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Remote in-situ detection of 2,4,6-trinitrotoluene and other explosives using fiber optics

Yunke Zhang
University of New Hampshire, Durham

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REMOTE IN-SITU DETECTION OF 2,4,6-TRINITROTOLUENE AND OTHER EXPLOSIVES USING FIBER OPTICS

by

Yunke Zhang
Bachelor degree, Shaanxi Normal University, 1982.
Master in Science, Shaanxi Normal University, 1984.

DISSERTATION

Submitted to the University of New Hamphshire
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in
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This dissertation has been approved.

Dissertation director, W. Rudolf Seitz
Professor of Chemistry

Sterling A. Tomellini
Assistant Professor of Chemistry

Colin D. Hubbard
Professor of Chemistry

Richard P. Johnson
Associate Professor of Chemistry

Donald C. Sundberg
Associate Professor of Chemical Engineering

3/22/89
Date

ABSTRACT

REMOTE IN-SITU DETECTION OF 2,4,6-TRINITROTOLUENE AND OTHER EXPLOSIVES USING FIBER OPTICS

by

Yunke Zhang
University of New Hampshire, May, 1989.

The goal of this research was to develop methods for the in-situ determination of 2,4,6-trinitrotoluene (TNT) and other polynitroaromatic compounds in groundwater. The first approach was to use an adsorbent to preconcentrate TNT for direct determination. Poly(vinyl alcohol) was crosslinked with glutaraldehyde to form a clear gel that is transparent into the ultraviolet. The volume and swelling of the gel can be controlled by varying the amount of glutaraldehyde. The coefficient for TNT partitioning between the gel and water is 1.4. The gel offers important advantages as a matrix for chemical sensor development, but is not suitable for determining TNT in groundwater because the partition coefficient is too small.

A TNT-sensitive membrane was prepared by dissolving the following in tetrahydrofuran: 0.50 g poly(vinyl chloride), 0.20 ml dioctyl phthalate, and 0.12 ml Jeffamine T-403, a polyoxyethyleneamine which reacts with TNT to produce colored

products. The membrane is formed by casting the solution into a glass Petri dish with a diameter of 8.8 cm and allowing the solvent to slowly evaporate. Trace amounts of 1,3,5-trinitrobenzene, 2,4,5-trinitrotoluene, and N-picryl-methylnitramine also reacted with the membrane to produce a reddish brown color but their visible absorption spectra differ from that of TNT. Application of the membrane to groundwater sample analysis is comparable with previously developed HPLC methods. Recoveries from groundwater spiked to contain 0.1 to 4.0 ppm TNT ranged from 95% to 105%.

Single fiber optical measurement of membrane absorption was developed for remote in-situ detection of TNT. Refractive index matching to reduce stray light and a reflector behind the membrane to increase reflected intensity were essential to keep the stray light levels small relative to the signal of interest. To compensate for drift, the reflected intensity at 500 nm is measured relative to the reflected intensity at 824 nm, a wavelength where intensity is not affected by color formation in the membrane. The rate at which the ratio of reflected intensity at 824 nm to reflected intensity at 500 nm increases is a function of TNT concentration.

This dissertation is dedicated to my parents.

Acknowledgments

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Special thanks go to Dr. D. C. Sundberg who provided valuable suggestion throughout of the project. Dr. C. L. Grant also provided valuable suggestions

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Finally, I would like to thank my family for their continuous support and understanding for my study here.

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CHAPTER 1

INTRODUCTION

1. Background

2,4,6-Trinitrotoluene (TNT) is a common explosive used as the main bursting charge in shells, mines, and bombs. It is also a potent poison for the liver, blood, and blood forming organs. Fatalities have been recorded from toxic anemia, toxic jaundice, and acute yellow atrophy of the liver.[1] TNT contaminated water has been shown to be very toxic to aquatic organisms, with acute lethal concentration below 1 ppm to species like minnows, water fleas, bluegills and green algae.[2] Because large amounts of TNT are required for military applications, large volumes of TNT-containing water are produced. "Red water " is formed during the Sellite (sodium sulfite) purification process, in which the raw products are washed with sodium sulfite solution, the acidic impurities, such as dinitrotoluenesulfonic acids, form soluble sodium salts and are washed away. Small amounts of TNT react with sodium sulfite to form colored products which reddens the water. "Pink water" is formed when partially purified TNT is finally water-washed following the Sellite purification step. The most serious waste water is the wash-down water used to clean the equipment and the interior of the finishing plant building. As much as 500,000 gallons

per day of such water can be generated at a single plant. The "red" and "pink" water have normally been incinerated, but in the past the wash-down water was discharged into nearby rivers or streams. In addition to TNT, this water contains lesser amounts of other nitro containing compounds, such as dinitrotoluene and isomers of 2,4,6-trinitrotoluene.[3-5] These unsound disposal practices have led to contamination at military sites and created the need for a method to assess the extent of TNT pollution in groundwater.

Besides detecting TNT pollution in groundwater, there is also a need for a method to determine TNT concentration in waste water used in the treatment process. Current practice is to pass TNT wash water through a carbon or diatomaceous earth adsorption column prior to discharge to surface waters.[6] Although adsorption technology can reduce the TNT concentration to the low ppb levels, which meet present discharge limitations, these columns can be saturated and have finite lifetimes. Continuous monitoring of the TNT content of the discharge is required to identify possible breakthrough.

In addition to detecting TNT in the environment, there is long-standing interest in determining TNT for forensic purposes. The most obvious difference between the two applications is that the former usually involves trace TNT detection while the latter deals with small amounts of sample, usually explosive residues. Unless they are important for environmental analysis, forensic methods

for TNT identification are not discussed here.

The second section of this chapter will briefly review different methods used to detect TNT, especially methods related to water contamination. The fast growth of remote monitoring by fiber optic spectroscopy is reviewed in the third section, and the goal of this research is presented at the end of the chapter.

2. Determination of TNT

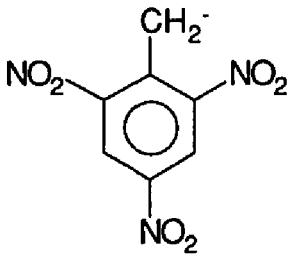
A variety of different techniques have been used for the analysis of TNT and other related polynitroaromatic compounds. Jehuda Yinon extensively reviewed various methods used for analysis of explosives in 1977.[7] Although many methods are possible, the most widely used for water pollution is reversed phase high performance liquid chromatography (HPLC), which was reviewed by Krull and Camp in 1980.[8] While HPLC is ideal for the separation of nitro compounds in water samples, detection based on ultraviolet absorption is difficult because TNT absorbs at 230 nm where many other organic compounds also absorb strongly.[9]

In order to improve the sensitivity and selectivity of the reversed phase HPLC method, Maskarinec et al. developed a method in which water contaminants are initially preconcentrated on a Porapak resin and then desorbed by acetone for HPLC analysis with electrochemical detection at a gold-mercury electrode.[10]

Because TNT has a fairly high vapor pressure, it is also amenable to separation by gas chromatography. Richard et al. have applied gas chromatography to detecting TNT contamination in water by first adsorbing TNT on an XAD-4 resin and then eluting the resin with ethyl acetate. [11] The concentrated eluant was then separated by gas chromatography and detected by an electron capture detector which is selective for nitro groups.

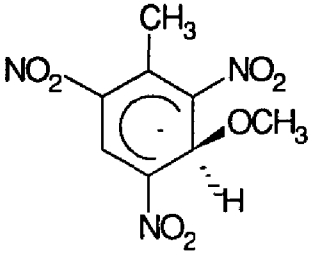
It has been known for a long time that nitroaromatic compounds can react with bases to produce colored species. [12,13] Further study has shown that the reaction actually gives rise to a variety of colored products, including charge transfer complexes, Meisenheimer complexes by base addition, radical ions by electron transfer, and, in cases where there is a substituent alkyl group, carbanions by proton subtraction. [14-22]

Fyfe et al. have used high-resolution nuclear magnetic resonance spectroscopy to investigate the reaction of TNT with methoxide ion in mixtures of Me_2SO-MeOH. [20] While transient charge-transfer complexes formed as well as possibly radical ions, the main products were three relatively stable compounds as shown in Figure 1.1. The characteristic NMR and UV-visible spectra of each species have been established. The maximum absorption wavelengths are presented in Figure 1.1 along with the molar absorptivities. It has been observed that the Meisenheimer complex **2** and dianion **3** tend to decay by forming the carbanion **1**. All of the species absorb



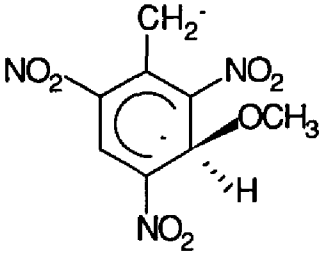
1

Absorption peak: 520 nm.
Molar absorptivity: 17000.



2

Absorption peaks: 433nm, 505 nm.
Molar absorptivities: 18000, 17000.



3

Absorption peak: 550 nm.
Molar absorptivity: 15000.

Figure 1.1. Products of the reaction of TNT with methoxide and their absorption properties.

in the visible range, and the carbanion **1** absorbs strongly at 520 nm in Me_2SO with a molar absorptivity of 17,000. Similar species were observed by an NMR study of the reaction of TNT with ammonia.[22]

Methods based on the reaction of TNT with base have been developed for the selective detection of TNT in the presence of other species which may otherwise interfere with the measurements. These methods have found applications in analysis of explosive residues, [23,24] in thin layer chromatographic detection[25,26] and ground water contamination analysis.[27] The selectivity of the reaction for polynitroaromatic compounds is very important for forensic analysis because qualitative identification of the explosive is of cardinal importance. The color from TNT in base is unstable in water. This can be partially overcome by running the samples and standards under similar conditions using automated flow injection analysis. Glover and Kayer have reported a quantitative spectrophotometric method for polynitroaromatic compounds by reacting them with ethylenediamine in dimethylsulfoxide[28] where the colored species are more stable than in other solvent systems.

Polynitroaromatic compounds can also form Jackson-Meisenheimer complexes with sodium sulphite.[29-32] Both 1:1 and 1:2 addition complexes are formed. These products also absorb in the visible and this reaction has also been used for the detection of TNT in ground water. [33]

TNT can also be determined by oxidation to nitrate with $(NH_4)_2S_2O_8$, followed by nitrate determination by the cadmium reduction method.[34]

Wannlund and DeLuca reported a sensitive bioluminescent immunoassay method for TNT detection in 1982. [35] They covalently bonded a trinitrophenyl (TNP) group to firefly luciferase by reacting the protein with trinitrobenzenesulfonic acid. The TNP-enzyme conjugate is incubated with immobilized anti-TNT antibody. Antigen-antibody complex formation causes the TNP-enzyme conjugate to combine with the immobilized antibody. TNT in the solution competes with TNP-enzyme conjugate for binding sites on the antibody. The amount of enzyme bound at equilibrium is inversely proportional to the TNT concentration. Subsequent determination of the enzyme bound to the antibody provides sensitive and selective detection of TNT in the sample. The same principle can be applied with other enzymes.

Perhaps the most interesting methods are those which combine preconcentration and chemical detection. Heller et al. reported a method for TNT detection based on a fluorescent ion-exchange resin. [36] Dowex-2 resin was first treated with NaCN to change the original counterion Cl^- to CN^-. Since CN^- is a base, the resulting resin causes the pH of a water sample to increase. Then uranine (fluoresceine disodium salt) was adsorbed onto the resin to make it fluorescent. The fluorescent resin was then packed into a glass

column. TNT was determined by pumping water sample through the column while illuminating the resin with a tungsten light. Fluorescence is detected with a photomultiplier tube. Although a slow decrease in fluorescence intensity was observed even with distilled water, the presence of TNT caused a much larger decrease in fluorescence intensity as TNT reacted to form colored products which adsorb on the resin surface and quench fluorescein emission. Because preconcentration and detection are combined in a single step, the method is relatively simple and has high sensitivity, but the lack of selectivity and fluorophor leaching hinder its application to complex environmental samples. Besides, NaCN used to treat the resin is a notorious poison.

A field detection method was reported in 1982.[37] Here a bisectional cylinder was developed as a TNT indicator tube. The first section of the cylinder was filled with glass beads coated with CaO powder. As water passes over the slightly soluble basic oxide powder, hydroxide is produced which then reacts with TNT in the sample. Glass beads were used to increase the porosity and speed up the analysis. The second section was filled with an anion exchange resin in the chloride form. When TNT-containing water passes through the column, colored anionic products are produced in the strong basic oxide layer and subsequently adsorb onto the ion-exchange resin surface. When the flow rate is held constant, the length of the second section of the tube which is colored is

proportional to TNT concentration. This indicator tube is portable and provides rapid analysis with small sample volumes.

From the methods available, we can see that selective, and sensitive detection of TNT can be achieved by combining preconcentration and selective detection. But none of the methods developed to date are suitable for long term monitoring because none provide a reagent layer which can last for a significant period of time.

3. Fiber Optic Spectroscopy in Remote Measurements

There has been great interest in the in-situ detection of water pollutants using fiber optic spectroscopy in recent years.[38-41] Methods developed involve both fluorescence and absorption measurements. Fluorescence, especially laser induced fluorescence, is preferred in that fluorescence and excitation can be resolved by the difference in their wavelengths. A significant advantage is small background signal and high sensitivity for trace analyte detection. Instrumentation for both single-fiber and two-fiber fluorimetry measurements have been developed. The concept is shown in Figure 1.2a and Figure 1.2b, respectively. With the two-fiber instrument, excitation and emission are transmitted by different optical fibers. The background signal is relatively small since only scattering and reflection at the sensor surface or in the sample solution contribute

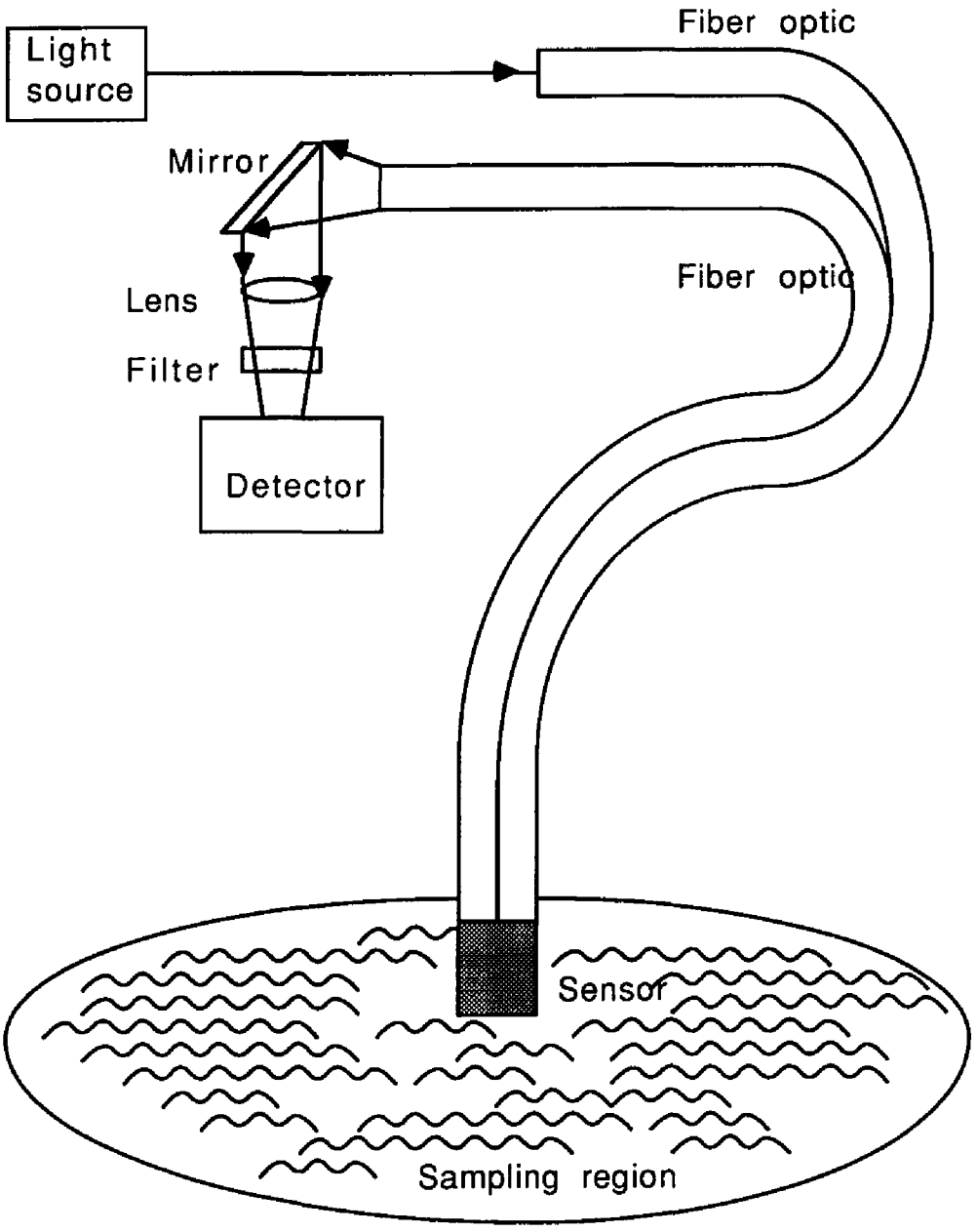


Figure 1.2a: Schematic of two-fiber remote fluorimetry.

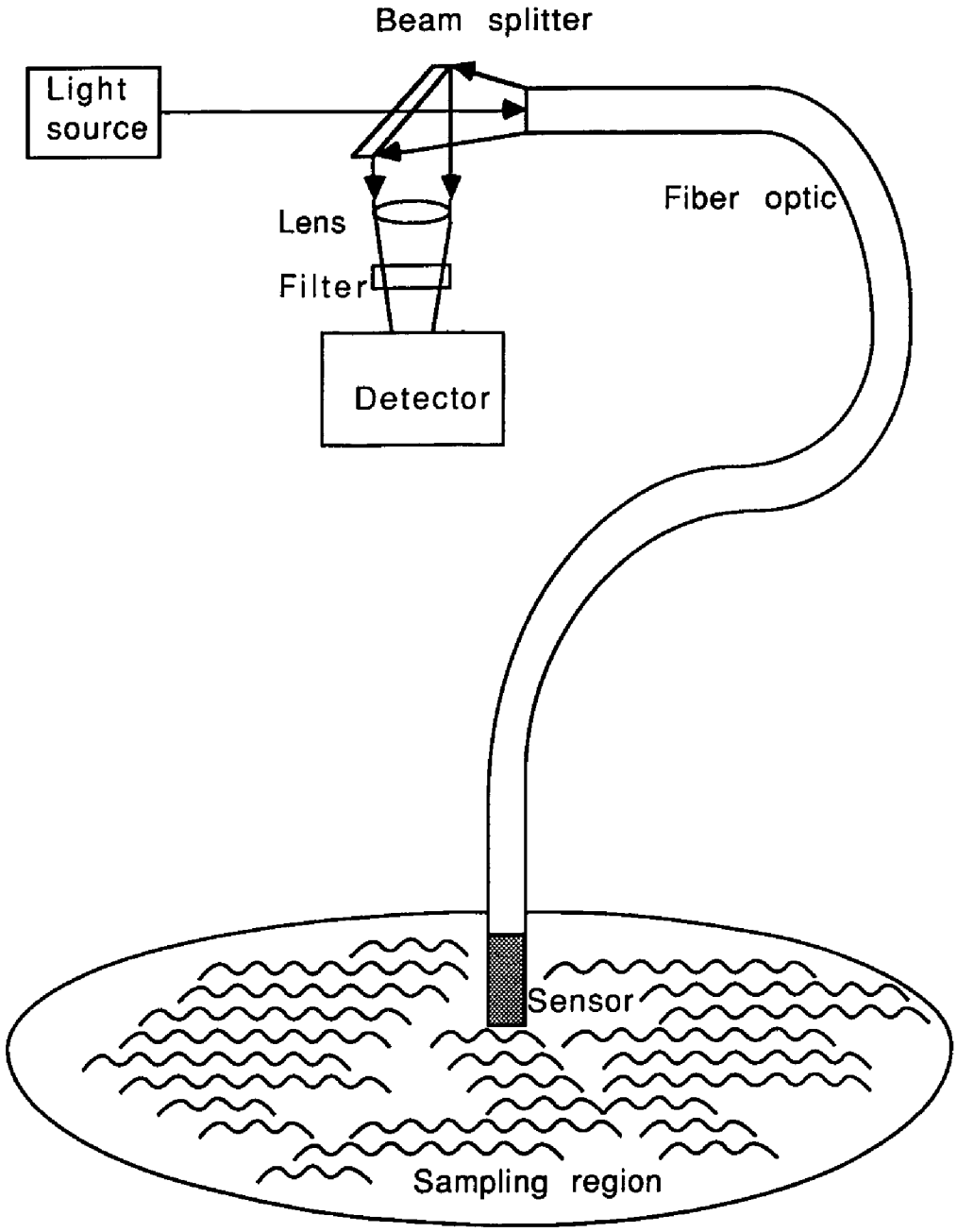


Figure 1.2b: Schematic of single-fiber remote fluorimetry.

to the the background. This kind of instrument has been used by Chudyk et al. for direct detection of weakly fluorescent aromatic organic compounds in groundwater at a distance of 25 meters.[42] This distance is close to the depth of many aquifers used as drinking water supplies. Only Raman scattering from water contributes to stray light in the measurement. This scattered light can be separated by a filter and serves as a reference intensity for monitoring drift of the instrument.

The one serious disadvantage of two-fiber fluorimetry is that only a small fraction of the fluorescence is collected by the emission fiber. Therefore, a strong source must be used to excite fluorescence. In the case of an indicator attached at the fiber end, this may increase the photodegradation of the sensing reagents. Also, the requirement of an intense source can limit the measurement distance as light is inevitably attenuated by the fiber. To improve the fluorescence collection efficiency, the single-fiber fluorimeter has been developed in which the fluorescence and excitation wavelengths are transmitted through the same fiber. A beam splitter at the proximal end of the optical fiber transmits excitation radiation to the fiber and reflects the light emerging from the fiber to a monochromator which resolves fluorescence from the reflected and scattered excitation light.[43] A dichroic mirror can be used in place of the beam splitter. The dichroic mirror can transmit the short wavelength excitation radiation

without reflection but reflects the longer wavelength fluorescence at 90^0 to the detector. Simple filters can be added to further reject the excitation light to get purer fluorescence.[44] This system can provide simultaneous measurements at two wavelengths without moving parts. Other advantages of single-fiber fluorimetry are the low instrumentation cost as the fiber needed is decreased by one half and increased convenience in placing the distal end of the fiber. One additional advantage of using a laser as the light source is that the laser light can be focused to an extremely small spot and can be efficiently coupled into the small entrance aperture of a fiber. [43]

Species that fluoresce can be directly measured with a bare fiber optic fluorimeter as illustrated above for aromatic organic compounds contamination in water.[42] But only a small fraction of all compounds are intrinsically fluorescent. Fluorescent tracers have been used to provide an analytical signal to monitor rare earth metal ion movement in groundwater.[45] Considerable research has been done to apply a reagent layer at the end of the fiber optic to make a chemical sensor. The fluorescence characteristics, such as intensity, lifetime, polarization, etc., of the reagent layer vary with analyte concentration in the sample.[40,46] A reversible reaction between the analyte and the reagent is always preferred for sensor development to avoid depletion of the sensing reagent by reaction with the analyte. These sensors have been developed for monitoring the concentration of oxygen, halothane and metal ions. [40] But with

trace contaminants in complex environmental systems, a reversible reaction with the desired sensitivity and selectivity may be difficult to find. Sensors based on an irreversible reaction with a reagent reservoir separated from the sample by a semipermeable membrane have also been developed. An example of such a system is the remote detection of organochlorides by the Fujiwara reaction.[47] A reagent reservoir containing 10 M KOH and pyridine enclosed by a semipermeable membrane at the end of the fiber optic is used as a sensing layer. The membrane is impervious to water diffusion but allows the vapor of organochlorides to go through. Diffusion of the organochlorides into the membrane results in a red fluorescent product which then gives a detectable signal proportional to the organochloride concentration. The reaction is presented in Figure 1.3.

Inorganic halides have also been determined by attaching silver fluoresceinate at the fiber optic end as a sensing reagent.[46] The reagent itself is not fluorescent due to the quenching effect of the heavy silver ions. But contact with solution containing halide will form a silver halide precipitate and release the fluorescent fluorescein anion. The increase in fluorescence intensity is a measure of halide concentration. The silver fluoresceinate reagent is consumed in this detection scheme. The signal is also sensitive to variations in the mass transfer of fluorescein away from the surface.

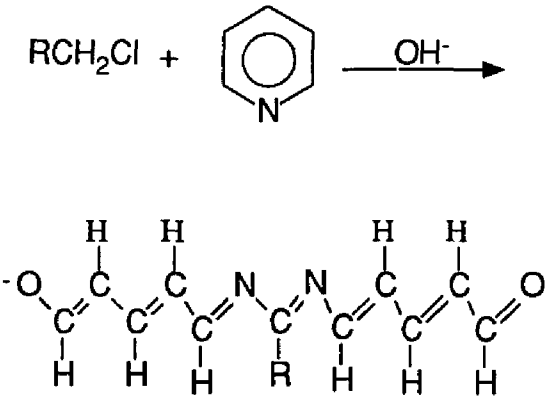


Figure 1.3. Chemical basis of the Fujiwara reaction.

As the number of species that can be selectively detected with fluorimetry is limited compared to absorption, remote fiber optic photometers for UV-Vis-NIR absorption measurements have also been developed in recent years.[48-50] A system for remote determination of copper in industrial baths with a fiber optic absorption cell has been described recently.[51] Two optical fibers were used to transmit the source light and signal light, respectively. The fixed distance between the ends of the fibers in the sample defines the absorption path. The transmitted light is measured and compared with the recorded light intensity of the blank measurement to give the absorbance in much the same way as a conventional single beam absorption photometer. Reflectance measurements using a single fiber to transmit source and reflected light over a length of 30 meters of optical fiber have also been developed.[52] The instrumentation is similar to the single fiber fluorometer except the emission monochromator is not needed. An obvious problem in this mode of measurement is that the stray light has the same wavelength as the signal light. There are two principal sources of stray light when making a single fiber absorption measurement. One is the light reflected at the proximal end of the fiber optic as light is focused into the fiber. The second is the light reflected at the interface where the light emerges from the fiber optic into the sample. Because the second source of stray light is attenuated by the fiber, the first source is the most important. The

intensity reflected at an interface when the incident light is perpendicular to the interface can be expressed by equation (1.1):

$$F = \frac{(n_2 - n_1)^2}{(n_2 + n_1)^2} \qquad (1.1)$$

where F is the fraction of light reflected at the interface of the two media with refractive indices of n_1 and n_2 respectively. A coupling gel with similar refractive index to that of the optical fiber has been used to significantly decrease this part of the stray light.[52] Also, because light reflected at the proximal end of the optical fiber reaches the detector earlier than the signal light, which has to pass through the fiber twice, modulating the light source at a frequency that matches the transit time through the fiber can provide a 90^0 phase difference between the stray light and the signal. A lock-in amplifier can be used to resolve the signal from the stray light. The total background, which can be measured with a blank solution without any reflection device, can be recorded before the sample measurements and used to determine the absorbance. A problem with the bare fiber remote absorption measurements is that the collected light signal decreases quickly as the light path for absorption increases, especially when the fiber has a small diameter, because the light coming out of the optical fiber spreads out in a cone. This limits the light path for absorption and the sensitivity of the

technique.

4. Goal of the Research

The purpose of this research is to develop a method suitable for detecting trace amounts of TNT in groundwater and then adapting the method to remote fiber optic spectroscopy. The system will meet the needs for in-situ detection of TNT contamination in groundwaters and also for monitoring waste water treatment processes in munition plants. Investigation of the possibility of making a chemically reversible fiber optic sensing system is described in the second chapter. The third chapter will describe an irreversible sensing membrane that responds to TNT and other polynitroaromatic compounds. The fourth chapter will describe preliminary tests for in-situ monitoring using single fiber absorption measurements coupled to the membrane described in Chapter 3.

CHAPTER 2

PREPARATION AND CHARACTERIZATION OF A CROSSLINKED POLY(VINYL ALCOHOL) GEL AND ITS USE IN CHEMICAL SENSOR DEVELOPMENT

This chapter describes experiments to develop a TNT sensor based on partitioning of TNT between groundwater and a suitable reagent phase. Because this system involves a partitioning equilibrium rather than an irreversible reaction, it was expected to give a reversible chemical sensor with a long lifetime.

1. Theory and Background

A schematic of the sensor concept is shown in Figure 2.1. Two separate fused silica optical fibers serve to transmit light to and from the reagent phase, respectively. There is a reflective material at the end of the reagent layer to prevent the incident light from interacting with the groundwater and to redirect the incident light towards the fiber which transmits light to the detector. The diagram includes a porous size exclusion membrane to hold the reagent in place while allowing dissolved substances to be extracted into the reagent phase. The ideal reagent should have a high partition

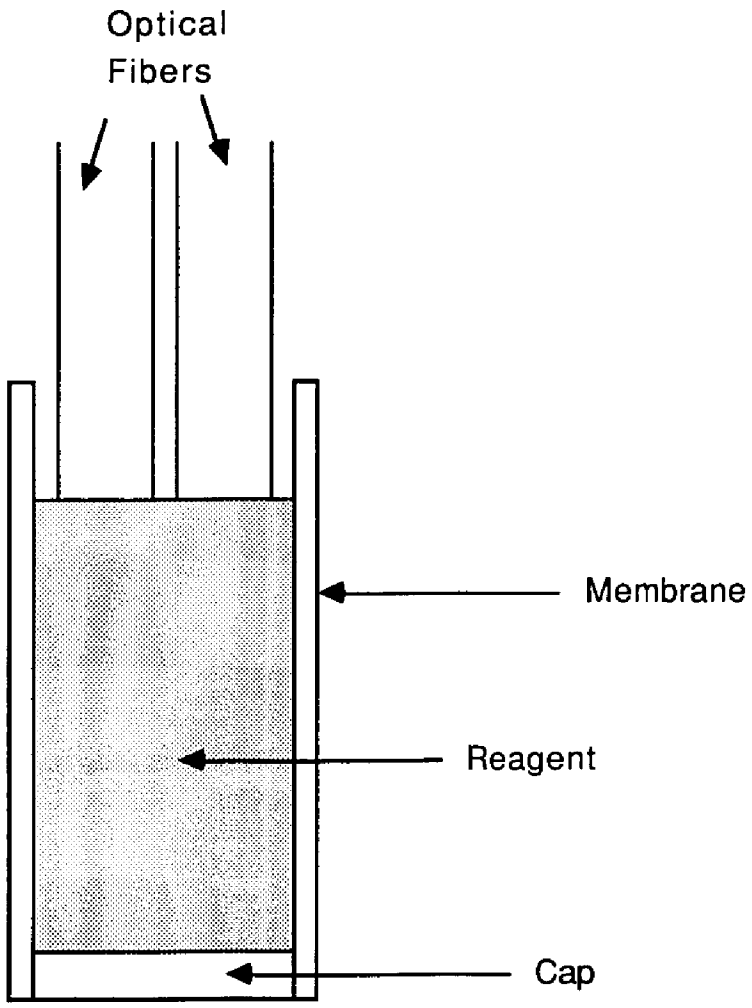


Figure 2.1. Diagram of reagent phase end of proposed sensor.

coefficient towards TNT and selectively concentrate TNT in the reagent phase while excluding naturally occurring hydrophilic organic compounds that absorb in the ultraviolet and would otherwise interfere. The reagent itself should not absorb strongly in the UV range where TNT absorption is measured. The concentrated TNT in the reagent phase can be measured by the increase of absorbance due to TNT partitioning. In addition to holding the reagent, the membrane would exclude colloidal materials that might otherwise interfere by absorbing in the spectral range of interest.

If Beer's law holds for the absorbance measurement shown in Figure 2.1, then

$$A=ab[TNT]_r \qquad (2.1)$$

where A is the absorbance due to TNT in the reagent layer, a is molar absorptivity of TNT, b is the pathlength which is twice the reagent phase thickness, and $[TNT]_r$ is the concentration of TNT in the reagent layer. At equilibrium $[TNT]_r$ depends on the concentration of TNT in the groundwater $[TNT]_w$ and the distribution between two phases as expressed by Equation (2.2).

$$K_d = \frac{[TNT]_r}{[TNT]_w} \qquad (2.2).$$

Where K_d is the distribution coefficient. Substituting (2.2) into (2.1) yields

$$A=abK_d[TNT]_w \qquad (2.3).$$

This shows that absorbance in the reagent phase is proportional to TNT concentration.

The absorption spectrum of TNT in aqueous solution is shown in Figure 2.2. The molar absorptivity of TNT is approximately 19,000 L/mol·cm at 227 nm.[53] The solubility of TNT in water is small, about 0.013g/100g water at 20^{0}C. But TNT is quite soluble in organic solvents. Solubility in 95% ethanol at 20 ^{0}C is 1.23g/100g solvent, 95 times greater than in water.[54] The high solubility difference promises a large K_d when a suitable reagent layer is used. A large K_d would make it possible to obtain a measurable UV absorption spectra for the low levels of TNT groundwater contamination that are of concern environmentally.

2. Choice of Reagent

The above discussion shows that an ideal reagent should (1) have high K_d for concentrating TNT from groundwater, (2) selectively extract TNT and avoid interference from other UV-absorbing organic compounds in water, and (3) transmit in the UV at wavelengths where TNT absorbs.

Earlier work on this project by Shane et al.[55] used a concentrated dextran solution confined by a dialysis membrane as

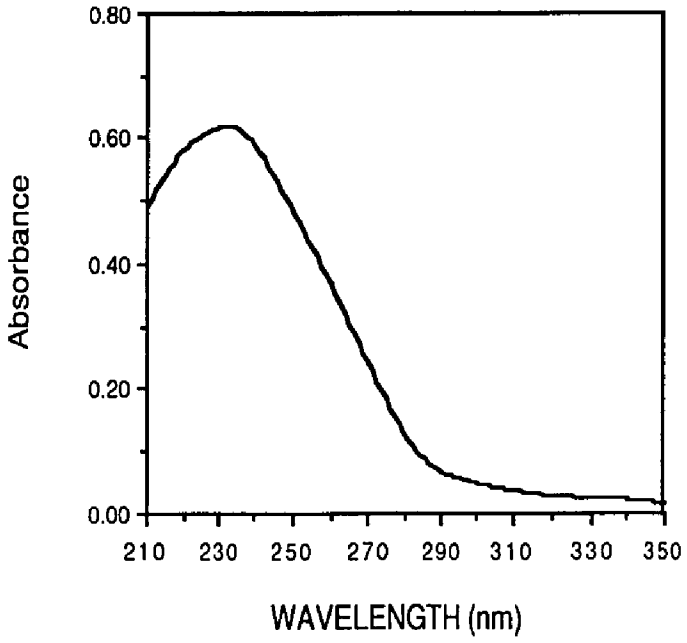


Figure 2.2. Absorption spectrum of TNT.

the reagent phase. Although spectral changes due to TNT partitioning into the sensing solution were observed, several unanticipated problems were also encountered: (1) osmotic pressure caused the dialysis membrane to swell, (2) some of the dextran leaked through the dialysis membrane and (3) an unidentified UV-absorber leached from the membrane and interfered with the absorption measurement. As a consequence, a crosslinked polymer gel was proposed to replace the dextran solution.

Poly(vinyl alcohol) (PVal) was chosen because it has a structure similar to ethanol. PVal crosslinking by reaction with glutaraldehyde has been reported in the literature by Higuchi and Iijima.[56] In their procedure, a dry PVal membrane was prepared by dissolving 1 g PVal in 10 ml water at 100 ^{0}C for 4 hours and then aging at 21 ^{0}C for 48 hours to allow air bubbles to escape. This was followed by casting the solution on a glass plate and drying at temperatures around 21 ^{0}C for a week. The membrane was crosslinked by swelling the dry PVal in 20% Na_2SO_4, followed by reaction with 0.1% glutaraldehyde in 1% H_2SO_4 and 20% Na_2SO_4 solution. The crosslinking reaction is shown in Figure 2.3. The membranes prepared by Higuchi and Iijima were around 0.4 to 0.6 mm thick. This procedure is only good for preparing membranes that are thin enough so that a homogeneous gel can be readily obtained by swelling a dry membrane and the reagents for the crosslinking reaction can quickly reach the center of the gel. This procedure was

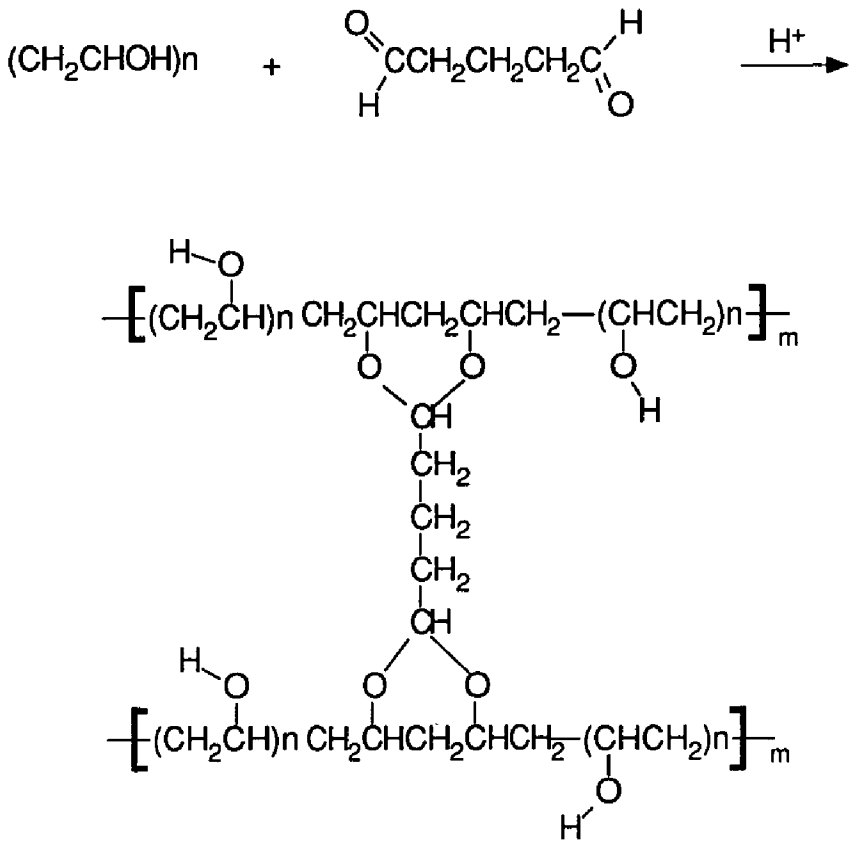


Figure 2.3. Reaction for crosslinking PVal with glutaraldehyde.

modified to produce crosslinked gels which were sufficiently thick for the UV absorption measurement required to detect TNT.

3. Experimental

(1). Chemicals and Equipment

PVal (100% hydrolyzed) with average molecular weights of 14,000 and 86,000 respectively were purchased from Aldrich. Glutaraldehyde (50% w/w) was obtained from Fisher Scientific. Standard reference TNT was obtained from the U.S. Army Toxic and Hazardous Materials Agency. A standard TNT solution was prepared by dissolving 40.0 mg of solid TNT in 10 to 15 ml of methanol in a 25 ml beaker. The methanol solution was then quantitatively transferred to about 400 ml of distilled water while vigorously stirring the water. This solution was then transferred to a 500.0 ml volumetric flask and brought to volume. The TNT concentration was 80.0 ppm. It was stored in a refrigerator at 4 ^{0}C and diluted to the required concentration before use. TNT used for the solubility measurements was purchased from Kodak. Other reagents were also obtained from the available commercial sources. All materials were used as received.

A Shimadzu Spectronic 200 spectrophotometer was used for all the absorption measurements.

(2). Gel Preparation

A convenient procedure was developed for making a thick crosslinked PVal gel by mixing the polymer solution with glutaraldehyde and a suitable amount of acid and letting the crosslinking reaction occur slowly. Control of the reaction speed is crucial in getting a clear gel. If crosslinking is too rapid, local precipitation can cause inhomogeneities which make the resulting gel appear cloudy. Also, air bubbles can be trapped in the gel. Both effects render the gel unsuitable for optical measurements.

The following procedure yields a clear gel. 1.0 g PVal (average molecular weight 86,000) is dissolved in 18 ml water by gently heating the solution to about 90 ^{0}C and stirring for one hour. The solution is cooled to room temperature, and 0.16 ml 10% glutaraldehyde (diluted from 50% glutaraldehyde with water) is added and mixed well. Then 0.4 ml of 1.2 M HCl (prepared by diluting concentrated HCl 1:9 in water) is added and the resulting solution is mixed. The solution is then covered and allowed to sit at room temperature for 12 hours. The resulting clear gel is then soaked in pH 6 phosphate buffer for 2 days to neutralize the acid in the gel.

Gels were usually prepared in disposable 1.00 cm pathlength plastic cuvets. After the gel had set, the cuvets were broken apart yielding a clear gel with a well defined geometry. These blocks of gel were used for UV absorption measurement.

(3). Absorption measurements

The absorption spectrum of the gel is measured by holding the block of gel in a disposable plastic cuvet whose two opposite walls has been removed to allow UV light to be transmitted. The absorption spectrum of TNT in the gel is obtained using another gel soaked in the blank solution as the reference.

(4). Swelling index measurement

The swelling index of the gel is defined by equation (2.4).

$$SI = \frac{W_2 - W_1}{W_1} * 100 \qquad (2.4)$$

where SI is the swelling index and W_1, W_2 are the weights of the gel before and after soaking in water for 24 hours. Slices of gel about 0.5 cm thick were used for the measurement as thinner slices tend to reach equilibrium at shorter time periods.

(5). Measurement of TNT solubility

Solubilities of TNT in various ethanol-water mixtures were measured by adding solid TNT to a series of ethanol-water mixtures and shaking for 48 hours at room temperature. After centrifuging to remove excess solid TNT, the TNT concentrations were determined by UV absorption.

4. Factors Influencing Gel Properties

(1). PVal Concentration

As the concentration of PVal increases, two problems developed: (1) the aqueous PVal solution becomes so viscous that air bubbles entrapped in the mixing steps escape very slowly and (2) the crosslinking reaction tends to go so rapidly that mixing of HCl with the glutaraldehyde/PVal solution causes local precipitation and entrapment of air bubbles. To prepare a gel from a 10% (w/w) PVal solution the procedure described in the experimental section has to be modified. PVal with an average molecular weight of 14,000 was used to replace the high molecular weight PVal. Aqueous glutaraldehyde/PVal and the concentrated HCl reagent were added to separate containers. Both containers were placed in a covered 500 ml beaker. The HCl slowly transfers through the vapor phase, initiating crosslinking. Once gel starts to form on the surface of the glutaraldehyde/PVal container, the concentrated HCl solution is removed from the covered 500 ml beaker and 0.05 M HCl solution is added to the beaker. The HCl solution is allowed to be in direct contact with the gel surface and gel formation continues to completion.

In another approach the HCl solution was replaced by 0.26 ml $(CH_3)_2SO_4$. This reagent itself has no catalytic effect on the crosslinking reaction. When all the reagents are mixed, the solution

is heated to about 80 ^{0}C to hydrolyze $(CH_3)_2SO_4$ which produces the catalyst H^+ homogeneously in the solution.

$$(CH_3)_2SO_4 + 2\,H_2O \longrightarrow H_2SO_4 + 2\,CH_3OH$$

The gel made by this procedure has no apparent cloudiness but the absorption at wavelengths shorter than 250 nm is increased.

(2). Glutaraldehyde Concentration

The final water content of the gel depends on the degree of crosslinking. This is measured in terms of the swelling index which is defined in the previous section. Figure 2.4 shows how varying the amount of glutaraldehyde affects the swelling index for the gel. All the plots in this work are drawn by interpolating between the measurements. The lines do not have a theoretical basis and are included for illustration purpose only. The data shown are for gels made by combining 1.0 g PVal in 18 ml water and 0.4 ml 1.2 M HCl with variable amounts of 10% glutaraldehyde in water. As indicated in Figure 2.4, the gel formed using 0.16 ml 10% glutaraldehyde has a swelling index of 0. This means the gel, as formed, neither gains nor loses water when added to an aqueous solution. This formulation was used to measure TNT partitioning. Gels with higher amounts of glutaraldehyde tend to lose water by shrinking and the ones with lesser amounts of glutaraldehyde tend to gain water by swelling.

The ratio of PVal to water in the final gel is increased by using higher levels of glutaraldehyde to increase the degree of

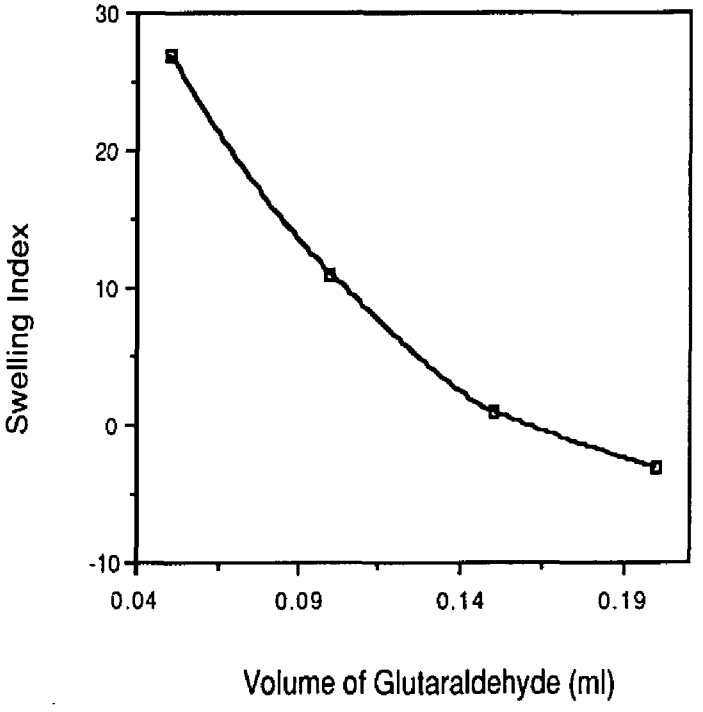


Figure 2.4. Swelling index for PVal gel as a function of the volume of 50% (w/w) glutaraldehyde added to 1 g PVal.

crosslinking. Higher PVal levels should increase the partition coefficient of TNT in the gel. However, it was found that increased glutaraldehyde levels caused increases in the background absorption or scattering in the UV which would interfere with the TNT measurement.

(3). HCl Concentration

Solutions prepared by combining 1.0 g PVal in 18 ml water and 0.16 ml 10% glutaraldehyde were reacted with 0.1, 0.4, and 0.8 mls of 1.2 M HCl. No gel is formed even after a week with 0.1 ml HCl. Both 0.4 and 0.8 ml HCl yielded clear gels in a reasonable period of time. Higher HCl concentration cause quick precipitation and traps air bubbles in the gel. Concentrated HCl can not be directly used due to quick local precipitation during mixing.

(4). Temperature

Gels were prepared at 12 ^{0}C, room temperature (about 27 ^{0}C) and 60 ^{0}C. Gel formation is accelerated at the higher temperature. However, the gel formed at 60 ^{0}C had significantly higher background absorption. There was no significant difference between the gels prepared at room temperature and at 12 ^{0}C.

5. Properties of the PVal Gel

(1). Absorption

Figure 2.5a and 2.5b show UV absorption spectra for PVal in

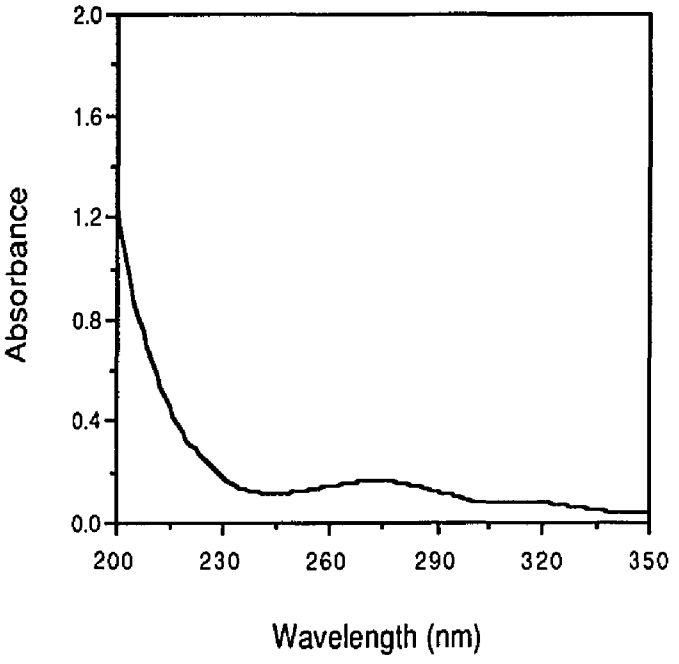


Figure 2.5a. Absorption spectrum for 5% (w/w) PVal in water.

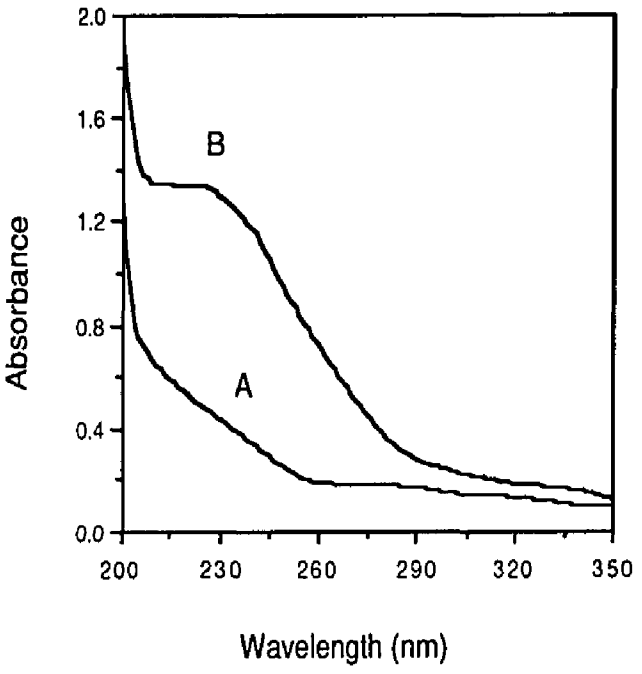


Figure 2.5b. Absorption spectra for PVal gels before (A) and after (B) soaking in 10 ppm TNT for 10 hours.

solution, PVal gel soaked in blank solution and PVal gel soaked in 10 ppm TNT solution for 16 hours. Crosslinking leads to broadening of the major PVal absorption band in the range from 220 to 250 nm. There is also a weak band centered at 280 nm. This band, which may due to residual carbonyl absorption, actually decreased after crosslinking. The gel is sufficiently transparent to permit measurement of TNT absorption.

(2). Stability with Time

The absorption spectra of gels soaked in water or pH 6.0 phosphate buffer do not change over a week. However, when the gel is soaked in 0.1 M NaOH, absorbance increases slowly at wavelengths shorter than 260 nm.

(3). Response to TNT

1.0 cm thick gels prepared in 1.00 cm disposable plastic cuvets were soaked in aqueous TNT solutions. Absorption spectra measured at various times show that it takes at least 35 hours for the TNT concentration in the gel to reach equilibrium. Results are shown in Figure 2.6.

Figure 2.7 shows the decrease in TNT absorbance with time for a 1.0 cm block of gel pre-exposed to a 10 ppm aqueous TNT solution for 17 hours and then placed in water. The data confirm that the gel responds reversibly to variations in TNT concentration.

(4). Partition Coefficient

The partition coefficient for equilibrium partitioning of TNT

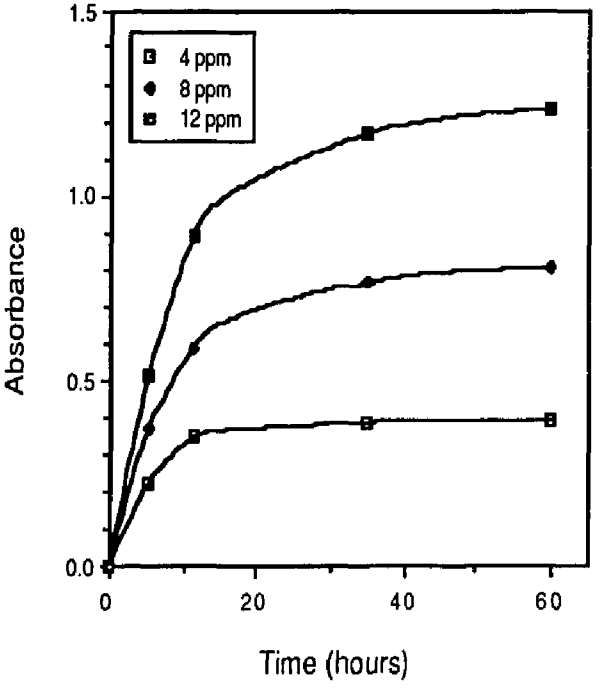


Figure 2.6. Absorbance at 230 nm for PVal gels exposed to 4, 8, and 12 ppm aqueous TNT standard solutions.

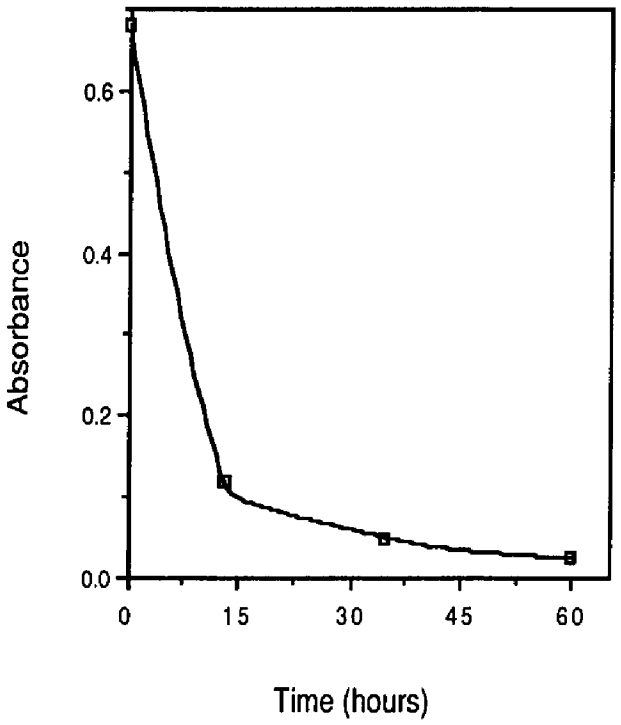


Figure 2.7. Absorbance of TNT treated PVal gel in water as a function of time.

from aqueous solution into the gel was determined by measuring the relative magnitude of TNT absorption in aqueous solution and in the gel for a block of gel at equilibrium with an aqueous TNT solution. For a gel prepared from 1.0 g of PVal in 18 ml water and 0.16 ml 10% glutaraldehyde, the partition coefficient was determined by exposing blocks of gel to 4, 8, and 12 ppm TNT solutions for 59 hours, then measuring the absorbance of TNT at 230 nm in the gel and the solution. The partition coefficient was calculated from the ratio of TNT absorbance in the gel to the absorbance in the solution. Results for the measurements are given in Table 2.1. The average value for the partition coefficients is 1.43.

6. Prospects for Increasing the Partition Coefficient

Because the partition coefficient is too low, TNT can not be preconcentrated to the extent required for in-situ sensing of TNT in groundwater at the sub-ppm level. Several approaches to increasing the partition coefficient were considered. The ratio of PVal to water in the gel can be increased by starting with a higher initial PVal concentration or by increasing the level of glutaraldehyde to get a higher degree of crosslinking. However, higher levels of glutaraldehyde lead to higher background absorption, and formation of a clear gel is more difficult at higher PVal levels because of the increased viscosity of the aqueous PVal solution and the tendency

Table 2.1.

Partitioning for TNT between PVal gel and water.

TNT concentration	Absorbance in gel	Absorbance in solution	Partition coefficient
12 ppm	1.25	0.858	1.46
8 ppm	0.814	0.582	1.40
4 ppm	0.394	0.274	1.44

for the gel to form more rapidly.

Rather than attempt the difficult task of forming gels with higher PVal levels, a simple experiment was performed to get an estimate of the PVal level required to significantly increase the partition coefficient. The solubility of TNT was measured as a function of the volume percentage of ethanol added to water. Because ethanol and PVal are both aliphatic alcohols, the increase in solubility as a function of added ethanol should be similar to the increase in preconcentration factor with an increased percentage of PVal in the gel.

Solubility data are shown in Figure 2.8. Although TNT is much more soluble in pure ethanol than in pure water, small amounts of added ethanol do not significantly increase the solubility of TNT. The concentration of added ethanol must be greater than 50% by volume to significantly enhance TNT solubility. Since formation of gels containing PVal approaching 50% does not appear to be feasible, it seems impossible to prepare gels that will adequately preconcentrate TNT for the sensing application.

The solubility of TNT in concentrated dextran solutions was measured by the same procedure used to measure TNT solubility in ethanol-water mixtures. It was found that TNT is 2.1 times more soluble in a solution prepared by dissolving 4.0 g of dextran in 10 ml of water than in pure water. This indicates that concentrated dextran solution will not adequately preconcentrate TNT. The

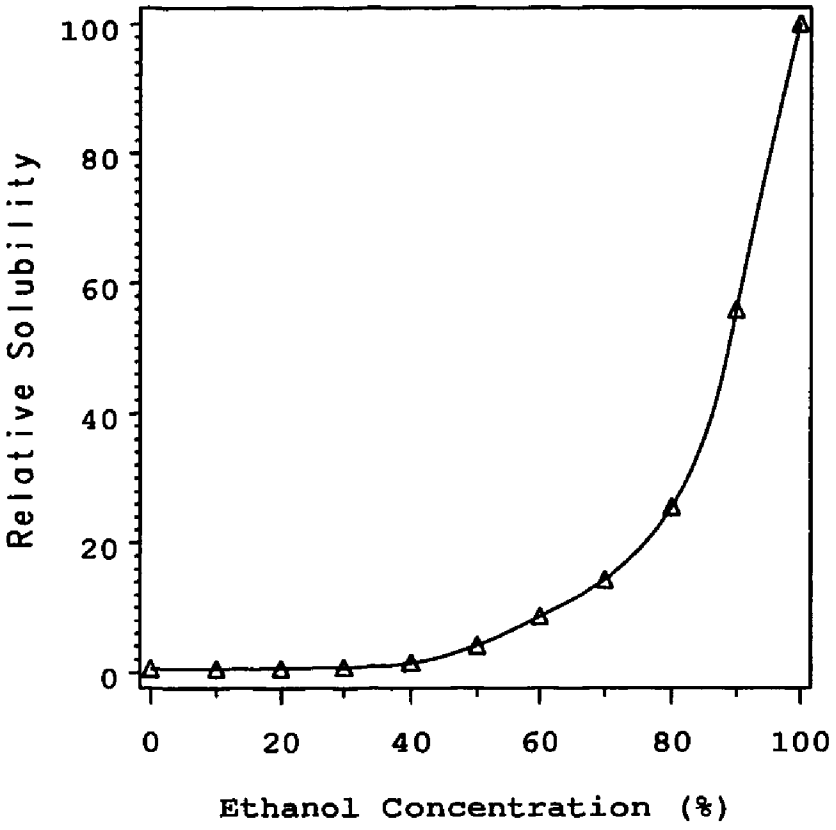


Figure 2.8. Relative solubility of TNT in ethanol-water solutions.

apparent high partition coefficients measured in the previous study[55] were an artifact of membrane leakage and associated problems.

The possibility of increasing the hydrophobicity of the gel to produce increased partitioning was also investigated. The gel was reacted with monofunctional aldehydes including formaldehyde, acetaldehyde and butyraldehyde. The resulting gels tend to shrink after formation and become very cloudy. They are completely unsuitable for UV absorption measurements.

7. Application of the Gels

While PVal gels do not have the necessary characteristics for in-situ measurements of TNT, they are useful media for optical measurements. Although no further attempts were made to use the gel for TNT sensor development, other research within the group has proven that the gel is a desirable support material for immobilizing reagents for other sensing applications. Immobilization of fluoreseinamine and calcein for pH and calcium sensing, respectively, have been reported.[57,58]

CHAPTER 3

A CLEAR, AMINE-CONTAINING POLY(VINYL CHLORIDE) MEMBRANE FOR OPTICAL DETECTION OF TNT

Reactions of TNT with amines have been described in Chapter 1. Measurements based on these reactions offer important advantages relative to measurements in the ultraviolet below 300 nm. Less expensive optical components can be used, including an incandescent source and glass optical fiber. Optical transmission is much higher in the visible than in the ultraviolet. Furthermore, background absorption is less likely to be a problem in the visible region of the spectrum. Because of these advantages, a plasticized poly(vinyl chloride) (PVC) membrane doped with amine has been prepared and successfully used for sensitive detection of TNT and other related polynitro aromatic compounds in groundwater. Preparation and properties of the membrane are described in this Chapter.

1. Background

PVC has been widely used for preparing reagent-loaded plasticized membranes for analytical applications, especially for making ion selective membrane electrodes. [59-62] The unique

characteristic of PVC is that it accepts large amounts of plasticizers gradually changing in physical properties from a rigid solid to a soft gel or viscous liquid. Most other resins undergo this transformation with slight increases in plasticizer content or temperature. [62]

Amines are known to be good stabilizers for PVC.[64] Trioctylamine-loaded PVC particles have been used to extract metal ions from water.[65] These applications indicate that PVC is compatible with some amines.

PVC is also compatible with nitroaromatic compounds. Nitrobenzene has been used as a plasticizer for making a soft PVC membrane.[66] Derivatives of nitrobenzene such as *o*-nitrophenyl phenyl ether and *o*-nitrophenyl octyl ether have been used as plasticizers for making ionophore-impregnated PVC membranes for ion selective electrodes.[66,67] These reports suggest that TNT will have a high affinity for PVC, thus, the coefficient for TNT partitioning into the membrane from water will be high.

TNT solubilities in methyl acetate and benzene are $5.5X10^3$ and $5.2X10^3$ higher than in water, respectively.[54] This suggests that the commonly used PVC plasticizer, dioctylphthalate (DOP), a benzene derivative with two hydrogen atoms substituted by two ester groups, may also be a good solvent for TNT and thus help to efficiently extract TNT from water into the membrane.

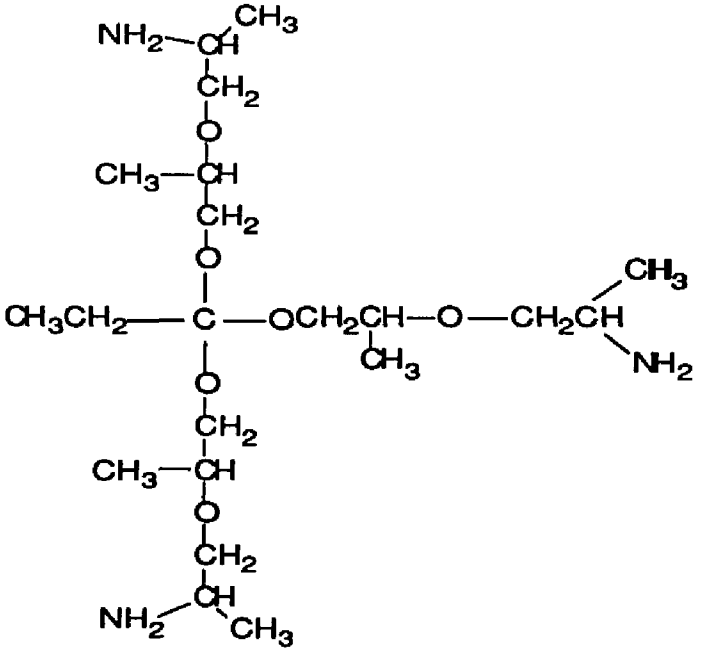
Another important reason for expecting the membrane to be

highly sensitive to TNT is that the reaction in the membrane is irreversible, rather than reaching an equilibrium, TNT will continue to enter the membrane, reacting to form colored products that accumulate in the membrane until the amine is depleted. The accumulated products can then be detected by measuring the extent to which they absorb light.

2. Experimental

(1). Chemicals and Equipment

Geons 142 and 143 PVCs manufactured by B. F. Goodrich were obtained from Geon Vinyls. Jeffamine T-403 was obtained from Texaco Chemical Company as a pure liquid. It has the structure shown below:



The average molecular weight of Jeffamine T-403 is 403. The primary amine content is 6.1 meq g^{-1}. Most other amines were from Aldrich, except for 2-methyl-1,5-diaminopentane which was obtained from Dupont. All amines are used as received.

Anhydrous tetrahydrofuran (THF) was used as the solvent for casting PVC membranes. It was obtained from Aldrich or by distilling reagent grade THF prior to use.

Explosives were obtained from the U.S. Army Toxic and Hazardous Materials Agency (THAMA) as reference materials for evaluating analytical methods.

Membrane absorption spectra were measured using the Spectronic 200 Spectrophotometer described in Chapter 2. A membrane exposed to a blank solution was placed in the reference cell. The nitrogen content of the membranes was measured with a Perkin-Elmer 240B Elemental Analyzer by the stuff at the Instrument Center in the department.

(2). Procedures

Membranes were prepared by solvent casting from THF. The procedure which yields the best membrane is as follows. Bulk polymerized PVC (0.5 g) is placed in a 25 ml glass beaker which has been predried at 130 ^{0}C. After adding 11 ml of anhydrous THF, the beaker is covered with a piece of Parafilm to exclude atmospheric moisture. The PVC gels in about two hours. After it has gelled, the beaker is gently heated with stirring to make a homogeneous

solution. DOP (0.20 ml) and Jeffamine T-403 (0.12 ml) are added. Gentle warming and stirring are continued until all components are well mixed and the solution appears clear and completely uniform. The solution is then added to a warm, dried Petri dish (8.8 cm inner diameter) and covered with a layer of dry Kimwipes to let THF evaporate. After three days or more, a clear membrane has formed and is ready for use. Prior to use membranes are stored in sealed, dry vials. Analogous procedures were used to prepare other membranes with different amine contents and thicknesses.

Preparation of standard TNT solutions has been described in Chapter 2. All other explosives were prepared in the same way and have concentrations ranging from 20 to 80 ppm.

The pH 5.0 buffer was prepared by dissolving 16 g of sodium acetate in about 80 ml of water, adding acetic acid to bring the pH to 5.0 and then adding water to bring the final volume to 100 ml. The pH 6.0, 7.0, and 8.0 buffers were prepared by adding varying amounts of 0.1 M NaOH to 0.1 M NaH_2PO_4 and adjust the final volume to 100 ml. The pH 9.0 buffer was prepared by adjusting the pH of 50 ml of 0.10 M trishydroxyaminomethane to pH 9.0 with 0.10 M HCl and diluting to a final volume of 100 ml.

Measurements of the TNT concentration in were made by placing two 0.5X3.0 cm^2 pieces of membrane in 200 ml of an aqueous TNT sample in a container sealed to prevent evaporation. The reaction was allowed to proceed for a fixed period of time

without any stirring. The membrane was then removed. The spectrum of the membrane was measured by attaching the membrane at the outer surface of a quartz cuvet masked so that the width of the light path is 0.2 cm. Surface tension is sufficient to hold the moist membrane on the cuvet. Another piece of membrane, soaked in the blank solution, was attached to a second cuvet to serve as the reference. The cuvets were filled with distilled water to reduce the reflection at the quartz-air interface. The absorbance at 800 nm was adjusted to zero to compensate for the variation due to the difference in membrane thickness.

Leaching of amine from membranes in distilled water was evaluated by titration. A piece of fresh membrane with an area of 60 cm^2 (corresponding to 0.80 g) was placed in 30.0 ml of distilled water. After exposure for a known time, the leached amine was titrated with 0.012 M HCl which had been standardized against Jeffamine T-403 using methyl orange as the indicator.

Membrane nitrogen contents were measured by the staff at the UNH Instrumentation Center using a Model 240B Elemental Analyzer. For the membranes stored in air for different periods of time, small pieces of the membrane were washed with distilled water quickly to remove the amine that had leached onto the surface, then the membrane was dried with Kimwipe paper and analyzed. Data for membranes stored in air for different periods of time were measured using membranes prepared at different times. All

measurements were made at the same time. Membranes exposed to 0.01 M phosphate buffer solution were dried in the same way before analysis. Measurements were made after various exposure times. Membranes with different initial amine concentrations were all measured at the same time .

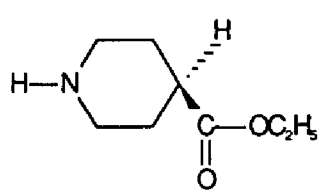
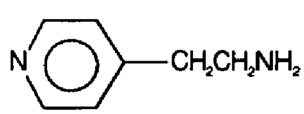
3. Results and Discussion

(1). Choice of Amine

Membranes were prepared using a variety of amines. Table 1 qualitatively summarizes the results. Chemical compatibility of the amine with the plasticized PVC is important for getting clear, tough membranes. As noted in Table 3.1, tris(2-aminoethyl)amine and 4,9-dioxa-1,12- dodecaneamine fail to yield clear membranes. Instead they seem to partition into a separate phase leading to microdomains of amine within the membrane which manifest themselves as cloudiness. Ethyl isonipecotate forms a much less tough membrane and soon becomes cloudy and yellow in the air. These cloudy membranes can not be used for the measurement and were not studied further.

When the clear membranes with different amines were exposed to aqueous TNT solutions it was found that N,N-diethylethylenediamine, 2-methyl-1,5-diaminopentane,

Table 3.1. Effect of amine structure on membrane response.

Amine	Structure	Response
Ethylenediamine	$H_2NCH_2CH_2NH_2$	Weak color
2-Methyl-1,5-diaminopentane	$H_2NCH_2CH(CH_2)_3NH_2$ CH_3	Medium color
Dibutylamine	$(C_4H_9)_2NH$	Weak color
Triethylamine	$(C_2H_5)_3N$	No color
Trioctylamine	$(C_8H_{17})_3N$	No color
N,N-Diethylethylenediamine	$(C_2H_5)_2N(CH_2)_2NH_2$	Strong color
Tri(2-aminoethyl)amine	$(H_2NCH_2CH_2)_2NH_2$	Cloudy membrane
3-Diethylaminopropylamine	$(C_2H_5)_2N(CH_2)_3NH_2$	Strong color
3-Dibutylaminopropylamine	$(C_4H_9)_2N(CH_2)_3NH_2$	Strong color
4,9-Dioxa-1,12-dodecaneamine	$H_2N(CH_2)_3O(CH_2)_4O(CH_2)_3NH_2$	Cloudy membrane
Ethyl isonipecotate		Medium color
4-Aminoethylpyridine		Medium color

3-diethylaminopropylamine, 3-dibutylaminopropylamine and Jeffamine T-403 gave strong color, dibutylamine give medium color, aminoethylpyridine also gave medium color and the membrane itsef slowly became yellow in storage, and trioctylamine and triethylamine did not give any color. These results tell us that only primary and secondary amines yield observable color. Low molecular weight amines with high water solubility yield relatively weak color because the amines are rapidly extracted into the aqueous phase where no reaction occurs. A lipophilic primary or secondary amine is required for maximum sensitivity.

Of the amines evaluated, Jeffamine T-403 proved to be the most satisfactory because it combined sensitive response to TNT with stability towards loss of nitrogen. Jeffamine T-403 is unique among the amines tested in that it can serve as a plasticizer. Clear elastic membranes are formed by solvent casting PVC with Jeffamine T-403. However, greater stability is achieved with formulations that also include DOP as a plasticizer. The unique characteristics of Jeffamine T-403 may result from the good compatibility of the ether chains in the molecule with PVC. THF, as an ether, can mix with PVC in any proportion. Crown ethers have been loaded into PVC membranes to produce lithium ion selective electrodes. [67,68] Furthermore, the highly branched geometry of Jeffamine T-403 and its large molecular weight may limit its diffusion in the membrane and help to keep it from leaching into the

aqueous solution.

(2). Choice of Poly(vinyl chloride)

The PVC used for the initial membrane preparation and evaluation was prepared by emulsion polymerization. This type of PVC contains residual surfactant with ionic functional groups, [69] which act as sites for water accumulation in the membrane. As a result, membranes became cloudy when exposed to water for several hours.

When the problem with the emulsion polymerized PVC was recognized, four other types of PVC were evaluated: Geons 142 and 143 from B. F. Goodrich, reagent grade PVC from Aldrich, and 1.8% carboxylated PVC from Aldrich. The 1.8% carboxylated PVC is least suitable for this application. Membranes prepared from this material rapidly become cloudy in contact with water because the carboxyl groups act as hydrophilic sites that attract water. The best PVC for our purpose is Geon 143, which is prepared by a bulk polymerization process that does not require added surfactant. Membranes prepared from this material remain clear for months in contact with water. They can be exposed to aqueous TNT for longer periods of time, allowing more of the brown product to accumulate, resulting in lower detection limits.

(3). Spectral Properties and Response to Other Explosives

Besides TNT, the membrane also was found to react with 2,4,5-trinitrotoluene, (2,4,5-TNT) methyl-2,4,6-trinitrophenyl-

nitramine (tetryl) and 1,3,5-trinitrobenzene (TNB) to form colored products. However, the absorption spectra of the products differ for different explosives. Figure 3.1 shows absorption spectra for 2 cm^2 pieces of membrane exposed to 200 ml of each explosive for 24 hours. The concentrations were 5 ppm for TNT and TNB, and 2ppm for 2,4,5-TNT and tetryl. Differences in the absorption spectra mean that the membrane can be used to discriminate among different polynitroaromatic hydrocarbons.

The membrane does not react with hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) to form colored products. Weak yellow color was observed for membranes exposed to 2-amino-4,6-dinitrotoluene (2-amino-DNT) and 4-amino-2,6-dinitrotoluene (4-amino-DNT). The spectra corresponded to the solution spectra of the amino-DNT's. Thus amino-DNT's are physically partitioning into the membrane, but do not react with the amine. RDX and HMX may also enter the membrane. However, their absorption is at shorter wavelengths where it is obscured by the intrinsic absorption of the membrane.

Comparing the spectra of the four trinitro compounds with the spectra obtained by Fyfe et al. for the TNT anion, TNT-base 1:1 and 1:2 addition complexes, [20] we conclude that the observed TNT spectrum is mainly due to TNT anion absorption. The anion probably exists as an ion-pair with protonated Jeffamine cation. Otherwise, it would be expected to leach from the membrane. The single TNB

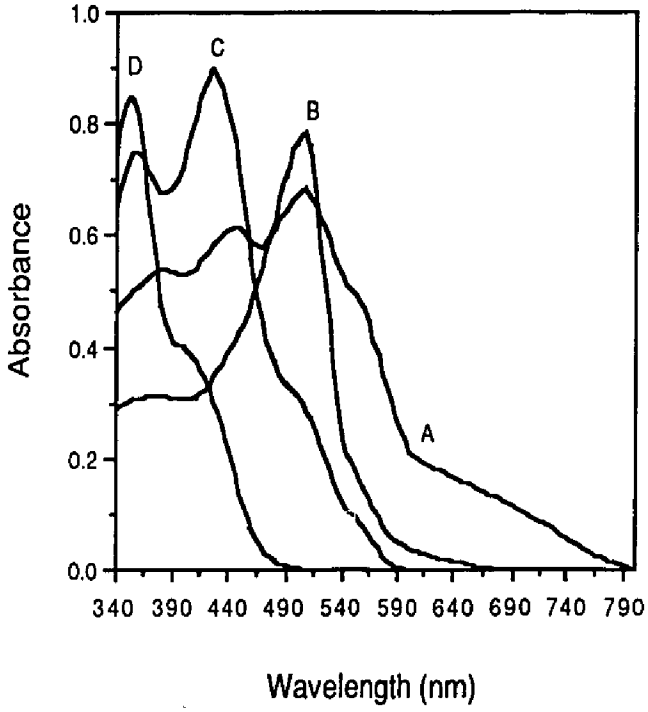


Figure 3.1. Absorption spectra of membranes exposed to TNT (A), TNB (B), TETRYL (C), and 2,4,5-TNT (D).

absorption peak shape agrees well with the spectrum of the 1:2 addition complex of TNT with base. The spectra of 2,4,5-TNT and tetryl resemble the spectrum of TNT-base 1:1 addition complex with 2,4,5-TNT absorption shifted towards the shorter wavelength. This may be because 2,4,5-TNT is less acidic and because steric effects with these two compounds prevent formation of the 1:2 addition complex.

The absorption spectra of membranes exposed to TNT for long periods of time showed shifts in spectral distribution with short wavelength bands increasing relative to the main band at 510 nm. In very highly exposed membranes the band at 510 nm becomes only a shoulder and a new peak with maximum absorption at 432 nm is observed.

(4). Effect of Plasticizer

Dioctyl phthalate (DOP) serves as a plasticizer to keep the membrane soft and elastic which facilitates handling. Increasing the percentage of DOP in the membrane increases the rate of diffusion for both TNT and amine leading to faster response. DOP may also help to keep the amine inside the membrane while excluding water. Membranes formed from PVC in the absence of DOP were hard and rigid and unsuitable for optical measurements. If too much DOP was added, membranes tended to become cloudy in water. A 2:5 DOP:PVC ratio was found to be optimum and was used for most of the results reported here.

As noted above, Jeffamine T-403 can serve as a plasticizer for PVC yielding membranes that respond sensitively to TNT. However, the properties of these membranes were found to change with time because Jeffamine T-403 was subject to relatively rapid extraction into water when present in concentrations high enough for it to serve as a PVC plasticizer. This caused the membranes to lose flexibility.

Because DOP was quite satisfactory, other plasticizers were not evaluated. However, other plasticizers are likely to work just as well.

(5). TNT Partitioning into the Membrane

To confirm the roles of DOP and amine, the following experiment was performed. Three membranes with similar weights and surface areas were prepared: one with 0.80 g PVC, one with 0.50 g PVC and 0.30 ml DOP and one with 0.50 g PVC, 0.20 ml DOP and 0.10 ml Jeffamine T-403. 15 cm^2 (equivalent to 0.2 g) pieces of each membrane were put into separate 30 ml solutions containing 2 ppm TNT and into separate 30 ml blanks. For all three samples, the solution absorbance of TNT at 230 nm was monitored as a function of time and compared to the absorbance of an aqueous 2.0 ppm TNT solution prepared at the same time. No apparent change in TNT absorbance was observed for the solution containing the pure PVC membrane. TNT did not partition into PVC to a significant extent in the absence of a plasticizer.

Figure 3.2 shows the decrease in TNT concentration with time for solutions in contact with plasticized membranes with and without Jeffamine T-403. The concentration of the solution containing the plasticized membrane without amine decreased rapidly at first, then more gradually, approaching a constant value after 90 hours. If it is assumed that the decrease in TNT concentration in solution is due only to partitioning into the membrane, one can calculate the partition coefficient to be 272 (= [TNT] in membrane / [TNT] in water).

The decrease in absorbance with time is greater for the amine containing membrane because the reaction with amine depletes free TNT in the membrane, causing more to diffuse in.

(6). Effect of pH on response

The influence of pH on response to TNT was evaluated by exposing 1.5 cm^2 pieces of membrane to 200 ml of 2.0 ppm aqueous TNT solutions buffered at the following pHs: 5.0, 6.1, 7.0, 7.9 and 8.8 by adding 20 ml of each buffer solutions to 20 ml of 4 ppm TNT solutions. Figure 3.3 shows absorbances measured after 12 and 24 hours. For the pH range from 6.1 to 8.8, the 12 hour response was not significantly influenced by pH. However, at pH 5, initial color formation is weaker and gradually disappears with time. At the lower pH, there is a stronger tendency for the Jeffamine T-403 to be protonated. Furthermore, the acetic acid used in the buffer system is a neutral molecule which may be able to enter the membrane and

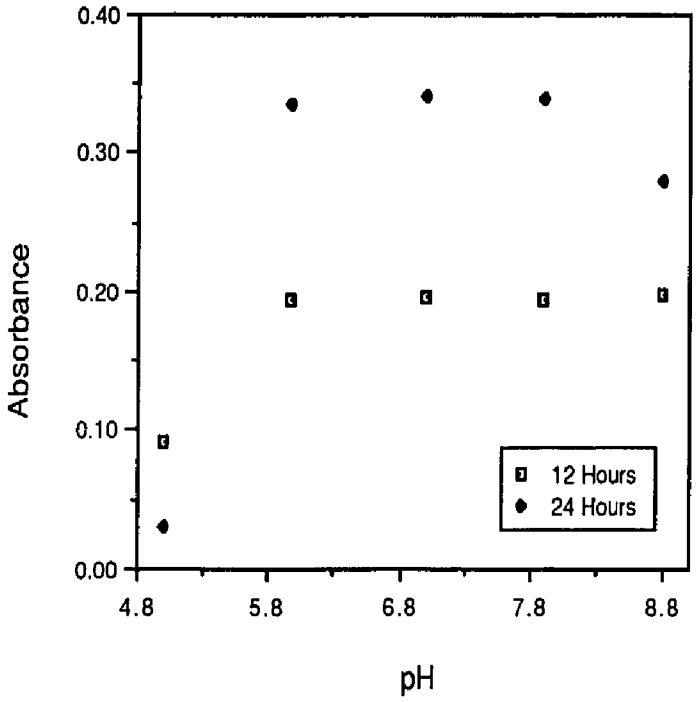


Figure 3.3. Effect of pH on membrane response to 2 ppm TNT after 12 and 24 hours.

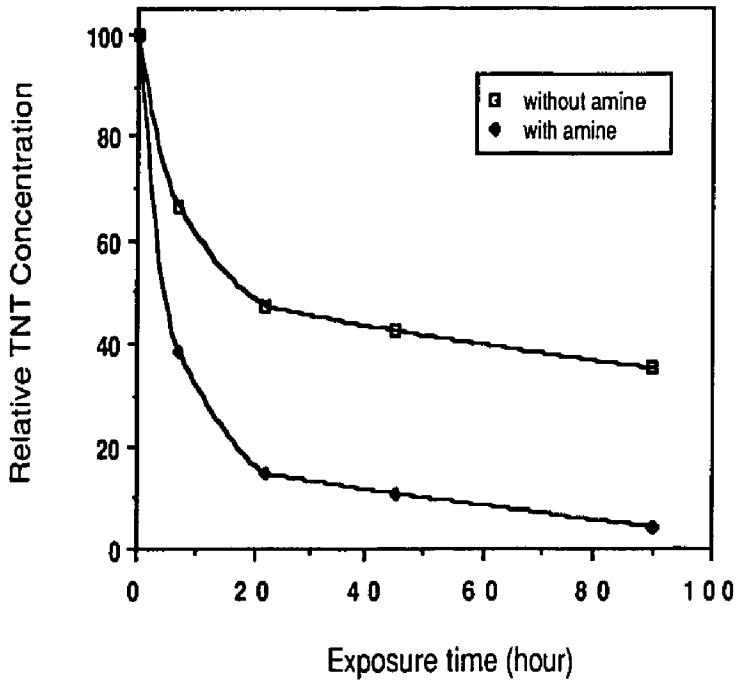


Figure 3.2. Absorption of TNT by membranes with amine and without amine.

interact with Jeffamine, interfering with its ability to react with TNT. In any event the membrane may not be suitable for analysis of acidic environmental samples. The points in Figure 3.3 were not connected because it was suspected that the nature of the buffer may also have a significant effect on the response.

The decrease in absorbance at pH 5 at long exposure times may also indicate that the reaction of TNT with amine is reversible at short time periods within the membrane. The membrane may stabilize the reaction intermediates by limiting the rate of diffusion of species within the membrane.

(7). Effect of Membrane Thickness

Increasing the thickness of the membrane leads to an increase in sensitivity as shown in Figure 3.4. However, membranes thicker than 0.72 mm are less flexible and tend to become slightly cloudy. Most measurements were made with 0.12 mm membranes.

(8). Membrane Stability

Membranes prepared from Geon 143 with the optimum amount of DOP remained clear in water several weeks. However, although membranes prepared from Jeffamine T-403 were found to be far more stable than membranes prepared from any other amine, they were still found to be subject to instability due to amine leaching from the membrane. Therefore, experiments were undertaken to characterize the rate of the leaching process. Loss of Jeffamine T-403 from membranes stored in air was determined by elemental

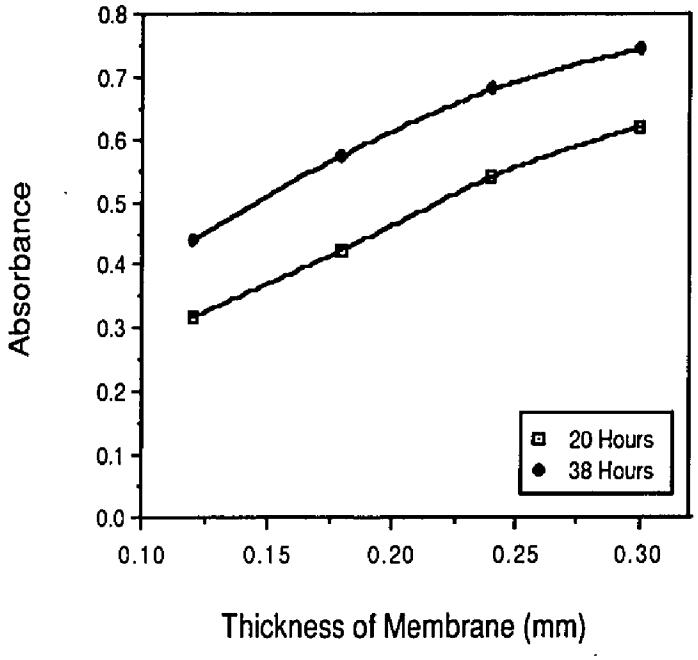


Figure 3.4. Effect of membrane thickness on the absorbance of membranes exposed to 2 ppm TNT solution for 20 hours and 38 hours.

analysis for nitrogen. The nitrogen percentages (w/w) of membranes stored for different times in closed containers are shown in table 3.2. Figure 3.5 shows the decrease as a function of storage time. While there is measurable loss of nitrogen, over 80% of the nitrogen remains in the membrane after storage for 80 days. Furthermore, the rate of nitrogen loss appears to decrease with time.

Leaching of amine from membranes in water as determined by titration is shown in Figure 3.6. The initial leaching rate was quite high, but decreased with time. At the point where 50% of the amine had left the membrane, the leaching rate was less than 1% of the amine per day.

The initial high leaching rate may be influenced by several factors. One is that there may be excess amine at the membrane surface prior to exposure to water. The second is that the fresh membrane may be oversaturated with amine. Therefore, an experiment was undertaken to determine whether membranes with lower nitrogen contents were more stable.

Leaching of amine in pH 7.0 0.010 M phosphate buffer solution at long time periods was studied by determining the nitrogen content. Pieces of membranes prepared with 0.6 g PVC, 0.24 ml DOP and varying amounts of Jeffamine T-403 from 0.20 ml to 0.05 ml in 0.03 ml intervals were put into pH 7.0 buffer solution, and the nitrogen contents of the membranes were measured as a function of

Table 3.2

Nitrogen content of membranes in storage for different times.

Time in storage (day)	Nitrogen concentration (%)	
0	1.16	1.15
22	1.09	1.05
42	1.02	1.06
64	0.91	0.99
79	0.88	0.97

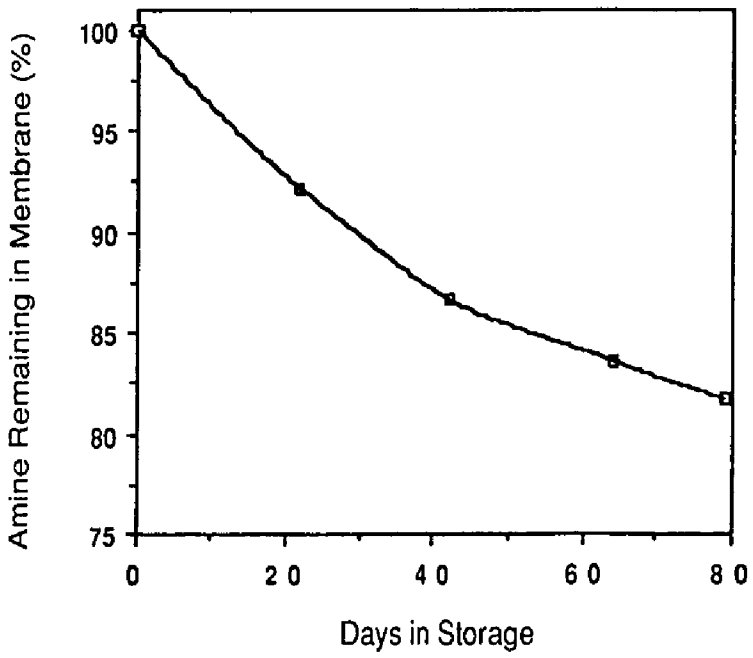


Figure 3.5. Amine loss upon storage.

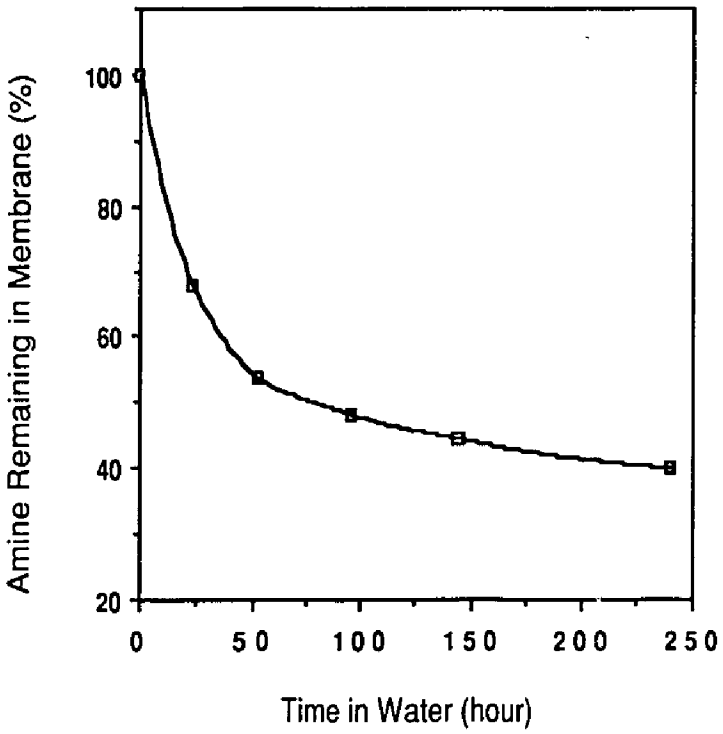


Figure 3.6. Amine loss in water.

time. The nitrogen percentages measured after different exposure times are shown in Table 3.3. Figure 3.7 shows plot of the results of 3 membranes with initial amine contents of 0.20 ml, 0.14 ml and 0.08 ml. The final amine contents of the membranes were confirmed by determining the response to a 10 ppm TNT solution after soaking in pH 7.0 buffer in 46 days. The response was less sensitive than for fresh membranes, but all the membranes exhibited absorbance values greater than 1.1 after two days exposure time.

An important conclusion from the results in Table 3.3 is that the rate of loss is less for membranes prepared to have low initial nitrogen contents. The crossing of the curves in Figure 3.7 indicates that membranes have high initial amine contents actually contain less amine than membranes with low initial amine contents after long exposure times. Determination of low TNT concentrations over long exposure times may be more sensitive with membranes prepared to initially have less amine.

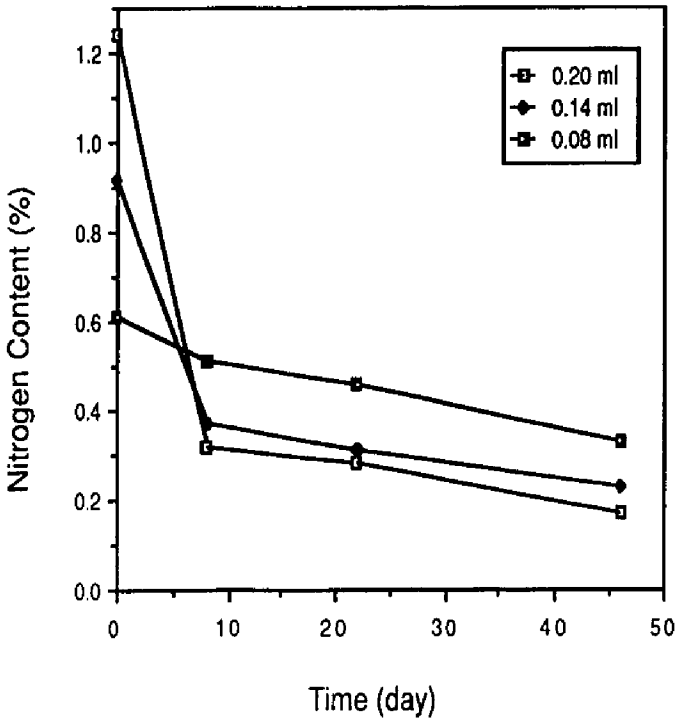
The response of fresh membranes with different initial amine contents to 2 ppm TNT is shown in Figure 3.8. The exposure time was 24 hours. It can be seen that membranes with higher amine contents respond more sensitively. But the response is not proportional to amine content.

Experiments were also performed in which membranes were soaked in distilled water for 10 days and then exposed for a week in the dark to a well water sample spiked to contain 0.10 ppm TNT. The

Table 3.3

Nitrogen percentage (w/w) of membranes with different initial amine contents after exposure to pH 7.0 buffer solution for different periods of time.

Amine added	1st day	8th day	22th day	46th day
0.20 ml	1.24	0.38	0.28	0.17
0.17 ml	1.07		0.29	0.23
0.14 ml	0.92	0.37	0.31	0.22
0.11 ml	0.81		0.44	0.33
0.08 ml	0.61	0.51	0.46	0.34
0.05 ml	0.44	0.38	0.33	0.26



Figur 3.7. Leaching of amine in solution as a function of initial amine concentration.

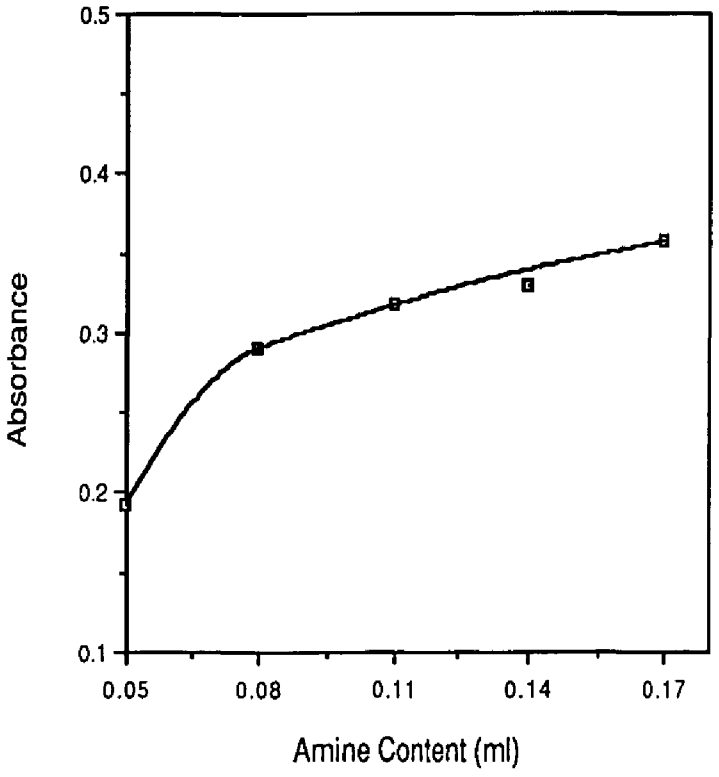


Figure 3.8. Relative membrane sensitivity vs. amine content.

absorbance after ten days was 0.15. A fresh membrane under the same conditions gave an absorbance of 0.24.

In practice, membranes should not be preexposed to water and should be stored in a sealed environment to minimize amine loss.

(9). Response to TNT

Figure 3.9 shows the response as a function of TNT concentration for membranes exposed to TNT for 1, 5, and 10 days. Absorbance is proportional to TNT concentration for a constant exposure time, provided the total absorbance remains low. At higher absorbance there is noticeable curvature in the response function. The slope of the response curve increases with exposure time although not proportionally. By controlling the response time the membrane can be used to detect a wide range of concentrations. Although 1 day exposure is appropriate for samples with TNT concentrations around 1 or 2 ppm, 10 ppm TNT gives discernible color in a couple of minutes. Membranes exposed to aqueous 10 ppb TNT solutions for a week in the dark had visually detectable color.

The absorbance over long exposure time is greater if samples are protected from light. This suggests that response is influenced by product photodecomposition. This effect is significant for detection of TNT at low levels and over prolonged exposure time. Most of the experiments did not take special care to avoid room light except where indicated.

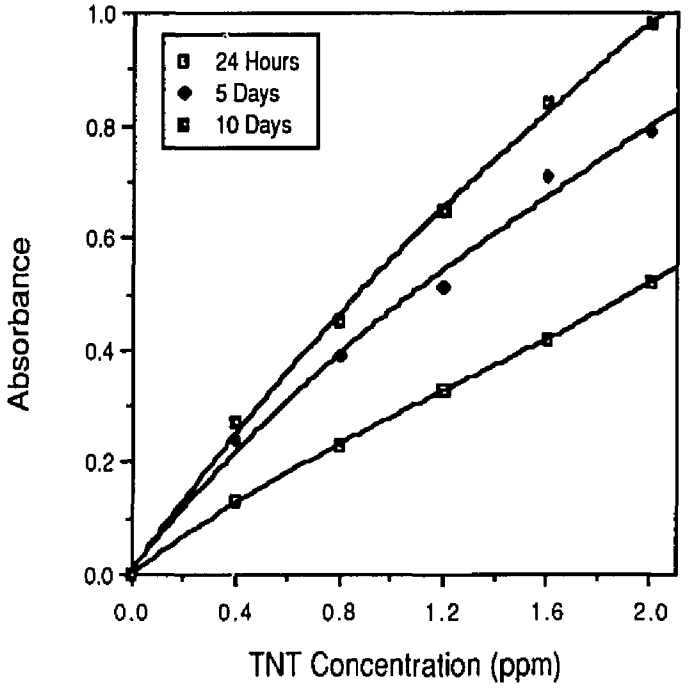


Figure 3.9. Response curves for membranes exposed to aqueous TNT standards for 24 hours, 5 days, and 10 days.

(10). Application to Field Samples

Membrane response was first evaluated using an uncontaminated groundwater sample obtained from a well near Hanover, NH. The pH of the groundwater was 7.6. Membranes exposed to this untreated groundwater sample did not develop any observable color. Portions were then spiked to contain 0.10, 1.0, and 4.0 ppm TNT. The absorbances of membranes exposed to the spiked groundwater samples were compared to the absorbances for a series of membranes exposed to standard TNT solutions in phosphate buffer at pH 7.0. Calculated percentage recoveries were 105%, 103% and 95% for 0.10, 1.0 and 4.0 ppm TNT, respectively.

Four munitions wastewater samples were obtained from the Cold Regions Research and Engineering Laboratory (CRREL) in Hanover, NH. The pH values of the samples were 7.07, 6.72, 7.23, and 7.08, respectively. Each sample had a total volume around 250 ml. 20 ml of each sample was taken for a preliminary test with a piece of membrane. Sample 1 and 4 developed color very quickly, while samples 2 and 3 showed no color development at all. Accurate measurements were made by exposing 100 ml of sample 2 and 3 to a piece of membrane each in the dark for a week. Sample 1 and 4 were diluted by factors of 20 and 4, respectively, with distilled water to a final volume of 20 ml. Other pieces of membrane were exposed to a blank, 5 ppm, and 10 ppm TNT standard solutions under the same conditions. Absorbances were measured in duplicate as presented in

Table 3.4. Diluted samples 1 and 4 both had absorbances close to the 5 ppm standard solution. The TNT concentration was calculated by comparison with the 5 ppm standard absorbance based on the assumption that Beer's law is obeyed for the measurement. The precision of the measurements is limited by the variations in membrane thickness. Pieces of membrane cut near the side of the big piece are thicker than pieces from the center and thus more sensitive. This may due to the surface tension developed near the wall of the Petri dish when the membranes are formed. The average TNT concentrations measured by the membranes are compared to concentrations measured by reverse phase HPLC at CRREL[6] in Table 3.5. *Absorption spectra run on membranes exposed to samples* 1 and 4 were identical to spectra for membranes exposed to aqueous TNT standards confirming that the observed membrane color was due only to TNT. The samples as received were slightly colored and turbid; however, this did not interfere with the TNT measurement.

The absence of a detectable signal for sample 2 and 3 was quite encouraging since it indicates that the membrane will not give false positive results when exposed to samples that do not contain polynitro aromatic compounds. This is important since we project that an important application for the membrane will be for rapid screening of samples to establish whether they should be subjected to further analysis.

Table 3.4

Measurement for the sample TNT determination.

Sample	Absorbance		Mean
5 ppm TNT	0.720	0.710	0.715
10 ppm TNT	1.120	1.180	1.150
Sample 1	0.618	0.632	0.625
sample 4	0.638	0.610	0.633
	TNT concentration		mean
Sample 1	17.3	17.7	17.5
Sample 2	89.2	85.3	87.2

Table 3.5

Comparison of groundwater TNT analysis by membrane and HPLC.

Sample No.	concentration (ppm) Membrane	HPLC
1	87.2	103
2	<d	<d
3	<d	<d
4	17.5	17.7

Note: <d indicates below limits of detection.

4. Conclusions

We have developed a membrane formulation that is reasonably stable and responds sensitively and selectively to polynitroaromatic hydrocarbons. The membrane is suitable for in-situ detection of TNT contamination using simple visual detection. The membrane can also be coupled to fiber optics for remote in-situ optical measurements in groundwater. This is described in the next chapter.

CHAPTER 4

SINGLE FIBER ABSORPTION MEASUREMENTS FOR REMOTE DETECTION OF TNT

1. Background

The advantages of single fiber measurements have been described in Chapter 1. However, these measurements are subject to high levels of stray light. This makes the absorption measurement particularly formidable because the stray light and signal light have the same wavelength. Chemical sensors based on single fiber measurements of indicator absorption have yet to be reported.

Investigations of single fiber absorption measurements in solution have been reported recently. As mentioned in Chapter 1, Skogerboe et al. have minimized the effect of stray light by using refractive index matching to decrease interface reflection and by modulating the light source with lockin amplifier detection to temporally reject part of the stray light. [52] Another approach is to increase the magnitude of the collected signal intensity by using cell formed by a special needle with highly reflective walls and a reflective mirror which directs light back into the fiber.[70] In both cases the pathlength for absorption can not be much longer than the

diameter of the optical fiber to insure that the collected signal light is much larger than the stray light.

An indirect approach to single optical fiber absorption measurements has been developed by Jordan et al. [71] Absorption is measured by its effect on emission intensity from a co-immobilized fluorophor. This idea is exemplified by a chemical sensor for pH measurement. The sensor is prepared by coimmobilizing phenol red (an absorber) and eosin (a fluorophor) in a polymer gel at the distal end of the fiber. The fluorescence intensity varies with pH because the absorption spectrum for the basic form of phenol red overlaps the eosin emission, leading to quenching via Foster energy transfer. Provided a suitable absorber/fluorophor system can be found, such an approach is less subject to stray light because the incident and measured intensities are at different wavelengths.

In the work described in this chapter the techniques used to alleviate the influence of stray light in single fiber solution absorption measurements were combined with the TNT sensitive membrane described in the preceeding chapter to develop a system for remote in-situ TNT measurement. The possibility of using an absorber/fluorophor system for indirect absorption measurement of TNT was also investigated.

2. Experimental

(1). Reagents

Membranes were prepared as described in Chapter 3. To prepare fluorescent membranes, the fluorophor was also dissolved in THF prior to membrane casting.

Coumarin 314 was obtained from Kodak, perylene was from Aldrich, and the other fluorophors were from Molecular Probes.

(2). Equipment and Measurement Operation

Fluorescence spectra were measured on an SLM 8000 spectrofluorometer with a double excitation monochromator and a single emission monochromator. The bandpass for all measurements was 2 nm.

Single fiber absorption measurements were made with components of the SLM 8000 spectrofluorometer. The fiber optic arrangement is shown in Figure 4.1. Light from the excitation monochromator of the SLM spectrofluorometer is focused into one arm of a four port fiber optic coupler with 200 micrometer core diameter glass-on-glass fiber (ADC Corporation, Westborough, MA). This is accomplished with a specially machined hollow aluminum cylinder. One end of the cylinder fits over the lens housing in the SLM sample compartment. The other end accommodates an SMA style fiber optic connector on the coupler. In practice the aluminum cylinder is adjusted to the position which gives the greatest

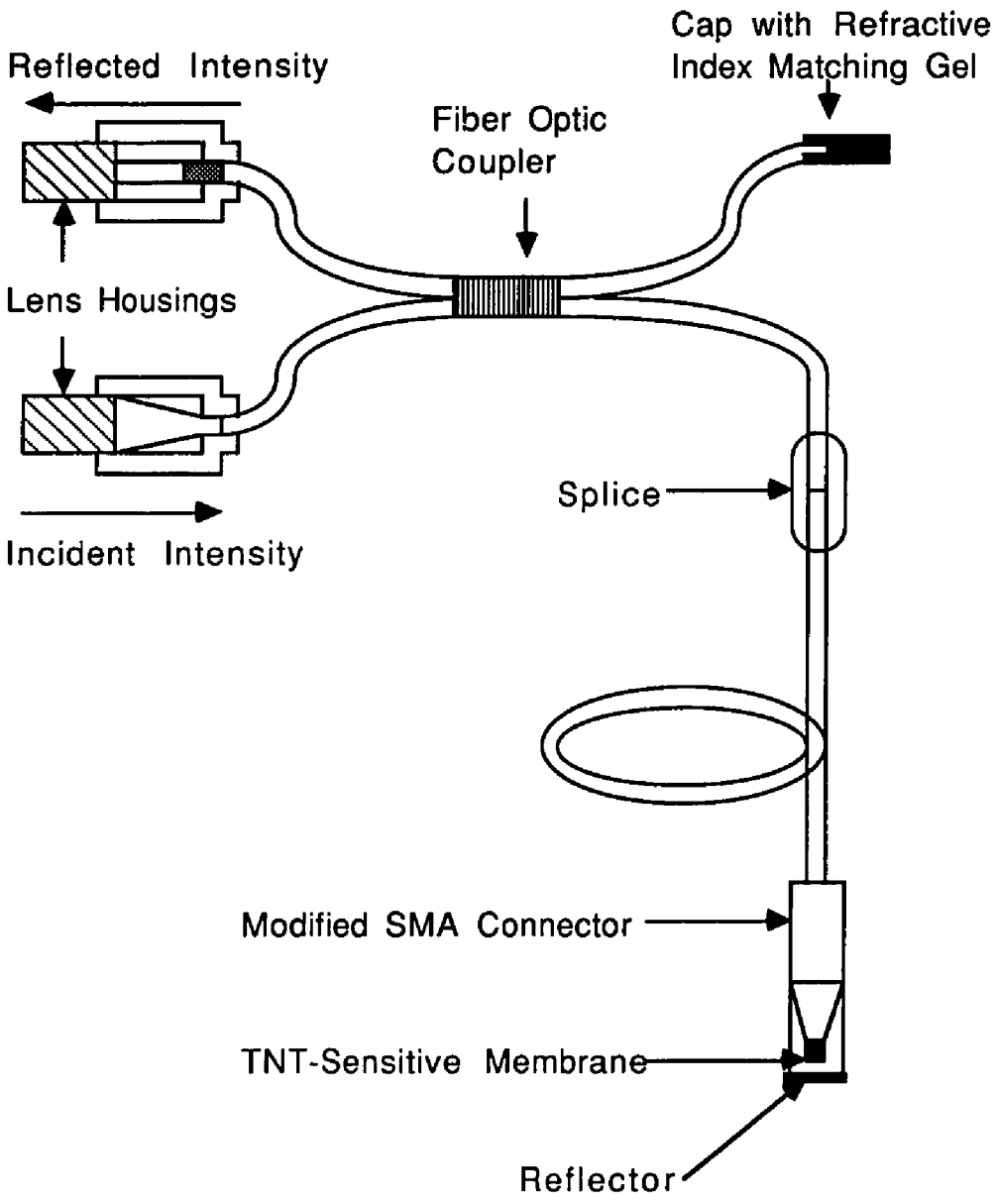


Figure 4.1. Experimental arrangement for coupling fiber optics to SLM 8000 spectrofluorometer.

intensity on the detector and is then secured in place with a set screw.

Light is transmitted through the coupler and a splice to a 200 micrometer core diameter HCS plastic clad silica fiber (Ensign-Bickfold, Avon, CT), which leads to the TNT sensitive membrane. The end of the unused arm of the coupler is immersed in a refractive index matching gel and covered with a black connector cap to minimize the amount of reflected light. Refractive index matching gel is also put in the splice to minimize reflection. For reflectance measurements, light from the TNT-sensitive membrane returns through the coupler directly to one of the photomultipliers of the SLM 8000, bypassing the emission monochromator. A graded index of refraction (GRIN) lens is used to focus light onto the detector. The bandpass for all single fiber measurements was 2 nm.

For many measurements the plastic clad silica fiber was omitted and the TNT-sensitive membrane was mounted on one arm of the coupler. Fiber ends were polished until a shiny surface could be observed using a microscope.

The membrane was attached to the end of the fiber as shown in Figure 4.2. A 905 style SMA connector (Ensign-Bickford, Avon, CT or OFTI, Billerica, MA) was attached to the end of the fiber. The end of the connector was tapered with a file until it was only slightly larger than the fiber diameter. This makes the end of the fiber more accessible to the sample solution, enhancing sensitivity by

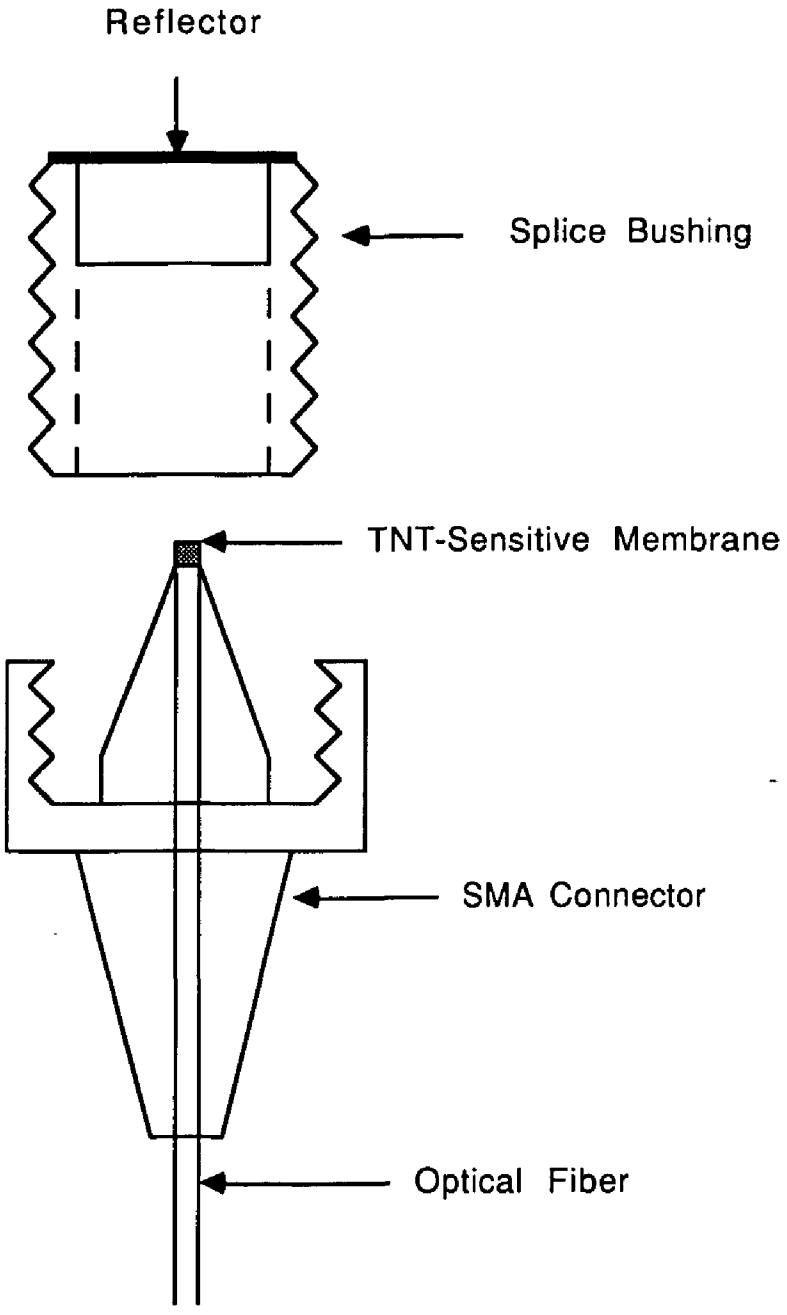


Figure 4.2. Schematic of TNT sensor tip.

promoting mass transfer from the bulk solution to the membrane surface. Optically clear epoxy resin, Norland optical adhesives, tetrahydrofuran (THF) and a series of greases were evaluated as media for coupling the membrane to the fiber. Epoxy resins, THF, and optical adhesives need to be cured for more than one day to get the membrane to adhere to the end of the optical fiber. THF may also cause the membrane to become cloudy when it is used as an adhesive. Because it best combines satisfactory adhesion with convenience, Thomas Lubriseal 8690-B20 was used for most measurements. For best results a thin layer of the grease is applied to the end of the optical fiber. It is then put into water for a couple of minutes to saturate the grease with water. A small disk of TNT-sensitive membrane is cut from a larger piece with a borer made by a No.18 syringe needle with its tip cut off squarely to give a clean cut. The small piece is then pressed against the end of the fiber. The resulting sensor is placed in distilled water for several minutes and reflected intensity is monitored. A stable signal confirms satisfactory attachment between the membrane and the fiber.

To enhance the observed intensities, a reflector was incorporated into the sensor behind the membrane. Reflectors included 200 mesh gold grids (Polysciences, Inc.) and a strip of stainless steel. The reflector was glued onto an SMA splice bushing with epoxy resin, as shown in Figure 4.2, much of the distal end of

the splice bushing was filed away leaving only two 2 mm thick supports to hold the reflector. This allows the sample greater access to the membrane, leading to improved sensitivity.

When the gold grid was used as the reflector, the splice bushing was inserted into the SMA connector until the gold grid pressed against the membrane helping to hold it in place. When the stainless steel strip was used as the reflector, the splice bushing was inserted in the connector until the reflector was approximately 1 mm from the membrane.

A long term drift measurement experiment was performed on a fiber optic photometer with an incandescent source and interference filters for wavelength selection. In this measurement 660 nm was used as the reference intensity.

3. Results and Discussion

(1). Fluorescent Membrane Measurements

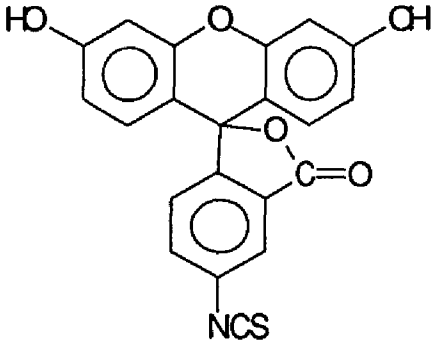
The fluorescence energy transfer/inner filter effect approach to single fiber absorption measurements was tried as the initial approach to remote single fiber absorption measurements. The goal was to incorporate a fluorophor into the PVC membrane such that fluorescence intensity would decrease as the colored product of the reaction between TNT and Jeffamine T-403 was formed in the membrane. Beyond that, we hoped to find a system such that

fluorescence would be attenuated more at one wavelength than at another so that the measured parameter could be an intensity ratio which would be insensitive to drift. Several fluorophors were incorporated into PVC membrane in attempts to develop such a system. Structures and spectral properties of the reagents tried are given in Table 4.1.The first fluorophor to be tried was fluorescein. The isothiocyanate derivative of fluorescein was covalently coupled to Jeffamine T-403 by the following reaction, which was carried out in THF:

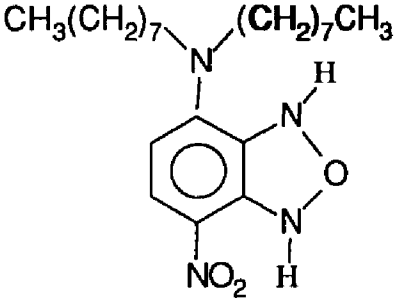
$$R{-}NH_2 + S{=}C{=}N{-}R' \longrightarrow R{-}NH{-}\overset{\overset{S}{\|}}{C}{-}NHR'.$$

RNH_2 represents Jeffamine T-403 molecule and R'NCS represents fluorescein isothiocyanate. When this fluorescent derivative was incorporated into the PVC membrane, the fluorescence efficiency was significantly reduced. Membranes containing large amounts of fluorescein (25 mg fluorescein isothiocyanate per 0.5 g PVC) fluoresced strongly. However, the fluorescence intensity did not change significantly when the membrane was exposed to TNT. This may be caused by two factors: one is that the membrane itself is colored by fluorescein and the absorption characteristics were dominated by the fluorecein rather than the product of the reaction between TNT and amine; the second reason is that much of the fluorescence we observed is due to the surface fluorescein

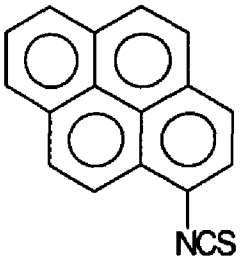
Table 4.1. Structure and fluorescence properties of fluorophors tested for TNT measurements.



Fluorescein isothiocyanate.
Emission peak: 530 nm.
Excitation peak: 488 nm.

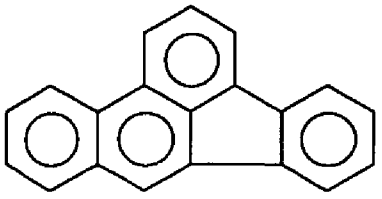


4-(N,N-Dioctylamino)-7-nitrobenz-2-oxa-1,3-diazole.
Emission peak: 538 nm.
Excitation peak: 473 nm.

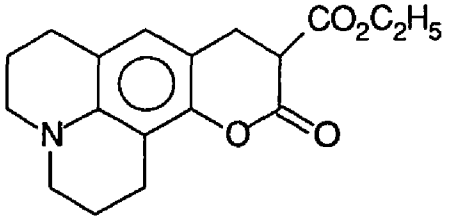


Pyrene isothiocyanate.
Emission peak: 460 nm.
Excitation peak: 400 nm.

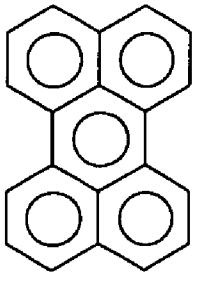
Table 4.1. (Continued).



Benzofluoranthene.
Emission peak: 448 nm.
Excitation peak: 370 nm.



Coumarin 314.
Emission peak: 480 nm.
Excitation peak: 402 nm.



Perylene.
Emission peaks: 452 nm, 478 nm.
Excitation peaks: 410 nm, 440 nm.

emission, which was not absorbed by the colored products formed inside the membrane.

Next, 4-(N,N-dioctylamino)-7-nitrobenz-2-oxa-1,3-diazole was directly incorporated into membranes. However, as with fluorescein, very high amounts were required to get measurable fluorescence.

Neither pyrene isothiocyanate nor benzofluoranthene showed significant fluorescence when incorporated into PVC membranes. Stronger fluorescence was observed for coumarin 314. However, the accumulation of brown product did not lead to significant changes in spectral distribution.

The only fluorophor that showed promise was perylene. Intensities were greater than for the other fluorophors and the formation of the reddish-brown product did lead to observable changes in the ratio of intensities of the perylene emission bands that could be related to TNT concentration. However, perylene is not stable in PVC. Instead the fluorescence signal decreases during membrane storage. A typical result is given in Figure 4.3. 0.040 mg perylene was added to 0.50 g PVC and other constituents to make the fluorescent membrane. The two curves are fluorescence spectra of a membrane before and after exposure to 1 ppm TNT solution for 12 hours, respectively. It can be seen that fluorescence intensities at all wavelengths decrease when the membrane was exposed to TNT. Besides absorption by the brown product formed in the membrane,

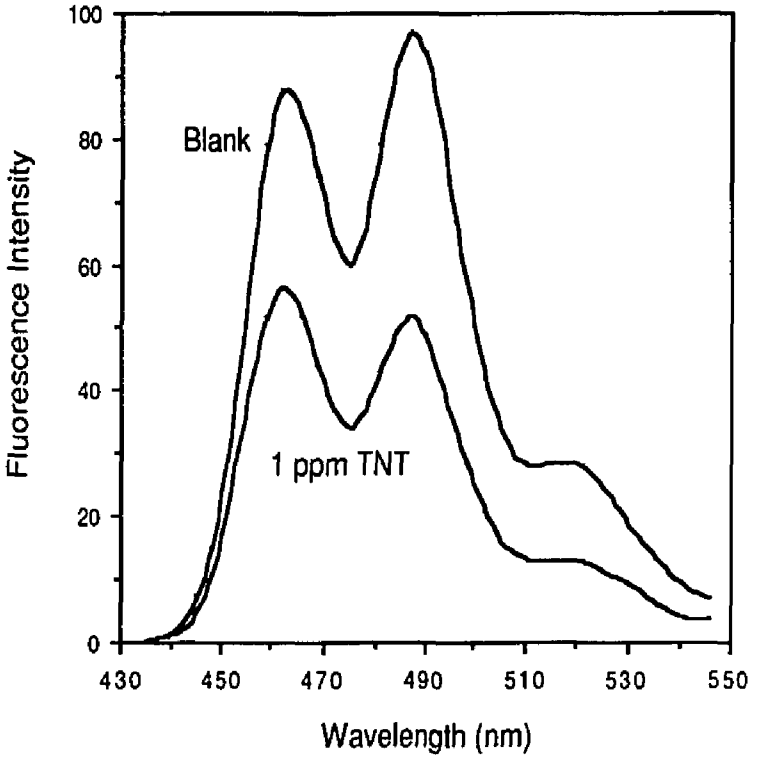


Figure 4.3 Fluorescence spectral change for perylene treated membrane upon exposure to TNT solutions.

fluorescence quenching may also contribute to this decrease since nitro compounds are known to be efficient quenchers. Fluorescence intensites also decrease when membranes are stored. We attribute this to perylene aggregation in the membrane.

Because of the failure to develop a suitably fluorescent PVC membrane and reports of successful single fiber absorption measurements in the literature, the fluorescence energy transfer/inner filter effect approach was abandoned in favor of direct absorption measurements.

(2). Choice of Reflector

Although light reflected at the interface between the PVC membrane and the aqueous sample provides a signal, it is weak because of the relatively small refractive index difference between the two media. To minimize the effect of stray light on signal, it is important to use a reflector. The use of a gold grid as the reflector leads to a fivefold increase in reflected intensities while still allowing contact between the membrane and the sample. Although the gold grid was used successfully, it is subject to several disadvantages: (1) gold has reduced reflectivity at 560 nm and below, (2) the grid blocks part of the surface reducing the rate of membrane response, and (3) the epoxy resin used to glue the grid to the bushing can slowly spread over the grid, blocking contact with the sample after a week or so and preventing color development.

A 1000 mesh nickel grid was evaluated as a reflector and

found to be unsatisfactory due to poor reflectivity and lack of mechanical strength.

When a strip of stainless steel is used as a reflector, intensities increase by factors as large as 19. Sensitivity to TNT was enhanced by keeping the reflector away from the membrane so that it did not block contact with the sample. However, this arrangement is subject to error if the sample absorbs at the wavelengths used to probe the membrane. Unlike the grid arrangement, the stainless steel strip does not help to hold the membrane in place.

The distance between the stainless steel reflector and the membrane was adjusted to be about 1 mm. It was found that this allows the sample sufficient access to the membrane to get sensitive response to TNT. When a membrane was changed, the distance was adjusted to give approximately the same level of reflected intensity.

(3). Single Fiber Absorption Spectra

Figure 4.4 shows single fiber absorbance spectra for a membrane before and after exposure to 5 ppm TNT for 24 hours. Because this is a single beam instrument, the initial spectrum is simply the power spectrum of the xenon lamp modified by the various optical elements that the beam traverses en route to the detector. After the exposure to TNT, reflected intensity decreases at the wavelengths where the reddish-brown product of the reaction

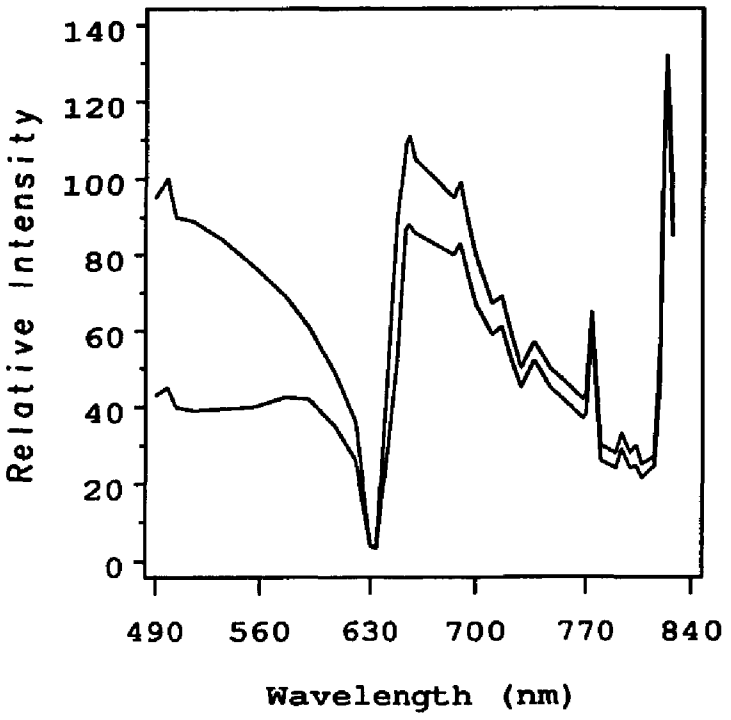


Figure 4.4. Single fiber absorption spectra for the membrane prior to and after exposure to 5 ppm TNT solution for 24 hours.

between TNT and membrane absorbs.

Because it provides a strong signal at a wavelength far from the absorption band of the reddish-brown product, the band at 824 nm was chosen as a reference intensity. The TNT signal was measured at 500 nm, close to the absorption maximum for the reddish-brown product. The measured parameter was the ratio of intensity at 824 nm to the intensity at 500 nm.

(4). Stray Light in the Measurement

Stray light levels were measured as the remaining reflected intensity at 500 nm for membranes exposed to high concentrations of TNT for extended periods of time such that the membrane transmittance was less than 1%. The lowest stray light levels were observed when the membrane was attached directly to one arm of the fiber optic coupler and the stainless reflector was used. Under these conditions stray light could be as little as 7.7% of the initial intensity observed before the membrane was exposed to TNT. When the gold grid was used as the reflector, stray light levels were typically on the order of 15% of the initial intensity for unexposed membranes. Stray light levels remained constant when the room lights were switched off, indicating that ambient room light does not significantly contribute to the stray light. But direct exposure of the plastic clad fiber to sunlight should be avoided as this increases the observed stray light and influences the observed spectrum.

When the membrane was placed on the end of 200 nm

micrometer core diameter plastic clad fused silica optical fiber, which was spliced to the fiber optic coupler, the relative stray light levels increased significantly. Typical stray light levels were 18% of the initial intensity with the stainless steel mirror as the reflector and 28% with the gold grid membrane. Two factors contribute to this increase. One is reflection at the interface in the splice, since the glass-on-glass fiber optic coupler and plastic clad silica fiber have different refractive indices. The other is the intensity loss at the splice. Both factors can be improved if the same type of optical fiber is used throughout instead of coupling glass-on-glass fiber with a low numerical aperture (0.20) to plastic clad fused silica with a higher numeric aperture (0.37).

Corrected absorbances can be calculated from known stray light levels.[72] However, because stray light levels can change when a new piece of membrane is placed on the end of the fiber, we did not attempt this.

(5).Intensity Levels

Reflected intensity levels are typically 500 times greater than the dark current of the photomultiplier. This is a significant advantage of single fiber absorption measurements relative to single fiber fluorescence measurements. Single fiber fluorescence measurements are reduced in intensity for several reasons including: (1) indicator levels are usually too low to absorb all incident radiation, (2) fluorescence is emitted randomly in all directions,

only a small fraction of the total emitted intensity is collected by the fiber, and (3) fluorescence quantum yields are often significantly less than unity. As a consequence, single fiber fluorescence measurements often require a high incident intensity which can lead to problems due to photodegradation. In contrast, single fiber absorption measurements are possible with much weaker incident intensities.

(6). Response to TNT

Experimentally, it was found that the initial values of the intensity ratio varied by as much as 7% when separate membranes were used on different days. Therefore, response to TNT was measured in terms of the percent increase in intensity ratio to provide a common initial point for all measurements. Figure 4.5 shows the percent increase in intensity ratio as a function of exposure time for separate pieces of membrane exposed to 0, 1, 2, 4, 8 and 16 ppm aqueous TNT. These data were measured with the membrane attached directly to one arm of the fiber optic coupler. The curves level off at the higher TNT concentrations. This is expected given the logarithmic relationship between absorbance and concentration. For 16 ppm TNT, response levels off quickly at long exposure times as the membrane absorption becomes large, and stray light becomes the main contribution to the intensity at 500 nm. The data for the plot is given in Table 4.2.

Figure 4.6 shows the intensity ratio at 3, 9, and 23 hours as a

Table 4.2

Percent increase in the ratio of intensity reflected at 824 nm to the intensity reflected at 500 nm for various TNT concentration at different times.

Time	Ratio increase at different TNT concentration					
(hour)	0	1 ppm	2 ppm	4 ppm	8 ppm	16 ppm
0.00	0	0	0	0	0	0
1.00			4.9	5.6	14.8	37.1
1.50		5				
2.00				14.4		78.6
2.33			11		31	
2.42		13.1	14.1	22.5		127
3.00					45.1	
3.20						202
4.00					61.3	
4.20		20.6	27	40		295.5
5.00					85.8	
5.70				63.1		
7.00						449.4
8.33					145.2	
8.43						
9.00	3.5					
9.15		36.9				
20.00			99.4			
21.47		76.3				
21.50			109.8			
22.00	4.9					575.3
23.00				203.8	409.7	

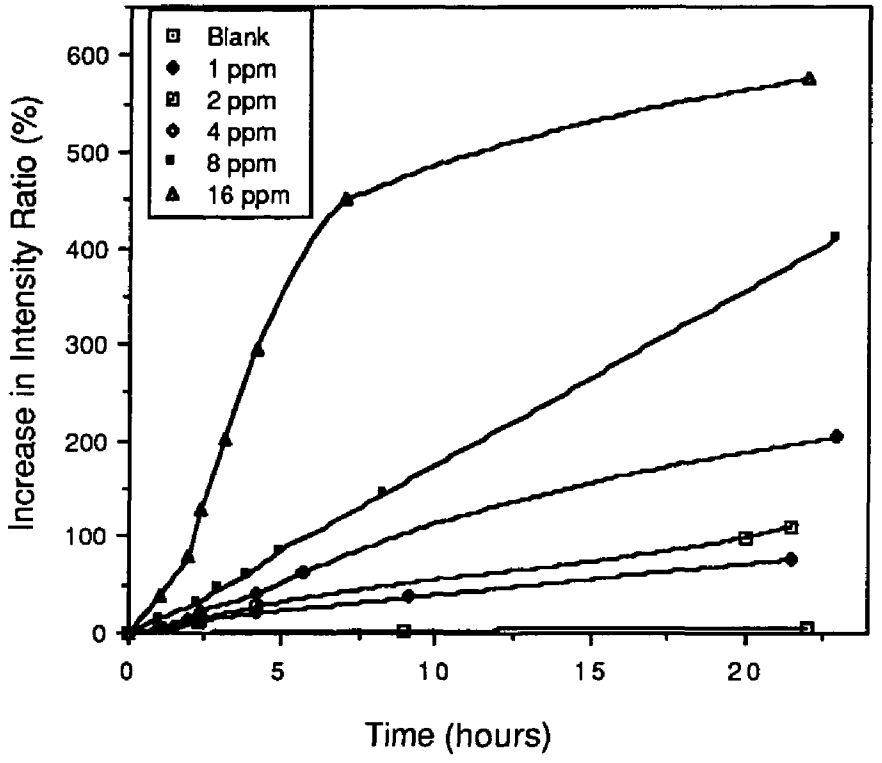


Figure 4.5. Percent increase in the ratio of intensity reflected at 824 nm to the intensity reflected at 500 nm with time for various TNT concentrations.

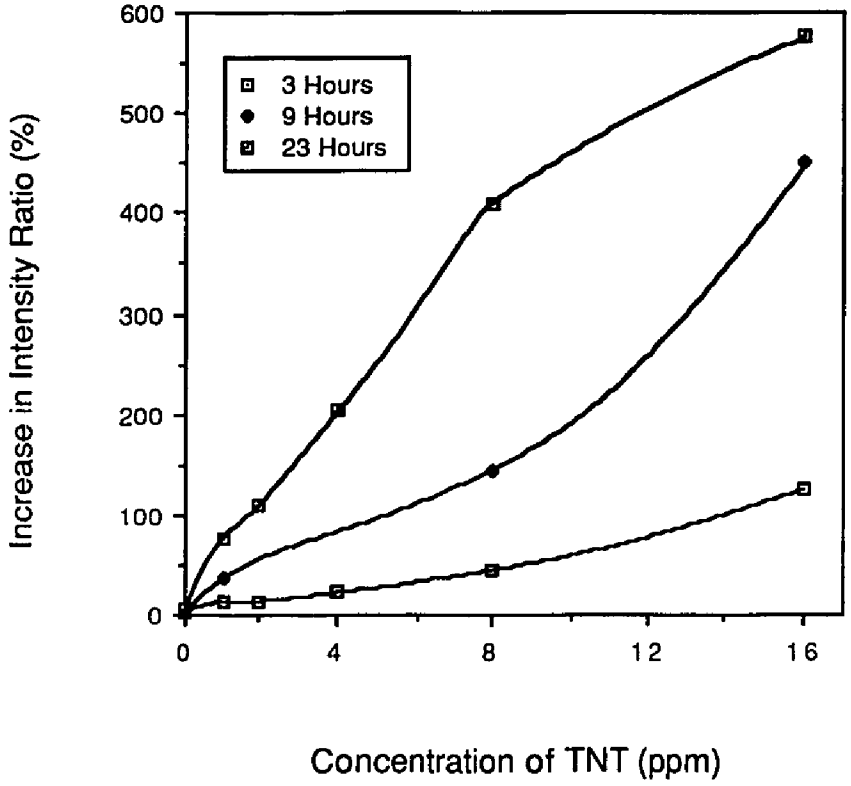


Figure 4.6. Percent increase in intensity ratio as a function of TNT concentration for membranes exposed to TNT for 3, 9, and 23 hours.

function of TNT concentration. Response approaches linearity, falling off at high concentrations which corresponds to high membrane absorption.

TNT detectability is limited by drift in the intensity ratio. The intensity ratio with pH 7.0 buffer solution increased by an average of 3%/day for 9 days. Since the increase for 1 ppm aqueous TNT with fresh membrane was measured to be 76%/day, the detection limit for TNT should be less than 0.1 ppm TNT.

4. Conclusions

We have demonstrated that direct single fiber absorption measurements are conveniently implemented using a reflector to maximize reflected intensity and refractive index matching to reduce stray light. Remote single fiber absorption techniques have been used to detect aqueous TNT.

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