

# Structural characterization of new Cd<sup>2+</sup> fluorescent sensor based on lumazine ligand: AM1 and *ab initio* studies

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## Abstract

6-Thienyllumazine (TLM) is synthesized as a new fluorescent sensor that is capable of indicating selectively the presence of Cd<sup>2+</sup> ion via a fluorescence signal. Experiment has been performed in the presence of Ni<sup>2+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup>, Ag<sup>+</sup>, Mn<sup>2+</sup>, Hg<sup>2+</sup>, Zn<sup>2+</sup>, Pb<sup>2+</sup>, and Mg<sup>2+</sup> metal ions in aqueous solutions. The product was characterized by elemental analysis, mass, and NMR spectra. The spectral characteristics (maxima, quantum yields, Stokes shift, and lifetimes) of TLM in organic and aqueous solvents have been studied with the help of absorption and fluorescence spectroscopy, as well as, using time dependent spectrofluorimetry (single photon counting technique). The fluorescence dependence of TLM on the pH has also been investigated. The experimental results indicate that TLM exists in two ionic forms: neutral (acid) and anion (base). Electronic structure calculations of TLM were carried out using Semiempirical Austin Model 1 (AM1) and *ab initio* Hartree–Fock (HF) with 6-31G\* basis set and using Gaussian 03 program. Absorption energies for TLM have been calculated using ZINDO method. The theoretical results confirm the presence of the thiophene and pteridine rings in two conformations: twisted at angle of about 35° in the excited state and coplanar in the ground state.

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**Keywords:** Fluorescence spectra; Time-resolved fluorescence spectra; Solvent effects; Theoretical calculations; Lumazine; Fluorescent sensor

## 1. Introduction

Metal ions can be toxic to life when present at certain concentrations in the environment, water supplies, food chain, and industrial chemicals and products. Consequently, an intensive effort has been devoted to develop various sensory molecular receptors capable of recognizing, sensing, and selectively removing these toxic positively charged substrates from the environment [1]. Cadmium is one of the most toxic metal environmental pollutants [2]. It may also replace zinc in some zinc enzymes, thereby altering the stereostructure of the enzyme and impairing its catalytic activity [3]. These factors make the detection and quantification of cadmium in the environment a significant area of research.

It has been stated that pteridines and their structurally related flavins and pterins are biochemically important molecules, which attracted the attention of researchers [4]. The role

developed by these heterocycles is directly related with the establishment of coordinative bonds to biologically essential metal ions in some metalloenzymes [5]. Lumazine (2,4-(1H,3H)pteridinedione, LM after herein) (Fig. 1A) is a naturally occurring pteridine derivative known as a substrate in the oxidative hydroxylation reaction catalyzed by xanthine oxidase resulting in the product violapterin (2,4,7-(1H,3H,8H)pteridinetrione) [6]. The structures and spectral properties of several metal complexes of LM and its methylated derivatives, which usually coordinate to the metal ions in a bidentate pattern with N5 and O4 atoms (Fig. 1), have been studied by several researchers [7–11].

Han and Kim et al. [12] reported the involvement of LM in the selective detection of zinc ion. In an accompanying work, we report 6-thienyllumazine, TLM, as a selective fluorescent sensor for Cd<sup>2+</sup> ion. The chemical structure of the newly synthesised derivative, TLM, is shown in Fig. 1B. This paper is aimed at characterizing the structural properties of the ground and excited states of TLM in different media using steady and time-resolved molecular spectroscopy, as well as, theoretical calculations. The results might help us in understanding the structural and envi-

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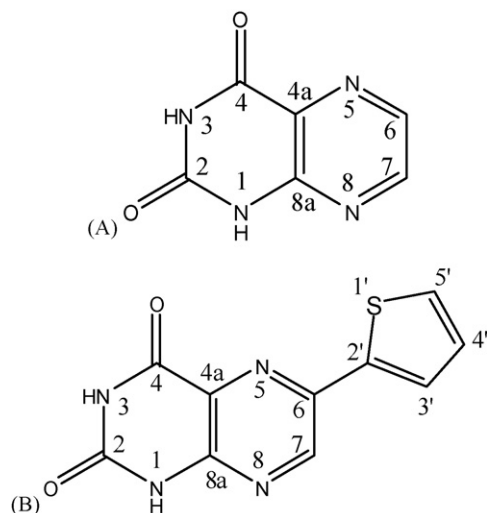


Fig. 1. Molecular structures of: (A) lumazine (LM) and (B) 6-thiellumazine (TLM) showing the atom numbering scheme.

ronmental factors that affects the fluorescent sensing properties of TLM to  $\text{Cd}^{2+}$  ion.

## 2. Experimental

### 2.1. Synthesis of TLM

As shown in Scheme 1, suspension of 5,6-diaminouracil (**1**) (2.0 g, 13.98 mmol) and 2-oxo-2-(thiophen-2-yl)acetaldehyde oxime (**2**) (2.6 g, 16.78 mmol) in EtOH (40 ml) was heated under reflux for 3 h, after cooling, the crystals were filtered off, washed with hot water and the crude product was recrystallized from EtOH to afford a yellow powder of TLM (2.72 g, 79%); mp 282 °C (from EtOH);  $^1\text{H}$  NMR (DMSO- $d_6$ ; 250 MHz;  $\text{Me}_4\text{Si}$ ):  $\delta_{\text{H}}$  10.61 (br s, 2H, NH); 9.21 (s, 1H, H-7); 7.94 (d, 1H,  $J=4.0$  Hz, H-5'); 7.21 (d, 1H,  $J=3.8$  Hz, H-3'); 7.19 (dd, 1H,  $J=4.0$  Hz, H-4');  $^{13}\text{C}$  NMR (DMSO- $d_6$ ; 250 MHz;  $\text{Me}_4\text{Si}$ ):  $\delta_{\text{C}}$  161.3 (C-4); 150.3 (C-2); 150.10 (C-7); 146.9 (C-8a); 144.9 (C-2'); 143.9 (C-6); 129.8 (C-4a), 129.1 (C-3', C-4'), 126.7 (C-5'); Anal. Calc. for  $\text{C}_{10}\text{H}_6\text{N}_4\text{O}_2\text{S}$  (246.25): C, 48.78; H, 2.46; N, 22.75. Found: C, 48.58; H, 2.34; N, 22.56;  $m/z$  (EI) 246 (80%).

### 2.2. Steady spectroscopy

The absorption spectra were collected using Labomed Double PC UV–vis spectrophotometer, while the fluorescence

spectra were measured using Edinburgh FS-900CDT fluorometer with excitation and emission bandwidth of  $4 \pm 0.5$  nm, the instrument full description is available elsewhere [13]. The absorption of each chromophore was checked to be less than 0.1 absorbance unit at the excitation wavelength ( $\lambda_{\text{excitation}} = 375$  nm). The solvents were of a spectroscopic grade. Prior to spectral measurements, solvents were degassed by bubbling  $\text{N}_2$ . NMR spectra were measured with Bruker WM-250 with TMS as internal standard and on a  $\delta$  scale in ppm. EI mass spectra were recorded on a MAT 312 mass spectrometer. The melting point was uncorrected. Quantum yields ( $\phi_f$ ) were measured using coumarin1 (7-diethylamino-4-methylcoumarin) in ethyl acetate as a standard ( $\Phi = 0.99$ ) [14].

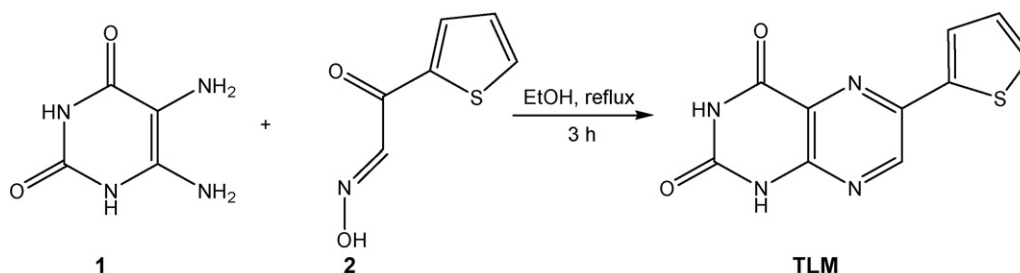
### 2.3. Time-resolved spectroscopy

The time-resolved data were measured using time correlated single photon counting (TCSPC) apparatus: Edinberg Instrument Model 199. The sample was excited with a flash lamp filled with  $\text{N}_2$  at 1.5 bar operated at 8 kV of  $\sim 3 \pm 0.5$  ns duration (half-width) with low-level light signals of repetition rate at  $\sim 20$  kHz. Excitation and emission bandwidth was  $10 \pm 1$  nm. The emission intensity was reduced (800 counts/s) such that only one photon is detected for each excitation period [15].

### 2.4. Computational methods

Ground state gas-phase molecular geometries were optimized at two different levels, Semiempirical Austin Model 1 (AM1), and *ab initio* Hartree–Fock (HF) computed at the HF/6-31G\* level of theory. To compute the structure of the excited state, the ground state structure was first used as the input file and freezing the dihedral angle (atoms, N5-C6 -C2'-S1') to a random number, then the structure was optimized at the HF/6-31G\* ground state level of theory. Afterward, the angle constrains was removed and the excited states structure was carried out using single-excitation CI (CI-Singles-CIS) at 3-21G, 6-31G and 6-31G\* basis set, including all singly excited state configurations.

The gradients and vibrational frequencies were determined analytically, and no imaginary frequency modes were found at the ground state optimized structure. All calculations were carried out using Gaussian 03 software [16].



Scheme 1.

Table 1

Absorption and fluorescence maxima, quantum yields ( $\phi_f$ ) and Stokes shifts ( $\nu_A - \nu_F$ ) of TLM in solvents with different properties<sup>a</sup> at 25 °C and pH 5.0

Number	Solvent	$\epsilon$	$\eta$	$\lambda_{\text{abs}}$ (nm)	$\epsilon$ (cm <sup>-1</sup> )	$\lambda_{\text{em}}$ (nm)	$\phi_f$	$\nu_A - \nu_F$ (cm <sup>-1</sup> )
1	Ethyl acetate	6.0	0.44	374	21,446	431	0.34	3536
2	Butanol	17.5	2.50	376	26,523	435	0.10	3607
3	Ethanol	24.6	1.10	374	39,245	434	0.19	3696
4	Methanol	32.7	0.54	374	24,884	434	0.09	3696
5	Ethylene glycol	37.7	16	380	21,117	442	0.07	3691
6	Dimethyl sulfoxide (DMSO)	46.5	2.14	378	26,470	440	0.06	3728
7	Water	78.3	0.89	378	33,373	452	0.04	4331

<sup>a</sup>  $\epsilon$ : Solvent dielectric constants;  $\eta$ : solvent viscosities in centipoises at 25 °C.

### 3. Results and discussion

#### 3.1. Photophysical properties of TLM in organic and aqueous solvents

Table 1 summarizes the photophysical properties of TLM at pH 5 and 25 °C in several organic solvents and in water solvent. Fig. 2, as an example, shows the absorption and emission spectra of TLM in methanol at 25 °C. The initial observation of this table and figure established that the absorption spectrum shows a broadband located near UV region with high molar extinction coefficients, while the fluorescence spectrum appears as structured band in bluish-green region. It is also seen (Table 1) that the polarity or viscosity of the organic solvents has no influence on the Stokes shift or fluorescence quantum yield values of TLM. In fact, most of the solvent parameters, such as Dimroth parameter ( $E_T$ ), polarity and polarizability parameter ( $\pi^*$ ), and Kosower parameter ( $Z$ ) were tested and neither one could correlate with the fluorescence or absorption maxima. Nevertheless, absorption and fluorescence maxima change only slightly with solvents variation.

Fig. 3 depicts the pH-dependent (titration) fluorescence of TLM in water in order to characterize its acid/base behavior, which is of interest to its application as metal sensor. Observation of well-defined isosbestic point at around 460 nm confirms complete conversion upon addition of each equivalent of NaOH. The

fluorescence emission maximum shifts from 445 nm ( $\phi_f = 0.02$ ) to 483 nm ( $\phi_f = 0.05$ ) with the increase in pH from 2 to 11. The excitation was made at 230 nm, which is the isosbestic point measured using UV–vis spectroscopy. The pH dependence of fluorescence intensity has been analyzed by the following equation:

$$\text{pH} - \text{p}K_a = \log \left( \frac{F_{\text{max}} - F}{F - F_{\text{min}}} \right) \quad (3.1)$$

where  $F_{\text{min}}$  and  $F_{\text{max}}$  are the integrated fluorescence intensities between 380 and 650 nm of the acid and base forms of the species involved in the acid–base equilibrium and  $K_a$  is the dissociation constant. The calculated  $\text{p}K_a$  value is  $5.5 \pm 0.3$  ( $R = 0.999$ ). No significant differences between the  $\text{p}K_a$  values determined by fluorescence titration and that obtained from spectrophotometric titration were observed.

Table 2 shows the fluorescence decays for TLM fluorescence in water at pH 5.0. Notice that, changing the emission upon which the biexponential decay is monitored from 450 to 510 nm increases the contribution of the second lifetimes ( $\tau_{F2}$ ).

It is interesting to compare the spectral characteristics of TLM to those corresponding to LM (the parent compound) in DMSO [17], and in water [12,18]. The UV–vis maxima of LM in DMSO were 328 nm for the neutral form and 380 nm for the anion form. It has been known that LM exists in three

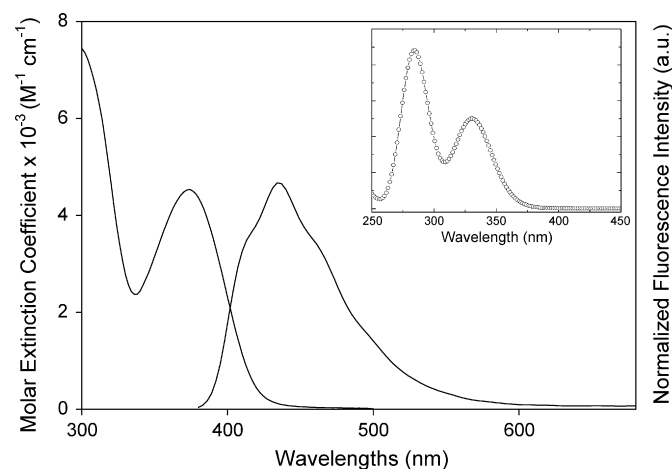


Fig. 2. The absorption and emission spectra of 3  $\mu\text{M}$  TLM in MeOH solution at 25 °C and pH 5.0. Excitation was made at 375 nm. Inset: Shows the UV–vis absorption spectrum obtained by the ZINDO method.

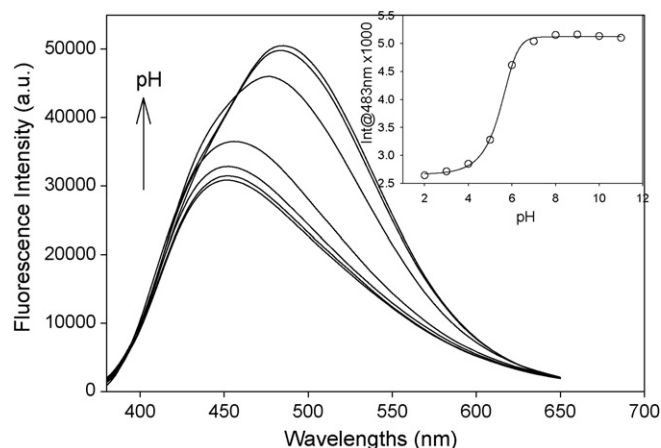


Fig. 3. The evolution of the fluorescence spectra of 7  $\mu\text{M}$  TLM in  $\text{H}_2\text{O}$  with pH. Inset: Shows the pH titration plots at 483 nm. The estimated  $\text{p}K_a$  is  $5.5 \pm 0.3$  ( $R = 0.999$ ). pH was adjusted by adding small aliquots of aqueous solutions of strong acids HCl or bases NaOH to achieve the desired ionic states. Excitation was made at 375 nm.

Table 2

The fluorescence lifetimes ( $\tau_F$ ) in nanoseconds (ns) and the corresponding amplitudes ( $A$ ) of TLM in water at 25 °C and pH 5.0. Excitation was made at 337 nm

Monitored emission	$A_1$ (%)	$\tau_{F1}$ (ns)	$A_2$ (%)	$\tau_{F2}$ (ns)	$\chi^2$
450	61	1.6	39	5.3	1.109
470	55	1.6	45	5.3	1.078
510	45	1.6	55	5.3	1.156

ionic states (neutral, anion, and dianion) (Scheme 2) [6,17]. The monoanion form arises from deprotonation of the (3)NH proton, located between the carbonyl groups, while the dianion from results from dissociation of the (1)NH and (3)NH protons. The optical and fluorescence characteristics of LM in water were as follows: below pH 6.0, the absorption spectrum of LM has well developed maximum at 228 ( $\epsilon = 12,400 \text{ cm}^{-1}$ ) and 325 nm ( $\epsilon = 8500 \text{ cm}^{-1}$ ), while above pH 10, the spectrum exhibits three peaks at 234 ( $\epsilon = 13,600 \text{ cm}^{-1}$ ), 270 ( $\epsilon = 11,500 \text{ cm}^{-1}$ ), and 345 nm ( $\epsilon = 600 \text{ cm}^{-1}$ ). The fluorescence properties of LM in water, however, was reported to show bluish-green fluorescence at pH 7.0 but it turns to green when the solution becomes alkaline, which is ascribed to the deprotonated LM that is generated by base. At pH 10, LM is present predominantly in the form of monoanion in the aqueous solution. The monoanion shows a fluorescence emission maximum at 467 nm with a quantum yield of 0.24 when excited at 370 nm. The titration of aqueous LM reveals a  $pK_a$  of 7.9 and another above 12. The lower  $pK_a$  was assigned to the (3)NH proton, located between the two carbonyl groups [12,18] (Scheme 2). In short, it is seen that the absorption and emission spectral features of TLM are red shifted comparing to those of LM in the same solvent at given pH. For example, by the introduction of thiophene group at position-6, the absorption maximum at low pH shifts from 328 to 378 nm in DMSO, and from 325 to 378 nm in water, in addition, the fluorescence maximum in water at pH 10 shifts from 467 to 483 nm. It is also noted that TLM is more acidic than LM compound. For example, the first  $pK_a$  shifts from 7.9 in LM to 5.5 in TLM molecule.

### 3.2. AM1 and *ab initio* calculations of TLM structures

Both ground state structures optimized at a Semiempirical Austin Model 1 (AM1) or at *ab initio* Hartree–Fock (HF)/6-31G\* level of theory show a planar geometry. The potential energy diagram of the optimization (Fig. 4) reveals that the ground state has two local minimas (a and b), the two of them are flat but a has the S-1' in the same side of N-5, while b minimum

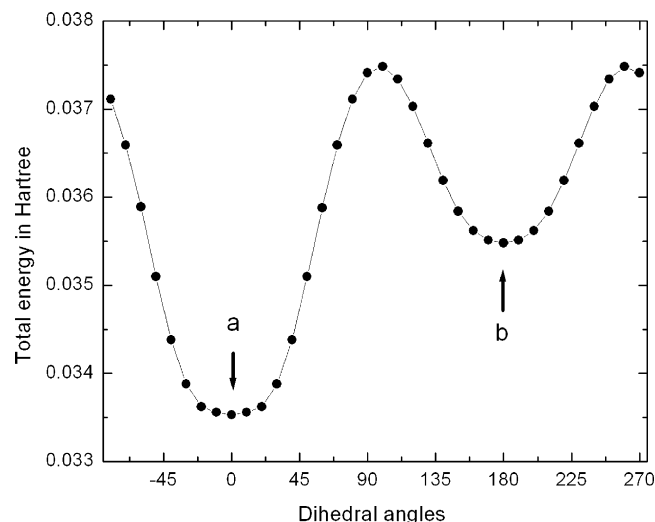
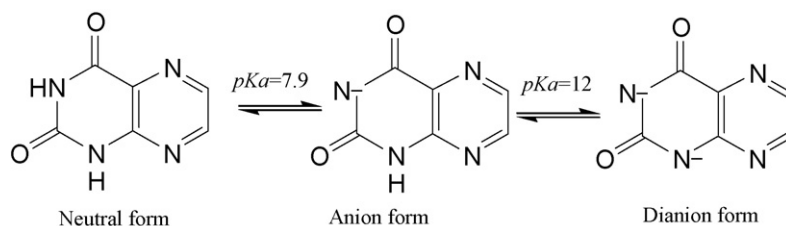


Fig. 4. Potential energy diagram of the ground state optimization of TLM obtained at the AM1 level by altering the dihedral angles angle between the thiophene and the lumazine groups.

has the two atoms opposite to each other. The former minimum turns to be the global minimum. The planar geometry gives the molecule the appropriate space for  $\pi$  delocalization. Such interaction is will explained by looking at the HOMO orbital (Fig. 5A). It is obvious that the  $\pi$  delocalization is stronger than the eclipsing energy between H-3' and H-7, thus forcing the molecule into a planar geometry. The LUMO orbital of the molecule (Fig. 5B) clearly indicates the absence of delocalization on the thiophene moiety, such that, the molecule will twist itself in an attempt to release the torsional energy between H-3' and H-7. Simulation of the UV–vis absorption spectra (Fig. 2, inset) was conducted by the Zerner's Intermediate Neglect of Differential Overlap (ZINDO) method which shows a great resemblance of the measured spectrum with about 30 nm differences. This might indicate that the calculated geometry of the ground and the excited state agrees with the actual structures.

### 3.3. Influence of metal ions on the fluorescence intensity of the TLM in aqueous solutions

The sensing properties of TLM to metal ions were measured using fluorescence technique in aqueous solutions as follows: a 2.5 ml water solution containing 4  $\mu\text{M}$  of TLM at pH 5.0 was prepared, and an initial fluorescence measurement was made. Aliquots of Cd(II) from stock solution of  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  were added into the solution to give a final concentrations of 2.5 mM,



Scheme 2.



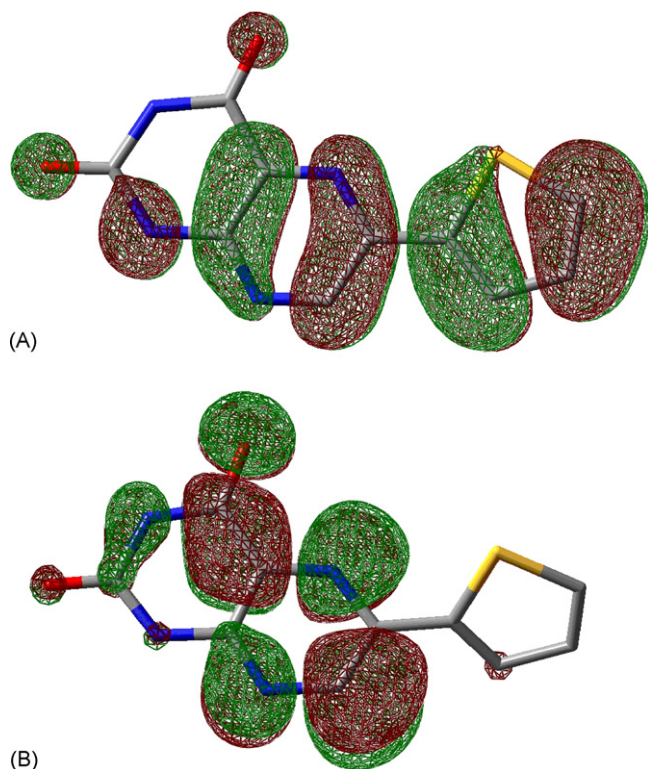


Fig. 5. (A) HOMO orbital of the TLM molecule calculated at the HF/6-31G\* level. (B) LUMO orbital of the TLM molecule calculated at the HF/6-31G\* level.

and the fluorescence spectra were recorded again. Fig. 6 shows enhancement and blue shift of fluorescence with the addition of aqueous solutions of cadmium nitrate salt. In contrast, fluorescence quenching (without shift) was observed with the addition of similar concentrations from nitrate or chlorides salts of the following ions:  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ag}^{+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$ , and  $\text{Mg}^{2+}$ . All fluorescence spectra were acquired by exciting at 375 nm. The indicated ions were selected since ligands which complex  $\text{Cd}^{2+}$  usually also complex these ions.  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$

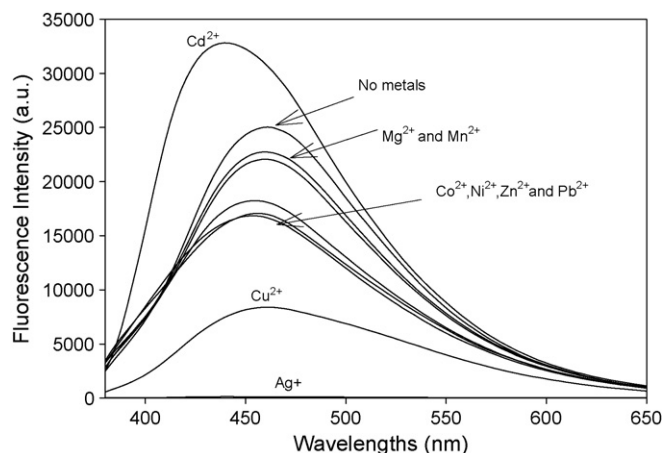


Fig. 6. Fluorescence emission spectra at pH 5.0 obtained by the addition of 2.5 mM aqueous solutions of  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{Pb}(\text{NO}_3)_2$ ,  $\text{CoCl}_2$ ,  $\text{NiCl}_2$ ,  $\text{CuCl}_2$ ,  $\text{ZnCl}_2$ ,  $\text{AgNO}_3$ ,  $\text{MnCl}_2$ , or  $\text{MgCl}_2$  salts to 5  $\mu\text{M}$  aqueous solution containing TLM. Excitation was made at 375 nm.

were selected in particular because they have similar toxic properties, such as  $\text{Cd}^{2+}$ , while  $\text{Zn}^{2+}$  has been chosen due to its electronic configuration that makes it similar to  $\text{Cd}^{2+}$ .

#### 4. Conclusions

The fact that the excited state of TLM is twisted and the ground state is planar might explain the deviation of its absorption and fluorescence from mirror image relationship, as well as, the observed vibronic structure in its emission spectrum. Moreover, the slight influence of solvents variation on the observed absorption and emission maxima of TLM seems to suggest absence of electron transfer or (eximer) formation. Therefore, it might be proposed that the observed spectral bands of TLM are associated with the local (monomer) absorption or emission of pteridine ring. Consequently, the delocalizations of  $\pi$  electrons in TLM might explain the red shift in its absorption and emission bands in comparison to those of LM compound.

The change of TLM fluorescence in water with pH and the fact that it shows biexponential decays suggests the existence of two ionic species similar to those species reported for LM: neutral (acid) and anion (base) forms; both in the ground and excited states. It can be said that the introduction of thiophene group enhances the delocalization of  $\pi$  electrons. Such delocalization together with the presence of sulphur atom (empty d orbital) in the same side of (4) $\text{C}=\text{O}$  group might explain the decrease in the acidity of the (3) $\text{NH}$  group in TLM compound compared to that in LM (the parent molecule).

In general, the result of this work indicates the importance of investigating the effect of pH on the fluorescence of  $\text{Cd}^{2+}$  probe in order to estimate the pH at which the maximum enhancement of the fluorescence is obtainable. This issue, which is under investigation in our laboratory, is important for the practical application of the proposed sensor in the quantitative determination of  $\text{Cd}^{2+}$  concentration in real water samples. We also plan in fully characterizing the overall geometry around the cadmium center using X-ray crystallography in view of the present results, if single crystals of the TLM/ $\text{Cd}^{2+}$  complex could be obtained.

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