10 ml triethylammonium phosphate solution each of **NT1** and **NT2** (0.1 mmol) was prepared. Thereafter, 5 ml each of the prepared solutions was electrochemically analyzed using cyclic voltammetry.

Chemo-sensor UV-visible study of NT1 and NT2

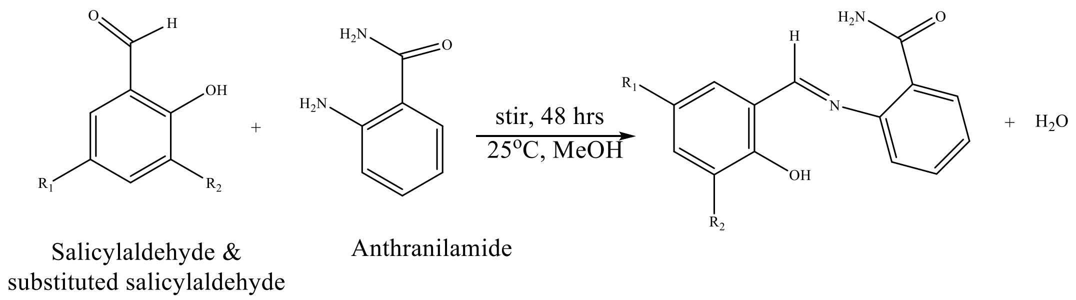
A 0.01 mmol of each of the ligand was dissolved in 2 mL acetonitrile. Then, 20 µl of the ligand solutions was diluted with 3 ml acetonitrile to make a final concentration of 33 µM (3.02 ml). UV-vis spectra were recorded from 200-800 nm.

For the titration study, (0.04 mmol, 6.8 mg) of copper chloride dihydrate ($CuCl_2.2H_2O$) was dissolved in 2 mL of tris-buffer. Then, 0.2, 0.4, and 0.6 ml of copper chloride dihydrate solution was added to each of the prepared Schiff base (**NT1** and **NT2**) solutions. The titration solutions were analyzed using UV-vis spectroscopy.

RESULTS AND DISCUSSION

Synthesis of Schiff bases

The general synthetic scheme for **NT1** and **NT2** is provided in **Scheme 1**



NT1: R_1 = H, R_2 = H

NT2: R_1 = Br, R_2 = H

Scheme 1: General synthetic scheme for NT1 and NT2

The Schiff bases were obtained in good yields from the reactions of anthranilamide with salicylaldehyde and 5-bromosalicylaldehyde in a 1:1.4 stoichiometric ratio (**Scheme 1**). The formation of the desired ligands was confirmed by ESI-MS and FTIR. The physical properties of the synthesized Schiff bases **NT1** and **NT2** are presented in **Table 1**. The compounds have sharp melting points and gave a good yield in the range of 65-70%.

Table 1. Physical data of Schiff bases NT1 and NT2

Schiff bases	**Colour**	**Melting Point (^{0}C)**	**Yield (%)**
NT1	White	188-190	65
NT2	White	195-197	70

Characterization of Schiff bases

The FTIR spectra of the Schiff bases represented in **Figures 1** and **2** are summarized in **Table 2**. The spectra showed bands in the range expected for the ligands. Both compounds displayed bands at 1607-1614 cm^{-1}, 3409-3411 cm^{-1} and 3192-3288 cm^{-1} which are characteristic of the -HC=N-, -OH and N-H groups respectively. Carbonyl stretch for amide group was observed at 1645 and 1646 for **NT2** and **NT1** respectively. Absence of the band at 1720-1740 cm^{-1} due to C=O of aldehyde indicates the formation of the Schiff bases.

ESI-MS spectra confirmed the exact mass of ligands **NT1** ($C_{14}H_{12}O_2N_2$) $[M + 1H]^{+1}$ m/z = 241.09, found 241.09, and **NT2** ($C_{14}H_{11}N_2O_2Br$)$[M+1H]^{+1}$ m/z = 319.00, found 319.00, confirming the purity and structures of **NT1** and **NT2** ligands (**Figures 3 and 4**).

Table 2:FTIR bands (cm^{-1}) of Schiff bases NT1 and NT2

Compound	***v*(O-H)**	***v*(N-H)**	***v*(C=O$_{amide}$)**	***v*(C=N)**
NT1	3411	3203, 3288	1646	1614
NT2	3409	3192, 3281	1645	1607

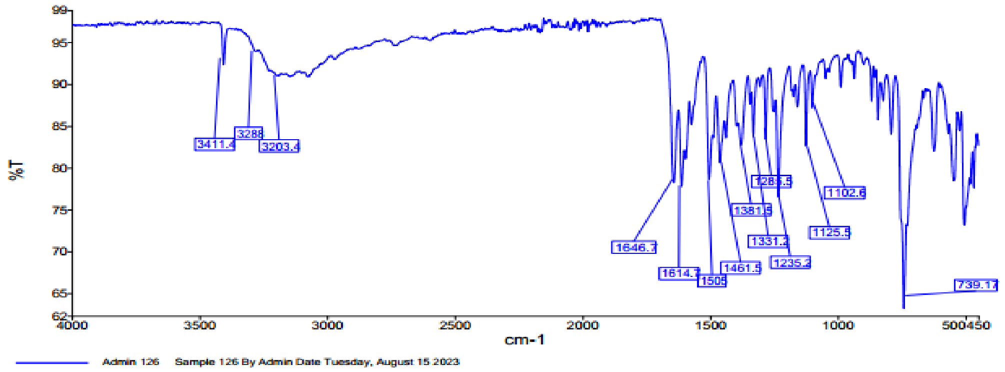


Figure 1: FTIR spectra of Schiff base NT1

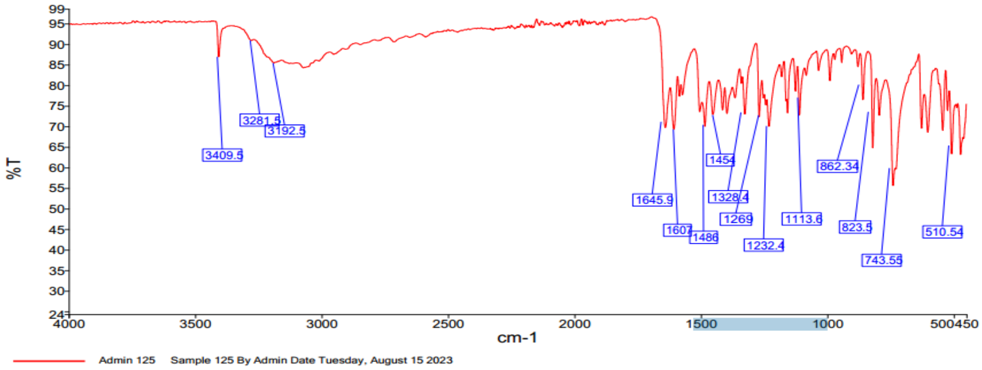


Figure 2: FTIR spectra of Schiff base NT2

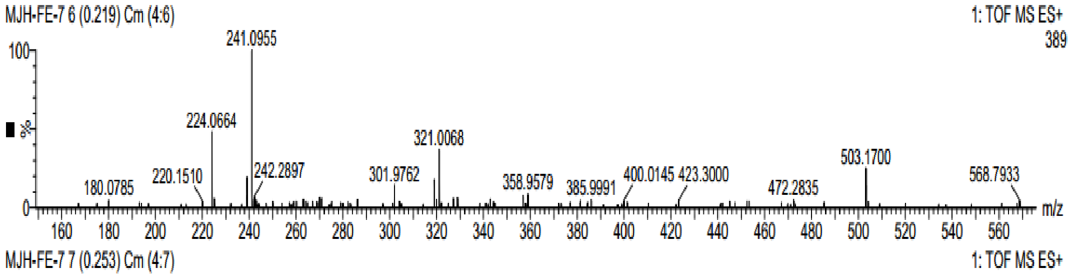


Figure 3: ESI-MS spectra of NT1 $[M + 1H]^{+1}$ m/z = 241.09

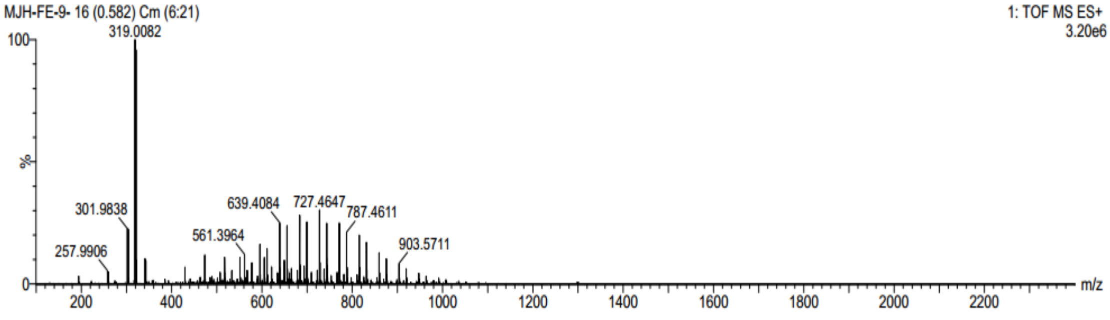


Figure 4: ESI-MS spectra of NT2 $[M + 1H]^{+1}$ m/z = 319.00

Electrochemical behavior of NT1 and NT2

The voltammogram of **NT1** and **NT2** shows that the free ligands are electroactive due to observed redox processes in the forward and reverse scan (**Figures 5a-b**). The voltammogram for **NT1 Figure 5a** revealed an irreversible process with anodic peak around 500 mV. For **NT2** ligand, a reversible process showing both anodic and cathodic peaks was observed. Redox process within the potential range of -550 to +550 mV **Figure 5b** is evident. A peak separation beyond 59 mV between the anodic and cathodic process is indicative of a quasi-reversible process (Abayneh *et al.*, 2018). But, similarity in the anodic and cathodic peak heights is indication that the process is fully reversible (Bard *et al.*, 2022). The ratio of the peak currents (i_{pa}/i_{pc}) of a cyclic voltammetry is important and usually employed to determine the reversibility of electrode reaction (Massot, 2009, Bard & Faulkner, 2001). Also, if the ratio obtained tends towards unity, this indicates redox reversibility and the electro-active species of the electrochemical reaction (Wu *et al.*, 2008). Analyzing the redox performance of **NT2**, i_{pa}/i_{pc} is equal to 1. This reversible process can be attributed to the effects of bromo, an electron withdrawing substituent, which has the ability to remove electrons from the system (Ejiah *et al.*, 2024).

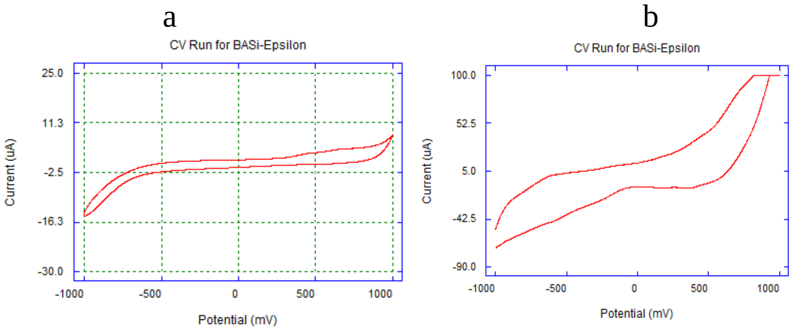


***Figure 5**: (a-b) Cyclic voltammogram for 50 mM NT1 and NT2 in acetonitrile containing triethylammonium phosphate (TEAP) as a supporting electrolyte at a scan rate of 100 mV s^{-1}*

Chemo-sensor activity of Schiff bases using UV absorption spectroscopy

The UV-visible measurement was carried out between 200-800 nm. The UV-vis spectrum of free**NT1** and **NT2** are shown in **Figures 6** and **7**. The solution of the free ligands gave peaks at 390 nm for **NT1** and **NT2** which is attributed to n → π* transitions of the imine functional group.

Upon complexation with Cu^{2+}, a red shift was observed. For the titration studies (**Figure 8**), varying volumes of the metal salt solution, 0.2 ml, 0.4 ml and 0.6 ml were added to the solution of the ligand (**NT1**). At low concentration of Cu^{2+}solution, a pale blue solution was formed, and a peak at 620 nm was observed. The peak observed at 620 nm can be attributed to *d-d* transition that occur within the complex.

A similar trend of interaction was observed for **NT2** (**Figure 9**). Absorption wavelength was also observed at 390 nm for **NT2**. A red shift occurred in the absorption wavelength of the probe upon complexation. On addition of $Cu^{2+}$of varying concentrations, peak around 690 nm was observed. The peak observed around 690 nm can be attributed to *d-d* transition that occur within the complex. This also confirms that both ligands **NT1** and **NT2**would exhibit fluorescence property. An increase in the volume of the metal ion even at low concentration led to an increase in absorbance and invariably increases the molar absorptivity.

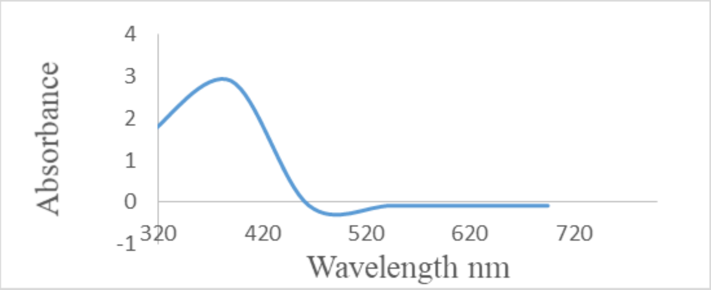


***Figure 6**: UV spectra of free NT1*

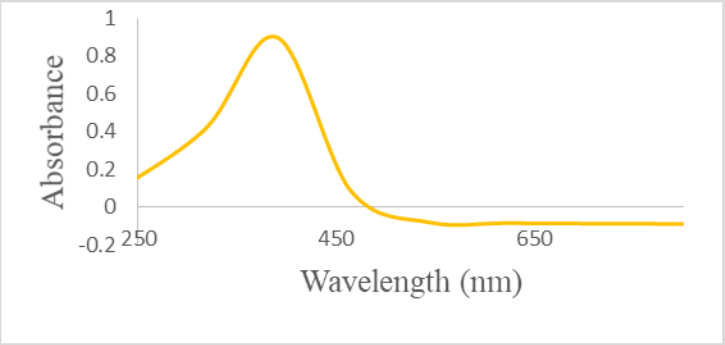


Figure 7: *UV spectra of free NT2*

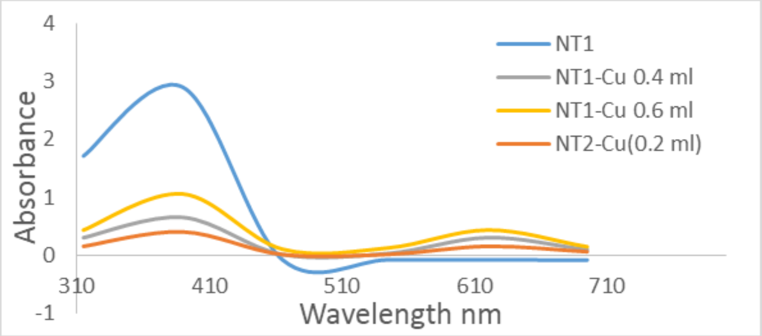


Figure 8: *UV titration of NT1 with Cu^{2+} at various concentrations (NT1-Cu (0.2ml) = 2.5 mM, NT1-Cu(0.4ml) = 0.625 mM, NT1-Cu(0.6ml) = 0.156 mM*

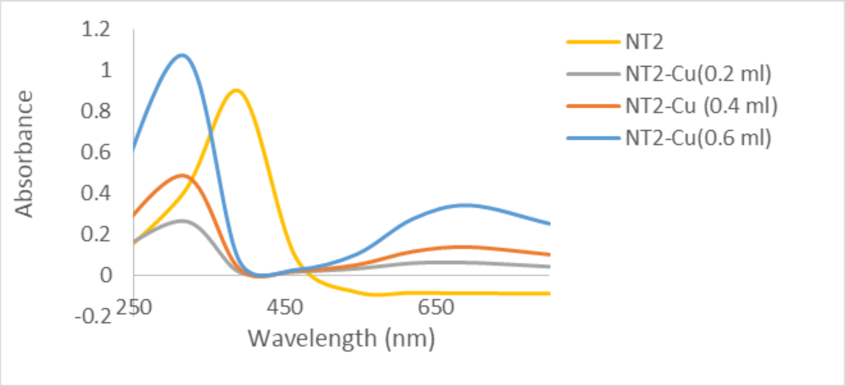


Figure 9: *UV titration of NT2 with Cu^{2+} at various concentrations (NT2-Cu(0.2ml) = 2.5 mM, NT2-Cu(0.4ml) = 0.625 mM, NT2-Cu(0.6ml) = 0.156 mM*

Conclusion

We have successfully synthesized new anthranilamide Schiff bases **NT1** and **NT2** using spectroscopic techniques such as FTIR and ESI-MS. Electrochemical results confirmed the electro-activeness of both ligands, with bromo substituent exhibiting a better redox process. The chemo-sensor study revealed the ability of both probes to detect copper ions in solution which is evident from the red shift in the electronic spectra. Also, increase in the volume of the metal ion at low concentration led to an increase in absorbance and invariably this increased the molar absorptivity. This work has shown the ability of these new ligands to act as chemo-sensor even at low concentrations, hence we would employ them to detect other important metals of biological importance. The use of other simple spectroscopic techniques would be employed.

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References

Abayneh, A. Gebretsadik, T. Tadesse, S. Thomas, M. (2018). Synthesis, spectroscopic, structural characterization, conductivity and electrochemical studies of a Schiff base ligand and its copper complexes, Adv Chem Eng Sci., 8:241-254

Alfimov, V. Fedorova, A. Gromov, S. (2003). Photos witchable molecular receptors Photochem Photobiol A: Chem., 158:183-198

Atwood, J. Holman, K. Steed, J. (1996). Laying traps for elusive preys: recent advances in non-covalent bonding anions. Chem Commun., 1401-1407

Bard, A. J. Faulkner, L. R. White, H. S. (2022). Electrochemical methods: fundamentals and applications, 3rd Edition, John Wiley & Sons., 1104 pp

Bard, A. J.Faulkner, L. R. (2001). Electrochemical methods: fundamentals and applications, New York, Wiley, 2nd ed., Russ J Electrochem., 38:1364-1365 (2002)

Callan, F.De Silva, P.Magri, D.(2005). Luminescent sensors and switches in the early 21st century, Tetrahedron., 61:8551-8588

De Vries**,** W. Romkens, M. Schutze, G. (2007). Critical soil concentrations of cadmium, lead, and mercury in view of health effects on humans and animals. Rev Environ Contam Toxicol., 191:91-130

Duraisamy, U. Inbaraj, V. (2020). Review on Schiff base fluorescent chemosensors for cell imaging applications. J Fluoresc., 30:1203-1223.

Ejiah, F. N. Rofiu, M. O. Akitoye, A. A. Fasina, T. M. (2024). Synthesis, characterization and electrochemical studies of Schiff bases containing 4-aminobenzoic acid moiety. J Chem Soc Nigeria., 49:172-186

Ejiah, F. N. Rofiu, M. O. Oloba-Whenu, O. A. Fasina, T. M. (2023). Schiff bases as analytical tools: synthesis, chemo-sensor, and computational studies of 2-aminophenol Schiff bases, Mater Adv, 4, 2308-2321

Fu, F. Wang, Q. (2010). Removal of heavy metal ions from wastewaters: a review. J Environ Manag., 92:407-418

Fu, Y. Mu, L. Zeng, X. Zhao, J. Redshaw, C. Yamato, T. (2013). An NBD-armed thiacalix [4]arene-derived colorimetric and fluorometric chemosensor for Ag+: a metal-ligand receptor of anions. Dalton Trans., 42:3552-3560

Gerdan, Z. Saylan, Y. Denizli, A. (2022). Recent advances of optical sensors for copper ion detection. Micromachines-Basel., 13:1298

Huang, S. Du, P. Min, C. Liao, Y. Sun, H. Jiang, Y. (2013). Poly (1-amino-5-chloroanthraquinone): highly selective and ultrasensitive fluorescent chemosensor for ferric ion. J. Fluoresc., 23:621-627

Kim, H. Ren, W. Kim, J. Yoon, J. (2012). Fluorescent and colorimetric sensors for detection of lead, cadmium, and mercury ions. Chem Soc Rev., 41:3210-3244

Kubo, K. Sakurai, T. (2000). Molecular recognition of pet fluoroionophores. Heterocycles., 52: 945-976

Lehn, J. (1995). In:supramolcular chemistry-concept and perspective. VCH, Weinheim, x, 271 pp

Li, M. Zhang, D. Liu, Y. Ding, P. Ye, Y. Zhao, Y. (2014). A novel colorimetric and off-on fluorescent chemosensor for Cr^{3+} in aqueous solution and its application in live cell imaging. J Fluoresc., 24:119-127

Li, Y. Wang, H. Tang, H. (2020). Chemo/bionanosensors for medical applications, in micro and nano technologies. Nanosensors for Smart Cities., pp 483-500

Massot, L. Chamelot, P. Cassayre, L. Taxil, P. (2009). Electrochemical study of the Eu(III)/Eu(II) system in molten fluoride media. Electrochim Acta., 54:6361-6366

Mcrae, R. Bagchi, P. Sumalekshmy, S. Fahrni, C. (2009). In situ imaging of metals in cells and tissues. Chem Rev., 109: 4780-4827

Mitra, S. Chakraborty, A. J. Tareq, A. M. Emran, T. B. Nainu, F. Khusro, A. Idris, A. A. Khandaker, M. U. Osman, H. Alhumaydhi, F. A. Simal-Gandara, J. (2022). Impact of heavy metals on the environment and human health: novel therapeutic insights to counter the toxicity, J King Saud Univ Sci., 34, 101865

Montalti, M. Prodi, L. Zaccheroni, N. (2006). Handbook of photochemistry and photobiology. 3rd ed. Boca Raton, 3, pp 271

Myint, Z. W. Oo, T. Thein, K. Z. Tun, A.M. Saeed, H. (2018). Copper deficiency anemia: review article. Ann Hematol., 97:1527-1534.

Padhan, S. Murmu, N. Mahapatra, S. Dalai, M. Sahu, N. (2019). Ultrasensitive detection of aqueous Cu^{2+} ions by a coumarin-salicylidene based AIEgen. Mater Chem Front., 3:2437-2447

Piwowarska, D. Kiedrzyńska, E. Jaszczyszyn, K. (2024). A global perspective on the nature and fate of heavy metals polluting water ecosystems, and their impact and remediation. Crit Rev Environ Sci Technol., 1-23

Qasem, N. A. A. Mohammed, R. H. Lawal, D. U. (2021). Removal of heavy metal ions from wastewater: a comprehensive and critical review. NPJ Clean Water., 4, 36

Sall, M. L. Diaw, A. K. D. Gningue-Sall, D. *et al.* (2020). Toxic heavy metals: impact on the environment and human health, and treatment with conducting organic polymers, a review. Environ Sci Pollut Res., 27, 29927-29942

Sharma, N. Sodhi, K. K. Kumar, M. Singh, D. K. (2021). Heavy metal pollution: insights into chromium eco-toxicity and recent advancement in its remediation. Environ Nanotechnol Monit Manag., 15:100-388

Silva, A. Gunaratne, H. Gunnlaugsson, T. Huxley, A. McCoy, C. Rademacher, J. Rich, E. (1997). Signalling recognition event with fluorescent sensors and switches. Coord Chem Rev., 97:1515-1566

Singh, S. Kumar, V. Dhanjal, D. S. Datta, S. Prasad, R. Singh, J. (2020). Biological biosensors for monitoring and diagnosis. Micro bBiotechnol: Basic Research and Applications., pp 317-335

Spichiger-Keller, U. (2008). Chemical sensors and biosensors for medical and biological applications. John Wiley & Sons, New York, 425 pp

Stern, B. (2010). Essentiality and toxicity in copper health risk assessment: overview, update and regulatory considerations. J Toxicol Environ Health A., 73:114-127

Valeur, B. Leray, I. (2000). Design principles of fluorescent molecular sensors for cation recognition. Coord Chem Rev., 205:3-40

Wang, Y. Hao, X. Liang, L. Gao. L. Ren, X. Wu, Y. Zhao, H. (2020). A coumarin-containing Schiff base fluorescent probe with AIE effect for the copper (II) ion. RSC Adv., 10:6109-6113.

Wazir, S. M. Ghobrial, I. (2017). Copper deficiency, a new triad: anemia, leucopenia, and myeloneuropathy. J Community Hosp Intern Med Perspect., 7:265-268

Witkowska, D. Słowik, J. Chilicka, K. (2021). Heavy metals and human health: possible exposure pathways and the competition for protein binding sites. Molecules., 26:6060

World Health Organization. (2008). Guidelines for drinking-water quality (3rd ed) vol 1, Geneva., pp 188

Wu, W. Guo, S. Zhang, J. (2018). Electrochemical behaviors of Cr (III) in molten LiF-NaF-KF Eutectic. Int. J Electrochem Sci., 13:225-234

Zhang, D. Wang, M. Wang, C. Li, M. Ye, Y. Zhao, Y. (2013). Two highly sensitive and selective colorimetric “off-on” rhodamine-based fluorescent chemosensor for Hg(II) in aqueous media. J Fluoresc., 23:1045-1052