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A Water Soluble Fluorescent Chemosensor Based on Asp Functionalized Naphthalimide for Successive Detection Fe$^{3+}$ and H$_3$PO$_4$\textsuperscript{-}

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Abstract:
The selective recognition of target ions in water is very important and the development of novel water soluble chemosensor is still an intriguing challenge. Herein, a novel water soluble fluorescent sensor based on aspartic acid (Asp) functionalized 1,8-naphthalimide derivative (Asp-NI) has been designed and synthesized. The sensor Asp-NI could dissolve in water and successively detect Fe$^{3+}$ and H$_2$PO$_4^-$ in water solution with high selectivity and sensitivity. The detection limits are $4.97 \times 10^{-7}$ M for Fe$^{3+}$ and $5.27 \times 10^{-6}$ M for H$_2$PO$_4^-$. Other coexistent competitive metal ions (Hg$^{2+}$, Ag$^+$, Ca$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, Cd$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, Cr$^{3+}$ and Mg$^{2+}$) showed no interference in the Fe$^{3+}$ detection process. The sensor Asp-NI could act as a Fe$^{3+}$ and H$_2$PO$_4^-$ controlled “On-Off-On” fluorescent switch. More interestingly, the Fe$^{3+}$ induced fluorescence quenching process could be totally reversed by the addition of H$_2$PO$_4^-$, this “On-Off-On” switching process could be repeated several times with little fluorescence loss. Notably, the actual usage of sensor Asp-NI was further demonstrated by test kits.

Keywords:
water soluble fluorescent sensor; 1,8-naphthalimide derivative; Fe$^{3+}$ recognition; H$_2$PO$_4^-$ recognition; successive fluorescent detection.
1. Introduction

As we all know, iron ions play a fundamental role in many chemical, biological, medical, environmental and technological processes\(^1\). Most of all, in the human body, iron, the most abundant and versatile transition metal in cellular systems\(^2\), is present in numerous numbers of enzymes and proteins and it is essential for various biological processes\(^3\), such as oxygen uptake, oxygen metabolism, electron transfer, and transcriptional regulation\(^4\). In addition, recognition of bioactive ions has come into limelight since they play significant roles in bioprocesses\(^5\). In particular, phosphates are crucial anions since they are used in energy storage devices and signal transducers. Besides, phosphates are also well known anions used in industrial processes\(^6\). Hence, the development of new methods for efficient monitoring of iron and \(\text{H}_2\text{PO}_4^-\) is very necessary. Recently, a large number of methods have been reported for detecting \(\text{Fe}^{3+}\) and \(\text{H}_2\text{PO}_4^-\), such as, colorimetry\(^7\), atomic absorption spectroscopy\(^8\), voltammetry\(^9\), and spectrophotometry\(^10\). Among of these methods, colorimetric and fluorimetric sensors have attracted considerable interest owing to their simple, inexpensive, rapid implementation and high sensitivity\(^11\). However, there is still a vexing problem. Generally, most of biological or environmental procedures are carried out in water system, and due to the poor water solubility of chemosensors, most of those couldn’t work in aqueous solution or water\(^12,13\). Therefore, much attention has been paid to developing \(\text{Fe}^{3+}\) and \(\text{H}_2\text{PO}_4^-\) sensors that work in the aqueous phase. But, most of the sensors often employ sophisticated structures and complicated synthetic method, and the progress of its targeted detection need high temperature and long reaction time\(^14\).

In view of this and as a part of our research effort devoted into ions recognition\(^15\), we want to develop a fluorescent chemosensor for efficient detection \(\text{Fe}^{3+}\) and \(\text{H}_2\text{PO}_4^-\).
in water. Herein, we report the aspartic acid (Asp) functionalized 1,8-naphthalimide
derivative (Asp-NI, Scheme 1), which act as a hydrosoluble fluorescent sensor for the
detection of Fe$^{3+}$ and H$_2$PO$_4^-$. The strategy for the design of sensor Asp-NI as follows.
Firstly, we introduced aspartic acid (Asp) into the sensor molecular as a hydrophilic
group, which can enhance the water-solubility of sensor Asp-NI. Moreover, the
aspartic acid moiety employ two carboxyl groups act as binding sites for iron ions.
Secondly, owing to the excellent optical properties of 1,8-naphthalimide derivatives,
we introduced 1,8-naphthalene dicarboxylic anhydride groups as the signal groups to
achieve fluorescent recognition. Finally, the sensor Asp-NI is designed easy to make,
the synthesis process of the sensor only need one step reaction. As a results, the nice
thing here is that the sensor Asp-NI could successively detect Fe$^{3+}$ and H$_2$PO$_4^-$ in
water solution with high selectivity and sensitivity.

( Insert Scheme 1 )

2. Experimental

2.1 Materials and instruments

All reagents and solvents were commercially available at analytical grade and were
used without further purification. Fresh double distilled water was used throughout
the experiment. $^1$H NMR spectra were recorded on a Mercury-400BB spectrometer at
600 MHz and $^{13}$C NMR spectra were recorded on a Mercury-400BB spectrometer at
150 MHz. Chemical shifts are reported in ppm down field from tetramethylsilane
(TMS, δ scale with solvent resonances as internal standards). Infrared spectra were
performed on a Digilab FTS-3000 FT-IR spectrophotometer. Mass spectra were
recorded on an esquire 6000 MS instrument equipped with an electrospray (ESI) ion
source and version 3.4 of Bruker Daltonics Data Analysis as the data collection
system.
2.2 General procedure for fluorescence experiments

All fluorescence spectra were recorded on a Shimadzu RF–5301 fluorescence spectrometer after the addition of perchlorate metal salts in water, while keeping the ligand concentration constant (2.0 × 10\(^{-5}\) M). The excitation wavelength was 330 nm. Solutions of metal ions were prepared from the perchlorate salts of Fe\(^{3+}\), Hg\(^{2+}\), Ag\(^{+}\), Ca\(^{2+}\), Cu\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\), Cd\(^{2+}\), Pb\(^{2+}\), Zn\(^{2+}\), Cr\(^{3+}\) and Mg\(^{2+}\) (4 × 10\(^{-4}\) M). Solutions of anions were prepared from tetrabutylammonium salt of anions (F\(^{-}\), Cl\(^{-}\), Br\(^{-}\), I\(^{-}\), AcO\(^{-}\), H\(_2\)PO\(_4\)^\(-\), HSO\(_4\)^\(-\) and ClO\(_4\)^\(-\)) and sodium salt of anions (CN\(^{-}\) and SCN\(^{-}\)).

2.3 Synthesis and characterization of sensors Asp-NI

A mixture of 1,8-naphthalenedicarboxylic anhydride (1.98 g, 10 mmol) and aspartic acid (1.331 g, 10 mmol) in DMF (20 mL) was stirred at 140 ℃ reflux for 24 h. After cooling to room temperature, water (50 mL) was added and the precipitate was filtered, washed three times with hot DMF, then get light yellow powder product Asp-NI \(_1\) (2.31 g). Then, the Asp-NI \(_1\) (5 mmol, 0.156 g) and sodium hydroxide (10 mmol, 0.40 g) were dissolved in water (50 mL). The reaction mixture was stirred for 30 minutes under room temperature. Then the water (50 mL) was evaporates by drying oven to afford the desired product; N,N-bis (1,8-naphthalene dicarboxamide) aspartic sodium salt, that is the sensor molecule Asp-NI.

Asp-NI \(_1\): yield: 74%; m.p. , 254 ℃; \(^1\)H NMR (D\(_2\)O, 400 MHz, ESI, Fig. S1†) d 8.37-8.35 (2H, ArH), d 8.19-8.18 (2H, ArH), d 7.65-7.64 (2H, ArH) 5.82-5.79 (1H, CH) 3.09-3.06 (1H, CH\(_2\)) 2.84-2.80 (1H, CH\(_2\)). \(^{13}\)C NMR (D\(_2\)O, 400 MHz, ESI, Fig. S2†) d 179.89, 176.99, 165.54, 134.98, 134.57, 134.54, 129.94, 127.98, 127.56, 126.95, 125.68, 125.06, 121.36, 53.89, 38.43. IR (KBr, cm\(^{-1}\), ESI, Fig. S3†) v: 3437,
(-OH); 1699, (COOH, C=O); 1656, (C=O); 1238, (-N-CH) cm$^{-1}$. In the IR spectra of
Asp-NI, upon the addition of Fe$^{3+}$, the stretching vibration absorption peaks of
carboxyl shifted from 1699 cm$^{-1}$ to 1651 cm$^{-1}$ as show in ESI, Fig. S4† ESI-MS m/z:
[Asp-NI +H]$^+$. Cold for C$_{16}$H$_{11}$NO$_6$ 313.0586; Found 313.0540, (ESI, Fig. S5†).

3. Results and Discussion

In order to investigate the ions recognition capacity of the sensor Asp-NI in pure
water, a series of recognition experiments were carried out. The recognition profiles
of the chemosensor Asp-NI toward various metal ions, including Fe$^{3+}$, Hg$^{2+}$, Ag$^+$,
Ca$^{2+}$, Cu$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Cd$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, Cr$^{3+}$ and Mg$^{2+}$ were primarily investigated
using fluorescence spectroscopy in water. As shown in Fig. 1, in the fluorescence
spectrum, the emission of Asp-NI appeared at the maximum emission wavelength
was 385 nm in pure water when excited at 330 nm. When 20 equivalents of Fe$^{3+}$ (4 ×
10$^{-4}$ M) and the other cations were added to the water solution of sensor Asp-NI,
respectively. Only the Fe$^{3+}$ could induce the solution of sensor Asp-NI fluorescent
quenching, other competitive metal ions couldn’t induce similar response. Moreover,
as shown in Fig. 2a, the photographs show the change in the fluorescence of Asp-NI
after the addition of various metal ions in water.

(Insert, Fig. 1 and Fig. 2a)

Interestingly, the quenched fluorescence of Asp-NI in solutions by Fe$^{3+}$ was turned
on after addition of H$_2$PO$_4^-$ To evaluate the H$_2$PO$_4^-$ selective nature of the
Asp-NI-Fe$^{3+}$ complex, the influence of other anions such as F$^-$, Cl$^-$, Br$^-$, I$^-$, AcO$^-$, CN$^-$,
HSO$_4^-$, ClO$_4^-$, CN$^-$ and SCN$^-$ was investigated. (Fig. 2b) Interestingly no significant
change in fluorescence emission was observed with other anions. The resulting
Asp-NI-Fe$^{3+}$ complex showed high selectivity for H$_2$PO$_4^-$ in the same media as well
The binding affinity of sensor Asp-NI with Fe$^{3+}$ was estimated by performing emission titration experiments when excited at 330 nm. The sensor Asp-NI showed strong fluorescence at 385 nm. As shown in Fig. 4, upon the addition of increasing concentrations of Fe$^{3+}$ (0-20 equiv.), the emission peak at 385 nm gradually diminished intensity, and the fluorescence of Asp-NI was essentially quenched by 20 equiv. of Fe$^{3+}$, moreover the signal saturation was observed. The detection limit of the sensor towards Fe$^{3+}$, obtained according to fluorescence titration on the basis of $3S_B/S$, was $4.97 \times 10^{-7}$ M$^{[16]}$ (Fig. S6 in the ESI). The binding constants for the formation of the respective complexes were evaluated using the Benesi–Hildebrand (B–H) plot$^{[17]}$.

$$\frac{1}{(I - I_0)} = \frac{1}{K(I_{\text{max}} - I_0)C} + \frac{1}{I_{\text{max}} - I_0}$$

$I_0$ is the emission intensity of Asp-NI at emission maximum ($I = 396$ nm), $I$ is the observed emission intensity at that particular wavelength in the presence of a certain concentration of the metal ion ($C$), $I_{\text{max}}$ is the maximum emission intensity value that was obtained at $I = 396$ nm during titration with varying metal ion concentration, $K$ is the binding constant (M$^{-1}$) and was determined from the slope of the linear plot, and $C$ is the concentration of the Fe$^{3+}$ ion added during titration studies. The binding constant for the formation of the Asp-NI-Fe$^{3+}$ complex was calculated on the basis of the change in emission at 385 nm by considering a 1 : 1 binding stoichiometry. The binding constant ($K$) determined by the B-H method was found to be $2.9 \times 10^4$ M$^{-1}$.

To further exploit the utility of the chemosensor Asp-NI as an ion-selective sensor for Fe$^{3+}$, competitive experiments were carried out in the presence of 20 equiv. of Fe$^{3+}$.
and 20 equiv. of various cations in pure water. As shown in Fig. 5, it is noticeable that the miscellaneous competitive cations did not lead to any significant interference. The fluorescence selectivity was examined at an excitation wavelength of 330 nm, and none of the competing cations interfered in the detection of Fe$^{3+}$. This result displays the highly selectivity of the sensor Asp-NI toward iron over the other analytes mentioned above. Further, the interference experiments were carried out to study the selectivity of Asp-NI. The fluorescence spectroscopy was carried out just after the addition of KCl and NaCl in water solution. As shown in Figure S7, the fluorescence emission peak at 385 nm was no obvious changed compare with the solution of free Asp-NI. The result indicated that the existence of Na$^+$, K$^+$ have not interference with identification process of Asp-NI to Fe$^{3+}$. Then we added the solutions of KHCO$_3$ and KNO$_3$ to Asp-NI + Fe$^{3+}$. As shown in Figure S11, the sensor behavior of H$_2$PO$_4^-$ to Asp-NI + Fe$^{3+}$ could not be influenced even existence the HCO$_3^-$ and NO$_3^-$.

(Insert Fig. 5)

Moreover, the pH dependence of the Asp-NI in the HEPES buffer system was verified using fluorescence spectroscopy, as shown in Fig.6, Fe$^{3+}$ detection was successful in the pH ranged from 2.0 to 10.0 in HEPES buffered solution.

(Insert Fig. 6)

The reversibility of the sensor was tested by addition of various anions to the Asp-NI-Fe$^{3+}$ solution. The addition of H$_2$PO$_4^-$ to the solution of Asp-NI-Fe$^{3+}$ showed the detection process of the sensor Asp-NI for Fe$^{3+}$ is reversible, and the reversible
process could be repeated at several times with the little fluorescent efficiency loss. (Fig. 7) Thus, the sensor Asp-NI could act as not only a reversible Fe\(^{3+}\) and H\(_2\)PO\(_4\)\(^{-}\) sensor, but also a Fe\(^{3+}\) and H\(_2\)PO\(_4\)\(^{-}\) controlled “On-Off-On" fluorescent switch. Besides, the reversibility of the chemosensor is a very important aspect for its practical application. To further investigate the practical applications of chemosensor Asp-NI, test strips were fabricated by immersing filter paper into water of Asp-NI (2 × 10\(^{-3}\) M) followed by drying them in air. The test strips containing Asp-NI were utilized to sequential sense Fe\(^{3+}\) and H\(_2\)PO\(_4\)\(^{-}\). As shown in Fig. S8, when Fe\(^{3+}\) was first added to the test strips, an obvious color change was observed. After the addition of H\(_2\)PO\(_4\)\(^{-}\) the color of the test strips recovered, which served as a convenient and efficient sequential Fe\(^{3+}\) and H\(_2\)PO\(_4\)\(^{-}\) test kits, which indicated that the test strips could act as a recyclable test kits for sequential detection of Fe\(^{3+}\) and H\(_2\)PO\(_4\)\(^{-}\).

(Insert Fig. 7)

As Asp-NI showed specific selectivity for Fe\(^{3+}\) and H\(_2\)PO\(_4\)\(^{-}\) in water, a series of experiments such as Job’s method, IR, and MS were carried out to investigate the Fe\(^{3+}\) and H\(_2\)PO\(_4\)\(^{-}\) recognition capability and mechanism of the sensor Asp-NI. To gain an insight into the stoichiometry of the Asp-NI-Fe\(^{3+}\) complex, the method of continuous variations (Job’s method) was used (Fig. 8). As expected, when the molar fraction of sensor Asp-NI was 0.50, the absorbance value approached a minimum, which demonstrated the formation of a 1:1 complex between the sensor Asp-NI and Fe\(^{3+}\).

(Insert Fig. 8)

The practical utility of the probe was carried out by detection of Fe\(^{3+}\) in tap water. As shown in Fig. 9 and S9, firstly, the standard curve of the Asp-NI for Fe\(^{3+}\) was obtained by fluorescent titration of Asp-NI solutions with different concentrations Fe\(^{3+}\) (5.0×10\(^{-7}\) M, 1.0×10\(^{-6}\) M, 1.5×10\(^{-6}\) M, 2.0×10\(^{-6}\) M, 2.5×10\(^{-6}\) M, 3.0×10\(^{-6}\) M,
3.5×10⁻⁶ M, 4.0×10⁻⁶ M). Then, the concentration of Fe³⁺ in tap water was investigated by standard curve method and obtained as 1.42×10⁻⁶ M. In order to verify this result, we also used inductively coupled plasma (ICP) experiments to measure the concentration of Fe³⁺ in tap water. The ICP results show that the concentration of Fe³⁺ in tap water is 1.09×10⁻⁶ M, which supported the result obtained by standard curve method. Moreover, the special advantage of this sensor is water soluble. A comprehensive comparison on synthetic method of probe, suitable solvents, and LOD (limit of detection) of the probe Asp-NI with reported probes were listed in Table S1.

(Insert Fig. 9)

The recognition mechanism of the sensor Asp-NI with Fe⁵⁺ was also investigated by IR spectra. In the IR spectra of Asp-NI (ESI, Fig. S3†), upon the addition of Fe⁵⁺, the stretching vibration absorption peaks of carboxyl shifted from 1699 cm⁻¹ to 1651 cm⁻¹ as show in ESI, Fig. S4†, which indicated that Asp-NI complexed with Fe⁵⁺ via the carboxyl group of Asp-NI (Scheme 2). In addition, the ESI Mass results are in good agreement with this finding. The peak at m/z 312.0940 (Fig. S5) is corresponding to [Asp-NI + H]^+ and the peak at m/z 736.9666 demonstrate the presence of [Asp-NI + Fe⁵⁺ + 3(ClO₄⁻) + DMF] (ESI, Fig. S11). According to the Job’s method, IR and ESI Mass, the ions response mechanism of Asp-NI with Fe⁵⁺ and H₂PO₄⁻ could be presumed as Scheme 2.

(Insert Scheme 2 )

4. Conclusions

In conclusion, we rationally design and synthesis a water-soluble, easy-to-make and efficient Fe⁵⁺ fluorescent sensor Asp-NI. By rationally introduce carboxyl moiety into 1,8-naphthalimide-based chemosensor molecular, the obtained chemosensor shown well solubility in water and specific fluorescent selectivity for Fe³⁺ and H₂PO₄⁻ in
water. The detection limits are \(4.97 \times 10^{-7}\) M for Fe\(^{3+}\) and \(5.27 \times 10^{-6}\) M for H\(_2\)PO\(_4^-\) (ESI, Fig. S11†). Other ions had nearly no significant influence on the sensing behavior, which indicated that the sensor Asp-NI may be useful as a sensor for monitoring Fe\(^{3+}\) levels in biological, medical, environmental and technological systems. Interestingly, the sensor Asp-NI could act as not only a reversible Fe\(^{3+}\) sensor, but also a Fe\(^{3+}\) and H\(_2\)PO\(_4^-\) controlled “On-Off-On” fluorescent switch. The distinct color changes under UV light (365 nm) and rapid reversible fluorescent changes can be detected by the naked eye. More interestingly, the Fe\(^{3+}\) induced fluorescence quenching process could be totally reversed by the addition of H\(_2\)PO\(_4^-\), this “On-Off-On” switching process could be repeated several times with little fluorescence loss. Notably, the actual usage of sensor Asp-NI was further demonstrated by test kits.

Acknowledgements

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Reference


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**Scheme 1** Synthesis of the chemosensors Asp-NI.

**Fig.1** Fluorescent emission ($\lambda_{ex} = 330$ nm) spectra of Asp-NI ($2 \times 10^{-5}$ M) with various cations in water in response to Fe$^{3+}$ (20 equiv).
Fig. 2 The change in the fluorescence of (a) Asp-NI \((2 \times 10^{-5} \text{M})\) after the addition of cations \((4 \times 10^{-4} \text{ M})\) in water. (b) Asp-NI-Fe\(^{3+}\) \((2 \times 10^{-3} \text{M})\) after the addition of various anions \((2 \times 10^{-5} \text{ M})\) in water under 365nm UV light.
Fig. 3 Fluorescent emission ($\lambda_{ex} = 330 \text{ nm}$) spectra of Asp-NI-Fe$^{3+}$ ($2 \times 10^{-5}$M) with various anions in water in response to H$_2$PO$_4^-$ (20 equiv.).

Fig. 4 Fluorescence emission spectra of Asp-NI ($2 \times 10^{-5}$M) in the presence of different concentration of Fe$^{3+}$ anions (0.00–20.00 equiv.) in pure water. Inset: A plot
of fluorescent intensity depending on the concentration of cyanide in the range from 0 to 20 equivalents. ($\lambda_{em} = 385$ nm)

**Fig. 5** Fluorescence of sensor Asp-NI ($2 \times 10^{-5}$ M) at 385 nm with addition of 20 equiv. of Fe$^{3+}$ in the presence of 20 equiv. of other anions in water solution. ($\lambda_{ex} = 330$ nm)
**Fig. 6** Effect of pH on the fluorescence spectra (385 nm) of Fe$^{3+}$ (20 equiv.) from 2.0 to 13.0 in water solution. ($\lambda_{ex} = 330$ nm)
Fig. 7 Fluorescent emission ($\lambda_{ex} = 330$ nm) spectra showing the reversible complex between Asp-NI ($2 \times 10^{-5}$ M) and Fe$^{3+}$ (20 equiv.) by introduction of H$_2$PO$_4^-$ (20 equiv.) in water solution.
Fig. 8 The Job's plot examined between Fe$^{3+}$ and Asp-NI, indicating the 1:1 stoichiometry, which was carried out by fluorescence spectra ($\lambda_{ex} = 330$ nm, $\lambda_{em} = 385$ nm).

Fig. 9 The Fe$^{3+}$ concentration and fluorescence intensity function diagram. ($\lambda_{em} = 385$ nm).
Scheme 2 The proposed mechanism of Asp-NI bonding with the Fe$^{3+}$ in DMF solution.