Application Note: Ocean Optics in the Teaching and Research Laboratories
Susquehanna University
Department of Chemistry
July 2014

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Introduction

In 2013, the Chemistry Department was awarded a $6000 grant for spectroscopic equipment by the Spectroscopy Society of Pittsburgh. The Department, in recent years, has revamped its laboratory curriculum at all levels to include experiments that are more project-based and connected to real-world problems will the goal of enhancing learning and preparing students for the undergraduate research experience and the real world. The Department has an active undergraduate research program and students are encouraged to do research as early as their first year.

With these goals in mind, the Department has been working towards expanding its instrument holdings and adding smaller, portable instruments that can be used by multiple students in a laboratory setting, and be easily moved from one room to another for use in multiple classes and research projects. As a result, two USB650 Red Tide spectrometers were purchased for use in multiple courses and for undergraduate research. To date, these spectrometers have been used in the following courses: Biochemistry of Proteins and Enzymes (Dr. Thomas, Fall 2013), Physical Chemistry II (Dr. Basu, Spring 2014), Instrumental Analysis (Dr. Johnson, Spring 2014). The spectrometers have also been used for a summer research project on nanoparticle synthesis and applications, supervised by Dr. Basu.

Physical Chemistry II (Spring 2014, 7 students)

Physical Chemistry II is an upper-level course predominantly taken by junior (and some senior) chemistry majors. Experiments range from the basics of spectroscopy (Beer's Law, quantum yield) to computational chemistry to applications (lasers, infrared and Raman spectroscopy). The Ocean Optics spectrometers were used for two experiments. In the first experiment, the quantum mechanical "particle in a box" model was studied using a series of cyanine dyes (Figure 1). This experiment was based on an article by Moog (*J Chem Ed*, vol. 68, 1991).

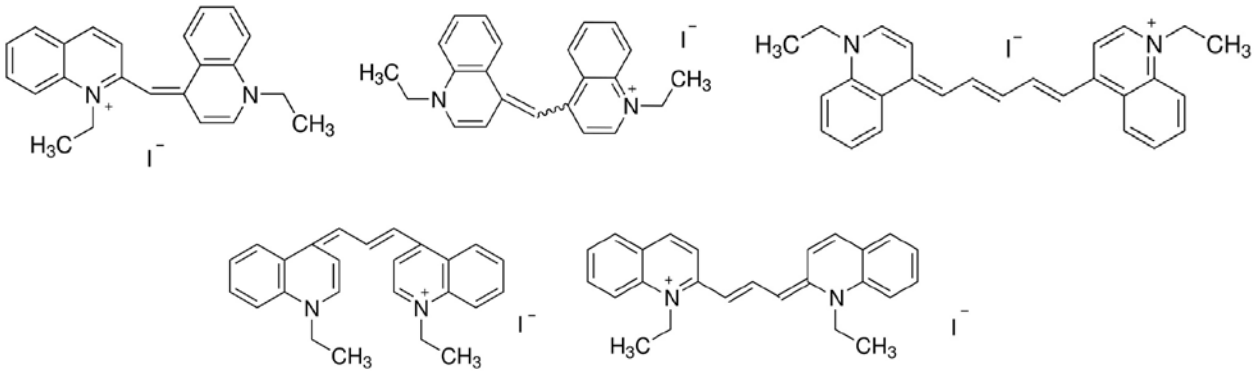

Figure 1. Structures of cyanine dyes. Clockwise from top left: 1,1'-diethyl-2,4'-cyanine iodide, 1,1'-diethyl-4,4'-cyanine iodide, 1,1'-diethyl-4,4'-dicarbocyanine iodide, 1,1'-diethyl-2,2'-carbocyanine iodide, 1,1'-diethyl-4,4'-carbocyanine iodide.

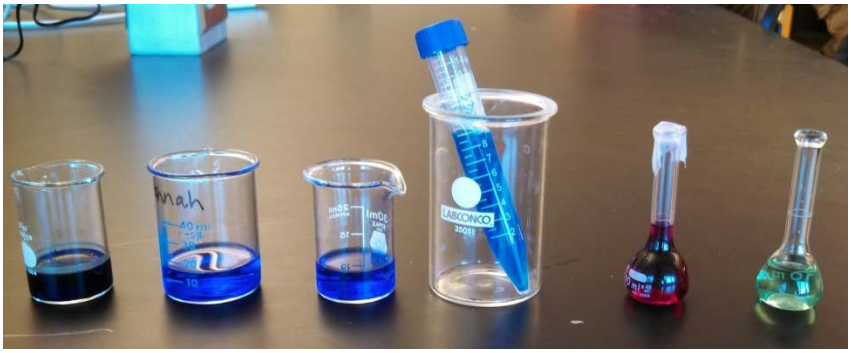

Figure 2. Solutions of various cyanine dyes.

Students prepared a series of dye solutions in methanol (Figure 2). The absorption spectra of the dyes were measured using the spectrometers in order to determine (a) the absorption maxima and (b) the appropriate dilution steps necessary to obtain absorbances in the 0.5-0.6 range (Figure 3).

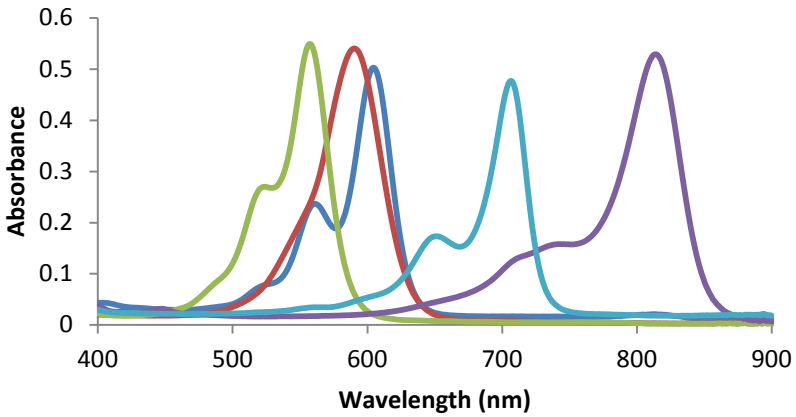

Figure 3. The absorption spectra of the cyanine dyes. Green: 1,1-diethyl-2,4-cyanine iodide, red: 1,1-diethyl-4,4-cyanine iodide, dark blue: 1,1-diethyl-2,2-carbocyanine iodide, light blue: 1,1-diethyl-4,4-carbocyanine iodide, purple: 1,1-diethyl-4,4-dicarbocyanine iodide.

In the "particle in a box" model, the absorption maximum is directly proportional to the number of π electrons in a conjugated chain. In these cyanine dyes, the conjugated chain links the two nitrogens. The experimental absorption maximum is used to calculate the experimental "box length", which is the sum of the bond lengths from one nitrogen to the other. This is compared to the theoretical "box length", which is also used to determine the theoretical absorption maximum for each dye. The results for one group of cyanine dyes are summarized in Table 1.

Table 1: Experimental and theoretical data for the "particle in a box" experiment.

Compound	Number of π electrons	Box lengths (nm)		Absorption Maxima (nm)		Color
		Experimental	Calculated	Experimental	Calculated	
1,1'-diethyl-2,4'-cyanine iodide	8	1233	864	557	273	reddish purple
1,1'-diethyl-2,2'-carbocyanine iodide	8	1285	857	605	266	blue
1,1'-diethyl-4,4-cyanine iodide	10	1403	1140	590	389	blue
1,1'-diethyl-4,4'-carbocyanine iodide	12	1670	1567	707	624	blue
1,1'-diethyl-4,4'-dicarbocyanine iodide	14	1923	1716	814	647	green

In the second experiment, the effect of metallation on the optical properties of porphyrins was studied. The spectrometers were used to measure the absorption of the porphyrin Q bands (Figure 4) and plan dilutions for fluorescence experiments that were required for quantum yield calculations. The experiments were based on articles by Fery-Forgues (*J Chem Ed*, vol. 76, 1999) and Quimby/Longo (*J Am Chem Soc*, vol. 97, 1975).

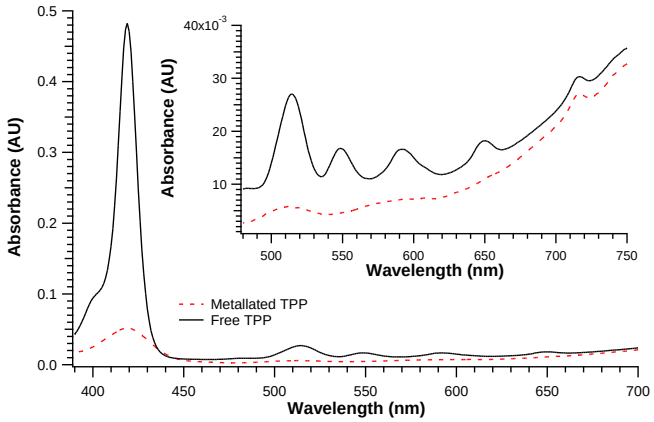

Figure 4. Typical UV-Vis absorption spectrum of a porphyrin (student data). The intense Soret band appears around 420 nm and the weaker Q-bands (inset) appear in the 500-700 nm range.

Biochemistry of Proteins and Enzymes (Fall 2013, 9 students)
Biochemistry of Proteins and Enzymes is a 400-level course taught at Susquehanna University to junior and senior Biology, Biochemistry and Chemistry majors. The majority of the semester was devoted to an experiment involving catalase. Students researched and wrote their own protocols to purify catalase, confirm the identity of the enzyme and reproduce literature values for Michaelis-Menten kinetics (Beers and Sizer, *J Biol Chem*, vol. 195, 1952). Catalase activity was monitored spectroscopically as a decrease in hydrogen peroxide concentration over time. Different groups used different spectrophotometers for this method. One group used the Ocean Optics Spectrophotometer with the SpectraSuite software.

The absorbance at 240 nm was recorded every ten seconds until the starting value decreased by half (one half-life). Hydrogen peroxide concentration at each time point was calculated using Beer's Law (Equation 1). The absorbance (A) is related to the concentration (c), path length of the cell (l) and the molar extinction coefficient (ε).

$$A = \varepsilon c l \qquad (1)$$

The specific activity of catalase was reported as U/mg enzyme where U (unit) is concentration of hydrogen peroxide consumed per minute. Values reported by students ranged from 0.005 U/mg to 2000 U/mg over the course of enzyme purification for the entire class. The group using the Ocean Optics Spectrophotometer reported specific activity from 2.90 U/mg to 20.5 U/mg.

Instrumental Analysis (Spring 2014, 7 students)
Instrumental Analysis is a 400-level course taken by senior Chemistry majors. A significant component of the course is UV-vis spectroscopy. This particular experiment involved the molecule fluorescein (Figure 5), a common dye. Fluorescein has a strong absorbance in the visible region (450-480 nm), is frequently used as a water flow monitor colorant for streams and in the laboratory as a stain.

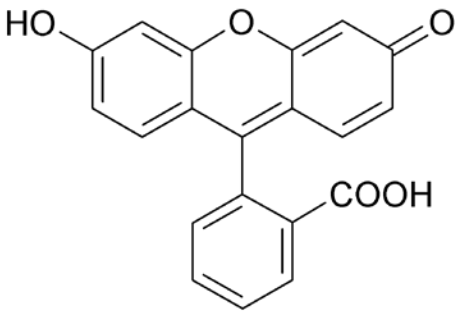

Figure 5. Structure of fluorescein.

The experiment involved measuring a standard concentration curve of fluorescein to determine the concentration at which the spectrometer begins to deviate from Beer's law (Equation 1). Several types of instruments were used: a Varian Cary 4000, Beckman DU 730, Genesys 10, and the Ocean Optics USB650 Red Tide UV-vis spectrometer. Each instrument was chosen based on its differences as a spectrometer. The Cary is a highly-sensitive double-beam spectrometer, the Beckman a typical bench-top diode-array, and the Genesys 10 is a modern version of a Spec20.

Both the Cary and Beckman are expensive laboratory instruments and effectively immobile. Each instrument demonstrates Beers Law linearity to greater than 2 O.D. (Figure 6). The Genesys 10 is an inexpensive but relatively large, shoebox sized, single wavelength spectrometer. At 480 nm the linearity of the standard curve began to break down just above 1 O.D. and continued to deviate further as the absorbance increased.

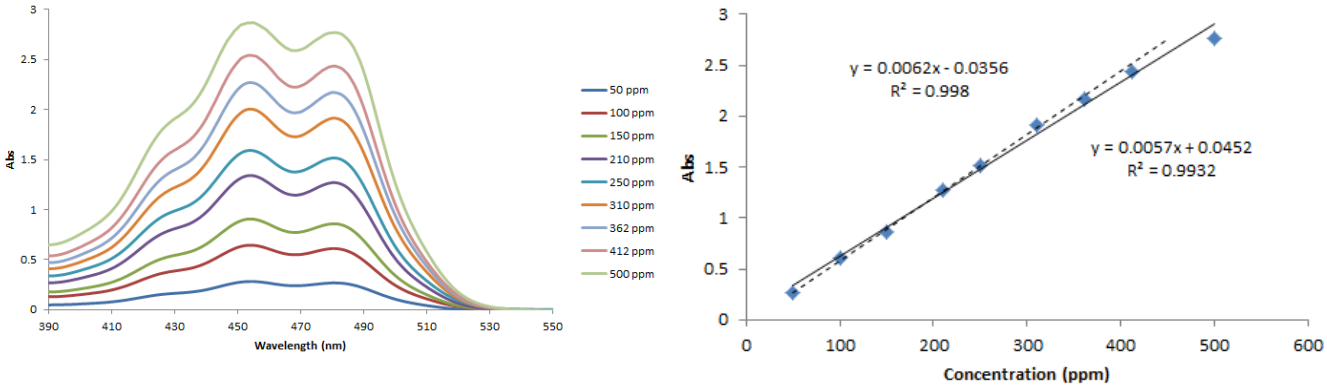

Figure 6. Left: Fluorescein absorption from 390 to 550 nm as a function of concentration recorded with the Cary 4000. Right: Beer's Law plot for fluorescein at 480 nm. The absorption of fluorescein increases linearly with concentration to greater than 2 O.D.

The Ocean Optics USB650 Red Tide performed better than the larger Genesys instruments and rivalled the performance of the Cary and Beckman (Figure 7).

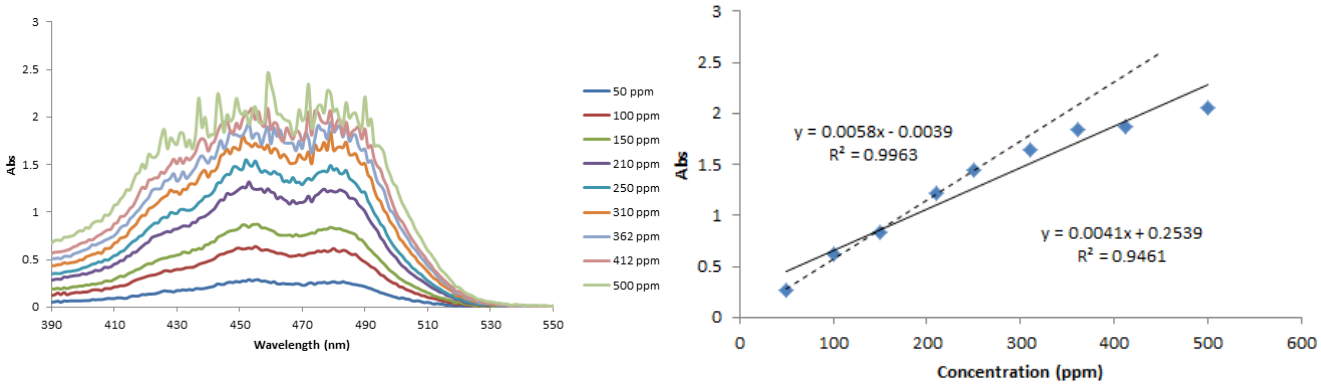

Figure 7. Left: Absorption spectra of fluorescein as a function of concentration from 390 to 550 nm recorded with the Ocean Optics USB650 Red Tide. Right: Beer's Law plot for fluorescein at 480 nm. The absorption of fluorescein increases linearly with concentration to approximately 1.5 O.D.

Overall, the Ocean Optics USB650 Red Tide UV-vis spectrometer performed well. Both the Beckman and Red Tide have diode array detectors with similar resolution and have a linear response well past 1 O.D. But the Red Tide unit is a small fraction of the size of the shoe box size Beckman and bench top Cary.

Research Project (Summer 2014)

The spectrometers are currently being used in an undergraduate summer research project. Gold and silver nanoparticles of various shapes and sizes are being synthesized for applications ranging from surface-enhanced Raman scattering (SERS), singlet-oxygen generation and fluorescence lifetime experiments. Gold and silver spheres with diameters in the 10-20 nm range have been synthesized using published methods. Solutions of gold nanospheres show a strong absorption band in the 510-530 nm range (Figure 8) and silver nanospheres show a similarly intense band around 400 nm (Figure 9). The presence of these peaks is used to confirm the presence of nanospheres prior to proceeding with various applications.

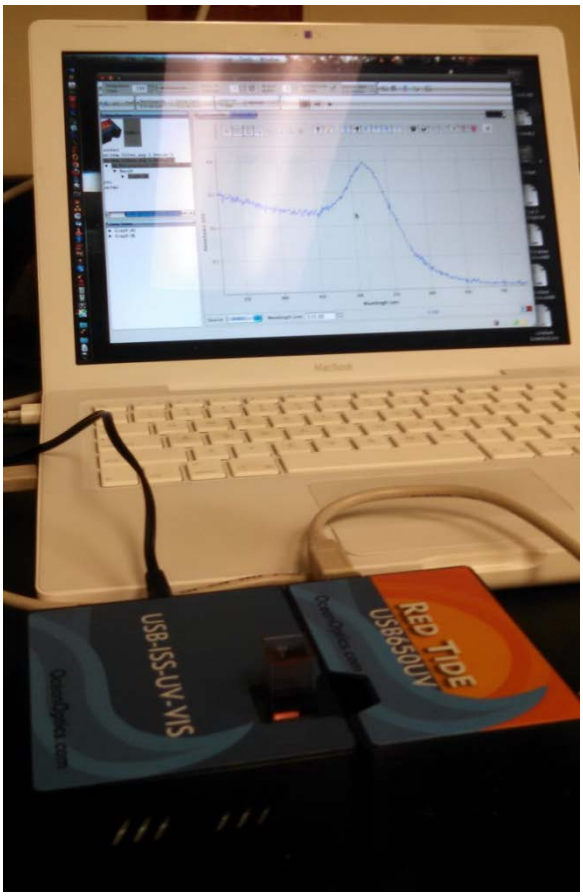

Figure 8. Experimental setup for the determination of the absorption spectrum of gold nanospheres.

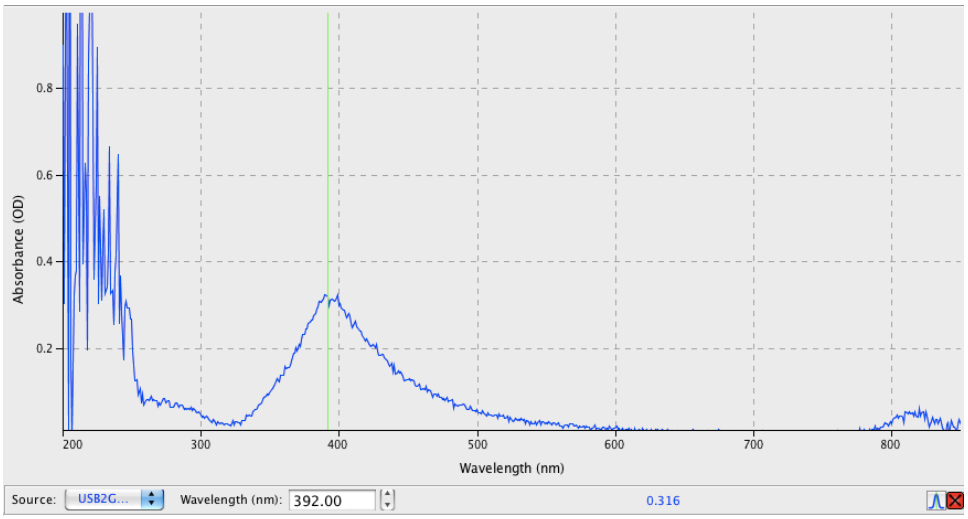

Figure 9. Absorption spectrum of silver nanospheres.

Conclusions

In conclusion, the Ocean Optics USB650 Red Tide spectrometers have already been used in three upper-level classes and an undergraduate research project during the 2013-2014 academic year and Summer 2014. Students have had the opportunity to compare the ease of use of these spectrometers to other instruments as well as the ability to get "instant spectroscopic feedback" as they prepare solutions for various experiments, allowing for experimental planning and redesign "on the fly". Faculty members now have more flexibility with laboratory scheduling and experimental design. These spectrometers will continue to be used in these classes and new experiments will be incorporated in these classes as well as other classes in the future.