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Development of a new fluorescent probe: 1,3,5,7-tetramethyl-8-(4′-aminophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence for the determination of trace nitrite

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Abstract

A new fluorescent probe, 1,3,5,7-tetramethyl-8-(4′-aminophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence (TMABODIPY) has been developed for the determination of trace nitrite in terms of the reaction of nitrite with TMABODIPY first in acidic solution and then in alkaline solution to form diazotate, a stable and highly fluorescent reagent. The method offered the advantage of specificity, sensitivity and simplicity. The linear calibration range for nitrite was 8–300 nmol l^{-1} s with a 3σ detection limit of 0.65 nmol l^{-1}. The proposed method has been applied to monitor the trace nitrite in drinking water and vegetable without extraction.

Keywords: 1,3,5,7-Tetramethyl-8-(4′-aminophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence (TMABODIPY); Fluorescent probe; Nitrite; Spectrofluorimetry

1. Introduction

Nitrite plays an important role in environmental, food, industrial and physiological systems. On one hand, as it is one of the stable metabolites of nitric oxide involved in the regulation of numerous physiological and biological processes as a messenger molecule [1] and nitric oxide can be transformed into nitrite, a greater stable ion, through the reaction with oxygen, the measurement of nitrite can therefore provide a reliable measurement of nitric oxide action within the body and can be used as a biomarker that enables physicians to gauge the health of an individual [2]. On the other hand, nitrite is a widespread contaminant in environmental, food, industry and human body. Passage of nitrite into the blood stream results in the irreversible conversion of hemogloblin to metahemogloblin with oxygen uptake and transportation compromised [3]. Furthermore, nitrite can produce nitroamine, a carcinogenic material within the acidic conditions of the stomach and then subsequent implication in the pathology of gastric cancer [4].

Due to the importance of nitrite, great interests in trace nitrite determination have increased in recent years. The techniques mainly include spectrophotometry [5,6], chemiluminescence [7], eletrometry [8], chromatography [9,10], kinetic methods [11] and spectrofluorimetry [12–19]. Among all of the techniques, spectrofluorimetric protocol is widely used because of its simplicity, sensitivity and low cost. Although a number of different fluorescent reagents have been developed, such as 5-aminofluorescein [12], 2-amino-4-chloro-1-hydroxybenzene-6-sulphonicbenzidine [13], resorcinol [14], 4-hydroxycoumarin [15], 5,6-diamino-1,3-naphthalene disulfonicacid [16], 2,3-diaminonaphthalene (DAN) [17], tryptophan [18] and 2,6-diaminopyridine [19], difluoroboradiaza-*s*-indacences (BODIPY) have not been found to be used in nitrite determination. BODIPY are an important class of highly rigidized and polymer-like fluorescent dyes that have found widespread applications in biochemistry and molecular biology [20]. They present many advantageous photonic properties, such as high extinction coefficients, good photostability, and can be exited at wavelengths around or over 500 nm. Due to the small Stokes shift, the fluorescence quantum yield of BODIPY is high. What is more, they can be used as the mediators to form many kinds of ex-

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tremely fluorescent materials [21]. Since much of the literature on them is patent material designed to restrict unlicensed commercial uses. BODIPY dyes tend to be sold prohibitively expensive even in small quantities. Therefore, it is highly desirable to synthesize and exploit this kind of reagents suitable for various analytical application. For this purpose, a new BODIPY reagent, 1,3,5,7-tetramethyl-8-(4′-aminophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence (TMABODIPY) has been developed to determine trace nitrite in terms of the reaction of nitrite with TMABODIPY first in acidic solution and then in alkaline solution to form diazonate, a stable and extremely fluorescent product, at the light of the work of Axelrod and Enge [12]. The method presented the advantage of specificity, sensitivity and simplicity. The proposed method has been used to monitor the trace nitrite in real samples such as drinking water and vegetable without extraction.

2. Experimental

2.1. Apparatus

Mass spectra were obtained by means of a VG ZAB-3F GC–MS instrument (Manchester, UK). FT-IR spectra were obtained for the products in KBr disks by means of a Bruker IFS48 instrument (Karlsruhe, Germany). Fluorescence spectra were recorded with a Shimadzu RF-5000 spectrofluorimeter (Kyoto, Japan). pH was determined by means of a DF-801 accurate acidimeter (Zhongshan University, Guangzhou, China).

2.2. Reagents

Unless otherwise specified, all reagents were of analytical reagent grade without further purification. All solutions were prepared in double-distilled water.

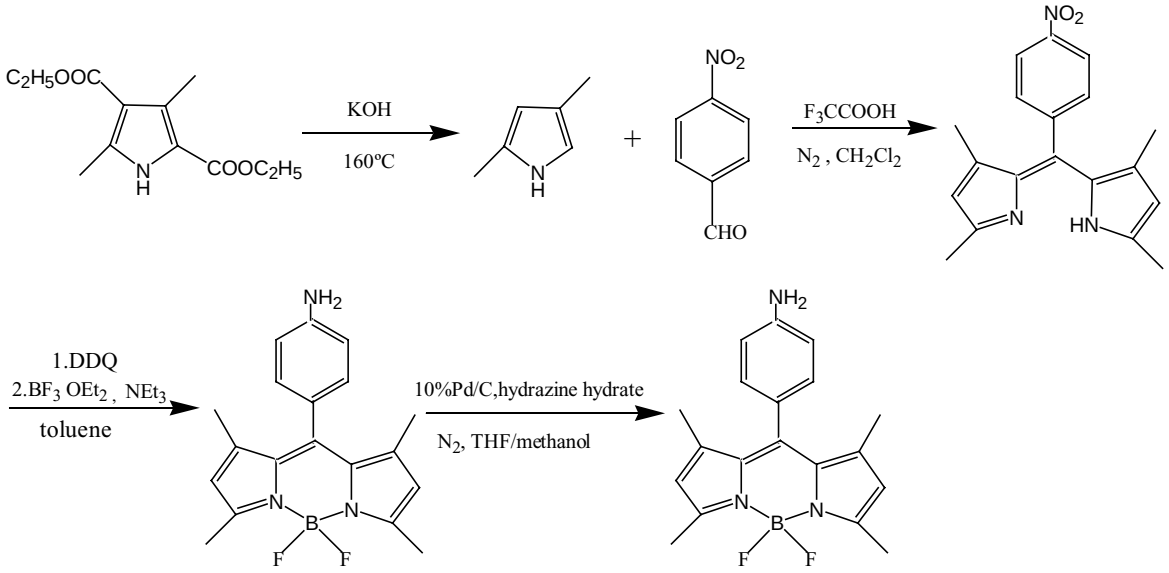
TMABODIPY was synthesized in our laboratory and its 1.0×10^{-4} mol l^{-1} solution was prepared with ethanol and stable at least for a month kept under 4 °C.

KH_2PO_4–Na_2HPO_4 buffer was prepared by mixing 0.78 mmol l^{-1} KH_2PO_4 solution and 0.78 mmol l^{-1} Na_2HPO_4 solution to appropriate pH value. A standard nitrite solution (2.5×10^{-4} mol l^{-1}) was prepared by drying sodium nitrite at 110 °C for 4 h and dissolving it in water. Twenty drops of chloroform and a pellet of sodium hydroxide were added to prevent liberation of nitrous acid and to inhibit bacterial growth and thus make the nitrite solution stable. The standard solution was prepared weekly and kept in refrigerator. The working solution was prepared daily by appropriate dilution.

2.3. Synthesis of 1,3,5,7-tetramethyl-8-(4′-aminophenyl)-4,4-difluoro-4-bora-3a, 4a-diaza-s-indacence (TMABODIPY)

The synthetic route employed to obtain the new probe is outlined in Scheme 1. 2,4-Dimethylpyrrole was synthesized according to the literature [22]. Ninety-five grams of 2,4-dimethyl-3,5-dicarbethoxypyrrole was mixed with 145 g of 85% potassium hydroxide and heated in a steel bomb under 160 °C for 4–5 h. The reaction mixture was steam distilled and the distillate was extracted several times with ether. The extract was dried over potassium carbonate. The ether was removed and the residue was distilled under vacuum, bp 72 °C (3.333×10^3 Pa).

4-Nitrobenzalhade 0.7556 g (5 mmol) and 2,4-dimethylpyrrole were dissolved in dry CH_2Cl_2 (50 ml) at room temperature. The solution was purged with N_2 for 30 min. Several drops of CF_3COOH were added to initiate the condensation. After 1.5 h, TLC (silica, CH_2Cl_2) showed that all of the aldehyde had been consumed. The reaction mix-



Scheme 1. Synthesis of TMABODIPY.

ture was washed with saturated aqueous $NaHCO_3$ solution and water, dried with Na_2SO_4, filtered and rotary evaporated. The product was dissolved in dry toluene (50 ml) and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ, 1.0 g) was added in the solution. After 15 min, triethylamine (6.0 ml) was added, followed immediately by BF_3-etherate (6 ml). After stirred for 1.5 h at room temperature, the mixture was washed with water, dried with Na_2SO_4, filtered and rotary evaporated.

The compound was dissolved in warm THF (30 ml). Ethanol (30 ml) was added as a co-solvent. After purged with N_2, 10% Pd/C (1.0 g) and 1.0 ml hydrazine were added. The solution was stirred at reflux under N_2 for 30 min, cooled to 20 °C and poured into water. The aqueous mixture was extracted with CH_2Cl_2. The extract was washed with water, and the solvent was removed on a rotary evaporator. The residue was taken up in CH_2Cl_2 and applied to a silica gel column chromatography using hexane–CH_2Cl_2 (1:2) to afford the desired compound.

Mass spectra: *m*/*z*: 339 (*M*), 301 (*M*–2F). IR: (KBr, $\nu\,cm^{-1}$): 3441.3, 3201 (–NH_2), 1091.9 (–B–F), 1713.3 (–C=N), 2924.8 (–CH_3).

2.4. Fluorometric analysis

Relative fluorescence (RF) quantum efficiencies were obtained according to the literature [23] by comparing the area under the corrected emission spectrum of the test sample at 492 nm with that of the solution of fluorescein in 0.1 mol l^{-1} NaOH whose quantum efficiency is 0.85. The slit width was both 1.5 nm for excitation and emission, respectively.

2.5. Determination of nitrite

Two milliliters of TMABODIPY solution (2.5×10^{-6} mol l^{-1}) was transferred to a 10 ml test-tube. And a working solution of 1 ml of nitrite (1.0×10^{-6} mol l^{-1}) was added followed by 1.0 ml of HCl solution (3.6 mol l^{-1}). The mixture was diluted to 5.0 ml with water and stood at 40 °C for 30 min. After that, 2.0 ml of NaOH solution (2.6 mol l^{-1}) was added. Then, the solution was diluted to the mask with water. Five minutes later, the relative fluorescence intensity was measured at 510 nm with excitation at 497 nm. The slit width was 3 and 5 nm for excitation and emission, respectively.

2.6. Preparation of samples

Drinking water was determined according to the procedure described above directly without further treatment. One milliliter of the water was transferred to a 10.0 ml test-tube, and the following operations were as in the procedure in Section 2.

Fresh vegetable was cleaned and dried, then 10 g of which was mortared and immerged in 100 ml water. After being filtered, the solution was transferred to 100 ml volumetric flask with water and diluted to the mark. Two milliliters of the solution was transferred to a 10 ml test-tube, and was tested as described in Section 2.

3. Results and discussion

3.1. Spectrofluorimetric and spectrophotometric properties

The fluorescence and absorption are shown in Figs. 1 and 2, respectively. The excitation and emission maximum of TMABODIPY was at 497/510 nm, respectively. The fluorescence intensity increased when nitrite was added. Under the same condition, the maximum absorbance of TMABODIPY was at 497 nm, while that of the product was at 507 nm,

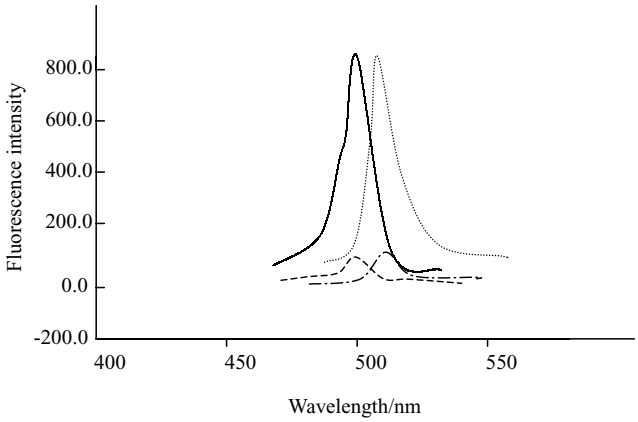


Fig. 1. Fluorescence spectra of TMABODIPY and the product, $C_{TMABODIPY} = 2.0 \times 10^{-6}$ mol l^{-1}, $C_{NO_2^-} = 2.0 \times 10^{-6}$ mol l^{-1}, $C_{HCl} = 0.36$ mol l^{-1}, $C_{NaOH} = 0.52$ mol l^{-1}; (—) excitation spectrum of the product, (···) emission spectrum of the product, (- - -) excitation spectrum of the TMABODIPY, (-··-) emission spectrum of the TMABODIPY; slit width of exitation and emission: 3 and 5 nm, respectively.

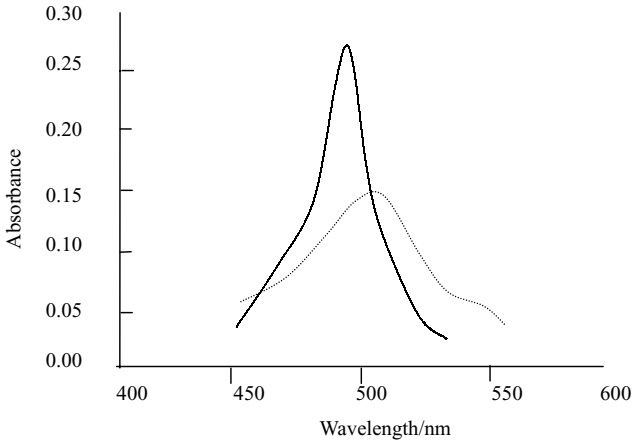
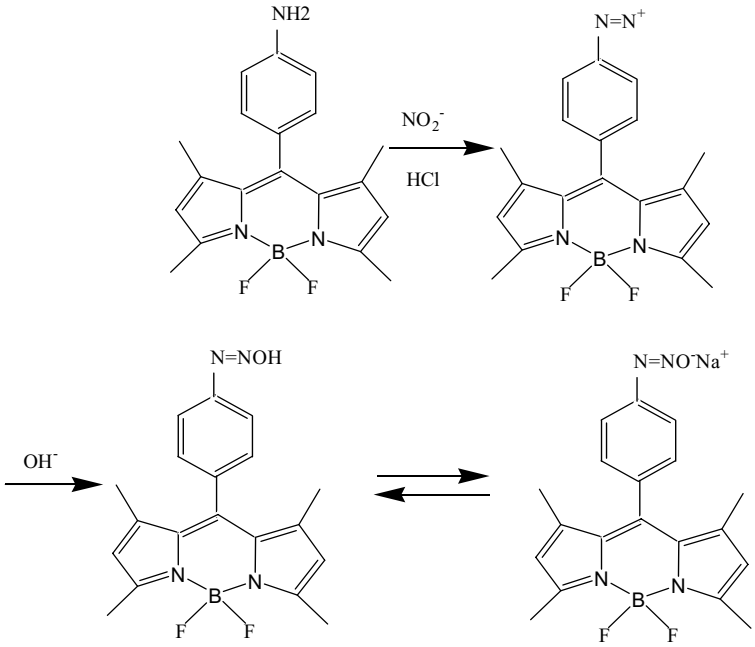


Fig. 2. Absorption spectra of TMABODIPY and the product. $C_{TMABODIPY} = 2.0 \times 10^{-6}$ mol l^{-1}, $C_{NO_2^-} = 1.0 \times 10^{-3}$ mol l^{-1}, $C_{HCl} = 0.36$ mol l^{-1}, $C_{NaOH} = 0.52$ mol l^{-1}; (—) absorbance spectrum of TMABODIPY, (···) absorbance spectrum of the product.



Scheme 2. TMABODIPY reacts with nitrite.

with a red shift of 10 nm, showing that there is a new reagent formed. The proposed product should be a diazonate derivative according to the literature [12]. The reaction scheme of TMABODIPY and nitrite is shown in Scheme 2. The diazotate extends the conjugated system compared with TMABODIPY and then the maximum absorbance of which has a bathochromic shift. Diazotate in alkaline is electron rich as it carries positive charge. The electron donation of diazotate leads to the fluorescence intensity of the product increasing greatly compared to TMABODIPY. What is more, the fluorescence intensity of TMABODIPY keeps stable over a wide pH range, as indicated in Fig. 3, from 6.0 to 12.0 (Table 1).

Table 1
Spectroscopic properties of TMABODIPY and the product

Solvent	$\varepsilon_{max\,(abs)}$ $\times 10^4$ M^{-1} cm^{-1}	$\lambda_{abs,max}$ (nm)	$\lambda_{em,max}$ (nm)	ϕ_f
Water	1.11	497	510	0.32
Ethanol	1.23	473	507	0.31
Acetonitrile	1.41	501	505	0.29
Dichloromethene	1.04	505	509	0.33
THF	1.10	503	508	0.32
Toluene	0.94	505	510	0.37
Water[a]	0.64	507	521	0.84

[a] The spectroscopic properties of the product.

3.2. Effect of TMABODIPY concentration

Fig. 4 indicates the effect of TMABODIPY concentration on the relative fluorescence intensity. TMABODIPY concentration varied from 2×10^{-7} to 8×10^{-7} mol l^{-1}. In this concentration range, there was a linear relationship between

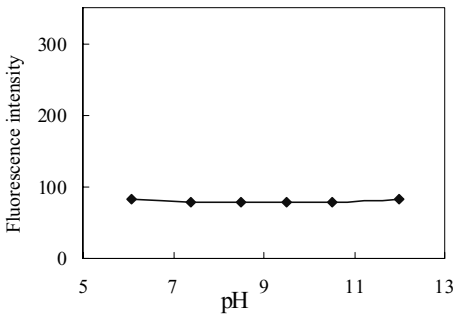


Fig. 3. Effect of pH on TMABODIPY, $C_{TMABODIPY} = 5.0 \times 10^{-7}$ mol l^{-1}.

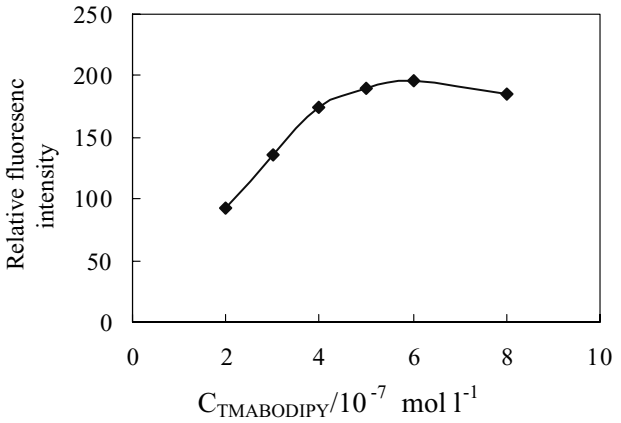


Fig. 4. Effect of TMABODIPY amount, $C_{NO_2^-} = 1.0 \times 10^{-7}$ mol l^{-1}, $C_{HCl} = 0.36$ mol l^{-1}, $C_{NaOH} = 0.52$ mol l^{-1}; $T = 30\,^{\circ}C$; reaction time: 60 min.

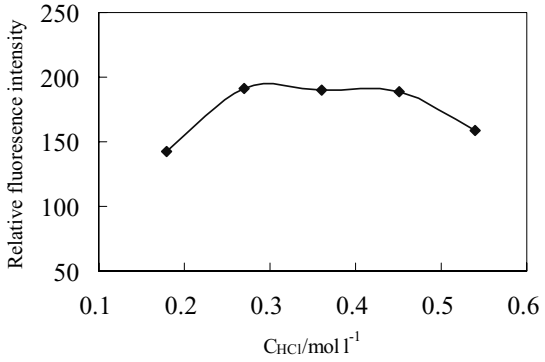


Fig. 5. Effect of HCl amount, $C_{TMABODIPY} = 5.0 \times 10^{-7}$ mol l^{-1}, $C_{NO_2^-} = 1.0 \times 10^{-7}$ mol l^{-1}, C_{NaOH}–$C_{HCl} = 0.08$ mol l^{-1}; $T = 30\,^{\circ}$C; reaction time: 60 min.

relative fluorescence intensity and the concentration of nitrite solution. A 5×10^{-7} mol l^{-1} of TMABODIPY solution was chosen as optimal for at which the highest sensitivity was obtained.

3.3. Effect of HCl concentration

As diazonium salt must be prepared in acidic condition, proper HCl concentration should be selected to ensure TMABODIPY react with nitrite as completely as possible. The effect of HCl concentration was studied in the range of 0.25–0.54 mol l^{-1}. It was found that the highest relative fluorescence intensity of TMABODIPY was obtained at HCl concentration of 0.36 mol l^{-1} with a tolerance range of 0.27–0.45 mol l^{-1}, as can be seen in Fig. 5.

3.4. Effect of NaOH concentration

Proper alkaline solution is needed to change diazonium salt to diazotate, a stable and extremely fluorescence product. The effect of NaOH concentration is shown in Fig. 6.

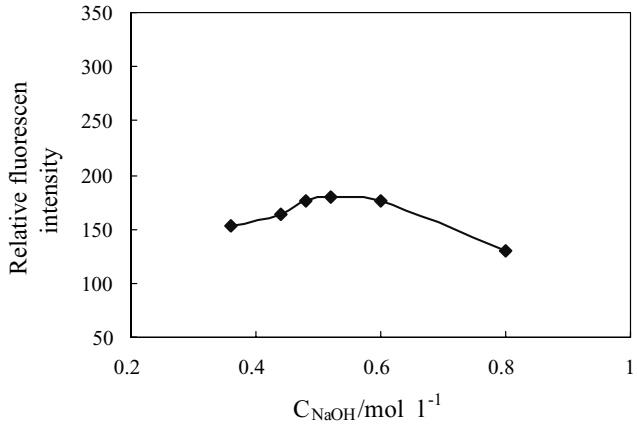


Fig. 6. Effect of NaOH amount, $C_{TMABODIPY} = 5.0 \times 10^{-7}$ mol l^{-1}, $C_{NO_2^-} = 1.0 \times 10^{-7}$ mol l^{-1}, $C_{HCl} = 0.36$ mol l^{-1}; $T = 30\,^{\circ}$C; reaction time: 60 min.

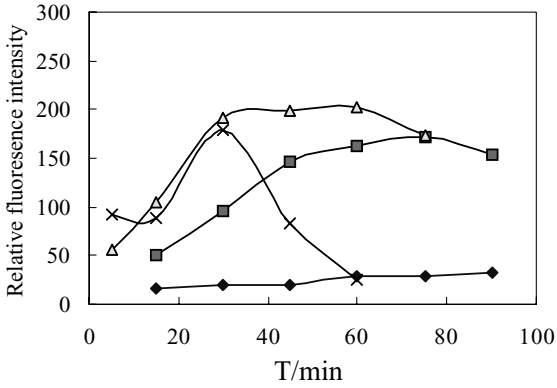


Fig. 7. Effect of time and temperature, $C_{TMABODIPY} = 5.0 \times 10^{-7}$ mol l^{-1}, $C_{NO_2^-} = 1.0 \times 10^{-7}$ mol l^{-1}, $C_{HCl} = 0.36$ mol l^{-1}, $C_{NaOH} = 0.52$ mol l^{-1}; (◆) 20 °C, (■) 30 °C, (Δ) 40 °C, (×) 50 °C.

The highest determination sensitivity was obtained at NaOH concentration of 0.52 mol l^{-1}. Therefore, the final concentration of NaOH concentration was chosen as 0.52 mol l^{-1}.

3.5. Effect of time and temperature

Time and temperature are both critical factors in reaction of TMABODIPY and nitrite. According to the literature, the TMABODIPY reacts with nitrite first in acidic solution to form diazonium salt then in alkaline, the diazonium salt transforms to diazotate soon. The effect of time and temperature during the first reaction period was investigated. The effect of time and temperature was indicated in Fig. 7. It was found that it needed a longer time to reach the maximal relative fluorescence intensity at low temperature (20–30 °C) than at high temperature (40 °C). Furthermore, high temperature (40 °C) resulted into more relative fluorescence intensity than low temperature. But as temperature climbed up to 50 °C, the relative fluorescence intensity began to decline and was hard to keep stable because diazonium salt is apt to decomposed at too high temperature, while the reaction temperature of 40 °C offered the RF stable at half an hour. Therefore, the reaction was first performed at 40 °C for 30 min in acidic solution, then at room temperature for only 5 min in alkaline solution. After formation, the product kept stable for at least 24 h.

3.6. Linearity, sensitivity and precision

Under the optimal conditions described above, the linear calibration curve, detection limit and precision were obtained. The linear calibration curve was as follows: $Y = 1914.9X - 3.904$ ($\gamma = 0.9987$, $n = 10$; X: nitrite concentration (μmol l^{-1}); Y: RF between the blank and the standard sample). Linear range was from 0.08×10^{-7} to 3.00×10^{-7} mol l^{-1} and the detect limit was 0.65×10^{-9} mol l^{-1} with a signal to noise ratio of 3. The repeatability of the method was tested by the analysis of a standard nitrite solu-

Table 2
Interference of foreign ions

Foreign ions	Tolerance ($\mu g\,l^{-1}$)	Foreign ions	Tolerance ($\mu g\,l^{-1}$)	Foreign ions	Tolerance ($\mu g\,l^{-1}$)
Ca^{2+}	100000	Pb^{2+}	1500	CO_3^{2-}	20000
Mg^{2+}	30000	Mn^{2+}	150	NO_3^-	50000
Al^{3+}	20000	Ni^{2+}	300	EDTA	30000
Fe^{3+}	300	Cu^{2+}	1000	Citric	20000
Fe^{2+}	150	PO_4^{3-}	4000	Br^-	2000
Hg^{2+}	300	$C_2O_4^{2-}$	2000	I^-	0.2
Zn^{2+}	40000	SO_4^{2-}	10000	I^- [a]	2500

[a] Masked by Fe^{3+}.

Table 3
Analytical result of nitrite in samples with TMABODIPY

Sample	Added	Found	R.S.D. (%) ($n = 6$)	Recovery (%)
Drinking water ($\mu mol\,l^{-1}$)	0.0000	0.0251	1.65	
	0.0500	0.0726	1.68	95.0
	0.1000	0.1239	2.34	98.8
Vegetable (cole)* ($ng\,mg^{-1}$)	0.0000	9.0390	1.15	
	11.5000	20.4000	3.93	98.8
	23.0000	33.2810	2.34	105.0

tion over a period of 10 days ($n = 6$). The relative standard deviation was found to be 4.53% at $1.00 \times 10^{-7}\,mol\,l^{-1}$.

3.7. Interference of foreign ions

The interference of a number of foreign ions was investigated according to the recommended procedure at a nitrite concentration of $1.00 \times 10^{-7}\,mol\,l^{-1}$. The tolerance limit of an ion was taken as the maximum amount causing an error of $\leq$5% in the fluorescence of sample. As Table 2 indicated, most of the ions studied did not interfere with the determination, even when present in large excess. Only I^- had obvious interference, but could be well masked by Fe^{3+}.

3.8. Sample analysis

The method has been applied to the determination of nitrite in real samples such as drinking water and fresh vegetable (cole). Drinking water was tested without further treatment. Fresh vegetable (cole) was prepared according to the procedure described above. Both samples were analysis in the recommended procedure. The results were shown in Table 3.

4. Conclusion

A new fluorescence probe, TMABODIPY, which provides high quantum efficiency, modest extinction coefficients, rigid and tolerate wide pH range, was developed to determine trace nitrite in real samples. The method offered the advantages of specificity, sensitivity and simplicity. The sensitivity of the method is more highly than other spectrofluoremetric methods (see Table 4).

Table 4
Comparison of detection limits for spectrofluorimetric determinations for nitrite with different reagents

Reagents	Detection limit ($ng\,ml^{-1}$)	References
2,3-Diaminonaphthalene	<0.3	[17]
2,6-Diaminopyridine	2	[19]
Resorcinol	33	[14]
4-Hydroxycoumarin	3	[15]
5-Aminofluorescein	0.5	[12]
Tryptophan	1	[18]
5,6-Diamiono-1,3-naphthalene disulfonic acid	0.09	[16]
TMABODIPY	0.03	This paper

Acknowledgements

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