Coumarin Appended Calix[4]arene as a Selective Fluorometric Sensor for Cu^{2+} Ion in Aqueous Solution

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There is an increasing demand to selectively sense some heavy metal ions such as Hg^{2+}, Pb^{2+}, Cu^{2+} because of their high toxicity.[1] Many analytical methods have been applied for this purpose including atomic absorption spectrometry (AAS), ion selective electrodes (ISE), flame photometry. However, they require high cost, large amount of samples and do not allow continuous monitoring. Recently, considerable attention has been paid on fluorometry due to its simplicity, selectivity, sensitivity and response time.[2] Most fluorometric sensors are designed to adopt photo-physical changes produced upon complexation including photo-induced electron transfer (PET),[3] photo-induced charge transfer (PCT),[4] excimer/exciplex formation and extinction,[5] or fluorescence resonance energy transfer (FRET).[5]

PET sensors are the most commonly exploited because of a large change in fluorescence intensity upon addition of metal ions.[7] There can be an enhancement or a quenching dependent on ions coordinated with the ligand. In general, the former phenomenon is observed because the complexation causes an increase in the redox potential of the donor so that the relevant HOMO's energy decreases to a level lower than that of the fluorophore. Consequently, the excited state energy of the fluorophore is dumped as a visible emission. Such enhancement of fluorescence intensity upon cation binding is called CHEF (CHelating Enhancement Fluorescence) effect.[3d,8] In some cases, complexation of metal cations, particularly seen with Cu^{2+} and Ni^{2+} ions,[9] does not induce CHEF effect but causes fluorescence to be quenched *via* two well defined mechanisms: electron transfer (eT) and energy transfer (ET) that undergo rapid non-radiative decay followed by a quenching in fluorescence intensity.

In previous paper,[10] we reported a calixarene with two coumarin groups on the lower rim (**1**) acting as a fluoride-selective sensor in CH_3CN solution. From its structure, we believe that a PET process may occur from the carbonyl oxygen atoms to the coumarin in the excited state. Besides, amide nitrogen atoms were found to be capable of chelating with some transition metal ions.[11] Keeping this in mind, we thought **1** could be used as a sensor for some heavy metal ions based on ET or eT process. This paper presents the fluorescence studies of **1** and its complexes of cations in the mixture of H_2O/DMSO.

In order to search for a sensor that can be applied in aqueous solution, fluorescence changes with ratio of H_2O/DMSO were conducted. As seen in Figure 1, the fluorescence intensity decreases with increasing ratio of H_2O/DMSO. This can be explained by the two facts that (a) solvation by polar solvents causes the energy of the polar excited state to decrease and reduces the gap between the excited state and the ground state, thus non-radiative deactivation is favored;[12] (b) the formation of intermolecular H-bonding between amide hydrogen atom and oxygen atom in water molecule induces an increased negative charge in the amide oxygen atom in a new form of $-NH^{-}=C(R)-O^{-}$ so that PET from this oxygen to coumarin group is strengthened. From the ratio of 1:1 up, fluorescence intensity decreases considerably, therefore next experiments were carried out at the ratio of 2:3. In addition to a quenching of fluorescence intensity, increasing water also induces a red shift of emission band. As known, a fluorophore has a large dipole moment in the excited than in the ground state. When excited, the solvent dipoles can orient or relax around the moment in the excited state, lowering the energy of this state.[13] As water is more polar than DMSO, increasing water results in emission band at lower energies or longer wavelengths. In addition to the fluorescence emission at *ca.* 430 nm, increasing water also induces a new band at *ca.* 490 nm, tentatively arising from tautomerization between amide and hydroxyimine.

Fluorescence of **1** bearing amide moieties might be affect-

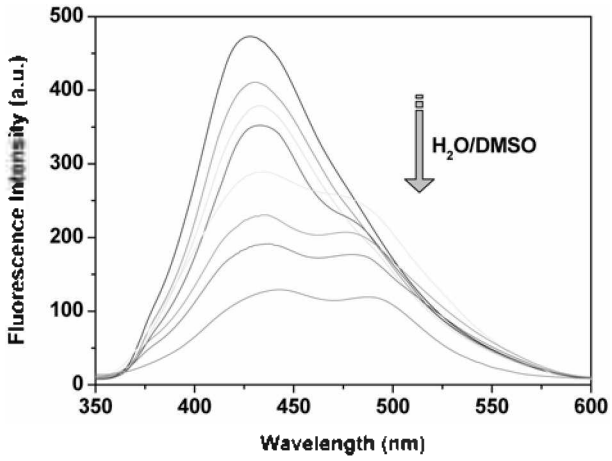


Figure 1. Fluorescence spectra of **1** (5.0 μM) in H_2O/DMSO mixture with an excitation at 335 nm. Ratios of H_2O/DMSO: DMSO only, 1/4, 3/7, 2/3, 1/1, 3/2, 7/3 and 4/1.

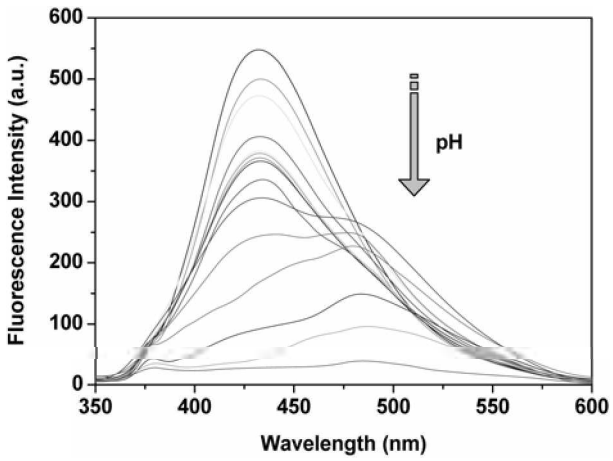


Figure 2. Fluorescence spectra of **1** in mixture of H_2O/DMSO = 2/3 at different pH conditions: 3.1, 3.5, 3.9, 4.3, 4.6, 5.4, 5.9, 6.4, 6.8, 7.5, 7.9, 8.3, 9.0, 10.5, 11.9, and 12.5.

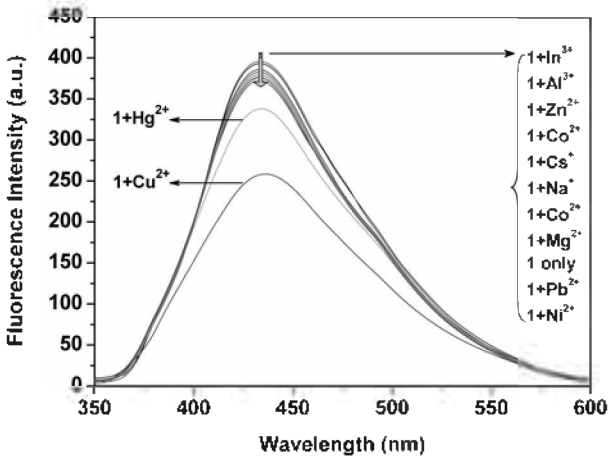


Figure 3. Fluorescence spectra of **1** (5.0 μM) in mixture of H_2O/DMSO = 2/3 at pH 6 upon addition of different metal ions (50 eq) with an excitation at 335 nm.

ed by proton concentration in the solution. Therefore the investigation on pH-dependent fluorescence was conducted to find out the appropriate pH range for complexation study. Figure 2 shows that the fluorescence intensity strongly increases upon addition of H^+, which can be explained by the fact that the amide nitrogen atoms are protonated, lowering the negative charge density on the carbonyl oxygen atoms. As a result, the lone-pair electrons on these atoms are less flexible, reducing PET process from these electrons to coumarin groups followed by a considerable enhancement of fluorescence intensity. In contrast, upon addition of OH^- into solution of **1**, fluorescence is quenched, obviously due to the enhancement of negative charge density on the carbonyl oxygen atoms as well as the phenolate formation followed by an increased PET process. However, there is no significant change of fluorescence intensity in the range of pH 5-7, suggesting that the compound exist mainly in neutral form in this range. Next experiments were then carried out at pH 6, using KH_2PO_4/NaOH as a buffer solution.

Figure 3 displays fluorescence changes upon addition of various metal ions to **1** in H_2O/DMSO mixture at pH 6. Generally, fluorescence intensity increases slightly when metal ions are added, owning to CHEF effect from complexation. Whereas, complexation of **1** with Hg^{2+}, or Cu^{2+} induces a quenching of fluorescence intensity that can be explained as a result of either an electron transfer (eT) or an electronic energy transfer (ET) from coumarin moieties in the excited state to these metal ions. Ni^{2-}, Hg^{2-}, Cu^{2+} were reported to have the ability to quench the emission intensity of some fluorophores due to eT or ET process,[14] but in our study only Cu^{2-} ion causes a significant change. This is probably due to the geometrical difference between **1**·Cu^{2+} complex and other metal ion complexes. With an electron configuration of d^9, Cu^{2+} is favorably coordinated in a square plane geometry according to *Jahn-Teller* effect.[15] Moreover, **1** with two nitrogen atoms and two of four oxygen atoms at the lower rim of calixarene can provide accommodation for Cu^{2-} to form a square plane complex. The effect of pH on fluorescence intensity of **1**·Cu^{2-} complex was also investigated. As seen in Figure 4, in the range of pH 5-9, the difference between the fluorescence intensity of **1** and that of **1**·Cu^{2-} is almost unchanged, suggesting that the complex be stable in this range. At low pH, the intensity returns to the original value of **1** with decreasing pH, indicative of a complete dissociation, and thus Cu^{2+} no longer affects the fluorescence intensity of **1**. With the increasing pH after 9, the fluorescence intensity of **1** and that of **1**·Cu^{2+} become closer, probably because of the formation of hydroxo-complex of Cu^{2+} is favored in this condition. Figure 4 shows the fluorescence intensities of **1** and **1**·Cu^{2+} as plateau from pH 5-7, therefore the pH 6 chosen above is a good choice for experiments.

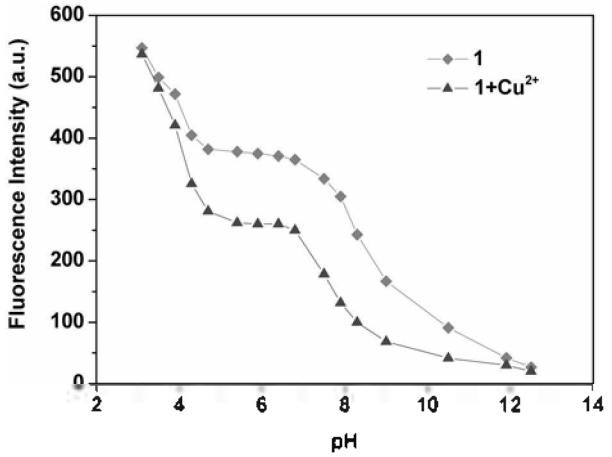


Figure 4. Fluorescence intensity of **1** (5.0 μM) and **1** (5.0 μM) + Cu^{2+} (50 eq) recorded at 434 nm at different pH values.

To figure out the complexation ratio between **1** and Cu^{2+} ion under the condition of an invariant total concentration, the Job plotting experiment was also conducted (see the inset of Fig. 5). As a result, the **1**·Cu^{2-} complex concentration approached the maximum when the molar fraction of [**1**]/([**1**] + [Cu^{2-}]) was about 0.5, indicating that it formed a 1:1 complex of **1** and Cu^{2+} as shown below.

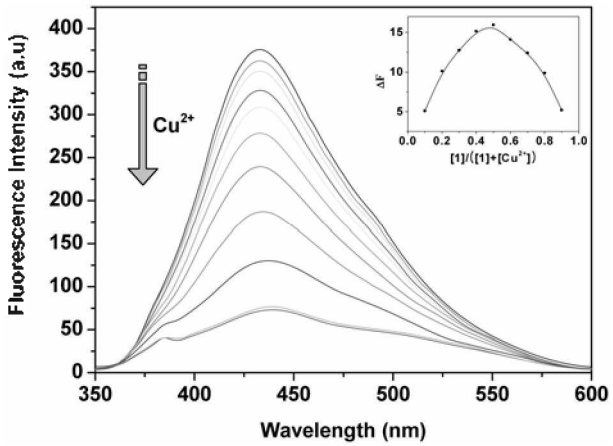
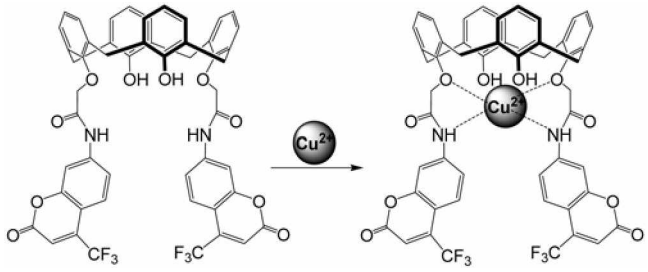


Figure 5. Fluorescence changes of **1** (5.0 μM) in mixture of H_2O/DMSO = 2/3 at pH 6 upon addition of Cu^{2+} with different concentrations. Inset: Job's continuous variation plot for **1**·Cu^{2+} at 434 nm.

Figure 5 displays fluorescence changes of **1** with Cu^{2+} ion concentration. The fluorescence intensity was gradually decreased by the addition of Cu^{2+} ion up to 900 equiv. and then saturated. From this titration experiment and using ENZFITTER Program, the association constant of **1** for Cu^{2+} ion complexation in mixture of H_2O/DMSO = 2/3 at pH 6 was found to be $1.47 \times 10^3\ M^{-1}$.



In conclusion, a coumarin-appended calixarene **1** was studied as a fluorometric sensor for metal cations in aqueous medium. **1** displays a high selectivity towards Cu^{2+} ion over other metal ions tested in a mixture of H_2O/DMSO = 2/3 and pH 6 controlled by KH_2PO_4/NaOH as a buffer solution. The selectivity of **1** towards Cu^{2+} is dominated by (a) the eT or ET process from the excited coumarin moieties to Cu^{2+} ion as well as (b) the favorable formation of a square plane complex of Cu^{2+}.

General Procedure for Fluorescence Measurement. Fluorescence spectra were recorded using a RF-5301PC spectrofluorophotometer. For all measurements, the excitation was 335 nm; excitation and emission slit widths were both 5 nm. Fluorescence experiments were performed using 5.0 μM of **1** and various equivalents of Cu^{2+} perchlorate. After calculating the concentrations of the Cu^{2+} complex and free ligand **1** from fluorescence titration experiment, the association constant was obtained using the computer program ENZFITTER.[16]

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