

MEASUREMENT OF HYDROGEN SULPHIDE GAS USING FLUORESCENCE QUENCHING

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ABSTRACT

The quenching of the fluorescence of thionine by hydrogen sulphide is reported. The extremely efficient quenching process is attributed to reversible reduction of the thionine dye both in the ground and the excited state leading to the formation of a non-fluorescent semi/leuko thionine. Other toxic gases, namely SO_2 , HCl and NH_3 , were found to have no effect on thionine. Potential analytical applications of this discovery, including development of a fiberoptic H_2S sensor, are discussed.

1. INTRODUCTION

Hydrogen sulphide (H_2S) which is best recognized by its characteristic smell of "rotten eggs" is one of the primary air pollutants. Concentrations of 10 ppm in air have been recommended as threshold limit value (TLV) for human exposure. Major anthropogenic sources of H_2S include chemical processes and sewage treatment. Natural sources include volcanos and biological action in swamps. Hydrogen sulphide is ultimately converted into sulphur dioxide in the atmosphere and is therefore a source of sulphur dioxide.

Exposure to H_2S results in irritation of eye and upper respiratory tract. Prolonged exposure may result in pulmonary edema. Large dosages produce headache, dizziness, excitement, staggering and gastroenteric disorders, followed by bronchitis and bronchial pneumonia. Exposure to concentrations above 600 ppm may lead to respiratory paralysis and thus can be fatal.

Both off-line and on-line methods for quantitatively measuring H_2S concentrations are known. The conventional discontinuous methods depend on the spectroscopic measurement of the dye complexes formed by reagents and H_2S or iodometric titration. Optical methods of H_2S measurement have been known. These include measurement of reflectance of an indicator due to the presence of H_2S and the infra-red absorbance measurement. Although infra-red absorption measurements can be applied in on-line measurement, these tend to be bulky for several applications. Furthermore, these devices are expensive. Reflectance measurement lacks the desired sensitivities and has strong interferences from other species, especially when used in liquid samples.

In continuation to our work on the novel molecular phenomenon, we report in this paper on our discovery that thionine fluorescence is selectively and very efficiently quenched by H_2S . The findings are likely to lead to the development of a fluorescence quenching based fiberoptic chemical sensor/probe for H_2S .

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2. EXPERIMENTAL

2.1. Materials.

Thionine and other chemicals employed in this investigation were from Aldrich Chemical Co. and were used without further purification. Methanol was doubly distilled and dried over CaCl_2 . Pure dry H_2S was used for making the stock solution.

2.2. Instrumentation.

Absorption spectra were recorded using a Perkin-Elmer $\lambda 5$ spectrophotometer in a 1 cm path length quartz cuvette. The fluorescence spectra were recorded on a Perkin-Elmer LS-5 spectrofluorimeter which was equipped with a Hamamatsu photomultiplier R928 as detector.

Fluorescence quenching measurements were made by recording a series of standard solutions, in which the concentration of H_2S ranged from 0 to 120 μM . The concentration of thionine was kept constant at 1 μM to avoid self-quenching or inner-filter effects. The excitation wavelength was 596 nm for these spectra. A 2 nm bandpass was used throughout for both the excitation and emission monochromators. All measurements were made in a standard quartz sample cell (1 x 1 cm) at room temperature ($22 \pm 1^\circ\text{C}$).

2.3. Preparation of H_2S Solutions

The H_2S stock solution in methanol was prepared by bubbling dry H_2S gas through methanol over a period of 1 h. Thereafter, it was exposed to air to see the loss, if any, of H_2S . The H_2S solution was then assayed by iodometric titration. The stock solution was found to be 0.58 M in H_2S . Desired dilutions were made from the stock solution to perform further experiments.

3. RESULTS AND DISCUSSION

The fluorescence of thionine is characterized by broad excitation (ca. 400-640 nm with peak at 596 nm and a shoulder at 560 nm) and emission spectra (ca. 580-700 nm with peak at 620 nm). Figure 1 shows these spectra for a 1 μM solution of thionine in methanol which are in agreement with the literature¹⁻².

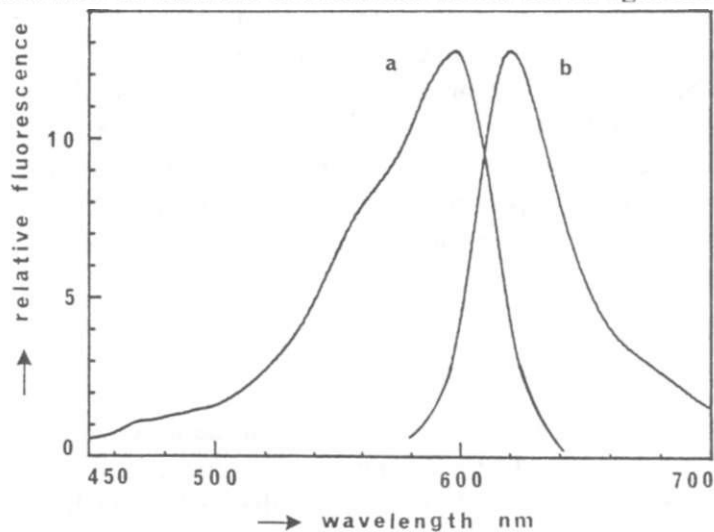


Figure.1 Excitation and fluorescence spectra of thionine in methanol

The ability of H₂S to quench the fluorescence of thionine was demonstrated by the series of thionine emission spectra which revealed that the fluorescence intensity of thionine decreases as the concentration of H₂S increases. These spectra also suggested that the position of the thionine emission band is independent of the H₂S concentration. The fluorescence quenching data have been analyzed according to the Stern-Volmer relationship:

$$I_0 / I = 1 + K_{sv} [Q]$$

where I₀ and I are the fluorescence intensities in the absence and presence of the quencher, K_{sv} is the Stern-Volmer quenching constant and [Q] is the concentration of the quencher. Figure 2 shows the Stern-Volmer plot for H₂S quenching, data of which was collected with an emission wavelength setting of 630 nm.

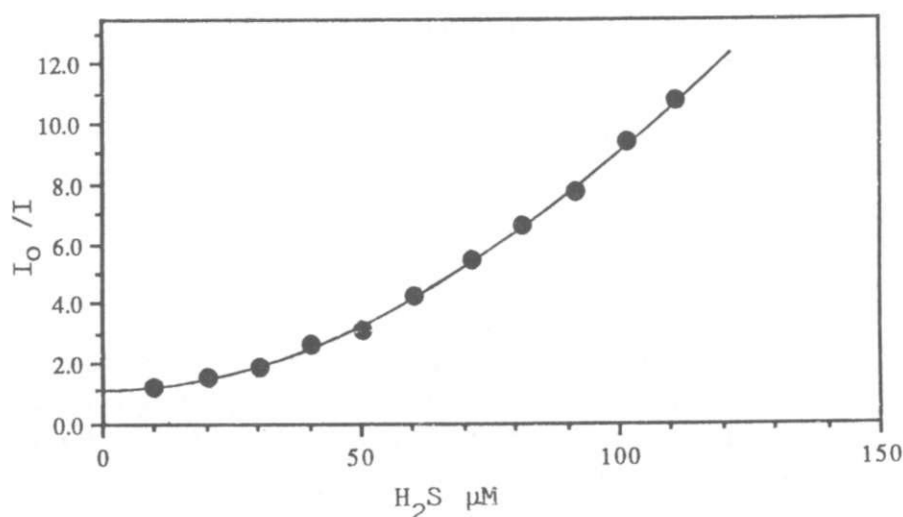


Figure 2. Stern-Volmer plots for the quenching of thionine by hydrogen sulphide

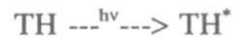
It is clear from this plot that the quenching process does not follow the Stern-Volmer relation. An upward curvature in this plot indicates existence of more than one mechanism responsible for the observed quenching. The extent of quenching efficiency can be estimated by calculating K_{sv} (appx). This can be defined as the reciprocal of the quencher concentration which corresponds to the half quenching (I₀/I=2). In the present case this is 47.2 uM which results in a K_{sv}(appx) value of 2.1 X 10⁴M⁻¹. The observed high efficiency of fluorescence quenching indicates that a process other than dynamic quenching is operative in this case. In dynamic quenching the process is limited by molecular diffusion, and the diffusion limited bimolecular collisional quenching rate constant (k_q) is related to the overall quenching constant (K_{sv}) by the following expression:

$$K_{sv} = k_q * \tau$$

where τ is the fluorescence decay time of the fluorophore in the absence of the quencher. A value for k_q can be calculated by using 2.1 x 10⁴ M⁻¹ for K_{sv} and 350 (± 20) x 10⁻¹² s for τ¹. The resulting value for k_q is 0.6 x 10¹⁴ M⁻¹ S⁻¹. This high value of k_q establishes that H₂S quenching of thionine is not diffusion controlled and suggests therefore that dynamic quenching is not the principal mechanism for this process.

Results from a preliminary experiment suggest that the quenching mechanism is based on formation of a non-fluorescent exciplex between H₂S and thionine and can be understood in terms of a photochemical reaction between the excited thionine (TH*) and H₂S which results in non-fluorescent (leuko/semi) thionine.

a. Light reaction



b. Dark reaction



Hence, upon excitation, a photo-reaction between thionine and H₂S is initiated. This results in a reduced population of the excited thionine, thus in a decrease in the fluorescence intensity. During the reverse reaction, the non-fluorescent form of thionine recovers. This suggests that formation of a non-fluorescent exciplex is involved in the quenching process.

In a separate experiment, the absorption spectra of 1 μM thionine was recorded both in the presence and absence of 1mM H₂S. As expected, change in absorbance of thionine was observed, but spectral features remained unchanged. Interestingly, the solution of thionine got de-colourised, but it recovered when nitrogen is bubbled through it for some time. This confirms that a ground state interaction is also involved. However, changes in the absorption spectra were very small as compared to those observed in case of fluorescence. The above experiments and the high efficiency of this quenching process support the conclusion drawn by Sharma³, which suggests that thionine undergoes a reversible photo-reduction by H₂S.

The finding that H₂S is an extremely efficient quencher of the fluorescence of thionine can be exploited for the quantitative measurement of H₂S in industrial and environmental applications. The possibility of making H₂S measurements in air, food and sweetening plants in oil industry is of particular interest. The measurement of thionine fluorescence, and consequently the quenching of this fluorescence by H₂S, is possible. This is especially attractive, since other major pollutant gases, such as SO₂, HCl and NH₃, do not interfere in the measurement. A simple device to monitor H₂S, dissolved in liquids/gases based on H₂S quenching of thionine, is possible. Optical properties of thionine are well suited for the use of simple, inexpensive and durable solid state optoelectronic components. In addition, the broad features of the excitation and emission spectra provide a great deal of flexibility in designing the optics for a particular application. These features suggest that a compact and inexpensive instrumentation can be readily developed for H₂S measurements based on thionine fluorescence quenching. Corresponding work is in progress.

4. ACKNOWLEDGMENTS

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5. REFERENCES

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