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A reversible chemosensor for nitrite based on the fluorescence quenching of a carbazole derivative

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Abstract

The carbazole derivative, with an amino group in 9-position (9-methylacryloylamino carbazole (MAC), has been utilized to prepare a fluorescent sensor and used for the determination of NO_2^- based on the reaction between nitrite (NO_2^-) and excess I^- to form I_3^-, which can quench the fluorescence of carbazole derivative. MAC, as a fluorescent carrier, has a terminal double bond and is covalently immobilized on a quartz glass plate surface by photo-polymerization to prevent the leakage of the dye. The sensor shows sufficient repeatability, selectivity, operational lifetime of 8 weeks, and a fast response of less then 30 s. NO_2^- can be determined in the range between 1.0×10^{-6} and 1.0×10^{-4} mol l^{-1} with a detection limit of 8.0×10^{-7} mol l^{-1} at pH of 2.0. The quenching mechanism is discussed. Most commonly coexisting ions do not interfer with the NO_2^- assay.

Keywords: Chemosensor; Carbazole derivative; Fluorescence quenching; Covalent immobilization; Nitrite

1. Introduction

In a search of new fluorescent carriers used in the fabrication of chemical sensors [1–9], the carbazole derivatives attracted our attention as an important charge transferring materials. Many carbazole derivatives have been synthesized and been widely applied as photo-conductor materials. But this category of compounds has rarely been used as fluorescent carriers for chemosensors [10]. From carbazole, one of the present authors synthesized 9-aminocarbazole [11]. In this compound, an amino group in 9-position as an electron-donating group can enhance the charge transfer ability in the molecule, which can make the fluorescence of carbazole be greatly enhanced. Taking advantage of excellent fluorescence properties of 9-aminocarbazole, it is interesting to explore the possibility of using it as a fluorescent carrier for optical chemo-sensing. Among various ways of fluorescent carriers, covalent immobilization seems to be the most efficient method which can effectively prevent the leakage of the fluorescent dye form the sensor membrane, a phenomenon that shortens the long-term stability of many chemosensors [12,13]. It is desirable to introduce into 9-aminocarbazole a terminal double bond capable to co-polymerize with a monomer on the sensor surface. Reacting 9-aminocarbazole with methylacryloyl chloride to form 9-methylacryloylamino carbazole (MAC) could realize the introduction of terminal double bond. Under UV radiation MAC could be photo-copolymerized on the quartz glass surface treated with a silanizing agent. The sensing membrane prepared in such a way is expected to show strong fluorescence, which can be quenched by some agents. NO_2^- reacts with excess I^- to form I_3^- in solution of pH of 2.0, and I_3^- formed [14] can quench the fluorescence of MAC membrane. The fluorescent sensor based on MAC through covalent immobilization could be used as a very simple and sensitive tool for indirectly NO_2^- assay, which is reported in this paper.

Our interest was focused on the detection of nitrite ion. Nitrite ion is one of the parameters involved in the estima-

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tion of nitrogen fixation, denitrification, sedimentation and many other anthropogenic processes that have impact on the quality of natural, surface and underground waters. Nitrite is also known as preservative of meat and fish products. Monitoring of nitrite level in these products is of great importance as it undergoes chain reactions leading to formation of carcinogenic nitrosamines. Several methods have been reported for the quantitative determination of NO_2^-, including kinetic methods [15], chromatography [16], potentiometry [17], amperometry [18], polarography [19], spectrophotometry [20], and colorimetric methed [21]. An alternative more robust and accurate analytical procedure would be desirable for determining NO_2^-. It has been shown experimentally that nitrite can be detected by the proposed sensing membrane containing MAC.

2. Experimental

2.1. Apparatus

The fluorescence measurements were performed on a Hitachi F-4500 spectrofluorimeter (Hitachi, Japan) controlled by a personal computer data processing unit. The light source is a 150 W Xe lamp and the detector is a R928F red-sensitive photomultiplier tube. By using a specially designed flow-through cell (Fig. 2) and pumping the sample solutions at a flow rate of 2.0 ml min^{-1} using a peristaltic pump (Guokang Instruments, Zhejiang). The glass slide (diameter 25 mm) with the sensing membrane fabricared on it by a photo-polymerization procedure (vide infra) was mounted in the flow-through cell. The membrane side is facing the cell chamber with the circulating sample sweeping over the membrane and the opposite side of the glass disc tightly matching light source. A PHS-3C pH meter (Shanghai Analytical Instruments, Shanghai) was used for pH measurements. All fluorescence measurements were made under ambient temperature at 25 °C. ^{1}H NMR spectra were recorded on a INOVA-400 (Varian) spectrometer with DMSO as the solvent. MS spectra were obtained on a GC-17A, QP-5000 (SHIMADZU) spectrometer.

2.2. Materials

Carbazole was obtained form Zuzhou Pharmaceuticals (Hunan). 3-(Trimethoxysilyl)propyl-methacrylate (TSPM) was purchased from ACROS (Sweden). Sodium nitrite and potassium iodid were from Haoda Pharmaceuticals (Guangdong). A standard sodium nitrite solution was prepared by drying sodium nitrite by dissolving it in double-distilled water to give a 1.0×10^{-2} mol l^{-1} solution. This standard solution was prepared weekly and kept in refrigerator, and further dilution was made daily as required. Methylacryloyl chloride was prepared and purified in this laboratory. For measurement of pH of aqueous solutions, a series buffer solutions were prepared as follows: pH 1.7–12.0, Britton–Robinson buffer solution (0.2 M acetic acid, phosphate, borate, and 0.2 M NaOH were mixed and used). All

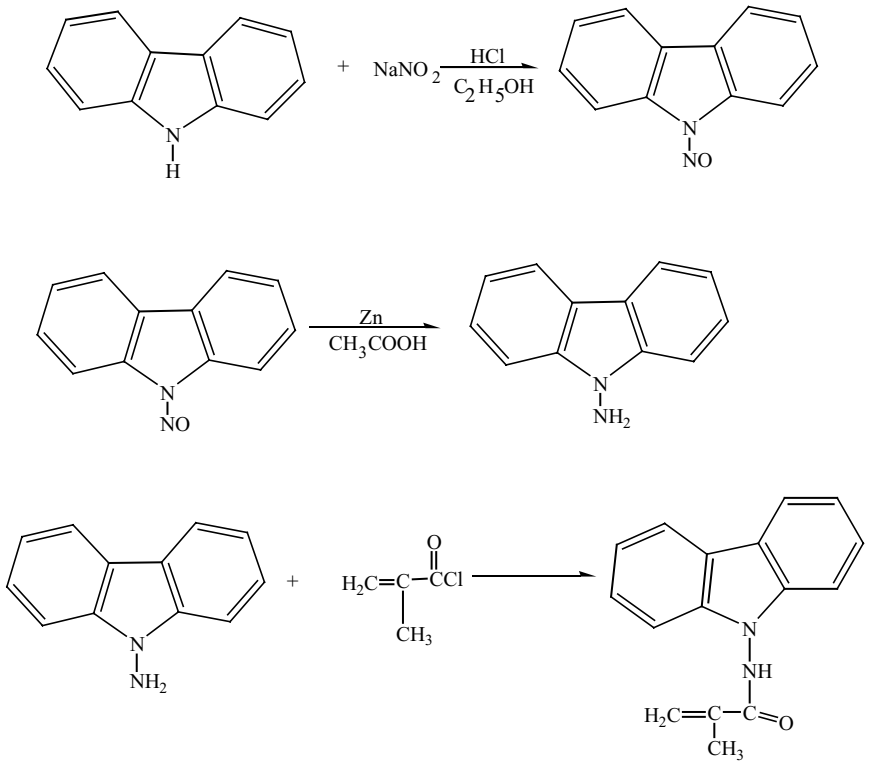

Fig. 1. Synthesis scheme of 9-methylacryloylamino carbazole.

other reagents and solvents were of analytical regent grade unless otherwise stated. Double-distilled water was used throughout.

2.3. Synthesis of 9-methylacryloylamino carbazole

The general scheme of the synthesis is shown in Fig. 1.

Carbazole (10.0 g) was reacted with sodium nitrite (4.2 g) by refluxing for 2 h in 150 ml of ethanol. To this reaction mixture, 12 ml of concentrated hydrochloric acid was added drop-wise. The yellow solid obtained was filtrated, dried, recrystallized with ethyl acetate to give 9.0 g of yellow powder solid of 9-nitrosyl carbazole with a nominal yield of 77% mp = 160 °C.

9-Nitrosyl carbazole (9.0 g) was reacted with zinc powder (5.0 g) by refluxing for 2 h in acetic acid at 0 °C. The filtrate was poured into about 2 l of water. The gray powder obtained was filtrated, dried, recrystallized with ethanol to give crystallic 9-aminocarbazole of 7.2 g with a nominal yield of 40% mp = 138–140 °C. $M^+ = 182$. ^{1}H NMR (DMSO) (ppm) 7.15–8.11 (8H, carbazole), 2.49–2.51 (2H, $–NH_2$).

Compound 9-aminocarbazole (0.5 g) and methylacryloyl chloride (0.1 mol) were mixed with 0.2 ml of triethylamine and refluxed 2 h at room temperature in 10 ml of THF. The reactive mixture was left overnight and filtrated to give a gray product of 0.42 g of 9-methylacryloylamino carbazole with a nominal yield of 38.2%. $M^+ = 250$. ^{1}H NMR (DMSO) (ppm) 7.15–8.11 (8 H, carbazole), 3.56 (1H, –NH–), 1.18 (2H, $=CH_2$), 2.51–2.52 (3H, $–CH_3$).

2.4. Preparation of the sensing membrane

For convalently immobilizing MAC on the quartz glass surface, a terminal double bond should be introduced in the quartz glass surface of the sensor. The quartz glass surface was modified by silanization as described in the literature [17,18] with some modifications. The surface was silanized according to following steps. The conventional quartz glass plates (diameter = 25 mm) were immersed in 3% HF and 10% H_2O_2 for 30 min each and washed with water. The glass plates were submerged in a TSPM solution for 3 h which was prepared by mixing 0.6 ml of TSPM, 6 ml of 0.2 mol l^{-1} HOAc-NaOAc buffer solution (pH 3.6) and 24 ml of double-distilled water. The quartz glass plates were washed with double-distilled water and dried at room temperature.

A gray transparent membrane cocktail was prepared under ultrasonic agitation by dissolving acrylamide (400 mg), benzoine ethyl ether (90 mg), benzophenone (60 mg), 1,2-cyclohexanediol biacrylate (0.1 ml), DMF (0.2 ml) and MAC (10 mg). About 3.0 ml of the cocktail solution was put onto a poly (tetrafluorethylene) plate, then a 25 mm diameter quartz glass plate treated as above was covered onto the solution drop. Under the UV radiation for about 5 h, the glass plates with the membrane formed were washed with double-distilled water in order to remove any unreacted species, then dried and stored for use.

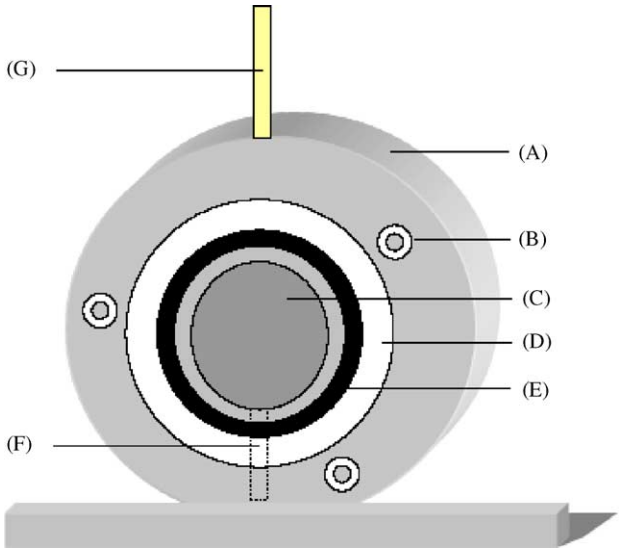

Fig. 2. Schematic diagram of the flow cell system. (A) Cell body; (B) mounting screen nut; (C) flow chamber; (D) quartz glass; (E) seal ring; (F) solution inlet channel; and (G) solution outlet channel.

2.5. Fluorescence measurements

The sensing membrane prepared as described in Section 2.4 and a 25 mm diameter glass plate were mounted in a homemade flow-through cell (Fig. 2). Fluorescence measurements were taken using the flow-through cell under an appropriate position to guarantee the intensity of fluorescence emission without interference from the excitation source. The sample solution was driven through the flow-through cell by a peristaltic pump at a flow rate of 2.0 ml min^{-1}. The sensor membrane was equilibrated with the sample solution for obtaining a stable fluorescence signal. The excitation and emission fluorescence spectra of MAC are recorded with the emission and excitation wavelengths fixed at their peaks of 367 and 290 nm, respectively. After each measurement, the fluorescence intensity of the sensing membrane was recovered by pumping the blank solution through the cell prior to the next measurement.

3. Results and discussion

3.1. Spectral characteristics

Fig. 3 shows the fluorescence spectra of the MAC membrane in buffer solutions of pH 2.0 containing various concentrations of NO_2^- with excess I^- (1.0×10^{-2} mol l^{-1}). The excitation and emission fluorescence spectra of MAC are recorded with the emission and excitation wavelengths fixed at their peaks 367 and 290 nm, respectively. Various concentrations of NO_2^- solutions with excess I^- (1.0×10^{-2} mol l^{-1}) at pH of 2.0 were pumped through the flow-cell with a flow rate of 2.0 ml min^{-1}.

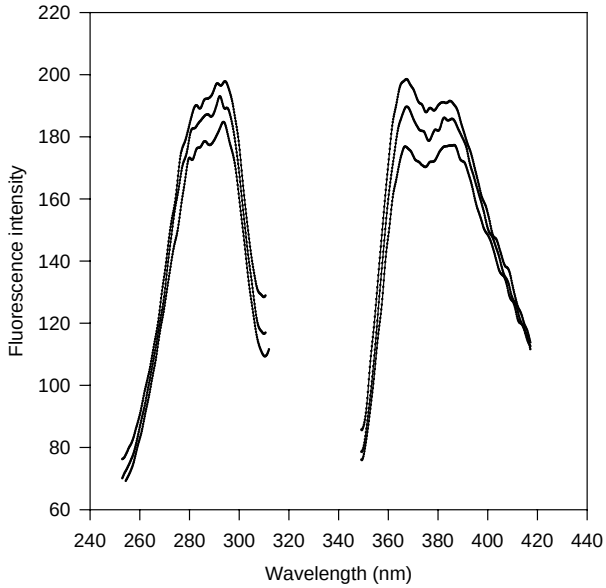

Fig. 3. Fluorescence excitation (left) and emission (right) spectra of the MAC sensor on exposing to NO_2^- solutions of different concentrations with excess I^- at pH of 2.0. From top to bottom: blank solution, 1.0×10^{-5} and 4.0×10^{-5} mol l^{-1}.

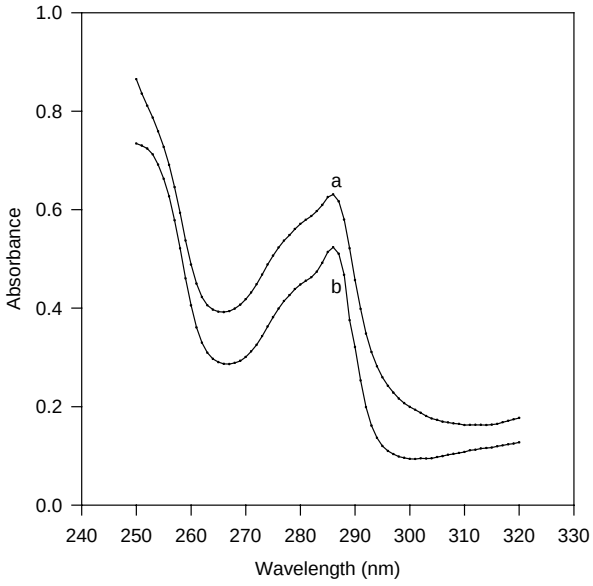

Fig. 4. The UV spectra of MAC (1.0×10^{-5} mol l^{-1}) in 10% ethanol solution (spectrum a) and MAC (1.0×10^{-5} mol l^{-1})/NO_2^- (1.0×10^{-6} mol l^{-1}) with excess I^- at pH of 2.0 in 10% ethanol solution (spectrum b).

3.2. The quenching process and the basis of quantitative assay

In order to explore the principle of the fluorescence quenching of MAC in this experiment, we noticed that the fluorescence of MAC did not change when I^- or NO_2^- separately reacted with MAC at pH of 2.0. When NO_2^- is present with excess I^- (1.0×10^{-2} mol l^{-1}) in solution of pH of 2.0, it can significantly quench the fluorescence of MAC. Based on above experiment, one could deduce the following reactions taking place

$$2NO_2^- + 3I^- + 4H^+ = I_3^- + 2NO + 2H_2O \quad (1)$$

$$I_3^- + MAC = [MAC][I_3^-] \quad (2)$$

Fig. 4 shows the UV-Vis spectra of MAC (1.0×10^{-5} mol l^{-1}) in 10% ethanol solution (spectrum a) and MAC (1.0×10^{-5} mol l^{-1})/NO_2^- (1.0×10^{-6} mol l^{-1}) and I^- (1.0×10^{-2} mol l^{-1}) in 10% ethanol solution (spectrum b) at pH of 2.0. The fluorescence of MAC is significantly quenched by addition of different concentration of NO_2^- with excess I^- (1.0×10^{-2} mol l^{-1}) at pH of 2.0 (Fig. 3). The absorbance spectra also changed obviously (Fig. 4), which indicates that MAC and I_3^- are likely to form a ground state complexes.

When the MAC sensing membrane contacts with various concentrations of NO_2^- with excess I^- solutions of pH of 2.0, I_3^- formed reacts with MAC in the membrane phase. An equilibrium between I_3^- in the aqueous solution phase (aq) and MAC in membrane phase (mem) is established with the formation of a *m*:*n* complex

$$mI_3^-(aq) + nMAC(mem) \overset{K}{=} (I_3^-)_m(MAC)_n(mem) \quad (3)$$

Here, K is the equilibrium constant. The difference between the activities and concentrations is neglected. According to the law of mass action, K can be expressed as

$$K = \frac{[(I_3^-)_m(MAC)_n]_{mem}}{[I_3^-]^m_{(aq)}[MAC]^n_{(mem)}} \quad (4)$$

The relative fluorescence intensity α is defined as

$$\alpha = \frac{F - F_s}{F_0 - F_s} \quad (5)$$

Here, F is fluorescence intensity when the sensor is contacted with the solution of I_3^- formed; F_s is the F when the complete complexation of MAC in the membrane by I_2 formed takes place. F_0 is the F when the membrane is contacted with the blank solution. Combining Eqs. (4) and (5), one obtains

$$\frac{a^n}{1-a} = \frac{1}{nK[MAC]^{n-1}_{(mem)}[I_3^-]^m_{(aq)}} \quad (6)$$

By changing the ratio of *m*:*n* and adjusting the value of equilibrum constant K, one can fit the experimental data to Eq. (6), with the fitting results shown in Fig. 5. The complex ratio of 1:1 and a reasonable K value of 6.2×10^3 provides the best fit for the experimental data points.

3.3. Effect of pH

Though the fluorescence intensity of the sensing membrane was found to be pH-dependent, NO_2^- react with excess I^- (1.0×10^{-2} mol l^{-1}) forming I_3^- only at pH of 2.0, which can quench the fluorescence of MAC. A pH 2.0 B–R buffer solution was used in subsequent experiments.

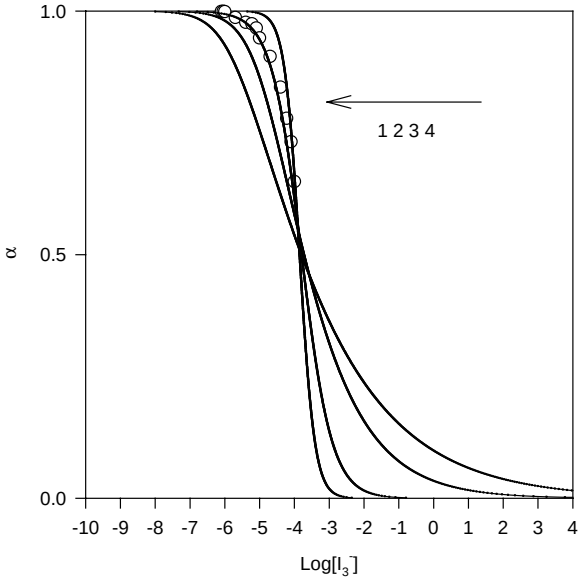

Fig. 5. Relative fluorescence intensity values (α) as a function of log[I_3^-]. The curves fitting the experimental data calculated from Eq. (6): (1) $m{:}n = 1{:}5$, $K = 2.0 \times 10^{12}$; (2) $m{:}n = 1{:}3$, $K = 7.0 \times 10^{7}$; (3) $m{:}n = 1{:}1$, $K = 6.2 \times 10^{3}$; and (4) $m{:}n = 2{:}1$, $K = 5.0 \times 10^{7}$.

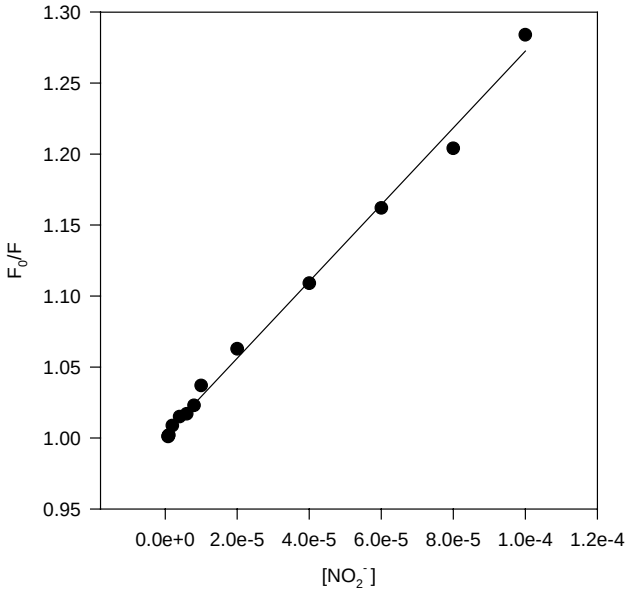

Fig. 6. A plot of F_0/F as a function of the concentration of NO_2^-.

3.4. Selectivity

Table 1 shows the effect of different possible interferents. For many common inorganic salts less than 3.11% relative error was obtained for the concentration level specified. Fe^{3+}, $Cr^2O_7^{2-}$, and MnO_4^- which were redox species showed interference at pH of 2.0 as expected, these redox compounds seem not to co-exist with NO_2^- in common situation. The sensor has a sufficient selectivity for NO_2^-.

Table 1
Effect of different interferents on the relative fluorescence intensity of the sensing membrane[a]

Interference	Relative fluorescence change value (%) $(F_2 - F_1)/F_2 \times 100$[b]
$AlCl_3$	0.55
$CdCl_2$	−0.62
$Co(NO_3)_2$	−0.28
$Cr_2O_7^-$	33
$Cu(NO_3)_2$	−3.11
$FeCl_2$	0.55
$FeCl_3$	29
Kl	−0.62
KCl	0.21
$MgSO_4$	−0.75
$Mn(Ac)_2$	0.68
MnO_4^-	30
NaCl	0.32
$NaNO_3$	0.20
NH_4Cl	1.05
Ni_2SO_4	−1.78
$Zn(NO_3)_2$	0.28

[a] Each solution contains a fix NO_2^- concentration of 1.0×10^{-5} mol l^{-1}/I^- (1.0×10^{-2} mol l^{-1}) and an interferent concentration of 1.0×10^{-3} mol l^{-1} at pH of 2.0.

[b] F_1 and F_2 are the fluorescence intensities of the optical membrane contacted with the 1.0×10^{-5} mol l^{-1} NO_2^- solution without and with 1.0×10^{-3} mol l^{-1} interferents, respectively.

3.5. Quantitative determination, measurement range and detection limit

The quenching efficiency is given by the Stern–Volmer equation based on the $m{:}n = 1{:}1$ complex

$$\frac{F_0}{F} = 1 + K[NO_2^-] \quad (7)$$

The F_0/F values show a quasi-linear relationship with the I_3^- formed in the concentration range of 1.0×10^{-6} to 1.0×10^{-4} mol l^{-1} with the regression equations of the form $F_0/F = 1.002 + 2704.244\ [NO_2^-]$ ($r = 0.9951$) (Fig. 6). This provides the quantitative basis for NO_2^-. The detection limit was evaluated to be 8.0×10^{-7} mol l^{-1} as corresponding to three times of the fluorescence intensity value of the blank solution. The determination of NO_2^- in some samples shows satisfactory veracity and recoveries (Table 2).

3.6. Repeatability, reversibility, and response time

The repeatability and reversibility of the sensor were evaluated by exposing alternatively to NO_2^- solutions of 1.0×10^{-5}, 2.0×10^{-5}, 8.0×10^{-5}, and 1.0×10^{-4} mol l^{-1} of pH 2.0 with excess I^- solution and blank buffer solution (Fig. 7). The mean fluorescence intensity values at 365 nm with the standard deviation were found to be 197.5 ± 0.78 ($n = 9$, blank buffer solution), 188.5 ± 0.10 ($n = 2$, 1.0×10^{-5} mol l^{-1} NO_2^-), 175.3 ± 0.21 ($n = 2$, 2.0×10^{-5} mol l^{-1} NO_2), 165.5 ± 0.15 ($n = 2$, 8.0×10^{-5} mol l^{-1} NO_2^-), 154.7 ± 0.14 ($n = 2$, 1.0×10^{-4} mol l^{-1} NO_2^-). The response time of the sensor was less than 30 s and the recovering time was about 40 s.

Table 2
Determination results of NO_2^- in samples solution[a]

Sample	Present (mol l^{-1})	Griess (mol l^{-1})	Added (mol l^{-1})	Total found (mol l^{-1})	Recovery (%)	R.S.D. (%)
Top water[b]	3.62×10^{-6}	3.57×10^{-6}	1.00×10^{-5}	1.34×10^{-5}	98.7	3.1
Pond water	1.42×10^{-6}	1.40×10^{-6}	5.00×10^{-6}	6.45×10^{-6}	100.4	2.7

[a] The mean values and standard deviations of the three determination.
[b] Top water was 10 times concentrate.

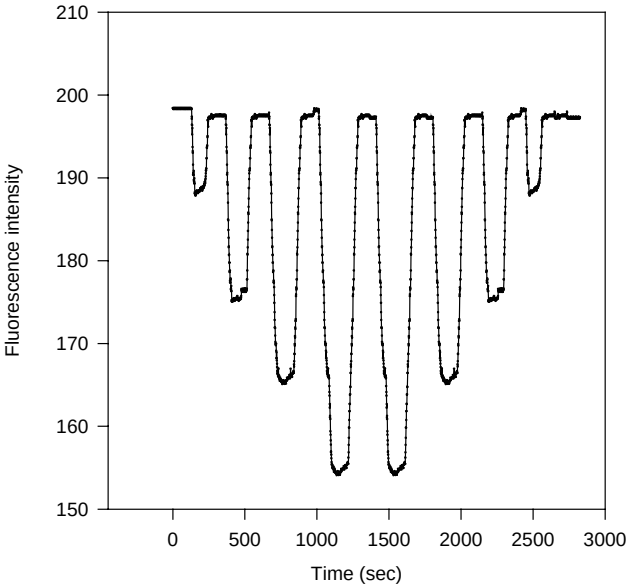

Fig. 7. Fluorescence intensity vs. time when the sensor is exposed alternatively to blank solution and 1.0×10^{-5} mol l^{-1}, 2.0×10^{-5} mol l^{-1}, 8.0×10^{-5} mol l^{-1}, 1.0×10^{-4} NO_2^- solution with excess I^- at pH of 2.0.

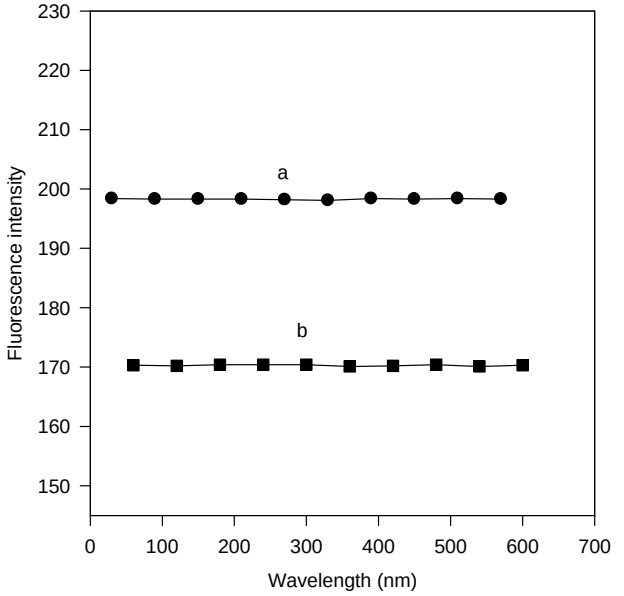

Fig. 8. The stability of the sensor exposed to NO_2^- (5.0×10^{-5} mol l^{-1}) of pH of 2.0 with (a) buffer solution and (b) excess I^- solution. Fluorescence intensities were recorded with intervals of 30 min.

Table 3
The figures of the sensor's merit

Response time (s)	30–40
LOD (mol l$^-$)	8.0×10^{-7}
Precision (S)	0.15–0.25
Accuracy(R.E.)	0.16–0.3
Dynamic range (mol l$^-$)	1.0×10^{-6} to 1.0×10^{-4}

3.7. Short-time stability and lifetime

In order to examine the short-time stability of the sensor, the later is alternatively exposed to the blank buffer solution and NO_2^- (5.0×10^{-5} mol l^{-1}) of pH 2.0 with excess I^- solution in a period of 10 h. The fluorescence quenching intensity was recorded at interval of 30 min (Fig. 8). The fluorescence response remains stable even after 8 weeks usage. The carrier immobilized on glass plates by covalent bonding effectively prevented the leakage of carrier. A newly prepared sensor can be used at least 8 weeks.

4. Conclusions

The classical fluorescence agent carbazole has been modified by introducing a terminal double bond which makes it possible to covalently immobilize the dye on the surface of the modified quartz glass. The sensor prepared using the carbazole containing membrane shows excellent analytical characteristics (Table 3). The fluorescence quenching effect can be utilized for nitrite (NO_2^-) assay. The life-time of the sensor is guaranteed due to the prevention of carrier leakage by covalent immobilization.

Acknowledgements

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