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2-Amino-5,7-dimethyl-1,8-naphthyridine as a fluorescent reagent for the determination of nitrite

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Abstract

A new fluorescent reagent 2-amino-5,7-dimethyl-1,8-naphthyridine (ADMND) was proposed for the determination of trace nitrite. The reaction is based on the diazotization of naphthyridine amine with nitrite to form a diazonium salt that hydrolyzed when boiling to give hydroxyl group substituted naphthyridine. Fluorescence quenching degree of ADMND by nitrite ion is linear in the nitrite concentration range of 1×10^{-7} to 2.5×10^{-6} mol l^{-1} with a detection limit of 4.06×10^{-8} mol l^{-1}. Reaction and determination acidity for nitrite is the same which made the method much simpler compared with the widely accepted fluorescence method with DAN as a fluorescence reagent.

Keywords: 2-Amino-5,7-dimethyl-1,8-naphthyridine; Nitrite; Fluorescence quenching; Determination

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1. Introduction

Nitrite is ubiquitous within environmental, food, industrial and physiological systems. Highly carcinogenic *N*-nitrosoamines could be formed by the reaction of nitrite with secondary amines and amides, which are generally present in meat and fish products, where nitrite is used as a food preservative. Nitrogen dioxide, which is one of the most important atmospheric pollutants, can be dissolved in triethanolamine (TEA) solution, and exists as nitrite [1]. It is recommended that the nitrite level in drinking water should not exceed 0.2 mg l^{-1} [2]. Thus, developing sensitive, specific, simple and low-cost methods for the determination of nitrite is of great importance.

Various methods have been reported for the determination of nitrite, including spectroscopic and electrochemical detection, chromatography and capillary electrophoresis [3]. Among these methods, spectrofluorimetry is widely used due to its high sensitivity and selectivity.

Fluorescence determination of nitrite is usually based on the reaction with a fluorescent reagent, such as 2,3-diaminonaphthalene (DAN) [4], 2,6-diaminopyridine [5], 4,4′,4″,4‴-tetrasubstituted amino aluminium phthalocyanine [6]. Among them, DAN is the most widely used reagent for fluorescence determination of nitrite. But the reaction and determination processes are complicated, including requirement of different pH conditions in the reaction and detection, poor water solubility of the product, and time-consuming.

2-Amino-5,7-dimethyl-1,8-naphthyridine (ADMND) is a new reagent synthesized in our laboratory. It can be synthesized by only one simple step and serve as a ratiometric fluorescent pH probe near neutrality [7]. This high-fluorescent reagent can react with nitrite to form a low-fluorescent compound, 2-hydroxyl-5,7-dimethyl-1,8-naphthyridine (HDMND), which can also serve as a fluorescent pH probe. Such fluorescence quenching process leads us to set up a new method for nitrite determination. In this paper, ADMND was proposed as a new fluorescent reagent for nitrite determination. Reaction and determination conditions were studied, the method has proven to be much simpler compared with those using DAN-related fluorescent reagents and has been successfully applied to the determination of nitrite in tap water samples.

2. Experimental

2.1. Apparatus

All fluorescence measurements were made with a JASCO FP-6500 spectrofluorimeter equipped with an 1 cm quartz cell.

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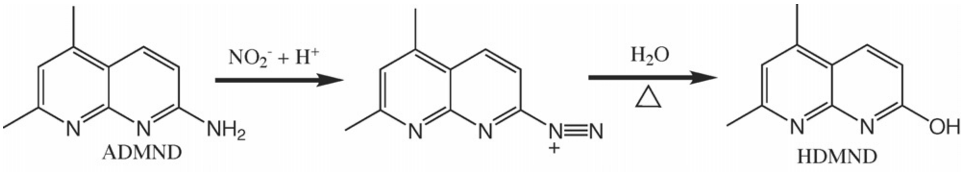

Scheme 1. Reaction between ADMND and nitrite.

Excitation and emission slits were set at 5 nm. The absorption spectrum was performed on a JASCO V-550 UV–vis spectrophotometer with bandpass as 1 nm.

2.2. Reagents

All reagents were of analytical reagent grade. All solutions were prepared with doubly distilled water.

Stock solution of nitrite (1.5×10^{-3} mol l^{-1}) was prepared by dissolving sodium nitrite (Beijing Chemical Reagent Co., China, dried at 105 °C for 2 h before use) in water. This nitrite solution was prepared every 2 weeks and kept in a refrigerator. The working solution was prepared freshly by appropriate dilution.

ADMND and HDMND were synthesized as described previously [7,8]. A 1.5×10^{-3} mol l^{-1} ADMND stock solution was prepared by dissolving the dye in 1 mol l^{-1} sulfuric acid and diluted with water as required.

2.3. General procedure

The 1 ml of 1×10^{-5} mol l^{-1} ADMND and 1 ml of 2.5 mol l^{-1} H_2SO_4 were transferred into a 5 ml volumetric flask, final concentration of the sulfuric acid is 0.5 mol l^{-1} and thus $[H^+] = 1$ mol l^{-1}. Then a standard solution of sodium nitrite was added and the resulting solution was diluted to 5 ml with water. The flask was placed into a boiling water bath for 20 min and then cooled to room temperature. The relative fluorescence intensity was measured at 403 nm when excited at 346 nm.

3. Results and discussion

3.1. Spectral characteristics and proposed mechanism

Fluorescence determination of nitrite with ADMND is typically based on the reaction of naphthyridine amino group with nitrite to form a diazonium salt that hydrolyzed when boiling to give hydroxyl group substituted naphthyridine HDMND. The proposed reaction mechanism is shown in Scheme 1 as reported in the literature when aromatic amine was used [3,7].

The absorption and fluorescence emission spectra of ADMND and its reaction product HDMND are shown in Figs. 1 and 2. The excitation and emission maxima were found to be at 346 and 403 nm, respectively. Decrease of fluorescence intensity, blue shift of the fluorescence spectra were found when nitrite was added. In the absorption spectra, blue shift of the absorption maxima was also found in the presence of nitrite. As the electron donating power of hydroxyl group in HDMND was weaker than the primary amine in ADMND fluorescence quenching and blue shift of both absorption and emission spectra was observed. Similar spectra change was also found when ADMND serves as a ratiometric fluorescence pH probe [7].

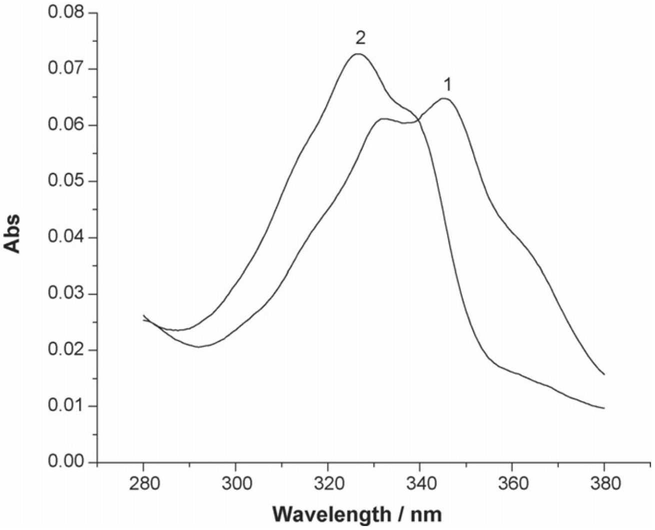

Fig. 1. Absorption spectra of ADMND (1) and ADMND in the presence of nitrite (2). $[ADMND] = 1 \times 10^{-5}$ mol l^{-1}, $[nitrite] = 5 \times 10^{-5}$ mol l^{-1}.

3.2. Effect of reaction acidity

The reaction between ADMND and nitrite is a diazonium procedure, which should occur in acidic medium. In the present

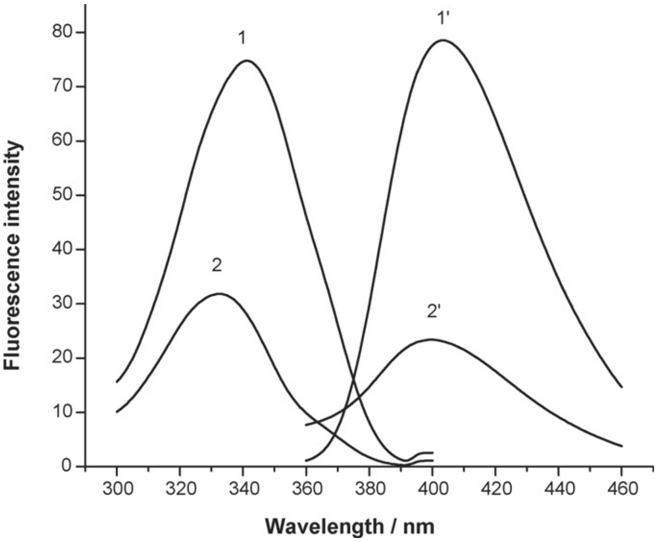

Fig. 2. Fluorescence excitation and emission spectra of ADMND (1) and ADMND in the presence of nitrite (2). $[ADMND] = 1 \times 10^{-5}$ mol l^{-1}, $[nitrite] = 5 \times 10^{-5}$ mol l^{-1}.

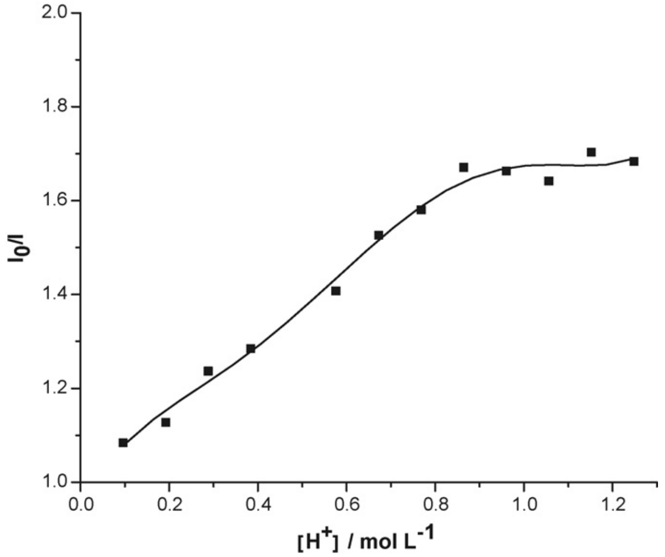

Fig. 3. Effect of H^+ concentration on fluorescence intensity change of ADMND after reaction. [ADMND] = 1×10^{-5} mol l^{-1}, [nitrite] = 5×10^{-5} mol l^{-1}. Boiling water bath for 60 min reaction. I_0 and I refer to the fluorescence intensities of ADMND solution before and after the reaction with nitrite ion.

work, we choose H_2SO_4 to obtain an acidic medium. To study the effect of acidity on the reaction, the concentration of H^+ was varied in the range of 0.1–1.2 mol l^{-1}. As can be seen in Fig. 3, a final concentration of 1 mol l^{-1} H^+ was chosen for the reaction.

3.3. *Choice of detection pH*

It was found that the fluorescence of ADMND was sensitive to pH, especially near neutrality [7]. To study the pH dependence of the reaction product of nitrite with ADMND, HDMND was also synthesized. Fig. 4 shows the pH dependence of the fluorescence intensity for both ADMND and HDMND. It is found that the fluorescence intensity difference between ADMND and HDMND was the largest when detecting at the pH around 4–6 and thus the detection sensitivity would be the highest in this pH range. However, as can be seen in Fig. 4, the fluorescence intensity of ADMND itself changes abruptly in this pH range. So it is hard to control pH at a fix value in this pH range to get a stable fluorescence emission, causing poor reproducibility. From Fig. 4 it is seen that fluorescence quenching of ADMND by HDMND was also large enough when detecting at more acidic condition. Detecting at the pH just the same as reaction condition in the present work has some advantages like, much simpler determination procedure compared with the most reported works that needed adjusting pH from acidic in the reaction condition to basic for fluorescence detection [9,10], eliminating most of the influence caused by foreign ions. In this work, the detecting pH condition was chosen as 1 mol l^{-1} H^+ solution as the same pH condition of reaction and satisfied sensitivity was obtained as indicated in Section 3.4.

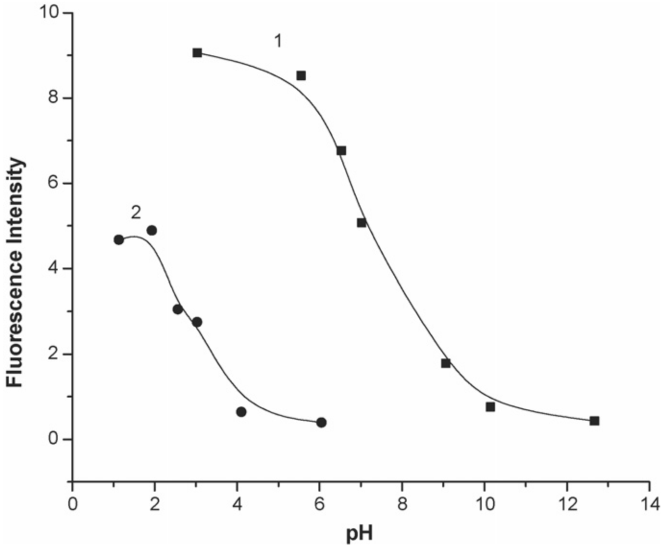

Fig. 4. pH dependence of ADMND (1) and HDMND(2). Concentration of 1 and 2 was the same, 2×10^{-7} mol l^{-1}.

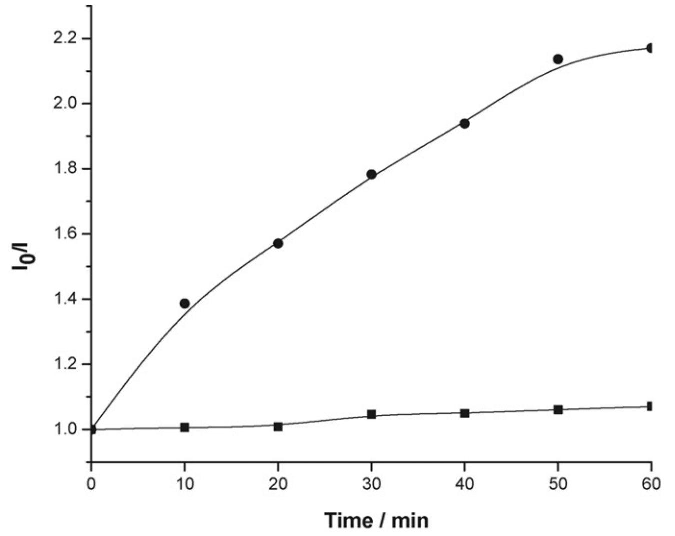

Fig. 5. Effect of reaction time and temperature. [ADMND] = 1×10^{-5} mol l^{-1}, $[H^+]$ = 1 mol l^{-1}, [nitrite] = 5×10^{-5} mol l^{-1}. I_0 and I refer to the fluorescence intensities of ADMND solution before and after the reaction with nitrite ion: (■) room temperature and (●) boiling water bath.

3.4. *Effect of reaction time and temperature*

As shown in Scheme 1, the last step of reaction is the hydrolysis of diazonium salt that needs a high temperature condition. The effect of reaction time and the reaction temperature were examined and the results are shown in Fig. 5. It is found that

Table 1
Effect of foreign ions

Ions	Tolerance limit (molar ratio)
Mg^{2+}, Zn^{2+}, Cu^{2+}, Li^+, Mn^{2+}, Fe^{2+}, NH_4^+, K^+, tartrate, SO_4^{2-}, Cl^-, EDTA, CO_3^{2-}, Br^-, $HCOO^-$, $B_4O_7^{2-}$, CH_3COO^-, $H_2PO_4^-$	1000
Fe^{3+}	10
NO_3^-	100

Table 2
Determination of nitrite in water samples

Sample	Nitrite added (nmol)	Nitrite found[a] (nmol)	R.S.D. (%)	Recovery (%)
Deionized water	0.00	0.328	7.3	–
	2.00	2.408	0.5	104
	3.00	3.680	1.4	111
	5.00	5.408	0.8	102
Tap water in Tsinghua University[b]	0.00	13.90	2.48	–
	20.00	31.80	2.25	89.5
	40.00	53.94	8.28	100.1
	60.00	69.05	6.29	91.92
	80.00	92.76	2.89	98.58

[a] Average of three determinations.
[b] In the presence of 1×10^{-3} mol l^{-1} EDTA.

a boiling water bath was necessary to get obvious fluorescence response upon nitrite reaction. In sample analysis lower nitrite concentration was expected and when the concentration of nitrite is chosen as 1×10^{-5} mol l^{-1} 20 min reaction in boiling water bath was enough. This condition was used in the following experiments.

3.5. *Effect of diverse ions*

The effect of diverse ions on the fluorescence determination of nitrite with ADMND was studied and the results are summarized in Table 1. The tolerance limit was taken as the concentration of a diverse ion causing less than 5% relative error on the nitrite determination. As can be seen in the table, most of the usual ions have no influence on the determination of 1×10^{-5} mol l^{-1} nitrite. Influence of high concentration of iron(III) can be eliminated by addition of a masking agent like EDTA and effect of nitrate can also be avoided by proper reduction to nitrite as reported [11].

3.6. *Linearity, sensitivity, and precision*

A linear calibration curve was obtained in the nitrite concentration range of 1×10^{-7} to 2.5×10^{-6} mol l^{-1} with correlation coefficient R as 0.996. The correlation equation is $I_0/I = 1.7 \times 10^5$ [nitrite] + 0.99 where I_0 and I are the fluorescence intensities in the absence and presence of nitrite, respectively, at 403 nm. The relative standard deviation ($n = 3$) is 0.23% at 1×10^{-7} mol l^{-1} nitrite. With a signal to noise ratio of 3, the detection limit is calculated as 4.06×10^{-8} mol l^{-1} which is comparable to the existed fluorescence method with aromatic amine but needs more complicated determination procedure [9,12].

3.7. *Determination of nitrite in water samples*

The present method was applied to the determination of nitrite in water samples. Deionized water was analyzed directly by transferring 0.5 ml of the water into a 5 ml volumetric flask. Tap water was analyzed in the presence of 1×10^{-3} mol l^{-1} EDTA to avoid the influence of Fe^{3+} and 1 ml sample was taken. The samples were then reacted with ADMND in 1 mol l^{-1} H_2SO_4 according to the reaction and detection procedure described in Section 2. The results are shown in Table 2. The final concentration of nitrite in deionized water and tap water are 6.56×10^{-7} and 1.39×10^{-5} mol l^{-1}, respectively. Nitrite concentration in tap water is about three times higher than the drinking water tolerance [2] therefore it is not suggested to drink the tap water in China directly except for stated specially.

4. Conclusion

Based on the pH dependent fluorescence property of 2-amino-5,7-dimethyl-1,8-naphthyridine (ADMND) and 2-hydroxyl-5,7-dimethyl-1,8-naphthyridine (HDMND), a new fluorescence reagent ADMND for nitrite determination was proposed. The reaction of nitrite with ADMND and fluorescence determination of the product HDMND were all performed in 0.5 mol l^{-1} H_2SO_4 solution, which greatly simplified the process but still with sufficient detection sensitivity compared with the existing method by using DAN as a fluorescence reagent. The method is simple and effective, and is expected to be in practical use in chip based in situ determination of nitrite, nitrogen dioxide and related compounds.

References

[1] Y.L. Wei, M. Oshima, J. Simon, S. Motomizu, Talanta 57 (2002) 355.
[2] World Health Organization, 2004, http://www.who.int/water_sanitation_health/dwq/gdwq3/en/index.html.
[3] M.J. Moorcroft, J. Davis, R.G. Compton, Talanta 54 (2001) 785.
[4] P. Damiani, G. Burini, Talanta 33 (1986) 649.
[5] L.J. Dombrowski, E.J. Pratt, Anal. Chem. 44 (1972) 2268.
[6] X.Q. Zhan, D.H. Li, H. Zheng, J.G. Xu, Anal. Lett. 34 (2001) 2761.
[7] Y. Xiang, A.J. Tong, Chem. Lett. 34 (2005) 926.
[8] J. Bernstein, B. Stearns, E. Shaw, W.A. Lott, J. Am. Chem. Soc. 69 (1947) 1151.
[9] M.C. Carre, B. Mahieuxe, J.C. Andre, M.L. Viriot, Analusis 27 (1999) 835.
[10] H. Wang, W. Yang, S.C. Liang, Z.M. Zhang, H.S. Zhang, Anal. Chim. Acta 419 (2000) 169.
[11] S. Biswas, B. Chowdhury, B.C. Ray, Talanta 64 (2004) 308.
[12] Revanasiddappa, K. Kumar, M. Bilwa, Mikrochim. Acta 137 (2001) 249.