THE VISIBLE SPECTROSCOPY EXPERT WITNESS PROBLEM
INTRODUCTION

Description

This is a two-week lab in which students explore some of the basics of spectrophotometric analysis and then apply what they have learned to solve a forensic science problem. In week one, students investigate absorbance as a function of wavelength and as a function of concentration and use this information to identify the concentration of the solution. Students are then given a spectrophotometric method for determining salicylates in blood and are asked to determine the validity and reliability of this method. To do this, students must test a modified version of the procedure. The modification is mandated by the nature of the equation and solutions which are deliberately provided to the students. The modifications to be done are decided by the students, but include the size of the sample and how the sample is made.

Goals for This Experiment

The goals for this experiment are to have students:

1. practice calculations needed to make dilutions and perform those dilutions,
2. practice measuring volumes with pipets and/or burets (whichever they request),
3. report data to the proper number of significant figures,
4. experience measurements and techniques used in spectroscopic analysis,
5. evaluate and understand a standard procedure for the determination of salicylate in an unknown,
6. modify the standard procedure to run on the model of spectrometer made available to them, and
7. write a report that addresses the validity and reliability of the modified visible spectroscopy method for analyzing a blood sample for the amount of salicylate present.

Recommended Placement in the Curriculum

The Visible Spectroscopy Expert Witness Problem is best implemented after the students have performed at least one lab using the Spec 20 visible spectrometer or a similar spectrophotometer. Our students previously completed an investigation of visible spectroscopy that included obtaining three spectra, one spectrum each of the two colored solutions and a spectrum of a mixture of the two solutions. They also developed a Beer's Law plot for each solution and analyzed an unknown concentration of a mixture of the two solutions.

THE EXPERT WITNESS PROBLEM

SCENARIO

A case involving a drug poisoning/overdose has come up in your local county court. The toxicology evidence has been handled by the state crime laboratory. The prosecution claims the victim was poisoned by the suspect with curare and aspirin (aspirin was used to make it look like the victim overdosed instead of being poisoned). The crime laboratory determined the amount of salicylate in blood serum using visible spectroscopy. The prosecution knows you are studying chemistry at a nearby state college and that visible spectroscopy is part of the laboratory course of study. The prosecution has asked you to testify regarding the *validity and reliability of the method* used to analyze the blood serum sample for the amount of salicylate.

YOUR TASK

Understand the method used to determine a salicylate unknown well enough so that you will be prepared to testify, when asked, to its validity and reliability for identifying the concentration of aspirin in an aqueous solution. You will turn in a written report of your work.

PROCEDURE

You need to understand the basic procedures and principles of spectroscopic analysis. You will follow through the procedures of Part I, making sure that you understand *what you have done, why you have made the measurements as you did*, and *how the measurements can be used to determine the concentration of a solute in a solution.* You will work in pairs for this experiment.

I. Learning about Spectrophotometric Analysis

A spectrophotometer is used to make absorbance measurements. Light passes into a monochromator where only the desired wavelength, or a very narrow range of wavelengths, can pass through. From there, light passes through the sample and on to a phototube, where the light energy is converted to an electrical current that is registered on a meter. The instruments we will use are capable of quite precise measurements if used properly. Thus, it is essential that solutions are made carefully and the directions for making the measurements are followed precisely. Our instruments work best within the wavelength range of 375–600 nm. Solutions should be of a concentration that produce absorbance measurements within the range of 0.1–0.9. You need to be sure that you use the same instrument for all of your work. Directions for using the spectrophotometers appear in the front section of your Chemistry 144 written materials.

As you do the following procedures, keep careful written notes of what specific steps you took in carrying out these procedures. You will need to compare the way you actually carried out these steps with the crime lab's procedure.

A. Absorbance as a Function of Wavelength for a Colored Solution

- You will be assigned a solution of a specific ion of known concentration. Record these data.

- Fill one cuvette about 40% full with the sample solution. Fill a second cuvette with the same amount of distilled water to be used as a blank. (The blank needs to contain all substances *except* the one whose color you are studying.)

Note: Cuvettes look like regular test tubes, but they are not. The tubes have been precisely made to have a composition that is transparent to light of the visible range and a shape that is a constant 1 cm diameter, perfectly round. Cuvettes must be handled with care. Be sure to touch them only near the top, and to wipe off the outside with a piece of lintless paper every time before putting the cuvette into the instrument. To prevent scratching, cuvettes should be stored in wooden or plastic test tube racks when not in use Students who break cuvettes will be responsible for the cost of replacing them, so **be careful!**

- Prepare a spectrum for the given solution.
  1. Record the absorbance and percent transmittance for every 20 nm throughout the range 370–600 nm.
  2. Review your results. Repeat the scanning every 5 nm in the range of greatest absorbance.
  3. The optimum wavelength for use with this solution will be one near or at the maximum absorbance.

- Compare the colors and optimum wavelengths of the solutions used by you and your classmates.

B. Absorbance as a Function of Concentration for a Single Component Solution

1. Label 4 clean, dry containers that will hold at least 10 mL of solution.

2. Make the dilutions listed in the table below. Use pipettes to measure the stock solution and a buret to measure water. As each solution is made, stir it well with a clean, dry stirring rod to ensure thorough mixing.

DILUTED SOLUTIONS FOR STUDY

solution label	volume stock solution (mL)	volume water (mL)
A	2.0	3.0
B	1.0	4.0
C	1.0	9.0
D	5.0 mL of solution # C	5.0

3. Set the wavelength on the spectrophotometer to the wavelength that you have chosen as the optimum absorbance for your solution.

4. Using water as a blank, determine the absorbance of the four dilutions.

C. Identification of the Concentration of a Solution

Each student pair should obtain an unknown sample of the solution being studied. Measure the absorbance of your sample.

D. Data Manipulation and Analysis

1. Calculate the concentrations of the diluted sample.
2. Prepare a properly labeled graph with absorbance (y-axis) as a function of concentration (x-axis).
3. Plot the data for the stock solution and the four dilutions (*five* points total.)
4. Draw the "best straight line" through the data points. (*Your instructor can explain what this is, if you need help.)*
5. Use your graph and the absorbance value of your unknown solution to determine the concentration of your unknown sample.

II. Applying Spectroscopy to a Specific Problem

A. Reviewing the Procedure

Find another pair of classmates to work with and discuss the following ideas. Make sure you all understand the procedures you have just performed. If you have questions, ask your instructor.

- the purpose of measuring the absorbance of a solution at different wavelengths
- what makes a wavelength "optimum" for a particular analysis
- You made 5.0 and 10.0 mL of the diluted solutions you used. Consider reasons for making so much when the cuvettes only require approximately 2.0 mL.
- Because the instrument is useful over a specific range of absorbance, what changes in procedure would need to be made if the absorbance value was > 0.9? What if it was <0.1?
- how the graph was used to determine the concentration of the unknown
- how you could tell that your procedure was accurate and precise

B. Planning the Next Step

Once you and your partner believe you understand the basic principles and decision making processes of visible spectroscopy, look at the procedure given to you by the Crime Laboratory.

The laboratory procedure used by the crime laboratory is attached. In addition, an unknown which must be analyzed will be available in the laboratory. The data should be used as evidence to support the statements about the method that you make in your report. THE UNKNOWN IS NOT A REAL BLOOD SERUM SAMPLE. It is a simulated sample prepared for use in this laboratory.

Because you have been called to testify regarding a specific analytical method, it is important that the specific method be followed at least once. Crime laboratories are apparently better funded and stocked than educational laboratories because the directions call for preparing and mixing the solutions to make a standard curve directly in the cuvettes. Our laboratories aren't so generously stocked with cuvettes, so the solutions must be prepared in clean, dry containers and transferred to a cuvette at the time of use.

C. The Written Plan

Before you come to laboratory next week to do the work, you must fill out and submit the written plan of action for the experiment. This plan must show the modifications you plan make in the original procedure in order to be able to collect, successfully, the needed evidence for your testimony. This plan must be turned in to your instructor by the date and time specified.

D. Testing the Procedure

During the second week of the project, you and your partner will perform the analytical procedure according to your written plan. You will collect necessary data for your plan. Include concentration measurements of the unknown simulated blood sample.

Before leaving lab, be sure to get the actual concentration of salicylate present in your unknown sample so you can use it in your final report.

E. Final Report

You and your partner will submit a written report to answer the task. Include quantitative evidence from your laboratory work to support your opinions about the validity and reliability of the visible spectroscopic method for analyzing a blood sample for the amount of salicylate present. Where crime laboratory units are used in the analysis, convert them to standard units (such as moles per liter) to illustrate agreement with accepted chemical principles.

STUDENT REFERENCE SHEET

CRIME LABORATORY METHOD FOR SALICYLATE DETERMINATION

Theoretical Principle of Method

Visible spectroscopy is the measurement of the amount of light absorbed by a solution. The amount of light is proportional to the concentration of the compounds in the solution. Each compound has a select few wavelengths that it will absorb in preference to other wavelengths. Therefore, by carefully selecting the wavelengths, it is often possible to measure the amount of one compound in the presence others.

Salicylates react with iron (III) salts to produce a violet color, which is proportional to the concentration of the salicylate. A wavelength of 540 nm is preferable for the detection of salicylates by this method. Because of the simplicity of this salicylate procedure, the assumption of salicylate poisoning may be verified within minutes, especially as severe poisoning produces a very strong and easily visible violet color with the color reagent.

Analytical Procedure

1. *Place seven spectrophotometer cuvettes in a small test tube rack.*
2. *Pipet the amounts of material into six cuvettes as shown in Table 1, and shake to mix thoroughly.*
3. *Turn on the spectrophotometer and allow it warm up for 10 minutes. Set the wavelength to 540 nm.*

***Table 1** Preparation of Salicylic Acid Standards*

Cuvette #	*mL of 50 mg/dL Standard*	*mL of distilled H_20*	*mL of dilute Iron (III) nitrate*	*mL of 0.039 M HNO_3*
Blank 1	*0.0*	*1.0*	*0.0*	*1.0*
2	*0.1*	*0.9*	*1.0*	*0.0*
3	*0.3*	*0.7*	*1.0*	*0.0*
4	*0.5*	*0.5*	*1.0*	*0.0*
5	*0.7*	*0.3*	*1.0*	*0.0*
6	*1.0*	*0.0*	*1.0*	*0.0*

4. *Measure the absorbance of the known (concentration) salicylic acid standards. If a sample has an absorbance reading outside the optimum range for the instrument, remake the sample with a more dilute concentration.*

5. *Prepare the unknown sample by pipetting 1.0 mL of the unknown solution into the cuvette, and mix with 1.0 mL of dilute iron (III) nitrate solution. Measure the absorbance of the unknown sample. If the absorbance reading is outside the optimum range for the instrument, remake the solution, adjusting the relative amounts of sample and iron (III) nitrate as needed.*

6. *Calculate the salicylate concentration (in mg/dL) for each prepared standard solution.*

7. *Plot the absorbance of the standard solutions as a function of concentration of standard. Draw the best line through these points. Use the graph you constructed to determine the concentration of salicylate in the unknown sample.*

EXPERT WITNESS INVESTIGATION
Pre-Lab Assignment for Week 2

I. Group members' names:

II. Review of the Procedure

As a check of your understanding of this procedure, answer the following questions:

1. What is the independent variable?

2. What is the dependent variable?

3. What other experimental conditions must you be sure to keep invariant?

4. Why does one have to find an "optimum" wavelength for each compound used in a spectrophotometric analysis?

5. How do you decide what volume of each (the standard and the iron (III) nitrate) must be used in making the set of standards?

6. What are the most important observable characteristics of the crime lab method that will help you determine whether or not the method is *valid* and *reliable*?

III. Plan of action:

Write responses for the following questions:

1. What are the changes you believe are necessary in the Crime Lab procedure to make it work in your laboratory with the equipment you have?

2. What is your method of attack, if you should find the following conditions:
   a) the standards give too great an absorbance
   b) the standards give too small an absorbance
   c) the prepared unknown sample has an absorbance reading outside the range of the standards

YOU MUST TURN IN THIS PROCEDURE REVIEW AND PLAN OF ACTION TO YOUR INSTRUCTOR AT THE BEGINNING OF THE LABORATORY PERIOD TO GAIN PERMISSION TO WORK IN THE LABORATORY. Feel free to talk with your instructor before class meets about your plan.

THE VISIBLE SPECTROSCOPY EXPERT WITNESS PROBLEM
INSTRUCTOR NOTES

Time Required
The laboratory experiment takes approximately $2^1/_2$ hours of lab time and requires approximately 30 minutes for a pre-lab discussion. In addition, students spend approximately 30 minutes outside of class to design their laboratory procedure. Inform students the week before the experiment that modifications need to be made and that they need to come to lab with a written "plan of attack." Since they have had prior experience with a Spec 20, they have enough background in theory and skills needed to devise their initial method. (Revisions to the method mostly concern the sample size—the standard method calls for 2 mL of sample. A larger sample of at least 4 mL is needed when using the Spec 20.)

Group Size
Students work in teams of two or three to design their lab procedure and evaluate it. Each team then submits a single written report. Students receive a team grade.

Materials Needed
per team:
- 3 cuvettes
- test tube rack for the cuvettes
- buret
- one each of 1, 2, 3, and 5 mL pipets

available to the class (20 students):
- 1 L of 0.5 g/L salicylic acid: Weigh 0.5 g of salicylic acid, sodium salt and add to 550 mL of distilled water. Add 0.1 M NaOH (weigh 0.4 g of sodium hydroxide and dissolve in 100 mL of distilled water); stir in 5 mL increments until the salicylic acid dissolves completely. Bring up to volume with distilled water. Store the solution in a brown bottle and label as "50 mg/dL."
- 1 L of 0.33% (w/v) iron (III) nitrate hydrate: Weigh 3.3 g of $Fe(NO_3)_3 \cdot 9H_20$ and dissolve in 1 L of 0.07 M nitric acid (see below).
- 1 L of 0.07 M nitric acid (for the $Fe(N0_3)_3$ solution):
  - From concentrated acid: Slowly and carefully add 4 mL of concentrated (18 M) HNO_3 to 1000 mL of distilled water while stirring.
  - From 6 M acid: Slowly and carefully add 12 mL of 6 $MHNO_3$ to 1000 mL of distilled water while stirring.
- 200 mL of 0.07 M nitric acid (for student dilutions)

- unknown solutions (10 mL of an unknown per pair with a few extras in case students decide they want to do more than one unknown)
  Dilute the stock salicylic acid solution with distilled water as 9:1, 8:2, 7:3, etc., down to no less than 2:8. An example is shown below.

Unknown group	Dilution (Acid:water)	Directions
1	7:3	Add 3 mL of distilled water to 7 mL of salicylic acid for each unknown made.

Safety, Disposal, and Special Handling
Review the Material Safety Data Sheet (MSDS) of any chemical used in the experiment for information regarding safety and handling. Dispose of waste according to your local ordinances.

Points to Cover in Pre-Lab
- Look over each team's plan for lab if this has not been done previously.
- Remind students to record data and report results using the proper number of significant figures.
- You may want to ask students questions to get them to think about what they will do in lab. Examples of questions include:
  - ➢ What is the task for this scenario?
    To determine whether or not the method using visible spectroscopy is valid and reliable.
  - ➢ What are the variables that are important?
    The concentration of the salicylic acid (it is actually a complex) and what they will measure (the absorbance).
  - ➢ What will be the blank in this method? (Again, if students have had a previous lab regarding spectroscopy they will understand the function of a blank.)
    A cuvette filled with the dilute nitric acid.
- Point out the limitations of the visible spectroscopy instrument. To obtain accurate data on a Spec 20 instrument, the absorbance readings need to be between 0.03 and 1.0 absorbance units (this translates to a reading of between 10 and 90% transmittance).
- Explain to students that the cuvettes are special cells, not test tubes (even though they look like test tubes). The cuvettes are specially made to ensure that they all have a pathlength of 1 cm and that there are no optical distortions in the glass. The students need to be careful with the cuvettes so that they do not scratch them and should wipe them only with Kimwipes or other non-lint tissues. Explain that the cuvettes are not cleaned with a test tube brush, but simply pre-rinsed with whatever solution they will measure in the spectrometer.

- Show the students an example of the purple solution they will measure. Tell them to allow the complex 10 minutes to form (and the resulting purple color to develop), but once developed, the complex (and its resulting color) is stable for up to 30 minutes.
- Draw the structure of salicylic acid for the students and give them the molecular weight (138.1 g/mole).

Likely Play-Out of Lab

The students probably will not realize that the preparation table for the salicylic acid standards that they have been given yields sample sizes that are too small (the sample's total volume is only 2 mL). They should know from experience that at least 5 mL of sample are needed to rinse and fill the cuvette properly. Students will need to make corrections in their procedure before they begin their lab work if they have made this mistake.

You should note that the students' solutions need to be within a given concentration range as denoted by the color intensity of the solutions. The students' solutions should be approximately the same intensity of purple as the sample shown to them in the pre-lab. If the solution is lighter (e.g., too dilute), the sample will not absorb enough radiation to be detected. If the sample is too dark (e.g., too concentrated), the absorbance will fall outside the linear range of the instrument.

Encourage students to plot their calibration curve before they analyze their unknown. Students tend to fail to realize how careful they must be in making the dilutions for their standard solutions in order to obtain good data. Their success or failure to pipet well will be immediately apparent when they plot their calibration curve. If the plot is not linear, they will have to make new solutions and remeasure the absorbencies. Usually students are much more careful in making the dilutions on their second attempt.

Once a good calibration curve is obtained, students should attempt to analyze their unknown. Students may not realize that the unknown has to be diluted in order to fall within the range of their calibration curve. Once they obtain the absorbance of the unknown, ask them where the unknown absorbance falls on their calibration curve. If it falls outside the linear region, ask them how they can determine its concentration. Lead them to the realization that the unknown absorbance must fall on their "best-fit" line for their standards. You may want to ask questions such as:

➢ Approximately, what should the concentration of the unknown be to fall on the "best-fit" line?
The concentration of the unknown must fall within the range of their standards.

➢ Approximately what is the concentration of the unknown?
The concentration can be estimated from the calibration curve even though it is outside the range. The idea is to get the students to realize that the unknown is more concentrated than their standards.

➢ What can be done to the unknown to get its concentration to fall with the range of the standards?
Dilute the unknown.

Students may also forget to add the coloring agent. Once students calculate the concentration for their unknown, they should ask you for the actual concentration of the unknown. In their written report, they should address the validity and reliability of their visible spectroscopy method in determining the concentration of the salicylic acid unknown.