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The characterization of methylene-blue-doped sol gels and the development of a fluorenscence-based fiber-optic sensor for sulfite

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THE CHARACTERIZATION OF METHYLENE-BLUE-DOPED SOL GELS AND THE DEVELOPMENT OF A FLUORESCENCE-BASED FIBER-OPTIC SENSOR FOR SULFITE

By

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ABSTRACT

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The purpose of this research was to develop a fiber-optic sensor for sulfite via immobilizing Methylene Blue (MB) within a sol-gel matrix.

MB is a redox-indicating dye. In the oxidized form, the dye is a blue color, but reduction causes a change to the colorless, leuco form. This property is advantageous for the detection of reducing agents, such as sulfite. In solution, leuco-MB reverts to its blue form in the presence of an oxidizing agent.

The method of MB immobilization is through the use of sol gels. The environment of a sol gel is chemically inert and small analytes can easily diffuse into a thin-film matrix. In this study, the sol-gel matrix was formed via the hydrolysis and polycondensation of tetraethoxysilane (TEOS) as well as organically-modified orthosilicates.

Blue MB is converted to leuco-MB in both solution and in the thin-film coated fiber upon exposure to sulfite. In the sol gel, however, blue-MB is not regenerated in the presence of oxidizing agents. Hence, the sensor cannot be regenerated and is currently only satisfactory for a single use.

Fundamental studies of the spectroscopic properties, including absorbance and fluorescence spectra, of the sol gel environment were also performed as the sol gel aged.

ACKNOWLEDGEMENTS

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I would also like to thank the Union College Machine Shop for their help in the design and construction of the cuvette holder used in this research.

I am also indebted to the friends that I have been so lucky to make at Union. I am grateful for precious memories that will remain with me in years to come.

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Tara K. Morcone

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CHAPTER 1

INTRODUCTION

The purpose of this project was to study the chemical behavior of Methylene Blue in a sol-gel environment and its applications in fluorescence sensing. The goals of the research discussed here are (1) to tailor sensors in order to lower detection limits for sulfite, SO_3^{2-}, (2) to study the reversibility of Methylene Blue within the sensors, and (3) to investigate applications for these sensors.

1. METHYLENE BLUE (MB)

Methylene Blue (MB) is a thiazine-derived dye that is found most commonly in its hydrated form, $C_{16}H_{18}ClN_3S \cdot 3H_2O$. In its chloride salt form, MB is a cationic species which is soluble in water, ethanol, and ethylene glycol [1]. The dye is most commonly used as a redox indicator, a medicinal antiseptic, and as a biological stain [1]. MB is fluorescent and is excited at 662 nm, as seen from its absorption spectrum in Figure 1-1.

It is the ability of MB to act as a redox indicator that renders MB useful for some interesting applications in fluorescence sensing. The oxidized form of the species is blue and fluorescent. Reduced MB exists as the 'leuco' form and is colorless and non-fluorescent. A representation of this half-reaction, as well as the structure of the dye in each of these states is shown in Figure 1-2 [1]. Previous studies have shown that when blue MB is excited at 670 nm (the far-red region) with a diode laser, it subsequently fluoresces [2]. Thus, one should expect that in the presence of an appropriate reducing agent, the fluorescence intensity will decrease as the blue, fluorescent MB is converted

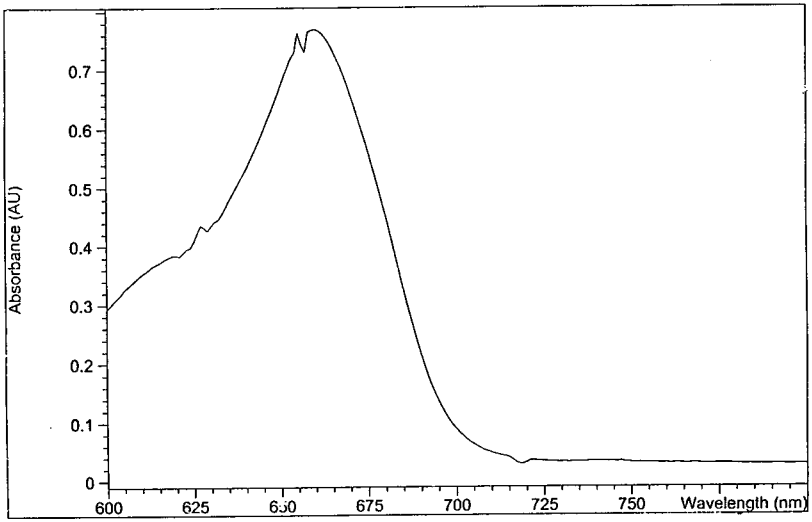

Figure 1-1. Absorption spectrum of Methylene Blue

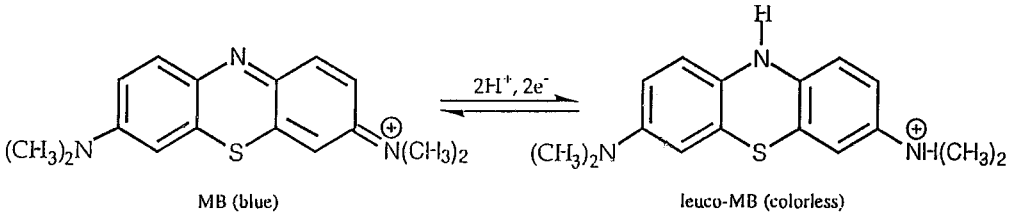

Figure 1-2. Reversible half-reaction of MB to leuco-MB
Reprinted from [1]

into the leuco form of the species. A common analyte that produces this effect is sulfite, which has the following oxidation half reaction [3]:

$$SO_3^{2-} + 2OH^- \Leftrightarrow SO_4^{2-} + H_2O + 2e^- \qquad E^o = 0.93V$$

MB is very stable at room temperature and is unaffected by its exposure to air upon prolonged storage because the presence of oxygen will allow MB to remain in its blue, oxidized form, with only small levels of photodecomposition. MB is also easily re-oxidized following the reduction described earlier. Possible agents which have the ability to oxidize the leuco form are $O_2(g)$ (including aerated $H_2O(l)$) and $H_2O_2(l)$.

2. SOL-GELS

The formation of a sol-gel is a two-step process of hydrolysis and condensation of low molecular weight semi-metal alkoxide monomers such as tetramethoxysilane (TMOS) or tetraethoxysilane (TEOS). The first step of the process, which occurs at room temperature, is the formation of the "sol" [4]. In this step, water causes silanol (Si-OH) groups to develop [4]. Condensation and polycondensation then occur: the silanol groups are deprotonated and Si-O-Si bonding occurs [4]. With this, a three-dimensional silica network, or "gel," is formed. The gelation process is initiated by the introduction of an acid catalyst such as HCl [4]. The formation of the "gel" has two states: first, the development of a wet gel and second, solvent and water evaporation to form a "xerogel" [4]. The following is a representation of the gelation process [5]:

$Si(OR)_4 + nH_2O \Rightarrow Si(OR)_{4-n}(OH)_n + ROH$ hydrolysis

$\approx Si\text{-}OH + RO\text{-}Si\approx \Rightarrow \approx Si\text{-}O\text{-}Si\approx + ROH$ condensation

$\approx Si\text{-}OH + HO\text{-}Si\approx \Rightarrow \approx Si\text{-}O\text{-}Si\approx + H_2O$ polycondensation

The silica network that is formed in this process has been shown to be a chemically inert environment and suitable for the entrapment of chemical species [4]. Several studies [5, 6, 9, 10, 12, 13, 14] have been done in which organic molecules are introduced to the sol-gel solution in the initial stages of hydrolysis and become entrapped within the matrix but are able to rotate freely. Figure 1-3 is a representation of a protein being integrated into the sol-gel matrix [6]. The sol-gel matrix provides a suitable environment, therefore, for the immobilization of species used in chemical sensing. For instance, in fluorescence-based sensing, analytes can penetrate the matrix and react with the immobilized species in order to produce a change in fluorescence signal.

The entrapment of molecules within a silica matrix has advantages over attaching the molecule to an organic polymer support. For instance, studies have shown that covalent attachment fixes the orientation of the species whereas the sol-gel matrix allows for mobility of the species [6]. Additionally, while a sol-gel environment (as explained above) is one that is chemically inert, electrostatic immobilization can change the chemical nature of the immobilized species. Studies have shown that the immobilization of MB by electrostatic attachment has been successful, but the indicating (redox) ability of the species was inhibited: no change in absorbance was observed in the presence of reducing agents [7]. Another important property of the matrix is the lack of swelling which occurs

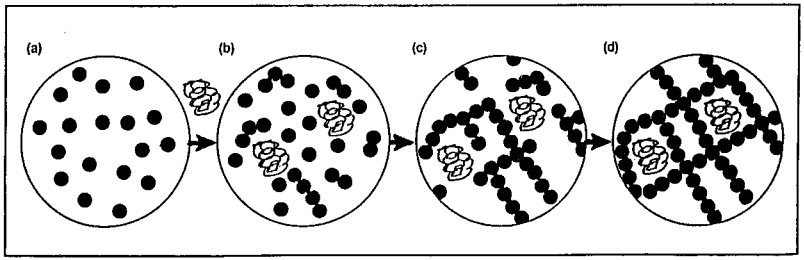

Figure 1-3. Entrapment of proteins within a sol-gel matrix
Reprinted from [6]

= protein

● = semi-metal alkoxide monomer

when exposed to aqueous and organic solutions. This prevents leaching which is often apparent in attachment to a polymer support [8] as well as electrostatic attachment [7]. For instance, when MB is electrostatically immobilized on a polystyrene surface and placed in a reactant solution, it leaches, or becomes lost to the solution. The practical applications of sensors of this type, therefore, are very limited. The physical and chemical stability of the species immobilized within a sol-gel, as well as the thermal, photochemical, and biodegredational stability of the sol-gel environment allow for practical applications of sol-gels in chemical sensing [4].

Specific studies have been done to observe species within the sol-gel environment. For instance, thin films doped with Rhodamine 6G (R6G) have been cast on glass microscope slides [9]. Like MB, R6G is a common dye which has a high quantum yield in solution and is relatively photostable. After R6G was isolated within the sol-gel matrix, steady-state and time-resolved fluorescence as well as absorbance measurements were taken at varying concentrations. From this data, information regarding the actual photostability and kinetics of the species within the matrix was deduced [9]. Studies regarding the behavior of an immobilized species as a function of the age of the sol-gel were also done through anisotropy measurement: the observation of the changing rotational property of the immobilized species [5].

There are several physical configurations of sol-gels. A monolith is a three-dimensional piece which, upon formation, takes the shape of the container (such as a cuvette) in which it was allowed to dry. The viscosity of the sol-gel also allows for the formation of thin films, that are normally less than 1 μm in thickness. The recipe used in

thin-film fabrication can be identical to that of monolith preparation, but the film adheres to a support upon gelation. For sensor applications, thin films have a clear advantage over monoliths because the thin layer allows for short diffusion pathways into the silica matrix as well as greater analyte surface contact. Other, less common, applications of sol-gels are in the production of powders, monodispersions and fibers for analytical detection [4].

The modification of sol-gels is possible by changing the alkoxide precursor from TMOS or TEOS to one with organic, Si-OR substituents such as methyltriethoxysilane (MTEOS). These modified sol-gels are called ormosils. They tend to enhance sensor performance by making the interface of the sensor increasingly hydrophobic [10]. Also, ormosils increase the thin film affinity for its polymer support (i.e.: an optical fiber core) [4].

3. FIBER OPTIC SOL-GEL SENSORS

The fluorescence sensing system in this research relies on the utilization of fiber optics. A fiber optic system relies on total internal reflection. Incident light passes through the fiber and is totally internally reflected at the core/cladding interface (see Figure 1-4). The core and cladding have refractive indices n_1 and n_2, respectively. The incident light enters the fiber within an acceptance cone, which has a half-angle, α, that is

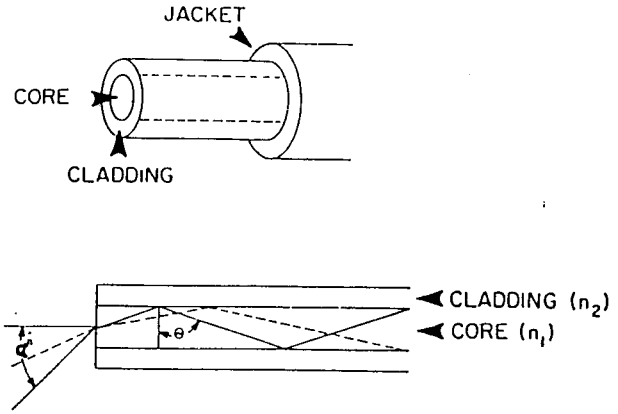

Figure 1-4. Representation of an optical fiber. Light transfer through an optical fiber. Reprinted from [11]

dependent on n_1 and n_2, as well as the refractive index of the outer medium (such as air), n_0. This relationship is as follows:

$$\sin\alpha = (n_1^2 - n_2^2)^{1/2} / n_0$$

The sensor used in this research utilizes fiber optics for transmission of the laser excitation light to the sensor. At the fiber tip, the cladding is removed and, on the core surface, a thin film, MB-doped, sol-gel coating is placed (as described earlier). This type of sol-gel fiber modification, shown in Figure 1-5, has proven very effective in reducing analyte diffusion lengths (as described earlier), providing a long optical pathlength, and minimizing photobleaching, which is frequently caused by laser radiation [4].

Experimentation in this research relies upon a fiber optic system in which indicator chemistry (the MB-analyte redox reaction) is the method of analysis. Thus, we classify this system as a second-generation sensing system. Second-generation sensors rely on a change in analyte to produce a change in the optical property of the immobilized indicator [11].

Fluorescence-based sensing is not a new analytical approach. Many biological and chemical sensors have relied on fluorescence as a means of analytical detection. For instance, extensive studies have been done in which proteins such as bovine serum albumin (BSA) act as biorecognition elements at or within the interfaces of fluorescence sensors. Through the observation of changes in fluorescence patterns upon interaction with analytes, changes in the rotational mobility of proteins can be observed [12].

Figure 1-5. Sol-gel-coated optical fiber sensor
1, source; *2*, cladding; *3, 6*, core; *4*, unclad section; *5*, doped sol-gel film; *7*, photodetector
Adapted from [4]

4. APPLICATIONS OF MB SENSORS

Other studies focused on sensing methods that rely on the indicating ability of MB. Also, sensing methods have been developed for sulfite detection. This research, however, incorporates the two methods.

MB-based detection methods have been studied previously. For example, flow injection analysis (FIA) coupled with MB fluorescence detection has been used to determine the presence of sulfide in solution. In this method, known as the 'Methylene Blue Method,' MB is formed via an on-line reaction in an acidic carrier stream [2, 13].

Sensing methods have also been developed specifically for sulfite. Through the use of FIA, sulfites can be detected in wine samples using amperometric detection. This method relies on the redox reaction between iodine and sulfite. The limits of detection were found to be 0.05 mg/L, making this a very suitable method for the analysis of dilute wine samples [14].

The objective of this research is to create a MB-based sol-gel sensor for sulfite. This sensor could have important applications, since sulfur dioxide is used as a preservative in wines and foodstuffs. Sulfur dioxide is frequently found in the ionized form, sulfite (SO_3^{2-}), which is potentially toxic [14]. MB fluorescence emission intensity change in a sulfite-containing environment is studied. Modifications of the sol-gel thin-film sensor are also done in an attempt to optimize MB-sulfite interaction within the matrix. Ultimately, practical use of this sensor would require a sulfite detection limit at a level competitive with current methods of analysis (such as FIA).

CHAPTER 2

EXPERIMENTAL

1. EXPERIMENTAL PREPARATION

Preparation of Stock Solutions: Two 0.001 M solutions of Methylene Blue (83%, purchased from Aldrich) were prepared in deionized water. One solution was prepared in 0.001 M HCl, in order to prevent the photodegradation of MB [15].

A 1.00 M sulfite solution was prepared using sodium sulfite, Na_2SO_3, in deionized water. Fresh solutions were prepared at the beginning of each day of sulfite experimentation, as suggested in the literature [14].

A 1.00 M citrate solution was also prepared using sodium citrate in deionized water.

2. INITIAL STUDIES OF METHYLENE BLUE IN SOLUTION

A. Varying MB Concentration: A series of solutions, varying in concentration between 1 μM and 60 μM MB, were prepared in deionized water. Fluorescence emission spectra were taken of each solution using the PTI Quantamaster Fluorometer. The instrumental parameters are listed in Table 2-1 below:

Table 2-1: PTI Quantamaster Instrumental Parameters

Excitation Monochromator Slitwidth	2 nm
Emission Monochromator Slitwidth	4 nm
Excitation Wavelength	662 nm
Emission Wavelength Scan	670-800 nm

Absorption spectra were also taken of MB solutions varying in concentration from 1 μM to 10 μM MB.

B. MB Response to Sulfite: A 3-mL solution of 10-μM MB was prepared in a polystyrene cuvette. Fluorescence was monitored (using the parameters in Table 2-1) as successive 30-μL aliquots of 1.00-M SO_3^{2-} were added to the cuvette.

C. MB Response to Citrate: The citrate experiment was performed, also, by adding 30-μL aliquots of a 1.00 M citrate solution (in deionized water) to a 3-mL solution of 10 μM MB. Fluorescence data were collected as in the sulfite experiment above.

D. MB-Protein Interaction: The SLM48000 MHF multifrequency phase-modulation fluorometer with a xenon-arc lamp was utilized for this experiment.

A solution containing 2.00 μM MB and 2.00 μM HCl, and separate solutions of 50.0 μM bovine serum albumin (BSA), 59.0 μM IgG, and 5% w/v Nafion®, diluted 1:10 in deionized water, were prepared for these analyses.

Fluorescence Anisotropy was measured in each experiment using the SLM48000 instrument. In the first experiment, the MB/HCl solution was titrated with microliter aliquots of BSA while anisotropy was measured. The second and third analyses were done using the same method, but replacing BSA with the IgG and Nafion® solutions, respectively.

E. Changing the Polarity of the MB Environment: MB was prepared at 10 μM in eleven solutions. The solutions varied in ratio of H_2O:ethanol from 0:10 to 10:0.

3. PREPARATION OF MONOLITHS

A. Preparation of TEOS Monoliths: Pre-sol-gel solutions were prepared by using a molar ratio of 1:2:1.6x10^{-4} of TEOS (4.5 g) to H_2O (1.4 g) to 0.1 M HCl (100 μL). TEOS is first added to a scintillation vial. The H_2O/HCl mixture is added to the TEOS drop-wise while stirring. The solution is then covered and allowed to stir for a hydrolysis time of at least eight hours.

Upon completion of hydrolysis, the solution becomes monophasic. It is separated into two, 3-mL portions in two separate, plastic cuvettes. The solution in each cuvette is then doped to the desired concentration of MB, covered with Parafilm®, and capped.

Monolith gelation times vary, but are on the order of days.

B. Preparation of Ormosil Monoliths: Monoliths using an ormosil precursor are prepared similarly to the TEOS monoliths described above. The ormosil recipe is altered to a 1:0.1:2:2:1.6x10-4 molar ratio of TEOS to ormosil to ethanol to H_2O to 0.1 M HCl. Table 2-2 represents the types of ormosils and the amounts of each part of the solution used for each recipe:

Table 2-2: Ormosil Monolith Recipes

Type of monolith (ormosil)	Amount of TEOS (g)	Amount of ormosil (g)	Amount of H_2O (g)	Amount of Ethanol (g)	Amount of 0.1M HCl (μL)
methyltriethoxysilane, Me-TriEOS	4.5	0.3851	0.7776	1.9872	35
ethyltriethoxysilane, Et-TriEOS	4.5	0.4154	0.7776	1.9872	35

The sol-gel preparation is almost identical to that of TEOS, with the only difference being the presence of ethanol in the H_2O/HCl mixture which is added dropwise to the orthosilicate (TEOS/Me-TriEOS) solution.

The solution is separated into two 3-mL portions in plastic cuvettes. Each aliquot is doped to the desired concentration of MB and treated with 100 μL of a 0.01 M phosphate buffer (pH 7.00). The cuvettes are covered and the solution is allowed to gel over several days.

4. OBSERVATION OF MONOLITHS

Monolith Aging: Absorbance and fluorescence data were collected for 10-μM doped MB monoliths over a five-month period. TEOS, Me-TriEOS, and Et-TriEOS monoliths were each monitored.

5. PREPARATION OF SOL-GEL-COATED FIBERS

A. Pre-Treatment of Fibers: Prior to coating a fiber with sol-gel, the fiber is first unclad and soaked in concentrated KOH.

B. Dipping of Fibers: Sol-gel solutions are prepared using the protocol, described earlier, for monolith preparation. The fiber is placed into the solution (pre-doped with the desired concentration of MB) at a uniform rate, and allowed to remain suspended (manually) for thirty seconds. The fiber is then removed from the solution at a uniform rate and allowed to dry upside-down, uncovered, at room temperature for at least eight hours.

6. DEVELOPMENT OF OPTICAL SENSING PLATFORM

In the original sensing platform (shown in Figure 2-1), a 670 nm diode laser was directed through an iris and into an optical fiber. This fiber was connected to the sensing fiber, which was positioned close to the entrance slit of a monochromator. In order to reduce the interference of scattered light in the presence of MB emission, a Roscolux® filter (#81, Primary Blue) was placed over the monochromator entrance slit. The percent transmittance of the filter at the laser wavelength is about ten percent.

The results obtained using this sensing method, however, were not precise since the optical fiber was not mechanically stable upon addition and removal of

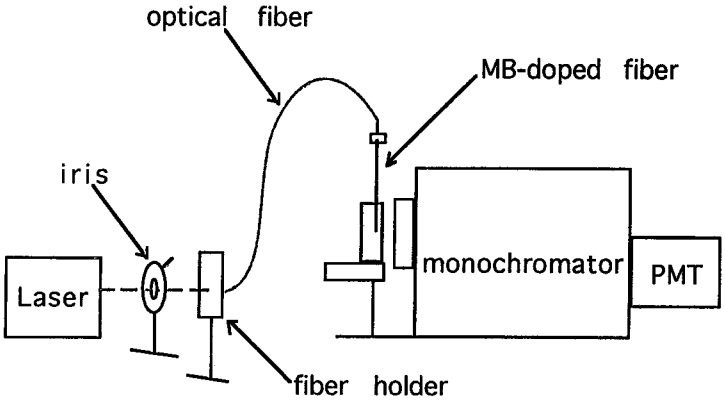

Figure 2-1. Original optical sensing platform

solutions from the cuvette within which it was placed. A cuvette holder was then constructed which provides a more stable platform for analysis and is used in the current sensor/detection system, represented in Figure 2-2. Signal transfer to the detector was also optimized by the introduction of optical lenses (F/#'s: 1 and 4, respectively).

The computer monitor was set as a blue screen during the analysis to prevent extraneous red light from the monitor from reaching the detector.

7. MB-DOPED SOL GEL FIBER ANALYSES

A. MB-Doped Fibers in the Presence of Sulfite: MB-doped optical fibers were placed in a cuvette filled with 3 mL of deionized water. TEOS fibers doped at 10 μM MB were tested.

Fluorescence emission spectra between 680 and 737 nm were collected for each fiber in water. Subsequently, 30-μL additions of 1.00 M SO_3^{2-} were added to the cuvette, and a measurement of fluorescence followed each addition.

B. Regeneration Using Aerated H_2O: The regeneration of MB by re-oxidation was attempted using aerated deionized water. Following the series of sulfite addition, the sulfite-containing solution was removed from the cuvette and fresh deionized water was added. The solution was allowed to remain standing, open to the atmosphere, and fluorescence emission spectra were collected over time.

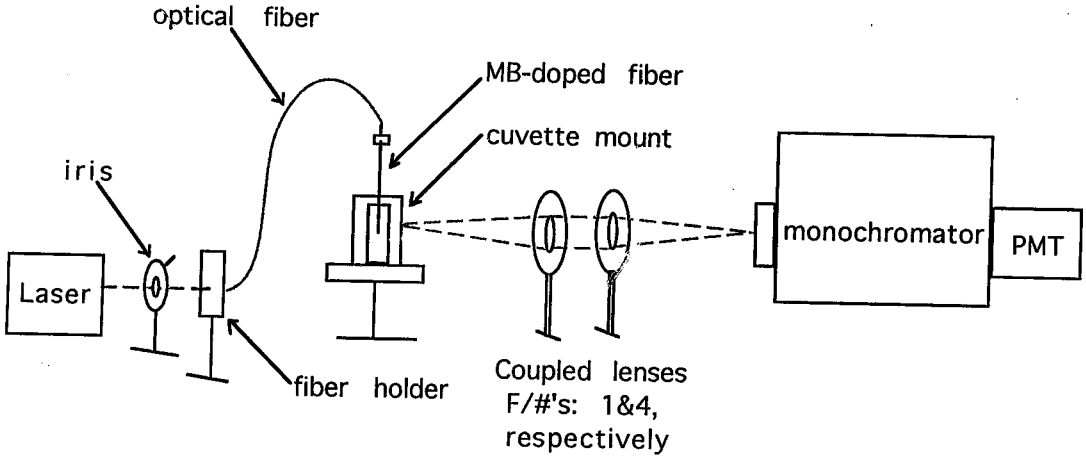

Figure 2-2. Modified optical sensing platform

C. Regeneration Using 3% H_2O_2: The procedure for this experiment was done using a similar procedure as in Section B, but replacing the sulfite-containing solution with 3% H_2O_2 and monitoring fluorescence over time.

8. POST-DOPING OF FIBERS

A blank fiber was soaked in a 10-μM MB solution within a cuvette. After ten minutes, the fiber was rinsed with deionized water, the solution was removed, and 3 mL of deionized water was added to the cuvette and the fluorescence was measured. A 60-μL aliquot of 1.00 M sulfite solution was then added to the cuvette and the fluorescence signal was measured again.

The fiber was then soaked, for a second time, in a 10-μM MB solution, rinsed, and treated with sulfite. A fluorescence spectrum was taken before and after the second sulfite addition.

This process was repeated in a series of three post-doping/sulfite addition cycles.

CHAPTER 3

RESULTS

1. CHARACTERIZATION OF MB IN SOLUTION

MB was initially studied within an aqueous environment (deionized water). The maximum excitation wavelength was confirmed to be 662 nm, and the spectral emission between 670 and 800 nm. The maximum emission wavelength is 691 nm.

The concentration of MB in deionized water was varied and the fluorescence spectra of these solutions indicate, as expected, an increase in relative intensity as the concentration of MB in solution increases. The spectra in Figure 3-1 show the linear increase in spectral intensity as the concentration of MB is increased in solutions of low concentrations of MB (1-6 μM). The spectral intensity, however, does not increase linearly at higher concentrations. Solutions prepared at concentrations varying between 1 and 60 μM MB exhibit a roll-off effect at and beyond 10 μM-MB. This is shown in the plot of maximum fluorescence intensity as a function of MB concentration (Figure 3-2).

The absorption of a series of MB solutions was also observed (Figure 3-3). Beer's Law was followed by these solutions, which cover a MB concentration range of 1-10 μM.

2. MB RESPONSE TO REDUCING AGENTS IN SOLUTION

A. Sulfite: 30-μL aliquots of 1.00 M Na_2SO_3 were added to a 10-μM MB solution in H_2O. Fluorescence intensity of blue-MB in solution was observed to decrease as the concentration of SO_3^{2-} in solution increased, as shown in the plot of maximum

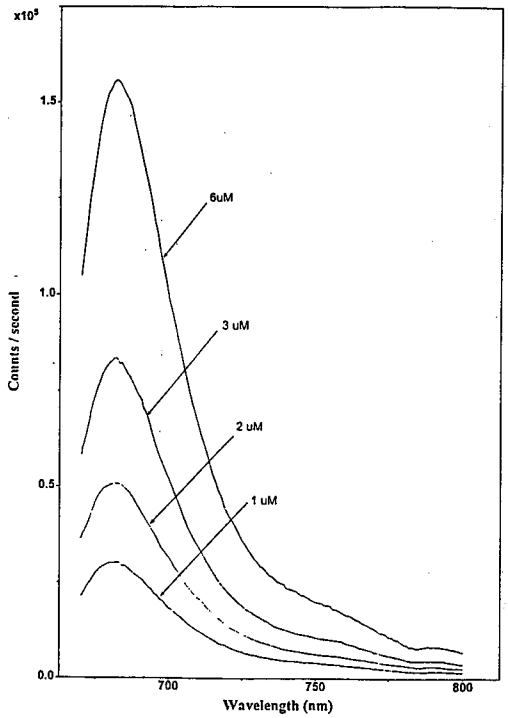

Figure 3-1. Fluorescence spectra of MB solutions in H_2O

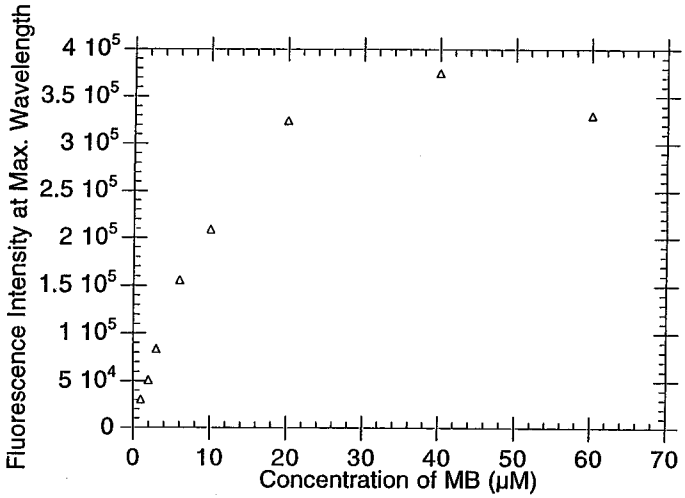

Figure 3-2. Maximum MB fluorescence intensity plotted as a function of MB concentration

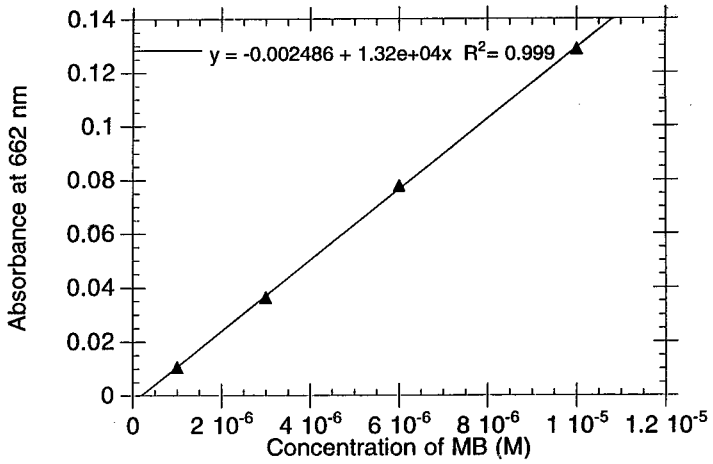

Figure 3-3. Beer's Law test for MB solutions:
Absorbance plotted as a function of MB concentration (1-10 μM)

fluorescence intensity versus sulfite concentration (Figure 3-4). This corresponds to a decrease in concentration of MB as reducing agent is added. The linear fit to the curve, however, is relatively poor.

B. Citrate: Fluorescence was also measured upon addition of 30-μL aliquots of 1.00 M citrate to a solution of 10-μM MB in H_2O. The maximum fluorescence emission intensity of MB was, again, observed to decrease as the reducing agent was added to the solution (see Figure 3-4).

3. MB-PROTEIN INTERACTION

A. BSA: Steady-state anisotropy measurements were taken as BSA was added in micromolar-additions to a 2 μM MB solution in 2 μM HCl. There was no significant change in anisotropy as the protein was added to the solution, indicating that the chemical environment of MB is unaffected by BSA and, therefore, an apparent lack of binding of MB to BSA.

Steady-state fluorescence and absorbance measurements were also made as a function of BSA addition. As shown in Figure 3-5, the fluorescence emission maximum and the relative fluorescence intensity does not change significantly as the protein is added to the MB solution. This further implies that the chemical environment of MB is not varying significantly with the introduction of the protein. The slight decrease in fluorescence intensity as the BSA solution is added to the cuvette is likely due to a dilution effect.

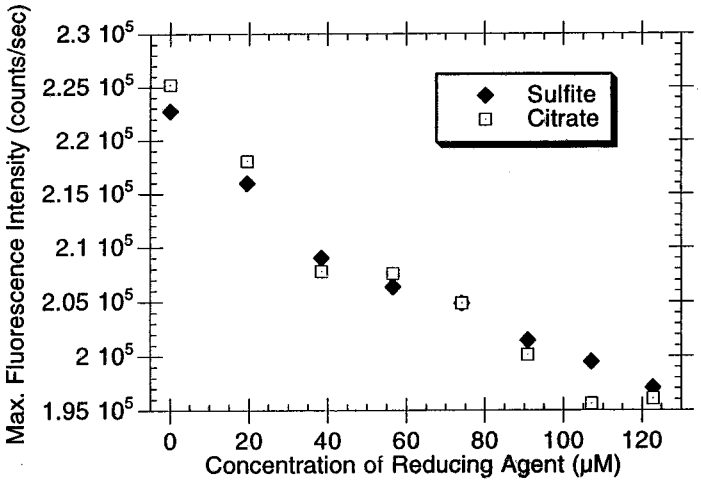

Figure 3-4. Sulfite and citrate addition to a 10 μM MB solution: maximum MB fluorescence intensity plotted as a function of reducing agent concentration

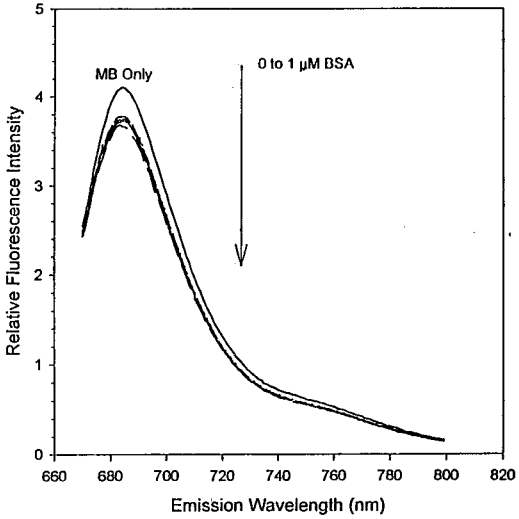

Figure 3-5. Fluorescence spectra of a 2 μM-MB solution with BSA addition

B. IgG: The above anisotropy experiment was also performed using the protein IgG. The resulting data showed no significant variance in anisotropy as the protein was added, once again indicating negligible binding of the protein to the species.

C. Nafion®: Nafion® is a perfluorinated polymer that electrostatically interacts with ions and has a tendency to aggregate in the presence of ionic regions [15]. Anisotropy was measured as a 5% Nafion® solution was added to a 1-μM solution of MB. The anisotropy did, in fact, increase as increasing concentrations of Nafion® interacted with the free-MB in solution, as seen by the plot in Figure 3-6.

4. POLARITY EFFECTS ON MB SPECTRA

Fluorescence and absorbance measurements were taken for solutions of 10 μM MB in solutions of differing polarity, varying from pure H_2O to the less polar ethanol. As the percent volume of ethanol in the solutions was varied from 0-100%, the maximum fluorescence intensity of MB increased, as shown in Figure 3-7. The molar absorptivity of MB increased and the wavelength of maximum absorbance shifted from 662 nm to a lower wavelength, 656 nm, as ethanol concentration in the solvent increased (see Figure 3-8).

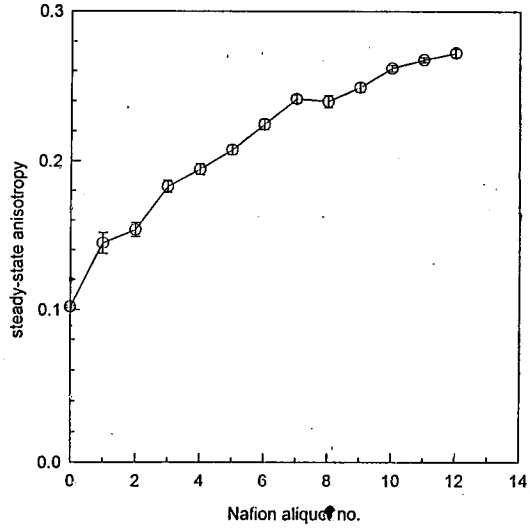

Figure 3-6. Steady-state anisotropy measurement of a 1 μM-MB solution with Nafion addition (30μL/aliquot)

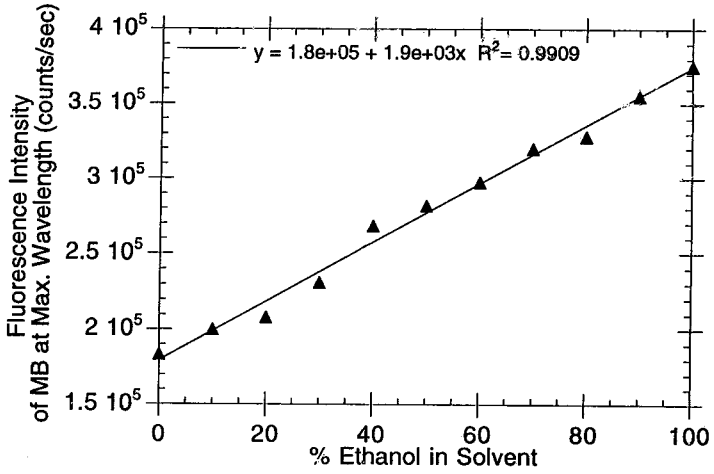

Figure 3-7. Fluorescence emission of MB plotted as a function of %EtOH

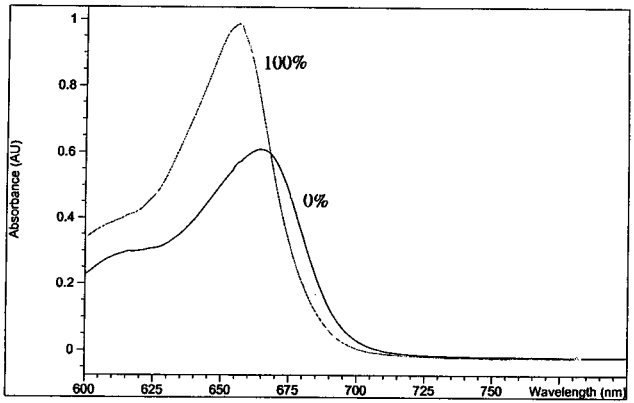

Figure 3-8. Absorption spectra of MB solutions in 0% and 100%EtOH

5. SPECTRAL OBSERVATION OF MB-DOPED MONOLITHS

Absorption spectra of MB-doped sol-gel monoliths were collected over time for TEOS monoliths prepared using the original recipe described in Chapter 2 (molar ratio of $1:2:1.6x10^{-4}$ of TEOS (4.5 g) to H_2O (1.4 g) to 0.1-M HCl (100 μL)). The data plotted in Figure 3-9 depicts the changes in absorbance at 662 nm observed over time for 20- and 40-μM MB monoliths. It is interesting to note, however, that the maximum absorbance of MB occurs at 630 nm, instead of 662 nm, after the monolith is allowed to age four months (see Figures 3-10 and 3-11).

Monoliths were also prepared (as described in Chapter 2) using the organically modified precursors Me-TriEOS and Et-TriEOS. These sol-gel solutions were made using a similar recipe as that of the TEOS monoliths, but with the addition of ethanol. In order to eliminate any variation in the polarity of the monolith environment between the TEOS sol-gel and the ormosil sol-gels, each monolith (including TEOS) was prepared using ethanol with the recipes given in Table 2-2. The absorbance value at 662 nm for these monoliths was observed as a function of monolith aging time and plotted in Figure 3-12.

Figures 3-13 and 3-14 depict the absorption spectra of a Me-TriEOS monolith aged one day and four months, respectively. The wavelength at maximum absorbance shifts from 662 nm to 635 nm, a similar effect to that seen with the TEOS monolith (see Figures 3-10 and 3-11).

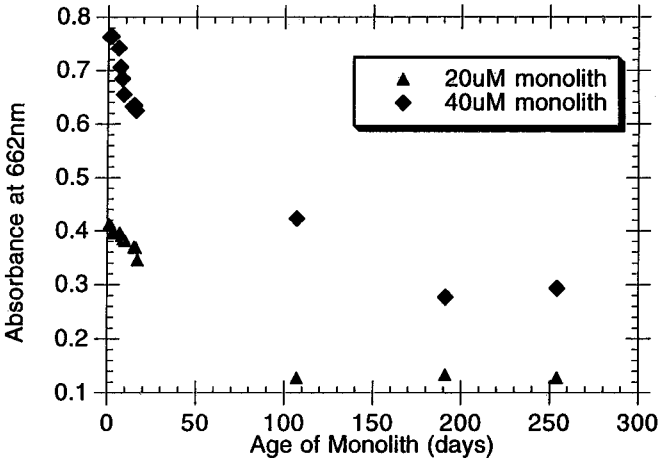

Figure 3-9. Absorbance at 662 nm of 20 and 40 μM MB-doped TEOS monoliths observed over time

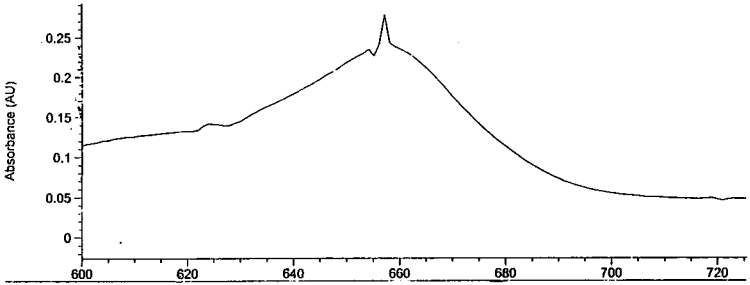

Figure 3-10. Absorption spectrum of a 10 μM MB-doped TEOS monolith: aged 0 days

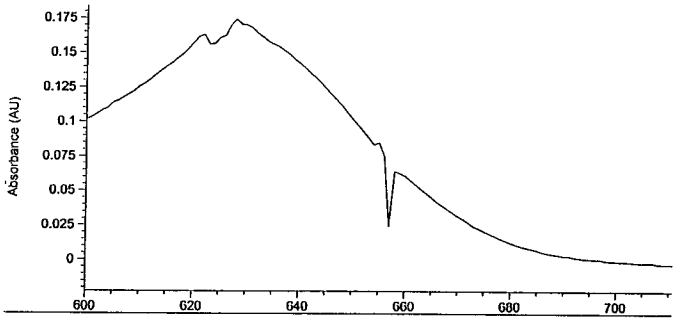

Figure 3-11. Absorption spectrum of a 10 μM MB-doped TEOS monolith: aged 4 months

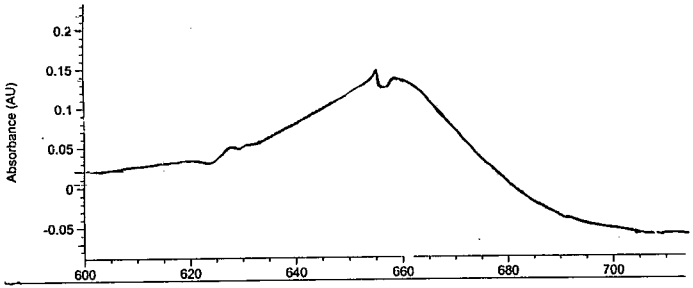

Figure 3-12. Absorption spectrum of a 10 μM MB-doped Me-TriEOS monolith: aged 1 day

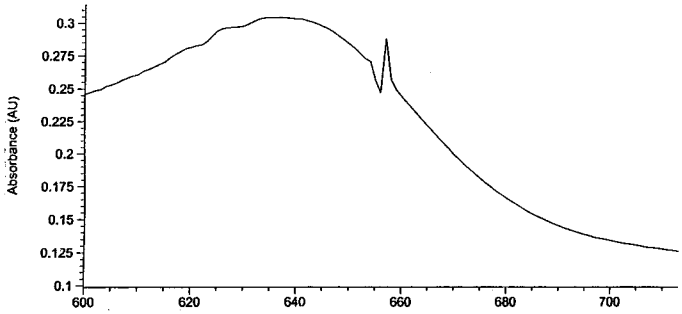

Figure 3-13. Absorption spectrum of a 10 μM MB-doped Me-TriEOS monolith: aged 4 months

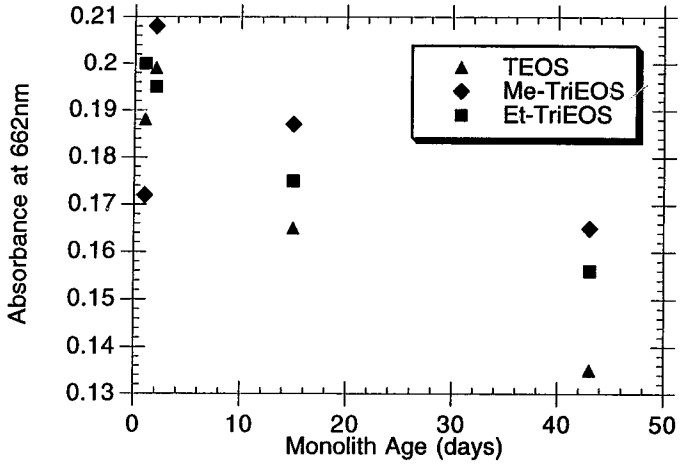

Figure 3-14. Absorption of 10 μM MB-doped TEOS, Me-TriEOS, and Et-TriEOS monoliths plotted as a function of time

6. RESPONSE OF MB-DOPED FIBER IN SULFITE

Fluorescence spectral data was obtained for the MB-doped sol-gel fibers using the instrumental system detailed earlier (Figure 2-1).

A. Sensor Response as a Function of Sulfite Concentration: A 10-μM MB-doped TEOS fiber was first placed in a cuvette containing 3 mL of deionized water. Aliquots (30 μL) of 1.00 M SO_3^{2-} were then added to the solution and the fluorescence emission from the doped fiber was measured two minutes after each addition[1]. Fluorescence signal decreased, as expected, following each addition. The spectra are shown in Figure 3-15. A plot of the intensity at λ_{max} (697 nm) versus the concentration of added sulfite (Figure 3-16) gives a non-linear curve.

Regeneration was attempted by replacing the sulfite-containing solution with a solution of 3% H_2O_2 and also with deionized H_2O. In both cases, there was no significant regeneration of fluorescence signal observed.

B. Sulfite Effects as a Function of Time: In order to study the kinetics of MB reduction within the thin film, a blank fiber was post-doped for ten minutes in a solution of 10 μM MB. The fiber was then rinsed and placed in deionized water. Next, a single, 30-μL addition of 1.00 M sulfite was added to the solution and the fluorescence emission was monitored over time. The spectral data and the plot of MB fluorescence intensity at

[1] Note that the two-minute equilibration time was chosen arbitrarily.

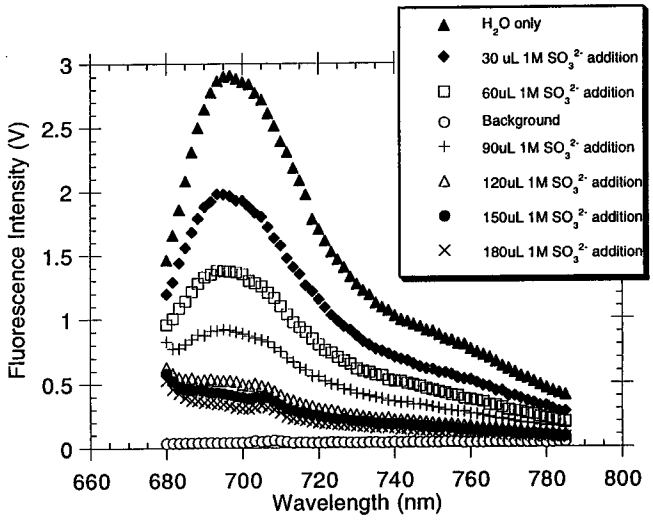

Figure 3-15. Fluorescence emission spectra from a 10 μM MB-doped fiber upon addition of sulfite

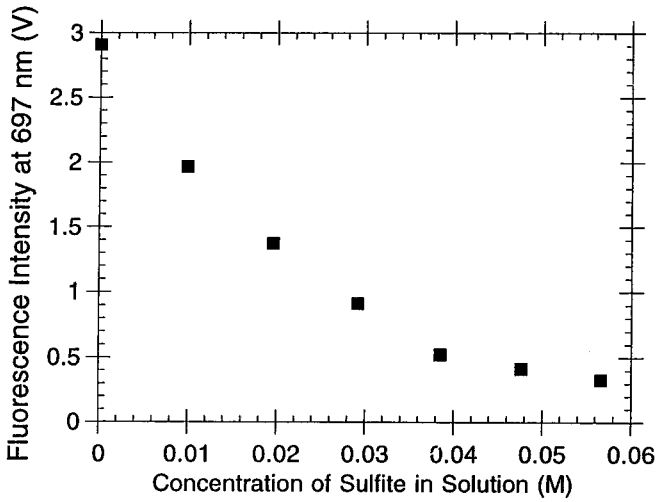

Figure 3-16. Fluorescence intensity of MB at 697 nm plotted as a function of sulfite concentration

697 nm as a function of time are given in Figures 3-17 and 3-18, respectively. The sulfite kinetics data were fitted to a single exponential curve (see Figure 3-18).

7. EFFECTS OF MB ADDITION TO A PRE-USED SENSOR

A study was also done in order to determine the capacity of the thin film for blue-MB through post-doping. A blank, sol-gel-coated fiber was soaked in a solution of 10 μM MB for ten minutes. The fiber was then rinsed and placed in 3 mL of deionized water. Fluorescence intensity of the MB-doped fiber was measured before and after the addition of a 30 μL-aliquot of sulfite. The reacted sensor was then soaked, again, in a solution of 10 μM MB for ten minutes. After placing the fiber in fresh deionized water, the MB fluorescence emission spectrum was collected. The post-doping/sulfite-addition procedure was repeated twice. Figure 3-19 depicts the fluorescence emission spectra collected throughout the experiment. Following each initial addition of sulfite, the post-doping does not result in a return to the initial fluorescence intensity.

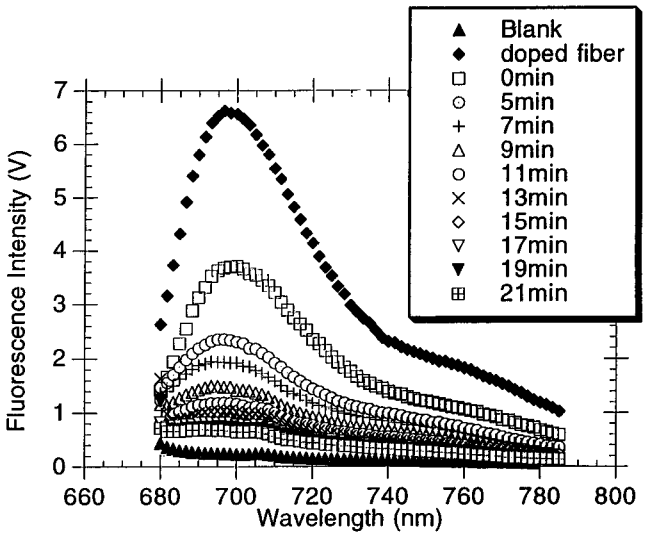

Figure 3-17. Fluorescence emission spectra from a 10 μM MB-doped fiber taken in two minute increments following a single 30-μL addition of 1.00 M sulfite

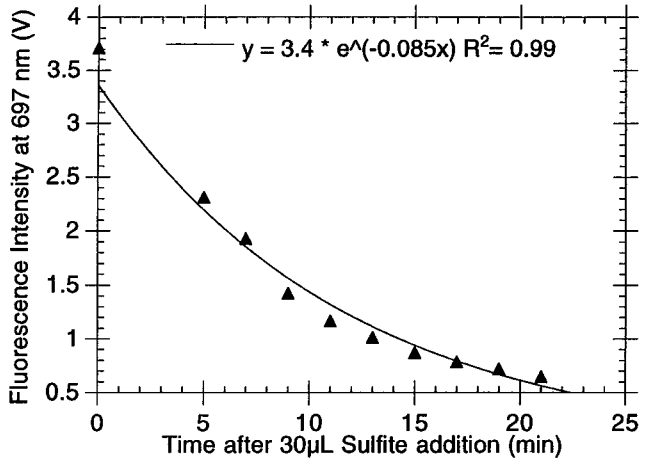

Figure 3-18. Fluorescence intensity of MB at 697 nm plotted as a function of time following a single 30-µL addition of 1.00 M sulfite

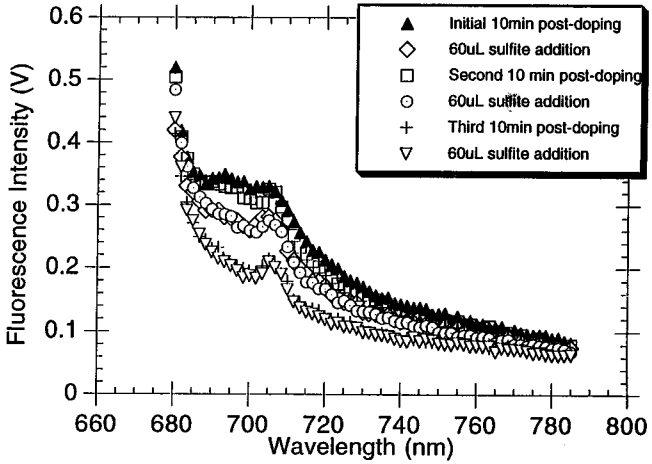

Figure 3-19. Fluorescence emission spectra of a 10 μM MB-post-doped fiber: sulfite addition followed by post-doping, repeated twice

CHAPTER 4

DISCUSSION

1. MB STUDIES IN SOLUTION

A. Self-Absorption of MB: Preliminary studies of MB in solution show that relative fluorescence intensity is dependent on the concentration of blue-MB in solution. The linear relationship between fluorescence intensity and MB concentration, however, only occurs when the solutions are below a MB concentration of 10 μM. (see Figures 3-2 and 3-3). At higher concentrations, the fluorescence emission intensity decreases as the amount of MB in solution increases. The exhibited "roll-off" effect (Chapter 3, 1) is likely due to (1) self-aggregation and/or (2) self-absorption of MB at higher concentrations. The first effect results from the formation of dimers and trimers of the MB species at high concentration, which causes a decrease in concentration and, therefore, in the fluorescence intensity, of the MB monomer. Self-absorption of MB is caused by the absorption of MB photon emission by neighboring species. This, in turn, inhibits the number of photons that reach the detector. Since self-absorption is dependent on the presence of neighboring species, the effect becomes more prominent as the concentration of species increases and, as a result, a decrease in fluorescence emission intensity occurs.

In order to minimize the effects of MB self-aggregation and self-absorption on these experiments, the concentrations of MB used in the preparation of solutions, monoliths, and fibers for most studies in this research were chosen at levels at or below 10-μM MB.

B. Sulfite and Citrate Effects on MB Fluorescence Intensity: Sulfite and citrate aliquots were added to aqueous MB solutions and the relative fluorescence intensity of blue-MB in solution decreased upon each addition (Chapter 3, 2A and 2B). This observation is expected due to the conversion of blue-MB to leuco-MB upon reduction, as described in the Introduction. Both sulfite and citrate are reducing agents and, hence, cause this conversion to occur. In effect, the observed decrease in intensity is due to the changing ratio of fluorescent MB to the non-fluorescent, leuco-MB form.

C. Anisotropy Studies of MB and Proteins: As described in Chapter 3, 3A and 3B, the anisotropy data obtained for MB in the presence of either BSA or IgG showed that each protein resulted in a negligible change in the anisotropy of MB in solution. In addition, there was negligible change in the fluorescence intensity of MB as BSA is added (see Figure 3-5). This lack of apparent interaction is likely due to an electronic repulsion between the MB and proteins and, as a result, the rotational environment of MB remains unaffected in the presence of either BSA or IgG.

As described in Chapter 3, 3C, Nafion® was tested for its effect on the anisotropy of MB in order to show a contrast to the lack of MB-protein interaction described above. Nafion® normally exists as depicted in Figure 4-1, where X represents a sulfonic or carboxylic functional group, and M represents a metal which is neutral, unless in acidic form (where it has a positive charge) [16]. Thus, since this experiment was conducted in a weakly acidic environment (MB in 0.001 M HCl), Nafion® existed largely as a negatively charged species. The interactions between Nafion® and MB, therefore, would be favorable because of the attractive forces between positively-charged MB and

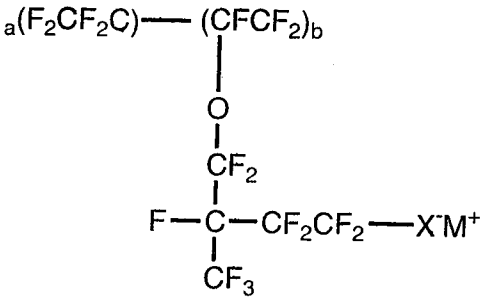

Figure 4-1. Nafion® structure
Adapted from [16]

negatively-charged Nafion®. Figure 3-6 is the anisotropy of MB plotted as a function of Nafion® addition. As a result of Nafion®-MB interactions, an increase in binding causes the rotational environment of MB to be affected, and the anisotropy of the species to ultimately increase.

D. Ethanol Effects on MB in Solution: The polarity of the MB environment was varied by changing the ethanol concentration (Chapter 3, 4). An decrease in solution polarity (increase in ethanol concentration) caused an increase in the maximum intensity of MB fluorescence (Figure 3-7). The wavelength at maximum MB absorption shifted to a lower wavelength, from 662 to 653 nm, as the concentration of ethanol increased in solution (see Figure 3-8); however, there was no significant trend observed in absorbance at 662 nm.

The shift in the absorption spectrum described above is likely due to a decrease in the polarity of the solvent surrounding the absorbing species as more ethanol is added. As a result, a blue shift (shift to lower absorption wavelengths) in the MB spectrum occurs as the solvent polarity decreases (greater concentrations of ethanol), and the excitation wavelength becomes longer [16]. The blue shift likely occurs due to a decrease in the interaction between MB and the solvent, which results in an increase in the energy of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of MB.

2. MB STUDIES IN MONOLITHS

As described in Chapter 3, 5, the absorbance intensities of MB in TEOS, Me-TriEOS, and Et-TriEOS monoliths studied decreased as the monoliths aged (see Figure 3-9). This effect may be explained by the decrease in pathlength as the monolith shrinks away from the cuvette. In observation of Beer's Law (A = abC), a decrease in pathlength should lead to a decrease in absorbance. The shrinking of the sol-gel piece, however, should cause an increase in the concentration of MB per unit volume. As a result, there would be an increase in relative MB absorbance as the monolith aged and detached from the cuvette wall. The experimental trend for the three monoliths studied here may be due to a combination of both effects, although further data should be collected as the monoliths age.

It was necessary to minimize spectral changes due to variations in sol gel polarity between TEOS and ormosil monoliths in order to compare changes in absorbance of the two sol gel types as they aged. Therefore, sol gel preparation using both organically substituted and un-substituted silane precursors were made using an equivalent amount of ethanol. Monolith aging experiments have, thus far, only been done to observe absorbance changes at 662 nm for ormosil and TEOS monoliths. The absorbance at 662 nm decreases for both TEOS and Me-TriEOS monoliths as they age (see Figures 3-9 and 3-14). This trend, however, may be due to the change in the wavelength of maximum MB absorbance that occurs over time. As seen from the differences between the spectra in Figures 3-10 and 3-11, and 3-12 and 3-13, the wavelength of maximum absorbance shifts left as both the TEOS and Me-TriEOS monoliths age. The evaporation of ethanol

and, more slowly, water, from the sol-gel may cause an overall decrease in MB-solvent interaction, which would result in the observed blue shift.

In the future, the monitoring of relative changes in the absorption profiles of both TEOS and ormosil monoliths should be done in order to observe the changes in polarity of the sol gels as they age.

3. FIBER STUDIES

A. Sulfite Effects on MB-Doped Fibers: As described in Chapter 3, 6A, the fibers were soaked in deionized water and the concentration of sulfite in the cuvette was increased while MB fluorescence was observed. The fluorescence emission spectrum of MB decreases in intensity upon addition of sulfite (see Figure 3-15). The decrease, however, is not linear (see Figure 3-16) as is observed for MB in aqueous solution (see Figure 3-4). A reason for the non-linear change may be caused by a near total conversion of blue-MB to its leuco form upon the first few additions of 1.00 M sulfite aliquots. Later sulfite additions, therefore, will cause little or no change in fluorescence intensity, since most of the blue-MB in the sol gel will have already been converted to the leuco form. In the future, similar studies may be performed using smaller concentrations of sulfite per aliquot.

The equilibration time for MB-sulfite interaction within the thin film was studied by the addition of a single aliquot of sulfite to the cuvette followed by the measurement of the MB fluorescence emission spectrum at two-minute time intervals (Chapter 3, 6B). The spectra in Figure 3-17 are evidence that an equilibration time is necessary for the full reduction of MB within the sol gel thin film. The plot of the MB fluorescence emission

at 697 nm as a function of time (Figure 3-18) shows that the time required to fully reduce 10 μM MB with a single 30 μL aliquot of 1.00 M sulfite is at least sixteen minutes. It is possible that the implementation of a stirring apparatus within the system would increase the speed of MB reduction via an enhancement of sulfite transport.

B. Regeneration Attempts: Attempts at the regeneration of blue-MB from leuco-MB in the sol gel fiber via the addition of H_2O_2 were unsuccessful. In the future, studies of the regeneration of blue-MB in solution may prove useful in determining an appropriate oxidizing agent for sensor regeneration.

It is possible that leuco-MB is unable to be re-oxidized when entrapped within the sol gel. The leuco form has one additional hydrogen present in the MB structure (relative to blue-MB) (see Figure 1-2), which may increase the opportunity for hydrogen bonding of MB to the matrix. As a result, the binding of leuco-MB to the matrix might cause the oxidation of leuco-MB to become thermodynamically unfavorable. Another possible reason for leuco-MB irreversibility may be a reaction of leuco-MB within the matrix to form another species, thus making the reformation of blue-MB impossible. A third hypothesis for the irreversibility of leuco-MB is the possibility that the species, during or following reduction, is leaching from the sol gel (at concentrations too small to detect).

C. Post-Doping Experiment: The fiber was successfully post-doped, as described in Chapter 3, 7, by simply placing a "blank," sol-gel-coated fiber (without apparent MB fluorescence signal) in an aqueous solution of 10-μM MB. The MB fluorescence emission intensity obtained from this method was smaller than that obtained

by the normal doping method. The signal, however, was real since a decrease in emission intensity occurred upon addition of sulfite. The fiber was then soaked in another solution of MB. The MB fluorescence emission, as depicted in Figure 3-19, did not reach its initial intensity prior to sulfite addition. The procedure was repeated for a third time, and MB emission of the post-doped fiber again decreased.

The preliminary data supports the hypothesis that MB may have an increased affinity for the sol-gel matrix. If the leuco-MB were leaching from the sol-gel matrix, one would expect that the initial concentration of MB should be obtained following each post-doping. On the other hand, if leuco-MB or the product of reacted leuco-MB remains within the matrix, then post doping will yield a fluorescence emission intensity which is lower than that caused by the initial post-doping. Since the initial concentration of MB was unable to be reached via post-doping, the data supports the possibility of either the retention of leuco-MB or a reacted form of the species within the matrix. Evidence of leaching, therefore, is not apparent.

D. Ormosil Thin-Films: The use of organically-modified sol gels thin films for sulfite sensing is currently under investigation. It is possible that an organically-modified sol-gel thin film would provide fewer opportunities for hydrogen bonding of leuco-MB to the matrix. This is of particular interest in examining the source of leuco-MB irreversibility within the sol-gel matrix. Also, if leaching does occur from the TEOS thin film, an organically-modified environment might show an enhanced affinity for the MB species. Moreover, an organically-modified environment might increase the mobility of

sulfite into the matrix, enhancing the sensitivity of the sensor. There is the possibility, however, that organic substituents may act as steric barriers to the polar analyte.

4. CONCLUSION

The detection of sulfite using MB-doped sol-gel thin films has been demonstrated using a fiber optic-based sensing system. MB appears to have a strong affinity for the thin film, but the reduction of the species in the sol gel is irreversible, hindering the reuse of the sensor.

Future studies should be done in order to improve the sensor. For instance, the determination of an appropriate oxidizing agent for MB or the organic modification of the sensor (via ormosil precursors) may prove to be beneficial in creating a regenerable sulfite sensor. Organic modification of the sensor may aid (or sterically hinder) analyte diffusion into the matrix, improving the sensitivity and response time of the sensor. Also, the aging time of the thin film should be further studied in order to determine whether there is a time-window for the usability of the sensors, in order to avoid the spectral variations which occurred with monolith age.

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