

An indolium croconine-based colorimetric and fluorescent chemosensor for detection Fe^{3+} and Cu^{2+}

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A colorimetric and fluorescent chemosensor for Fe^{3+} and Cu^{2+} based on indolium croconine (**ICR**) was synthesized and characterized. It showed sensitive recognition and highly selective to Fe^{3+} and Cu^{2+} in $\text{C}_2\text{H}_5\text{OH}/\text{H}_2\text{O}$ solution. In the presence of Fe^{3+} , fluorescent maximum emission band of **ICR** at 782 nm blue-shifted to 766 nm, and the fluorescence of **ICR** was completely quenched. The color of solution changed from olive green to yellow. In the presence of Cu^{2+} , the color of solution changed from olive green to pale pink. The fluorescent maximum emission band of **ICR** at 783 nm blue shifted to 771 nm.

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1. Introduction

Design of chromogenic or fluorescent chemosensors for the selective detection of cations, especially for metal ions with biological interest, has always been of particular significance due to their potential applications in many fields including chemistry, biology, medicine and the environment. Iron and copper are the most abundant transition metal ions in biological systems, which are important to the metabolic processes. Cu^{2+} ions play an important role in fundamental physiological processes in human body, and it is considered as an essential element for human life [1], which serves as an essential cofactor for enzymes in all living organisms due to its edox-active nature. However, low level of Cu^{2+} in the cells will affect the corresponding enzyme activity, inhibit cell normal metabolism, whereas the exceeding concentration of Cu^{2+} can promote the direct oxidation of protein side chains and has also shown to nucleate protein aggregation associated with many amyloid-based diseases, such as Alzheimer disease, Parkinson disease and dialysis-related diseases [2]. Iron in trivalent state is essential which provides oxygen-carrying capacity of heme and acts as a cofactor in many enzymatic reactions to human. It participates in many biological processes at the cellular level ranging from oxygen metabolism to DNA and RNA synthesis. The deficiency of Fe^{3+} causes anemia, hemochromatosis, liver damage, diabetes, Parkinson's disease and cancer [3]. Therefore, detection of Fe^{3+} and Cu^{2+} ions efficiently has now become an emergency for the sake of environment and human health. Among the numerous analytical methods for the detection of Fe^{3+} and Cu^{2+} ions, the techniques based fluorescent sensors have attracted much

attention due to their distinct advantages of sensitivity and tunableness [4-8]. Though some fluorescent chemosensors for both Fe^{3+} and Cu^{2+} were thereby developed, few of them could successfully distinguish between Fe^{3+} and Cu^{2+} [9,10]. Therefore, it is necessary to develop the colorimetric chemosensors with “naked eye” capability have more advantages over the fluorescent chemosensors. Recently, many research groups have drawn to design the colorimetric sensors which focused on selective and sensitive detection of both Fe^{3+} and Cu^{2+} . Narayanaswamy and Govindaraju have developed novel aldazine-based colorimetric chemosensors for Cu^{2+} and Fe^{3+} ,² while Rathinam et al. have proposed a highly sensitive fluorescent and colorimetric chemosensor for the detection of Cu^{2+} and Fe^{3+} by rhodamine-triazole conjugate [11].

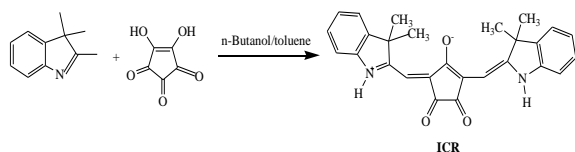
Croconines are a particularly promising class of organic NIR dyes that exhibit unique photophysical properties, namely, a sharp and intense absorption band in the red to NIR region. The croconines can be designed and applied for the optical detection of metal ions due to their absorption and emission properties are sensitive to the surrounding medium. However, there are no studies on croconine-based chemosensors designed for the metal ions recognition. In the present study, we have report an indolium croconine-based colorimetric and fluorescent chemosensor which shows excellent selectivity toward Fe^{3+} and Cu^{2+} .

2. Experimental

All the reagents and metal salts were purchased and used without further purification. ¹H NMR was recorded at

300 MHz on an AVANCE III spectrometer with TMS as an internal standard. The Mass spectra were carried out on a LCMS-2020 Mass Spectrometer. UV-vis spectra were performed using a UV759-vis spectrophotometer. Fluorescence spectra were measured with an F-280 spectrometer. Morphologies of the samples were observed on a SUPRA 55 field emission scanning electron microscope (FESEM, Germany).

The studied 1,3-bis [(3,3-dimethylindolin-2-ylidene) methyl]croconine (indolium croconine (**ICR**)) was synthesized according to a reported procedure [12]. The synthesis route is shown in Scheme 1. A solution of 0.1 g (0.0007 mol) of croconic acid and 0.22 g (0.0014 mol) of 2,3,3-trimethyl-3H indole was refluxed in 15 ml of benzene and 15 ml of 1-butanol for 6 h, during which a olive-green precipitate was formed. After cooling to room temperature, the precipitate was filtered and then washed several times with ethyl acetate. The obtained product was purified by column chromatography. $^1\text{H NMR}$ (300 MHz, DMSO) δ 7.50-7.59 (q, 4H, ArH), 7.35-7.40 (t, 2H, ArH), 7.21-7.26 (t, 2H, ArH), 5.96-6.0 (d, 2H, $-\text{CH}=\text{C}$), 1.50 (s, 12H, $-\text{CH}_3$). FTIR (KBr): 1721 (C=O), 1558 (C=C) and 2361 (amide in indolium croconine). MS (EI): $m/z = 425.1$ $[\text{M}+\text{H}]^+$; calcd $m/z = 424.5$ for $\text{C}_{27}\text{H}_{24}\text{N}_2\text{O}_3$.



Scheme 1 Synthesis route of indolium croconine (**ICR**).

Stock solutions (1.0×10^{-2} M) of Na^+ , Mn^{2+} , Ca^{2+} , Zn^{2+} , Fe^{3+} , Ba^{2+} , Cu^{2+} and K^+ were prepared from their corresponding salts. The indolium croconine-based chemosensor was prepared in a concentration of 1.0×10^{-5} M used the ethanol/water (4:1) as solvent. Titration was done by adding an appropriate metal ion solution into a test tube with 1.0 equiv of solution. All of optical measurements were operated after the test solution was prepared.

3. Results and discussion

Fig. 1 shows the absorption spectra of chemosensor **ICR**, which were studied in ethanol/water (4:1) upon addition of various metal ions such as Na^+ , Mn^{2+} , Ca^{2+} , Zn^{2+} , Fe^{3+} , Mg^{2+} , Ba^{2+} , Cu^{2+} and K^+ . It was found that the compound **ICR** solution showed the absorption band at 750 nm. Upon addition of Fe^{3+} , the absorption intensity decreased and the color of solution changed from olive green to yellow. The absorption intensity also decreased and the color of solution changed from olive green to pale pink with addition of Cu^{2+} . In the presence of other metal

ions (Na^+ , Mn^{2+} , Ca^{2+} , Zn^{2+} , Mg^{2+} , Ba^{2+} and K^+), the compound **ICR** showed either no change or slight decrease in the absorption intensity. There was no change in the solution color of compound **ICR** with addition of these metal ions (inset of Fig. 1) under the same condition. The fact indicated the compound **ICR** exhibited high selectivity for Fe^{3+} and Cu^{2+} over other various metal ions.

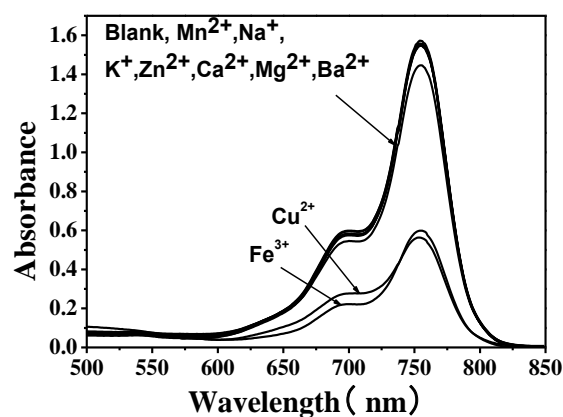


Fig. 1. UV-vis absorption of **ICR** (1.0×10^{-5} M) upon the titration of different metal ions (1.0 equiv for Na^+ , Mn^{2+} , Ca^{2+} , Zn^{2+} , Fe^{3+} , Mg^{2+} , Ba^{2+} , Cu^{2+} and K^+) in ethanol/water (4:1, v/v) solution at 750 nm. (Inset: color changes of **ICR** upon addition of respective metal ions).

We further investigated the effect of increasing concentration of Fe^{3+} and Cu^{2+} on the absorption behavior of **ICR**, respectively. Figs. 2a and 2b show the effect of the continuous addition of Fe^{3+} and Cu^{2+} on the absorption behavior of **ICR** chemosensor, respectively. As shown in Fig. 2a, the successive addition of Fe^{3+} into the **ICR** solution caused the original absorption centered at 750 nm gradually decreased and a prominent enhancement at 450 nm. When the concentration of Fe^{3+} was up to 0.6 mM, the absorption band at 750 nm disappeared. According to the linear Benesi-Hildebrand expression, the measured absorbance $[A_0/(A_0-A)]$ at 755 nm shows a linear relationship with a change of $1/[\text{Fe}^{3+}]$ ($R=0.99$) (inset of Fig. 2a). The association constant K_a of **ICR** and Fe^{3+} in ethanol/water (4:1, v/v) was found to be $0.53 \times 10^4 \text{ M}^{-1}$. The detection limit of **ICR** for Fe^{3+} was estimated about 0.6×10^{-3} M. Similarly, the absorption intensity at 750 nm had also gradually decreased upon addition of Cu^{2+} (Fig. 2b), and the intensity turned to stable when the concentration of Cu^{2+} was up to 0.6 mM. As shown in the inset of Fig. 2b. The detection limit was calculated to be 0.6×10^{-3} M. The association constant K_a of receptor **ICR** for Cu^{2+} ($R=0.986$) was calculated on the basis of the Benesi-Hildebrand plot and it was found to be $0.25 \times 10^4 \text{ M}^{-1}$.

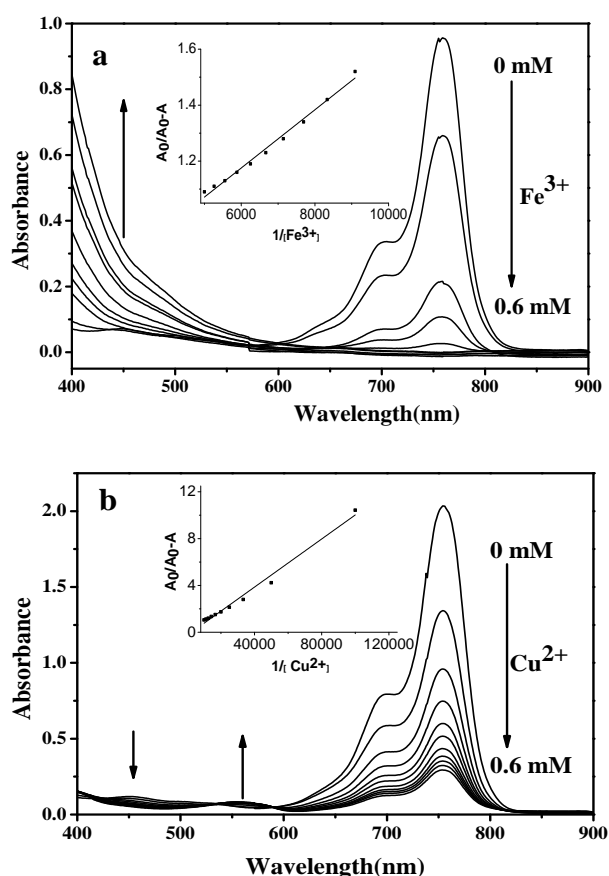


Fig. 2. Absorption of **ICR** (1.0×10^{-5} M) in ethanol/water (4:1, v/v) solution upon addition of Fe^{3+} (0–0.6 mM) (a) and Cu^{2+} (0–0.6 mM) (b), respectively. (inset: a plot of $[A_0/(A_0-A)]$ as a function of the $1/[\text{Fe}^{3+}]$ (a) and $1/[\text{Cu}^{2+}]$ (b)).

To further evaluate the Fe^{3+} -responsive and Cu^{2+} -responsive natures of **ICR**, fluorescence titration with Fe^{3+} and Cu^{2+} ions in varying concentration was conducted, respectively. As shown in Fig. 3, fluorescence spectrum of **ICR** showed the maximum wavelength at 782 nm. Dramatic decrement of emission intensity could be observed as the concentration of Fe^{3+} increased, and the maximum emission wavelength gradually blue-shifted to 766 nm. The fluorescence was completely quenched as the concentration of Fe^{3+} was up to 0.6 mM. This result can be also observed from fluorescence changes upon **ICR**- Fe^{3+} complexation (inset of Fig. 3), which is attributed to the coordination of Fe^{3+} to the ligand of **ICR**. The deprotonation of heteroatom-N of **ICR** allows the charge transfer from ligand to Fe^{3+} , which caused the fluorescence quenching behavior of ligands upon Fe^{3+} complexation.

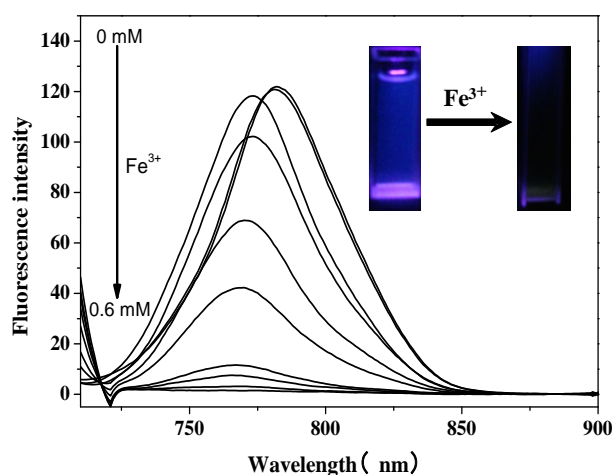


Fig. 3. Changes in the fluorescence spectra of **ICR** (1.0×10^{-5} M) with gradual addition of different amounts of Fe^{3+} (0–0.6 mM). (Inset: fluorescence change of **ICR** upon addition of Fe^{3+}).

Fig. 4 shows the fluorescence titration of **ICR** upon addition of Cu^{2+} in ethanol/water (4:1). The fluorescence spectrum of **ICR** showed the maximum wavelength at 783 nm. Dramatic decrement of emission intensity could be observed as the concentration of Cu^{2+} increased, and the maximum emission wavelength gradually blue-shifted to 771 nm. As the concentration of Cu^{2+} was up to 0.6 mM, the fluorescence was also quenched 49.1% of its initial intensity. The fluorescence change upon **ICR**- Cu^{2+} complexation is shown in inset of Fig. 4.

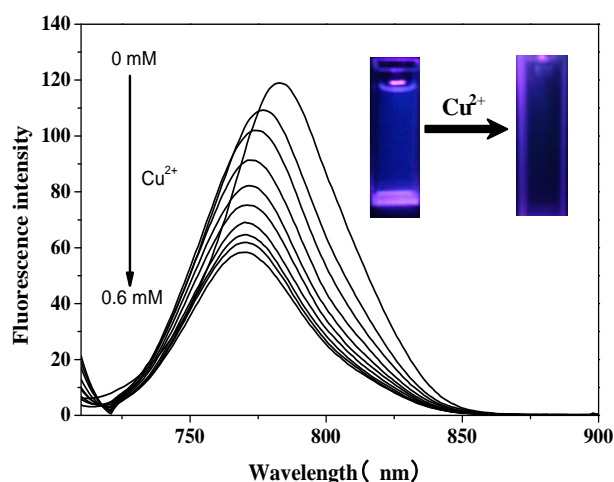


Fig. 4. Changes in the fluorescence spectra of **ICR** (1.0×10^{-5} M) upon addition of different amounts of Cu^{2+} (0–0.6 mM). (Inset: fluorescence change of **ICR** upon addition of Cu^{2+}).

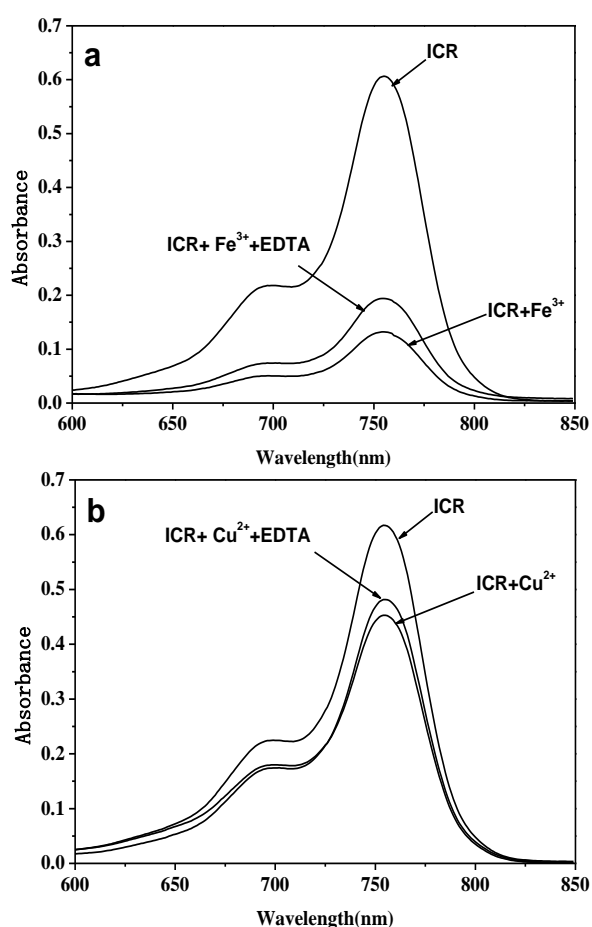


Fig. 5. UV-vis absorption of **ICR** (1.0×10^{-5} M) in the presence of Fe^{3+} ($66 \mu\text{M}$) (a) and Cu^{2+} ($66 \mu\text{M}$) (b) and/or EDTA ($33 \mu\text{M}$) in ethanol/water (4:1).

In order to observe whether the spectra of **ICR** could be regenerated upon the addition of cation-chelating agents, EDTA was added to the **ICR**- Cu^{2+} and **ICR**- Fe^{3+} solution, respectively. Fig. 5a exhibit that the addition of EDTA can not make solution return to the metal-free spectrum. However, addition of EDTA resulted in partly reversible in absorption spectrum. This indicated that the chelate ability of the EDTA and Fe^{3+} higher than that of **ICR** and Fe^{3+} . Therefore, the absorption spectrum of **ICR**- Fe^{3+} solution increased with the addition of EDTA. The interaction between **ICR** and Cu^{2+} was same as that of **ICR** and Fe^{3+} , which can be verified by the introduction of EDTA into the system containing **ICR** and Cu^{2+} (Fig. 5b).

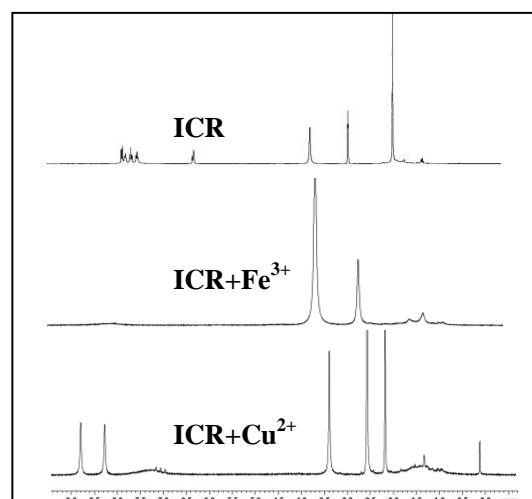


Fig. 6. ^1H NMR spectra of **ICR**, **ICR**- Fe^{3+} and **ICR**- Cu^{2+} in DMSO. (Note: for the complexes, 1.0 equiv of $\text{Cu}^{2+}/\text{Fe}^{3+}$ were added to the **ICR**.)

^1H NMR titration in DMSO was carried out in order to clarify the binding mechanism of Fe^{3+} and Cu^{2+} with sensor **ICR** (Fig. 6). It was found that the peaks near 7.0-7.5 ppm were broadened upon coordination with Fe^{3+} . Because of the imine hydrogen was slightly down field and broadened, the broadening at 6.0 ppm was not observed in **ICR**- Fe^{3+} . Therefore, the change in the imine hydrogen might be attributed to the coordination of **ICR** with Fe^{3+} . At a relatively higher concentration of metal ions, the peaks were fully shifted and broad due to the paramagnetic nature of metal ions. What's more, addition of 1.0 equiv of Cu^{2+} resulted in shifting and broadening of the peak at 7.0-7.5 ppm, corresponding to the imine hydrogen. And simultaneously, phenyl groups were broadened and shifted at 8.5-9.0 ppm due to the formation of complexes, which might indicate a decrease in electron density at imine nitrogen resulting from coordination of **ICR** with Cu^{2+} .

In order to further investigate the coordination behavior of metal ions to the ligand of **ICR**, the morphologies of **ICR** solution, **ICR**- Fe^{3+} complexation and **ICR**- Cu^{2+} complexation were sampled and studied by FESEM. It was found that there were neat **ICR** dispersed particles with regular round balls (Fig. 7a). Fig. 7b and 7c show the morphologies of **ICR**- Fe^{3+} complexation and **ICR**- Cu^{2+} complexation, respectively. It can see that the dispersed particles tend to form multi-particle aggregates and the morphology of the **ICR**- Fe^{3+} become more tightly associated than **ICR**. Based on Fig. 7c, it was observed that the dispersed particles take on a large number of agglomeration for as-prepared **ICR**- Cu^{2+} , which may be attributed to the more efficient complexation of **ICR**- Cu^{2+} .

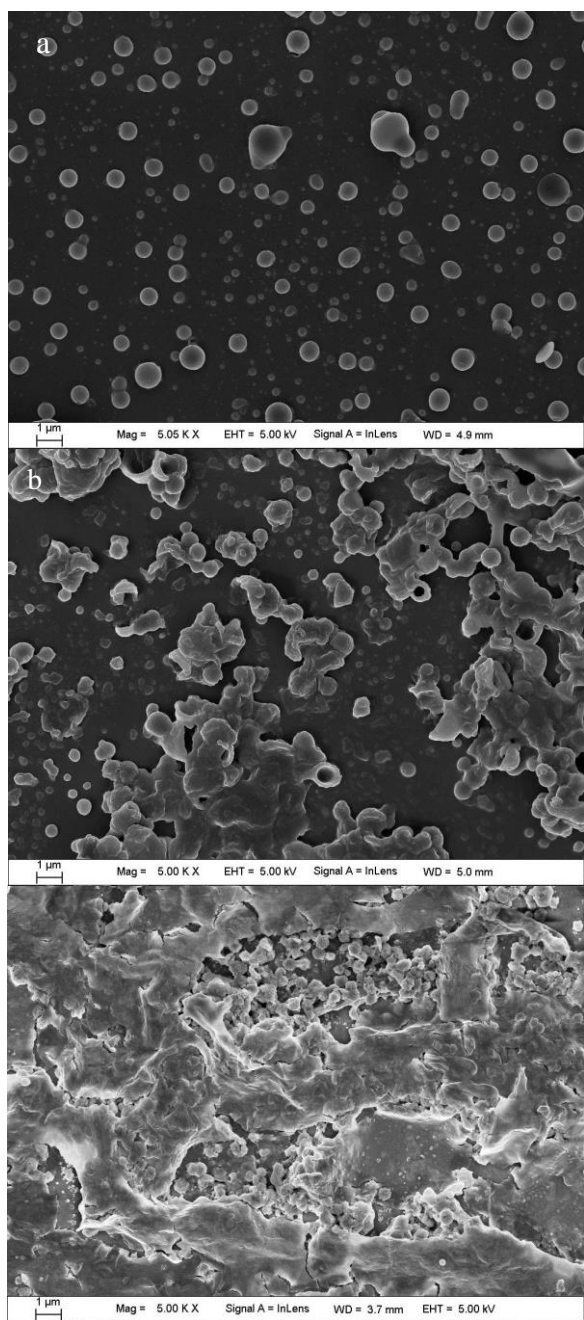


Fig. 7. FESEM images of **ICR** (a) (1.0×10^{-5} M) in ethanol/water (4:1, v/v), **ICR-Fe³⁺** (b) and **ICR-Cu²⁺** (c).

4. Conclusions

In conclusion, we have developed a colorimetric and fluorescent chemosensor based on an indolium croconine that can bind with Fe³⁺ and Cu²⁺ exhibiting completely different spectral features. The selective recognition of Fe³⁺ and Cu²⁺ is possible with sensor **ICR** based on UV absorption and fluorescence data. The coordination phenomena were further investigated by ¹H NMR spectra. Moreover, the FESEM results indicated that the

coordination behavior of Fe³⁺ and Cu²⁺ to the ligand of **ICR** owned characteristic of multi-particle aggregates.

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