A Highly Selective and Sensitive Fluorescent Chemosensor for Fe^{3+} in Physiological Aqueous Solution

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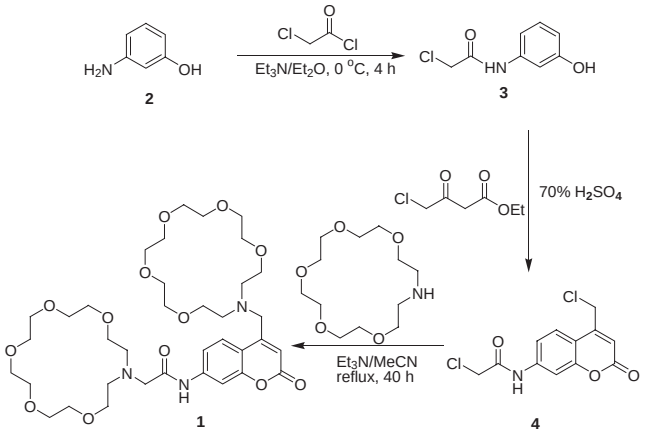
A novel fluorescent chemosensor in which two aza-18-crown-6 moieties are linked to a coumarin fluorophore has been synthesized for sensing Fe^{3+}. The selective fluorescence enhancement was observed upon binding Fe^{3+} at physiological conditions ($pH = 7.4$, $[NaCl] = 0.135\,M$, $[KCl] = 0.01\,M$), whereas other metal ions, such as Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Hg^{2+} and Pb^{2+} produced no effect.

Considerable research interest has been focused on the development of chemosensors for the selective and efficient detection of chemically and biologically important ionic species in biological and environmental science.[1] The design and synthesis of a sensitive and selective fluorescent chemosensors is a basic aim for organic and analytical chemists.[2] An important area within this field is the development of new fluorescent chemosensors for many heavy and transition metal ions, particularly in critical analysis of biological (blood and serum) samples[3] and real-time detection of live cells.[4] The metal–ligand coordinative interaction displays its superiorities to hydrogen bonding and electrostatic interaction in a polar environment.[5] Therefore, the metal–ligand complexes have been reported for effective and selective recognition of metal ions. Crown ether derivatives are known to form complexes with various cations and anions selectively. There are numerous examples in the literature of crown ethers used as sensors for a broad range of metal ions.[1,6]

Iron is an essential element for human and plays an important role in biochemical and nutritional processes. Many proteins and enzymes contain ferric ions either for structural purposes or as part of a catalytic site.[7] Therefore, the development of selective as well as sensitive fluorescent sensors for ferric ions presents a challenge. However, only a few example of fluorescent chemosensor for Fe^{3+} has been described.[8]

Coumarins generally show good spectral features such as large Stokes shifts and high quantum yields and have been successfully employed as PET chemosensor devices.[9] Coumaryl crown ether based chemosensors were reported to be able to detect selectively saxitoxin in the presence of sodium and potassium ions.[9a] Mizukami et al.[9c] designed and synthesized a fluorescent sensor for anions, which contains 7-amino-4-trifluoromethylcoumarin as a fluorescent reporter and Cd(II)-cyclen (1,4,7,10-tetraazacyclododecane) as an anion host. Suzuki[4d] reported Mg^{2+}-selective fluoroionophores based on a coumarin derivative and used for Mg^{2+} measurement in a living cell. Here, we would like to report a novel and selective fluorescent chemosensor for Fe^{3+}.

We designed a sensor molecule **1**, in which two aza-18-crown-6 moieties are linked to a coumarin fluorophore, and hope this molecule to adopt a semirigid V-shaped conformation, which might be able to bind selectively with a metal ion. The primary test showed that **1** possesses a highly selective and sensitive response of fluorescence enhancement (FE) toward Fe^{3+} in neutral buffer aqueous solution.



Scheme 1. Synthesis of the chemosensor **1**.

The synthesis of chemosensor **1** is shown in Scheme 1. Atkins' method was used for the preparation of 4-chloromethyl-7-chloroacetamidocoumarin (**4**) from 3-aminophenol (**2**).[9a,10] The acetylation of the amino group of **2** afforded chloroacetamidophenol (**3**) in 90% yield. A Pechmann condensation of **3** with ethyl 4-chloroacetoacetate gave **4** in 60% yield. Alkylation of 1-aza-18-crown-6 with **4** afforded compound **1** in 42% yield.[11]

The photochromic and fluorescence properties of chemosensor **1** were investigated by absorption and fluorescence measurements. After a preliminary survey with various solvent systems, we have focused our attention on aqueous solution for possible applications of the present system in the metal ion analysis of biological samples.

Fluorescence emission titrations of $1 \times 10^{-6}\,M$ of **1** with 100 equiv. of group I (Li^+, Na^+, K^+), 1 equiv. of group II (Mg^{2+}, Ca^{2+}), and heavy and transition metal ions group III (Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Fe^{3+}, Hg^{2+}, Pb^{2+}) (as their Cl^-, NO_3^-, or ClO_4^- salts) are performed in buffered HEPES solution (0.1 M, pH 7.4) (Figure 1). The results showed that the alkali and alkaline earth metal ions did not cause significant changes in fluorescence emission spectra of **1**. However, chemosensor **1** has different degree of fluorescence enhancement with heavy and transition metal ions. In fluorescence titration studies, chemosensor **1** displayed 15-, 2.5-, and 1.9-fold fluorescence enhancements for Fe^{3+}, Cu^{2+}, and Hg^{2+} among 13 metals investigated, respectively. Furthermore, red shift absorption was observed on the addition of Fe^{3+}. It is worth noting that the complex **1**·Fe^{3+} exhibits 8-nm-red shift. The red shift may be due to the intramoleucular excimer formation that is caused by

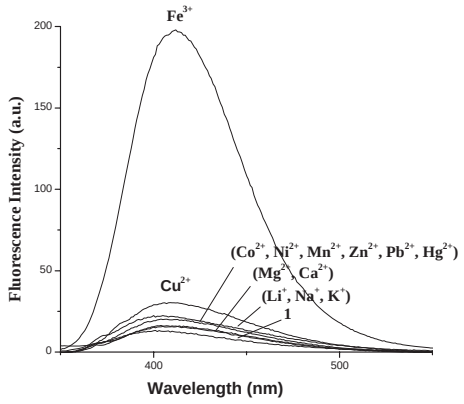


Figure 1. Fluorescence spectra of free **1** (1×10^{-5} M) and **1** in buffered HEPES solution (0.1 M, pH 7.4) in the presence of different metal ions (1×10^{-5} M) ($\lambda_{ex} = 336$ nm).

the decrease in the distance between two crown ethers when ferric ion is bound. These results demonstrated that the selectivity of chemosensor **1** for Fe $^{3+}$ is very high.

We also tried to find the possibility of practical applicability of this chemosensor in the analysis of biological samples by studying the Fe^{3+}-selective response of the fluorescence spectra in the presence of background metal ions of physiologically important Na^+ (0.135 M), K^+ (0.01 M), Mg^{2+} (0.001 M) and Ca^{2+} ions (0.001 M). The chemosensor **1** showed a selective response toward Fe^{3+} ions and was found to have an almost identical response and detection limit comparable with the results obtained in the absence of other background metal ions.

The stoichiometry of the **1**·Fe^{3+} complex system was determined by the changes in fluorescence emission spectra of **1** in the presence of varying concentrations of ferric ions at 412 nm. The results indicated that the complex has 1:1 (host **1**: guest Fe^{3+} ion) stoichiometry.

The association constant K_a for the interaction of **1** with Fe^{3+} ions was estimated by the nonlinear curve fitting procedure of the fluorescence titration data. The K_a values of the 1:1 complex formation for **1**·Fe^{3+} and **1**·Cu^{2+} system were found to be 1.5×10^5 and 2.4×10^3 M^{-1}, respectively.

To explore further the utility of **1** as an selective fluorescence chemosensor for Fe^{3+}, the competition experiments were conducted in the presence of Fe^{3+} at 1×10^{-5} M mixed with Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Hg^{2+}, and Pb^{2+} at 5×10^{-5} M as well as the mixture of the metal ions in buffered HEPES solution (0.1 M, pH 7.4), respectively; no significant variation in its fluorescence intensity was found by comparison with that without the other metal ions besides Fe^{3+}. Moreover, no obvious interference was observed in its fluorescence while performing the titrations with Fe^{3+} in the physiological important metal ions. The above results implied that its selectivity for Fe^{3+} was remarkable in physiological conditions.

In conclusion, we have designed and synthesized a new fluorescent chemosensor **1**, which can detect Fe^{3+} with an excellent selectivity and molecular sensitivity at physiological conditions.

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11 Compound **1**: mp>300 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 2.78 (t, $J = 5.28$ Hz ,8H), 3.51–3.57 (m, 40H), 3.61 (s, 2H), 3.89 (s, 2H), 6.50 (s, 1H), 7.58 (dd , $J = 2.18$ Hz, 1H), 7.88 (s, $J = 2.18$ Hz, 1H), 7.89 (s, $J = 2.18$ Hz, 1H), 10.16 (s, 1H); ^{13}C NMR (DMSO-d_6): δ 54.7, 56.2, 59.8, 66.7, 69.7, 70.1, 70.6, 70.9, 106.3 112.4, 114.7, 116.2 126.6, 142.2, 154.2, 155.1, 161.2, 171.7; HRMS-ESI (m/z): Calcd for $C_{36}H_{57}N_3O_{13}Na$ ([M + Na]$^+$) 762.3789, Found 762.3777.

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