ISSN: 2472-3703
DOI: 10.6911/WSRJ.202105_7(5).0024

Development of DPA Modified Squaraine Probe for Fluorescence Quenching Detection of Cu^{2+} ions

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Abstract

Metal ions play important functions in the chemistry and biology field which have attracted great interests by researchers. A DPA modified squaraine probe has been developed which has shown high selectivity for Cu^{2+} ion other than metal ions by color change and fluorescence quenching phenomenon. The limit of detection was quite low and the detection mechanism was studied by FT-IR spectrum which revealed that non-covalent bonding reaction occur between SQDPA probe and Cu^{2+}.

Keywords

Squaraine dyes; Copper ion; Fluorescent quenching; Limit of detection.

1. INTRODUCTION

Fluorescent sensor for active metal ion detection was attracted by researchers since it has wide applications in chemistry, biology and environmental field [1-3]. Among the cation ions, copper ion is considered as the essential trace element which playing the very important role in the environmental analysis, biological process, chemical reaction and etc. Both overdose and deficiency of the ion may cause serious damage [4, 5]. Different methods have been developed to rapid and accurate analysis of the substrate, such as absorption spectroscopy, inductively coupled plasma, biological sensor, and electrochemical analysis [6-9]. However, these methods are time consuming, high cost and highly rely on the instrument. It's urgent for developing rapid and simple method. Recently, colorimetric and fluorescent molecular sensing method became a new research area. Different absorption and fluorescence probes have been developed for the heavy metal detection [10-12]. Also there are a few examples on the copper ion detection [13-24]. Inspired by these exciting findings. We have decide to focus our attention on the new sensor or probe for copper ion with more excellent performances.

Squaraines are a class of near infrared dyes with intense absorption and efficient fluorescence emission in the near infrared region [25]. The structures can be versatile and its optical properties are very stable and excellent [26], which making it suitable as fluorescent group in part of sensor or probe. Another part of sensor or probe is target acceptor. The good acceptor could be simple in structure and well fitted with target. Pyridine based heterocyclic group can be considered one of the types, which has proven by quite a few literature examples [27-30]. Nitrogen atom in the group can provided tunable non covanlent bonding interaction with electron deficient metal ions.

In this work, a symmetrical squaraine based organic sensor with pyridine modification (DPA) has been developed and been applied for copper analysis.

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2. EXPERIMENTAL SECTION

2.1. Materials and Instrumentation

All the reagents were purchased from energy-chemical corporation, China. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker AV-400 spectrometer (TMS as internal standard). High resolution electrospray ionization mass spectrometry analysis was performed on a exactive mass spectrometer (Thermo Fisher Scientific, USA). Absorption and fluorescence spectra were measured on M5 spectrometer.

2.2. General Procedures for Absorption and Fluorescence Measurements

SQDPA stock solution was prepared with concentration of 1 mM in DMSO. Further dilution was prepared by adding in different solvents. Stock metal ion solution was prepared in 10 mM. 2 μL of SQDPA in stock solution and 2 μL of metal ions in stock solution were mixed together with 196 μL solvents, thus in total 200 μL. The solution was transferred into 96 well plates and measured by absorption and fluorescence properties by Molecular Device Spectrometer 5 (Molecular device corporation, USA).

2.3. Compound Synthesis (SQDPA)

The synthetic pathway was illustrated in Scheme 1. Intermediate 1 (0.1921g, 0.5mmol) and 2 (0.1795g, 0.5mmol) were mixed and dissolved in 10 mL of ethanol and toluene (volume 1:1) solution and refluxed for 5 hours. After reaction, the solvent was removed and the crude mixture was purified by flash column using mixture solvent of DCM and Methanol (volume 1:1) to obtain intermediate 3. Next the obtained intermediate 3 was further reacted with DPA intermediate by amide coupling reagent in DCM solution for 24 hour in room temperature. The final product was gained in the yield of 74.6% by flash column in methanol and DCM 10:1. ^{1}H NMR (500MHz, $CDCl_3$) δ 8.54-8.58 (d, *J* = 10 Hz, 2H), 7.68-7.74 (d, *J* = 10 Hz, 2H), 7.56-7.59 (t, *J* = 5 Hz, 2H), 7.52 (s, 1H), 7.35-7.36 (d, *J* = 2.5 Hz, 1H), 7.29-7.32 (t, *J* = 5 Hz, 1H), 7.22 (s, 3H), 7.14-7.17 (t, *J* = 5 Hz, 1H), 7.01-7.02 (d, *J* = 2.5 Hz, 1H), 6.89-6.91 (d, *J* = 5 Hz, 1H), 5.92-5.98 (d, *J* = 10 Hz, 2H), 4.91 (s, 2H), 4.75 (s, 2H), 4.08(s, 2H), 3.97 (s, 2H), 1.76 (s, 6H), 1.64 (s, 6H), 1.37-1.40(t, *J* = 5 Hz, 3H), 1.31-1.34(t, *J* = 5 Hz, 3H).^{13}C NMR (125MHz, $CDCl_3$) δ 181.15, 172.47, 170.88, 156.62, 149.83, 148.24, 143.53, 142.40, 142.04, 141.76, 137.79, 136.93, 130.34, 127.91, 127.69, 124.22, 123.41, 122.68, 122.42, 121.74, 109.51, 108.48, 86.86, 86.69, 54.78, 50.75, 49.61, 48.83, 38.65, 38.34, 29.69, 26.99, 26.83, 12.11, 11.91.ESI-MS calculated for: 677.3366; found: 678.3429.

3. RESULTS AND DISCUSSION

3.1. Absorption Properties study of SQDPA

The SQPDA was synthesized followed by the pathway illustrated in scheme 1. To evaluate the sensing properties of SQDPA, the absorption properties of itself in different solvents were firstly investigated. Three types of aqueous solutions including (pure water, Tris-HCl buffer, PBS buffer) and three other organic solvents which are soluble in water (EtOH, MeCN and Acetic acid) were applied to prepare SQDPA solution with final 10 μM concentration. As shown in Figure 1, there were significant differences of absorption performances in terms of absorption wavelength and intensities when SQDPA was dissolved in solution. SQDPA has shown broad peaks and low intensities as in aqueous solutions while exhibited sharp peaks in 635 nm and high intensities in organic solvent which indicating the strong solvent effect absorption properties. As SQDPA exhibited best absorption properties in MeCN, thus MeCN was chosen to be optimized solvent for the following study.

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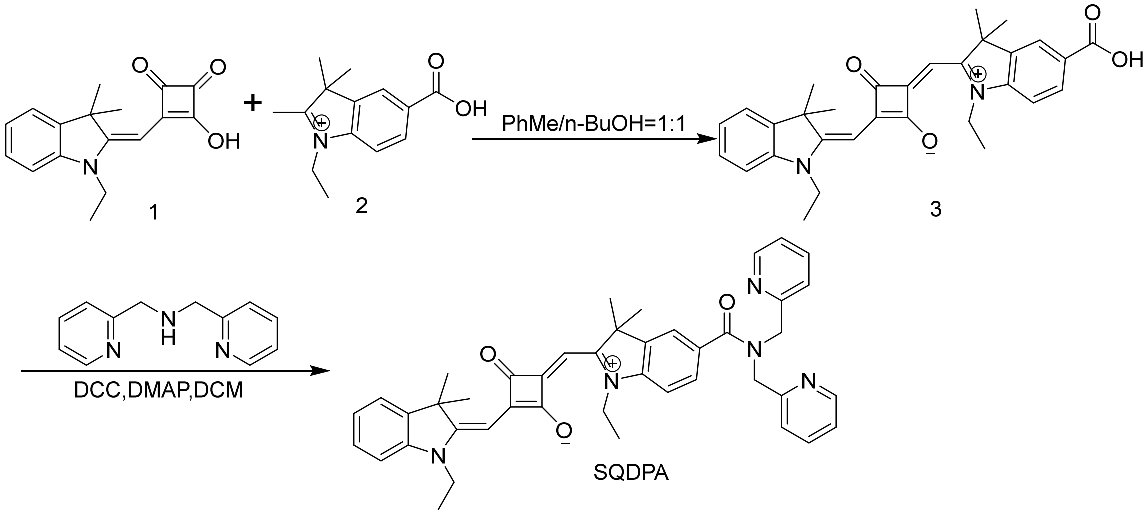

Scheme 1. Synthetic pathway of SQDPA

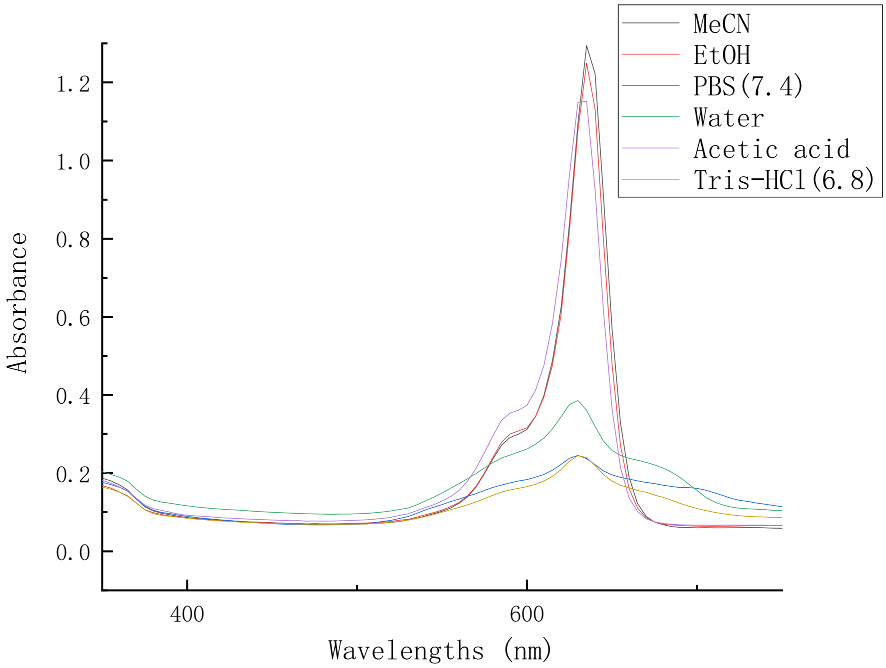

Figure 1. Absorption spectrum of SQDPA (10μM) in different solvents.

3.2. Selective Detection of Copper ion by SQDPA

To further investigate the selectivity of SQDPA in the detection of metal ions, the absorption spectra were observed by SQDPA in MeCN (final concentration of SQDPA: 0.01mM) once the metal ions were added. Various metal ions were applied including K^+, Zn^{2+}, Li^+, Fe^{3+}, Hg^{2+}, Cd^{2+}, Fe^{2+}, Ag^+, Co^{2+}, Na^+, Ca^{2+}, Cu^{2+} etc with final concentration of 0.1 mM. As shown in Figure 2, there were no obvious change of absorption in terms of wavelength and intensity when serious of metal ions were added into the MeCN solution except for Cu^{2+}. The absorption intensity of SQDPA was dramatically decreased in 635 nm from 1.3789 to 0.1676 once Cu^{2+} was in the solution which resulting in the solution colour change from blue to light yellow at once. There were no similar phenomenon observed when other metal ions presenting in the solution.

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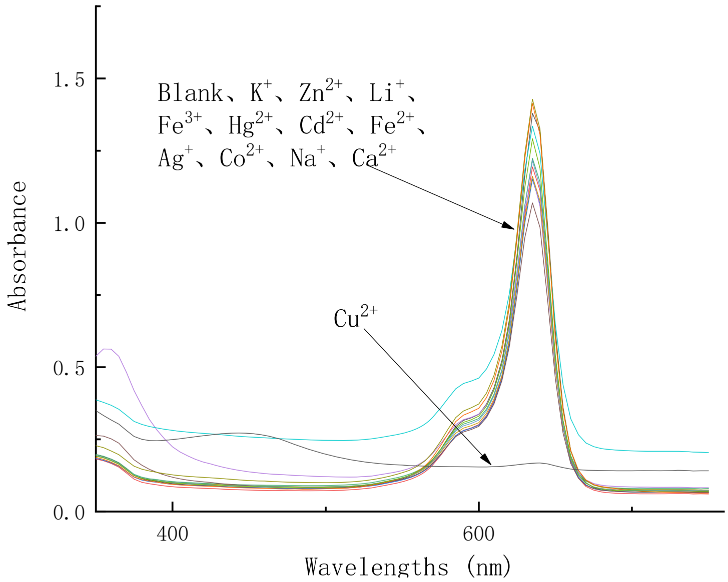

Figure 2. Absorption spectrum of SQDPA (10 μM) in the presence of metal ions

The fluorescence spectra were also conducted to investigate the selectivity for detecting metal ions and the data has shown in Figure 3. The excitation wavelength was set to 600 nm and the emission wavelength was ranged from 640 nm to 700 nm. The maximum emission wavelength was found to be 650 nm. SQDPA with final concentrations of 0.01 mM in MeCN has shown strong fluorescence in the absence of different metal ions. The fluorescence remained even if serious metal ions existed in the solution except for Cu^{2+}. The addition of other metal ions have no effect on the fluorescence of SQDPA. The fluorescence was quenched only if Cu^{2+} was added into the SQDPA MeCN solution.

Both data in Figure 2 and Figure 3 have shown that the absorption and fluorescence spectrum can be changed only if Cu^{2+} was in the SQDPA MeCN solution. This indicated that SQDPA as near infrared probe has selective detection ability for specific Cu^{2+} rather than other metal ions.

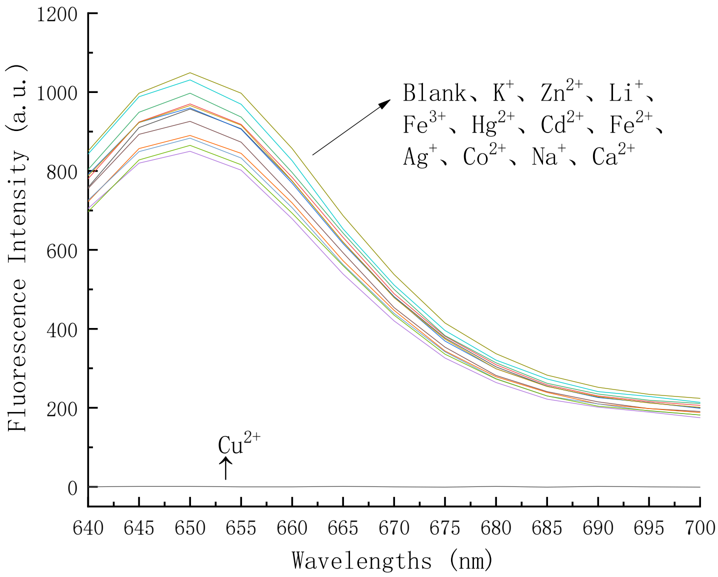

Figure 3. Fluorescence spectrum of SQDPA (10 μM) in the presence of metal ions

3.3. Solvent Effect on the SQDPA Selective Detection of Cu^{2+}

Considering solvent environment may have effect on the selectivity for detection of Cu^{2+}, the solvent was optimized in this stage. As shown in Figure 4, those solvents applied in the

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absorption study of SQDPA itself were again used for the solvent effect study on the selective detection of Cu^{2+}. The fluorescence quenching of SQDPA has shown most obviously in MeCN compared to other solvents once the Cu^{2+} was added in. This indicated that MeCN was the best optimized solvent for Cu^{2+} detection which was consistent conclusion as that in the previous study.

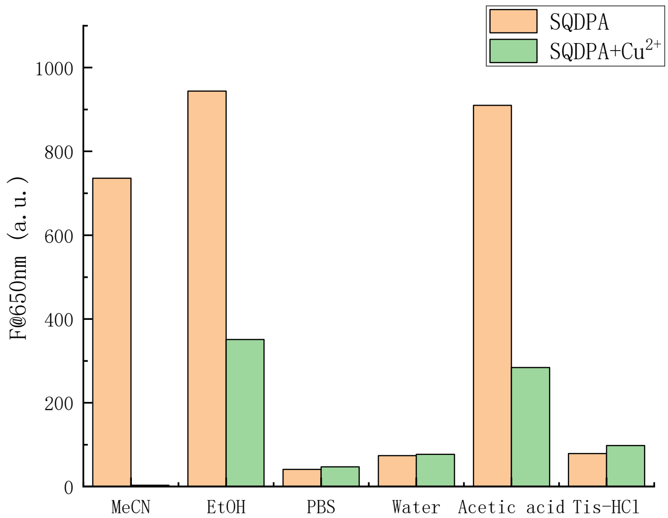

Figure 4. Solvent effect on the SQDPA selective detection of Cu^{2+}.

3.4. Limit of Detection for Cu^{2+}

To get further insight into sensing performance of SQDPA in the detection of Cu^{2+}, a quantative titration study of SQDPA with Cu^{2+} was conducted. As shown in Figure 5, the fluorescence intensity of SQDPA was gradually decreased with increment concentration of Cu^{2+} in the reaction solution. The fluorescence was absolutely quenched once the Cu^{2+} reached to 15 μM. The fluorescence decreasing of SQDPA was corresponded to the concentration of Cu^{2+} in a linear manner in the range of 5-15 μM(R^2= 0.999, Figure 6). The limit of detection of Cu^{2+} was calculated to be 109 nM in accordance with the equation of detection limit = 3σ/k, where σ was standard deviation of SQDPA, and also k was the slope of standard curve between the fluorescence decreasing at 650 nm versus the concentration of Cu^{2+}. The limit of detection was far lower than that value in US EPA regulation for Cu^{2+} content level in drinking water. This has proven excellent performance of SQDPA in the selective detection Cu^{2+} ion.

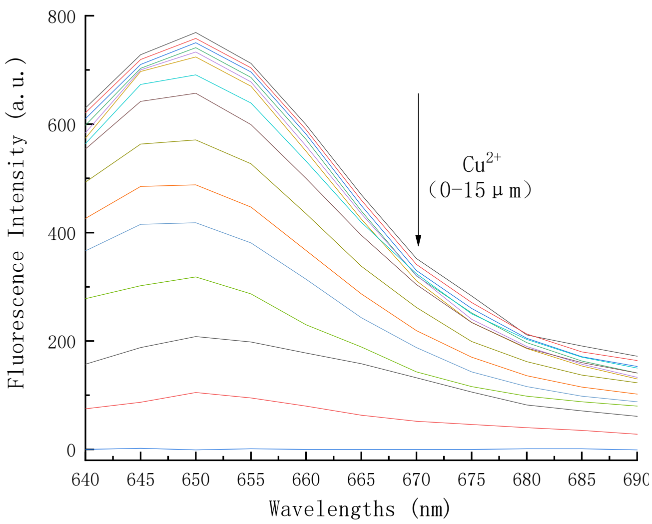

Figure 5. Fluorescence spectra of SQDPA in different concentrations of Cu^{2+}

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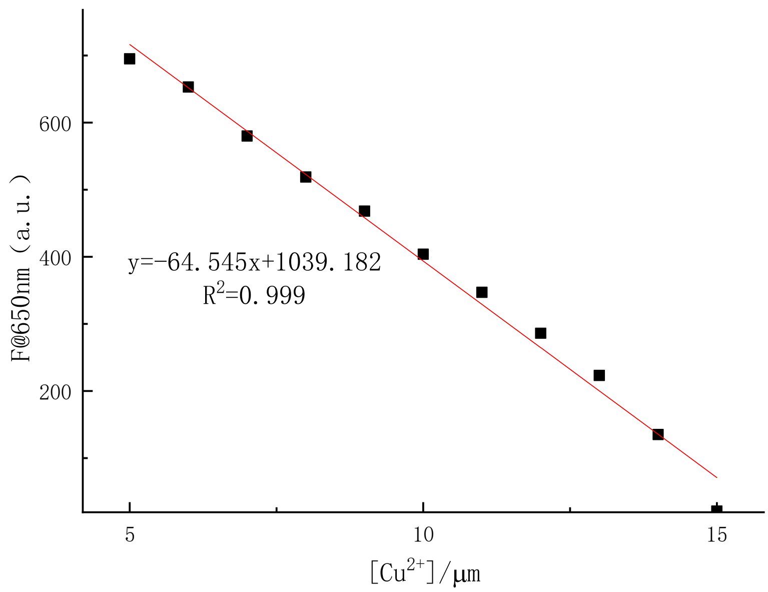

Figure 6. Fitted line between concentration and corresponding fluorescence intensity.

3.5. Competitive Experiments for Cu^{2+}

Different metals may co-exist in the real samples, which could lead to the interference in the detection of Cu^{2+}. In hence, the competitive sensitivity investigation was carried out towards other different metal ions in the present of Cu^{2+}. 5 equivalents of other metal ions as interference were pre-added to SQDPA solution followed by the addition of Cu^{2+} to observe if the fluorescence intensity of the solution would be changed once the interference was presented. And at the same time, the column fluorescence data of SQDPA was also listed as reference in Figure 7. The results have shown the extra amount addition of other metal ions did not affect the fluorescence of SQDPA, while the existing of Cu^{2+} could lead to the quenching of SQDPA. It revealed that the presence of the other metal ions did not interfere the detection of Cu^{2+}, which has proven high selectivity and stability for SQDPA.

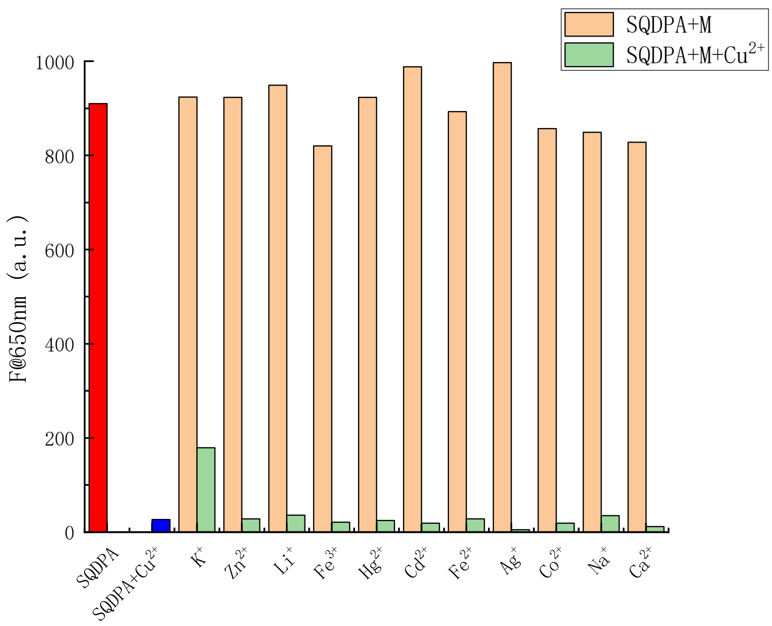

Figure 7. Interference effect on detection of Cu^{2+}

World Scientific Research Journal Volume 7 Issue 5, 2021
ISSN: 2472-3703 DOI: 10.6911/WSRJ.202105_7(5).0024

3.6. Mechanism Study of SQDPA Towards Cu^{2+} Detection

To better understand the binding interaction between SQDPA and Cu^{2+}, the FT-IR experiment was carried out and the spectrum of SQDPA and SQDPA together with Cu^{2+} were presented individually in Figure 8 and Figure 9. The characterized peak of 1594.812 cm^{-1} showed in both IR spectrum which indicated the carbonyl group in squaric acid core not involving the binding to the Cu^{2+}. However, the vibration peaks of 2919.643 cm^{-1} and 2850.220 cm^{-1} in -CH2- of DPA side chain were disappeared after the Cu^{2+} ions were added (Figur8,9). It was presumed that the nitrogen atom in pyridine ring was involved in the binding reaction and became more fixed. In addition, the shown up of -OH characterized peak in 3731.512 cm^{-1} in Figure 9 indicated that the carbonyl group in amide bond may also involved the binding reaction with Cu^{2+}.

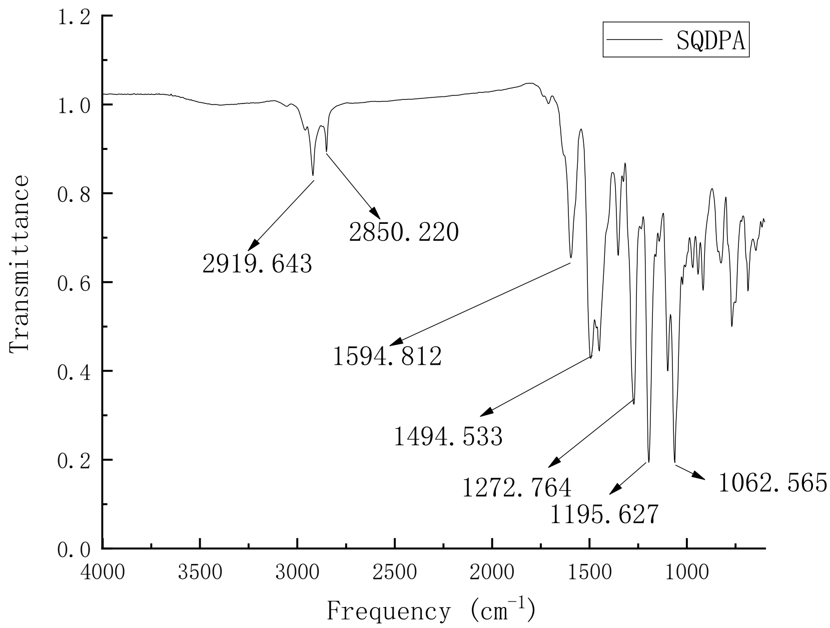

Figure 8. FI-IR spectrum of SQDPA.

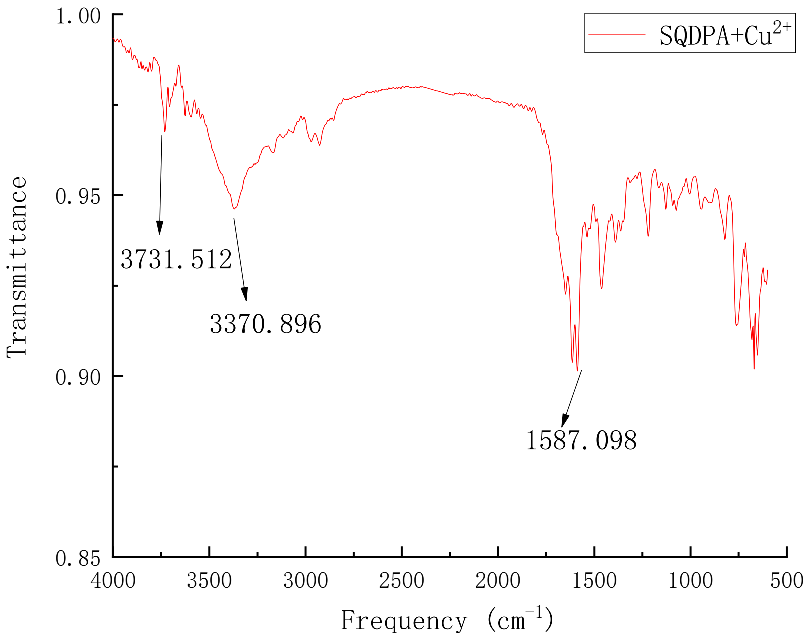

Figure 9. FI-IR spectrum of SQDPA-Cu^{2+}

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4. CONCLUSION

In summary, DPA including pyridine moiety as metal ion biding site was attached to the squaraine dye through amide coupling reaction to obtain final probe. SQDPA has shown specific recognition and low limit of detection towards Cu^{2+} instead of other metal ions by colour change and fluorescence phenomenon. The binding mechanism of SQDPA towards Cu^{2+} was further studied by FT-IR spectrum. It revealed that cabonyl group in amide bond and nitrogen atom in pyridine group may involve in the non-covalent bonding interaction.

5. CONFLICTS OF INTEREST

There are no conflicts to declare.

ACKNOWLEDGMENTS

This work was financially supported by Foundation of Jiangsu High Education Committee for Natural Science Research Projects (18KJB150005), the Postdoctoral Science Foundation in Jiangsu province (2018K275C), Changzhou technology programme (CJ20190057), the Natural Science Foundation of Jiangsu Province (BK20190933) and Natural Science Fundamental Research Project of Jiangsu Colleges and Universities (18KJD150005).

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