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Facile synthesis of anthracene-appended amino acids as highly selective and sensitive fluorescent Fe^{3+} ion sensors

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ABSTRACT

We designed new fluorescent chemical sensors for Fe^{3+} ion detection, by conjugating amino acids as receptors into an anthracene fluorophore. The conjugates were synthesized in solid phase by Fmoc-chemistry. Fluorescence sensors containing Asp (**1**) and Glu (**2**) both had exclusive selectivity for Fe^{3+} in 100% aqueous solution and in a mixed organic–aqueous solvent system. Other metal ions did not interfere with the detection ability of the sensors for Fe^{3+}. The sensors detect Fe^{3+} ions via a chelation-enhanced fluorescent quenching effect. The binding affinity, reversible monitoring, and pH sensitivity of the sensors were investigated. In addition, detection of fluoride ion among halide ions was done by a chemosensing ensemble method with **1**–Fe^{3+} and **2**–Fe^{3+} complexes.

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A variety of metal ions plays pivotal roles in structural, catalytic, and regulatory aspects of biological and environmental systems.[1] Thus, detection of metal ions is of great interest and importance in the fields of chemical, biological, and environmental sciences. As fluorescent chemosensors for metal ions provide distinct advantages of sensitivity, rapid response, low cost, and easy monitoring, a large number of fluorescent chemosensors have been reported.[2,3]

Fluorescent chemosensors consist of a receptor and a fluorophore. The receptor is responsible for the recognition of analytes, and the fluorophore converts the recognition events into optical signals. Several fluorophore-based sensors such as dansyl, rhodamine, anthracene, naphthyl, and nile blue were reported.[2] Various type of scaffolds such as crown ether, cryptand, calixarenes, steroid, and peptides have been used as receptors for the recognition of target analysts in chemical sensors.[3] In most cases, fluorescent chemosensors have required tedious synthesis to prepare, and have not worked well in 100% aqueous solution due to the low binding affinity for the target metal ions, as well as their poor solubilities of the sensors.

In the present study, we tried to synthesize simple fluorescent chemical sensors for Fe^{3+} ions by conjugation of natural amino acids into anthracene as a fluorophore, which would avoid tedious synthesis and would help to construct chemical sensor libraries in the future. In consideration of building chemical sensor libraries, we synthesized the chemical sensors using solid-phase synthesis. We designed chemosensors that would work well in aqueous phase, selective for Fe^{3+} ions because of that ion's importance to biological systems. Fe^{3+} is one of the most important intracellular metal ions and plays a vital role in a variety of cell functions.[4] For example, Fe^{3+} provides the oxygen-carrying capacity of heme and acts as a cofactor in many enzymatic reactions involved in the mitochondrial respiratory chain, and both its deficiency and excess can induce a variety of diseases. Moreover, iron homeostasis is an important factor involved in neuroinflammation and progression of Alzheimer's disease.[5] However, relatively few examples have been reported as chemosensors for Fe^{3+}.[6] Most of the reported Fe^{3+} sensors did not work in 100% aqueous solution due to their hydrophobicity and low binding affinity, or suffered from interference from either Al^{3+}, Cr^{3+}, Cu^{2+}, or Fe^{2+}. Therefore, fluorescent chemical sensors that sensitively and selectively detect Fe^{3+} ion in aqueous solution are of great importance.

On the basis of the information of previously reported Fe^{3+} chemosensors,[6] we focused on the development of small fluorescent chemosensors with asymmetric carboxylate sites for the Fe^{3+} receptor. As shown in Scheme 1, anthracene compounds containing L-aspartic acid or L-glutamic acid were synthesized in solid phase synthesis in 2-Cl trityl chloride resin. After Fmoc-protected amino acid was introduced into the resin, the coupling of anthracene acid was performed. To the resin bound amino acid (0.05 mmol), DMF solution (3 mL) containing 9-anthracenecarboxylic acid (0.15 mmol, 3 equiv), DIPC (0.15 mmol, 3 equiv) and HOBt (0.15 mmol, 3 equiv) were added and then were shaken for 24 h at room temperature. Deprotection and cleavage were achieved by treatment with a mixture of TFA/TIS/H_2O (9.5:0.25:0.25, v/v/v) at room temperature for 4 h. After precipitation of the crude product

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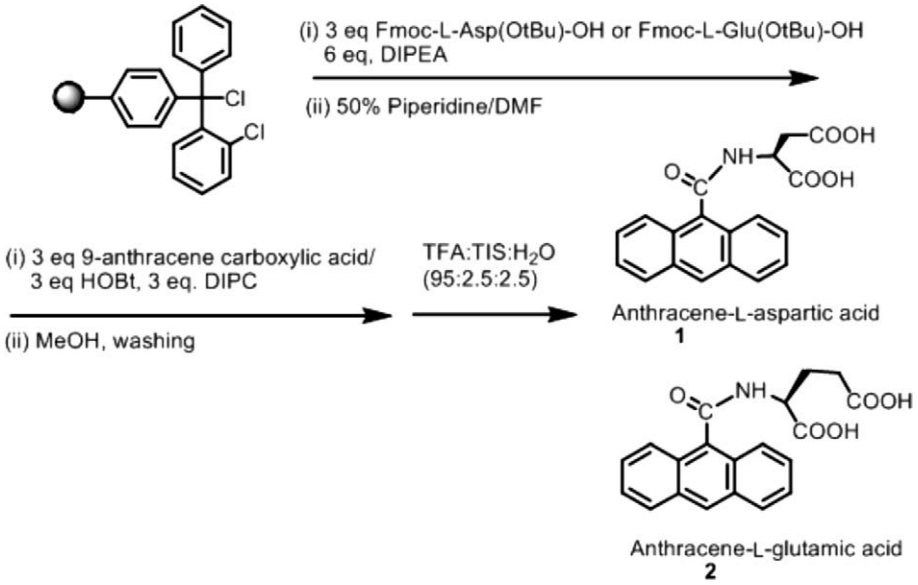


Scheme 1. Synthesis scheme for **1** and **2**.

in diethyl ether solution, the product was analyzed by analytical C_{18} HPLC. One major peak corresponding to the target compound was observed in HPLC. For the fluorescent experiments, each crude product was purified by semi-prep HPLC with a C_{18} column using a water (0.1% TFA) and acetonitrile (0.1% TFA) gradient. Successful synthesis and purity (>95%) were confirmed by analytical HPLC with C_{18} column and ESI mass spectrometer (**1** $[M-H]^-$ calcd 335.10; obsd 335.94 **2** $[M-H]^-$ calcd 349.10; obsd 349.95).

Fluorescence spectra of **1** and **2** were measured in the presence of each metal ion (Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Cr^{3+}, Fe^{2+} , Fe^{3+}, and Zn^{2+} with perchlorate anion and Na^+, Al^{3+} and K^+ with chloride anion) in different solvent systems, including 100% aqueous buffer solution. As shown in Figure 1, **1** and **2** exhibited selective response to Fe^{3+} among various metal ions in MeOH–water and 10 mM HEPES buffer solution (pH 5). We also investigated the fluorescent response of the sensors to metal ions

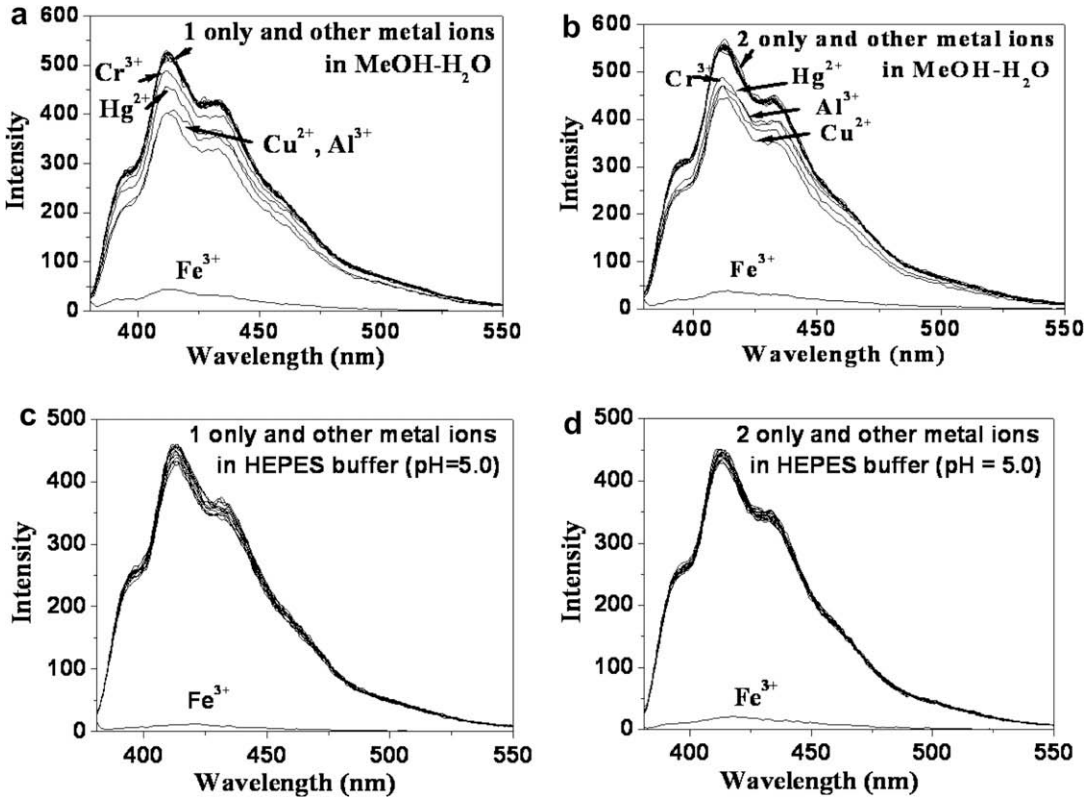


Figure 1. Fluorescent spectra of **1** (5 μM) and **2** (5 μM) in the presence of metal ions (4 equiv) in MeOH–water (1:1 v/v) and in the presence of metal ions (7 equiv) in 10 mM HEPES buffer pH 5.0. (λ_{ex} = 363 nm, slit: 10 nm/5 nm).

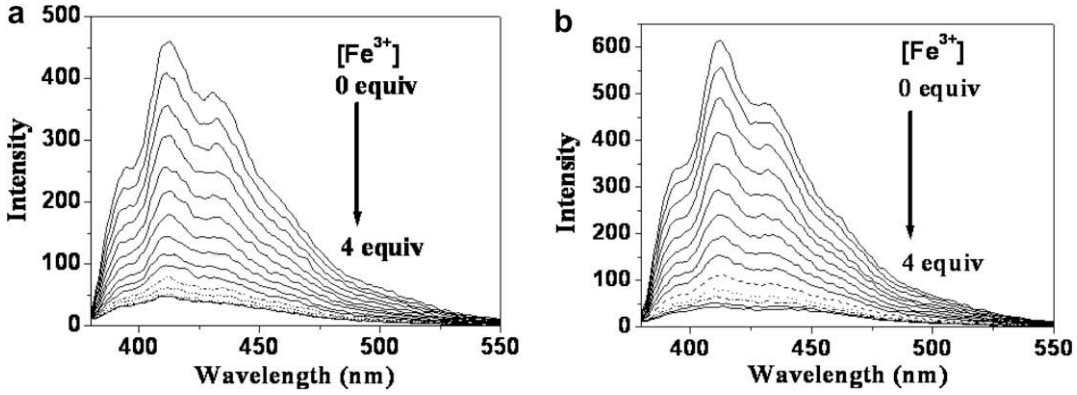


Figure 2. Fluorescent spectra of **1** and **2** (5 μM) with the addition of various concentration of $Fe(ClO_4)_3$ (1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 13.5, 15, 16.5, 18, and 19.5 μM) in MeOH–water (1:1 v/v) (λ_{ex} = 363 nm, slit: 10 nm/5 nm).

in various solvent systems. Both sensors exhibited selective response to Fe^{3+} among 13 metal ions in pure organic solvent system (MeOH and CH_3CN), 100% aqueous solvent system, and mixed organic–water solvent system such as MeOH–HEPES buffer solution (pH 5), MeOH–water, MeCN–HEPES buffer solution (pH 5), and MeCN–water (data not shown). Figures 2 and 3 show detailed fluorescent change of **1** and **2** upon gradual titration of Fe^{3+} ion in MeOH–water and 10 mM HEPES buffer solution (pH 5). With increasing concentration of Fe^{3+}, the emission intensity at 412 nm decreased. Four equivalents of Fe^{3+} ion is enough for the complete quenching of the sensors in MeOH–water, whereas 7 equiv of Fe^{3+} ion is sufficient for the complete quenching of the sensors in HEPES buffer solution (pH 5). Compounds **1** ($\varepsilon = 9.0 \times 10^3\ M^{-1}\ cm^{-1}$) and **2** ($\varepsilon = 9.3 \times 10^3\ M^{-1}\ cm^{-1}$) each showed absorption maxima at 363 nm in 10 mM HEPES buffer solution (pH 5). The anthracene-based sensors **1** and **2** have similar quantum yields (Φ = 0.20, Φ = 0.21) with reference to an anthracene standard.[7] Job plot analysis was carried out to determine the binding stoichiometry of **1** and **2** (data not shown). The 0.5 mol fraction at the maximum intensity change revealed that **1** and **2** each might form a 1:1 complex with the ferric ion. On the basis of 1:1 stoichiometry, the association constants of **1** and **2** with Fe^{3+} ion in buffer solution were calculated to be 60 μM (R^2 = 0.9917) and 70 μM (R^2 = 0.9866), respectively, using the ENZFITTER program based on the titration curve.[8] This indicates that both sensors have potent binding affinities for Fe^{3+} ion compared

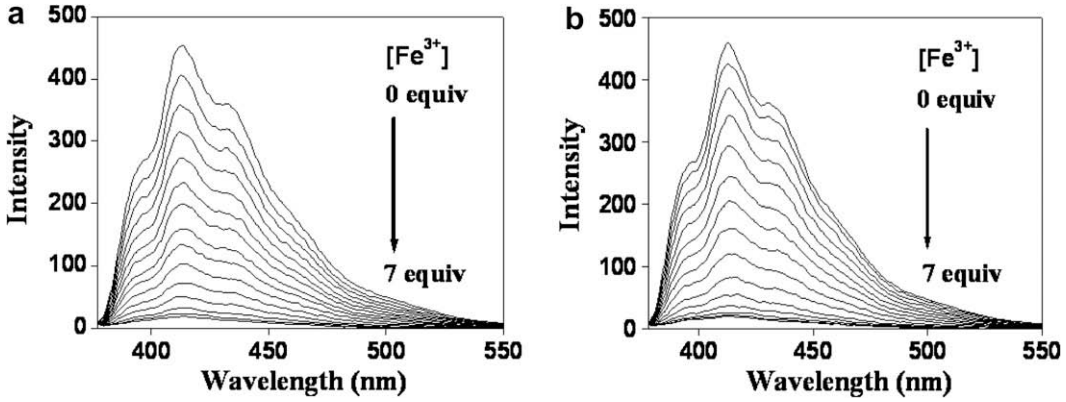


Figure 3. Fluorescent spectra of **1** and **2** (5 μM) with the addition of various concentration of $Fe(ClO_4)_3$ (2.5, 5, 7.5, 10,12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, 32.5, and 35 μM) in 10 mM HEPES buffer pH 5.0 (λ_{ex} = 363 nm, slit: 10 nm/5 nm).

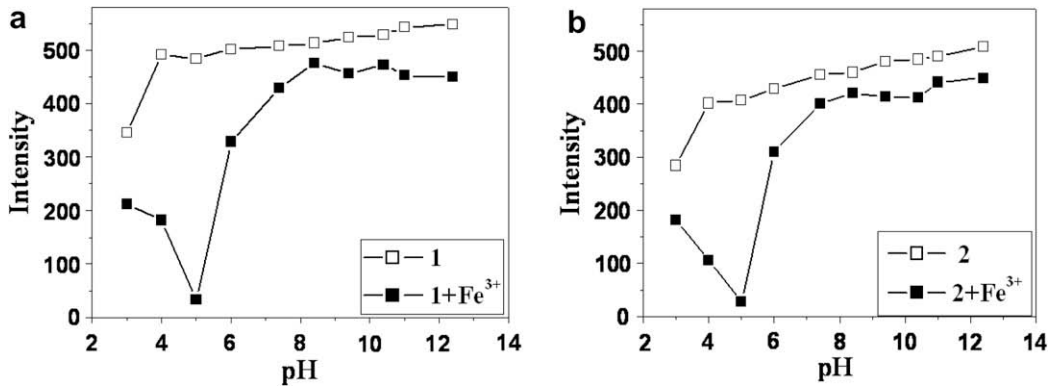
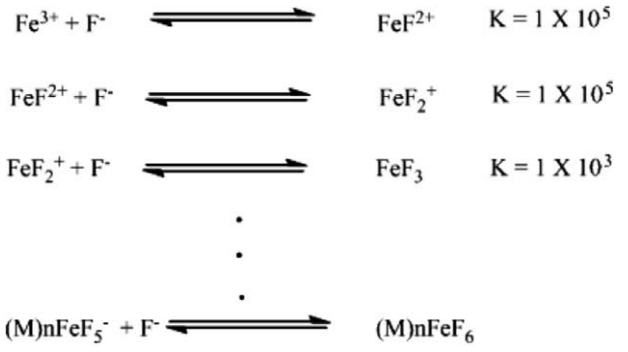


Figure 4. Fluorescence response of **1** and **2** (5 μM) in the presence and absence of $Fe(ClO_4)_3$ (7 equiv) at different pH values (λ_{ex} = 363 nm, slit: 10 nm/5 nm).



Scheme 2. The reaction scheme between ferric ion and fluoride ion.

to those of the previous reported Fe^{3+} sensors. To test the reversibility, EDTA was added to the sensor–Fe^{3+} ion complex that exhibited little fluorescent emission intensity. This addition of EDTA instantly resulted in the increase of the emission intensity. Addition of about 40 equiv of EDTA returned the emission almost to the original, Fe^{3+}-free spectrum, which demonstrates the readily reversibility of the signaling mechanism of the probes (data not shown).

We investigated the detection ability of the sensors in buffer solutions with different pH (Fig. 4). At acidic pH, both sensors showed sensitive response to Fe^{3+}; large intensity changes were observed in the presence of Fe^{3+}, while the sensors did not respond to Fe^{3+} ion sensitively in neutral and basic pH. Unexpectedly, the sensitivity of the sensors to Fe^{3+} seems to be the best at pH 5. Considering the pK_a values (3.86 and 4.25) of Asp and Glu residues, the carboxyl acid groups of the sensors must be in the carboxylate form at pH $\geqslant$ 5. Thus, the sensors were expected to have a greater binding affinity to Fe^{3+} at pH $\geqslant$ 5. However, the solubility of ferric ions decreases at pH >4 with formation of insoluble ferric hydroxides from Fe^{3+}.[9,10] We suppose that the sensitivity of the sensor is best at pH 5 because the acid group of the sensors is converted to a negatively-charged form and the formation of ferric hydroxide is not considerable at pH 5, whereas even though the acid group of the sensors is fully negatively charged, the formation of ferric hydroxide might increase at pH 6, resulting in the decrease of the sensitivity of the sensors at pH 6.

A chemosensing ensemble method has been recently reported in which the fluorescence of the sensors is either quenched or enhanced by noncovalent interactions of indicators. When the analytes replace the indicators, the fluorescence of the sensors is recovered. The chemosensing ensemble method using metal–chemosensor complexes has been reported to detect specific amino acids and anions.[6,11] In the chemosensing ensemble method, the binding affinity difference between indicator–receptor and analyte–receptor is the most important factor in analyte detection. The high affinity of Fe^{3+} to fluoride ion among other halide ions is well documented.[9,12] Thus, we investigated the application of **1**–Fe^{3+} and **2**–Fe^{3+} complex as chemosensing ensemble probes for monitoring fluoride ion. Fluoride is toxic, although EPA standards allow it in drinking water below 4.0 mg/L.[13] The chemosensing ensemble probes of **1**–Fe^{3+} and **2**–Fe^{3+} complexes exhibited an increase of emission intensity for only fluoride ion among halide

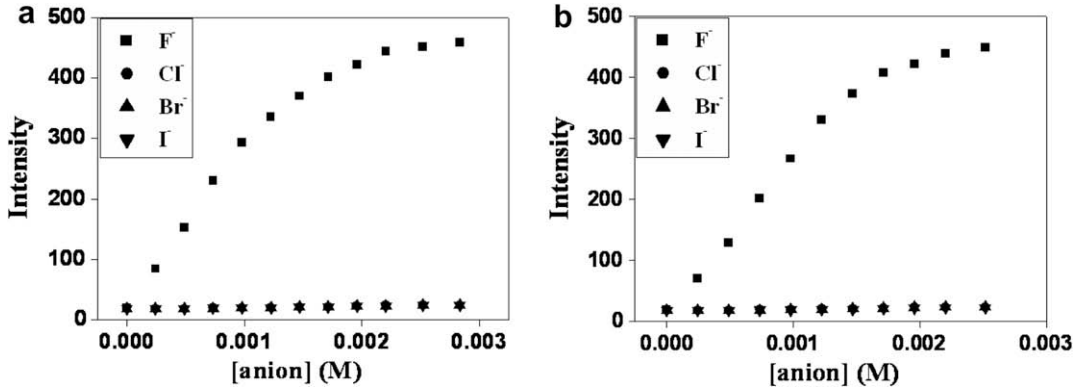


Figure 5. Emission intensity change of **1**–Fe^{3+} complex (a) and **2**–Fe^{3+} complex (b) at 10 min after the addition of various concentrations of halogen ions. Each sensor–Fe^{3+} complex was formed by mixing 5 μM of sensor with Fe^{3+} (7 equiv).

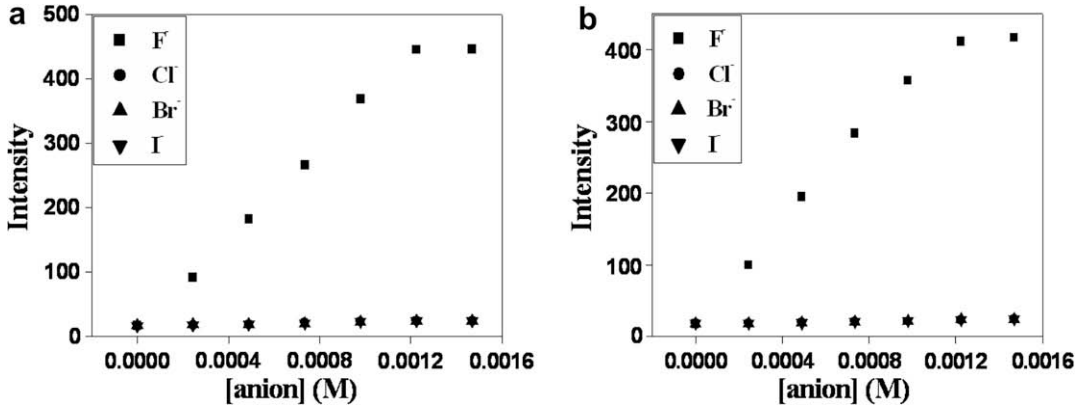


Figure 6. Emission intensity change of **1**–Fe^{3+} complex (a) and **2**–Fe^{3+} complex (b) at 6 h after the addition of various concentrations of halogen ions. Each sensor–Fe^{3+} complex was formed by mixing 5 μM of the sensor with Fe^{3+} (7 equiv).

ions in 100% aqueous solution. The reactions between ferric and fluoride ion are step by step, and the association constants of the first two reactions are high (Scheme 2). However, some of the reaction rates may not be fast, so the dose–response curve is time-dependent, and an excess of fluoride may be required for the saturation of intensity. After the sensor/ferric ion complex was mixed with fluoride ion, an intensity change was measured at different time intervals. The emission intensity was not changed after 6 h, suggesting that the reactions might reach an equilibrium state. Upon measuring after 10 min, the response curve looked like the standard saturation curve (Fig. 5). Upon measuring after 6 h, a linear response curve was observed (Fig. 6). The sensitivity for fluoride ion was calculated on the basis of this linear relationship between the emission intensity at 412 nm and the fluoride concentration. The intensity of the ensemble probes at 412 nm increased linearly with the concentration of fluoride ion, and linear measurements with high accuracy are possible up to 240 equiv of fluoride ion. The sensors **1**–Fe^{3+} and **2**–Fe^{3+} have detection limits of 10.9 μM (0.21 mg/L) and 10.0 μM (0.19 mg/L), respectively, based on $3\sigma_{bi}/m$, where σ_{bi} is the standard deviation of blank measurements and m is the slope between intensity versus sample concentration. The detection limit for fluoride ion is much lower than the EPA's maximum allowable level of fluoride in drinking water.

Conclusion. We synthesized simple anthracene-based chemosensors containing aspartic and glutamic acids in solid-phase synthesis. The sensors selectively and sensitively detected Fe^{3+} ions among various metal ions in 100% aqueous solution, and in an organic–aqueous mixed solvent system. L-Acidic amino acids (Glu and Asp) with two asymmetric carboxyl groups as sensors were selective receptors for Fe^{3+} ions. We also showed the new chemosensing ensemble method using **1**–Fe^{3+} and **2**–Fe^{3+} complexes for detection of fluoride ion. Our results show that anthracene-based compounds containing L-amino acids can be used as fluorescent chemical sensors.

Acknowledgments

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