Cost-Effective Teacher

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Analytical Spectroscopy Using Modular Systems

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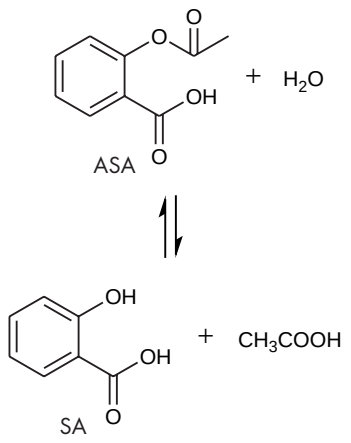
Analytical spectroscopy is typically taught in the classroom using a modular approach. For example, most spectroscopic systems are described as a source, monochromator, detector, and sampling configuration appropriate for each spectrometer type. In the laboratory however, the instruments exist in the form of black boxes, which make it difficult for students to conceptualize the instrument configuration and how it works. In the classroom, students learn several different methods such as UV–vis, fluorescence, infrared, and Raman spectroscopy, but an experimental comparison of these methods is seldom conducted. Consequently, students do not obtain a full understanding of how these methods compare to each other from an analytical standpoint. Analytical is meant to imply the required sample preparation, instrument configuration, and the qualitative and quantitative capacity of the method.

We have overcome these pedagogical problems by using modular miniaturized S2000 spectrographs *(1–3)* and accessories, purchased from Ocean Optics Inc. (OOI). Other suppliers of miniature spectrographs include: Acton Research, CVI Spectral Instruments, Instruments SA, and Oriel. Three experiments based on UV–vis, fluorescence, and Raman spectroscopy were developed to compare light absorbance, emission, and scattering approaches. Using a component approach, the students can quickly put together their own instruments and optimize them with respect to the desired measurement. The students conduct all three experiments, which gives them firsthand experience in prototyping instruments in addition to learning the analytical capabilities of the three methods. Pedagogically, we feel that this is a much better approach for students to learn since they can see how each of the instruments function and more importantly, get a feeling for what their analytical capabilities and limitations are. This discovery-based format has been shown to be extremely beneficial in the junior-level physical and analytical instructional laboratory.



Scheme I. Aspirin or acetylsalicylic acid (ASA) in tablets is slowly hydrolyzed by water in the air to salicylic acid (SA) and acetic acid.

In each experiment, the qualitative and quantitative determination of salicylic acid (SA) is made. The clinical assay of SA is pharmaceutically relevant. Aspirin or acetylsalicylic acid (ASA) in tablets is slowly hydrolyzed by water in the air to SA as shown in Scheme I. Since acetic acid is somewhat volatile, the main impurity that remains is SA, which is irritating to the stomach lining. Therefore, the Food and Drug Administration (FDA) has established a tolerance of 0.10% (mass) SA in unbuffered aspirin *(4)*. Accidental salicylate poisoning from an overdose of aspirin can occur at greater than 300 mg/L (ppm) SA in blood plasma *(5)*. A comparison of UV–vis, fluorescence, and Raman clearly shows which technique is valuable for quantitative work. A comparison of linearity and detection limit for SA by UV–vis, fluorescence, and Raman allows the student to choose one technique in particular for the determination of SA in aspirin.

Experimental Reagents and Solutions

The standard aqueous-based procedures cannot be used for the determination of SA in the presence of ASA owing to the premature hydrolysis of ASA during the sample preparation step. To avoid hydrolysis, nonaqueous solvents were employed in the investigation *(6)*. Previously a $CHCl_3$ solution was used, but this reagent was avoided since it is a suspected carcinogen. Other studies have hydrolyzed the ASA to SA and then measured the SA for ASA content using UV–vis absorption *(7, 8)* or by complexation with iron and colorimetric analysis *(9)*. These methods assumed that the original SA content was negligible, which may be appropriate if the tablet is new. However we wanted to look at expired or nearly expired tablets in which the SA content may not be negligible.

Absorbance standards were prepared by dissolving approximately 0.050 g of salicylic acid into 250 mL of *n*-hexane with constant stirring for approximately 30 min to help dissolution. Standard concentrations should be in the range of 10^{-4}–10^{-5} M. For the aspirin sample, two tablets were ground and approximately 0.10 g was dissolved into 250 mL of *n*-hexane.

Fluorescence standards were prepared by dissolving 0.10 g of salicylic acid into 100 mL of THF and diluting in the

Table 1. Spectrometer Characteristics

Characteristic	Spectrometer		
	UV–vis	Fluorescence	Raman
Serial number	US2J117	12J1430	I2J1217
Aperture (μm)	25	200	50
Bandwidth (nm)	200–850	200–850	510–795
Grating (lines)	600	600	1200
Blaze (nm)	300	300	750
Options	OFLV Detector	OFLV Detector, L2 lens	L2 lens

NOTE: Characteristics are set by the factory and cannot be changed by the user.

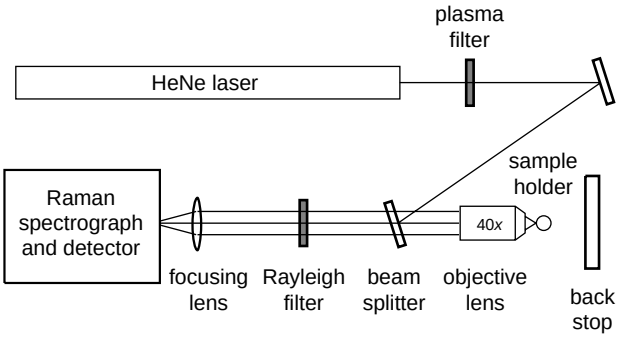


Figure 1. Layout of Raman instrument.

3.0×10^{-5} M to 6.0×10^{-6} M range. The unknown solution was made of 0.050 g of a ground aspirin tablet and diluted to 100 mL with THF.

Raman calibration standards were prepared by mixing KBr, a Raman inactive material, with SA. Standard concentrations ranged from 15 to 100% SA in KBr. All of the Raman samples were ground to a very fine powder and put into a standard melting point capillary tube. It is imperative to get as finely ground a homogeneous mixture as possible, since the laser spot size at the sample is approximately 10 μm.

Hazards

There are no hazards beyond the typical chemical hazards except to avoid shining light from the UV–vis source or the laser directly into the eye.

Instrumentation

All equipment for the absorbance and fluorescence instruments was purchased from OOI. For the Raman instrument, only the S2000 spectrometer was purchased from OOI, a breadboard was purchased from TMC, and the laser from Uniphase. The pertinent characteristics of the S2000 spectrometers for each experiment are given in Table 1. The S2000 spectrometer is composed of an entrance slit, a folded asymmetric Czerny Turner monochromator, and a linear CCD detector. Operational characteristics and noise dynamics of these spectrometers are described in detail elsewhere *(2)*. OFLV is a coating that is put on the detector by Ocean Optics to eliminate second- and third-order light. Ocean Optics software and information on the operation of the spectrometer are included with purchase, and are also available online at *http://www.oceanoptics.com* (accessed Sep 2003).

Absorbance measurements were carried out as follows. A pair of 200-μm diameter UV–vis optical fibers (OOI) attached a standard 1-cm quartz cuvette holder (OOI) to a DT-1000 Deuterium Tungsten Halogen light source (OOI) and to the S2000 spectrometer. The terminus of each of the optical fibers attaches to the cuvette holder with a 5-mm diameter *f*/2 collimating lens. Adjustment screws allow for the optimization of energy. For the absorbance spectrum, the integration time was adjusted such that the maximum intensity of the bright spectrum was about 75% near the 300-nm region of interest. This has the adverse effect of saturating some pixels at longer wavelength, leading to indivisible numbers and straight lines as the baseline. A diagram of this setup is available in the literature supplied with the spectrometer, and at the OOI Web site at *http://oceanoptics.com/technical/s2000.pdf* (accessed Sep 2003), p 92.

A DT-1000 Deuterium Tungsten Halogen light source was also used in the fluorescence measurements. The sample holder was mounted directly to the front of the light source. A single optical fiber mounted at 90° with a *f*/2 collimating lens connects the cuvette holder to the S2000 spectrometer. Excitation of SA systems is typically conducted at 308-nm *(10)*. However, a filter on this system will attenuate the source to a point that the fluorescence is no longer detectable. We have determined that acceptable data can be obtained without the filter in place. Mirrored screw plugs (retroreflectors) occupy sites directly across from the source and the optical fiber to improve signal. The fluorescence spectrometer, while operating in the same wavelength range as the absorbance spectrometer, utilizes a much larger aperture owing to a weaker signal (at a loss in resolution), and incorporates an L2 lens on the front of the CCD array to improve efficiency. A description of the accessory mounted onto the front of the light source is available at the same Web site as mentioned in the previous paragraph, on p 60 of the pdf file.

Raman measurements were conducted using a purpose-built spectrometer shown in Figure 1. A 35-mW HeNe laser (Uniphase) serves as the excitation source and is directed into a holographic notch filter (Kaiser Electro Optics), which serves as a beam splitter. Light reflected by the beam splitter is focused onto the sample using a 40×, 0.65 NA objective (Edmund Scientific). The Raman scattered radiation is collected 180° to the incident light using the same objective and is transmitted through the beam splitter and a second holographic notch filter. The beam splitter and the second holographic notch filter serve to reject the elastically (Rayleigh) scattered light. Light passing through the second holographic notch filter is then focused on the entrance slit of the S2000 spectrometer using a 15-mm diameter, 40-mm focal length plano-convex lens (Edmund Scientific). The entire setup is mounted on an optical breadboard (Technical Manufacturing Corporation) using micropositioners (Newport Corporation). A break down of the cost and important components necessary to build the spectrometer is provided in List 1. The open architecture of the instrument allows the students to see the vital components of the system and permits them to make adjustments for optimal performance. Typical data collection time is approximately 1 min (integration time of 5 s

List 1. Cost Breakdown of the Raman Spectrometer

Item	Cost/$
Breadboard	725
Uniphase HeNe 35-mW Laser	1700
Power supply	330
Spectrometer	1800
A/D card	490
Plasma line filter	92
Rayleigh filters (beam splitters)	1800
Mirrors	135
X–Y mount	133
Objective (NA 0.65)	91
Lens	20
Misc holders	343
Computer (Win 3.1)	900
Total cost	*8560*

and 16 co-adds) *(2)*. The Raman spectrometer has a theoretical resolution of 12 cm^{-1}. The system is capable of collecting both Stokes and anti-Stokes shifted wavelengths, thereby, providing a basis for several more physical chemistry experiments.

All calibration curves for SA are based on peak heights. Quantitative peaks used for absorbance, fluorescence, and Raman peaks were located at 315 nm, 450 nm, and 780 cm^{-1}, respectively.

Results and Discussion

The general procedure for the completion of the laboratory sequence is to have the students assemble the instruments and optimize them with a known sample, allowing four hours (one class period) per experiment with each instrument. During this procedure the students become aware of the optical geometry and requirement of each method. In addition to learning the important hardware characteristics for optimal performance, they learn the importance of signal-to-noise levels and how to optimize the experimental parameters related to data collection. In the written laboratory report, the students are asked to comment on the sensitivity, dynamic range, and detection limit for each method. The representative spectral data collected using each of the methods are shown in Figure 2. All the spectra are similar to those previously published or determined on research grade instruments. A SA spectrum in *n*-hexane run on a Hewlett Packard photodiode array instrument shows three peaks at 200 nm, 245 nm, and at 314 nm, each successively reduced in absorbance intensity. In our modular instrument, the DT-1000 source range is 250 nm to 800 nm, so the 200-nm peak is missing and the 245-nm peak is reduced in intensity. However, the 314-nm peak is still very useful for quantification. Our fluorescence spectrum does have more fine structure as shown by two peaks near the maximum fluorescence wavelength as compared to that previously published *(10)*. Our Raman spectrum shows all the dominant peaks, but some peak overlap at less than 600 cm^{-1} is noted. Calibration plots for each method and summaries of the relevant analytical values are shown in Figure 3 and Table 2, respectively. The data show

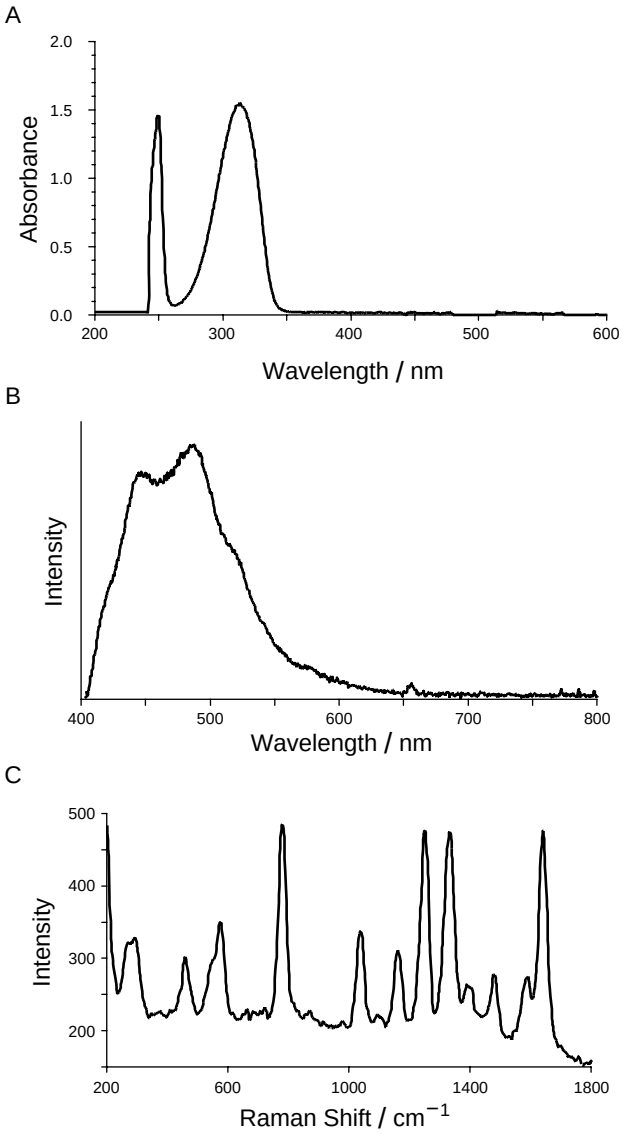


Figure 2. Sample spectra of salicylic acid: (A) absorbance spectrum of SA in hexanes, (B) fluorescence spectrum of SA in THF, and (C) Raman spectrum of pure salicylic acid.

Table 2. Experimental Data

Characteristic	Spectrometer		
	UV–vis	Fluorescence	Raman
Detection limit	7.55×10^{-5} mol/L	6.29×10^{-6} mol/L	15.62%
S/N ratio	12.99	30.12	21.97
Sensitivity	1594 L/(mol cm)	2.5×10^{5} (Counts L)/mol	3.024 Counts/%
Exp calc[a]	0.647	0.524	NA

[a]% grams of SA in aspirin (expired 1997). FDA approved standard for tolerance of SA in unbuffered aspirin is 0.10%

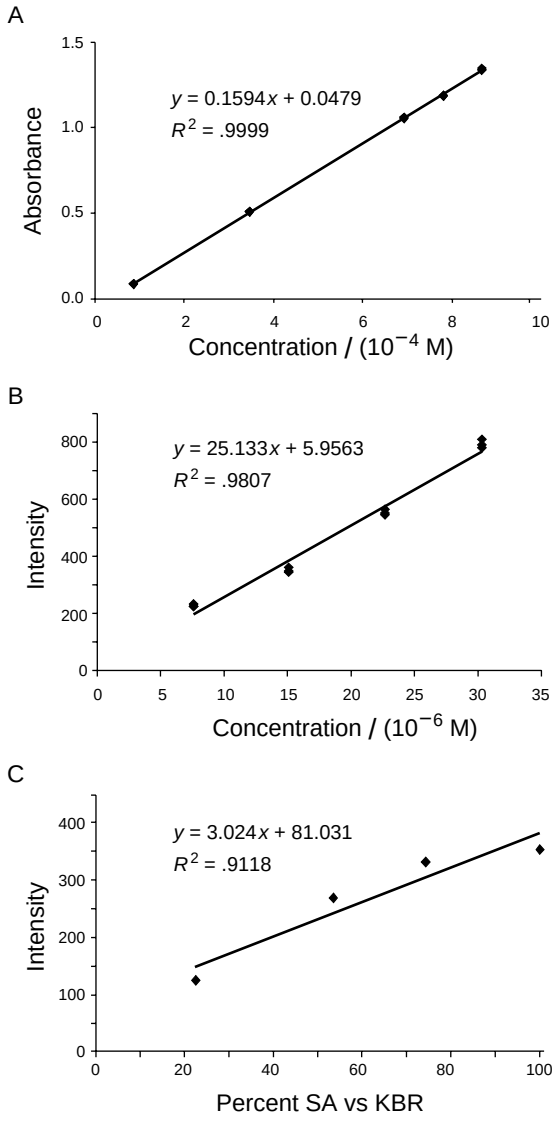


Figure 3. Calibration plots, average of 3 separate samples over concentration range: (A) absorbance spectrum of SA in hexanes, (B) fluorescence spectrum of SA in THF, and (C) Raman spectrum of pure salicylic acid.

the expected general trends: the limit of detection is optimal for fluorescence (μg/L), intermediate for UV–vis spectroscopy (10 μg/L), and poor for Raman spectroscopy (15% by weight). The dynamic ranges shown in Figure 3 for absorbance and Raman methods are reasonable and that for the fluorescence method agrees well with that of Schenks *(10),* which extended to 29×10^{-5} M.

In addition to these data interpretations, the students are asked to assign structural features to characteristic transitions observed in the spectra collected for each method. Although the limit of detection for the Raman method is high, Raman spectra of aspirin (ASA) and SA show extensive molecular information in direct contrast to the other methods. The Raman determination of ASA and SA in aspirin tablets has been reported previously *(11).* While the students can assign many functional groups to features in the Raman spectra, they find it difficult to assign more than one or two functional groups to the features observed in the fluorescence and UV–vis spectra. In addition, extensive sample preparation is required for fluorescence and UV–vis methods, whereas the Raman method allows the tablets to be examined directly with no sample preparation, The detection limit for SA is too high for it to show up in Raman spectroscopy. However, the quantitative determination of SA in old aspirin tablets was equally viable by either UV–vis or fluorescence spectroscopy (Table 2).

Conclusion

This suite of experiments clearly demonstrates a qualitative and quantitative comparison of three important analytical spectroscopy techniques, each based on a different principle of light measurement (absorbance, emission, and scattering). The students learn each method from a component standpoint and gain experience in instrument prototyping, the fundamentals of fiber optics, signal optimization, and the analytical capabilities of each method. In addition, although theory may be covered in a lecture course, Raman analysis is typically not performed in the laboratory because of the high instrument cost. Students have commented on the benefit of evaluating Raman spectroscopy. Finally, although the spectroscopic setups are not the ultimate research tools, they allow a wide variety of concepts to be investigated by the students at an economical cost to most chemistry departments.

Acknowledgments

We would like to acknowledge the Camille and Henry Dreyfus Foundation (Grant #SG-99-073) for the purchase of this equipment and Ocean Optics Inc. for an innovation in education spectroscopy grant (Grant #OOI-99-403). We would also like to acknowledge support from Miami University for the purchase of computer equipment.

[W]Supplemental Material

Instructions for the students, possible student answers to questions, and tips for success are available in this issue of *JCE Online*.

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