

Pigments and Dyes: Lab 1

This lab will consist of several parts. Different students will start on different parts and then do the other parts.

1. Preparation of pigments. These pigments will be used in another lab.
2. Analysis of Pro Chemical wool dyes. The supposition is that Pro Chemical uses several pure dyes to prepare all their other dyes, i.e., most of the dyes are mixtures. We want to find out which dyes are pure ones and which are mixtures. And for the mixtures, we want to know which pure dyes are present.
 - a. Visual spectroscopy using a light bulb light source, a spectroscope, and your eye (as detector).
 - b. UV-Visible instrumental spectroscopy
 - c. Paper Chromatography.

1. Preparation of Blue, White, and Yellow Pigments

IMPORTANT: Record all observations in your notebook in a neat table. This includes starting solutions, steps along the way, and the pigment at the end of the preparation. Later add the appearance of the fully dried pigment.

Blue pigment preparation:

1. 5 mL of BC solution - measure in graduated cylinder provided for BC solution and then pour into your beaker.
2. 5 mL of BA solution - measure in graduated cylinder provided for BA solution and then pour into your beaker which contains BC. Stir with a stirring rod.
3. Fold a filter paper circle into a cone and place it in a filter funnel. Pour and scrape your blue precipitate into the funnel and allow the excess liquid to drain into a beaker—a different beaker than the one the solid is in!
4. Place the filter paper with pigment on a watch glass that is labeled with your name. Allow the pigment to dry uncovered on the filter paper until next week.

Equipment: 1 30-50 mL beaker; 1 100-150 mL beaker, stirring rod, filter funnel and filter paper, rubber policeman, watch glass, labels

White pigment preparation:

1. Using the WA graduated cylinder, measure 20 mL of solution WA. Pour into a 100-mL beaker marked with your name.
2. Using the WC graduated cylinder, measure 20 mL of solution WC. Pour into the beaker and mix well.
3. Allow the precipitate to settle and decant the liquid into the drain. Allow the pigment to dry until next week.

Equipment: 1 100-150 mL beaker, labels

Yellow pigment preparation:

1. Measure 10 mL of the YA solution and pour into a beaker.
2. Measure 10 mL of the YC solution, pour into the same beaker, and stir.
3. Add about 3 mL of 6M NaOH to the beaker. Stir well. Note the colors and changes.
4. Filter the precipitate as with the blue pigment.
5. Allow the pigment to dry uncovered on the filter paper until next week. Use a watch glass as before, marked with your name.

Equipment: 1 30-50 mL beaker; 1 100-150 mL beaker, stirring rod, filter funnel and filter paper, rubber policeman, watch glass, labels

2. Analysis of Dyes using a spectroscope, a spectrophotometer (instrument), and paper chromatography

In this part of the laboratory you will analyze dyes using two common methods of analysis. UV-VIS (ultraviolet-visible) spectroscopy provides a plot of the intensity of the absorption of light by the dye as a function of wavelength. Paper chromatography is used to separate the individual components of a mixture. To help understand what the spectrophotometer does, you will also record your observations using a light bulb as the light source.

Use the same samples (pure dyes and dyes that are mixtures) in parts A1, A2, and B! You be assigned particular samples to study.

A. UV-VIS Spectroscopy

Molecules absorb electromagnetic radiation through the excitation of electrons from the ground state to an excited state. For some molecules the difference in energy between the ground and excited states corresponds to wavelengths of light which lie in the visible portion of the spectrum ($\Delta E=hc/\lambda$). When wavelengths of visible light are absorbed by the molecule they are not available to be transmitted to your eye. The color your eye sees is the color of the light (usually white light) minus the color of light absorbed. Due to the vibrations of the molecule, these absorption bands are not single wavelengths. The vibrational changes are also quantized but are very close in energy to each other as compared to the electronic levels (i.e., ground and excited states).

UV-VIS absorption involves measuring the amount of light that is absorbed as a function of wavelength. Our instrument shines UV or white light on the sample and measures the light that passes through the sample, i.e., some is absorbed and some is transmitted. By a mathematical analysis, the instrument determines and plots how much light is transmitted at each wavelength. A background sample, containing the solvent, is measured first. Then the colored sample is measured, and a correction is made for any absorption by the solvent.

The amount of light absorbed (A, absorbance) is directly related to how many molecules absorb the particular wavelength. This can be expressed as the concentration (c) of the solution (in moles/liter) times the pathlength (distance the light passes) of the cell (b). Typically cells with 1 cm pathlength are used. There is also a factor for the probability of a photon actually causing the excitation to occur. This term is the extinction coefficient (a or e). The equation obtained is referred to as Beer's law: $A=ebc$. If you added to your solution of dye an equal volume of water, how would the absorbance change?

Absorbances are additive. If dye 1 gave an absorbance of 0.5 for a 0.1 M solution and dye 2 gave an absorbance of 0.3 for a 0.1 M solution, then a 50-50 mixture of the two dyes should give an absorbance of $(0.5+0.3)/2=0.4$. The division by two comes from the dilution factor to each dye from the mixing. This should be observed with the dye mixtures.

IF the dyes are too concentrated, you will need to dilute them. Pipet, with assistance from the instructor or teaching assistant, 1 mL of the dye into a 25 mL volumetric flask, dilute to the mark with RO water, and mix thoroughly.

1. Spectroscope

Remember to use the same samples in parts A1, A2, and B! As demonstrated, raise and lower your sample in and out of the light (or, better, have a partner raise and lower the sample) and note which parts of the spectrum are being absorbed and which are being transmitted.

Chemists used both kinds of information, absorbance and transmittance. As noted above, absorbance has a simple mathematical definition, whereas transmittance is a logarithmic function. You could use whichever method makes most sense to you, but as I write this (January 3, 2007), transmittance makes more sense to me.

Place copies of the following chart in your notebook and record your observations for each sample. Use a

pencil to indicate those portions of the spectrum that are being transmitted (or those being absorbed). Colored pencils will be available if you choose to show the colors being transmitted. For transmittance, the unmarked regions will be those where the light is absorbed. For absorbance the unmarked regions will be those where light is transmitted. Record also what each sample is, and, if appropriate, the dilution, and what the color appearance is of the sample as a whole.

In your lab book you should record data in this format:

Sample _____ Dilution _____ Color _____

Violet	Blue	Green	Yellow	Orange	Red

400 450 500 550 600 650 700

2. Spectrophotometer HACH DR-2500

Remember to use the same samples in parts A1, A2, and B! Use the HACH DR-2500 spectrophotometer. Pour approximately 2 mL of a dye sample, pure or mixture, into a plastic cell. Obtain a UV-VIS absorption spectrum using the detailed instructions for the DR/2500 Spectrophotometer in the Wavelength Scan Mode, which are at the end of this experiment, page 5. Make a sketch of the spectrum in your notebook.

Repeat this process for the other dye samples you are working with.

The idea is to find out whether a given, pure dye is a component of the dye mixture. How will you do that? And if a pure dye is a component, can you estimate the percentage of the mixed dye that is made from that component?

[Enhancement] To examine the effects of pH on the color of the dye, pipet 1 mL of dye into each of two volumetric flasks. To one add 1 mL of concentrated acetic acid using a Brinkman dispensette (Warning corrosive) and dilute to the mark. To the other add 1 mL of 15 M ammonia using a Brinkman dispensette (Warning - add in the hood) and dilute to the mark. Run the UV-VIS spectrum visual-method spectrum of each.

3. Spectrophotometer HACH DR-2000

In this section you will observe how colors correspond to wavelengths and test the limits of your visual acuity. Make a table in your book to record the colors at the specified wavelengths; make another, small table to record the wavelengths of your cutoff point for red light and violet light. The detailed instructions for the DR/2000 Spectrophotometer are at the end of this experiment, page 6.

Write-up for part A. (in your lab book):

- Compare and contrast the visible and instrumental spectra obtained. Be sure to include a list of the peak positions (wavelengths). Are the wavelengths of light absorbed consistent with what your eye is perceiving? Explain.
- Which of the pure dyes that you examined are components of your dye mixture? Which are not?
- Sketch the emission and absorption spectra of a green solution. A red solution.

B. Paper Chromatography

Chromatography is a method used for the separation of the components of a mixture. In chromatography, each component of the mixture is partitioned between a stationary phase of high surface area and a mobile phase which flows through the stationary phase. For a pair of compounds, small differences in the fraction present in each phase can lead to good separations, for the partitioning is repeated thousands of times during the chromatography process. This difference is due to the relative affinity each component has for the stationary and mobile phases.

In paper chromatography, the stationary phase is a piece of filter paper.

You will start with two organic solvents and mixtures of these solvents for the mobile phase. The solvents will be assigned in lab. Two solvents that are sometimes used are methyl alcohol and acetone. Both of these solvents are polar, and they have similar dipole moments. Methyl alcohol most resembles water, because it has an OH group capable of forming hydrogen bonds. Acetone has lone pairs of electrons on oxygen, but it does not have a relatively positive hydrogen.

Paper Chromatography Procedure

For each sample:

- Obtain a piece of chromatography paper that is a little shorter than the test tube you will be using.
- Place a small amount of the solvent (or a mixture of solvents) in the test tube.
- Using a pencil, draw a line about 0.5 to 1.0 cm from the bottom. This line needs to be higher than the amount of solvent in the test tube!
- Apply a small dot of the dye/dye mixture on the pencil line using a capillary tube or a dropper. (N.B.! If the dye is not concentrated enough, place 50 mL of sample into a beaker and boil down to about 5 mL.
- Place the paper into the test tube; the bottom edge should be in the solvent. The top edge should be held in place by the rubber stopper that you now insert.
- The solvent should ascend, carrying the dye component(s) with it.
- When the solvent is near the top of the test tube, remove the stopper and the chromatography paper.
- With your pencil, mark the solvent front and the location of each component.
- Determine the R_F value for each component by measuring the distance from the starting line to the final component marking.

Evaluate the separation and select another solvent or solvent mixture to be tried. To make solvent mixtures, measure the amounts of each solvent using graduated cylinders. Each team should try the separation with each of the pure solvents and then at least two solvent mixtures.

To quantitatively describe a separation, an R_F value should be calculated for each component in a given mixture dye or dye mixture. The R_F value for a component in a separation is the ratio of the distance traveled by the component to the distance traveled by the solvent or mobile phase.

$$R_F = \frac{\text{distance}_{\text{component}}}{\text{distance}_{\text{solvent}}}$$

The distance traveled by each component is measured from the position where the sample is applied to its position in the developed chromatogram. The distance traveled by the solvent is measured from the position the sample is applied to the solvent front. For good separations, R_F values between 0.2 and 0.8 are desirable, with D R_F of 0.1 between components. Be sure to determine the R_F value for each component!

When other groups are using the same dye(s) but different solvents, be sure to get the data and observations from the other group(s).

Write-up for part B. (in your lab book):

- The dried paper chromatography strips.
- What are the conditions required for optimal separation of the dye/dye mixture?
- Is the order of elution always the same for the various components in the dye/dye mixture?
- Why were the particular solvent mixtures chosen? What were you trying to accomplish from each, and was it successful?
- What recipe would you use to produce the color of the commercially mixed dye by mixing the various pure dyes together, i.e., how much of each pure dye would you use to produce the same color as the mixture?

[Enhancement] Record and comment on the visual and instrumental absorption spectra of some or all of: prescription glasses, sun glasses, "glassblowers" didymium glasses, some of the filters we used in class,

supplies required for UV-VIS	supplies required for Chromatography(per team)
UV-VIS spectrophotometer	Strips of chromatography paper
UV-VIS sample cells (plastic)	4-8 test tubes with rack
Glacial acetic acid (small bottle 100 mL)	Capillary "tubes" for spotting the plate (pull pasteur pipets)
Ammonia (small bottle 100 mL)	Pencil
Per student (or team):	Plastic ruler
4 25 mL volumetric flasks	Assorted solvents-acetone, methanol, hexane, water....
3 1 mL pipets (2 may be communal, one for acid one for base)	

In your write-up, please also answer these questions:

- Comment on what you think would be the best order for the different parts of this experiment. We had different groups starting at different places so we could make best use of the equipment. But would you suggest that everybody do first, second, and third?
- Why do you suggest the order that you do?
- Which method gave you the most (useful) information about the components of your mixture?
- How well did the different methods agree with (complement) each other?

Procedure for using the DR/2500 Spectrophotometer in the Wavelength Scan Mode (Part A2):

You will use the DR/2500 in Wavelength Scan Mode for this part of the experiment. The Wavelength Scan Mode shows how light is absorbed by a solution over a range of wavelengths. The operation can display the collected data in graph form in units of absorbance.

Procedure

- From Main Menu, touch Wavelength Scan.
- Touch Options and Select
 - Wavelength Range: 380 – 750
 - Scale & Units: Auto Abs (0.000 – 2.000)
 - Cursor Mode: Track
- Use the blank and colored sample from the previous part of the experiment.
- Place the blank in the cell holder. Touch **Zero**. Wait for the instrument to perform the base line scan (be patient).
- Remove the blank from the cell compartment. Place the sample in the cell holder. Touch **Read**.
- Wait for the instrument to perform the scan. When the reading is done, the instrument will beep three times.
- You should sketch the resulting scan in your notebook.

Procedure for using the DR/2000 Spectrophotometer (Part A3):

For this part of the lab, you will use a Hach DR/2000 spectrophotometer. Two of these instruments will be located in the side room off of the lab. Since all groups will be using these two instruments, you will take a turn doing this part, as the instruments are available.

- Turn on the instrument by pressing the power key.
- The instrument will go through a self test and then indicate Method # ? Press number 0 and then the Enter key. If at any time the instrument stops producing light, you should repeat this step..
- Rotate the knob on the right of the instrument to change the wavelength to 410 nm.
- Place the white covered end of a popsicle stick down into the cell holder and slant it to the left. You will use this to observe the colors of different wavelengths of light.
- You will need to turn off the lights and shut the door to the room. Dark conditions are needed for you to see the color of light shining on the popsicle stick. A small flashlight is available for you to use.
- With the light turned off, look down into the cell holder. You may see the color violet. Now rotate the knob away from you and observe how the color changes as the wavelength increases. Allow your partner to do the same.
- Now with the aid of the flashlight, adjust the wavelength to 410 nm and record the color, if any, that you see that corresponds with that wavelength. Proceed to increase the wavelength in 40 nm units, recording the color for each wavelength until you reach 650 nm.
- Experiment to see what your visual limits are, move the wavelength to shorter and shorter wavelengths until the violet color disappears. Go back and forth across the 'boundary' (color to no color, no color back to color) to reproducibly pinpoint the wavelength value. Record the wavelength at which you last saw violet. Now, move the wavelength to longer and longer wavelengths until the red color disappears. Record the wavelength at which you last saw red. Compare your level of visual acuity to those of your lab mates.