ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal
www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2025, Vol. 41, No.(3):
Pg. 1032-1039

Achyranthes aspera Extract Mediated Synthesis of Cobalt Complex as an Efficient Fluorescent probe for Uric acid Detection

ELAKKIYA SUBRAMANI[1], KALAISELVI THANGARAJ[1], ABIRAMI MAHARAJAN[2] and SELVI GOPAL[1]*

1*Department of Chemistry, PSGR Krishnammal College for women, Coimbatore, India.
[2]Department of Chemistry, Sri Ramakrishna Engineering College, Coimbatore, India.
*Corresponding author E-mail: selvig@psgrkcw.ac.in

http://dx.doi.org/10.13005/ojc/410338

(Received: March 24, 2025; Accepted: May 03, 2025)

ABSTRACT

Plant-mediated synthesis is gaining popularity due to its toxic-free constituents, eco-friendly nature, and other advantages over conventional synthesis. In this work, a new strategy towards the synthesis of the Schiff base ligand (1) N-(2-hydroxyphenyl) -2-methoxy phenyl azomethine and its Cobalt complex (2), using the cold methanolic extract of the leaves of the plant species *Achyranthes aspera (Linn).* is successfully achieved. Synthesized ligand and the complex were characterized by spectral and analytical data. The size and the composition of the complex were confirmed by the SEM and SEM–EDAX analysis respectively. SEM analysis of the complex showed a nanorod like structure with an average particle size of 40-60nm. The nano cobalt complex thus obtained is further used as a fluorescent probe for the detection of Uric acid (UA). Uric acid is detected by the cobalt complex using UV-detector, UV-Visible spectrophotometer and PhotoLuminescence (PL) studies. The concentration of cobalt complex is taken as 1×10^{-6} M and the concentration of Uric acid, ranges from 50-500 µL. UV detector showed an intense fluorescence when irradiated with 254nm, the intensity of the colour increases with increase in concentration of Uric acid. The UV-Visible spectrum showed an enhanced absorption as the uric acid is added to the Cobalt Complex Probe and absorbance increases with increase in uric acid concentration at the same wavelength. The PL studies showed a remarkable quenching when Uric acid is added to the probe and the quenching increases with increase in concentration of Uric acid. Thus, the Cobalt complex synthesized can be used as an efficient Uric acid sensor.

Keywords: Cobalt complex fluorescent probe, *Achyranthes aspera*, Uric acid and photo luminescence.

INTRODUCTION

Recently, synthesis of novel compounds, as well new route for the already reported compounds has been continuously explored by adopting greener ways. In that series, synthesis of organic compounds using plant extract is of major attention. Schiff base ligands with nitrogen and oxygen donor atoms are efficient chelators for transition and non-transition metals[1]. The Schiff base ligands are prepared by the condensation reaction between different carbonyl compounds and amines to form azomethine (-C=N-) group[2]. Because of their ease of synthetic flexibility, these ligands are regarded as privileged ligands[3]. Considering their importance in both synthetic and structural study, Schiff bases have a wide range of applications and their metal complexes are widely synthesized due to its enhanced applications in various fields[4-7]. Cobalt complexes obtained from the Schiff bases found to be effective against various diseases such as antimicrobial[8,9], antiviral[10,11] anticancer[12,13] and antiproliferent[14]. One of the transition metal ions, cobalt plays an important role in many biological activities of multicellular organisms[15-18]. Cobalt is required for the nutritional process of humans and animals. It is essential for the synthesis of haemoglobin in the metabolism of vitamin B^{12} and iron, which is required for normal fatty acid metabolism[19-21]. Green synthesis of Cobalt complexes has received a lot of attention in fluorescence sensing applications[22,23]. The plant *Achyranthes aspera (Linn)* belongs to the Amaranthaceae family and is found primarily in Asian and African nations. It is a shrub with therapeutic applications and possesses a variety of biological and pharmacological activities[24]. Uric acid (UA) is an important biomolecule in the human body and an end product of purine metabolism[25]. Safety limit of uric acid in urine and serum is 1.49 to 4.46 mM and 0.15 to 0.42 mM respectively[26,27]. Increase in limiting value of uric acid leads to gout, renal illness, cardiovascular disease, hyperuricemia, kidney disorders and decrease in uric acid level would cause hypouricemia which leads to genetic illness[28-30]. Uric acid detection has been carried out in various methods including surface plasmon resonance[31], Colorimetry[32], fluorescence[33], capillary electrophoresis[34], electrochemistry[35], chromatography[36] and enzymatic methods[37].In this work we have attempted a facile synthetic route for the Schiff base and its cobalt complex by using the plant extract viz Achyranthes aspera. The cobalt complex thus obtained is further used for the detection of uric acid by a simple and cost-effective method viz. UV and photoluminescence study.

MATERIALS AND METHODS

Chemicals used were procured from Sigma Aldrich, Merk and of Analar grade. Fourier transform infra-red (FTIR) spectrophotometer were recorded by IR Affinity-1S (Shimadzu), UV-Visible spectra were recorded on UV Visible spectrophotometer (LABINDIA UV 3000+) ranges 200–800 nm at room temperature, NMR spectroscopy were recorded in Bruker Avance 400 MHz, Fluorescence detection was performed with UV detector lamp (DEEP VISION make), Photoluminescence spectrometer were recorded from JASCO-FP-8300 spectrofluorometer. The morphological nature of sample was identified by EVO 18 with ALTO 1000 cryo model of SEM.

Preparation of Cold methanolic of the plant *Achyranthes aspera*

Achyranthes aspera is collected from the Dharmapuri district and the leaves are shade dried, powdered and used further.

50 g of the powdered plant material is soaked with 150 mL of methanol and kept for three days. The mixture was shaked at regular intervals to ensure efficient extraction. The colour of the solvent turns dark green, completion of the extraction is confirmed, as there is no further change in the colour of the extract. The content of the mixture is filtered and the methanolic solution was concentrated the cold methanol extract thus obtained is further taken as the medium for the synthesis of Schiff base (1) and Cobalt complex (2).

Synthesis of N-(2-hydroxyphenyl)-2-methoxy phenyl azomethine (1)

To 20 mL of the methanolic leaf extract of *Achyranthes aspera*, was added, equal moles (49.810mmol) of o-anisidine and salicylaldehyde and stirred for 5 minutes. A yellow precipitate separates out. The excess extract was filtered and the precipitate was washed with petroleum ether and dried. Melting point: 59°C, (Lt.M.pt: 57°C)[38], yield: 85%. Infra red absorption spectrum of the ligand (1) Fig. 1 showed bands at 1612 cm^{-1} (CH=N), 3062 cm^{-1} (OH), 1278 cm^{-1} (OCH_3). ^{1}H-NMR (DMSO-d_6) of the ligand (1) Fig. 3 Showed a peak at δ 8.9 ppm (CH=N) singlet, δ 6.94-7.62 ppm (8 protons, for Aromatic protons), δ 2.6 ppm (s, 3H, OCH_3), δ 3.6 ppm (OH). ^{13}C-NMR (DMSO-d_6) of the ligand (1) Fig. 4: δ 60 ppm (OCH_3), δ 112, 117, 119, 121, 128, 132, 133, 136, 153, 161, 163 ppm. TGA- DTA Curve Fig. 7: 191.52°C (-77.889%), 274.42°C (-17.301%).

Synthesis of Cobalt complex (2)

To 20 mL of extract, added equal moles of N-(2-hydroxyphenyl)-2-methoxy phenyl azomethine and $CoCl_2.6H_2O$ was stirred for 2 hours. The colour change of the compound from reddish yellow to green was noticed. Completion of the reaction was further confirmed by TLC. The product obtained was recrystallized from ethanol and chloroform mixture, green colour solid was obtained. Melting point: 280°C, yield: 62%. IR spectrum of the complex (2) Fig. 2: 3334 cm^{-1} (OH), 1624 cm^{-1} (CH=N), 734 cm^{-1}. ^{1}H-NMR (DMSO-d_6) the complex (2) Fig. 5: δ 2.5 ppm (-OCH_3), δ 8.9 ppm (-CH=N) a broad peak at δ 4.4 ppm for OH of H_2O. ^{13}C-NMR (DMSO-d_6) Fig. 6: δ 60 ppm (-OCH_3), δ 110, 112, 117, 119, 120, 129, 133, 161, 163 ppm. TGA-DTA curve Fig. 8: 41.08°C (-5.720%), 203.9°C (-17.482%), 401.28 (-11.543%).

SEM–EDAX Analysis

SEM analysis Fig. 9 of the complex showed the size of the particle to be 40–60 nm. SEM-EDAX Fig. 10 showed the presence of Cobalt, oxygen, carbon and chlorine.

Fluorescence spectral studies

UV detector analysis

Stock solution of cobalt complex (2), (fluorescent Probe) 1x10^{-6} M concentration was prepared in ethanol and stock solution of uric acid was also prepared at 1x10^{-6} M concentration in distilled water.

To examine the ability of uric acid with fluorescent probe, the stock solution of the Uric acid is further diluted to 50 µL-500 µL, at regular intervals of 50 µL.

2 mL of uric acid of different concentration (50-500 µL) is added to 2 mL of the fluorescent probe (Cobalt Complex) and their fluorescence is noted for each concentration under irradiation of visible, short and long UV range respectively. The results are as shown in Fig. 11(a-c).

UV-Visible spectral analysis

2 mL of cobalt complex in ethanol and 2 mL of uric acid in distilled water was taken from the prepared stock solution at 1x10^{-6} M concentration mixed well, and irradiated under UV-Visible spectroscopy in Fig. 12.

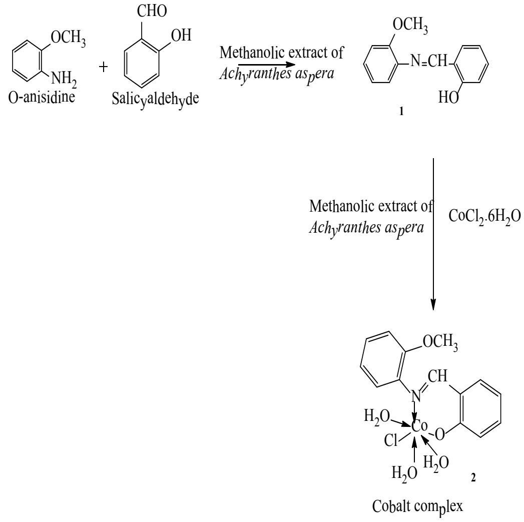
Photoluminescence spectral analysis

Similar to the UV-radiation, the samples are analysed in the photoluminescence spectroscopy at an excitation of 310nm, the results obtained are shown in the Fig. 14 and 15 respectively.

RESULTS AND DISCUSSION

A new synthetic approach using the plant extract *Achyranthes aspera* is adopted for the conventionally synthesised ligand and Cobalt complex39 towards the synthesis of rod like nano sized Cobalt complex with 40-60nm in size. Cobalt complex obtained by the synthesis of Schiff base which in turn prepared from the reaction of salicylaldehyde and o-anisidine using the cold methanolic extract of the plant *Achyranthes aspera* as reaction medium.

Accordingly, Equal moles of o-anisidine and Salicylaldehyde are stirred with the reaction medium viz., Cold methanolic extract of *Achyranthes aspera*. The Schiff base ligand (1) thus obtained was further stirred with $CoCl_2.6H_2O$ in the cold methanolic medium of the pant extract. The product obtained was rinsed with Petroleum ether and recrystallised from $CHCl_3$ and ethanol. The reaction sequence was represented in Scheme 1. In this novel approach, a remarkable decrease in reaction time and increase in yield for both the Schiff base and the complex is absorbed when compared to the reported methods[39].



Scheme 1. Green synthesis of Cobalt complex

Infra red Spectral studies

The IR spectrum of N-(2-hydroxyphenyl)-2-methoxy phenyl azomethine (1) showed absorption at 1612 cm^{-1} for azomethine and 3602 cm^{-1} for the phenolic OH, 1278 cm^{-1} for methoxy group of the Schiff base as illustrated in Fig. 1. IR spectrum of the complex (2) showed a broad absorption at 3334 cm^{-1} for OH, 1624 cm^{-1} for azomethine and 734 cm^{-1}. Disappearance of 3062 cm^{-1} band of the ligand in the complex confirms the formation of the complex through phenolic OH and a shift in stretching frequency from 1612 cm^{-1} to 1642 cm^{-1} confirms the linkage of nitrogen in the complexation, also a band at 734 cm^{-1} shows that N-Co bond is formed, thus formation of Cobalt complex through N-Co-O has been confirmed from the spectrum.

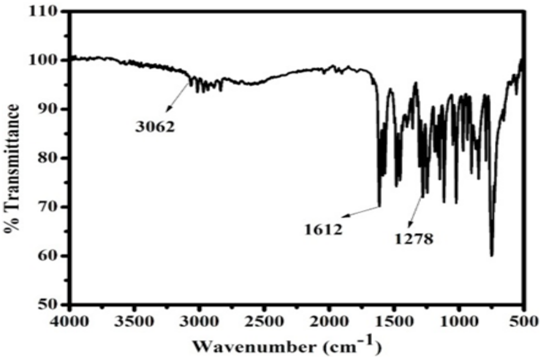


Fig. 1. FT IR spectrum of ligand (1)

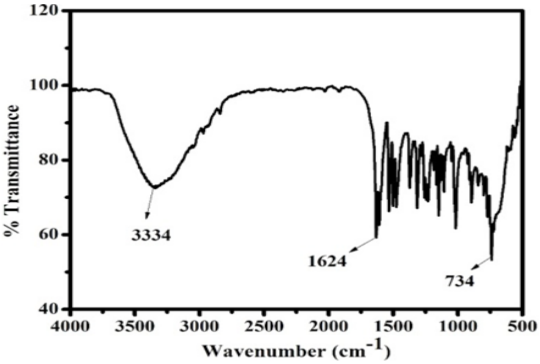


Fig. 2. FT IR spectrum of Cobalt complex (2)

Proton-NMR and Carbon-13-NMR spectral studies

Disappearance of a peak at δ 3.6 ppm due to phenolic OH of ligand (1), ^{1}H-NMR spectrum of the complex (2) and appearance of a broad peak at δ 4.42 ppm further confirms the formation of complex through N-Co-O as shown in Fig. 3 and 5.^{13}C-NMR spectrum of Fig. 6 also confirmed the formation of cobalt complex.

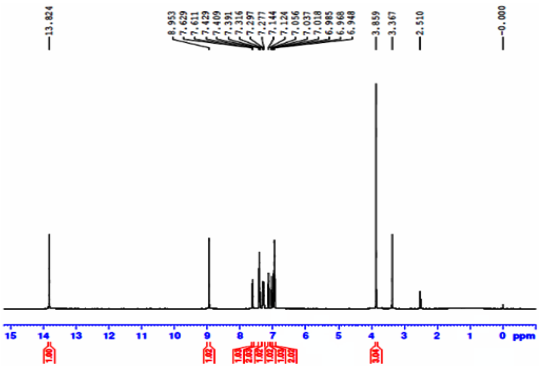


Fig. 3. ^{1}H-NMR spectrum of ligand (1)

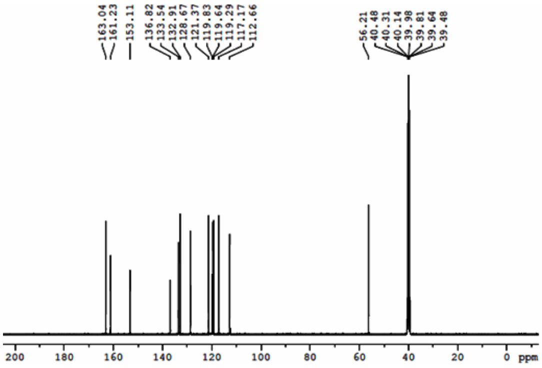


Fig. 4. ^{13}C-NMR spectrum of ligand (1)

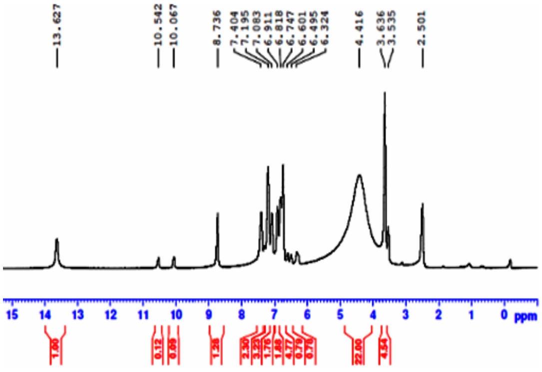


Fig. 5. ^{1}H-NMR spectrum of Cobalt complex (2)

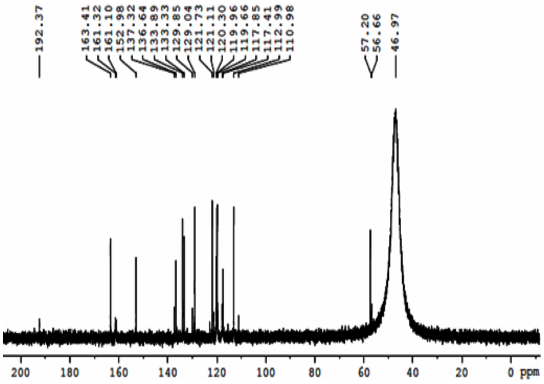


Fig. 6. ^{13}C-NMR spectrum of Cobalt complex (2)

TG-DTA studies

Fig. 7 displays TG-DTA of the ligand (1) with continuous decomposition starting from 40.9°C to 271.75°C with a loss of (77.889%) weight loss and at 274.42°C to 597.20°C with a loss of (17.30%) due to remaining organic moiety. Fig. 8 shows TG-DTA of the complex (2) decomposition from 41.08°C to 199.12°C with a loss of (5.750%) coordinated (H_2O) of the complex, second decomposition from 203.93°C to 398.61°C with loss of (17.482%) coordinated water molecule and final decomposition at 401.24°C to 598.90°C with loss of (11.543%) a complex decomposition was noticed. The TGA of the complex showed a smooth decomposition from 203.73°C to 596.9°C.

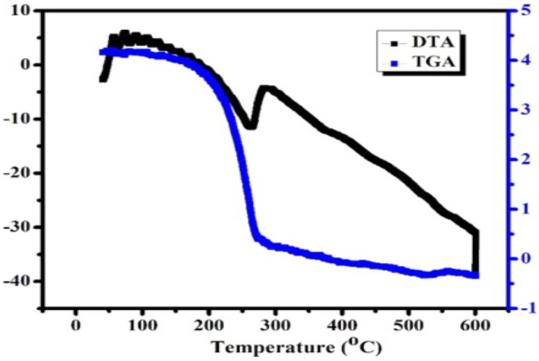


Fig. 7. TGA-DTA of ligand (1)

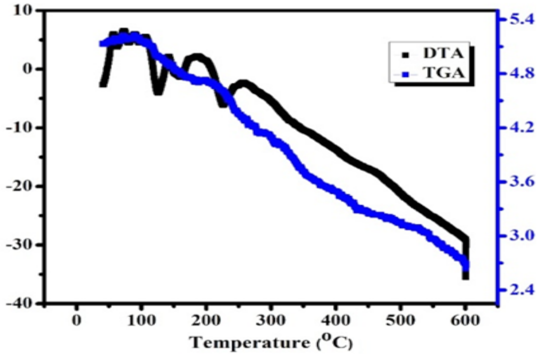


Fig. 8. TGA-DTA of complex (2)

SEM-EDAX analysis

Surface morphologies of the synthesized cobalt complex (2) at 2μm magnification, clearly showed the complex is rod shaped nano particle of size with the average particle size of 40–60 nm in Fig. 9. It confirms the particle size control in cobalt complex with the help of Achyranthes aspera leaf extract. SEM-EDAX Spectrum of the complex showed the presence of carbon, nitrogen, oxygen, chlorine and Cobalt in appropriate proportion as illustrated in Fig. 10.

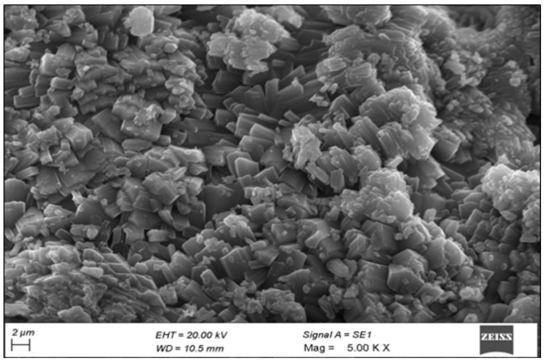


Fig. 9. SEM image of Cobalt complex (2)

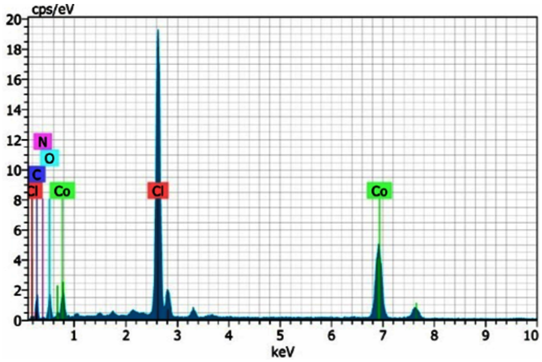


Fig. 10. SEM-EDAX spectrum of cobalt complex (2)

Fluorescence under UV detection

The cobalt complex (2) of 1x10^{-6} M is irradiated under the UV detector at visible, 254nm (Short UV) and 365nm (long UV) respectively. There is a no noticeable colour change for the complex (2) solution and fixing the solution as a fluorescent probe, the uric acid of various concentrations ranging from 50 μL to 500 μL is diluted from the stock solution and irradiated under UV detector. By increasing uric acid concentration from 50-500 μL a noticeable colour change was observed from pale yellow to dark yellow in visible light in Fig. 11(a), light green to dark green coloured fluorescence was observed at 254nm as shown in Fig. 11(b) and moderate colour change was detected under long uv light at 365nm under UV detector in Fig. 11(c) respectively. The vial labelled 0 μL consists of only the cobalt complex solution ie., the fluorescent probe, from the figure, it is noted that to visible light and short UV, showed a remarkable colour change and the short UV fluorescence on addition of uric acid to the fluorescent probe.

UV-Visible spectral analysis of uric acid detection

The stock solution of cobalt complex and uric acid was irradiated under UV-Visible spectroscopy. An absorption at 204nm and 324nm for cobalt complex absorbed was shown in Fig. 12.

When uric acid of 1x10^{-6} M concentration is added to the fluorescence probe an increase in absorption intensity is noticed. Hence the UV-Visible irradiation is extended to analyse the detection of uric acid with concentration ranging from 50-500 µL with an equal interval of 50 µL for each concentration is analyzed and the results are as shown in Fig. 13.

From the UV-Visible spectrum of Fig. 13 it was concluded that there was an increase in absorption on increasing the concentration of uric acid with constant concentration of the fluorescence probe.

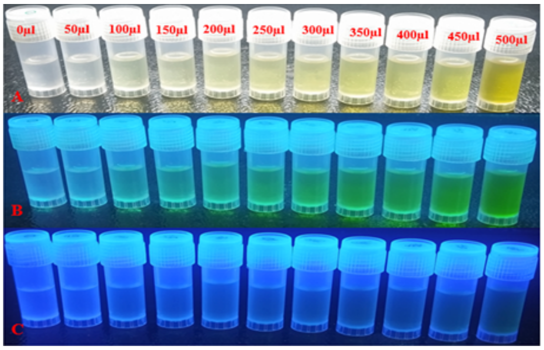


Fig. 11. Colour change of cobalt complex with different concentration (0-500 µL) of uric acid in UV light (a) Visible light, (b) short UV, (c) long UV

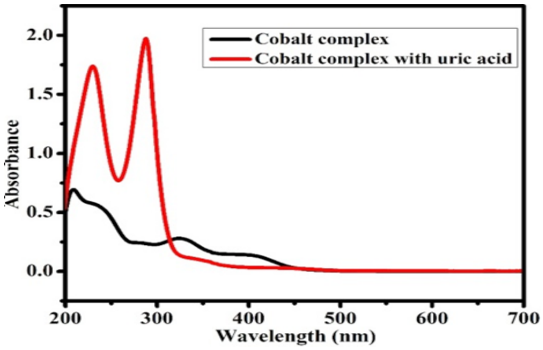


Fig. 12. Absorption response of cobalt complex and complex+uric acid

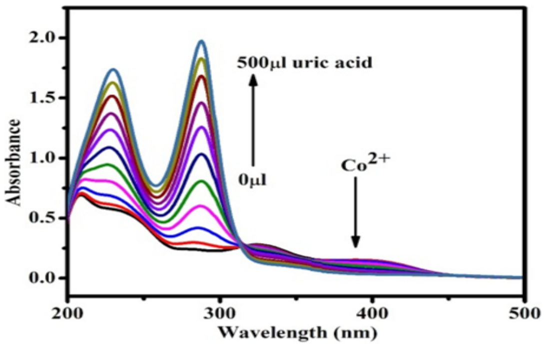


Fig. 13. UV-Visible spectra of cobalt complex on addition of different concentration of uric acid (0-500 µL)

Photoluminescence studies

2 mL of cobalt complex in 1x10^{-6} M concentration solution and 2 mL of uric acid in 1x10^{-6} M concentration solution was mixed to perform the photoluminescence spectrum study. After fixed upon the excitation wavelength at 310nm, cobalt complex intensity peak was observed at 348nm. on gradual addition of uric acid, fluorescence quenching was observed at the same excitation as shown in Fig. 14. The selective response of cobalt complex towards uric acid was carried out at by gradual addition of uric acid of increasing concentration from 50–500 µL with the solution of cobalt complex (1x10^{-6} M). As the concentration of uric acid increased the intensity peak at 348 nm was gradually decreased as shown in Fig.15(a). The corresponding linear function of the calibration curve $y=0.2928x+163.347$ with the correlation coefficient $R^2=0.9900$ which was calculated using the equation[40] $y=mx+c$ (1) Fig. 15(b). The observed intercept and slope from the graph was used to calculate the limit of detection and quantification (LOQ) using the formula 3 x Standard Deviation/slope and 10 x Standard Deviation/slope respectively[41]. The observed LOD and LOQ values are 49.7 µm and 16.52 µm.

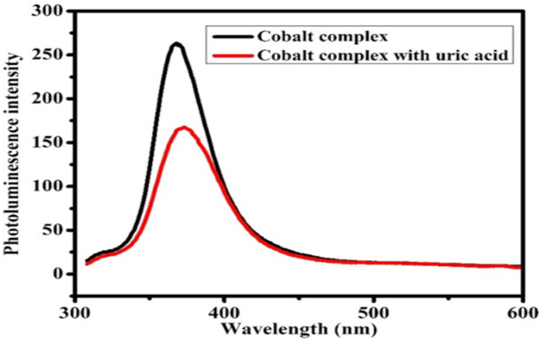
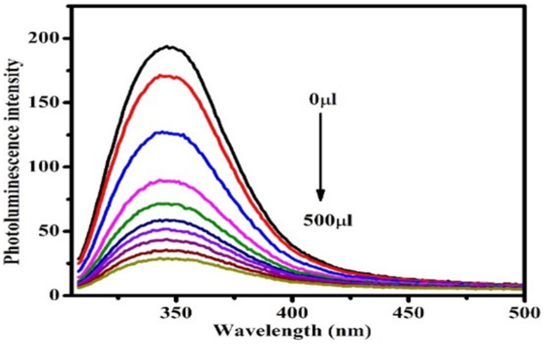
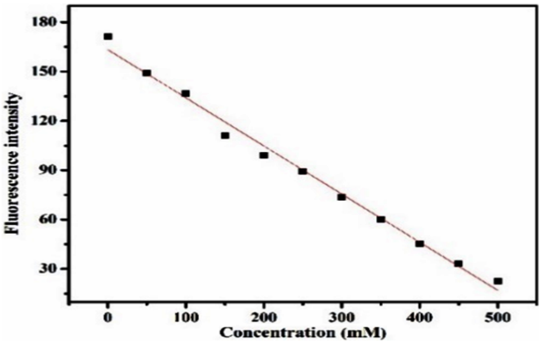


Fig. 14. Photo luminescence spectra of Cobalt complex and Complex with uric acid



(a)



(b)

Fig. 15(a). PL Spectra of Cobalt complex on addition of different concentration of uric acid (0–500 μL), (b) The linear relationship between fluorescence intensity and concentration of uric acid.

In this study, a simple, cost-effective and green approach for synthesis of the Schiff base (1) and Cobalt complex (2) using methanolic leaf extract of *Achyranthes aspera* is reported for the first time the structure of the ligand and the complex is confirmed by spectral analysis. SEM analysis showed rod like nano structure with average particle size of 40–60 nm which confirms the size of cobalt complex as nanoparticle. Detection of Uric acid is carried out with the cobalt complex as a fluorescent probe using UV detector, UV-Visible Spectroscopy and PL spectroscopy. From the results obtained, it was concluded that the Cobalt Complex (2) is an effective probe for the detection of Uric acid. Further this method is to be extended to examine the real time human samples, which would provide a convenient, fast and real-time sensor device for a day-to-day monitoring in clinical medicine.

ACKNOWLEDGEMENT

Authors Acknowledge the DST FIST, New Delhi for the infrastructure provided. The authors also duly acknowledge the Department of Nanoscience and Nanotechnology, Sri Ramakrishna Engineering College for the utilization the Photoluminescence spectroscopy.

Conflict of interest

No conflict of interest.

REFERENCES

1. Manjuraj, T.; Yuvaraj, T. C. M.; Jayanna, N. D.; Shreedhara, S. H.; Sarvajith, M. S., *J. of the Turk. Chem. Soc.*, **2020**, *7*, 449-462.
2. Manikshete, A. H.; Sarsamkar, S. K.; Deodware, S. A.; Kamble, V. N.; Asabe, M. R., *Inorg. Chem. Comm.*, **2011**, *14*, 618-621.
3. More, M. S.; Joshi, P. G.; Mishra, Y. K.; Khanna P. K., *Mater. Today Chem.,* **2019**, *18*, 100195.
4. Salahuddin, M. S.; Mazumder, A.; Abdullah, M. M., *Arab. J. Chem.*, **2017**, *10*, 503-508.
5. Zolezzi, S.; Spodine, E.; Decinti, A., *Polyhedron.*, **2002**, *21*, 55-59.
6. Ambike, V.; Adsule, S.; Ahmed, F.; Wang, Z.; Afrasiabi, Z.; Sinn, E.; Sarkar, F.; Padhye, S., *J. Inorg. Biochem.*, **2007**, *101*, 1517-1524.
7. Rahchamani, J.; Behzad, M.; Bezaatpour, A.; Jahed, V.; Dutkiewicz, G.; Kubicki, M.; Salehi, M., *Polyhedron.*, **2011**, *30*, 2611-2618.
8. Nagesh, G. Y.; Raj, K. M.; Mruthyunjayaswamy, B. H. M., *J. Mol. Struct.*, **2015**, *1079*, 423-432.
9. Salehi, M.; Amoozadeh, A.; Salamatmanesh, A.; Kubicki, M.; Dutkiewicz, G.; Samiee, S.; Khaleghian, *J. Mol. Struct.*, **2015**, *1091*, 81-87.
10. Shukla, S.; Srivastava, R. S.; Shrivastava, S. K.; Sodhi, A.; Kumar, P., *Med. Chem. Res.*, **2013**, *22*, 1604-1617.
11. Nejo, A. A.; Kolawole, G. A.; Nejo, A. O., *J. Coord. Chem.*, **2010**, *63*, 4398-4410.
12. Sangeetha Gowda, K. R.; BhojyaNaik, H. S.; VinayKumar, B.; Sudhamani, C. N.; Sudeep, H. V.; Ravikumar Naik, T. R.; Krishnamurthy, G. Spectrochim. Acta-A: Mol. Biomol., *Spectrosc.*, **2013**, *105*, 229-237.
13. Gupta, V. K.; Singh, A. K.; Mehtab, S.; Gupta, B., *Analytica Chimica Acta.*, **2006**, *566*, 5–10.
14. Pradeepa, S. M.; Bhojya Naik, H. S.; Vinay Kumar, B.; Indira Priyadarsini, K.; Atanu Barik.; Ravikumar Naik, T. R. Spectrochim., *Acta-A: Mol. Biomol. Spectrosc.*, **2013**, *101*, 132–139.
15. Alamgir, S.; Rhaman, M. D.; Basaran, I.; Douglas, R. P.; Hossain, A., *Polyhedron.*, **2020**, *187*, 114681.
16. Mahajan, P. G.;Dige, N. C.;Desai, N. K.; Patil, S. R.; Kondalkar,V.V.; Hong, S.; Lee., K. H., *Spectrochim. Acta-A: Mol. Biomol. Spectrosc.*, **2018**, *198*, 136-144.
17. Liu, Z.; Wang, W.; Xu, H.; Sheng, L.; Chen, S.; Huang, D.; Sun, F., *Inorg. Chem. Commun.*, **2015**, *62*, 19-23.
18. Lee, J. H.; Jeong, A. R.; Shin, I. S.; Kim, H. J.; Hong, *J. Org. Letters.*, **2010**, *4*, 764-767.

19. Abebe, F. A.; Eribal, C. S.; Ramakrishna, G.; Sinn, E., *Tetrahedron Letters*., **2011**, *52*, 5554-5558.
20. Sie, Y.; Wan, C.; Wu, A., *RSC Advances*., **2017**, *7*, 2460-2465.
21. Xiang, D.; Zhang, W.; Dong, Z.; Chen, W.; Wang, J.; Xu, H.; Lu, H., *Inorg. Chem. Commun*., **2019**, 107582.
22. Patra, D.; Kurdi, R. E., *Green Chem. Lett. Rev*., **2021**, *14*, 474-487.
23. Singh, A. K., *Curr. Res. Green Sustain. Chem*., **2022**, *5*, 100270.
24. Swetha, V.; Lavanya, S.; Sabeena, G.; Pushpalaksmi, E.; Jenson Samraj, J.; Annadurai, G. J., *J. Appl. Sci. Envir. Manag*., **2020**, *24*, 1161.
25. Matsoso, B. J.; Mutuma, B. K.; Billing, C.; Ranganathan, K.; Lerotholi, T.; Jones, G.; Coville, N. J., *J. Electroanal. Chem*., **2019**, *833*, 160-168.
26. Wang, H.; Lu, O.; Hou, Y.; Liu, Y.; Zhang, Y., *Talanta*., **2016**, *155*, 62-69.
27. Azmi, N. E.; Ramli, N. I.; Abdullah, J.; Hamid, M. A. A.; Sidek, H.; Rahman, S.A.; Ariffin, N.; Yusof, N. A., *Biosens. Bioelectron*., **2015**, *67*, 129-133.
28. Xu, P.; Li, R.; Tu, Y.; Yan, *J. Talanta*., **2015**, *144*, 704-709.
29. Choi, H. K.; Mount D. B.; Reginato, A. M., *Annals ofInternal Medicine*., **2005**, *143*, 499-516.
30. Wang, Q.; Wen, X.; Kong., *Crit. Rev. Anal. Chem*., **2020**, *50*, 359-375.
31. Misra, N.; Kumar, V.; Borde, L.; Varshney, L., *Sens. Actuators B: Chem*., **2013**, *178*, 371-378.
32. Mainak, G.; Jayasmita, J.; Anjali, P.; Tarasankar, P., *RSC Advances*., **2016**, *6*, 17683-17703.
33. Yanan, Y.; Zhengyan, Z.; Yang, Y.; Guanglan, L.; Ce, H., *New J. Chem*., **2019**, *43*, 9274.
34. Wang, Y.; Chen, H., *J. Chromatogr. A*., **2005**, *1080*, 192-198.
35. Sha, R.; Vishnu, N.; Badhulika, S., *Sens. Actuators B: Chem*., **2019**, *279*, 53-60.
36. Cox, G.B.; Loscombe, C. R., *Upfield, J. A. Analyst*., **1976**, *101*, 381-385.
37. Li, F.; He, T.; Wu, S.; Peng, Z.; Qiu, P.; Tang, X., *Microchem. J*., **2021**, *164*, 105987.
38. Wadhwania, P. M.; Panchal, V. K.; Shah., N. K., *RSC Adv*., **2016**.
39. Biradar, N. S.; Patil, B. R., *Monatshefte fur Chemie*., **1977**, *107*, 581-588.
40. York, D.; Evensen, N. M.; Martinez, M. L.; Delgado, J. D. B.; York, D.; Evensen, N. M.; Lo. M., *Am. J. Phys*., **2004**, *72*, 367-375.
41. Wahyuni, W.T., *Int. J. Electrochem. Sci*., **2020**, *16*, 210-221.