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DESIGN AND APPLICATION OF A COMPACT, MULTIPURPOSE, LASER-BASED SPECTROMETER

By

Andrew M. Leach

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Submitted in partial fulfillment
of the requirements for
Honors in the Department of Chemistry

UNION COLLEGE

June, 1996

ABSTRACT

LEACH, ANDREW M. Design and Application of a Compact, Multipurpose, Laser-Based Spectrometer. Department of Chemistry, June 1996.

A compact laser-based spectrometer has been designed and characterized. This design of this instrument gives it the ability to collect absorbance, fluorescence, and scattering signals simultaneously. A diode laser is used as the excitation for this instrument. Several optical arrangements have been tested in order to allow for the best possible signal collection. Electronics have been designed and constructed in order to facilitate the simultaneous collection of signals. This instrument has been characterized using several dyes and scattering agents. Fluorescence limits of detection for Methylene Blue and Nile Blue A have been calculated at 2 ppb.

The spectrometer has been applied to two different studies. A β-Cyclodextrin (β-CD) study has been conducted in order to determine whether recrystallization of β-CD reduces its aggregation and precipitation in water solutions near its solubility limit. Recrystallization has proven to reduce aggregation in β-CD by up to 70%. A dye-surfactant study has been performed to determine the interaction between Methylene Blue (MB, dye) and Sodium Dodecyl Sulfate (SDS, surfactant). The relationship between MB and SDS in solutions was studied at various concentrations with different solvents. The spectral characteristics of the MB-SDS interaction suggest that formation of a 1:1 or 1:>1 MB:SDS complex occurs. Hypothesis have been suggested to explain the results obtained for each application.

Future modifications to the spectrometer including the addition of spectral resolution and of a photomultiplier tube detector system have been outlined.

For Mom, Dad, and Chris

Acknowledgments:

I would like to first thank someone from my past. You often hear of how teachers love their jobs because of the effects they have on their students. I would like to thank one teacher in particular, my high school chemistry teacher Mary Klein. In Mrs. Klein's class I developed my love of chemistry and my interest in teaching. For both of those facts I feel I owe a lot to her.

I would also like to thank the Chemistry Department at Union College for the excellent education I have received, and the use of both its instrumentation and more importantly its outstanding minds. In particular I would like to thank Professor Mary Carroll. As both my academic and research advisor she has had a tremendous impact on the level of my knowledge of chemistry and my confidence. Thank you for giving me the tools and the room I needed to grow.

I would like to thank the Union College Summer Research Program and the Internal Education Fund for their financial support during my research.

Thanks as well to Roland Pierson and James Howard of the Union College Machine Shop for their help in the construction of all the little modifications I designed. I would also like to thank Gene Davison of the Union College Electrical Engineering Department who helped with the electronics. Further thanks to Professor Frank Bright and Christine Ingersoll of the University of Buffalo Chemistry Department for the use of their facilities and the opportunity to experience what graduate school is really like.

And of course I would like to show my unending appreciation for my family and friends. To my friends I would like to say thank you for your support and encouragement. To my family I would like to say thank you for making me who I am today. I owe you for the opportunities you have given me, the morals and drive which you have instilled in me, and for all your love.

Andrew M. Leach

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Chapter 1:

Instrument Design and Characterization

Instrument Design

Background:

The work described in the following chapter examines the optical and electronic evolution of a spectrophotometer originally built by Marc Unger in 1993 as a senior research project [1]. This instrument was designed with three major characteristics in mind.

The first characteristic of this instrument is that it is multipurpose. By this I mean that the instrument has the ability to collect absorbance, scattering, and fluorescence data simultaneously. This is accomplished through the use of the instrument's multichannel design (Fig. 1-1). The channel at 0° is used for the entrance of the excitation source. A similar channel at 180° is used for absorbance measurements. Channels at 90° and 270° are used for scattering and fluorescence measurements. The difference between the scattering and fluorescence channels is based on the type of filter placed within them. Rayleigh and Mie scattered light have the same wavelength as the excitation source. For this reason a band-pass filter is utilized to allow the transmittance of only excitation-wavelength light to the detector. Fluorescence occurs at a wavelength higher than the excitation source. For this reason a cutoff filter is placed in the fluorescence channel to allow the transmittance of light with wavelengths higher than the excitation source to reach the detector.

The second major characteristic of this instrument is the fact that its excitation source is a diode laser. Diode lasers are attractive excitation sources for routine analysis due to their low cost, small

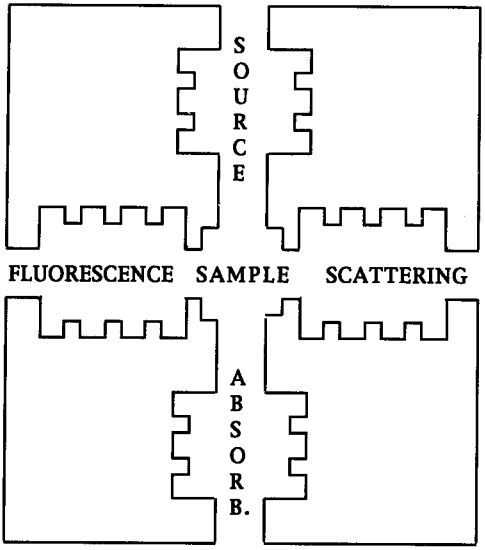


Figure 1-1. Layout of compact spectrophotometer instrument body. Fluorescence and scattering measurements are taken at 90 and 270 degrees from source, while absorbance signal is measured at 180 degrees from source.

size, and long service life. However, currently diode lasers are limited to the red and infrared region of the light spectrum due to the semiconductor materials of which they are made [2,3]. This severely limits the compounds and reactions which diode lasers may be used to monitor. Using a laser as an excitation source does allow for several improvements over other light sources. Lasers have the ability to be focused to extremely small spot sizes. This allows for good spatial resolution. Lasers produce highly monochromatic, coherent light which causes less destructive interference. These features add up to produce high photon flux which allows for increased sensitivity. Another feature possessed by lasers, which we are not utilizing currently, is the fact that lasers may pulsed for extremely short time durations (femto- to pico-seconds range) to allow for fluorescence lifetime measurements.

The third major characteristic of this instrument is the fact that it is compact. The main body of the instrument including the laser measures six and one-half inches by three and one-half inches and is three inches tall. The instrument is made of aluminum and has no moving parts, which renders it extremely rugged. The size and ruggedness of the instrument imply that the instrument may be made totally portable.

Optical Theory and Design:

This instrument's optical package has been developed through a series of lens and detector generations.

The first generation of optics was put forward by Mark Unger in 1993 and later characterized for Methylene Blue by Melissa Morris in the summer of 1994. They used the instrument's original design which utilized aluminum slats containing lenses and detectors which could be placed into set slots in the instrument's body. This configuration allowed for the use of f/f focusing theory [4]. In short, f/f focusing assumes that the light source (in this case the excited sample) is collimated. In f/f focusing the sample is placed at the negative focal length of the lens and the detector is placed at the lens's positive focal length, as shown in Figure 1-2.

The following problem arose from the use of the first-generation optical configuration. As the laser entered the sample cuvette it was tightly focused so that the excited sample appeared more as a point source than as a collimated source.

The first goal of my research was to improve the optical design for this spectrophotometer. I started by studying the laser beam as it traveled through the sample cuvette. The sample being excited by the laser would be the signal source for absorbance, scattering and fluorescence measurements. Since the laser was tightly focused in the sample cuvette, I decided to design the second-generation optical package around the idea that the source was a point instead of a collimated source. For this reason the second-generation optical package is based on the 2f/2f focusing theory [Fig. 1-3] [4]. In short, 2f/2f focusing assumes that the light source is a point. In 2f/2f focusing the sample is placed at two times the negative focal length of the lens and the detector is placed at two times the lens's positive focal length.

Figure 1-2. f/f focusing model

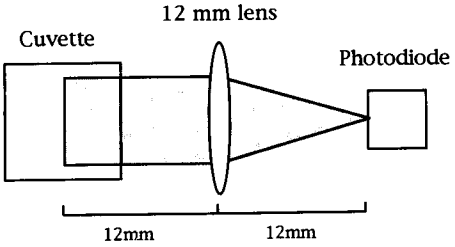
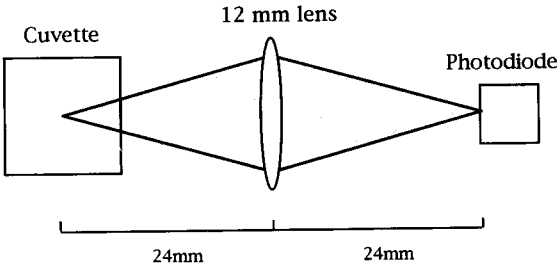


Figure 1-3. 2f/2f focusing model



Figures 1-2 and 1-3 are diagrams of common optical focusing theories. The f/f focusing theory is based on focusing a collimated light source to a point, while the 2f/2f theory is based on focusing a point light source to a point.

It was possible to implement the second-generation optical design in the existing absorbance channel. However this was impossible in the fluorescence and scattering channels using the instrument's original body. The main problem was that lenses needed to be placed in-between slots, while detectors needed to be placed outside the instrument's body. For this reason the second generation 12-mm lens slat and photodiode detector slat were developed [Figure 1-4]. The newly designed lens slat was 0.125 inches thicker than the original slats and allowed for inter-slot placement of lenses. The second-generation photodiode detector slat allowed for the detector to be externally mounted to the instrument in a vertically adjustable holder. The vertical adjustment was intended for precise aiming of the signal onto the photodiode detector.

The second-generation optical design was evaluated using the fluorescence signal of Nile Blue A Perchlorate (Eastman Kodak). We chose Nile Blue because of its strong fluorescence characteristics, and because it absorbs strongly at 636-nm. We used a 635-nm, 3-mW continuous wave diode laser (Power Technology Inc.) as the light source. A thin-film high-pass filter (#56, Roscolux) was placed in the fluorescence channel in order to block scattered light.

Nile Blue A Perchlorate

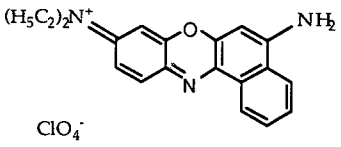
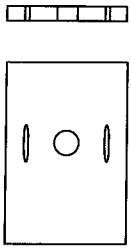


Figure 1-4. Optical Design Improvements

Top view

Side view

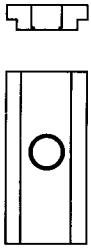


Photodiode detector slat (second generation)

Improvements: External attachment allows for increased focal length and vertical adjustmentofdetector.

Top view

Side view

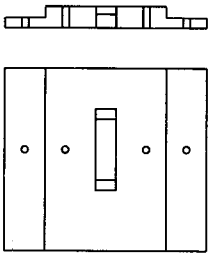


12mm Lens slat (second generation)

Improvements: 0.125 inch expansion allows for inter-slot placement of lens. This allows for adjustment of focal length.

Top view

Side view



Cylindrical lens slat (first generation)

Improvements: Holds a 6.35mm focal length cylindrical lens which allows for one dimensional focusing of light. Used to compensate for bar-shaped fluorescence emission.

Solution concentrations of Nile Blue in deionized water ranged from 4.08×10^{-9}M to 2.04×10^{-6}M. Three replicate measurements of each solution were made. Over this concentration range the fluorescence signal appeared linear in relation to concentration with a R^2 value of 0.999 [Fig. 1-5]. Limits of detection (LOD, three times the standard deviation above the background) and quantitation (LOQ, ten times the standard deviation above the background) were calculated and are presented in Table 1-1.

Table 1-1. Fluorescence data for Nile Blue A Perchlorate using second-generation optics

	Moles per liter	Parts per billion
Limit of Detection	2x10-8	10
Limit of Quantitation	8x10-8	30

These results showed good limits of detection and linearity. However upon further examination of the instrument it appeared that we were mis-directing between 60% and 80% of the light we were attempting to collect in the scattering and fluorescence channels. By this I mean that the optics in the channels at 90° and 270° from the source were refocusing a line from the sample onto a point for the detector [Fig. 1-6]. Since much of the signal did not fall on the detector, that light could not detected. These discoveries led to the third-generation optical package.

The third-generation lens package was developed only for the 90° and 270° channels. This package involved the placement of a cylindrical lens at the positive focal length of the 12-mm lens. The cylindrical lens was chosen for its ability to focus in one dimension [Fig. 1-7]. We hoped that this lens would convert the line we had

Figure 1-5. Fluorescence signal of Nile Blue A Perchlorate using second generation optics.

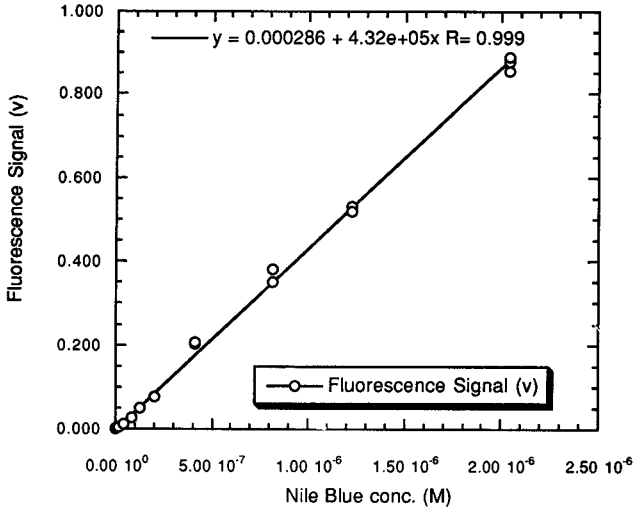


Figure 1-6. Laser beam appearance from 90, 180 and 270 degrees

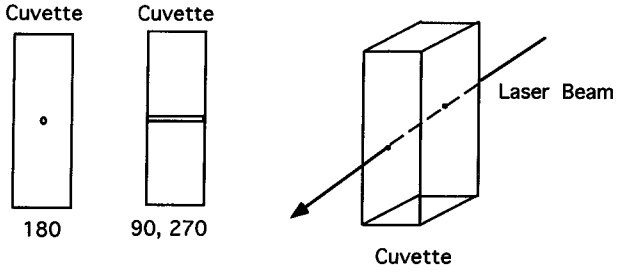
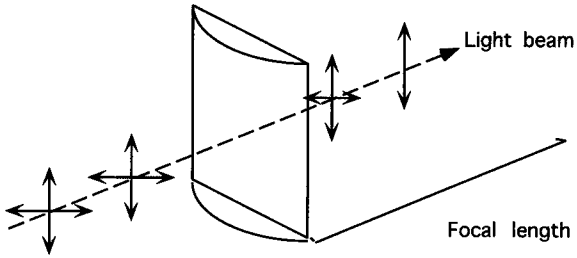


Figure 1-7. One dimensional focusing using cylindrical lens



been seeing into a point. The fluorescence signal of Nile Blue was used to characterize this optical package as well.

Solution concentrations of Nile Blue in deionized water ranged from 4.08×10^{-10}M to 4.08×10^{-7}M. Two replicate measurements of each solution were made. Over this concentration range the fluorescence signal appeared linear in relation to concentration with a R^2 value of 0.995 [Fig. 1-8]. Qualitatively, two times the amount of signal collected with the second-generation optical package was being collected. As before the LOD and LOQ were calculated. Use of the third-generation optical package greatly improved the sensitivity of the instrument [Table 1-2].

Table 1-2. Fluorescence data for Nile Blue A Perchlorate using third-generation optics

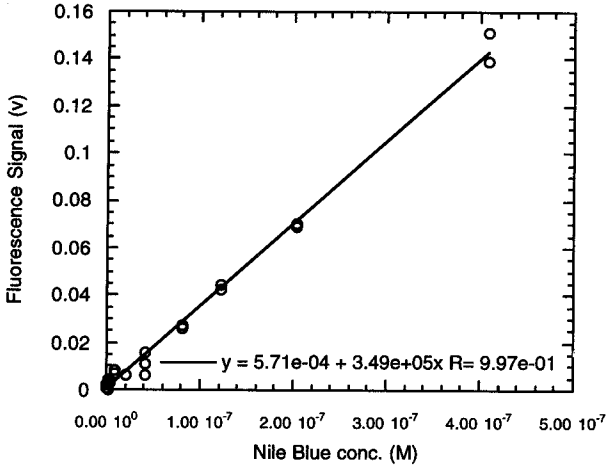
	Moles per liter	Parts per billion
Limit of Detection	5x10-9	2
Limit of Quantitation	2x10-8	8

Optical Characterization:

With the third-generation optical package in place it was time to test the measurement limits of this instrument. Characterization of the instrument's absorbance, scattering, and fluorescence signal collection capabilities was performed separately.

Absorbance characterization was performed using Methylene Blue (MB, Aldrich). MB's absorbance maximum in water is at 661-nm. For this reason a 670-nm, 7-mW continuous wave diode laser

Figure 1-8. Fluorescence signal of Nile Blue A Perchlorate using third generation optics.



(Power Technology Inc.) was used as the excitation source. A 671-nm band pass filter was placed in the absorbance channel in order to allow for the selective transmittance of laser light. MB solutions ranging from 1.10×10^{-4} M to 1.00×10^{-7} M were prepared in water. One measurement of each solution was made. The detection abilities of the multipurpose laser spectrometer were compared to those of a commercially available Hewlett Packard 8452 diode-array absorption spectrophotometer [Figures 1-9,1-10]. The results of this comparison and limits of detection and quantitation are listed in Table 1-3.

Table 1-3, Comparison of Absorbance limits for the Multipurpose Laser Spectrophotometer and a HP 8452A diode-array.

	Molar Concentration		Parts per Billion	
Instrument	LOD	LOQ	LOD	LOQ
MPLS	5x10-7 M	2x10-6 M	200	620
HP 8452A	1x10-7 M	4x10-7 M	40	150

*MPLS = Multipurpose Laser Spectrophotometer

As is evident from Table 1-3, the commercial diode-array instrument's statistical absorbance limits were five times lower than our compact instrument's limits. However it should be noted that our instrument is much smaller and cost a fraction of the HP 8452A price.

Scatter characterization was performed by placing a 671-nm band pass filter in the scatter channel to allow for the selective transmittance of scattered laser light. We used Ludox colloidal particles (DuPont) as our scatterer. Solutions of Ludox in deionized

Figure 1-9. Multipurpose Laser Spectrometer absorbance measurements for Methylene Blue

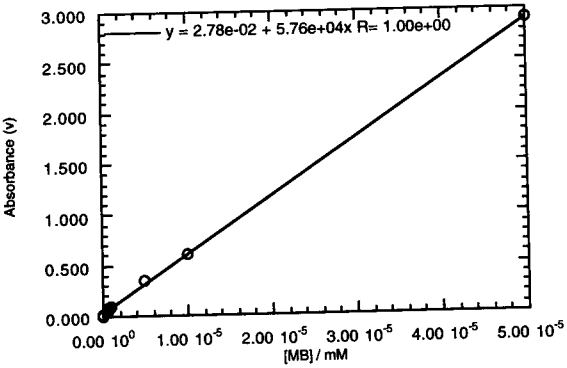
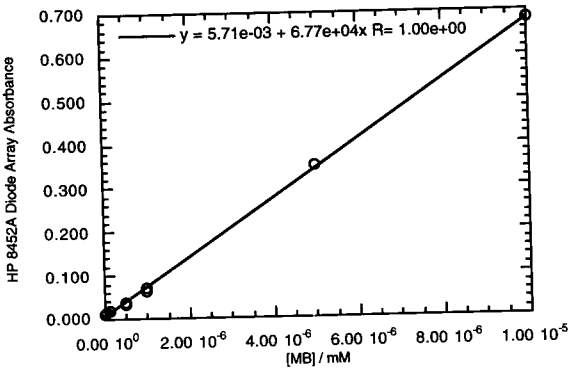


Figure 1-10. HP 8452A diode array absorbance measurements for Methylene Blue



water ranging from $1.0x10^{-4}$ to $2.0x10^{-1}$ µl scatterer per µl solvent [Fig 1-11]. One measurement of each solution was made. Limits of detection and quantitation were calculated at $6x10^{-4}$ and $1.9x10^{-3}$ µl scatterer per µl solvent respectively.

Fluorescence characterization for Methylene Blue was performed by placing a thin-film high-pass filter (#83, Roscolux) in the fluorescence channel in order to block scattered light. Solutions of MB were made with concentrations ranging from $5x10^{-5}$ M to $1x10^{-9}$ M in deionized water. One measurement of each solution was made. A linear relationship was found to cover two orders of magnitude [Fig. 1-12]. From this linear relationship a LOD and LOQ were calculated [Table 1-4].

Table 1-4. Fluorescence Characterization for Methylene Blue

	Moles per Liter	Parts per Billion
Limit of Detection	6x10-9	2
Limit of Quantitation	2.1x10-8	8

Electronics:

In order for the compact spectrophotometer to be able to collect data from all three channels simultaneously, a multichannel electronics system also had to be developed. Using simple electronics we designed a series of identical circuits which converted incoming current from the photodiode to voltage [5,6,7]. This voltage was then amplified by a switchable resistor. A switchable resistor in the amplification electronics provided a series of gain settings. This amplified voltage was then monitored using either a Fluke 77

Figure 1-11. Characterization of the Compact Spectrometer's Scatter Measurement Ability

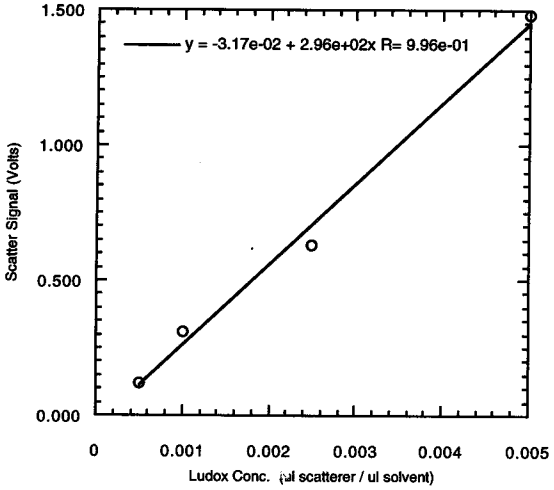
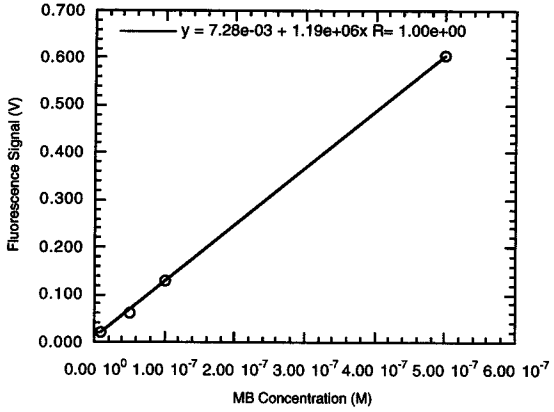


Figure 1-12. Characterization of the Compact Spectrometer's fluorescence measurement abilities for Methylene Blue.



battery-powered multimeter or a Macintosh IIci computer utilizing LABVIEW 2 software. Offset circuitry was built into this electronics package in order to subtract dark current produced by the circuitry itself. Dark current is current from the photodiode when no light is present (hence no "signal" should occur). After subtracting the dark current, we were able to amplify smaller signal voltages by increasing the circuit's gain setting. The final circuit diagram is seen in figure 1-13. This circuit board was hard-wired into a small aluminum box for protection and use around the lab. Currently this circuit board is powered by converted AC voltage, but with minimal adjustment the circuit could be powered by DC batteries allowing for total portability.

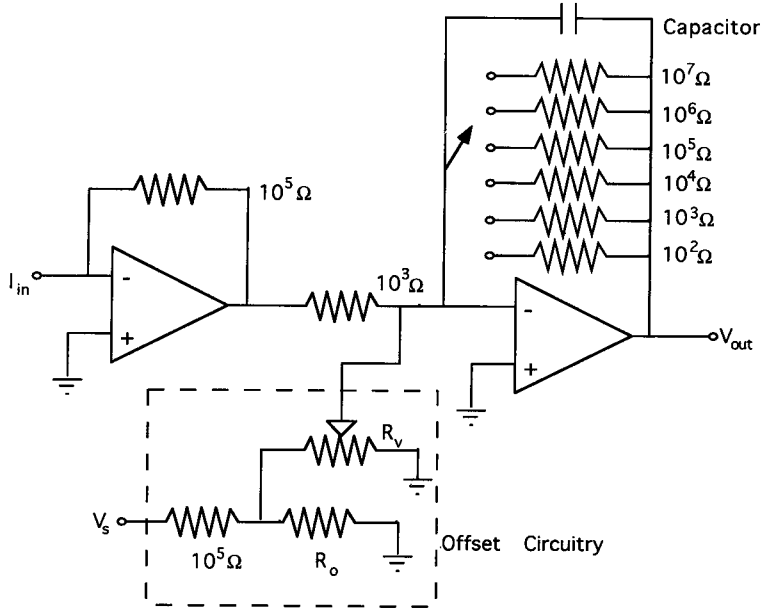


Figure 1-13. **Compact Spectrophotometer Electronics** (third-generation)

Three identical circuit systems were utilized for simultaneous collection of absorbance, fluorescence, and scattering signal. In this diagram V_S is either positive or negative 12V, depending upon the required offset direction. R_V is a variable resistor, and R_O is the offset resistor.

Literature Cited

1. Unger, M.A.; Senior Thesis, Union College, June 1993.
2. Ettenberg, M.; Laser Focus World, 27, May 1991, 137-138.
3. Nam, D.W.; Waarts, R.G.; Laser Focus World, 30, August 1994, 49-52.
4. Halliday, D.; Resnick, R.; Walker, J.; Fundamentals of Physics, John Wiley & Sons Inc., New York, 1993.
5. Skoog, D.; Leary, J.; Principles of Instrumental Analysis, 3rd ed., Saunders College Publishing, Orlando, 1992.
6. Horowitz, P.; Hill, W.; Art of Electronics, Cambridge University Press, Cambridge, 1989.
7. Malmstadt, H.; Enke, C.; Crouch, S.; Electronics and Instrumentation for Scientists, Benjamin/Cummings Publishing Company Inc., Reading, 1981.

Chapter 2:

Applications of a Compact Spectrophotometer

Part I: β–Cyclodextrin Scattering Study.

Part II: Dye-Surfactant Interaction Study

Part I: β-Cyclodextrin Scattering Study

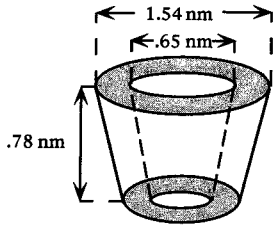
Introduction:

Cyclodextrins (CD) [1,2,3] are cylinder-shaped polysaccharides which form with an axial cavity. These cylinder-shaped macromolecules are used in a variety of chemical studies including chromatography and fluorescence experiments. The use of CDs is based on their ability to perform molecular encapsulation of organic, apolar compounds. This encapsulation occurs due to the inward-facing C-H groups that line the upper and lower edges of the cylinder. These groups cause a relatively apolar environment to form within the CD's cylinder.

This study is a continuation of an experiment started by Karen Colwell under the direction of T. C. Werner as part of her senior honors research [4]. The experiment looked at the scattering signal of β-Cyclodextrin. β-cyclodextrin [Fig. 2-1] has the potential to be the most useful type of CD due to it's cavity size, but it's low solubility in water limits it's use to dilute solutions. Colwell noticed that β-cyclodextrin in concentrations near its solubility limit (0.0163 M), aggregated in solution over time. This aggregation produced considerable scattering signal and caused problems during fluorescence studies. Colwell found that when the CDs were recrystallized, scatter signal was reduced by approximately 70 percent.

My goal in performing this experiment was to further characterize the relationship between recrystallized and

Figure 2-1. Simplified Stucture of b-Cyclodextrin



b-Cyclodextrin	
Molecular Weight	1135
Glucose Units	7
Cavity Volume mL/g	162.5

unrecrystallized β-cyclodextrins. I wished to determine whether or not recrystallizing the CDs reduced aggregation and also if these aggregates were precipitating out of solution.

Experimental Conditions:

β-cyclodextrin (American Maize-Products Company) and recrystallized β-cyclodextrin (recrystallized twice from deionized water) stock solutions were prepared in deionized water at a concentrations of 0.010 M. Two 1-cm quartz fluorescence cuvettes (Fisher) were filled with each stock solution, for a total of four cuvettes. I chose to fill two cuvettes with each stock solution so that one of each solution could be agitated prior to taking measurements. If the scatter signals for the agitated solution were higher than the signals for those solutions that were not agitated, I could determine that aggregates were precipitating out of solution.

The apparatus for this application is seen in Figure 2-2. A narrow band-pass filter (636-nm, Edmund Scientific) was placed in the scatter channel in order to block sample emission and to pass selectively the laser wavelength. The excitation source chosen was a 635-nm, continuous wave diode laser (Power Technology Inc). Scatter signal was collected at ninety degrees from the excitation source, using a 12-mm convex lens (Edmund Scientific) placed at twice the focal length from the cuvette. This signal was focused onto a 6.35-mm cylindrical lens (Melles Griot) and then onto a PIN photodiode (Honeywell). Current generated by the photodiode was

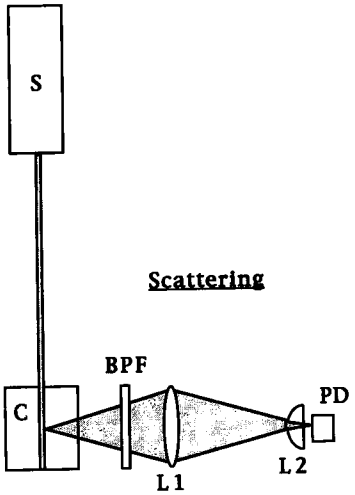


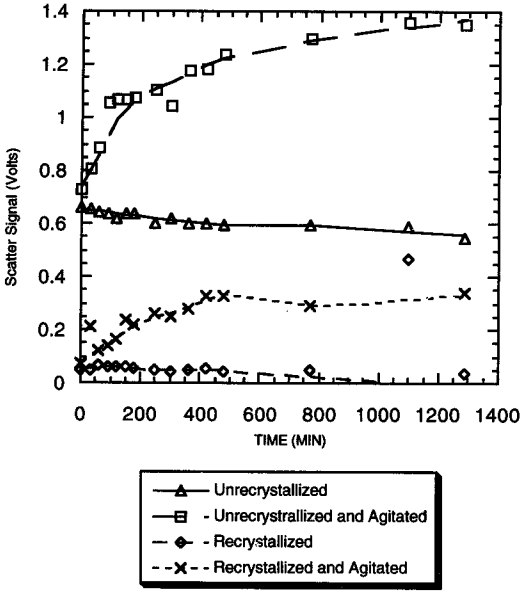
Figure 2-2. Depiction of cyclodextrin scattering study apparatus. **S** is the laser source (670 nm), **C** is a standard 1-cm fluorescence cuvette, **BPF** is a band pass filter (laser wavelength), **L1** is a 12 mm convex lens, **L2** is a 6.35 mm cylindrical lens, and **PD** is a photodiodedetector.

converted to voltage and amplified using DC electronics built in house. LABVIEW 2 software operated on a Macintosh IIci computer was utilized for data acquisition. Measurements were averaged over a period of 30 seconds to reduce noise interference. Data was collected for a total of two days. At the beginning of the study, data was collected for all four cuvettes every fifteen minutes. After two hours, the interval between measurements to one half hour. Then after four hours the measurement interval was increased again to an hour. Finally, after 8 hours the interval was increased to several hours.

Results and Discussion:

This study showed that the recrystallized CDs starting (t = 0) scatter signal was approximately 18% that of the unrecrystallized CD's signal [Fig. 2-3]. As time continued, the unrecrystallized non-agitated solution's scatter signal decreased slightly suggesting that something was precipitating out of solution. This was confirmed by the observation that the scatter signal from the agitated solution of unrecrystallized β-CD increased significantly with time, which suggested that aggregates which had fallen out of solution were being redistributed. The same precipitation of aggregates can be seen in the recrystallized CD solutions. In the recystallized CD solutions the non-agitated sample's scatter signal increased slightly while the agitated solution showed significant increase in scatter signal. Both the unrecrystallized and the recrystallized samples

Figure 2-3. Scatter study for B-Cyclodextrin as a function of Recrystallization and Time.



showed changes in their scatter signal up to 24 hours after solution preparation. After 24 hours, no significant changes were observed.

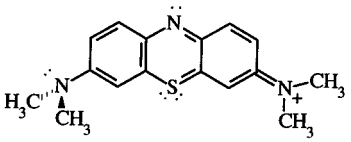
Overall, two things were learned from this study. First, the recrystallization of CDs significantly lowers the scatter signal. Second, that aggregates formed and precipitated out of solution in both the recrystallized and the unrecrystallized samples.

Part II. Dye-Surfactant Interaction Study

Introduction:

This is the study of the interactions between the cationic dye Methylene Blue (MB) and the surfactant Sodium Dodecyl Sulfate (SDS). This is an interaction which has been studied previously but is not understood. Several theories have been postulated about the interaction including; dye-dye dimer forming [5,6], dye-surfactant interaction [7], and dye-solvent interaction [8,9,10].

Methylene Blue

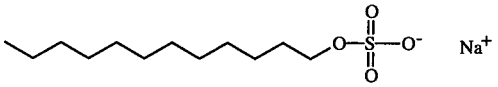


As mentioned previously, Methylene Blue is a cationic dye. Compared to many dyes, it is highly soluble in water (4 g/100 ml) [11]. MB is less soluble in ethanol (1.5 g/100 ml). Methylene Blue is commonly used as a redox indicator in titrations and has also seen use in ion-pair extractions of anionic ions.

Sodium Dodecyl Sulfate is an anionic micelle-forming surfactant. At low concentrations SDS exists in solution as a monomer, but at a critical point, the critical micelle concentration (CMC, 8.11×10^{-3} M in water), SDS forms micelles. A micelle is a spherical cluster of molecules with polar head-groups and non-polar hydrocarbon chain tails. In a micelle the non-polar tails interact

with one another to form a non-polar interior region while the polar head-group on the exterior of the micelle are in an aqueous environment.

Sodium Dodecyl Sulfate



Why study the interactions between dyes and surfactants? Both dyes and surfactants are amphiphiles. Amphiphiles are molecules containing both hydrophobic and hydrophilic moieties. The fact that amphiphiles have both hydrophobic and hydrophilic moieties plays a large role in the molecules' interaction with solvents and with other molecules. Often the amphiphile is forced to change its configuration (form micelles or bend) in order to reach a global minimum energy and therefore become stable.

In this study we are interested in the specific interactions of dyes and surfactants. When electrostatic forces cause a cationic dye to be attracted to an anionic surfactant, the region between the two molecules may undergo significant changes. These changes produce spectral shifts [12] which may be monitored optically. Through the use of spectroscopy it is possible to understand the interactions between these two amphiphiles in solution. The information gathered in this research of simple amphiphile interactions may be applied to more complex amphiphile systems, such as DNA.

Why use the compact multipurpose spectrophotometer to perform this experiment? First this instrument is the only instrument in our department which can be used for simultaneous measurement of absorbance, fluorescence, and scattering signals. Because we are unsure what effect the dye-surfactant interaction will have on the spectral characteristics of this system it may prove useful to collect several different types of spectral information. Secondly, the fact that Methylene Blue's absorbance maximum occurs at 661-nm in water (near the wavelength of our laser) makes this instrument particularly useful for absorbance and fluorescence studies of MB.

In this study, the relationship between MB and SDS under several different conditions has been explored. First an examination of the interactions between mainly monomeric MB ($1x10^{-6}$ M) and excess SDS was conducted. We investigated whether interactions occur in solutions containing a 1:1 ratio of MB:SDS. Finally, a study of the effect of solvent on the observed MB-SDS interaction was performed. Through these experiments a deeper understanding of dye-surfactant interactions will be produced.

Experimental Conditions:

Stock solutions of Methylene Blue (Aldrich Chemical Company) were prepared in various solvents at a concentration of $1.1x10^{-4}$ M. Solvents included 100% deionized water and mixtures of ethanol (absolute) and deionized water, with various percentages of ethanol. Sodium Dodecyl Sulfate (recrystallized twice over ethanol) solutions

were prepared in solvents identical to those used for MB at a concentration of 0.025 M.

In all of the experiments reported MB was kept at a concentration of 1.0 μM. This concentration was maintained to ensure that MB would exist primarily as monomers in solution. Concentrations of SDS ranged from 0 to 8 mM. In all but the 0 mM SDS solutions, SDS was in excess, compared to MB concentration. All solutions were prepared directly in 1-cm fused-silica fluorescence cuvettes (Fisher).

The apparatus for this application is seen in Figure 2-4. Simultaneous acquisition of fluorescence, scattering, and absorbance signals was achieved through the use of all three of our instrument's collection channels. The excitation source chosen was a 670-nm, 7-mW, continuous-wave diode laser (Power Technology Inc.). Third-generation optics were utilized in the fluorescence and scattering channels. Light was collected at 90° from the excitation source using a 12-mm convex lens (Edmund Scientific) placed at twice the focal length from the cuvette. This signal was focused onto a 6.35-mm cylindrical lens (Melles Griot) and then onto a PIN photodiode (Honeywell). The only difference between the scattering and fluorescence channels was the filter which was placed in each channel. A narrow band-pass filter (670-nm, Edmund Scientific) was placed in the scatter channel in order to block sample emission and to pass selectively the laser wavelength. In the fluorescence channel a high-pass thin-film filter (#83 Medium Blue, Edmund Scientific) was placed in order to block scatter signal while selectively passing sample emission. Second generation optics were used to collect

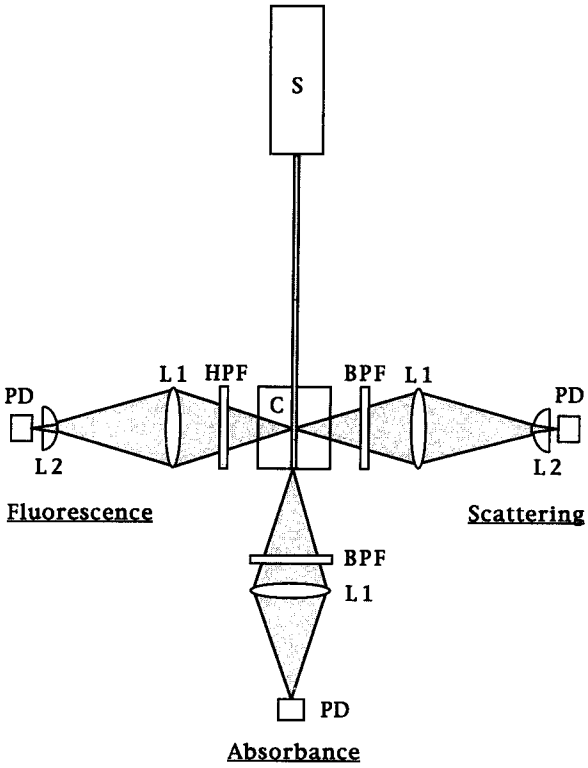


Figure 2-6. Depiction of simultaneous fluorescence, scattering, and absorbance measurements. **S** is the laser source (670 nm), **C** is a standard 1-cm fluorescence cuvette, **HPF** is a high pass filter, **BPF** is a band pass filter (laser wavelength), **L1** is a 12 mm convex lens, **L2** is a 6.35 mm cylindrical lens, and **PD** is a photodiode detector.

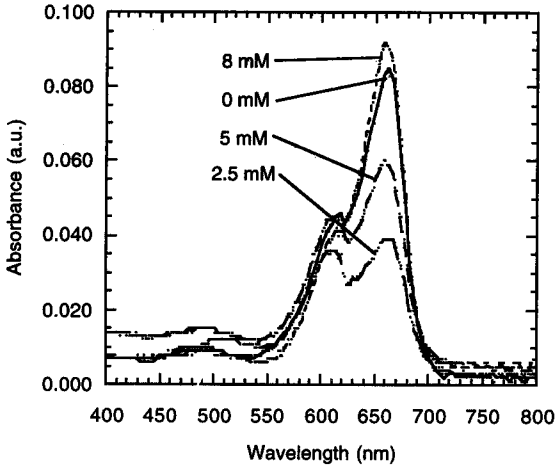
absorbance signal at 180° to the excitation source. Light was focused using a 12-mm convex lens onto a PIN photodiode. A band-pass filter was placed in the absorbance channel in order to block sample emission. Current generated by the photodiodes was converted to voltage and amplified using DC electronics built in house. LABVIEW 2 software, operated on a Macintosh IIci computer, was utilized for data acquisition. Measurements were averaged over a period of 30 seconds to reduce noise interference.

Due to low MB concentrations quantitative measurement of absorbance was not possible; therefore, all reported absorbance measurements were collected using a Hewlett Packard 8452A diode array instrument.

Results and Discussion:

This study started by monitoring the interaction between MB and SDS in a deionized water solvent. The absorbance spectrum of MB in water shows two peaks. The more significant peak at low MB concentration is the monomer peak at 661-nm. The secondary peak at 610-nm is due to MB dimers. In the presence of SDS, a dramatic decrease in monomer absorbance occurred well below SDS's critical micelle concentration [Fig. 2-5]. The decrease in monomer absorbance started as soon as SDS was added and reached a maximum decrease at 5 mM of SDS. At SDS concentrations above 5 mM, the monomer absorbance intensity increased to its original value. The fluorescence and scattering signals also showed a

Figure 2-5. MB absorbance as a function of SDS Concentration



initial decrease in intensity, reaching a minimum at 5 mM SDS, and then increasing past the original intensity as SDS concentration rose [Fig. 2-6 a,b].

The decrease in the MB monomer absorbance and fluorescence intensities suggest that MB monomers may be interacting with the SDS. Hamai [7] suggested that in a 1:1 ratio, dyes and surfactants form insoluble salts which caused shifts in MB's spectral characteristics. If insoluble salts were forming we would expect to see a precipitate or an increase in scattering. However, our experiment showed that the scattering intensity decreased as SDS concentration increased, and no precipitate was visible. Perhaps instead of forming an insoluble salt, the dye and surfactant form a non-absorbing ion-pair with a 1:1 or 1:>1 dye-surfactant ratio.

The second study we executed was based on work performed by Turner [8,9] involving the interactions of dyes and solvents. Turner's kinetic study showed that the presence of an alcohol as the solvent reduces dye dimer formation. We were keeping our MB concentration low in order to avoid MB dimer formation but we decided to try the alcohol solvent study to ensure that dye dimers were not affecting the spectral characteristics of MB. The solutions we prepared contained the same concentrations of MB and SDS as in the water solvent study; however, the solvent was altered by adding ethanol in increasing percentages.

The absorbance spectra produced by MB in presence of SDS with a solvent containing 25% ethanol proved interesting [Fig. 2-7]. The absorbance was no longer a function of SDS concentration.

Figure 2-6 a. Fluorescence intensity of MB as a function of SDS concentration

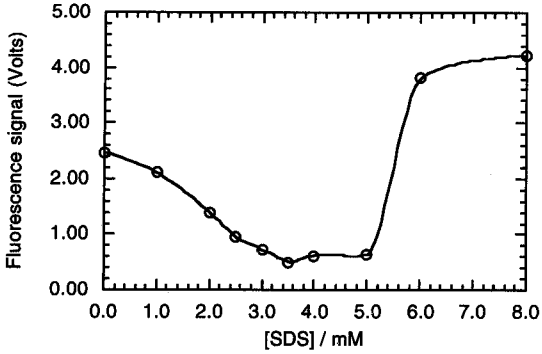


Figure 2-6 b. Scattering intensity of MB solution as a function of SDSconcentration

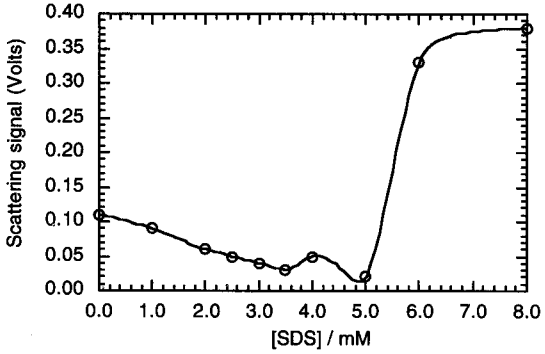
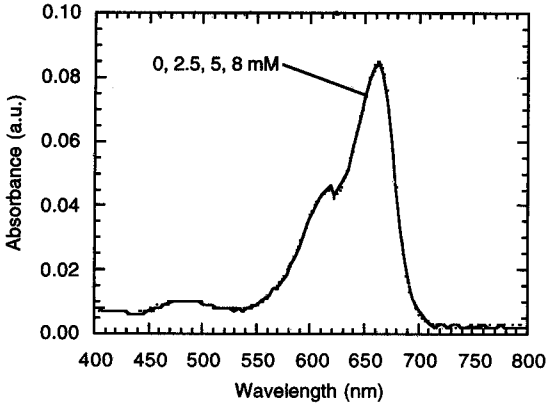


Figure 2-7. Absorbance of MB as a function of SDS concentration with 25% EtOH solvent



Instead, the MB absorbance spectrum remained constant. This is what we would expect if MB were alone in solution. A plot of MB fluorescence as a function of SDS concentration in 25% ethanol showed that the MB fluorescence was constant [Fig. 2-8a]. A constant fluorescence signal would be expected if a constant concentration of MB was not interacting with SDS in solution. A plot of MB solution scattering as a function of SDS concentration in 25% ethanol showed a linear increase in scatter signal [Fig. 2-8b]. That suggested that SDS scattering was increasing with increased SDS concentration. That is what would be expected if the SDS was not interacting with the MB in solution. A comparison of three different solvent mixtures can be seen in Figure 2-9 a,b. From Figure 2-9 it is possible to see the decrease in MB-SDS interaction as the solvent polarity decreases. It appears that the formation of aggregate MB-SDS salt decreases as ethanol concentration in the solvent increases.

Preliminary fluorescence lifetime measurements for solutions containing MB and SDS were collected in the Chemistry department at University of Buffalo. The instrumentation involved in these measurements included a 670-nm diode laser as the excitation source, a pockels cell for frequency modulation, and a 48000 MHF FT spectrofluorometer (SLM Aminco) for data collection and manipulation. Due to poor modulation of the low-power diode laser only rough trends in lifetimes were produced. The data showed that MB in aqueous solution had a short fluorescence lifetime similar to that reported by Fujimoto [13]. Fujimoto reported that MB in water had a fluorescence lifetime of 379 ± 7 ps. Our data also showed that when SDS was added to the solution the lifetime decreased. Since

Figure 2-8 a. Fluorescence intensity of MB as a function of SDS concentration with 25% EtOH solvent

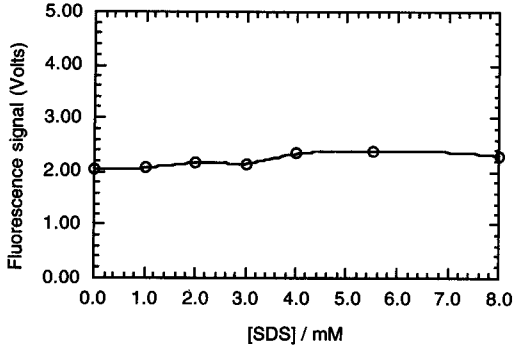


Figure 2-8 b. Scattering intensity of SDS as a function of concentration with 25% EtOH solvent

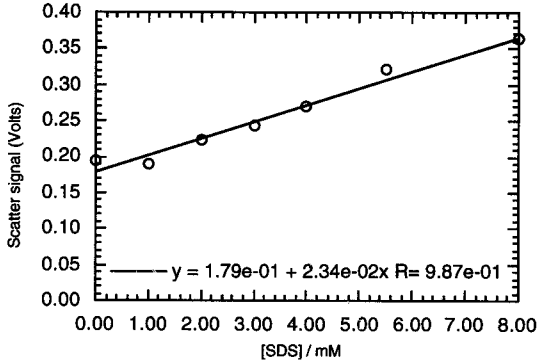


Figure 2-9 a. Comparison of Fluorescence intensities of MB-SDS interactions as a function of SDS concentration and solvents

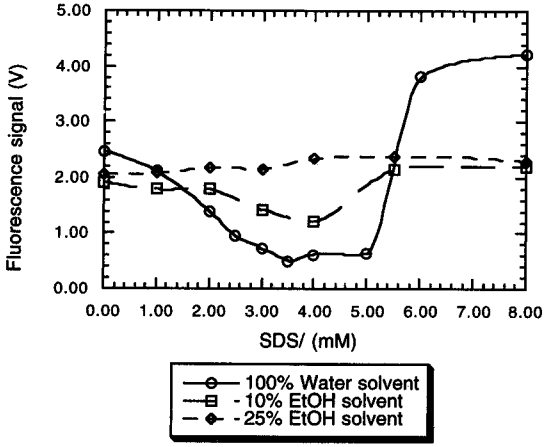
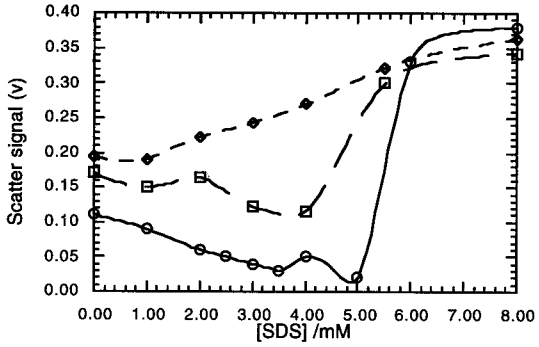


Figure 2-9 b. Comparison of Scatter intensities of MB-SDS as a function of SDS concentration and solvents.



fluorescence lifetime measurements give a sense of the environment around the fluorophore we are able to see that the environment of the MB ions changes as a function of SDS concentration.

It is still unclear what the exact relationship between MB and SDS is in solution. The formation of 1:1 MB-SDS ion-pairs may be a good explanation for the spectral changes observed. However, that does not explain why the most noticeable changes are seen at SDS concentrations between 3 and 5 mM. If ion pairs were formed, we would expect to see a linear relationship between SDS concentration and MB monomer absorbance but there is none. One possible explanation could be that the SDS concentration in solution is not equally distributed. Instead, the SDS could be aggregating around the MB at concentrations well below the CMC, and causing a decrease in MB absorbance. Then, as the SDS concentration approached the actual CMC, and the SDS reorganized into micelles, the MB was released into solution, and MB absorbance returned to its initial intensity.

Literature Cited:

1. Pharr, D.; Fu, Z.; Smith, T.; Hinze, W.; *Anal. Chem.*, 1989, 61, 275-279.
2. Szejtli, J.; Zsadon, B.; Cserhati, T.; Ordered Media in Chemical Separations, ACS, 1987.
3. Chatjigakis, A.; Donze, C.; Coleman, A.; *Anal. Chem.*, 1992, 64, 1632-1634.
4. Colwell, K. Senior Thesis, Union College, June 1995.
5. Mukerjee, P.; Ashish, K.; *J. Am. Chem. Soc.*, 92:22, 1970, 6403-6407.
6. Bergmann, K.; Konski, C.; *J. Phys. Chem.*, 67, 1963, 2169-2177.
7. Hamai, S.; *Bull. Chem. Soc. of Japan*, 1985, 58, 2099-2106.
8. Dewey, T.; Wilson, P.; Turner, D.; *J. Am. Chem. Soc.*, 1978, 100, 4500-4554.
9. Dewey, T.; Raymond, D.; Turner, D.; *J. Am. Chem. Soc.*, 1979, 101, 5822-5826.
10. Rabinowitch, E.; Epstein, L.; *J. Am. Chem. Soc.*, 1941, 63, 69-78.
11. Cheng, K.; Ueno, K.; Imamura, T.; CRC Handbook of Organic Analytical Reagents, CRC Press, Boca Raton, 1990.
12. Garcia, M.E.; Sanz-Medel, A.; *Talanta*, 1986, 33 (3), 255-264.
13. Fujimoto, B.S.; Clendenning, J.B.; Delrow, J.J.; Heath, P.J.; Schurr, M.; *J. Phys. Chem.*, 1994, 98, 6633-6643.

Chapter 3

Instrument Design Improvements

Design Improvements

The future for instruments like the compact multipurpose laser-based spectrophotometer is bright. The ability to perform routine chemical analysis in the field is an attractive option for analytical and environmental chemists, especially when sampling and/or sample degradation is a concern. However, most conventional instrumentation is restricted to the lab due to its size, cost, and relative delicacy. Our instrument was designed for just this type of operation. Instruments like this one may also be used for on-line industrial measurements. Several improvements should be made to this instrument to make it more practical.

Detectors:

Currently, the detectors used with compact spectrometer are PIN photodiodes (Honywell). These detectors are attractive due to their durability, size and cost. The silicon photodiodes used are constructed of the same *n*- and *p-type* materials mentioned previously. They produce current when light causes electrons and holes to recombine at the *p-n* junction. For their cost, these photodiodes have proved to be excellent detectors for this instrument, providing fluorescence limits of detection for both Methylene Blue and Nile Blue of 2 parts per billion. However there are more sensitive detectors than photodiodes.

One such detector is a photomultiplier tube (PMT). In short, a PMT is a detector which contains a photoemissive surface as well as several other electron sensitive surfaces [1]. When the photoemissive surface is struck by light, it emits electrons. These electrons then hit another electron sensitive surface which produces more electrons. In the end a cascade effect is produced which may generate up to 10^7 electrons for each photon collected. This significantly increases the sensitivity of the detector. However PMT's have drawbacks. PMT's are especially sensitive to thermal noise so for low intensity measurements they are maintained at sub-zero temperatures. PMT's are extremely sensitive to light so care must be taken to ensure that intense radiation is not focused onto the photoemissive surface. This means that special light shielding cases must be used with PMT's, making them bulky. A final draw back to PMT's is the fact that they rely on high voltages in order to produce the cascade effect with makes them so sensitive.

Until now photomultiplier tubes have not been an option for use with compact, portable instruments; however, recent improvements in PMT technology have produced compact PMT's. Recently a compact PMT (Hamamatsu) was acquired and plans are being made to use this detector with the compact spectrometer. In order to attach this PMT to the instrument a special adapter was built [Fig. 3-1]. This adapter contains as a shutter to protect the PMT's photoemissive surface from unwanted light. A new optical arrangement was also needed in order to use the PMT with the instrument. A f/f focusing theory similar to that used in the compact spectrometer's first generation optics was chosen. The light source

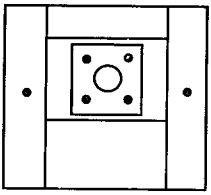
Figure 3-1. Photomultiplier Tube Adapter

Top view

Photomultiplier Tube Adapter

Improvements: Connects PMT to instrument. Contains a shutter to to limit the amount of light directed upon the PMT's sensor face.

Side view



for the fourth generation optics was still considered to be a point source, but instead of focusing back to a point as generations 2 and 3 had done, it was decided that a collimated emission beam would be used [Fig. 3-2]. This collimated beam allowed for the better use of the entire PMT sensor face, which has a substantially larger surface area than the photodiodes. The optical arrangement used with the PMT is seen in Figure 3-3.

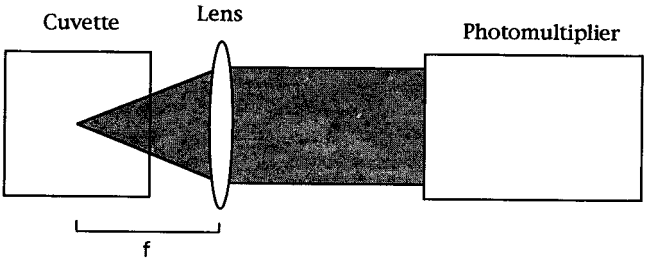
It is hoped that this more sensitive detector will allow for even lower limits of detection for this instrument. Characterization of the PMT for fluorescence and scattering measurements will be the next stage for this project.

Spectral Resolution:

Spectral resolution would add a whole new dimension to the use of this instrument. Currently this instrument is able to measure the combined emission signal for all wavelengths transmitted by a high-pass filter. Thus it is impossible to determine whether or not an analyte is emitting at 690-nm or at 720-nm. This is a major drawback to the instrument design. It is currently possible to tell that there is a species in solution which is emitting but there is no way of prove which species is producing the radiation. There are two ways of adding spectral resolution to the instrument.

The first and most costly method of creating spectral resolution would be the addition of a monochromator to the apparatus. Depending upon the size of the monochromator, the cost and the

Figure 3-2. Fourth Generation Optics "f/f Theory" with Photomultiplier Tube Detector



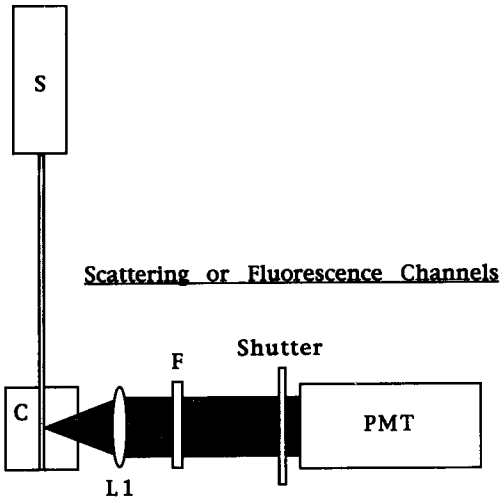


Figure 3-3. Depiction of photomultiplier tube detector apparatus. **S** is the laser source (670 nm), **C** is a standard 1-cm fluorescence cuvette, **F** is a filter (band-pass or high-pass), **L1** is a 12 mm convex lens, and **PMT** is a photomultiplier tube detector.

resolution will vary. Although a large costly monochromator will allow for superior resolution it will also decrease the practicality of having a compact instrument. The large monochromator would limit severely the portability and ruggedness for which this instrument was designed. A small monochromator (< 0.25 m) may be an acceptable compromise of resolution and size.

The second option for increasing the resolution of the instrument would be the use of a series of sequential band-pass filters. Although these filters would not have single-nanometer resolution, they would give a crude spectrum. As a second drawback, these filters would have to be inserted manually into the instrument, thus increasing the data acquisition time. Even with several drawbacks, this band-pass filter series would not limit the instrument's portability or ruggedness, or increase its cost significantly.

Semiconductor Lasers:

Semiconductor lasers are finding use in analytical chemistry as excitation sources for molecular spectroscopy because of their small size, low cost, and ease of maintenance. Diode lasers function in the same way as conventional gas or solid-state lasers. All lasers require a population inversion of excited state species over ground state species in order to allow for the spontaneous emission of laser light. The difference with semiconductor lasers is where this population inversion occurs. Semiconductor diodes consist of two types of

materials based on a silicon crystal containing four valence electrons per molecule. The first material, commonly known as *n*-type semiconductors contain five valence electrons. This means that *n*-type materials contain a surplus of free electrons. Examples of *n*-type semiconductors are arsenic and antimony. The second type of material, known as *p*-type semiconductors contain three valence electrons. These *p*-type materials have a shortage of electrons which may be referred to as electron holes. Examples of *p*-type semiconductors are aluminum and gallium. The region where *n*- and *p*-type materials meet is known as a junction. This is where population inversion and spontaneous emission occur in semiconductor lasers. Light is emitted when electrons and holes recombine and release energy equal to their band-gap energy.

Current development in the field of semiconductors has led to the commercial availability of III-V semiconductor lasers. These lasers are made of III-V semiconductor combinations such as gallium arsenide. The light emitted from these laser spans the region of the electromagnetic light spectrum from the infrared to the red region of the visible spectrum. The current wavelength limit of commercial diode laser technology is the AlGaInP/GaAs semiconductor mixture which produces red light of approximately 635-nm.

For the purposes of our experiments, our spectrometer has been equipped with both 670 and 635-nm diode lasers. The use of these lasers has led to both advantages and disadvantages for the instrument. The fact that the lasers are in the red region of the electromagnetic light spectrum ensures that there will be very few spectral interferences due the small number of molecules that will

absorb in this region. However, this fact also leads to the limited practicality of the instrument. Since so few molecules absorb in this region, few molecules may be monitored using a red laser as the excitation source.

This limitation to the instrument's capabilities may soon be changing. Research led be the telecommunications industry may soon produce commercially available blue-green diode lasers. These diode lasers are being produced using zinc-selenium semiconductors. The new lasers will allow us to expand the instrument's operating range and study many interesting species.

A further development in the use diode lasers will involve the application of time- or phase-resolved measurements. Diode lasers which can be pulsed at very high frequencies may be used for the measurement of time-resolved fluorescence life times for species in solution. Lasers which are able to be either internally or externally phase modulated may be used for fluorescence anisotropy measurements. Both anisotropy and lifetime data would assist in the understanding of the environment around the fluorophore.

Literature Cited

1. Skoog, D.; Leary, J.; Principles of Instrumental Analysis, 3rd ed., Saunders College Publishing, Orlando, 1992.