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A Modular Laser Apparatus for Polarimetry, Nephelometry, and Fluorimetry in General Chemistry W

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Occasionally, when instructing students, newer technologies are overlooked in favor of traditional exercises and examples that are proven commodities. Cutting-edge technology often comes to bear in the chemistry curriculum only in upper-level chemistry courses such as physical chemistry, instrumental or advanced analytical chemistry, or organic chemistry. In so doing, we may neglect our widest audience—students with whom we often only have a single opportunity to show the wonders of our discipline. These general chemistry students, many of whom are not chemistry majors, often do not see the spectacular advances and modern techniques in the field. One way to bring some of the "wow" in chemistry to students is to introduce lasers and their uses in the general chemistry laboratories.

Laser technology is becoming ubiquitous, not only throughout chemistry and other sciences, but also within society itself. One need not look further than one's desktop or car dashboard to find a laser at work. Consumer electronics are rife with lasers: CDs, DVDs, and communications devices. Inside medical offices, lasers can remove scars, unwanted tattoos and birthmarks, correct or repair vision, and even remove wrinkles. As many of our students are bound for a career in the health sciences, an introduction to lasers and their uses early in the undergraduate training is appropriate. For those students considering a career in chemistry, this early exposure to one of the most basic tools can capture their imagination of what a modern chemist does.

Design Criteria

The idea of introducing laser technology has its proponents. Schwenz and Moore *(1)* have discussed the importance of lasers in the undergraduate chemistry curriculum in their American Chemical Society-sponsored volume. Zare, Spencer, Springer, and Jacobson *(2)* have collected many exercises and demonstrations along with practical advice on using lasers for the beginning student. General descriptions of the uses of lasers in chemistry are also available *(3, 4)*. Further, several recent articles in this *Journal* have described numerous laser or simplified apparatus for investigating light scattering and refraction *(5–11)*, optical rotation *(12–14)*, and fluorescence *(15)*. We describe a novel apparatus that strengthens the connection between our research and teaching laboratories and serves multiple purposes. To do so, the apparatus should meet several criteria. First, each apparatus must be rugged enough to withstand student use and assembly for many years. Second, the apparatus must allow students to see the experimental layout of the instrument. Third, the components of the apparatus should allow for student assembly, alignment, and data collection in a single laboratory session of two to three hours. Fourth, the components should be easily assembled to do polarimetry, nephelometry (simple light scattering), or fluorimetry. Finally, the apparatus should be as affordable as possible without sacrificing the above goals to allow multiple setups to be available in a laboratory session.

The rationale for the design criteria addresses a pair of pedagogical and practical goals. First, in allowing the students to construct and align the instruments themselves, they develop a better understanding of the instrument, how it measures the property of interest, and exactly what the data mean. This method is in direct contrast to several of the commercial measurement kits and probes, such as those by Ocean Optics or Vernier. While these kits have definite application in many of our laboratories, they can contribute to the development of the "black-box syndrome" in students. Avoiding the idea that numbers miraculously appear from an instrument without further regard of their meaning, accuracy, or precision has been a paramount consideration in our design. Second, components rugged enough to withstand student use or abuse are often more costly. To offset the additional cost, the instrument should be used in more that one general chemistry lab, and in more than a single configuration. Making the apparatus appropriate also for the upper-level laboratories only enhances its value.

Beyond the simple introduction of the technology to the laboratory, these instruments greatly aid in the exploration of topics that are difficult or impossible to handle using classical methods. For example, general chemistry students can directly follow reaction kinetics nondestructively via fluorescence or polarimetry for much less cost than could be achieved measuring absorbance with a Spec 20 purchased for each group of students. These types of experiments demonstrate integrated rate laws and graphical approaches to studying kinetics. Students can also explore the Tyndall effect and use light scattering methods to determine sample concentrations of sulfate ion or other species. Finally, students begin to understand the components and techniques inherent to modern instrumentation and can avoid the black-box syndrome as they continue their studies in chemistry or develop a healthy skepticism in their endeavors outside chemistry.

Table 1. Description of Components Used in the Apparatus

Component	Source	Part Number
He–Ne Laser, 0.8 mW polarized output	Edmund Scientific	61338
Polarizer with rotary mount	Edmund Scientific	52574
Photodiode detector, 200 to 800 nm	ThorLabs	DET-200X
10-cm Quartz polarizer cell	NSG Precision Cells	34-G-100
4-in. Optical post	Newport	SP-4
Red cutoff filter, 1-in. diameter	Edmund Scientific	43-942

Basic Components

The stand-alone apparatus is designed to allow the student to configure the components as a simple polarimeter, a nephelometer, or fluorimeter. Relatively few components are needed to allow for this versatility: a small polarized He–Ne laser mounted on an optical post; a polarizer in a rotary mount with vernier markings of one degree or less; an amplified photodiode, red cutoff filter (or bandpass filter); and a digital voltmeter. Rather than using the more expensive optical rail system, a sturdy ring stand serves to keep the components aligned. The components are detailed in Table 1, while the instrument schematic is shown in Figure 1. Our initial cost of $500, which included the ring stand, posts, and clamps, can be reduced using a lower power or less rugged laser at the cost of sensitivity in the light scattering experiments. Further cost savings can be realized if the ring stands and other support equipment are already on hand. One instrument is appropriate for every two or three students in a laboratory session. More than three students per instrument will severely limit the experience of the student.

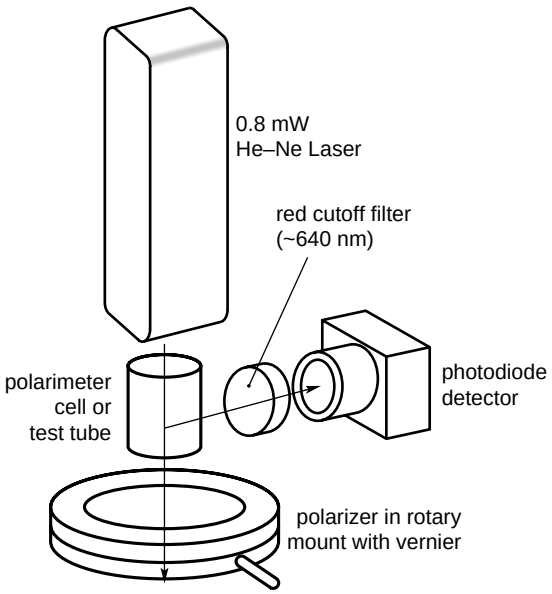

Figure 1. The apparatus schematic indicates all components, excluding clamps and posts, for use in the polarimeter, nephelometer, and fluorimeter. The polarimeter excludes the detector and filter, while the nephelometer and fluorimeter exclude the polarizer and mount. The filter is normally mounted directly on the detector. All the components are held in alignment with posts and clamps to a ring stand.

Polarimetry

The polarimeter apparatus is as shown in Figure 1 without the photodiode detector. The polarized laser light travels through a sample holder to the polarizer. The polarizer first is adjusted to a zero reading in the absence of sample. The sample holder is inserted into the laser beam, and the quantity of light rotation is found by adjusting the polarizer with the sample present. In both the zero reading and the reading with the sample present, the measurement is taken by adjusting the polarizer in such a way that the minimum of light is allowed to pass on a white card below the polarizer. Of course, the true polarization of the light would be 90° from the reading with minimum light, but the difference in the readings yields the optical rotation of the sample. We strongly suggest reading the polarizer in this way since it is easier to find the minimum rather than the maximum of the light passed through the polarizer. In those cases where the optical rotation is large and there might be some question about the direction of the rotation, the standard methods of changing the path length or concentration would reveal whether the rotation is truly dextrorotatory or levrorotatory.

We have tried a variety of sample holders. Initially, we purchased high quality quartz polarimeter cells for the measurements. This turned out to be unnecessary for most cases; a small (i.e., 4–6-in.) test tube with a rounded bottom proved to be sufficient for the general chemistry laboratories. Some error is introduced when the students measure the length of the tube, but the ease in handling compensates for the decrease in precision. We do not recommend a flat bottom test tube; the flat bottom tubes have a tendency to defocus the light, making the measurement more difficult. However, for measurement of accurate optical rotations, the polarimeter cell is still recommended.

There is a significant drawback to our apparatus; the specific rotations of substances are typically reported using a sodium lamp polarimeter at 589.3 nm *(16)*. Since the He–Ne laser emits at 632.8 nm and specific rotations are wavelength dependent, the specific rotations measured by our apparatus are different from literature values. When wavelength dependent optical rotations are reported, it is possible to interpolate these numbers to get a reasonable value for the 632.8 nm wavelength. For example, we have interpolated the wave-

length dependence of the specific rotation of sucrose using the data given in the CRC *(16)*. A linear least-squares fit of the data from 578 nm to 670 nm yielded a coefficient of determination (R^2) of .9968 and a value for the specific rotation of 55.66 deg/dm at 632.8 nm. Taking the average data obtained by our general chemistry students using a 1.0 M solution (34.23 g/100 mL), we find the specific rotation of sucrose using our apparatus to be 56.7 degree/dm with a 90% confidence interval of 1.1 degree/dm. At a minimum, this datum shows that the apparatus has sufficient accuracy and precision for a general chemistry exercise and is well suited for routine determinations in an organic laboratory setting. We have found the polarimeter apparatus to be useful in our organic and biochemistry laboratories; the apparatus is much easier to understand than the classical sodium lamp apparatus as the open construction allows the student to follow the light path through the instrument.

We have adapted a kinetics experiment for the polarimeter apparatus *(17)* to be suitable for the general chemistry laboratory in both content and duration. The polarimeter is zeroed by setting the vernier on the polarimeter to zero and rotating the polarizer to minimize the quantity of laser light passing through. The students prepare a solution containing 7 mL each of 1 M sucrose and 4 M hydrochloric acid. The experiment timing begins immediately upon mixing the solution. The students place the mixed solution in a 6-in. test tube and mount the test tube in a previously zeroed polarimeter. As soon as possible, a reading of the angle of rotation is taken by minimizing the laser throughput. This reading of the optical rotation is repeated every five minutes until four consecutive readings of the rotation are the same, about 90 min. The net rotation from this final value is used to create plots of rotation, natural log of the rotation, and inverse rotation versus time, corresponding to zero-, first-, and second-order kinetics, respectively. By choosing the plot with the best linear fit, the students can determine the reaction order. Further, since the acid-catalyzed hydrolysis of sucrose is first-order, the rate constant is given by the slope of the first-order graph without further conversion of the rotation to concentration units. Typical student results for the experiment are shown in Figure 2. This experiment has proven very effective in introducing graphical methods for the determination of reaction orders in kinetics in addition to highlighting the use of the laser in the direct measurement of the kinetics data.

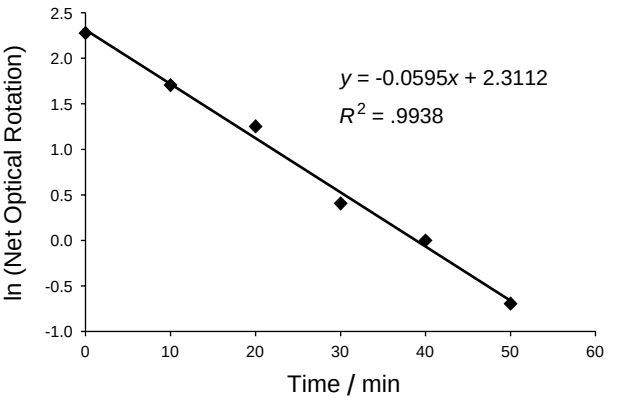

Figure 2. Typical student kinetics data for sucrose hydrolysis monitored with the polarimeter. The students monitor the net optical rotation of the solution (the products are slightly levrorotatory while sucrose is dextrorotatory) versus time. The plot shows the fit to first-order kinetics for the hydrolysis.

Nephelometry

The nephelometry apparatus is quickly assembled as in the polarimeter setup, but without the use of the polarizer. Instead of a polarimeter cell, the laser light travels into a 1-cm plastic square cuvette containing a scattering solution. The quantity of light scattered is measured using a photodiode at 90° to the incoming laser light. The signal is read directly from the photodiode using a digital voltmeter. We generally use a disposable cuvette as a sample holder. It is important to correct for any background light present (i.e., room lights) by measuring the voltage on the photodiode in the absence of any scattered laser light. The background interference is greatly reduced through the addition of a red cutoff filter to the photodiode detector and setting the instrument so the detector faces away from any outside light source. The apparatus could also be improved by the construction of a light-tight box around the sample; however, we have found that this is an unnecessary step in most circumstances. It is also critical that a digital voltmeter, with as high input impedance as possible, be used. The lower input impedances of cheap digital meters or analog meters drastically reduce the voltage response of the photodiode output.

We have adapted a standard nephelometry experiment *(18)* to measure the concentration of the sulfate ion in tap water. A series of standards (5 mL each) is prepared by diluting stock sulfate solutions (0.010 M and 0.0010 M) using a 10-mL graduated cylinder. We found that careful use of a 10-mL graduated cylinder was sufficient for this experiment; however, volumetric pipets could be used. The solution is made turbid by the addition of excess barium ion (0.10 M, 5 mL). After thorough mixing, a small quantity of the solution is transferred to the cuvette and placed in the He–Ne beam in front of the photodetector. The voltage reading on the multimeter is recorded. A small dark index card is used to block the laser beam and the voltage reading is recorded again to provide a zero reading for that solution. The procedure is repeated for each solution. A typical student calibration curve for a sulfate ion determination is shown in Figure 3. Three samples of water of three milliliters each are collected directly from the lab faucet and the turbidity is measured according to the above procedure. The spread in the results from nine teams of students showed 10% relative deviation.

This nephelometry experiment is suitable both for general and analytical chemistry laboratories. Any procedure based on a turbidimetric or nephelometric measurement could be easily adapted for use with this apparatus. Potentially, the apparatus could also be set up for turbidity measurements; in this case, one would measure the light that travels through the sample, rather than the light that is scattered by the sample. The turbidity measurements are more difficult owing to the intensity of the laser beam and are not recommended.

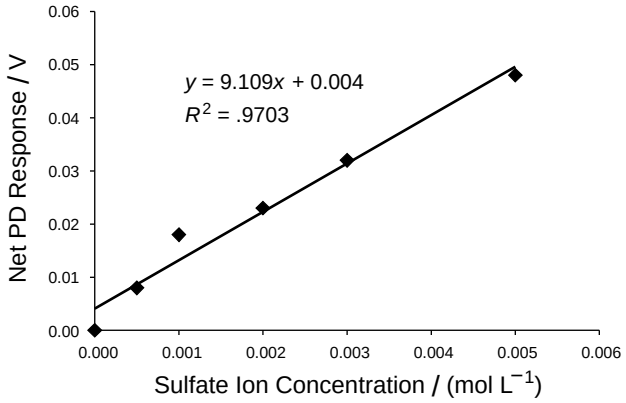

Figure 3. Typical student calibration data for determination of sulfate ion concentration by nephelometry. The photodiode (PD) voltages are read following addition of excess barium ion to the sulfate calibration standards. Each point represents the net photodiode voltage obtained by subtracting the background light reading from the reading of the total light (scattered plus background) from the calibration standards.

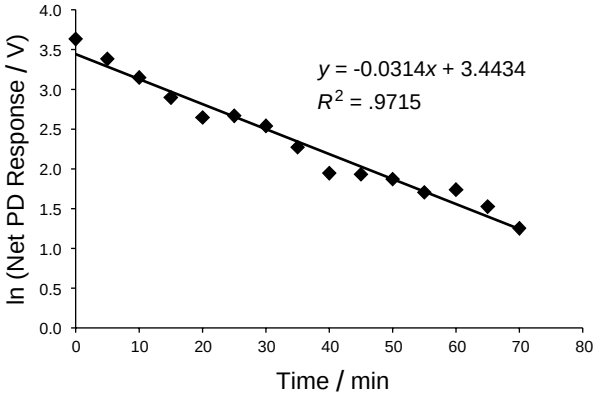

Figure 4. Student fluorescence data for phycocyanin destruction by thermal free radicals. First-order kinetics plot obtained from a solution containing: phycocyanin (60 μL of stock concentration high enough to give 0.10 absorbance); 2,2'-azobis(2-amindinopropane) dihydrochloride (thermal free radical generator, 200 μL of 40 mM solution); and 275 μL of 75 mM phosphate buffer at pH = 7.

Fluorimetry

The fluorimeter apparatus is assembled exactly as for the nephelometer. The laser light travels through a fluorescent sample that absorbs at 632.8 nm. Light is collected 90° to the incoming laser light. The collected light is passed through a cutoff filter and into the photodiode. The voltage is read directly from the photodiode using the digital voltmeter. As opposed to the nephelometry experiments, the construction of a lighttight sample box is necessary to obtain linear fluorescence data. Fortunately, a simple box made of card stock is sufficient for this purpose. The fluorimeter apparatus is of more limited utility than the other setups; only a limited number of substances fluoresce with 632.8 nm excitation light. Any substance with a blue solution (red absorption band) is a candidate; many laser dyes or food colorings may serve the purpose well.

We can suggest one experiment for the apparatus. Heller and Gindt have developed laboratories to study protein folding using the fluorescent protein phycocyanin *(19, 20)*. Phycocyanin has a maximum absorbance at 630 nm with a quantum yield of 60%, making it a prime candidate for the apparatus. The student procedure will be reported later, but an example first-order kinetics plot of the reaction of phycocyanin with excess thermal free radical generator is shown in Figure 4.

Hazards

The He–Ne lasers are Class III lasers; direct exposure to the laser should be avoided. No safety eyewear is required. In the polarimetry experiment, the acid-containing solutions should be neutralized and then present no further hazard. Any solutions containing barium in the nephelometry experiment should be collected and disposed of according to normal procedures. The chemicals in the fluorescence experiment present little, if any, hazard. Normal precautions should suffice.

Conclusion

An introduction to lasers as early as a general chemistry course can be of great advantage to both major and nonmajor students in chemistry, but often cost and other practical considerations prevent widespread use of lasers. We have designed a versatile laser apparatus suitable for the undergraduate teaching laboratory that may serve as polarimeter, nephelometer, or fluorimeter. Discussions with our faculty and surveys of students following the introduction of these instruments into our curriculum have been positive. Students state that the laboratories are more interesting with the use of laser technology. Several suggestions have been made for specific exercises using the apparatus in each of its three modes.

Acknowledgments

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[W]Supplemental Material

Instructions for the students and notes for the instructor are available in this issue of *JCE Online*.

Literature Cited

1. *Physical Chemistry: Developing a Dynamic Curriculum;* Schwenz, R. W., Moore, R. J., Eds.; American Chemical Society: Washington, DC, 1993.
2. Zare, R. N.; Spencer, B. H.; Springer, D. S.; Jacobson, M. P. *Laser Experiments for Beginners;* University Science Books: Sausalito, CA, 1995.

3. Andrews, D. L. *Lasers in Chemistry,* 3rd ed.; Springer-Verlag: New York, 1997.
4. Van Hecke, G. R.; Karukstis, K. K. *A Guide to Lasers in Chemistry;* Jones and Bartlett: Boston, MA, 1998.
5. Vaksman, M. A.; Lane, J. W. *J. Chem. Educ.* 2001, *78,* 1507–1509.
6. Neder, A. de V. F.; García, E.; Viana, L. N. *J. Chem. Educ.* 2001, *78,* 1481–1483.
7. Evans, J. J. *J. Chem. Educ.* 2000, *77,* 1609–1611.
8. Thomas, E. *J. Chem. Educ.* 1996, *73,* 470.
9. Betterton, E. A. *J. Chem. Educ.* 1991, *68,* 254–256.
10. Johns, N.; Longstaff, S. J. *J. Chem. Educ.* 1987, *64,* 449–451.
11. Das, I.; Das, S. S.; Pushkarna, A. A. *J. Chem Educ.* 1987, *64,* 729–730.
12. Stary, F. E.; Woldow, N. *J. Chem. Educ.* 2001, *78,* 644.
13. Levine, S. G. *J. Chem. Educ*. 1990, *67,* 1064.
14. Dorn, H. C.; Bell, H.; Birkett, T. *J. Chem. Educ.* 1984, *61,* 1106–1107.
15. Tran, Y.; Whitten, J. E. *J. Chem. Educ.* 2001, *78,* 1093–1095.
16. *Handbook of Chemistry and Physics*, 69th ed.; Weast, R. C., Ed.; CRC Press: Boca Raton, FL, 1988; p E423.
17. Daniels, F.; Alberty, R. A.; Williams, J. W.; Cornwell, C. D.; Bender F.; Harriman, J. E. *Experimental Physical Chemistry,* 7th ed.; McGraw-Hill Book Company: New York, 1970; pp 149–152.
18. *Standard Methods for the Examination of Water and Wastewater,* 18th ed.; Greenberg, A. E., Clesceri, L. S., Eaton, A. D., Eds.; American Public Health Association: Washington, DC, 1992; Part 4500-SO_4^{2-}.
19. Bowen, R.; Hartung, R.; Gindt, Y. M. *J. Chem. Educ.* 2000, *77,* 1456–1457.
20. Heller, B. A.; Gindt, Y. M. *J. Chem. Educ.* 2000, *77,* 1458–1459.