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Specific colorimetric detection of Fe³⁺ ions in aqueous solution by squaraine-based chemosensor[†]

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A new squaraine based chemosensor **TSQ** was developed for colorimetric detection of Fe³⁺ ions. A thymine moiety in **TSQ** was constructed to act as an ion acceptor. The sensor displayed an instant colorimetric response specific to Fe³⁺ over the other metal ions in 20% AcOH–H₂O solution. The limit of detection was much lower than that of the environmental protection agency guideline (5.37 μ M) in drinking water. A 1 : 1 binding between **TSQ** and Fe³⁺ ion was evidenced by Job's plot measurement, ESI-MS and Fourier transform infrared (IR) measurements. Moreover, the proposed sensing mechanism of the receptor towards Fe³⁺ was strongly supported by DFT calculation. Finally, the sensor has proven to be suitable in real sample applications.

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1 Introduction

For the past years, the development of highly sensitive and selective chemosensors has been attracting great interests for detecting heavy metals due to their importance in environmental, biology and chemistry domains.1-10 Iron, the third most abundant element on the earth, is not only the ubiquitous element in the environment, but also an essential element in living cells. It plays crucial roles in functional biological processes, such as cellular metabolism, enzymatic reactions, oxygen transport and gene expression.11-16 Both deficiency and excess can cause a series of diseases.17 Current qualitative and quantitative determination methods for Fe³⁺ ions include atomic absorption spectroscopy, inductively coupled plasma-atomic emission spectroscopy, mass spectrometry, electrochemical and fluorescence spectroscopic analysis.18-22 These methods suffer either from the sophisticated nature and high cost of instruments, or from tedious sample preparation procedures.^{23,24} It is imperative to develop simple, highly sensitive and selective chemosensors for Fe³⁺ detection. To date, a few chemosensors have been developed based on functioned nanoparticles or conjugated organic chromophores.²⁵⁻³² However, few of them can be used as

practical probes limited by their low sensitivity and selectivity, as well as incompatibility with aqueous environments.^{33–37} For example, Hg^{2+} , Zn^{2+} as well as Cu^{2+} , which exhibit many properties similar to those of Fe³⁺, could interfere the Fe³⁺ detection.^{38,39} Also it is noted that not many chemosensors with long absorption wavelength have been reported so far. Hence, to develop efficient colorimetric sensing materials still remains a big challenge.

Squaraines are versatile organic dyes which have shown intense absorption in near infrared region and have been widely applied in the optoelectronics fields such as light emitting diodes, field-effect transistors and chemosensors.^{40–46} Taken advantages of their unique properties, squaraine-based ion sensors can be constructed by a combination of squaraine chromophores with ligands. The intramolecular charge transfer will be occurred once metal ions have interacted with designed ion receptor. This could result in a rich modulation of color changes.

Thymine is one of the four nucleobases to perform base paring with adenine in constructing double helix DNA. It has proven to be one of the most effective ligands binding to the metal ions especially for mercury ion.⁴⁷ Therefore, we introduced a thymine group into the squaraine chromophore, leading to a new thymine-squaraine based chemosensor. To our surprise, the resulting chemosensor can specifically detect Fe³⁺ over the other metal ions by color change in aqueous solution. The complexation to Fe³⁺ and sensing mechanism were further investigated by Job's plot measurement, UV-vis titration, ESI-MS spectrometry analysis, IR analysis and theoretical calculation. The improvement in the color response, rapid accurate recognition of Fe³⁺ and low detection limit make this approach very promising for Fe³⁺ detection.

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2 Experimental section

2.1 Reagents and apparatus

All the reagents related to the starting materials for synthesizing **TSQ** in Scheme 1 and the inorganic salts together with buffer used in the properties study of **TSQ** were obtained from commercial available sources in analytical grade without purification. ¹H NMR (400 MHz) and ¹³C NMR (400 MHz) spectra were recorded on a Bruker AV-400 spectrometer using a deuterated solvent as the lock and TMS as an internal reference. Mass spectrometery analysis was performed on a Q exactive mass spectrometer (Thermo Fisher Scientific, USA). Absorption spectra were measured on Molecular Device Spectrometer 5 (Molecular Devices Corporation, USA).

2.2 Synthesis of TSQ

The synthetic pathway for TSQ was illustrated in Scheme 1. A mixture of A1 (1.351 g, 1.8 mmol) and A2 (0.375 g, 1.8 mmol) were dissolved in N,N-dimethylformamide (20 mL) together with amide coupling reagents of N,N-diisopropylethylamine (0.225 g, 3.6 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.342 g, 1.98 mmol) and 1-hydroxybenzotriazole (0.243 g, 1.8 mmol). The mixture was stirred for 4 h at room temperature. It was monitored by thin layer chromatography and purified by column chromatography using DCM : MeOH = 30 : 1 as eluents. The final compound TSQ was obtained as dark solid in the yield of 45.2%. The structure was confirmed by NMR and mass spectrometric analysis (Fig. S1 and S2^{\dagger}). ¹H NMR (400 MHz, CDCl₃) δ : 10.41 (s, 1H), 7.99 (s, 2H), 7.89-7.84 (m, 2H), 7.37-7.30 (m, 2H), 7.19–7.16 (m, 2H), 7.04–7.02 (m, 2H), 6.94–6.92 (d, I = 7.9 Hz), 5.97-5.92 (m, 2H), 4.37 (s, 1H), 4.09-4.07 (d, J = 6.6 Hz, 2H), 3.89 (s, 1H), 3.58 (s, 2H), 3.50 (m, 4H), 1.76-1.70 (m, 16H), 1.35-1.22 (m, 15H), 0.87-0.82 (m, 3H). ¹³C NMR (100 MHz, CDCl₃) δ: 181.28, 177.93, 171.20, 168.85, 168.02, 167.93, 164.66, 151.62, 145.46, 142.42, 141.66, 141.09, 129.93, 129.89, 128.54, 128.05, 127.98, 124.43, 122.44, 121.44, 111.07, 109.66, 108.75, 87.37, 86.77, 50.70, 49.72, 48.68, 43.77, 40.29, 38.72, 31.73, 29.70, 29.37, 29.33, 29.17 27.07, 27.00, 26.80, 22.59, 14.13, 14.09, 12.30, 12.13 ppm. ESI-MS calculated for TSQ 788.4261, found 789.5288.

2.3 UV-vis spectroscopy

A stock solution of **TSQ** was prepared 10 mM in DMSO. Further dilutions were made to prepare 100 μ M of **TSQ** by adding different types of solutions. 11 different metal salts were



Scheme 1 Synthetic pathway for chemosensor TSQ.

prepared 10 mM in distilled water and diluted further accordingly. After mixing **TSQ** and metal ion solutions, the absorption measurements were made in 96 well plates on Molecular Device Spectrometer 5 (Molecular Devices Corporation, USA) at the wavelength range of 350 nm to 750 nm.

2.4 Job's plot measurements

The stock solution of sensor **TSQ** (10 mM) in DMSO and FeCl₃ (10 mM) in distilled water were prepared, respectively. When the mole fraction of Fe³⁺ was equal to 0.1, the sensor **TSQ** solution (1.8 µL) and FeCl₃ solution (0.2 µL) were added to each 20% AcOH solution to make a total volume of 200 µL. After stirring for a few seconds, UV-vis spectra were recorded at room temperature. When the mole fraction gradually increased to 1.0, **TSQ** volume decreased by 0.2 µL and Fe³⁺ volume increased by 0.2 µL, respectively. The absorption spectra were recorded at absorption maximum wavelength. The plots were drawn by plotting $A_0/(A_0 - A) vs$. 1/[Fe³⁺], where A_0 equaled to absorption intensity of **TSQ** without Fe³⁺, *A* was corresponding to the absorption intensity of **TSQ** with different concentrations of Fe³⁺.

2.5 Competition tests

2 μ L of Na⁺, K⁺, Li⁺, Ag⁺, Zn²⁺, Hg²⁺, Cd²⁺, Fe²⁺, Co²⁺, Ca²⁺ ion solutions (10 mM) were extracted individually and mixed with 2 μ L Fe³⁺ (2 mM), 2 μ L **TSQ** (10 mM) and filled up with 20% AcOH–H₂O solution to total volume of 200 μ L. After stirring the solutions for a few seconds, UV-vis spectra were recorded at room temperature.

3 Results and discussion

3.1 Spectral properties of TSQ

The absorption properties of TSQ were investigated in different aqueous solutions, *i.e.* distilled pure water, phosphate buffer (PBS buffer, pH = 7.4), borate buffer (pH = 6.8) and acetic acid. The results were recorded as shown in Fig. 1a. Two broad absorption peaks of TSQ were observed at 605 nm and 660 nm once the water was present (distilled pure water, phosphate buffer and borate buffer). The sensor has shown a cyan color in the solution. Only one strong and symmetric peak was observed at 635 nm which has been shown as blue color in the presence of pure acetic acid. It is well known that squaraines have great tendency to aggregate in a different pattern which corresponding to the interesting spectroscopic response. A blue shifted absorption and broaden absorption peaks will be observed as in H-aggregation (paralleled oriented arrangement) and red shifted absorption and sharp absorption peaks will be shown as in Jaggregation (head to tail fashion). In this case, it is assumed that the different extent and H-aggregation occur in the presence of water by TSQ. To further validate the water induced aggregation effect, the different percentage of water was added into acetic acid to form acetic acid solution. As shown in Fig. 1b, as the percentage of water in acetic acid increased, the absorption performances were almost preserved even in the presence of 60% H₂O in acetic acid solution. With further increased water percentage to 80%, the intensity of new weak band at 650 nm



Fig. 1 (a) UV-vis spectra of TSQ (100 $\mu M)$ in different aqueous solutions and (b) in the mixture of AcOH–H_2O.

concomitantly increased (blue shift) which was consistent with results in Fig. 1a. For both PBS buffer (pH = 7.4) and borate buffer (pH = 6.8), the pH of the solvent system has no significant influence on the absorption spectrum.

3.2 Colorimetric sensing for Fe³⁺

Preliminary results have shown that **TSQ** could possess good absorption properties in acetic acid, which were selected for identifying the metal ions in the following experiments.

Colorimetric responses to various metal ions including Na⁺, K⁺, Li⁺, Ag⁺, Zn²⁺, Hg²⁺, Cd²⁺, Fe²⁺, Co²⁺, Ca²⁺, Fe³⁺ were investigated. It has shown in Fig. 2a that in 20% AcOH–H₂O solution of 100 μ M **TSQ**, the color change was dramatic and specific to Fe³⁺, while addition of other metal ions did not show any significant change. The Fe³⁺ induced a spontaneous color change from blue to green, which could be easily detected by the naked eyes. In the corresponding UV-vis spectrum, the intensity of strong absorption at 635 nm was decreased after the addition of Fe³⁺.

The solvent effects on detection of Fe^{3+} for TSQ in AcOH– H₂O solution were further evaluated (Fig. 3). By varying the portion of AcOH in the distilled water, the decreased absorption change of TSQ only can be observed in 20% AcOH–H₂O solution. There were no similar observations in other solutions, which indicating that TSQ was a highly sensitive and selective colorimetric sensor for Fe³⁺ ions.

3.3 Time-resolved colorimetric study

For the purpose of exploring the relationship between absorption intensity and response time, a dynamic study of **TSQ** in the detection of Fe^{3+} was monitored. The green color of **TSQ** was



Fig. 2 (a) Visualization and (b) absorption spectra for TSQ solution (100 μM) with/without addition of different metal ions (100 μM) in 20% AcOH–H₂O.

kept even after 4 hours which clearly indicated that the detection for Fe^{3+} was instant and the complexation reaction was stable (Fig. 4).

3.4 Binding constant K_a and limit of detection (LOD) for Fe³⁺

To get insight into the binding mode between **TSQ** and Fe³⁺, the UV-vis absorption spectra of 100 μ M **TSQ** in 20% AcOH–H₂O solution were recorded during the titration of various concentrations of Fe³⁺. The binding constant (K_a) was estimated using a Benesi–Hildebrand plot, which was calculated by absorption changes of consequent titration ($A_0/A_0 - A$) against 1/[Fe³⁺]. The magnitude of Ka was calculated from the intercept and slope of the straight line, and the estimated value is about 9.6 × 10⁵ M⁻¹ (Fig. 5). More importantly, the absorption change of **TSQ** corresponded to the concentration of Fe³⁺ in a linear manner in the range of 1–100 μ M. The high sensitivity of this sensor in 20% AcOH–H₂O solution allowed detection limit reaching to be 1 μ M. The detection limit of Fe³⁺ was much lower than the EPA guidelines for drinking water of 5.37 μ M.⁴⁸ The sensing ability of



Fig. 3 Bar chart showing the absorption changes of TSQ (100 μ M) in the different AcOH-H₂O solution with adding 100 μ M Fe³⁺ (red bar) or without Fe³⁺ (black).



Fig. 4 Dynamic study on the absorption change by mixing TSQ (100 $\mu M)$ and Fe $^{3+}$ (100 $\mu M)$ in 20% AcOH–H2O solution.



Fig. 5 Benesi–Hildebrand plot analysis of the absorption changes for the complexation between TSQ and Fe³⁺, $R^2 = 0.9947$.

TSQ towards Fe³⁺ was also compared to those for coumarin and rhodamine derivatives, which considering as other two types of promising near infrared colorimetric sensing probes. The LOD of present probe **TSQ** is superior to those of coumarin derivative^{49,50} and rhodamine based chemosensors³¹ (Table S1[†]).

3.5 Complexation mechanism of TSQ-Fe³⁺

Job's plot measurement was carried out to explore the binding ratio of **TSQ** and Fe^{3+} (Fig. 6). The plots were done by recording the absorption intensity A against $[Fe^{3+}]/[Fe^{3+}]+[TSQ]$. The results revealed that the TSQ probe bind with Fe^{3+} in 1:1 stoichiometry, which was further confirmed by ESI-MS data. A solution containing TSQ and 1 equiv. of FeCl₃ has shown a strong peak at m/z 905.5341, assigned to [TSQ + Fe³⁺ + CH₃-COOH + H^+ ion (Fig. S3[†]). IR spectra can provide valuable information on the complexation. To gain additional insight into the Fe³⁺ binding properties, the FT-IR spectra of TSQ and **TSQ**-Fe³⁺ complex were measured (Fig. 7). The shift of the carbonyl absorption band indicated the change of structure. The characteristic amide carbonyl (C=O) stretching vibrations at 1685 cm⁻¹ shifted to 1608 cm⁻¹ in the presence of Fe³⁺, while the characteristic carbonyl (C=O) stretching frequencies in thymine moiety appeared at 1584 cm^{-1} instead of 1596 cm^{-1} in the complex of TSQ-Fe³⁺. In addition, a new broad peak was generated at 3400 cm⁻¹ which corresponding to the N-H vibration. All these results strongly indicate the amide carbonyl O atom (C=O) and the carbonyl O atom in thymine moiety were



Fig. 6 Job's plot for the complexation of TSQ with Fe^{3+} in AcOH-H₂O (2 : 8, v/v) solution.

involved in the recognition of Fe³⁺. The density functional theory (DFT) study was further in proceed to get insight into details of **TSQ**-Fe³⁺ complexation. All calculations were carried out with the Gaussian 09 package.⁵¹ The density functional theory (DFT) hybrid model with the B3LYP was used for the gasphase geometry optimizations, Lanl2dz basis set with effective core potential (ECP) for Fe, and the 6-31G(d) basis set was used for all remaining atoms. Based on the calculations, the favourability binding mode between TSQ (a) and its Fe^{3+} complex (b) was depicted in Fig. 8. It is favourable for the thymine moiety, which was vertical to the planar of asymmetrical squaraine dyes. And the Fe³⁺ ion was tended to bind with one carbonyl group in thymine and another carbonyl group on the oxygen atom of amide bond. All conformations were calculated at the same level to confirm their stability (no imaginary frequencies).



Fig. 7 The IR spectra of TSQ (Black line) and TSQ-Fe³⁺ (Red line).



Fig. 8 The geometry-optimized structures of TSQ (a) and its Fe^{3+} complex (b) at the SMD (H₂O)-TD-PBE0/TZVP + LANL2DZ level.

3.6 Ions interference effects on the TSQ towards Fe³⁺

Counterion effects on the sensitive colorimetric sensor **TSQ** for Fe^{3+} have also been investigated. The results have shown that the anions with weak coordination abilities towards Fe^{3+} such as Cl^- , SO_4^{2-} , SO_3^{2-} , NO^{3-} , CO_3^{2-} had no predominant effect on the absorption change (Fig. 9). In addition, the colorimetric responses towards other environmentally relevant metal ions were recorded as well. Upon addition of 5 equiv. Na⁺, K⁺, Li⁺, Ag⁺, Zn²⁺, Hg²⁺, Cd²⁺, Fe²⁺, Co²⁺, Ca²⁺ in **TSQ**–Fe³⁺ complex solution, no apparent absorption changes were observed, indicating that extra amount of other cations have no significant interference to the response of **TSQ** towards Fe³⁺ (Fig. 10).

3.7 Reversible study of TSQ-Fe³⁺ complex towards EDTA

In order to test if the proposed TSQ-Fe³⁺ complex could be reversed, EDTA as a strong chelator was added to the solution containing of 1 equiv. TSQ and 1 equiv. Fe³⁺. As seen in Fig. 11, the addition of 1 equiv. EDTA restored the absorption signal of TSQ to its original level. Additional 1 equiv. Fe³⁺ was added in again to make another round of detection by colorimetric



Fig. 9 Absorption changes of TSQ-Fe³⁺ in the presence of various test anions in AcOH-H₂O (2 : 8, v/v).



Fig. 10 Absorption changes of $TSQ-Fe^{3+}$ in the presence of various test cations in AcOH-H₂O (2 : 8, v/v).



Fig. 11 Absorption spectra of TSQ (100μ M) + Fe³⁺ (100μ M) upon the addition of EDTA (1 equiv.) in AcOH-H₂O (2 : 8, v/v).

change from blue to green (Fig. S4^{\dagger}). All these results show that the process of titrating sensor **TSQ** with Fe³⁺ is reversible and that sensor **TSQ** could be used as an on-off-on switch chemosensor.

3.8 Preliminary analytical applications

In order to examine the capability of the proposed chemosensor in environmental samples, **TSQ** was applied to the determination of the Fe^{3+} in waste water from an electroplating factory. As shown in Table 1, the initial Fe^{3+} concentrations were measured by the atomic absorption spectrometry (AAS) method and three levels of standard concentrations of Fe^{3+} were added into real

| Table 1 Determination of Fe ³⁺ ion in waste water samples with TSQ | | | | |
|---|--------------|---------------|---------------|-----------------|
| Sample | AAS (µM) | Added (mM) | Found (mM) | Recovery (%) |
| Waste water 1 | 2.08 | 1.20 | 1.22 | 101.2 |
| Waste water 2 Waste water 3 | 3.78 6.02 | 2.31 3.62 | 2.38 3.58 | 103.3 99.0 |
| | | | | |

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samples. By applying our **TSQ** test, the content of Fe^{3+} in waste water was in good agreement with that added amount by a relative error in less than 5%. Therefore, the present **TSQ** was initially proven to be well used in the determination of Fe^{3+} in real samples.

4 Conclusions

In summary, we have successfully constructed a thyminesquaraine based colorimetric chemosensor for Fe³⁺ detection in aqueous solution. It has shown an excellent selectivity for Fe^{3+} over the other cations (blue to green). The limited of detection (1 µM) was determined lower than the EPA guidelines for drinking water of 5.37 µM and no other ion interferences have shown significant effects on the Fe³⁺ detection. Further study of mechanism by Job's plot measurement, ESI-MS indicated that 1 : 1 complex was formed between **TSQ** and Fe^{3+} . IR spectrum and DFT calculation further revealed the detailed binding mode of **TSQ**-Fe³⁺ complex in the way of binding on the amide carbonyl O atom (C=O) and the carbonyl O atom on thymine moiety towards Fe³⁺. Reversible study and preliminary analytical application study have shown strong potent of TSQ as a good chemosensor to Fe³⁺ in real sample applications. Considering that the developed thymine-squaraine based chemosensor TSQ was quite effective and selective, we expect that it will potentially serve as colorimetric probe for more Fe³⁺-related environmental studies.

Conflicts of interest

There are no conflicts to declare.

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