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Fiber-Optic Fluorescing Sensors for Nitrate and Nitrite Detection

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Fiber-optic sensors allow remote analyses of chemical substances and they now find many applications in chemistry and biology [1,2]. The purpose of this short report is to give our first results in the development of optical-fiber chemical sensors. Among the numerous known spectrometric methods, we chose the fluorometric one, generally described as a suitable method for determining substances at the parts per million or parts per billion level, with the objective of analyzing nitrate and nitrite anions, using modifications of the fluorescence emission of suitable dyes. The detection of nitrates is based on the irreversible nitration of fluorescein, which leads to a subsequent inhibition of fluorescence emission [3]; determination of nitrites corresponds to their addition on 2,3-diaminonaphthalene, which on the contrary, improves the fluorescence emission [4]. To set up simple instrumentation, we are developing fiber-optic sensors. This consists of (i) realizing an extrinsic active optical fiber by chemical linkage of suitable fluorescent dyes on silica fiber involving silanization reaction (APTES) and chemical methods and (ii) designing an optical device which is appropriate for measurements with optical fibers. The threshold of detection, coating efficiency, and stability with time are presented.

KEY WORDS: Fluorescence; fiber-optic; nitrate; nitrite; water pollutants.

INTRODUCTION

Both nitrates, which are used largely as chemical fertilizers, and nitrites, which are commonly used as food preservatives or come from nitrate reduction, are considered carcinogenic substances, because of their evolution to nitrosamines in human body. It becomes necessary to evaluate very low concentration of these anions in drinking water, even as traces. Our objective is to analyze nitrate and nitrite anions using modifications of fluorescence emission of suitable dyes.

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EXPERIMENTAL

Chemical Sensor Preparation

Before chemical treatment, the fibers were stripped of jacket and optical cladding. The cladding chemical stripping solutions was O. F. Stripper "S" (Lumer, Bagnolet, France). The surface of the fibers was activated in hot 1–2 *N* HNO_3 (50°C) for 1 h, washed with water and acetone, and dried at 100°C overnight. The activated fibers were placed in a flask containing APTES (3-aminopropyltriethoxysilane) in toluene. The mixture was gently stirred at 110–120°C for 5 h. Coupling of the amino groups of the surface was achieved with fluorescein isothiocyanate [5].

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Fluorescence Measurements of the Chemical Sensor

The system includes an argon laser (488 nm) as the exciting source. The light beam is focused on an optical fiber which guides the light to the chemical sensor. This sensor is a 3-cm-long silica multimode optical fiber (core diameter, 1mm; silicone as optical cladding; PCS 1000 W, Quartz & Silice, Paris, France). The fiber is immobilized in a quartz cell, containing a borate buffer, pH 8.6, inside a SPEX spectrofluorimeter whose detection system is used to determine fluorescence intensity levels.

RESULTS AND DISCUSSION

Chosen Probe for Nitrate

Only a few methods are available for direct determination of nitrate, and generally they require a concentrated sulfuric acid medium. Among them, we chose, nevertheless, the method which describes the quenching effect of nitrate on the fluorescence of fluorescein due to the formation of a nonfluorescent dinitrated compound [3] (see Fig. 1). We first reproduced the experiment following the process given elsewhere [6] (see Fig. 2).

Chosen Probe for Nitrite

Fluorimetric determination of nitrite is better described and it is often used as an indirect method for nitrate analysis after a reduction step. The retained process involves the reaction of nitrite with 2,3-diaminonaphthalene (DAN), giving the fluorescent 2,3-naphthotriazole (see Fig. 3). The method is generally described with a great excess of DAN ($5.5 \cdot 10^{-5}$ *M*, compared to the range of nitrite for analysis, $0–2 \cdot 10^{-7}$ *M*) [4,7,8]. As it is not possible to have such a concentration on the fiber surface, we first tried to improve the experimental conditions; indeed, we were able to perform the analysis by decreasing the DAN quantity by a factor of 100 (see Fig. 4).

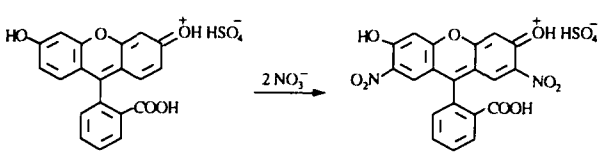


Fig. 1. Reaction of nitrate anions on fluorescein in 95% H_2SO_4.

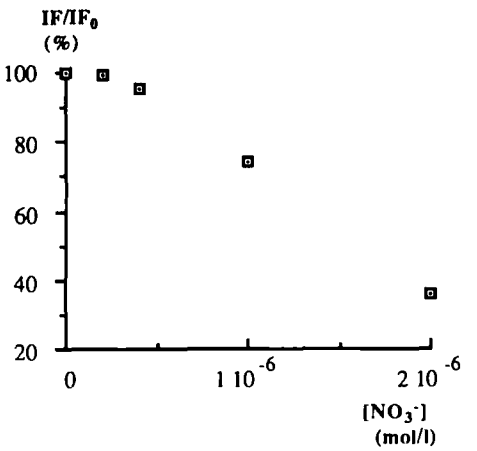
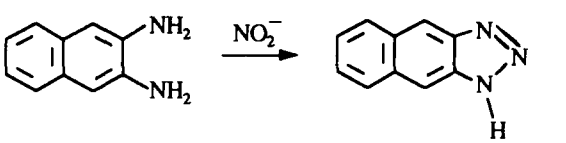


Fig. 2. Fluorescence intensity of fluorescein ($5 \cdot 10^{-7}$ *M*) in 95% H_2SO_4 versus $[NO_3^-]$.



2,3-diaminonaphthalene (DAN) — 1-[H]-naphthotriazole fluorescent

Fig. 3. Reaction of nitrite anions on DAN in 0.025 *N* HCl.

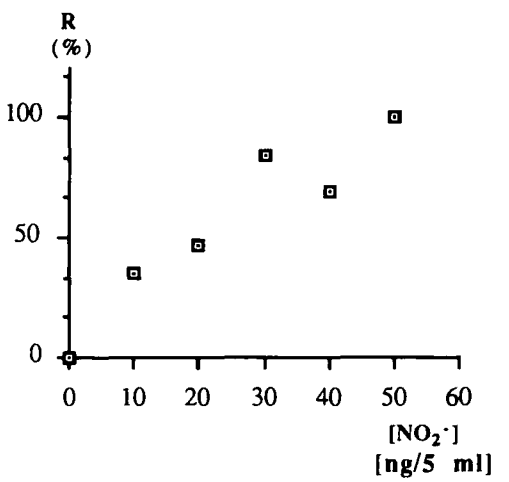


Fig. 4. Fluorescence intensity of naphthotriazole versus $[NO_2^-]$.

Fiber-Optic Chemical Sensor for Nitrate

Spectra. The emission spectrum of the immobilized fluorescein was found to be very similar to that of the

free fluorescein in alkaline medium (see Fig. 5), except that it is slightly red-shifted (520 instead of 515 nm). The sensor signal corresponds to an approximately $3 \cdot 10^{-7}$ *M* alkaline fluorescein solution.

Determination of Amount Bound. The amount of bound fluorescein was determined after stripping the probe from the fiber in alkaline hydrolysis (0.1 *N* NaOH) and measuring the fluorescein concentration by fluorimetry. The amount of indicator bound onto silica was estimated by comparing the fluorescence intensity of the sensor with that of a solution.

When the silanizing process is run without particular precautions, a polysiloxane network occurs on the silica surface, due to water hydrolysis of APTES. This leads to binding densities higher than $5 \cdot 10^{-10}$ mol cm^{-2}. Under strictly anhydrous silanizing conditions (distilled APTES and toluene; dry nitrogen atmosphere during the reaction), we obtained binding densities between $2 \cdot 10^{-11}$ and $1.2 \cdot 10^{-10}$ mol cm^{-2}.

The siloxane monolayer is preferable because of the better accessibility of nitrates to fluorescein and the probe's sensitivity, binding stability, reproducibility, and homogeneity.

Stability. A fiber was placed in a known volume of 95% sulfuric acid under vigorous stirring and the fluorescence of the solution was measured at 15-min intervals, then compared with a calibration graph; after each analysis, the acidic solution was changed. The amount of released fluorescein was determined over 3 h (see Fig. 6). The stability of these sensors in concentrated sulfuric acid is surprisingly high. After 45 min, which corresponds to the time required for nitration, the loss of the probe represents only 4% of the overall binding.

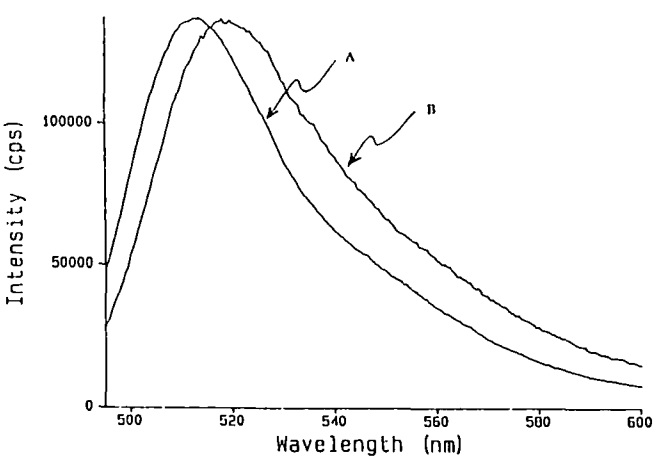


Fig. 5. Fluorescence intensity of fluorescein: (A) dissolved in 0.1 *N* NaOH; (B) bound on a silica fiber immerged in a borate buffer, pH 8.6.

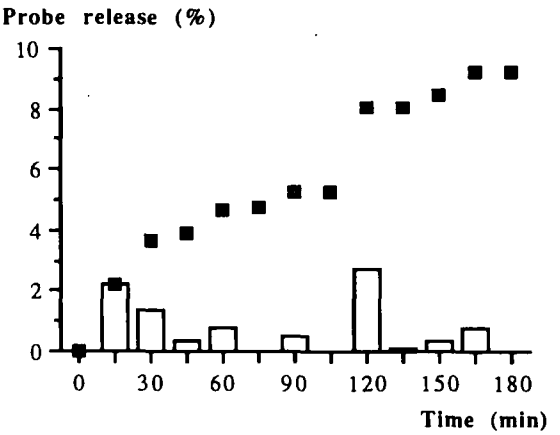


Fig. 6. Stability of bound fluorescein in 95% H_2SO_4. Histogram representation for each measure; cumulative values.

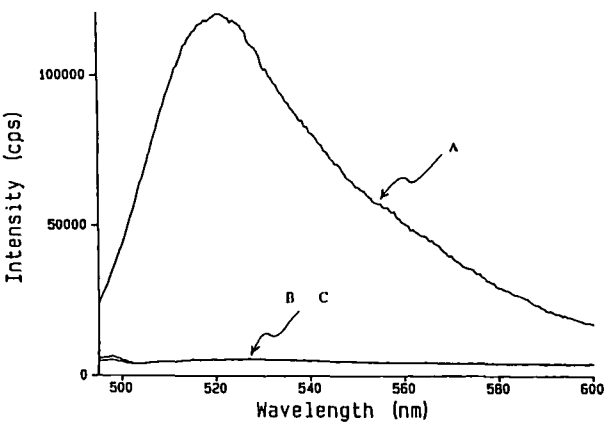


Fig. 7. Fluorescence of fluorescein bound on a silica optic fiber in 95% H_2SO_4. (A) Initial; (B) with $[NO_3^-] = 6 \cdot 10^{-5}$ *M* after 45 min; (C) with $[NO_3^-] = 6 \cdot 10^{-5}$ *M* after 1 h.

Response to Nitrate Ions. We have proved that immobilized fluorescein can still react with nitrates; this was shown by the appreciable change in fluorescence intensity (see Fig. 7). The fluorescence of the sensor was first measured in borate buffer, pH 8.6. Then the optrode was placed in the presence of nitrates in 95% H_2SO_4, during 45 min. The fluorescence was analyzed in borate buffer and compared with the first one. Total fluorescence quenching occurs at a high concentration of nitrate. In 95% H_2SO_4, without nitrate, a loss of 20% in the fluorescence intensity was observed, due to partial

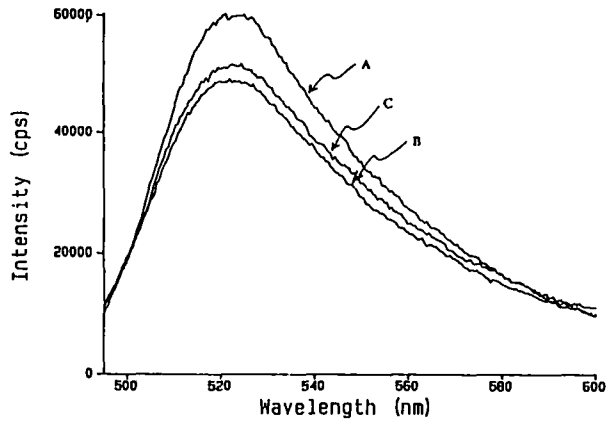


Fig. 8. Fluorescence of fluorescein bound on a silica optic fiber in 95% H_2SO_4. (A) Initial; (B) without NO_3^- after 45 min; (C) without NO_3^- after 1 h.

binding degradation and/or measurement reproductibility (see Fig. 8).

The Optical System's Limits. The optical device used until now cannot give reproducible measurements, because of poor connections between optical fibers and also with lasers, imperfect optical fiber cuttings, different curves in the laser beam optical fiber, laser instability, defective adjustment of the sensor in the cell, and heterogeneity of the surface binding on the optical fiber. All these drawbacks prevent any reliable analysis. At the present time, we are focusing on another optical setup, which would considerably improved the analysis measurements, especially concerning the potential reproducibility.

CONCLUSION

This study demonstrates the potential feasibility of a nitrate sensor based on immobilized fluorescein; as shown above, the fiber-optic nitrate sensor will be improved in several ways. The principal aim of this work was to demonstrate that bound fluorescein is still sensitive to nitrate. Maybe the determination of nitrate will be difficult, because of the necessity of a highly acidic medium; nevertheless, during this research we have acquired the basic knowledge required for binding chemical substances onto silica fibers, and as nitrate ions are often analyzed after their reduction into nitrite ions, the realization of a nitrite sensor might be an alternative.

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