A Sensitive Fluorescence Quenching Method for the Determination of Iron(II) with 1,10-Phenanthroline

ZHU, Guang-Hua(朱广华)   JU, Huang-Xian*(鞠熀先)   YE, Bao-Fen(叶宝芬)
Department of Chemistry, State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing, Jiangsu 210093, China

A sensitive and selective fluorescence quenching method for the determination of Fe^{2+} with 1,10-phenanthroline was developed. The fluorescence intensity of 1,10-phenanthroline at λ_{ex} of 266 nm and λ_{em} of 365 nm was constant in the range of pH 4.0 to 10.0 and decreased linearly upon addition of Fe^{2+} to its solution. This decrease was mainly due to a static quenching effect caused by the formation of a non-fluorescent complex of Fe^{2+} with 1,10-phenanthroline. The total amount of iron was determined by using hydroxylamine hydrochloride to reduce Fe^{3+} to Fe^{2+}. The linear range was from 5.0×10^{-7} to 2.0×10^{-5} mol/L with a detection limit of 2.4×10^{-8} mol/L at 3σ. The quenching constant of Fe^{2+} to 1,10-phenanthroline was calculated to be $(5.70 \pm 0.05) \times 10^4$ L/mol at 25 ℃. Effects of foreign ions on the determination of Fe^{2+} were investigated. The results of the new method for the determination of iron in tap water and natural water samples were in good agreement with those obtained by graphite atomic absorption spectrometry.

Keywords   fluorescence quenching, 1,10-phenanthroline, ferrous ion

Introduction

Fluorimetric technique has been widely applied in the determination of metal ions. Some fluorimetric methods are based on quenching phenomena by metal ions. Basically, the quenching process can be divided into two kinds of mechanism: static and dynamic quenching.[1] The static quenching mechanism involves the interaction between the fluorophore molecule and the metal ion, which takes place in the ground state of the fluorophore molecule and forms a non-fluorescent complex. The quenching efficiency is governed by the formation constant of the complex as well as by the concentration of metal ion. In dynamic quenching, the fluorophore molecule reacts with metal ion during the lifetime of the excited state of the fluorophore molecule. The quenching efficiency depends on the viscosity of the solution, the lifetime of the excited state of the fluorophore and the concentration of the metal ion quencher. Determinations of many kinds of metal ions, such as Co^{2+},[2] Cr^{2+},[3,5] Cu^{2+},[6,8] $Mo(VI)$[9,10] and $V(V)$,[11] inorganic anions,[12] organic[13,14] and biological materials[15] based on fluorescence quenching technique have been reported.

Iron is one of the most common elements. It plays a central role in a wide range of proteins[16] and is a significant factor in the evaluation of water quality. Spectrophtometry is one of the widely used methods for the determination of iron.[17,18] However, its sensitivity is poor. Other methods, such as inductively coupled plasma atomic emission method,[19] capillary zone electrophoresis[20] and chemiluminescence method,[21] still do not satisfy all requirements for routine analysis mainly due to their complicated procedures, time consumption or the need for expensive instruments. Mori et al.[22] used o-hydroxyhydroquinonephthalein as the fluorophore in the presence of Brij-58 to develop a method for the determination of Fe^{3+}. Cha and Park used both 2-pyridinecarbaldhyde-5-nitro-pyridylhydrazone and salicylic acid as fluorophore for this purpose.[23,24] Ragos et al.[25] presented a method to determine Fe^{3+} in bovine liver by using 4-hydroxyquinolion as fluorophore. Schulman proposed that aluminum 8-hydroxyquinoline can be used to measure clinically important ions, such as Fe^{3+}, Cu^{2+} and Co^{2+}.[26] However, no work on the determination of Fe^{2+} with a fluorescence quenching method has been proposed except one reported by Fink et al.,[27] in which they used 2,2',2''-terpyridyl to form a non-fluorescent complex with analyte Fe^{2+} with a detection limit of 7.5×10^{-7} mol/L and a linear range from 1.0×10^{-6} mol/L to 1.0×10^{-5} mol/L.

In this study, we used a novel fluorophore, 1,10-phenanthroline, to develop a more sensitive method for the determination of Fe^{2+} by the static fluorescence quenching of Fe^{2+} to 1,10-phenanthroline.[1] Under the optimum conditions the method could be used to detect Fe^{2+} in tap and mineral water samples with a detection limit of 2.4×10^{-8} mol/L. This method described here is sensitive, accurate and simple.

Experimental

Apparatus

A Perkin Elmer LS-50B spectrofluorimeter with a 150-W xenon lamp and a 1×1 cm^2 quartz cell was used for the spec-

tral recording and fluorescence measurements. Both of the excitation monochromator slit width and emission monochromator slit width were set to 10 nm. A Prekin-Elmer 3030 graphite atomic absorption spectrometer was used to determine Fe^{2+} in real samples for control.

Reagents

All reagents were of analytical reagent grade and used as received. 1.0×10^{-3} mol/L Fe^{2+} and 1.0×10^{-3} mol/L 1,10-phenanthroline stock solutions were prepared by dissolving $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ and 1,10-phenanthroline with water and 50% ethanol, respectively. Fe^{2+} (1.5×10^{-5} mol/L) and 1,10-phenanthroline (5.0×10^{-5} mol/L) working solutions were freshly prepared before experiments by diluting their stock solutions with water. Buffer solution (1.0 mol/L, pH 7.0) was obtained by dissolving ammonium acetate (77.1 g) with water and diluting to 1000 mL. Freshly double-distilled water was used throughout the experiments.

Analytical procedure

Fe^{2+} (1.0 mL) working solution, 1,10-phenanthroline (1.0 mL) working solution, buffer solution (2.0 mL, pH 7.0) and hydroxylamine hydrochloride (0.5 mL, 10%) were added sequentially to a 10 mL-standard volumetric flask. The mixed solution was heated up to 80 ℃ in water-bath for 10 min. After 20 min for cooling the solution down to room temperature (25 ℃) and diluting it to the mark with water, the fluorescence intensity was measured at λ_{ex} of 266 nm and λ_{em} of 365 nm against reagent blank.

Results and discussion

Spectral characteristics

Fig. 1 shows the fluorescence excitation and emission spectra of 1,10-phenanthroline. The excitation and emission peaks of the 1,10-phenanthroline appear at 266 and 365 nm, respectively. Upon addition of Fe^{2+} the fluorescence intensities of both excitation and emission spectra of 1,10-phenanthroline decrease. With an increasing Fe^{2+} concentration the decrease in fluorescence intensity increases, indicating the presence of fluorescence quenching due to the formation of Fe^{2+}-1,10-phenanthroline complex. It was also observed that the fluorescence intensity of 1,10-phenanthroline in the presence of Fe^{2+} fluctuates heavily (more than 10% of R.S.D.) when Fe^{2+} and 1,10-phenanthroline react at room temperature (25 ℃), while the intensity maintains at a constant value (less than 2% of R.S.D.) after the solution is heated to 80 ℃ in water-bath for 10 min and then cooled down to room temperature. The reaction rate between Fe^{2+} and 1,10-phenanthroline is relatively slow at room temperature and can be increased at higher temperature. On the other hand, the dissolved oxygen is removed from the Fe^{2+}-1,10-phenanthro-

line solution at higher temperature, which reduces the influence of dissolved oxygen on fluorescence quenching. Besides, as observed in Ref. 7, both fluorescence intensities of 1,10-phenanthroline solution alone and 1,10-phenanthroline solution in the presence of Fe^{2+} decrease slowly with time. However, the difference between these fluorescence intensities remains constant. Therefore, it is necessary to record the fluorescence spectra at a fixed time.
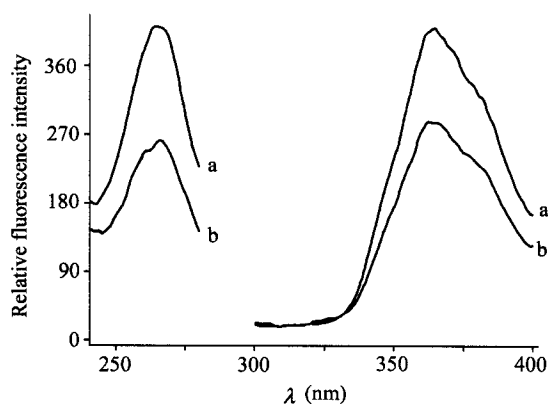

Fig. 1 Fluorescence excitation (left) and emission (right) spectra of 5.0 μmol/L 1,10-phenanthroline (a) and (a) + 0.75 μmol/L Fe^{2+} (b).

Effect of pH

Generally, the stability constant of a metal complex is dependent on pH of solution due to some by-reactions such as protonation of ligand and formation of hydroxide. Thus, the fluorescence quenching of fluorophore by metal ion depends on the pH of solution. In this work the effect of pH on the fluorescence intensity of 1,10-phenanthroline with Fe^{2+} ion solution is investigated in the solution containing 1.25 μmol/L Fe^{2+} and 5.0 μmol/L 1,10-phenanthroline. The relationship between the fluorescence intensity and pH is shown in Fig. 2. In the range of pH 4.0 to 10.0, the fluorescence intensity of 1,10-phenanthroline is almost constant. At pH less than 4.0, the free 1,10-phenanthroline is easily protonized, resulting in the decrease of the fluorescence intensity of 1,10-phenanthroline. Contrarily, when pH is higher than 10.0, the system shows a competition between the formation of Fe^{2+}-1,10-phenanthroline complex and ferrous hydroxide precipitation, which also results in a decrease of fluorescence intensity. Thus pH 7.0 is used for the following experiments.

1,10-Phenanthroline concentration

The degree of fluorescence quenching (ΔF) of 1,10-phenanthroline by Fe^{2+} is related to 1,10-phenanthroline concentration. A maximum fluorescence quenching magnitude appears in the range of 0.75—10 μmol/L 1,10-phenanthroline when the solution includes 1.5 μmol/L Fe^{2+}. ΔF obviously decreases when 1,10-phenanthroline concentration is lower than 0.75 μmol/L or higher than 10 μmol/L, which

means that the sensitivity to determine Fe^{2+} decreases. Thus, a 1,10-phenanthroline concentration of 5.0 μmol/L is chosen for this work.
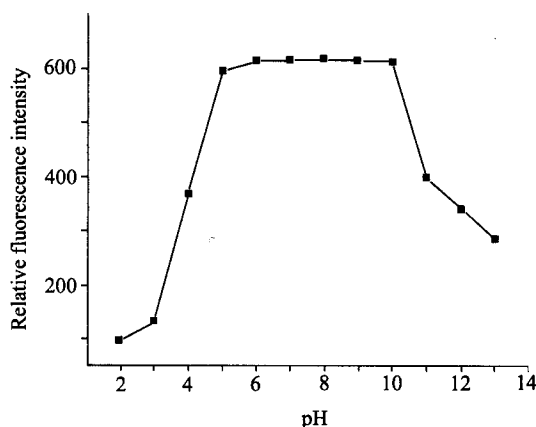

Fig. 2 Effect of pH on the fluorescence intensity of 5.0 μmol/L 1,10-phenanthroline solution in the presence of 1.25 μmol/L Fe^{2+}.

Interference

Many metal ions such as Al^{3+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Li^+, La^{3+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+} and Sr^{2+} exist in real sample solutions. Some of them can form complexes with 1,10-phenanthroline and affect the fluorescence quenching magnitude by Fe^{2+}. Some anions such as Br^-, Cl^-, CO_3^{2-}, F^-, NO_2^- and SCN^- also affect the fluorescence quenching magnitude due to the change of free Fe^{2+} concentration when they coexist in the solution. In order to observe the influences of these ions on the determination of Fe^{2+}, the fluorescence intensity of 1,10-phenanthroline is measured after adding different amount of these ions to the solution containing 0.50 μmol/L Fe^{2+} and 1.5 μmol/L 1,10-phenanthroline. The tolerance limit is described as the molar ratio of foreign ion to Fe^{2+} when the deviation of fluorescence intensity is less than 5%. The results are given in Table 1. It can be seen from Table 1 that Cu^{2+}, Co^{2+} and Ni^{2+} interfere with the determination of Fe^{2+}. Other cation and anion species examined in relatively high concentration do not interfere with the determination. Therefore, the method is of good selectivity.

Calibration curve

No change is observable upon addition of 0.5—20 μmol/L Fe^{3+} to 5.0 μmol/L 1,10-phenanthroline solution. In order to avoid the oxidation of Fe^{2+}, 0.5% hydroxylamine hydrochloride is added to this system for the determination of Fe^{2+}. The fluorescence intensity of 1,10-phenanthroline, measured at λ_{em} of 365 nm and λ_{ex} of 266 nm, decreases linearly with increasing Fe^{2+} concentration ranging from 0.5 μmol/L to 20 μmol/L. The linear regression equation is $y = 768 - 27.4x$ with a correlation coefficient of 0.9982 and a detection limit of 24 nmol/L at 3σ.

Table 1 Tolerance limits of interfering species for the determination of 0.5 μmol/L Fe^{2+} in 1.5 μmol/L 1,10-phenanthroline solution

Molar ratio[b]	Interfering species
1000	Al^{3+}, Ca^{2+}, Cr^{3+}, Zn^{2+}, Br^-, Cl^-, CO_3^{2-}, F^-, SCN^-
400	Cd^{2+}, La^{3+}, Mn^{2+}, Sr^{2+}, NO_2^-, SO_4^{2-}
200	Fe^{3+}, Li^+, Pb^{2+}
100	Mg^{2+}
10	Cu^{2+}
2	Co^{2+}, Ni^{2+}

[a] Tolerance limit is the maximum concentration in which there is less than 5% effect on fluorescence intensity. [b] Molar ratio of interfering species to Fe^{2+}.

Temperature effect

The quenching efficiency for a static quenching mechanism due to the formation of a non-fluorescent complex abides by the following equation:

$$F_0/F = 1 + K[Q] \tag{1}$$

where F_0 and F are the fluorescence intensities in the absence and presence of the quencher, respectively, K is the formation constant of non-fluorescent complex and $[Q]$ the concentration of the quencher. The plot of F_0/F versus $[Q]$ gives a straight line with a slope of K and a y-axis intercept of 1. In static quenching process, K is bigger at lower temperature than that at higher temperature.

Fig. 3 shows the plot of the Fe^{2+}-1,10-phenanthroline system at two temperatures. It can be seen that the plot of F_0/F versus $[Q]$ displays a straight line and the value of slope K is bigger at lower temperature.. Therefore it can be concluded that the quenching process of Fe^{2+} to 1,10-phenanthroline follows a static quenching mechanism and forms a non-fluorescent complex of Fe^{2+} with 1,10-phenanthroline.[28] The slope gives the quenching constant of Fe^{2+} to 1,10-phenanthroline to be $(5.70 \pm 0.05) \times 10^4$ and $(5.34 \pm$
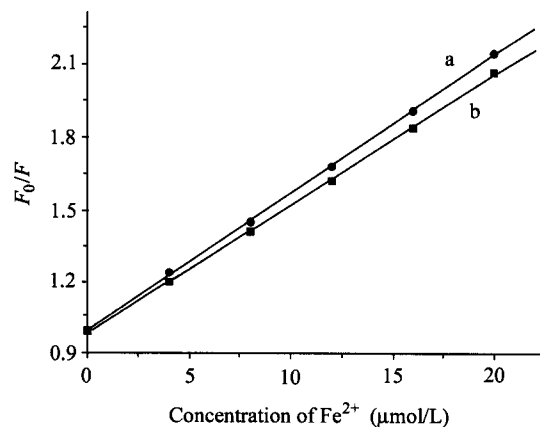

Fig. 3 Plots for the florescence quenching of 5.0 μmol/L 1,10-phenanthroline by 1.5 μmol/L Fe^{2+} at 25 ℃ (a) and 40 ℃ (b).

$0.06) \times 10^4$ L/mol at 25 and 40 °C, respectively.

Application

With the aid of hydroxylamine hydrochloride the method was applied to the determination of iron in tap water and natural water samples. Prior to determination the sample solutions were firstly filtered. The sample solution was analyzed for iron without a preconcentration process. The fluorescence intensity was measured at λ_{ex} of 266 nm and λ_{em} of 365 nm against reagent blank. The results are shown in Table 2. Obviously, the results obtained are in good agreement with those obtained by graphite atomic absorption spectrometry for comparison. Furthermore, this method shows an acceptable reproducibility.

Table 2   Determination of iron in tap water and natural water

Sample	This method ($\mu g/mL$)	Mean ($\mu g/mL$)	AAS ($\mu g/mL$)	Mean ($\mu g/mL$)
Tap water	0.12		0.13	
	0.12	0.12	0.13	0.13
Natural water	0.24		0.24	
	0.23	0.24	0.24	0.24

References

1   Schulman, S. G. *Molecular Luminescence Spectroscopy: Methods and Applications*, Part 1, Wiley, New York, **1985**, p. 11.

2   El-Daly, S. A. *Spectrochim. Acta, Part A* **1998**, *54*, 677.

3   Jie, N. Q.; Zhang, Q.; Yang, J. H.; Huang, X. R. *Talanta* **1998**, *46*, 215.

4   Jie, N. Q.; Yang, J. H.; Guo, J. *Anal. Lett.* **1992**, *25*, 1447.

5   Chen, T. S.; Chen, Z. G.; Wu, D. *Chin. J. Anal. Chem.* **1994**, *22*, 129 (in Chinese).

6   Manfred, A. K. *Anal. Chim. Acta* **1998**, *364*, 125.

7   Kim, H. S.; Choi, H. S. *Talanta* **2001**, *55*, 163.

8   May, R. *Anal. Chem.* **1961**, *33*, 1251.

9   Willard, H. H. *Anal. Chem.* **1952**, *24*, 862.

10   Hadelad, P. R. *Talanta* **1975**, *22*, 61.

11   Gao, J.; Zhang, X.; Zhao, B.; Yang, W.; Zhao, Y.; Teng, X. *Anal. Lett.* **1999**, *32*, 2127.

12   Jie, N.; Yang, Q. D.; Jang, Q.; Zhang, Q.; Wei, L. *Microchem. J.* **1999**, *62*, 371.

13   Ayala, J. H.; Afonso, A. M.; Gonzalez-Diaz, V. *Microchem. J.* **1998**, *60*, 101.

14   Hunt, A. L.; Alder, J. F. *Anal. Chim. Acta* **1999**, *387*, 207.

15   Ma, C. Q.; Li, K. A.; Tong, S. Y. *Anal. Chim. Acta* **1996**, *333*, 83.

16   Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, Wiley, New York, **1998**, p. 107.

17   Hill, A. G. *Analyst* **1978**, *103*, 391.

18   Mortatti, J.; Krug, F. J.; Pessenda, L. C. R.; Zagatto, E. A. G.; Jorgensen, S. S. *Analyst* **1982**, *107*, 659.

19   Shen, W. L.; Vela, N. P.; Sheppard, B. S.; Caruso, J. A. *Anal. Chem.* **1991**, *63*, 1491.

20   Pozdniakova, S.; Padarauskas, A.; Schwedt, G. *Anal. Chim. Acta* **1997**, *351*, 41.

21   Saitoh, K.; Hasebe, T.; Teshima, N. *Anal. Chim. Acta* **1998**, *376*, 247.

22   Mori, I.; Fujita, Y.; Nakashi, Y.; Kato, K.; Niwa, N. *Anal. Lett.* **1989**, *22*, 1969.

23   Cha, K. W.; Park, K. W. *Talanta* **1998**, *46*, 1567.

24   Cha, K. W.; Park, C. I. *Talanta* **1996**, *43*, 1335.

25   Ragos, G. C.; Demertzis, M. A.; Issopoulos, P. B. *Il Farmaco.* **1998**, *53*, 611.

26   Schulman, S. G. *Molecular Luminescence Spectroscopy: Methods and Applications*, Part 3, Wiley, New York, **1993**, p. 309.

27   Fink, D.; Pivnichny, J.; Ohnesorge, W. *Anal. Chem.* **1969**, *41*, 833.

28   Cha, K. W.; Park, K.; Korean, W. *J. Soc. Anal. Sci.* **1995**, *8*, 305.

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