



# Sensitive and selective detection of nitrite ion based on fluorescence superquenching of conjugated polyelectrolyte

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## ABSTRACT

In recent years, conjugated polyelectrolytes (CPEs) that feature good water-solubility have drawn great attention as optical transducers in high sensitive bio- and chemo-sensors due to their predominant optical/electronic properties and remarkable signal amplification. Herein, a sensitive and selective assay for nitrite ion has successfully been developed based on the fluorescence superquenching of an anionic CPE, PPESO<sub>3</sub>. With the sensor format composed of PPESO<sub>3</sub> and H<sup>+</sup>, Fe<sup>2+</sup> can easily be oxidized into Fe<sup>3+</sup> in the presence of NO<sub>2</sub><sup>-</sup>, and the later dramatically quenches the fluorescence of PPESO<sub>3</sub>. Indeed, the inclusion of conjugated polyelectrolyte into the sensory scheme can give rise to a notable enhancement of fluorescence response, which endows the newly proposed NO<sub>2</sub><sup>-</sup> probe with high sensitivity. Thus, nitrite ion within a relatively wide concentration range (0–70 μM) can be determined in a rather simple and sensitive manner with a detection limit of 0.62 μM (~28 ppb). Additionally, most other anions such as halogen ions, acetate, sulfate, carbonate, phosphate and even nitrate, show minor interference on the NO<sub>2</sub><sup>-</sup> detection.

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## 1. Introduction

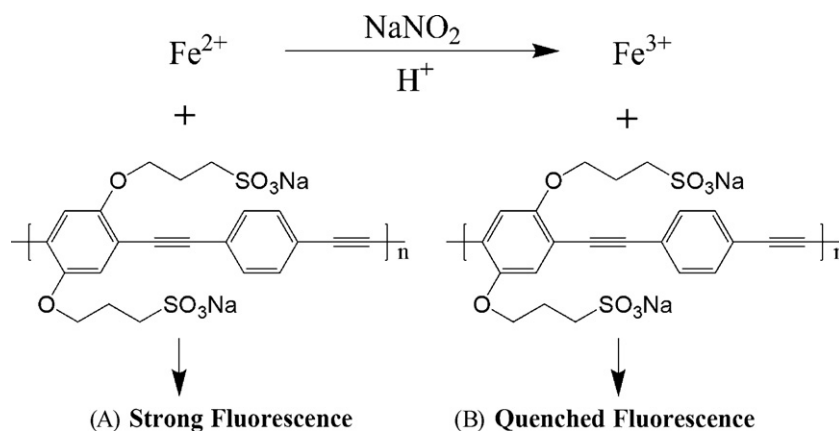
Quantitative determination of nitrite ion is of rapidly increasing interest in virtue of its great significance in many fields. Except for wide applications as additive and preservative in food industry, nitrite ion that serves as a critical intermediate in the nitrogen cycle can also be derived from many other sources, such as biological denitrification, fertilizers degradation, acid rain, industrial waste, and so on. Therefore, it is very important to monitor the content level of nitrite ion for the environmental reasons, as well as for public health, since its toxicity to induce methemoglobinemia and to form the highly carcinogenic N-nitrosoamines through the reactions with various amines and amides [1,2]. In parallel, nitrite ion is also of interest as one of the NO metabolites in biological systems and has recently been examined as a surrogate for in vivo NO, an important signaling compound in many physiological events, including neurotransmission, vasodilatation, inflammation, cell proliferation, and apoptosis [3,4]. To date, there have existed various methods for the detection of nitrite ion, and most important are spectrophotometry, cadmium reduction flow injection analy-

sis (Cd-FIA), ion chromatography (IC), ion selective electrode (ISE), capillary zone electrophoresis (CZE), and so forth [3,5–7]. However, limitations associated with these methods can be summarized as interference from both cationic and anionic ions, high detection limits, narrow dynamic ranges, and poor separation efficiencies [8]. Thus, there is still an urgent demand for simple, sensitive and specific assays for nitrite ion.

In recent years, conjugated polymers (CPs) that possess many prominent optical and electronic properties have attracted considerable attentions as optical transducers in high sensitive bio- and chemo-sensors. In comparison to typical fluorescent dyes, conjugated polymers consist of a large number of repeated absorbing units, and the unique π–π\* conjugated electronic structure of CPs allows for rapid migration of excitation along the whole backbone to energy/electron receptors, which results in remarkable amplification of optical signal through a collective response [9]. Undoubtedly, these advantages will endow the bio- and chemo-sensors based on CPs with high sensitivity. Thus, extensive efforts have recently been focused on the development of functional CPs and CPs-based sensors [10–13]. In particular, conjugated polyelectrolytes (CPEs) that take on ionic pendant groups have proven more favored in terms of their good water-solubility, as well as the integration of the outstanding quality of CPs with the behavior of polyelectrolytes. Thus far, a great variety of CPEs with diverse skeletons and pendant groups, e.g. sulfonate [14,15], phosphonate [16], carboxylate [17–19] and quaternary ammonium [20,21], have

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**Scheme 1.** Schematic illustration of the proposed nitrite ion assay.

already been developed and of special interest in fluorescent sensors to metal ions [22,23], saccharides [24,25], proteins [26–28], enzymes [29–31], DNA [32–35], and RNA [36,37].

In our previous work, an anionic water-soluble conjugated polyelectrolyte that bears sulfonated pendant groups, PPESO<sub>3</sub> (see Scheme 1 for chemical structure) [14], has successfully been prepared through a facile synthetic route [15]. And further investigations on the interaction between PPESO<sub>3</sub> and metal ions have also been carried out, with the original intention to evaluate the interference of metal ions when using PPESO<sub>3</sub> as sensing material. It is very interesting to find that Fe<sup>2+</sup> and Fe<sup>3+</sup> as a redox pair show different quenching effects on PPESO<sub>3</sub>, i.e., Fe<sup>3+</sup> (10 μM) can efficiently superquench the fluorescence of PPESO<sub>3</sub>, while Fe<sup>2+</sup> at the same concentration causes nearly no change. This is definitely an attractive phenomenon in view of signal transduction, which implies that a general sensing platform could be constructed based on a combination of the redox of Fe<sup>2+</sup>/Fe<sup>3+</sup> with the amplified fluorescence quenching of PPESO<sub>3</sub> [25]. In this work, a high sensitive and selective assay of nitrite ion has been demonstrated, through which nitrite ion within a relatively wide content range (0–70 μM) could easily be determined in a rather sensitive and specific manner, with a detection limit of 0.62 μM and minor interferences from most other anions, including halogen ions (X<sup>-</sup>), Ac<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, CO<sub>3</sub><sup>2-</sup>, PO<sub>4</sub><sup>3-</sup>, and even NO<sub>3</sub><sup>-</sup>.

## 2. Experimental

### 2.1. Materials and reagents

Conjugated polyelectrolyte PPESO<sub>3</sub> was synthesized according to a previous procedure reported by our group [15]. Sodium nitrite, ferric chloride and ferrous chloride were all purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sample NO<sub>2</sub><sup>-</sup> (100 mg/L according to the label) was obtained from Aladdin Reagent Inc. (Shanghai, China) and used by appropriate dilution. Without special mention, the rest chemicals, such as halogenides, acetate, sulfate, carbonate and nitrate, are all of analytical purity and used as received. DI water was prepared through a HFSuper-ROP Water Purification System (Heal Force Development Ltd., Hong Kong).

### 2.2. Methods for fluorescence measurements

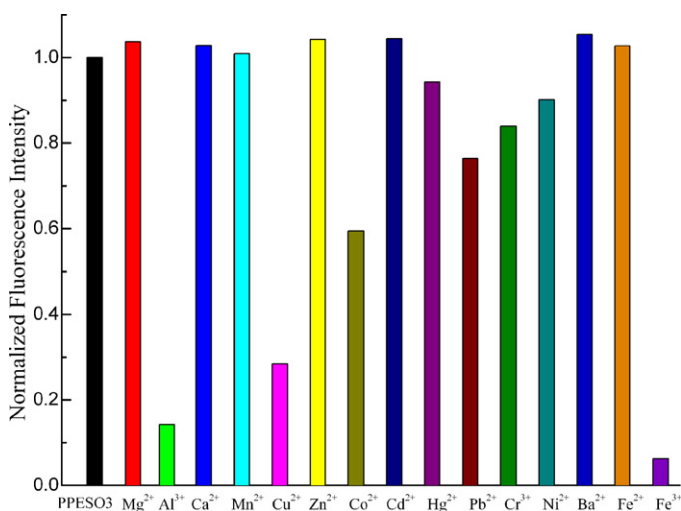
All fluorescence measurements were carried out in a 3-mL quartz cell at ambient temperature with a Shimadzu RF-5301 spectrometer. Unless otherwise specified, the excitation wavelength was 440 nm and the “fluorescence intensity” referred to the maximum emission of PPESO<sub>3</sub> at 530 nm.

For the assay of nitrite ion, the conjugated polyelectrolyte PPESO<sub>3</sub> was firstly diluted to 1 μM with water, followed by the addition of 20 μM Fe<sup>2+</sup> and 1 mM HCl. The resulting solution (3 mL) was kept at room temperature for 10 min. Then, various amounts of the stock solution of NO<sub>2</sub><sup>-</sup> (0.6 or 6.0 mM) were added, with the ultimate concentration of NO<sub>2</sub><sup>-</sup> covered a relatively wide range (0–70 μM). And for each NO<sub>2</sub><sup>-</sup> content, the fluorescence spectra were recorded every 5 min in a period of 30 min.

## 3. Results and discussion

### 3.1. Effects of metal ions on PPESO<sub>3</sub>

With the original intention to evaluate the effects of metal ions when using PPESO<sub>3</sub> as sensing material, the interactions between PPESO<sub>3</sub> and metal ions in aqueous solution have been examined in detail. As seen in Fig. 1, most of the metal cations (10 μM) show minor influence on the fluorescence of PPESO<sub>3</sub>, with the exception of Al<sup>3+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup> and Fe<sup>3+</sup> which quench the fluorescence notably. And it is worthy to note that these quenching effects could be remarkably minimized by the reduction of the concentration of metal ions (data not shown). Thus, the influence of metal ions on PPESO<sub>3</sub> might sometimes be negligible, especially for biological samples, since in these cases they are usually of trace amount at physiological level [38]. More interestingly, it is also noticed that Fe<sup>2+</sup> and Fe<sup>3+</sup> as a redox pair show different quenching effects on



**Fig. 1.** Effects of metal ions (10 μM) on the fluorescence of PPESO<sub>3</sub> in aqueous solution.

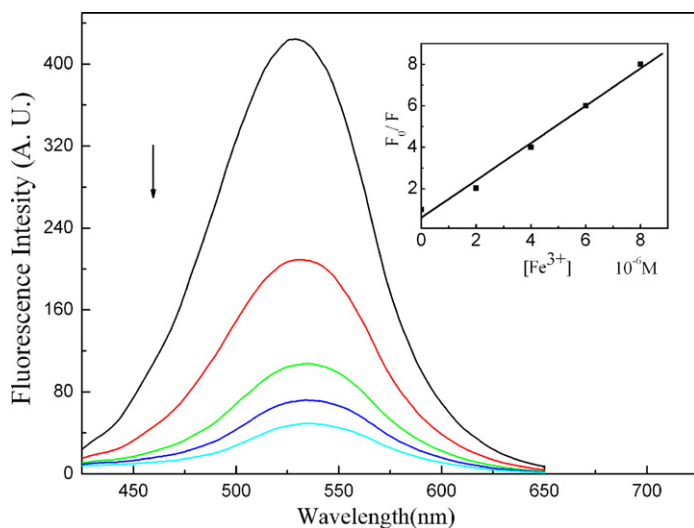


Fig. 2. Evolution of the fluorescence spectra of PPESO<sub>3</sub> (1 μM) upon adding Fe<sup>3+</sup>.

PPESO<sub>3</sub>. As shown in Fig. 1, Fe<sup>3+</sup> (10 μM) can effectively quench the fluorescence of PPESO<sub>3</sub>, while Fe<sup>2+</sup> at the same concentration causes nearly no change. One possible explanation has recently been ascribed to the fact that Fe<sup>3+</sup> can serve as an electron transfer (ET) center more effectively when comparing to Fe<sup>2+</sup> [25]. Anyway, it is definitely an attractive phenomenon that shows great potential as signal transducer in high sensitive bio- and chemo-sensors. For instance, a general sensing platform could easily be established for the detection of certain oxidants or reductants, as well as their related substrates. Herein, a sensitive and specific assay of nitrite ion has successfully been demonstrated.

### 3.2. Fluorescence superquenching of PPESO<sub>3</sub> caused by Fe<sup>3+</sup>

In aqueous solution, PPESO<sub>3</sub> exhibits a strong green emission at 530 nm, which is now believed to be quenched dramatically by Fe<sup>3+</sup> through electron transfer. And the efficiency of fluorescence quenching can be quantitatively evaluated by the Stern–Volmer equation [39].

$$F_0/F = 1 + K_{SV}[Q] \quad (1)$$

where  $F$  and  $F_0$  are the fluorescence intensity with and without the quencher, respectively,  $[Q]$  is the quencher concentration, and  $K_{SV}$  represents the Stern–Volmer constant. As shown in Fig. 2, the fluorescence of PPESO<sub>3</sub> can markedly be quenched by Fe<sup>3+</sup> even with very low concentration, and the linear region provides a  $K_{SV}$  value of  $9.8 \times 10^5 \text{ M}^{-1}$ , which is found to be 114-fold greater than that obtained from its small molecular counterpart [14,25]. Undoubtedly, this amplified fluorescence quenching is mainly derived from the intrinsic molecular-wire effects of conjugated polymer, through which a collective response of multiple repeat units on PPESO<sub>3</sub> can occur and give rise to remarkable amplification of the optical signal [9]. In addition, the aggregation of CPEs in aqueous solution that improves the inter-chain exchanges, as well as the electrostatic interaction between metallic cations and anionic PPESO<sub>3</sub>, can both contribute to the quenching efficiency in this case [40,41]. Thus, the assays that take advantage of the Fe<sup>3+</sup> induced fluorescence “superquenching” of conjugated polyelectrolyte PPESO<sub>3</sub> will intrinsically exhibit high sensitivity.

### 3.3. Assay for nitrite ion

The newly proposed nitrite ion assay is illustrated in Scheme 1. Conjugated polyelectrolyte PPESO<sub>3</sub> is used as signal reporter, which

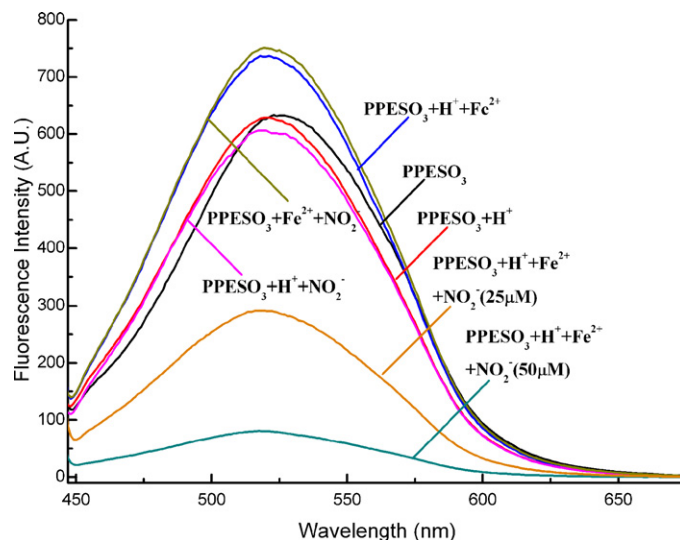


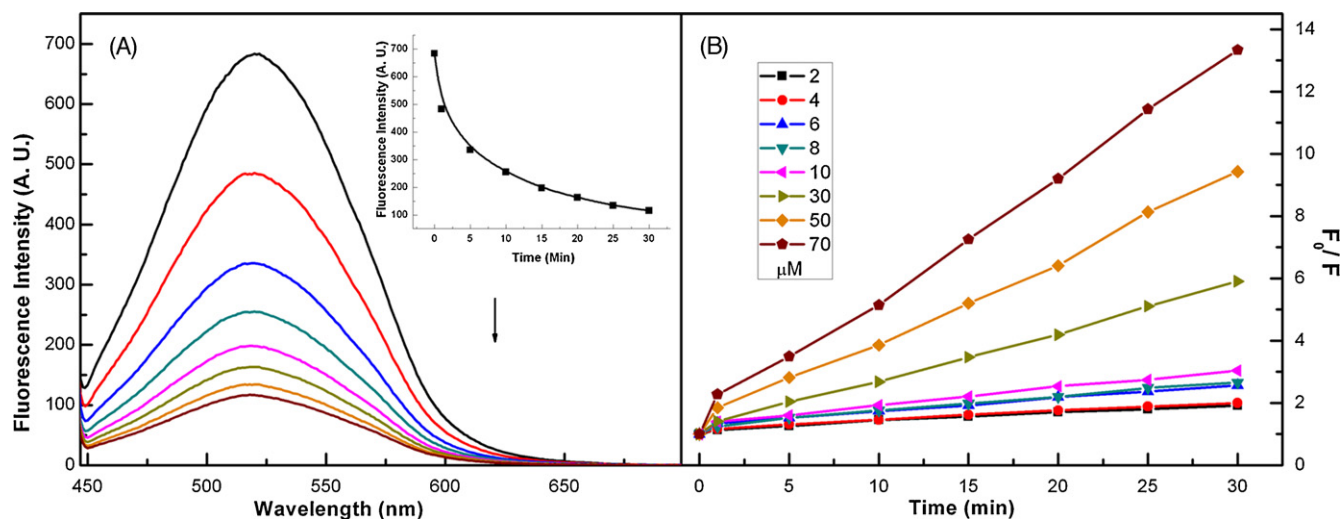
Fig. 3. Emission spectra of PPESO<sub>3</sub>, PPESO<sub>3</sub>-H<sup>+</sup> and PPESO<sub>3</sub>-Fe<sup>2+</sup> in the absence and presence of NO<sub>2</sub><sup>-</sup>. ([PPESO<sub>3</sub>] = 1 μM, [H<sup>+</sup>] = 1 mM and [Fe<sup>2+</sup>] = 20 μM).

emits strong fluorescence in the presence of H<sup>+</sup> and Fe<sup>2+</sup> (Situation A). However, once NO<sub>2</sub><sup>-</sup> is present in the system, Fe<sup>2+</sup> will be oxidized into the form of Fe<sup>3+</sup> rapidly, and the later can efficiently quench the fluorescence of PPESO<sub>3</sub> (Situation B). Thereupon, the quantification of nitrite ion can easily be achieved by measuring the fluorescence superquenching of PPESO<sub>3</sub>.

In order to testify the sensing scheme we proposed, several control experiments have been carried out. As seen in Fig. 3, neither H<sup>+</sup> nor Fe<sup>2+</sup> can quench the fluorescence of PPESO<sub>3</sub> separately or in a combination of the two species. However, upon sequential addition of H<sup>+</sup>, Fe<sup>2+</sup>, and NO<sub>2</sub><sup>-</sup> into an aqueous solution of PPESO<sub>3</sub>, fluorescence superquenching was observed. And it is also noticed that the diminished emission is highly dependent on the concentration of NO<sub>2</sub><sup>-</sup>, which to a large extent affords the feasibility to sense nitrite ion. Otherwise, the addition of NO<sub>2</sub><sup>-</sup> did not result in any fluorescence decrease without either Fe<sup>2+</sup> or H<sup>+</sup>. These results indicate that no oxidation of PPESO<sub>3</sub> can be induced by HNO<sub>2</sub>. And more importantly, NO<sub>2</sub><sup>-</sup> exhibits high oxidizability only in the acidic environment, which is thus very helpful to distinguish it from other oxidants in the same system.

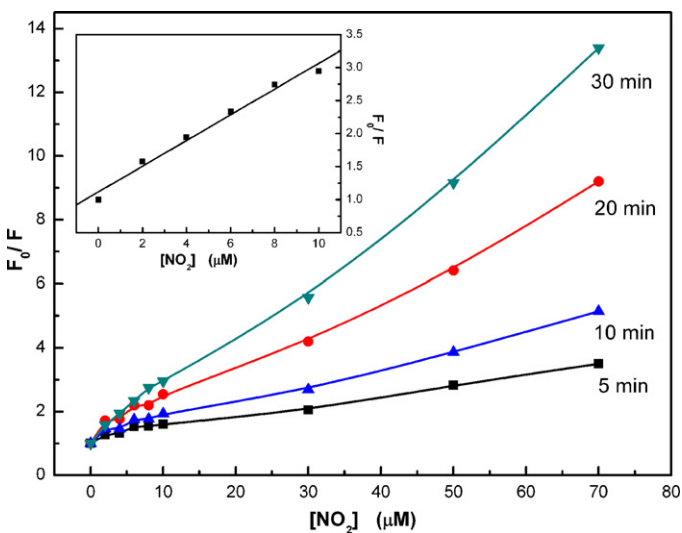
Fig. 4A displays the spectral changes of an aqueous solution containing PPESO<sub>3</sub>, H<sup>+</sup> and Fe<sup>2+</sup> with time after addition of NO<sub>2</sub><sup>-</sup> (30 μM). It reveals that the fluorescence of PPESO<sub>3</sub> is decreased more sharply at the initial stage, and then gradually levels off at about 30 min. Moreover, similar trends have also been observed for the rest contents of NO<sub>2</sub><sup>-</sup> ranging from zero to 70 μM. As a matter of fact, this emission profile not only depends on the oxidation rate of Fe<sup>2+</sup>, but also accords well with the process of static superquenching as depicted with the Stern–Volmer equation. Nevertheless, these results did not announce the necessity of a long time incubation (e.g. 30 min) for the assay of nitrite ion. As shown in Fig. 4B, from the very beginning there exists notable difference in the quenching efficiency in terms of the concentration of NO<sub>2</sub><sup>-</sup>, so that the quantification of nitrite ion can readily be achieved as long as the incubation time exceeds 5 min.

In Fig. 5, a series of representative quenching profiles for PPESO<sub>3</sub>-H<sup>+</sup>-Fe<sup>2+</sup> as functions of NO<sub>2</sub><sup>-</sup> contents are illustrated. These Stern–Volmer plots were established with various incubation times. As can be seen from this graph, even with the shortest period of incubation (5 min), the fluorescence quenching remains monotonously increased with the concentration of NO<sub>2</sub><sup>-</sup>, which is well consistent with the foregoing discussion and suggests a viable shortest time for the detection of nitrite ion. However, it is also

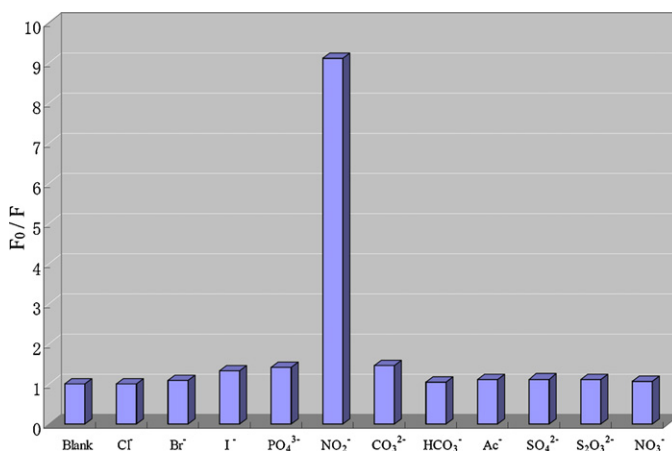


**Fig. 4.** (A) Time-dependent spectral changes of  $\text{PPESO}_3\text{-H}^+\text{-Fe}^{2+}$  upon addition of  $\text{NO}_2^-$  ( $30\ \mu\text{M}$ ). The inset highlights the emission intensity changes at  $530\ \text{nm}$ . (B) Time-dependent fluorescence quenching of  $\text{PPESO}_3\text{-H}^+\text{-Fe}^{2+}$  with various amounts of  $\text{NO}_2^-$ .

noticed that the assay sensitivity can be greatly improved through the adoption of a prolonged reaction time. As shown in Fig. 5, the quenching efficiency becomes nearly 4-fold higher once the incubation time is extended from 5 to 30 min. Plot in the inset highlights the quenching profile in the  $0\text{--}10\ \mu\text{M}$  range of  $\text{NO}_2^-$  with an incubation time of 30 min. It shows clearly a good linearity between the fluorescence quenching of  $\text{PPESO}_3$  and the content of  $\text{NO}_2^-$  with a correlation coefficient of 0.992. The limit of detection (LOD), defined as the minimum concentration corresponding to a signal above three times of standard deviation, is found to be  $0.62\ \mu\text{M}$  ( $\sim 28\ \text{ppb}$ ). And the relative standard deviation (RSD) for five parallel measurements of the same standard solution ( $5\ \mu\text{M}$ ) is only 4.2%, which implies the rational reproducibility of this method. Moreover, the recovery tests were conducted by adding three different amounts of  $\text{NO}_2^-$  to the sample solutions (Aladdin reagent) and each solution was repeated for three times. And the obtained results are well consistent with the labeled  $[\text{NO}_2^-]$ , with the mean recovery rate ranging from 98.3% to 108.6%. Evidently, these results are at least comparable with or even better than those obtained from most spectrometric methods [42,43]. In addition, it is worthy



**Fig. 5.** Stern–Volmer plots of  $\text{PPESO}_3\text{-H}^+\text{-Fe}^{2+}$  caused by  $\text{NO}_2^-$  with various incubation time (inset: scale-up of the  $0\text{--}10\ \mu\text{M}$  range with an incubation time of 30 min,  $R = 0.992$ ).  $[\text{PPESO}_3] = 1\ \mu\text{M}$ ,  $[\text{H}^+] = 1\ \text{mM}$  and  $[\text{Fe}^{2+}] = 20\ \mu\text{M}$ .



**Fig. 6.** Fluorescence quenching of  $\text{PPESO}_3\text{-H}^+\text{-Fe}^{2+}$  upon addition of  $\text{NO}_2^-$  and other anions ( $50\ \mu\text{M}$ ); the counterions are either  $\text{Na}^+$  or  $\text{K}^+$  (KI);  $[\text{PPESO}_3] = 1\ \mu\text{M}$ ,  $[\text{H}^+] = 1\ \text{mM}$  and  $[\text{Fe}^{2+}] = 20\ \mu\text{M}$ .

to note that the newly proposed  $\text{NO}_2^-$  sensor features a relatively wide dynamic range of  $0\text{--}70\ \mu\text{M}$ .

To examine the selectivity of the newly proposed sensing system, a variety of common anions have been investigated under identical conditions. Fig. 6 illustrates the fluorescence quenching of  $\text{PPESO}_3\text{-H}^+\text{-Fe}^{2+}$  upon the addition of  $\text{NO}_2^-$  and the other 10 anions ( $50\ \mu\text{M}$ ), including  $\text{X}^-$ ,  $\text{Ac}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{CO}_3^{2-}$ ,  $\text{PO}_4^{3-}$ , and  $\text{NO}_3^-$ . As can be seen, most of these species imposed nearly no influence on the emission of  $\text{PPESO}_3$ , with the exception that  $\text{I}^-$ ,  $\text{PO}_4^{3-}$  and  $\text{CO}_3^{2-}$  induced some observable quenching. However, these fluorescence decreases are all less than 5% relative to that caused by  $\text{NO}_2^-$ . Thus, the minor interference from these common anions reveals clearly the high specificity of the  $\text{PPESO}_3\text{-H}^+\text{-Fe}^{2+}$  system toward nitrite ion.

#### 4. Conclusion

In this work, a highly sensitive and selective assay for nitrite ion has successfully been developed. By taking advantage of the different effects that  $\text{Fe}^{2+}/\text{Fe}^{3+}$  imposed on an anionic CPE and the subsequent fluorescence superquenching, nitrite ion can be determined in a rather simple and sensitive manner, with a detection limit of  $0.62\ \mu\text{M}$  ( $\sim 28\ \text{ppb}$ ) and a relatively wide dynamic range

of 0–70  $\mu\text{M}$ . In addition, the minor interference from most concomitant anions, such as halogen ions, acetate, sulfate, carbonate, and even nitrate, demonstrates the high specificity of the newly proposed sensing system toward  $\text{NO}_2^-$ .

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