Polyhydric polymer-functionalized fluorescent probe with enhanced aqueous solubility and specific ion recognition: A test strips-based fluorimetric strategy for the rapid and visual detection of Fe$^{3+}$ ions

Zhiqiang Duan$^{a,b}$, Chunxian Zhang$^a$, Yuchun Qiao$^a$, Fengjuan Liu$^a$, Deyan Wang$^a$, Mengfan Wu$^a$, Ke Wang$^a$, Xiaoxia Lv$^a$, Xiangmu Kong$^{b,*}$, Hua Wang$^{a,*}$

$^a$ Institute of Medicine and Material Applied Technologies, College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu City, Shandong Province 273165, PR China

$^b$ College of Physics and Engineering, Qufu Normal University, Qufu City, Shandong Province 273165, PR China

1. Introduction

Recent years have witnessed the increasing attentions concentrated on the monitoring of iron levels in environmental water samples for the iron contamination and miner exploration [1]. Although iron element plays a vital role in human life activities, excessive Fe$^{3+}$ ions in body, which maybe accumulated from the over uptake of Fe$^{3+}$ ions from the environmental water and food, may exert some adverse effects on human physiologic systems [2–4]. Therefore, the quantitative evaluation of Fe$^{3+}$ ions in environmental water is highly important for preventing any Fe$^{3+}$-induced public health hazard. Also, the development of a rapid, sensitive, and onsite applicable detection method for exploring Fe$^{3+}$ ions may be of significant interest in the outdoor finding of the potential iron mines. Up to date, many analysis methods are available for the determination of Fe$^{3+}$ ions, such as inductively coupled plasma mass spectrometry (ICP-MS), absorption spectrometry, and reflection X-ray fluorimetry [5–7]. However, most of these methods can suffer from rapid detection inability, complex sample pretreatment, and bulky infrastructure requirement, which can render their ineffectiveness for the applications of in-field iron monitoring. Therefore, a facile, rapid, economical, and portable analysis method is highly desired for the onsite monitoring of Fe$^{3+}$ ions in environmental water and especially for the iron miner exploration.

It is well established that the fluorimetric methods with some fluorescent probes are the sensitive analysis strategies [8–15], which are especially applied for the detection of Fe$^{3+}$ ions [8–12]. Since the fluorimetric performances may largely depend on the probes, which can translate the recognition information into the fluorescence signals, a number of fluorescent probes have been synthesized for the selective detection of Fe$^{3+}$ ions. Nevertheless, most of these reported fluorescent
probes (i.e., organic chromophores and quantum dots) [8,9,11] are poisonous, biologically incompatible, and water-insoluble, which may greatly limit the detection of Fe³⁺ ions. Moreover, some probes of organic small molecules can generally encounter with evident drawbacks such as the synthetic difficulty, time-consuming response, and low water solubility for the examination of aqueous samples, which can restrict the practically aqueous applications on a large scale. Therefore, the development of highly selective, sensitive, and water-dispersible fluorescent probes is desired for the detection of Fe³⁺ ions. As a result, many efforts have been devoted to the development of polymer-based fluorescent probes due to their display several important advantages over the common organic fluorescent probes [16–19]. For instance, the fluorescent probes of recognition units-coupled polymers can be easily included in the devices toward the diverse and functional chemoprobes [16,19]. Moreover, it is well recognized that poly(vinyl alcohol) (PVA) as a common polyhydric polymer has concentrated many efforts of applications due to that it is biodegradable, water-soluble, inexpensive, and film forming [20–23]. On the other hand, the pyrene moieties feature the high fluorescence quantum yields, chemical stability, and long fluorescence lifetime, so that they have been widely applied as the fluorophores in the recognitions of a variety of chemical species [24–27]. Considerably low aqueous solubility of pyrene molecules or moieties might limit the wide applications, although they can display the monomer-eximer dual fluorescence [28]. Herein, the introduction of PVA with hydroxyl groups of PVA chains should improve the aqueous solubility of pyrene. Importantly, the combination of PVA and pyrene should enjoy the synergistic advantages of PVA and pyrene for the specific Fe³⁺ ions recognition.

In the present work, we have reported a facile and efficient synthesis method to prepare polyhydric polymer-functionalized fluorescent probe for the detection of Fe³⁺ ions in environmental water samples. Herein, 1-pyrenecarboxyaldehyde (Pyr) was chosen to be covalently bound onto PVA chains through the acetal reaction yielding the PVA-loaded pyrene (PVA-Pyr) probes, showing dramatically improved aqueous solubility and especially strong cyan fluorescence. More importantly, the fluorescence of the resulted fluorescent probes could be thus selectively quenched by Fe³⁺ ions, presumably resulting from the probe aggregation induced by the specific intermolecular hydrogen bonding between Fe³⁺ ions and the hydroxyl groups of PVA chains [29–31]. Furthermore, the PVA-Pyr probes were coated onto the test strips by taking advantage of the outstanding film-forming ability of PVA. Interestingly, a stable and uniform distribution of fluorescent probes on test strips could also be expected by introducing the host PVA matrix to depress the formidable “coffee-stain” effects generally occurred in test strips [32]. Subsequently, a test strips-based fluorimetric method was developed for the rapid and visual detection of Fe³⁺ ions in the environmental water, showing high detection sensitivity and selectivity.

2. Materials and methods

2.1. Materials and methods

Poly(vinyl alcohol) (PVA, MW 89,000), dimethyl sulfoxide (DMSO), p-toluenesulfonic acid, 1-pyrenecarboxyaldehyde (Pyr), and Whatman filters used for test strips were purchased from Sigma-Aldrich (Beijing, China). Hexadecyltrimethoxysilane (HDS) and aminopropyltriethoxysilane (APS) were bought from Sinopharm Chemical Reagent Co. (China). All other reagents were of analytical grade. Deionized water (> 18 MΩ) was supplied from an Ultra-pure water system (Pall, USA). The environmental water samples (wastewater, river water, seawater, drinking water, and rainwater) were applied to spike Fe³⁺ ions of different concentrations.

The fluorescence measurements were conducted using the fluorescence spectrophotometer (F-7000, Hitachi, Japan) operated at an excitation wavelength at 345 nm, with both excitation and emission slit widths of 5.0 nm. The photographs of different materials and reaction products were recorded under UV light at 365 nm. Fourier transform infrared (FTIR) spectra were obtained by FTIR spectrophotometer (Thermo Nicolet Nexus 4700FT, USA). UV–vis spectrophotometer (Shimadzu, UV-3600, Japan) and scanning electronic microscopy (SEM, Hitachi E-1010, Japan) were separately applied for the characterization of different materials or products. The wastewater and river water samples were comparably analyzed in the laboratory with the inductively coupled plasma-mass spectrometry (ICP-MS) of Agilent 7500ce (Agilent Technologies, Waldbronn, Germany).

2.2. Synthesis of polyhydric polymer-functionalized fluorescent probes

An aliquot of PVA (40.0 mg mL⁻¹) and Pyr (4.34 μM) were mixed in DMSO of 50 mL. Then, p-toluenesulfonic acid (5.80 μM) was dropwise added into the mixture to react at 80 °C for 6 h. After being cooled down to room temperature, the mixture was poured into CH₃OH (100 mL) to be precipitated, where the unbound Pyr was separated out. The so purified products of the fluorescent PVA-Pyr probes containing 1.85% Pyr and 98.15% PVA were further washed three times with CH₃OH (50 mL) to be dried under a vacuum (77% yield based on PVA).

2.3. Preparation of polyhydric polymer-functionalized fluorescent test strips

The preparation procedure of the polyhydric polymer-functionalized fluorescent test strips for probing Fe³⁺ ions were conducted simply by following the procedure reported previously in our group [33]. Typically, the filters papers were first cut into the slices of test strips (10 mm×10 mm) and then soaked into the above PVA-Pyr probes (5.00 mg L⁻¹) for 10 min. After that, the test strips were immediately placed onto the HDS-modified super-hydrophobic patterns to be dried in vacuum for 20 min. Furthermore, the resulted test strips were immersed into APS solutions for 30 s for the hydrophilic treatments. Subsequently, the APS-treated test strips were again placed onto the super-hydrophobic patterns to be dried in vacuum for 10 min to be dried and stored in dark for future usage.

2.4. Fluorimetric measurements of Fe³⁺ ions

The optimization of analysis conditions for the test strips-based fluorimetric detections of Fe³⁺ ions were conducted separately using different amounts of PVA-Pyr, pH values, ion strengths, and reaction time. Typically, an aliquot of PVA-Pyr probes by ions were calculated according to the following equation: quenching efficiencies=(F0–F)/F₀, where F₀ and F refer to the fluorescence intensities of PVA-Pyr (λex=345 nm) in the absence and presence of ions. The selective fluorimetric analysis with centrifuge tubes was performed for Fe³⁺ ions, together with the possibly interferential ions (200 μM) as the control tests, including Fe²⁺, Cu²⁺, Ni²⁺, Zn²⁺, Ag⁺, Cd²⁺, Pb²⁺, Sn²⁺, Cr³⁺, Al³⁺, PbCl₂, F, Cl⁻, Br⁻, CO₃²⁻, CH₃COO⁻, SO₄²⁻, NO₃⁻ and PO₄³⁻ ions. Under the optimized conditions, the developed test strips-based fluorimetric method with polyhydric polymer-functionalized fluorescent probes were subsequently applied for the detection of Fe³⁺ ions of different concentrations spiked in wastewater samples (0, 0.10, 0.50, 1.00, 5.00, 10, 25, 50, 75, 100, 125, 150, 175, 200, 300 μM). The calibration curves of the fluorimetric detections for Fe³⁺ ions were obtained, with the analysis results further compared with those obtained by the classic ICP-MS method.
3. Results and discussion

3.1. Synthesis of polyhydric polymer-functionalized fluorescent probes for the fluorimetric Fe$^{3+}$ analysis

The PVA-loaded pyrene (PVA-Pyr) probes were synthesized by the p-toluenesulfonic acid-catalyzed reaction procedure, as schematically shown in Fig. 1A. A facile one-step condensation reaction between PVA and Pyr occurred to yield the PVA-Pyr probes consisting of 98.15% hosting PVA chains and 1.85% pyrene moieties, showing the bright cyan fluorescence in aqueous system. As can be seen from Fig. 1B, when Fe$^{3+}$ ions were introduced, they would specially bind with the hydroxyl groups of PVA chains through the strong intermolecular hydrogen bonds. A conformational change in the structure of the PVA host would thus occur to further cause the aggregations of PVA-Pyr probes with the hydrophobic pyrene encapsulated inside the hydrophilic PVA chains. As a result, the pyrene fluorescence of the polymeric probes was quenched. Such a procedure was also described elsewhere for the interactions between amine-derivatized polyethyleneimine polymer and Sr$^{2+}$ ions. Subsequently, the developed fluorescent probes were coated onto the test strips by use of the unique film-forming ability PVA, which could additionally serve as a host matrix to achieve the uniform redistribution of fluorescent probes. Notably, Pyr in water could display a strong excimer emission at 355–410 nm and from 450 to 550 nm, respectively.

Moreover, the PVA-Pyr probes were prepared by different batch productions, which robustness and variability were tested with the results shown in Fig. 3. One can note that the obtained PVA-Pyr probes display no significant change in the fluorescent intensities (Fig. 3A). Also, they could be stored in dark with the fluorescent property well sustained over 12 months (Fig. 3B).

Fig. 4A shows the fluorescence spectra of PVA-Pyr probes in the presence and absence of Fe$^{3+}$ ions, taking native Pyr and PVA alone as the controls. One can find that the fluorescent probes can display the strong monomer emissions both at 376 and 396 nm (curve a), with the excimer peaks ranging from 355 to 410 nm and from 450 to 550 nm, respectively. Notably, Pyr in water could display a strong excimer emission at 472 nm, which would almost disappear after the conjugate formation of PVA-Pyr probes. Furthermore, the PVA-Pyr probes could present the largely improved aqueous solubility, in contrast to Pyr that might be aggregated in water, as illustrated in the corresponding photographs.
More importantly, the fluorescence intensities of the PVA-Pyr probes could be dramatically quenched upon the addition of Fe$^{3+}$ ions (curve d). Herein, Fe$^{3+}$ ions would specially bind with the hydroxyl groups of PVA chains through the strong intermolecular hydrogen bonds [29–31], thus leading to the aggregation of the PVA-Pyr probes towards the fluorescence quenching as aforementioned.

Moreover, UV–vis absorption spectra of PVA-Pyr probes in water were measured with and without the additions of Fe$^{3+}$ ions, taking native Pyr and PVA alone as the controls (Fig. 4). It was discovered that the PVA-Pyr probes could exhibit the absorption peaks in the 232–340 nm region (curve a). Compared to the typical absorption peaks of Pyr alone (curve b), however, they displayed apparently a blue shift of about 60 nm, indicating the covalent formation of the polyhydric polymer-based fluorescent probes. Remarkably, once the selective interaction between the PVA-Pyr probes and Fe$^{3+}$ ions took place, a significant change in the absorption spectra was observed showing a new broad band over the region in which the original pyrene absorption bands were emerged together (curve d). Of note, the Pyr fluorophore could display the poor aqueous solubility, as apparently manifested in the photographs with the amplified view (Fig. 4B, insert). In contrast, greatly improved aqueous solubility was obtained for the PVA-Pyr probes because of the introduction of hydrophilic PVA chains, so that the detection of Fe$^{3+}$ ions in water media could be expected.
Furthermore, scanning electronic microscopy (SEM) images were performed to comparably investigate the topological structures of the PVA-Pyr probes in the absence and presence of Fe³⁺ ions (Fig. 5). Again, it was found that the PVA-Pyr probes could be well mono-dispersed in the aqueous media (Fig. 5A), resulting from the carriers of hydrophilic PVA chains. Fig. 5B manifests that the addition of Fe³⁺ ions could cause the polyhydric polymer-functionalized fluorescent probes to largely aggregate through the PVA-Fe³⁺ interaction as mentioned in Fig. 1B, which should be responsible for the quenching of their fluorescence intensities.

3.2. Selective responses of polyhydric polymer-functionalized fluorescent probes to Fe³⁺ ions

The selective responses of the PVA-Pyr probes to Fe³⁺ ions were assessed with the fluorescence quenching efficiencies, in comparison to those of 22 kinds of common ions that might possibly co-exist (Fig. 6). As shown in Fig. 6A, the fluorescence of PVA-Pyr probes could be obviously quenched by Fe³⁺ ions, whereas no significant change in the fluorescence intensity of the probes occurred for other kinds of ions alone tested separately under the same conditions, as more clearly manifested in the corresponding photographs (insert). Furthermore, the polyhydric polymer-functionalized fluorescent probes could present basically the same responses to Fe³⁺ ions in the separately competitive co-existence of the tested ions (Fig. 6B). The data imply that the
prepared PVA-Pyr probes could possess the considerably high selectivity in sensing Fe\(^{3+}\) ions, with no significant interferences from these co-existing ions.

3.3. Optimization of the fluorimetric conditions for Fe\(^{3+}\) analysis

The test strips-based fluorimetric method with PVA-Pyr probes was employed to detect Fe\(^{3+}\) ions, where the test solutions containing Fe\(^{3+}\) ions would act as the media for the Fe\(^{3+}\)-probe reactions on the test strips resulting in the fluorescence quenching of the probes. The main conditions of the developed fluorimetric method for Fe\(^{3+}\) ions were explored (Fig. 7). Fig. 7A shows the quenching efficiencies of fluorimetric test strips depending on the concentrations of probes, indicating that 5.00 mg L\(^{-1}\) of the probes was the most suitable one to be chosen for the fluorimetric experiments. Moreover, it was surprisingly observed that the developed fluorimetric test strips could allow for the

![Fluorescence spectra of PVA-Pyr probes (1.00 mg L\(^{-1}\)) in the presence of different concentrations of Fe\(^{3+}\) ions spiked in wastewater samples (0 – 200 μM); (B) the relationship between the fluorescence quenching efficiencies versus the concentrations of Fe\(^{3+}\) ions; (C) fluorescence spectra of the test strips-based fluorimetric responses to Fe\(^{3+}\) ions of different concentrations spiked in wastewater samples (0–300 μM) (insert: the corresponding photographs of the developed test strips for Fe\(^{3+}\) ions under UV light as the standard colorimetric panel); (D) the relationship between the quenching efficiencies versus the concentrations of Fe\(^{3+}\) ions in wastewater samples, where the solid-state fluorescent intensities of the fluorescent test strips were measured at λ\(_{em}\)=392 nm.

Table 1
Comparison of analysis performances of the developed fluorimetric method with polymeric probes with the common analysis methods with other fluorescent probes for the determination of Fe\(^{3+}\) ions.

<table>
<thead>
<tr>
<th>Fluorescent probe</th>
<th>Detection limit (μM)</th>
<th>Linear range (μM)</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graphene Qdots</td>
<td>7.22</td>
<td>0–80</td>
<td>[8]</td>
</tr>
<tr>
<td>Benzimidazolium probes</td>
<td>2.80</td>
<td>350–2700</td>
<td>[9]</td>
</tr>
<tr>
<td>Au nanoclusters</td>
<td>3.50</td>
<td>5.0–1280</td>
<td>[10]</td>
</tr>
<tr>
<td>Metal-organic framework</td>
<td>0.90</td>
<td>3.0–200</td>
<td>[11]</td>
</tr>
<tr>
<td>Polymeric probes</td>
<td>0.73</td>
<td>5.0–300</td>
<td>This work</td>
</tr>
</tbody>
</table>

Table 2
Comparison of analysis results for Fe\(^{3+}\) ions in the wastewater and river water samples with ICP-MS analysis methods (n=5, confidence limit=95%).

<table>
<thead>
<tr>
<th>Sample numbers</th>
<th>Fe(^{3+}) ions in wastewater</th>
<th>Fe(^{3+}) ions in river water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This method (μM)</td>
<td>ICP-MS (μM)</td>
</tr>
<tr>
<td>1</td>
<td>73.2 ± 6.8</td>
<td>74.7 ± 4.6</td>
</tr>
<tr>
<td>2</td>
<td>102.5 ± 9.7</td>
<td>101.2 ± 7.3</td>
</tr>
<tr>
<td>3</td>
<td>152.9 ± 11.3</td>
<td>149.3 ± 8.9</td>
</tr>
</tbody>
</table>

Table 3
Analysis results of the developed fluorimetric method applied for detecting Fe\(^{3+}\) ions separately in seawater, drinking water, and rainwater samples (n=5).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fe(^{3+}) concentrations</th>
<th>Recoveries (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added (μM)</td>
<td>Found (μM)</td>
</tr>
<tr>
<td>Seawater</td>
<td>20.0</td>
<td>19.1 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>103.9 ± 9.7</td>
</tr>
<tr>
<td>Drinking water</td>
<td>20.0</td>
<td>21.4 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>104.7 ± 7.2</td>
</tr>
<tr>
<td>Rainwater</td>
<td>20.0</td>
<td>20.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>98.3 ± 5.3</td>
</tr>
</tbody>
</table>

prepared PVA-Pyr probes could possess the considerably high selectivity in sensing Fe\(^{3+}\) ions, with no significant interferences from these co-existing ions.

3.3. Optimization of the fluorimetric conditions for Fe\(^{3+}\) analysis

The test strips-based fluorimetric method with PVA-Pyr probes was employed to detect Fe\(^{3+}\) ions, where the test solutions containing Fe\(^{3+}\) ions would act as the media for the Fe\(^{3+}\)-probe reactions on the test strips resulting in the fluorescence quenching of the probes. The main conditions of the developed fluorimetric method for Fe\(^{3+}\) ions were explored (Fig. 7). Fig. 7A shows the quenching efficiencies of fluorimetric test strips depending on the concentrations of probes, indicating that 5.00 mg L\(^{-1}\) of the probes was the most suitable one to be chosen for the fluorimetric experiments. Moreover, it was surprisingly observed that the developed fluorimetric test strips could allow for the
Fe$^{3+}$ detections in the pH range from 2.0 to 12, showing almost invariable.

quenching efficiencies of probe fluorescence (Fig. 7B). Herein, no significant precipitation of Fe(OH)$_3$ would be formed even at pH 12, presumably due to the concentrations of Fe$^{3+}$ ions tested were too low to attain the solubility product of the precipitation. Also, no significant effect on the fluorimetric Fe$^{3+}$ analysis was observed for the ionic strengths of NaCl concentrations up to 150 mM (Fig. 7C). In addition, the response time of the test strips-based fluorimetric responses to Fe$^{3+}$ ions was evaluated (Fig. 7D). It was noted that the fluorescence intensities of the probes on test strips could tend to be stable within 3 min, so that the probe aggregation could be completed. These data indicate that the developed test strips-based fluorimetric method with the PVA-Pyr probes could facilitate the rapid detection of Fe$^{3+}$ ions under the widely applicable conditions.

3.4. Calibration curves of fluorimetric analysis for Fe$^{3+}$ ions in wastewater samples

Under the optimized conditions, the fluorimetric analysis with PVA-Pyr probes were firstly performed for detecting Fe$^{3+}$ ions spiked in wastewater using centrifuge tubes (Fig. 8A). One can observe that the fluorescence intensities of PVA-Pyr probes could reasonably decrease as Fe$^{3+}$ concentrations increased, as also exhibited in the photographs of the corresponding reaction products in centrifuge tubes. A calibration curve was thus obtained with the fluorescence quenching efficiencies versus different Fe$^{3+}$ concentrations (Fig. 8B). Accordingly, Fe$^{3+}$ ions in wastewater could be detected over the linear concentrations ranging from 1.00 μM to 200 μM (R$^2$=0.9993), with the limit of detection of 0.33 μM, estimated by the 3σ rule. Furthermore, the fluorimetric strategy with test strips was employed for the analysis of Fe$^{3+}$ ions spiked in wastewater by the dipping way, with the corresponding fluorimetric spectra shown in Fig. 8C. A relationship between the quenching efficiencies and Fe$^{3+}$ concentrations was attained showing the linear Fe$^{3+}$ concentrations ranging from 5.00 to 300 μM (R$^2$–0.9896), with a limit of detection of about 0.73 μM (Fig. 8D). Accordingly, the analysis results of the test strips-based fluorimetric method are comparable to those of the one using centrifuge tubes in term of detection sensitivity. Of note, the fluorimetric test strips could additionally enable the visual determination of Fe$^{3+}$ ions simply by the comparison of the tested strips with the standard colorimetric panel under UV light (Fig. 8C, insert).

Furthermore, the analysis performances of the developed test strips-based fluorimetric method were compared with those of some fluorimetric methods with other fluorescent probes (Table 1). It could be seen that the present fluorimetric strategy could exhibit better or comparable performances for the Fe$^{3+}$ detection in terms of linear concentration range and detection limit.

3.5. Evaluation of practical applications of the test strips-based fluorimetric method

To explore the feasibility of practical applications, the test strips-based fluorimetric method was employed to probe Fe$^{3+}$ ions separately in wastewater and river water samples (Table 2). The results were compared with those of ICP-MS using a t-test at 95% confidence limits, showing no significant difference (P > 0.05).

Moreover, the test strips-based fluorimetric method was employed to probe Fe$^{3+}$ ions separately in seawater, drinking water, and rainwater samples, with the results summarized in Table 3. After the additions of a known quantity of Fe$^{3+}$ ions into the testing solutions, the measured concentrations of Fe$^{3+}$ ions are in well consistent with the added ones. The recoveries so calculated are ranged from about 95.5–107.0%, indicating that the developed test strips-based fluorimetric method is feasible for detecting Fe$^{3+}$ ions in various environmental media, including for the finding of the potential iron mines.

4. Conclusions

In summary, a polyhydric polymer-functionalized fluorescent probe was successfully synthesized by covalently loading Pyr fluorophore onto the PVA chains using a facile one-step acetal reaction. The resulted PVA-Pyr probes were further coated onto the test strips towards a rapid and visual fluorimetric analysis method for Fe$^{3+}$ ions by taking advantage of the unique film-forming ability of PVA. The polyhydric PVA polymer chains can facilitate the Pyr fluorophore with dramatically improved aqueous solubility and strong cyan fluorescence, which could be specifically quenched by Fe$^{3+}$ ions through the PVA-Pyr interaction by triggering the probe aggregation. Moreover, the test strips-based fluorimetric method can enjoy the stable and uniform distribution of the polymeric probes on test strips. Particularly, it may promise the in-field applications for the onsite monitoring of Fe$^{3+}$ ions in environmental water samples including the outdoor finding of potential iron mines. Such a facile and effective probe fabrication route with polyhydric polymers may open a new door toward the design of a variety of organic fluorescent probes with the improved aqueous solubility and selective recognition for targeting ions.

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