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A selective fluorescent chemosensor based on chromone hydrazone ligand for Zinc ions

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Abstract

Chromone hydrazone ligand namely AFCTSC was synthesised and fully characterised using spectroscopy method of FT-IR, UV-Vis, ^{13}C & ^{1}H NMR. The fluorescence emission of free AFCTSC ligand was observed to be weak at 460 nm. The ligand showed high selectivity to Zn^{2+} by giving remarkable red shift of the emission to 505 nm, while other metal ions such as Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+} and Ni^{2+} quenched the fluorescence intensity of the ligand. Furthermore, Zn^{2+} addition into AFCTSC solution caused fluorescent colour change from blue to green when the solution is illuminated with a UV lamp (λ=365nm). The limit of detection obtained was 4.5×10^{-7} M making AFCTSC a promising fluorescent probe that could be an alternative to existing analytical methods for determination of Zn^{2+}. The binding study revealed each molecule of AFCTSC was coordinated to Zn^{2+} in mononegative tridentate manner via ONS donor atoms.

Keywords: *Chromone hydrazone; Zn^{2+}; fluorescent probe; ONS donor*

Introduction

In recent years, the development of fluorescent chemosensor for the detection of metal ions has been given a great attention from researchers around the world. Studies of fluorescent chemosensors proved this method could be used for metal ions detection with high selectivity, high sensitivity, real-time monitoring over other metal ions, rapid response time and versatility[1]. The detection of metal ions in water environment is important because of their harmful effects towards human health. There are already previous studies conducted in detecting metal ions such as Al^{3+} [2], Cu^{2+} [3, 4], Hg^{2+} [5] and Zn^{2+} ion [1, 6-8].

Chromones are suitable for formation of various type of compounds due to the three electron deficient sites of carbons (C-2, C-4) and aldehyde (CHO) which are ready for nucleophilic attack from any strong nucleophiles [9]. Chromone derivatives exhibited various biological activities such as anticancer, anticovalescent, antifungal, antimycobacterial, and antioxidants [10, 11]. Chromones has been reported to be used as a precursor for developing fluorescent chemosensor in detecting metal ions such as Co^{2+} [10], Mg^{2+} [12], and Zn^{2+} [8, 13].

Zinc is the second most copious metal ion that exists in a human body and plays important parts in biological processes such as gene expression, metalloenzyme function and neurotransmission [7, 14, 15]. The abnormality of zinc concentration in a human body may cause diseases such as Alzheimer'disease, amyotrophic lateral sclerosis and Parkinson's disease [8, 16-18]. Zinc is also considered as metal pollutant for environment which could lower the microbial activity of the soil and it is also a common pollutant in agricultural and food wastes [7]. Therefore, it is essential to design a new type of fluorescence-based chemosensor to detect the presence of zinc either in a human body or in the environment to prevent potential environmental and health problems. The concentration of zinc in water samples may range between 0.211 to 0.256 mg/L, while the permissible limit of zinc in water by WHO standard is set at 5.0 mg/L [19].

In this study, we report the fluorescent properties of synthesised chromone-based ligand namely 2-aminochromone-3-carbaxaldehyde thiosemicarbazone (AFCTSC) towards several divalent cations from the third-row elements in the periodic table (Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+}). Although the synthesised ligand has been previously reported [20], to the best our knowledge, there is no report on fluorescent activity towards these several divalent cations. The ligand exhibited great fluorescence behaviour towards zinc with limit of detection of 4.5×10^{-7} M compared to other listed metal ions, making AFCTSC a promising fluorescent probe that could be an alternative to existing analytical methods for determination of Zn^{2+}. The blue emission of free ligand changed to yellow-green emission when zinc was reacted with the ligand with a 7.5-fold increase in the fluorescence intensity.

Experimental

Materials and measurements

2-amino-3-formylchromone, thiosemicarbazide, metal acetates, acetic acid, dimethyl sulfoxide and ethanol were used without further purification and were acquired from ACROS and ALDRICH. Melting point was determined using Stuart Scientific Melting Point Apparatus SMP3. Thermo Nicolet Nexus spectrometer was used to collect FT-IR spectra at range of 4000-400 cm^{-1}. Cary 60 (Agilent) spectrophotometer was used to obtain electronic spectra of ligand and its complexes in DMSO. The ^{13}C-NMR and ^{1}H-NMR spectra were collected from JNM-ECX-500 (JEOL) spectrometer and the solvent used was dimethysulphoxide-d_6. Cary Eclipse (Agilent) spectrophotometer was used to record fluorescence spectra of ligand and its complexes in DMSO. Gas Chromatograph Mass Spectrometer (GCMS-QP2010) with acetone as solvent was used to collect the mass spectra of the ligand.

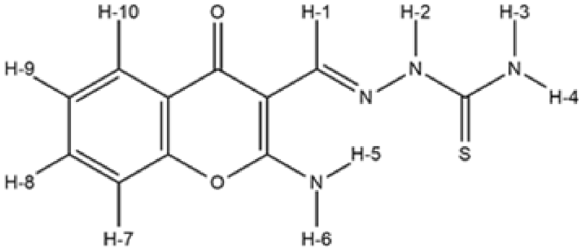

Figure 1: Structure of AFCTSC ligand.

Synthesis of ligand, AFCTSC

AFCTSC ligand or its IUPAC name N''-((2-Amino-4-oxo-4H-chromen-3-yl)methylene) thiocarbohydrazide was synthesised according to the previous reported procedure [21]. The synthesis was conducted by dissolving 1:1 molar ratio of 2-amino-3-formylchromone into acidified ethanol solution of thiosemicarbazide to produce AFCTSC ligand as proposed in **Figure 1**. The mixture solution was refluxed for 24 hours. Yellow precipitate was separated, washed with cold ethanol, and dried in desiccator. Yield, 85.4%, m.p: 317 °C. FT-IR (KBr, cm^{-1}): v = 3323 ($N-H_2$), 3214 (N-H), 1642 (C=O), 1619 (C=N), 1327 (C=S), 1094 (N-N). UV-vis: (DMSO): λ_{max}/nm (ε/$mol^{-1}L\ cm^{-1}$) 288, 348. MS (*m/z*): 267. 1H NMR (DMSO-d_6, 400 MHz): δ 11.06 (2H, br, $N-H_{3,4}$), δ 8.59 (3H, br, $CH_1=N$, $N-H_{5,6}$), δ 7.99 (1H, d, H_{10}), δ 7.89 (1H, br, $N-H_2$), δ 7.64 (1H, t, H_8), δ 7.34-7.38 (2H, m, $H_{7,9}$). 13C NMR (DMSO-d_6, 400 MHz): δ 176.6 ($C_1=O$), 173.9 ($C_{11}=S$), 162.3 (C_8), 153.1 (C_7), 142.7 (C_3), 133.6 (C_5), 125.6 (C_4), 125.4 (C_2), 121.9 (C_{10}), 117.05 (C_6), 92.3 (C_9).

Fluorescence property analysis

The stock solution of AFCTSC and the selected Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+} metal acetate salts (1.0 mM) were prepared by dissolving the ligand and metal salts in DMSO. The test solution was prepared by adding 250 µl of probe solution into a cuvette containing an appropriate aliquot of metal ions solution. The mixture was allowed to rest for 5 minutes and was diluted with DMSO to 3 ml. The slit widths for emission and excitation was set to 5 nm. 377 nm was selected as excitation wavelength during analysis. The investigation for the sensitivity of the ligand was further conducted by adding 2.0 eq. competing metal ions such Co^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, and Ni^{2+} to the mixture of ligand and Zn^{2+}. A study of time response of AFCTSC towards Zn^{2+} was carried out at an interval of 5 min. All fluorescence measurements of AFCTSC were recorded at 505 nm.

Results and discussion

Characterisation of the ligand

The explanation of ligand structure was determined by the IR spectra, UV-Vis spectra, ^{13}C NMR and ^{1}H spectra data. The absorption bands at 3323 cm^{-1} and 3214 cm^{-1} were designated to v(NH$_2$) and v(NH) respectively. The absorption bands for v(C=O$_{ketone}$), v(C=N) and v(C=S) were assigned at 1642 cm^{-1}, 1619 cm^{-1} and 1327 cm^{-1} respectively, comparable to the previously reported [22]. AFCTSC ligand showed absorption bands on its electronic spectra at 288 nm and 348 nm. AFCTSC structure was further supported by NMR spectra. From ^{1}H NMR spectra the synthesised ligand was confirmed with the signaling of proton from CH=N and N-H designated at δ 8.59 and δ 11.06 respectively. ^{13}C NMR spectra data showed the number of signals appeared for carbons were similar to the theoretical structure of AFCTSC. The mass spectra data for AFCTSC gave out 262 *m/z* which is similar to the calculated molecular weight of 262 g/mol.

Fluorescence study

AFCTSC chemosensor sensing ability was tested by the addition of different kinds of metal acetate salts such as metal ions of Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+}. Visible colour changes were observed. The colourless solution of AFCTSC changed to light yellow upon addition of Zn^{2+} metal ion (**Figure 2a**). However, the addition of metal ions such as Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+} and Ni^{2+} also showed colour changes. The visible colour changes are mainly due to the formation of L-M^{n+} coordination complexes, which was confirmed by the red shift in the absorption band from 348 nm to around 370 nm (**S1**). Therefore, AFCTSC was not a suitable naked eye chemosensor for detection of Zn^{2+} ion. On the other hand, when the solution is illuminated with a UV lamp (λ=365nm), only the addition of Zn^{2+} ion caused fluorescent colour change from blue to green (**Figure 2b**). Thus, the ligand showed a very promising result for detection of Zn^{2+} ion.

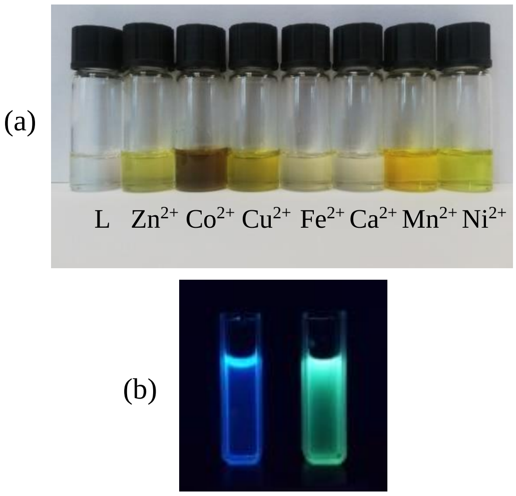

Figure 2: a) Visible colour changes of AFCTSC (1.0 mM) towards other metal ions (1.0 mM), (b) Fluorescence colour change of AFCTSC (blue) and AFCTSC-Zn (green)

The fluorescence property of AFCTSC towards Zn^{2+} ion was further investigated through its fluorescence spectra. AFCTSC showed great fluorescence selectivity when reacted with Zn^{2+} ion (**Figure 3a**).

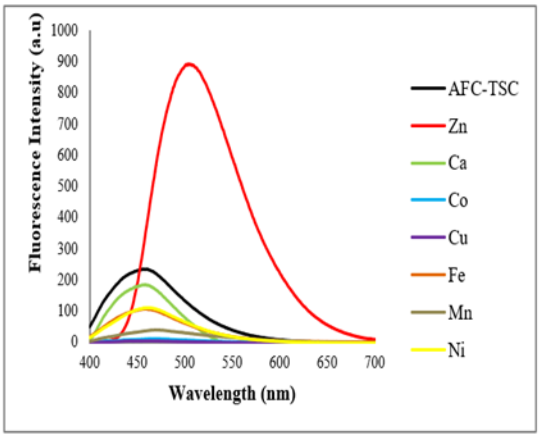

Figure 3a: The fluorescence spectra of AFCTSC (1.0 mM) in DMSO with different metal ions (λ_{excite} = 377 nm)

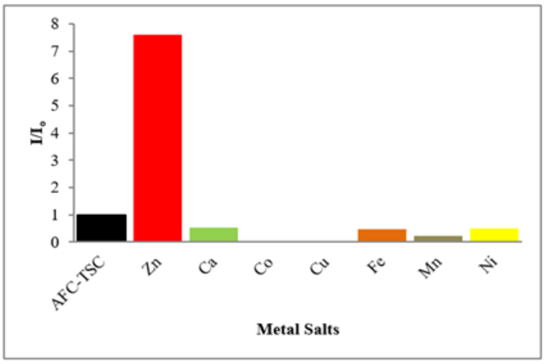

Figure 3b: Fluorescence response of AFCTSC towards 1.0 eq. of other metal ions. I = Max fluorescence intensity when reacted with metal ion, I_o = Max intensity of ligand

The emission peak of ligand was observed at 460 nm shifted to 505 nm upon the addition of Zn^{2+}. The addition of Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, and Ni^{2+} ions quenched the fluorescence emission of the ligand. The fluorescent activity could be seen in **Figure 3b** by comparing the value of I/I_o. Fluorescence enhancement with approximately 7.5-fold was observed in the presence of Zn^{2+}. This indicated AFCTSC could act as a selective chemosensor towards Zn^{2+} over other listed metal ions with a large responsive range of millimolar to picomolar level [22].

To further investigate the chemosensing properties, AFCTSC was fluorescence-titrated by adding 0-2.0 eq of Zn^{2+} ion. As Zn^{2+} ion was titrated gradually (**Figure 4**), the intensity of fluorescence emission for the compound gradually increased. The determination of the stoichiometry of plausible complexation was obtained from the mole-ratio method and also from Job's method of continuous variation [23].

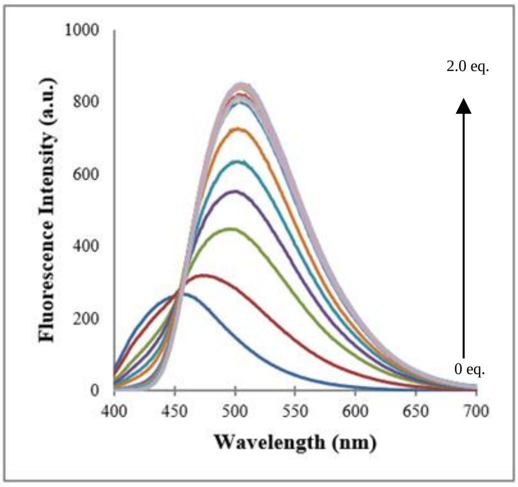

Figure 4: Titration fluorescence spectra of AFCTSC with Zn^{2+} ions (0-2.0 eq.)

The mole-ratio method is shown by the plot of metals to ligand ratio ($[Zn^{2+}]/[AFCTSC]$) versus fluorescence intensity (**Figure 5a**). Meanwhile the mole fraction of ligand was used in Job's plot by keeping AFCTSC under a constant concentration (1×10^{-3} M) and varying the concentration of Zn^{2+} (0 to 2×10^{-3} M) (**Figure 5b**). From the plot of metals to ligand ratio, the fluorescence signal started to remain at a plateau upon further addition of Zn^{2+} (>0.5 eq), reflecting the ligand to metal ratio was found to be 2:1. The stoichiometry was further confirmed by the value obtained from mole-ratio and Job's plot in **Figure 5a** and **5b**.

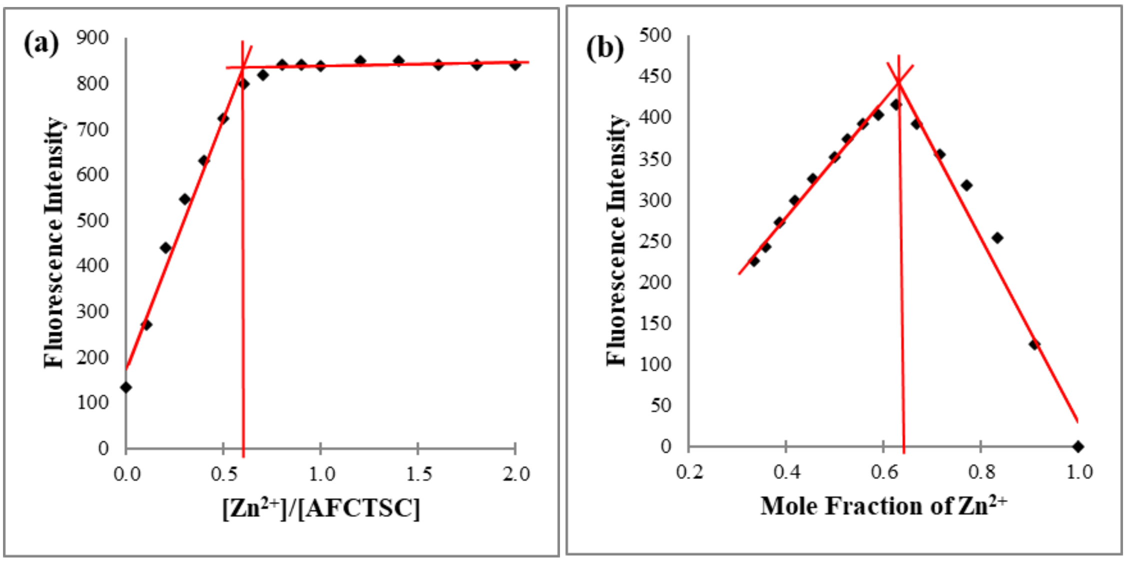

Figure 5: (a) Mole-ratio plot of interaction between AFCTSC and Zn^{2+}. (b) Job's Plot of interaction between AFCTSC and Zn^{2+}

A linear calibration plots in **Figure 6** which was performed using the basis of the titration data, showed the correlation coefficient (R^2) of 0.989. The limit of detection (LOD) of AFCTSC towards Zn^{2+} was calculated to be 4.5×10^{-7} M according to the equation of $3\times\sigma_{blank}/k$, where σ_{blank} was the standard deviation of the blank solution and k was the slope of the calibration curve **(S2)**. The detection limit is low and comparable with previously reported Zn^{2+} fluorescence chemosensor of hydroxyl pyrene-hydrazone-based chemosensor (3.29×10^{-7} M), functionalized porphyrin-based chemosensor (1.8×10^{-6} M) and quinolone-pyrazine-based chemosensor (1.11×10^{-6} M) [6, 7, 24].

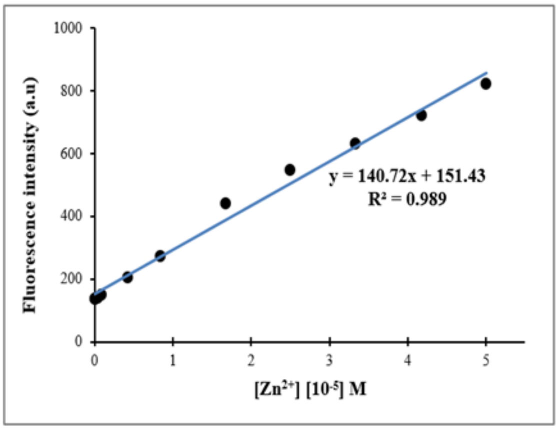

Figure 6: A linear calibration plot of AFCTSC with reaction towards Zn^{2+}

The time response study was conducted to determine the recognition response of AFTSC towards Zn^{2+} ions. **Figure 7** shows the reaction between ligand and Zn^{2+} ion was completed within 1 minute or less. Due to the prompt observable fluorescent emission [8] attest AFCTSC-Zn could be engaged as a fluorescent chemosensor in environmental analysis [25].

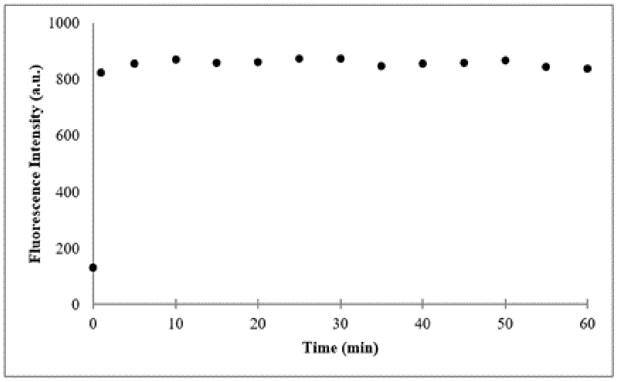

Figure 7: Fluorescence time response of AFCTSC in the presence of 1.0 equiv. of Zn^{2+}

Coordination of AFCTSC with Zn^{2+}

A binding study was carried out to figure out the fluorescence behaviour of AFCTSC towards Zn^{2+}, thus UV-vis, Infrared (IR) and 1H-NMR spectroscopy were employed. **Figure 8** showed the structure of AFCTSC and its binding proposition towards Zn^{2+}. The formation of AFCTSC-Zn was determined by comparing the absorption spectra before and after the addition of Zn^{2+} ion into the ligand solution **(S3)**. A very weak band at 288 nm and a strong band at 348 nm were observed in AFCTSC. The higher energy band was assigned to π- π^* transitions of the C=O, C=N and C=S groups while the lower energy band may be attributed to charge transfer transitions within the molecule [26]. The sequential addition of Zn^{2+} caused the gradual decrease in the absorption band of 348 nm accompanied by a red shift to 377 nm with increasing intensity, indicating the formation of complex AFCTSC-Zn [12].

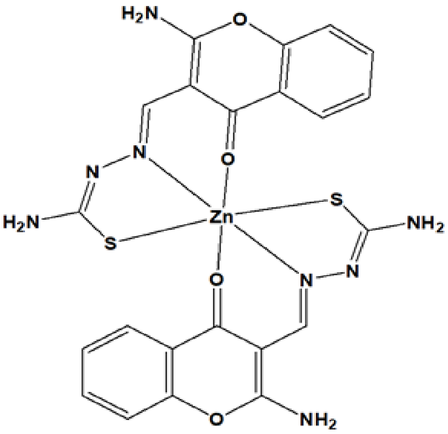

Figure 8: Proposed binding structure of AFCTSC with Zn^{2+}

The IR spectral data (**S4**) showed the addition of zinc to the ligand caused shifting in the v(C=O) and v(C=N) to 1635 cm^{-1} and 1602 cm^{-1} respectively, which indicates the coordination of zinc between carbonyl oxygen and azomethine nitrogen. The disappearance of v(N-H) and the shifting of v(C=S) to a lower value of 1259 cm^{-1}

indicate the loss of proton (H_1) or no N-H group are present, subsequently caused the thiolato sulphur to take part in the chelation. Thus, the coordination between AFCTSC and Zn^{2+} could be proposed as mononegative tridentate manner via ONS donor atoms [27]. The appearance of bands at 616 cm^{-1} and 410 cm^{-1} which were assigned to v(M-O) and v(M-N) respectively proved the formation of coordinative bonding of AFCTSC-Zn through carbonyl oxygen and azomethine nitrogen [21, 25].

^1H NMR data (**S5**) of AFCTSC and AFCFTSC-Zn. The signal at δ11.06 and δ8.59 were assigned for H_3 and H_4 ($N-H_2$) and H_1 (CH=N) respectively. Zn^{2+} addition caused the disappearance of the H_3 and H_4 signal completely, proved the ligand coordinate to Zn^{2+} in its amine-imine tautomer form (**Figure 7b**). Meanwhile, the signal for H_1 shifted to higher field of δ 8.69 confirmed the participation of azomethine nitrogen upon the coordination. The signals of remaining aromatic protons shifted downfield due to the coordination between ligand and Zn^{2+} [7].

Theoretically, AFCTSC acted as an ONS donor of tridentate ligand, which binds to the zinc atom through azomethine group of the Schiff base, γ-pyrone oxygen from 3-carbaldehyde chromone unit and thiolic sulfur of the thiosemicarbazone side chain. The fluorescence behaviour from blue to green emission signal in the presence of Zn^{2+} could be due to the formation of the amine-imine tautomer, giving chelation-enhanced fluorescence (CHEF) of AFCTSC towards Zn^{2+}. Moreover the excited state intramolecular proton transfer (ESIPT) and photo-induced electron transfer (PET) processes were inhibited during the chelation [2, 28].

Sensitivity study

Sensitivity study was performed to investigate the interference of other metals to the fluorescence behaviour of AFCTSC-Zn. The addition of interference metals such as Ca^{2+}, Co^{2+}, Mn^{2+} and Ni^{2+} showed decreasing but still significantly observable fluorescence intensity of AFCTSC-Zn, (**Figure 9**). The presence of Fe^{2+} had reduced one third of the fluorescence signal of AFCTSC-Zn, while Cu^{2+} completely quenched the fluorescence intensity. The great drop in the fluorescence intensity particularly Cu^{2+} was consistent with some previous reports [26, 29]. Therefore, the ligand was suitable for the detection of Zn^{2+} even with the presence of competing metal ions except for Cu^{2+}.

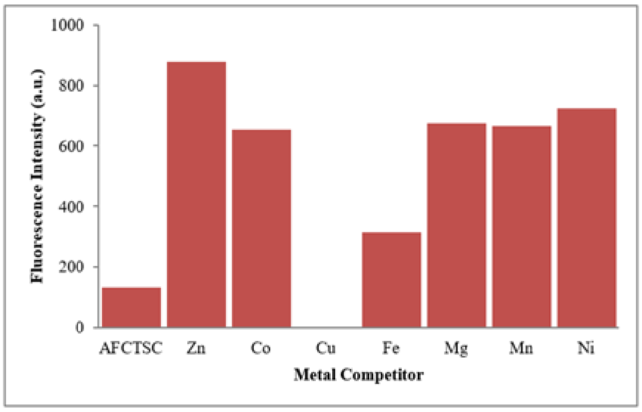

Figure 9: Fluorescence activity of AFCTSC and its complexation with Zn^{2+} in the presence of various competitor metal ions.

Conclusion

In summary, a chemosensor of AFCTSC showed a high selectivity towards Zn^{2+} in comparison with other listed metal ions with 7.5-fold fluorescence enhancement. Upon reaction with Zn^{2+}, the ligand exhibited a blue to green colour change when observed under UV light. AFCTSC also exhibited good sensitivity in detecting Zn^{2+} in the presence of most competing metal ions. The binding study explained the coordination between Zn^{2+} and AFCTSC was in a mononegative tridentate manner. The limit of detection of AFCTSC is below than the permitted level of Zn^{2+} by WHO which is 7.65×10^{-5} M. Thus, we believe AFCTSC can be a promising fluorescent probe for determination of Zn^{2+} contaminant in the environments and worthwhile, as it is easily synthesized via classical organic synthesis method and exhibits 4.5×10^{-7} M in low detection limit.

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