Microwave-assisted one-pot multicomponent synthesis of indole derived fluorometric probe for detection of Co^{2+} ions

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Abstract

Cobalt (Co^{2+}) is an essential constituent in the human body while excessive exposure leads to severe systemic toxic reactions which highlight the importance of developing effective methods to detect Co^{2+} ions. A simple and highly efficient fluorescence enhanced turn OFF-ON chemosensor was synthesized to detect the paramagnetic Co^{2+}. The ligand, *N*-((1H-indol-3-yl)(phenyl)methyl)aniline **(L),** was synthesized in 92% yield by means of hydrated ferric chloride catalyzed one -pot multicomponent microwave irradiation in the presence of Indole, benzaldehyde, and aniline as reactants. The major green principles of waste prevention, high atom economy (94.3%), green solvent, higher energy efficiency, and catalysis were the highlights of the ligand synthesis. The ligand exhibited remarkable fluorescence enhancement with Co^{2+} and a turn ON ratio of over 160-fold in $MeOH/H_2O$ (at pH 3.5) solution at an excitation wavelength of 369 nm in the Ultra-Violet range. The detection limit of **L-** Co^{2+} was 2.2 μM. The excitation and the emission spectra indicated stoke's shift of 93 nm which supports the fluorescence enhancement observed in L- Co^{2+} with respect to the free ligand. The Job's plot indicated fluorometric sensing of Co^{2+} ascribed to the complex formation with a stoichiometric ratio of 2:1 (L-Co^{2+}). Furthermore, the high linearity (r^2 =0.992) observed in the Benesi Hildebrand plot in a wide concentration range of $0.5 - 80$ μM confirmed the above stoichiometric ratio. The association constant (Ka) for the **L-**Co^{2+} was determined to be $8.382 \times 10^4\ \mathrm{M}^{-1} \pm 5.8 \times 10^3 \mathrm{M}^{-1}$.The prepared Co^{2+} fluorometric probe indicated long-term stability in -18 ℃ up to 45 days. Furthermore, the presence of Fe^{2+} and Fe^{3+} in the medium with Co^{2+} exhibited an interference effect in the fluorescence intensities. Upon further concentration studies, it was evident that the interference of Fe^{2+} and Fe^{3+} starts around 10.00 μM and rises exponentially.

Keywords: MCR, Green synthesis, Fluorescent Chemo-sensor, Turn OFF-ON, Cobalt (II), indole derivatives

1. Introduction

Cobalt is a 3d transition metal that is present in the human body as a trace element (Lee, 2007). Cobalt is the major constituent of vitamin B-12 (cobalamin), which is essential for making red blood cells in the body(Rickes et al., 1948). Cobalamin acts as a cofactor for two main human enzymes called methylmalonyl-CoA mutase and methionine synthase where both are highly important in maintaining health (Kräutler, 2012). Furthermore, cobalt is also involved in the biotin-dependent Krebs cycle which is the process that breakdown the sugars in the body (Yamada, 2013). However, excessive exposure to cobalt

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cause adverse health effects such as various systemic toxic reactions including severe effects to the cardiovascular system (Linna et al., 2020), peripheral and central nervous system (Catalani et al., 2012), endocrine system (Falfushynska et al., 2016), respiratory system(Linna et al. 2003), and skin diseases (Filon et al., 2013). Therefore, it is of great significance in monitoring its concentrations in the biological and environmental systems.

Zahir *et al.* reported a colorimetric method to determine trace levels of Co^{2+} in water bodies using complexation of Co^{2+} with 1-nitroso-2-naphthol-3.6-disulfonic acid disodium salt at a pH of 6.0. This method allowed the detection of Co^{2+} concentrations as low as 0.1 mg/L with no interference effects from the other cations at the provided conditions with greater accuracy (Zahir and Keshtkar, 1998). A highly sensitive colorimetric chemosensor based on coumarin conjugated thiocarbanohydrazone (CTC) was developed by Maity et *al*. The CTC chemosensor was developed as a staining agent for Co^{2+} in cellular micro-organisms and worked in the visible region as a naked eye colorimetric sensor. The chemosensor exhibited a detection limit as low as 1 µM in cobalt ion detection (Maity and Govindaraju, 2011).

Apart from the aforementioned techniques fluorometry is one of the spectrophotometric techniques with extraordinary sensitivity, higher specificity, and the ability to use in a wider concentration range (Karoui, 2016), which leads to metal ion detection through the development of a fluorometric probe. A typical fluorescent probe consists of at least one fluorescent core and a metal chelating moiety that will selectively bind with the metal ion of interest (Zheng et al., 2020). It was found that there is a lack of literature studies on the fluorescence chemosensors to detect cobalt via fluorescence enhancement due to the paramagnetic property of the Co^{2+} and the competitors such as Ni^{2+}, $Cu^{2+,}$ and Zn^{2+} (Pal et al., 2014). Hence, this research has its uniqueness in developing a turn OFF-ON chemosensor to detect Co^{2+} via fluorescence enhancement. Abebe *et al.* designed TURN-ON fluorescein-based chemosensors that are highly selective and sensitive for Co^{2+} and Ni^{2+} in both absorption and emission modes. This fluorescent chemosensor was stable for over a month. Reversibility of the binding in the Co^{2+} and Ni^{2+} was studied using EDTA (Abebe et al., 2011).

The main mechanism regulating fluorescence enhancement due to paramagnetic metal ion binding is the process of photoinduced electron transfer –PET (Figure 1). An internal redox reaction takes place between the excited state of the fluorophore and the species that can donate electrons (receptor) during the photoinduced electron transfer (PET) mechanism. This is due to the high energy in the excited fluorophore which gives rise to improved oxidant and reductant properties (Yang et al., 2015). The PET takes place from lone pair of electrons in coordinating atoms like N, O, S, and P to that of the Singly Occupied Molecular Orbital (SOMO) of the fluorophore (which was the HOMO before the excitation of the electron) in fluorescent metal ion complexes. The coordinating metal ion will result in lowering the energy of the lone pair preventing PET to take place (Formica et al., 2012).

Paramagnetic metal ions like Cu^{2+}, Co^{2+}, Ni^{2+}, Fe^{3+} speed up the intersystem crossing (ISC) which results in decreasing the fluorescence intensity in paramagnetic fluorescence quenching (Formica et al., 2012). This arises because once coordinated with the paramagnetic metal ion, the complex undergoes intersystem crossing (ISC) by excitation from S1 state to T1 state which is used to facilitate fluorescence quenching in biological and chemical sensors (Yang et al., 2015).

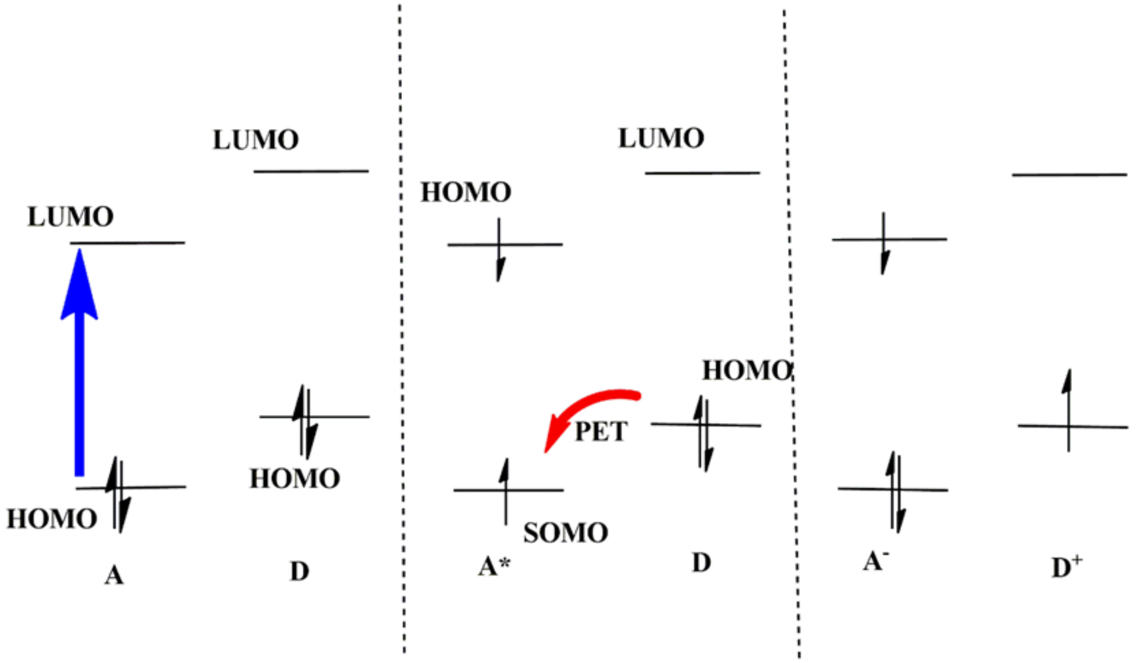

Figure 1. The energy level diagram from frontier orbital theory, illustrating PET mechanism for OFF state. A- acceptor (Fluorophore) D-donor(receptor).

Indole chemosensors are emerging in the field of chemosensors due to the Inherent fluorescent property of the indole chromophore (Dar et al., 2018). The substitution of electron-rich groups to an indole skeleton makes its affinity more towards cations rather than anions. The electron-donating ability and the extent of availability of the π electron cloud to complex with the metal plays a major role in the cation affinity towards the indole moiety (Zhu et al., 2008). Particularly, this type of chemosensor should have the ability to interact with metal ions in the solution and change the prevailing fluorescence properties either by fluorescence enhancement or by fluorescence quenching in the solution to be effective in action (Anger et al., 2006, Rani, 2015).

Dar et al. demonstrated the chemosensor activity of 3-(phenyl (phenylthio) methyl)-1H-indole ligand towards Cu^{2+} and Hg^{2+} via fluorescence enhancement. The ligand was synthesized as a one-pot multicomponent reaction using indoles, aromatic aldehydes, and thiols at room temperature using hydrated ferric sulfate as the catalyst. The rigid binding of the ligand to the cations prevented the non-radiative fluorescence compared to the free ligand (Dar et al., 2018).

Furthermore, Kaur et al reported tris(N-methylindolyl)methane ligand showing “TURN OFF” fluorescence sensing towards the two cations Cu^{2+} and Hg^{2+}. In this study, indole derivative was synthesized by the condensation reaction of N-methyl indole and N-methyl indole carboxaldehyde, catalyzed by ion-exchange resin amberlyst 15 (Kaur et al., 2011).

Shaik, Sharma et al synthesized N-((1H-indol-3-yl)(phenyl)methyl)aniline in water using polymer grafted ZnO nanoparticles as the catalyst while using indole, benzaldehyde and aniline as the starting materials. The reaction mixture was ultrasonicated to obtain a very high yield (i.e., 97%) within 10 minutes

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(Shaikh et al., 2019). Another research group introduced a novel approach to synthesize N-((1H-indol-3-yl)(phenyl)methyl)aniline using indole, benzaldehyde, and nitrobenzene in water using indium catalyst in aqueous HCL. The reaction was conducted at room temperature resulting in an excellent yield (i.e., 96 %) within 40 minutes (Das et al., 2013). Lu, Wang et al synthesized the same 3-substituted indole derivative using indole, benzaldehyde and aniline as the starting materials and the reaction was carried out at 75-90 ℃ for 3 hours and a yield of 58% was isolated (Lu et al., 2019). To the best of our knowledge, there have been no reports for the synthesis of N-((1H-indol-3-yl)(phenyl)methyl)aniline using hydrated ferric chloride catalyst under microwave irradiation which is described in this work.

Microwave-assisted organic synthesis (MAOS) is one of the major rising and revolutionizing areas in organic chemistry. MAOS has been identified as one of the major green synthesizing techniques (Ravichandran and Karthikeyan, 2011). Researches have been synthesizing these important derivatives of indole over the years using the conventional organic synthesis methods with major drawbacks such as side reactions, unnecessary byproducts, low yields, and time-consuming reactions (de la Hoz et al., 2005).

Lin et al synthesized 3- functionalized indole derivatives via a one-pot three-component domino reaction using anilines, arylglyoxal monohydrates, and 1,3- dicarbonyl compounds in a mixture of ethanol and water under MW irradiation. The reaction showed high regioselectivity (100%) and short reaction times (i.e., 40 min) which led to the generation of a large library of compounds with good to excellent yields (Lin et al., 2017).

We herein report a novel and efficient one-pot multicomponent green method to synthesis of a fluorometric probe, N-((1H-indol-3-yl)(phenyl)methyl)aniline from indole, benzaldehyde, and aniline under microwave irradiation (Scheme 1). The sensing ability of the synthesized ligand was investigated using fluorescence spectroscopy.

2. Experimental

2.1. Materials and methods

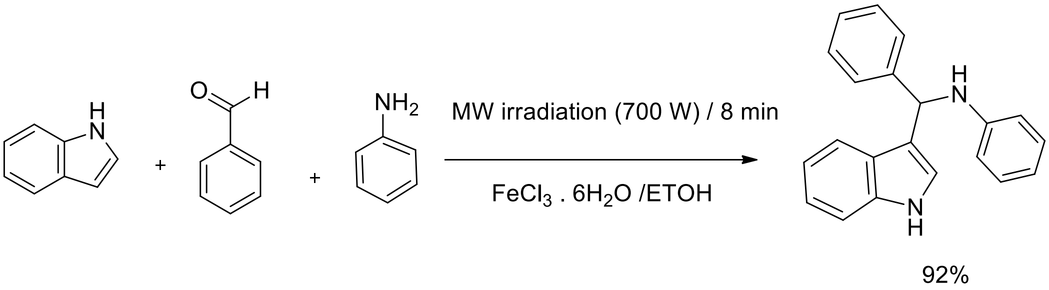
Indole, aniline, benzaldehyde, ethanol, hydrated ferric chloride ($FeCl_3.6H_2O$) CH_2Cl_2, silica for thin-layer chromatography, anhydrous sodium sulfate, silica for column chromatography, hexane, acetone, NaCl, KCl, $BaCl_2$, $CoCl_2.6H_2O$, $CdCl_2$, $MnCl_2$. $4H_2O$, $CuCl_2$, $ZnCl_2$, $AgNO_3$, $(NH_4)_2Fe(SO_4)_2\cdot6H_2O$, $NH_4Fe(SO_4)_2$ were purchased from Sigma Aldrich with analytical grade. All chemicals were used as received without any further purification. The solutions of metals ions from Na^+, K^+, Ba^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Fe^{2+}, Fe^{3+} were prepared using distilled water. Solutions of ligand were prepared in methanol.

Reactions were carried out in a modified domestic microwave oven with 700 W under medium-low power. The fluorescence emission spectra were recorded in Thermo Scientific Lumina Fluorescence Spectrometer. The ^1H NMR and ^{13}C NMR of the synthesized ligand were recorded using Bruker Ascend 400 spectrometer (Bruker, USA). Tetramethylsilane (TMS) was used as an internal reference and chemical shifts were reported as δ ppm units. Spectral data were presented as s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad, app=apparent , coupling constant (*J*) in Hertz (Hz). IR spectra are recorded by Attenuated Total Reflection- Fourier Transform Infrared (ATR-FTIR) spectroscopy on Thermo Scientific Nicolet S10 FT-IR spectrometer. Spectra were processed within 4000 -500 cm^{-1}.

2.2 Synthesis of N-((1H-indol-3-yl)(phenyl)methyl)aniline (L) under microwave irradiation

In a 25.00 ml round bottom flask, 3.0 ml of ethanol was added to a mixture of indole (0.117 g, 1 mmol) and benzaldehyde (0.01 ml, 1 mmol) at room temperature. The hydrated ferric chloride ($FeCl_3.6H_2O$, 0.027 g, 0.1 mmol) was then added to the reaction mixture and stirred well. To the stirring solution, aniline (0.11 ml, 1.2 mmol) was added, and the resulting mixture was microwave irradiated for 8 minutes (Scheme 1). The progress of the reaction was monitored by TLC.

The reaction mixture was then cooled to room temperature. The residual ethanol was removed using a rotary evaporator and the crude residue was extracted with dichloromethane (2 × 15.0 ml). The organic layer was washed with distilled water, dried over anhydrous sodium sulfate (Na_2SO_4), and it was concentrated in a rotary evaporator. Finally, the crude residue was purified by a silica gel column chromatography (dichloromethane: hexane =1:1) to afford a reddish-orange oil with a 92% yield.



Scheme 1. Synthesis of *N*-((1H-indol-3-yl)(phenyl)methyl)aniline.

2.3 Cation recognition studies

Exactly 30.00 μM solutions were prepared for Na^+, K^+, Mn^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Co^{2+}, Cu^{2+} and, Zn^{2+} in distilled water to determine the fluorescence intensity change. A solution of 30.00 μM *N*-((1H-indol-3-yl)(phenyl)methyl)aniline ligand (**L**) was prepared in methanol. Exactly 10.00 ml of each metal ion solution and the ligand (**L**) were mixed while maintaining the pH at 3.5. The fluorescence spectra were recorded at room temperature at an excitation wavelength of 369 nm.

2.4 Determination of ligand: Co^{2+} stoichiometric ratio

The stoichiometric ratio of Ligand: Co^{2+} was determined by Job's method of continuous variation. (Olson and Bühlmann, 2011). Stock solutions of **L** in methanol (30.00 μM) and Co^{2+} in distilled water (30.00 μM), was prepared at room temperature. Then 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00 and 9.00 mL of the stock solution of **L** were mixed with 9.00, 8.00, 7.00, 6.00, 5.00, 4.00, 3.00, 2.00 and 1.00 ml of Co^{2+} solution, to obtain molar fraction of **L** as 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 respectively,

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at pH 3.5. The fluorescence emission spectra were recorded after mixing the two solutions thoroughly. the stoichiometric coefficient between (**L**) and Co^{2+} was determined using Job's plot (Vashisht et al., 2019).

3. Results and discussion

3.1 Microwave-assisted synthesis of N-((1H-indol-3yl)(phenyl)methyl)aniline sensor

The ligand was synthesized from readily available starting materials using a one-pot multicomponent reaction under microwave irradiation and the optimum yield (i.e.,92%) was obtained after 8 minutes of MW irradiation. However, the reaction has not been completed even after five hours at room temperature and the product was obtained at a very low yield (i.e.,22%).

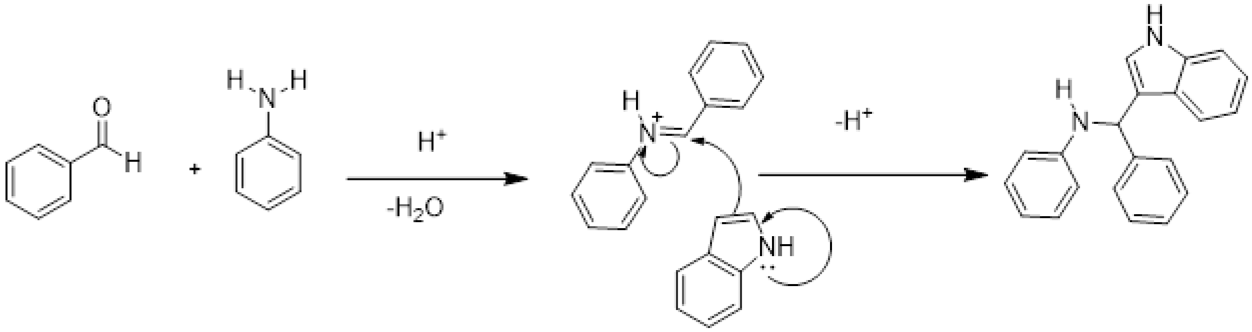
The only byproduct formed in this reaction is one equivalent of water which is an innocuous byproduct that does not require any further treatments. Due to the highly convergent nature of the multi-component reactions, maximum incorporation of the reactant materials in the product leads to a significantly high atom economy. A very high atom economy of 94 % was obtained for this synthesis which also indicates the minimal byproduct formation. According to the environment, health, and safety (EHS) approach (Capello et al., 2007) the solvent used in this synthesis, ethanol, provides a lower score which indicates low environmental and health hazards. Furthermore, the synthesis was performed in a modified domestic microwave oven. The reactions performed in microwave heating technology represent a green chemistry protocol by utilizing the reaction conditions, preventing waste, exhibiting energy efficiency, and minimizing the reaction time and accidents.

3.1.1 Spectral data for N-((1H-indol-3-yl)(phenyl)methyl)aniline

Reddish orange viscous oil;- yield :92% (0.275 g) R^f (Hexane: DCM 1:1)=0.51, FT-IR (ATR) ($\nu_{\max}/cm^{-1}$): 3408 (w), 3054 (b), 1616 (m), 1455 (w), 1336 (w), 1216 (w), 1174 (w), 1109 (w), 738 (m), 692 (m). ^1H NMR (400 MHz, Acetone-D6) : δ 4.55 (s,1H,NH) , 5.94 (s, 1H) 6.58 (ddt, J= 7.4, 6.3,1.0 Hz, 1H) , 6.71 – 6.61 (m, 2H), 6.86 – 6.75 (m, 1H), 6.91 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H), 7.12 – 6.98 (m, 3H), 7.26 – 7.15 (m, 1H), 7.32 – 7.26 (m, 2H), 7.51 – 7.32 (m, 4H), 10.00 (s, 1H, NH), ^{13}C NMR (101 MHz, Acetone-D6) δ 149.3, 146.0, 138.1, 129.5 (2C), 128.9 (2C), 128.1 (2C), 126.7, 124.6, 122.1, 120.3, 119.9, 119.3, 117.5, 115.2 (2C), 114.3, 112.12, 41.2. The spectroscopic data match with those reported in the literature (Lu et al., 2019, Shaikh et al., 2019).

3.1.2 The proposed mechanism for the synthesis of the ligand

The proposed mechanism for the synthesis of the ligand is shown in Scheme 2. An iminium ion intermediate is formed when the aniline is reacted with benzaldehyde in ethanol which is then attacked by the C-3 center of the indole to obtain the 3-substituted indole product. A new C-C bond and a C-N bond are formed during the reaction with the formation of a new chiral center (Srihari et al. 2009). The stoichiometric ratio of the reactants is highly important because the excess indole in the presence of aldehyde and catalyst leads to the formation of bis-indole methane derivatives (Khorshidi and Shariati, 2014, Tocco et al., 2017).



Scheme 2. Proposed reaction mechanism of synthesis of *N*-((1H-indol-3-yl)(phenyl)methyl)aniline sensor.

3.2 Selectivity of the ligand toward cations

The fluorescence measurements of the synthesized ligand were carried out using a fluorescence spectrometer. Ligand showed no considerable fluorescence activity in the range of 250-380 nm. A significant fluorescence enhancement was observed for the ligand in the presence of the Co^{2+} where other cations such as Na^+, K^+, Mn^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Ag^+ showed very low-intensity fluorescence enhancements. Fe^{2+} and Fe^{3+} showed mild fluorescence compared to the Co^{2+}. The relevant peak intensities to the **L**-metal ion complexes are shown in Figure 2. The maximum error value observed was less than 5%.

Based on the fluorescence intensity values for the different cations, the ionic radius of the Co^{2+} is in the optimum range among the tested cations indicating the optimal interaction of Co^{2+} with the ligand in the complex. Since the fluorescence changes occurred in the UV region (<400 nm), any visible color change was not observed throughout the fluorescence analysis. It was observed that the fluorescence of the ligand was enhanced by 160 fold with the 30.00 μM Co^{2+} solution.

The initial "OFF" fluorescence quenched state of the ligand can be described using photoinduced electron transfer (PET). The coordination of Co^{2+} and the receptor site of the ligand restrain the PET processes (Colenda et al., 2018). The redox potential of the donor is increased upon cation binding to the ligand. Hence fluorescence quenching is suppressed due to lowering of HOMO energy which makes PET not possible any further (Valeur and Leray, 2000). The changes in the charge density on the N-binding site of the ligand after coordinating with Co^{2+} inhibit the PET mechanism (Liu et al., 2019). The rigidity of the L-Co^{2+} complex is higher than to the free ligand, which gives rise to the Chelation enhanced fluorescence (Liu et al., 2019). The fluorescence enhancement of Co^{2+} with the ligand is a special case scenario because Co^{2+} is a paramagnetic metal ion with a d^7 configuration which normally quenches the intrinsic fluorescence.

This special phenomenon is explained by Ramachandram *et al.* for a few 1,8- naphthalimide derivatives induced by transition metal salts (Ramachandram et al., 2000). The interaction between the fluorophore and the Co^{2+} leads to fluorescence quenching whereas the interaction between the receptor site of the fluorophore and the Co^{2+} ions leads to fluorescence enhancement. Hence, the net effect of these opposing features is determined by the extent of quenching due to the fluorophore – metal ion interaction and the enhancement due to metal ion- receptor site interaction. This may be supported by the suitable

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coordination geometry of the *N*-((1H-indol-3-yl)(phenyl)methyl)aniline, the appropriate ionic radius of the $Co^{2+,}$ and adequate binding energy of the ligand to the Co^{2+} (Wang et al., 2018).

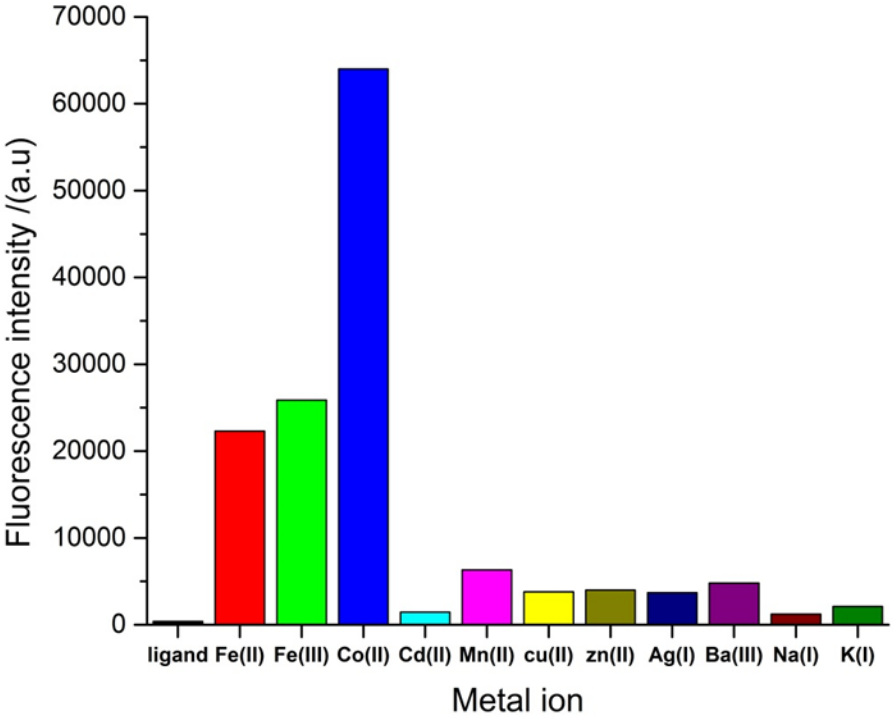

Figure 2. The fluorescence intensity of the ligand (30.00 μM) in the presence of various metal cations. *slit width =20 nm at 369 nm (error < 5%).

3.3 Determination of stoichiometric coefficient by Job's plot

A Job's plot was performed to determine stoichiometry between **L** and the Co^{2+} cation. The maximum fluorescence intensity was observed at 0.65 molar ratio confirming the 2:1 stoichiometric ratio of **L** to Co^{2+} (Figure 03).

The optical properties of the **L -** Co^{2+} complex showed that there is no overlap between the absorption and the fluorescence emission spectra. Stokes shift $\Delta\lambda$ was found to be 93 nm which was a considerably greater value for a single fluorophore. A greater stoke shift value minimizes self-quenching which will be significant for analytical purposes (Saleh et al., 2019).

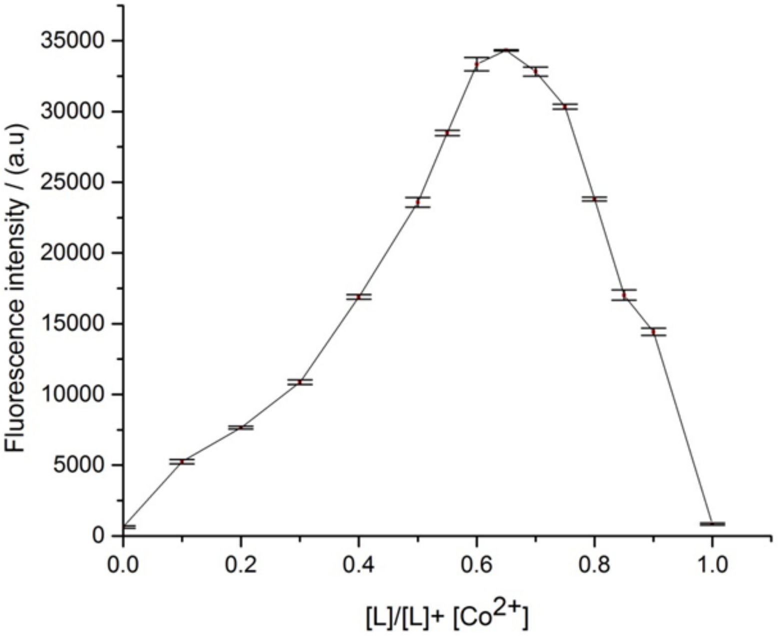

Figure 3. Job's plot depicting the binding ratio between the ligand and Co^{2+} ion.

3.4 Sensitivity studies

The sensitivity studies of *N*-((1H-indol-3yl)(phenyl)methyl)aniline sensor towards Co^{2+} were investigated by fluorescence titration experiments which were carried out by monitoring of fluorescence intensity changes upon the addition of various concentrations of Co^{2+} (from 0 – 70 µM) to the indole sensor (30.00 µM/ in MeOH/ at pH 3.5) at an excitation wavelength of 369 nm (Figure 4). It was observed that the intensity of the fluorescence enhancement increases with the increase of Co^{2+} concentration and showed a hyperchromic shift. The fluorescence intensity enhancements were leveled off closer to 70 µM. This can be due to the stronger suppression of the PET mechanism of the ligand by increasing the Co^{2+} concentration of the medium. (Ashokkumar et al., 2011, Gao et al., 2020).

The detection limit of the indole sensor was determined using the slope of a fluorescence intensity vs concentration of Co^{2+} and a threefold standard deviation of the lowest concentration of the calibration curve (blank solution)(Skoog et al. 2013). The detection limit is 2.2 µM (Figure 5) which is comparable to the reported values of Co^{2+} chemosensors such as coumarin (Vashisht et al., 2019), diethylenetriamine (Lee et al. 2016), oxadiazole (Wang et al., 2011), pyridine systems (Na et al., 2016), pyrazine coupled rhodamine(Liu et al. 2017) derivatives. The Co^{2+} induced fluorescence intensity enhancement showed excellent linearity with a correlation coefficient of $R^2 = 0.99$ in the range of 0.50 - 80.00µM. This indicates a wide linear range of chemosensor action in favor of detecting Co^{2+} in the actual quantitative measurements.

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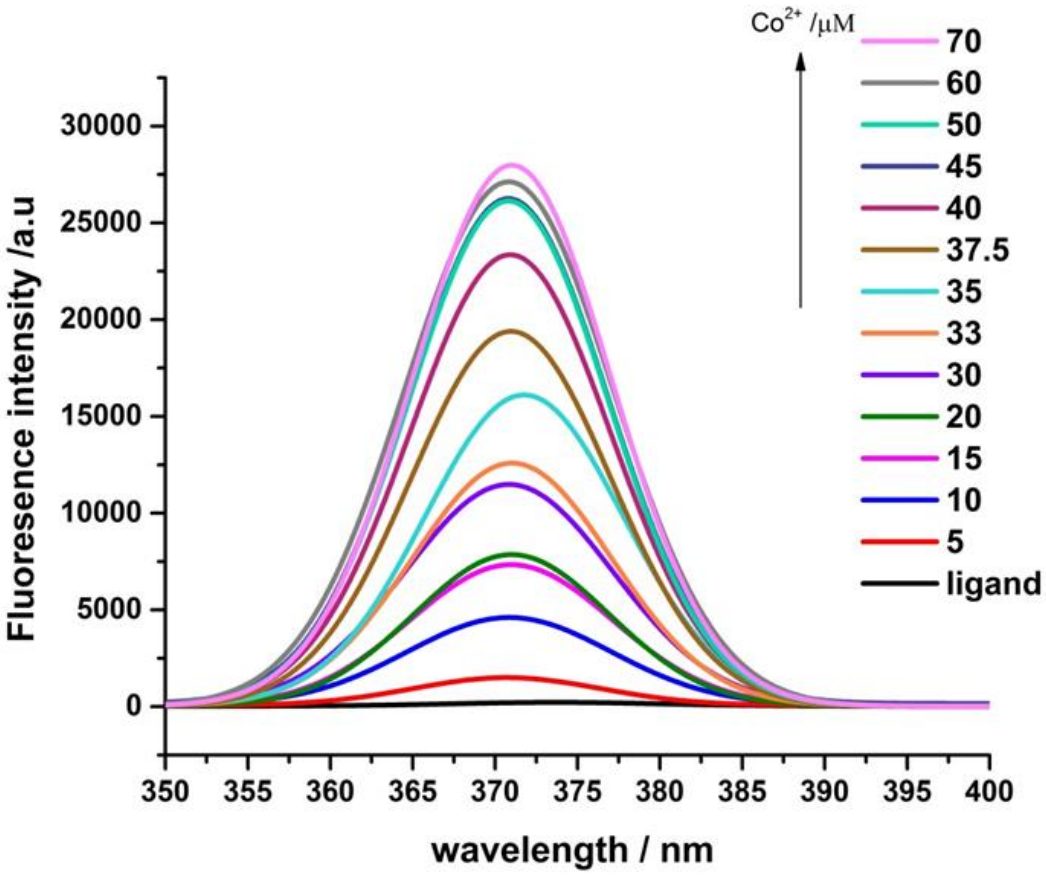

Figure 4. Fluorescence intensity change of the ligand (30.00 μM) with the increase of the Co^{2+} concentration.

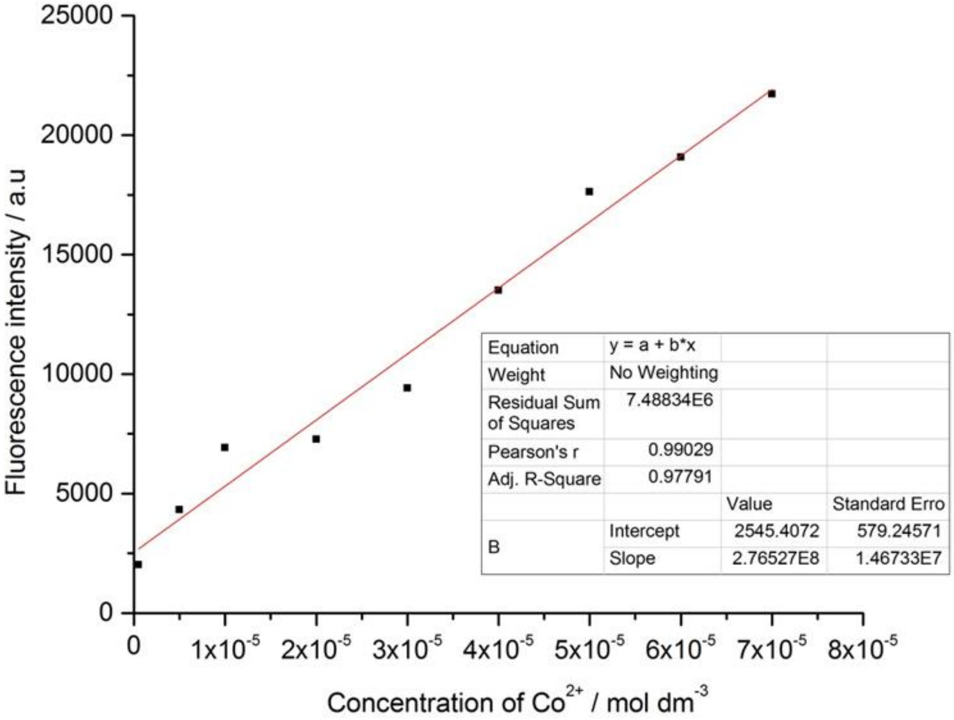

Figure 5. The calibration curve of the fluorescence intensity versus $[\mathrm{Co}^{2+]}$ at pH 3.5 (all the error values calculated were less than 1%).

3.5 Interference study

A competition ion study was performed to determine the interference effect from other cations to the **L-** Co^{2+} complex (Aich et al., 2015). A solution series were prepared in test tubes where each contains Co^{2+}: **L** in a 1:2 ratio (10.00 µM / pH -3.5). The fluorescence readings were measured after adding the cations Na^{+}, K^{+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+} of 10.00 µM separately to the ligand respectively. In this study, it was observed that the fluorescence intensities obtained for the **L**-Co^{2+} complex were unaffected in the presence of metal cations such as Mn^{2+}, Cu^{2+}, Cd^{2+}, Ba^{2+}, Ag^{+}, Zn$^{2+,}$ and Na^{+}. It was observed that fluorescence intensity enhancement was observed in the presence of Fe^{2+} and Fe^{3+} ions in the medium (Figure 6). Other metal ions caused ignorable perturbation on the fluorescence intensity measurements. Hence it can be concluded that there is an interference effect from Fe^{2+} and Fe^{3+} to the detection of Co^{2+}.

The extent of the interference effect from Fe2+ and Fe3+ was further investigated by measuring fluorescence intensities of 10.00 µM ligand and 10.00 µM Co2+ solutions with increasing Fe2+/ Fe3+ concentrations (Figure.7). It was observed that the interference starts from about 10.00 µM for both the Fe 2+ and Fe3+ up concentration and rises exponentially.

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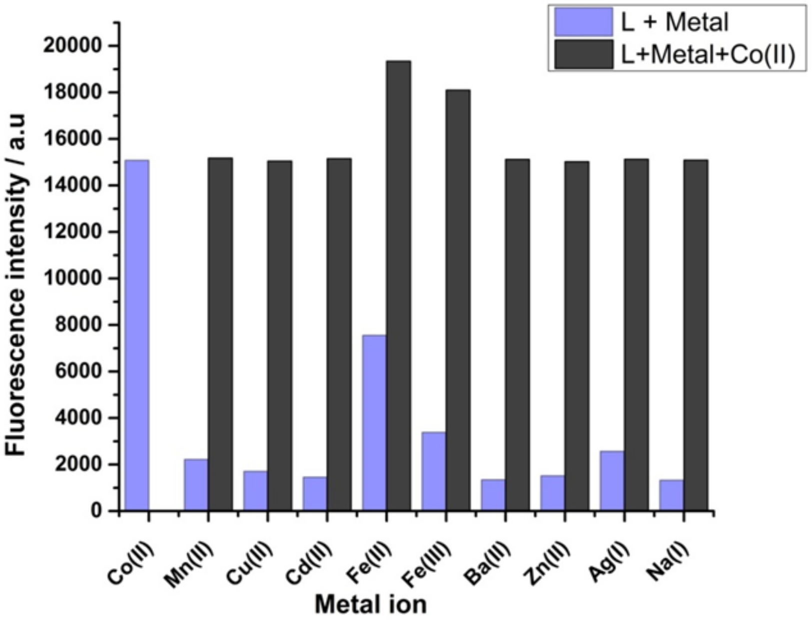

Figure 6. Metal ion selectivity of the **L** (10.00 μM) in MeOH solution. (the blue bars represent the fluorescent intensity of **L** in the presence of metal ion of interest (10.00 μM) in 2:1 ratio, and the black bars represent the changes of fluorescent intensity that occurs upon the subsequent addition of 10.00 μM Co^{2+} to the above solution).

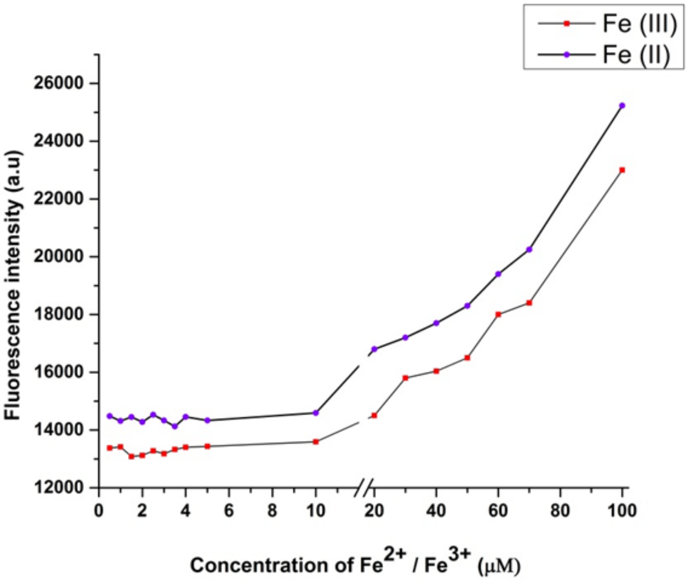

Figure 7. Fluorescence intensity variation of L- Co^{2+} in the presence of Fe^{2+} and Fe^{3+}.

4. Conclusion

An indole-based fluorometric turn OFF-ON chemosensor for the detection of Co^{2+} was synthesized using an efficient one-pot multicomponent, green method under microwave irradiation. An excellent yield (i.e.,92%) was obtained within 8 minutes of microwave irradiation.

The synthesized ligand, *N*-((1H-indol-3-yl)(phenyl)methyl)aniline showed a significantly high "TURN ON" fluorescence emission in the presence of Co^{2+} suppressing the prevailing PET mechanism. The extremely high linear dependency of the Benesi Hildebrand plot synchronized with the results of Job's plot analysis where it indicated a stoichiometric coefficient of 2:1. It was found that the low detection limit of the ligand is in the micro-molar range (2.2μM) which is in accordance with the previously developed chemosensors for Co^{2+}. Further, it was revealed that Fe^{2+} and Fe^{3+} are acting as interfering agents to the chemosensor activity of the ligand.

Acknowledgment

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