Fiber-Optic Sensor for Iodine Based on a Covalently Immobilized Aminobenzanthrone Schiff Base

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An aminobenzanthrone Schiff base has been synthesized as a new fluorescence carrier for the preparation of an optical chemical sensor for iodine. The response of the sensor is based on fluorescence quenching of the aminobenzanthrone Schiff base by iodine. The sensor shows a linear response toward iodine in the range of 1.0×10^{-5} to 1.0×10^{-3} mol l⁻¹, with a detection limit of 6.0×10^{-6} mol l⁻¹ at pH 8.0. Leaching of the fluorophore from the membrane is effectively hindered by covalent immobilization, resulting in an enhanced sensor lifetime. In addition to satisfactory reproducibility and reversibility, the prepared sensor exhibits sufficient selectivity toward iodine with respect to other coexisting ions. The sensor has been applied to the determination of iodine in common salt samples.

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Introduction

As an essential element in nutrition, iodine plays an important role in the growth and development of human beings. Both excessive and deficient iodine intake can give rise to different kind of diseases. Hence, there is a need for a simple, sensitive and reliable analytical method for the determination of iodine. In the published literature, a number of methods have been proposed to determine iodine, including colorimetry,¹ gas-liquid chromatography,² spectrophotometry,³ neutron activation analysis⁴ and ion chromatography.^{5,6} However, some of these methods require a cumbersome pretreatment of the samples, or sophisticated instrumentation, which restricts their widely practical application.

In recent years, much interest has arisen concerning the development of optical chemical sensors for analyte monitoring, since they can offer the advantages of simple preparation, reasonable sensitivity and selectivity. Many optical sensors have been reported for monitoring the pH,⁷⁻⁹ heavy metal ions^{11,12} and dissolved oxygen.¹³ Also, few attempts have been made to develop optical chemical sensors for iodine determination.¹⁴⁻¹⁶ Fluorescent dyes, such as fluoranthene and carbazole derivative, have been used as carrier compounds for iodine sensing, though the number of useful fluorescent carriers remains limited. Looking for new fluorescent carriers with excellent analytical characteristics is of interest.

Schiff bases, owing to their coordinating ability with the metal ions, have been the subject of analytical investigations for many years. They are a category of important organic compounds that have been employed as polymer laser dyes¹⁷ and polymer ultraviolet stabilizers.¹⁸ Recent attention has been focused on their application in antibacterial biological and anticancer activities.¹⁹ However, a Schiff base used as a fluorescence

carrier for preparing an optical sensor was rarely reported.²⁰ In order to extend the scope of useful fluorescent carriers of Schiff-base derivatives, an aminobenzanthrone Schiff base was synthesized by reacting 2-aminobenzanthrone with *p*-allyloxybenzaldehyde in our laboratory. Though Schiff-base complexes are usually relatively unstable, the aminobenzanthrone Schiff base synthesized is extraordinary stable, which seems to be associated with a dispersion effect of the phenyl group of *p*-allyloxybenzaldehyde with respect to the electron around the >C=N< bond in the Schiff base. As a fluorescence carrier, the aminobenzanthrone Schiff base with terminal double bounds was capable of copolymerization with a monomer on the modified sensor surface. Covalent immobilization^{21,22} can effectively prevent leakage of the carrier dye from the sensor membrane, and thereby provide super long-term stability. It has been shown experimentally that a sensing membrane containing an aminobenzanthrone Schiff base shows strong fluorescence, which can be quenched by iodine. The quenching effect can be utilized for the fluorometric determination of iodine.

In this paper, a new optical chemical sensor based on the fluorescence quenching of aminobenzanthrone Schiff base is described for the determination of iodine. The spectral characteristics and the analytical performance of the optical sensor are considered.

Experimental

Apparatus

Fluorescence measurements were conducted on a Hitachi F-4500 spectrofluorometer (Japan) with both excitation and emission slits set at 10 nm, controlled by a personal computer data-processing unit. The light source was a 150 W Xe lamp and the detector was an R928F red-sensitive photomultiplier tube. A homemade poly(tetrafluoroethylene) flow-cell and a bifurcated fiber were used for iodine sensing measurements.²³

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The excitation light was carried to the cell through one arm of a bifurcated optical fiber, and the emission light collected through the other. A glass plate (diameter 13 mm) covered with a sensing membrane was fixed on the top of the flow chamber by a mounting screw nut with the membrane in contact with the sample solution. The sample solution was driven through the flow cell by a peristaltic pump (GuoKang Instruments, Zhejiang, China) at a flow rate of 2.0 ml min⁻¹. All fluorescence measurements were made under ambient temperature at 25°C. A PHS-3C pH meter (Shanghai Analytical Instruments, Shanghai) was used for pH measurements.

Materials

2-Aminobenzanthrone was purchased from Shanghai Chemicals (Shanghai, China) and 3-(trimethoxysilyl)propyl methacrylate (TSPM) was from ACROS (Sweden). Allyl bromide was synthesized according to a method described elsewhere²⁴ and reacted with *p*-hydroxybenzaldehyde to form *p*-allyloxybenzaldehyde. 2-Hydroxyethyl methacrylate (HEMA), acrylamide and 1,2-cyclohexanediol diacrylate were of analytical reagent grade used in the membrane matrix preparation without further purification. Britton–Robinson (B–R) buffer solutions covering pH values of 1.7–12.0 were prepared by mixing appropriate amounts of phosphoric acid, acetic acid, and boric acid, and adjusting to the desired pH with 0.2 mol l⁻¹ sodium hydroxide.

A 2.0×10^{-2} mol l⁻¹ stock standard solution of iodine was prepared by dissolving 1.27 g of iodine in 100 ml of distilled water; then, 1.92 g of potassium iodide was added until the iodine dissolved completely. The resulting solution was diluted to 250 ml with doubly distilled water and stored in a brown glass reagent bottle. Iodine solutions of other concentrations were obtained by serial dilution with a pH 8.0 B–R buffer solution. The actual concentration of iodine was determined by iodimetry.

Unless otherwise stated, all other reagents and solvents were of analytical reagent grade. Doubly distilled water was used throughout.

Synthesis of Schiff base

Acetone (40 ml) was added into a 150 ml boiling flask, then *p*-hydroxybenzaldehyde (2 g), allyl bromide (2 ml) and potassium carbonate (2.64 g) were added. The mixture was stirred continuously and refluxed for 1.5 h at 85–90°C. After removing acetone, the brown *p*-allyloxybenzaldehyde was collected. The *p*-allyloxybenzaldehyde (0.8 g) dissolved in 40 ml of ethanol (40 mg) was added to a 250 ml flask, then 2-aminobenzanthrone (1.0 g) dissolved in 30 ml of ethanol was slowly dropped into the stirred solution from a funnel in 30 min. Subsequently, the mixture solution was stirred continuously for 30 min at room temperature, and then refluxed for 2.5 h at 90°C. After cooling, the mixture was distilled, and a purple crystal product (1.143 g) of aminobenzanthrone Schiff base was obtained with a nominal yield of 62%. (m.p., 148°C; MS, base peak, 245; M⁺, 389). The general scheme of the synthesis is shown in Fig. 1.

Preparation of the sensing membrane

The glass surface was modified by silanization according to the following steps, as described in the literature with some modifications.^{25,26} Conventional glass plates (diameter 13 mm) were immersed successively in 3% HF and 10% H₂O₂ for 30 min each, and then washed with doubly distilled water. A solution of TSPM was prepared by mixing 0.2 ml of TSPM, 2 ml of 0.2 mol l⁻¹ HOAc–NaOAc buffer solution of pH 3.6 and 8

Fig. 1 Synthesis scheme of an aminobenzanthrone Schiff base.

ml of double distilled water. After sufficient mixing, the glass plates were soaked in this mixture for 2 h, then washed with doubly distilled water and dried at room temperature.

The sensing membrane was prepared according to the following procedure. Acrylamide (200 mg) was dissolved in 0.20 ml of *N,N*-dimethylformamide (DMF), with a subsequent addition of HEMA (0.25 ml), 1,2-cyclohexanediol diacrylate (0.10 ml), aminobenzanthrone Schiff base (10 mg), benzoin ethyl ether (10 mg), and benzophenone (15 mg). Drops of the solution were taken onto a cleaned poly(tetrafluorethylene) plate, then silanized glass plates were placed over the droplets. After UV radiation (254 nm, 30 W, 10 cm over the glass plates) for about 2 h, glass plates with the membrane were washed with water and methanol to remove any unreacted species, then dried and stored for use.

Measurement procedure

Two arms of the bifurcated optical fiber were fixed in the detecting chamber of the spectrofluorometer to carry the excitation and emission light. The prepared sensing membrane was fixed on top of the flow chamber by a mounting screw nut with the membrane in contact with the sample solution. The sample solution was driven through the flow cell by a peristaltic pump (GuoKang Instruments) 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 aminobenzanthrone Schiff base were recorded with the emission and excitation wavelengths fixed at their peaks of 535 and 595 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.

Results and Discussion

Effect of acidity

The fluorescence intensity of the sensing membrane was found to be pH-dependent, though the effect is relatively weak. The properties of Schiff bases are sensitive to the pH of the solution, and the influence of acidity is, however, decreased after photopolymerization. Figure 2 shows the effect of the pH on the fluorescence quenching efficiency, F_0/F , where F_0 and F denote the fluorescence intensity in the absence and presence of iodine, respectively. The iodine solution was buffered at pH 2.0–12.0, and the maximum of the quenching efficiency was observed at pH 8.0. Since pH 8.0 is most favorable for

Fig. 2 Effect of the pH on F_0/F (iodine concentration: 2.0×10^{-4} mol l^{-1}).

fluorescence quenching, a pH 8.0 B-R buffer was used for the determination of iodine in aqueous solutions in subsequent experiments.

Spectrum characteristics

Iodine can strongly quench the fluorescence of the aminobenzanthrone Schiff base copolymer. Figure 3 shows the fluorescence spectra of the sensing membrane in buffer solutions of pH 8.0 containing different concentrations of iodine. The excitation spectra were recorded by fixing the emission wavelength at 595 nm, the peak of the emission spectrum. The emission spectra were recorded by fixing the excitation wavelength at 535 nm. Various concentrations of iodine solutions were driven through the flow-cell by a peristaltic pump at a flow rate of 2.0 ml min^{-1} .

Principle of operation

In order to gain insight into the principle of the fluorescence quenching of aminobenzthiazole Schiff base, the effects of the concentrations of iodine and I^- were examined independently. The experimental results show that the fluorescence of the aminobenzthiazole Schiff base in the membrane is quenched by iodine. Neither I^- nor Br^- shows any interference effect on the fluorescence quenching. While in contact with the Schiff base-containing membrane, iodine in aqueous solution was extracted into the membrane phase and interacted with the Schiff base to form a non-fluorescent ground-state complex. When this complex is excited by the absorption of a photon, it immediately returns to the ground state *via* a non-radiative process without the emission of fluorescence. As a result, significant fluorescence quenching of the Schiff base was observed. If the completed equilibrium between iodine in the aqueous solution phase (aq) and aminobenzthiazole Schiff base in the membrane phase is set up with the formation of an $m:n$ complex, the equilibrium can be described as follows:

Here, SB represents an aminobenzthiazole Schiff base. If the difference between the activities and the concentrations is neglected, the corresponding equilibrium constant (K) can be expressed by the law of mass action,

$$K = \frac{[(\text{I}_2)_m(\text{SB})_n]_{(\text{mem})}}{[\text{I}_2]_{(\text{aq})}^m [\text{SB}]_{(\text{mem})}^n}. \quad (2)$$

Fig. 3 Fluorescence excitation (left) and emission spectra (right) of the sensor on exposure to (from top to bottom). Blank solution, 2.5×10^{-4} , 6.0×10^{-4} , 8.0×10^{-4} , 1.0×10^{-3} , 1.5×10^{-3} , 1.8×10^{-3} , and 2.0×10^{-3} mol l^{-1} iodine solutions.

For investigating the influence of possibly varied experimental conditions and other factors, the value of α can be defined as the relative fluorescence intensity,

$$\alpha = \frac{F - F_s}{F_0 - F_s}. \quad (3)$$

Here, F is the fluorescence intensity of the sensing membrane exposed to iodine solutions of different concentration. F_0 is the fluorescence intensity when the sensing membrane is in contact with the blank B-R buffer solution. F_s is the fluorescence intensity when the Schiff base is completely complexed with iodine. According to Eqs. (2) and (3), the expression of α depends on the concentration of iodine in an aqueous sample solution:

$$\frac{\alpha^n}{1 - \alpha} = \frac{1}{nK[\text{SB}]_{(\text{mem})}^{n-1}[\text{I}_2]_{(\text{aq})}^m}. \quad (4)$$

Taking the logarithm of Eq. (4), the following equation is obtained:

$$\log\left(\frac{1 - \alpha}{\alpha^n}\right) = \log(n \cdot K \cdot [\text{SB}]_{(\text{mem})}^{n-1}) + m \log[\text{I}_2]_{(\text{aq})}. \quad (5)$$

From Eq. (5), it can be seen that when the stoichiometric ratio of the complex changes, the relative fluorescence value (α) has different functional relations with $\log[\text{I}_2]_{(\text{aq})}$. Equation (5) can be used as a basis for estimating the complex ratio between the iodine and aminobenzanthrone schiff base by using a curve-fitting procedure.²⁷ The experimental data were fitted to Eq. (5) by changing the $m:n$ ratio and adjusting the equilibrium constant (K). Figure 4 shows the fitting results; the curve corresponding to the 1:1 complex ratio and $K = 8200$ are best fitted to the experiment data points.

Quantitative determination and detective limit

Because fluorescence quenching originates from the 1:1 ground-state complex formation, the following equation can be derived for describing of the quenching efficiency:

$$\frac{F_0}{F} = 1 + K[\text{I}_2]_{(\text{aq})}.$$

Fig. 4 Curve fitting for the experimental data. (a) $m:n = 1:3$, $K = 1.85 \times 10^7$; (b) $m:n = 1:2$, $K = 1.3 \times 10^5$; (c) $m:n = 1:1$, $K = 8200$; (d) $m:n = 2:1$, $K = 1.5 \times 10^6$.

Fig. 5 Plot of F_0/F as a function of the concentration of iodine.

When the concentration of iodine was varied in the range of 1.0×10^{-5} – 1.0×10^{-3} mol l $^{-1}$, the experimental data fitted the following calibration equation: $F_0/F = 1.000265 + 254.928[I_2]$ ($r = 0.9934$). Figure 5 shows the results. The calibration equation provides a quantitative basis for the determination of iodine in sample solutions. A detection limit, defined as three-times of the standard deviation of the black readings, is 6.0×10^{-6} mol l $^{-1}$ ($n = 11$).

Reproducibility, reversibility and response time

The reproducibility and reversibility of the sensing membrane were evaluated by recording the value of fluorescence during alternatively pumping iodine solutions of 5.0×10^{-4} mol l $^{-1}$, 2.0×10^{-4} mol l $^{-1}$ and a blank buffer solution of pH 8.0. Figure 6 shows the fluorescence intensity changes vs. time for the sensing membrane. The relative standard deviations of the fluorescence intensities recorded from eight replicates of 5.0×10^{-4} mol l $^{-1}$ and 2.0×10^{-4} mol l $^{-1}$ were found to be 4.37% and 3.61%, respectively. The reproducibility and reversibility seem to be satisfactory. The response time of the sensor was about 2 min, and the recovering time was about 4.5 min.

Stability and lifetime

For evaluating the stability of the proposed sensor, the sensing membrane was in contact with a buffer solution and an iodine solution of 2.0×10^{-4} mol l $^{-1}$ over 6 h. Figure 7 shows the membrane fluorescence intensities for recorded at intervals of 30 min. The relative standard deviations were 2.54% and

Fig. 6 Fluorescence intensity vs. time by alternatively pumping blank solutions (a) and iodine solutions of 2.0×10^{-4} mol l $^{-1}$ (b), 5.0×10^{-4} mol l $^{-1}$ (c).

Fig. 7 Stability of the sensor exposed to buffer (A) and 2.0×10^{-4} mol l $^{-1}$ iodine solution (B). Fluorescence intensities were recorded with intervals of 30 min.

3.48%, respectively. Actually fluorescence intensities of the same sensing membrane remained reproducible for quite a long time period. The membrane showed satisfactory stability, and possessed a lifetime of one month. The covalent immobilization of the fluorescence carrier is favorable to guarantee a sufficient lifetime.

Selectivity

The effect of interferences on the fluorescence determination of iodine was also investigated. The sensing membrane was contacted with an iodine solution of 2.0×10^{-4} mol l $^{-1}$ containing each interference compound, such as common inorganic ions as well as some possible co-existing pharmaceutical species. The selectivity was evaluated by the relative error of the fluorescence intensity changes. The co-existing species is considered not show any significant interference if the relative error is less than $\pm 5\%$. The results are summarized in Table 1. I $^{-}$ at a high concentration of 6.0×10^{-2} mol l $^{-1}$ shows no interference for the determination of iodine. The sensor exhibits reasonable selectivity for iodine, making its application to the analysis of real samples feasible.

Table 1 Effect of interferents on fluorescence intensity of the optode membrane^a

Interferent	Relative fluorescence change value ^b , % ($\Delta F/F_0$) $\times 100$
NaCl	-1.86
KCl	-1.46
KBr	3.68
KI	-1.75
MgSO ₄	-2.63
ZnCl ₂	2.22
Cu(NO ₃) ₂	1.19
Chlorphenamine	3.55
Phenobarbital	-1.36
Aspirin	4.35
Phenytion sodium	1.96
Virugon	-2.50
Cefazolin sodium	-3.20
Benzylpenicillin sodium	2.63
Dexamethasone	4.02
Acetylspiramycin	3.65
Sufadiazine	2.14
Norfloxacin	4.33
Diprophylline	-3.51
Gentamycin suiface	-2.59
Furosemide	3.69
Vitamin B ₆	-2.07

a. Each solution contains a fixed iodine concentration of 2.0×10^{-4} mol l⁻¹ and an interferent concentration of 1.0×10^{-3} mol l⁻¹ except of KI (the concentration of KI is 6.0×10^{-2} mol l⁻¹).

b. $\Delta F = F - F_0$, F_0 and F are the fluorescence intensity of the optical membrane in contact with the 2.0×10^{-4} mol l⁻¹ iodine solution without and with interferents, respectively.

Table 2 Determination of iodine in common salt samples

Added/ mol l ⁻¹	Found/ mol l ⁻¹	Average/ mol l ⁻¹	Rec., %
8.0×10^{-5}	7.64×10^{-5}	8.28×10^{-5}	91.8
2.0×10^{-5}	1.74×10^{-5}	1.93×10^{-5}	93.7
6.0×10^{-5}	5.57×10^{-5}	5.30×10^{-5}	90.6

Recovery experiments

The proposed sensor was applied for the direct determination of iodine in common salt samples. A series of recovery experiments in salt samples were carried out by adding certain amounts of iodine solution to the tested samples. The sample solutions were diluted with buffer solution of pH 8.0 and analyzed using the proposed sensor by a calibration-curve method. As shown in Table 2, the recovery was satisfactory.

Conclusions

The newly synthesized aminobenzanthrone Schiff base has been utilized as a fluorescent carrier in the preparation of an optical sensor for iodine. The sensor shows sufficient performance in terms of selectivity, reproducibility and response time. The lifetime of the sensing membrane is guaranteed by covalent

immobilization. The new analytical method presented is simple and reliable, which provides an alternative approach for iodine assay.

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