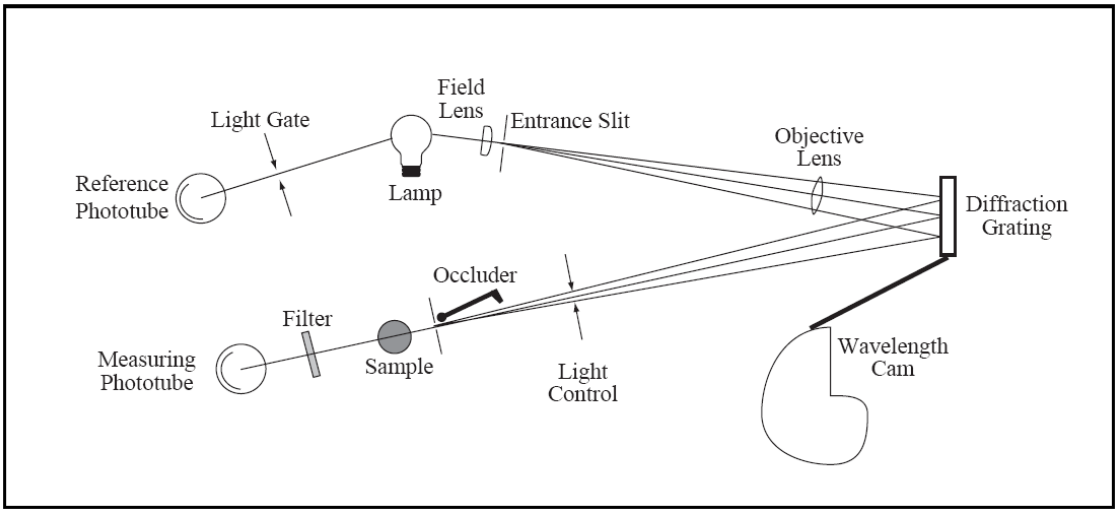
The Spectrophotometer

INTRODUCTION

Spectrophotometers are instruments that send electromagnetic radiation into a target and measure the resulting interaction of the energy and the target. You will use a UV/VIS spectrophotometer (one that operates in the ultraviolet and visible regions of the electromagnetic spectrum) to determine the absorption spectra of three substances in the 340 –600 nanometer wavelength region of the spectrum. You will graph the absorption versus wavelength data, and compare the appearance of the substance to the observed absorption pattern. The Spec-20 is a simple UV/VIS spectrophotometer. An image of the machine is shown on the last pages of this experiment. Here is a diagram of its light path:



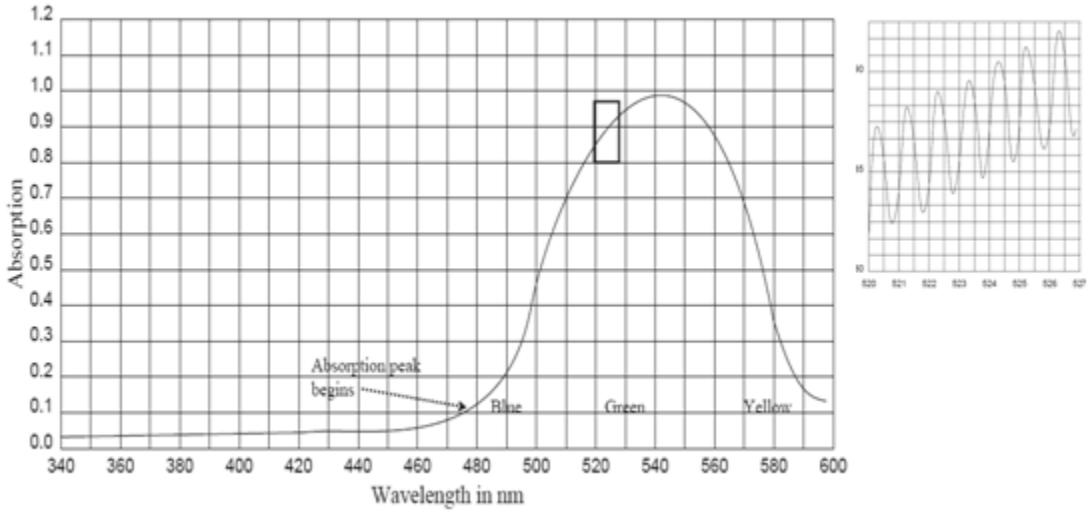
Notice the lamp and the photo tube to its left in the diagram. When the machine is turned on and the sample holder is empty, the light output of the bulb is measured by the reference photo tube and displayed on the meter. The first step in using the machine is to "zero" the light output. Zeroing the light output is a means of maintaining equal operating conditions on a day-to-day basis. Follow the light path from the bulb to the right and notice the grating attached to the wavelength cam. This is a reflecting diffraction grating, which resolves incident light into its spectrum. As the cam is moved, the grating moves, and the direction of the reflected spectrum of colors shifts. The wavelength of the part of the spectrum that gets through to the sample is indicated on the dial on top of the machine. These machines are set up to operate in the 340–600 nm range. Above 600 nm, a special filter is required. The “light control” shown in the diagram consists of a slotted piece of metal which can be moved back and forth in the light path. The bulb produces varying intensities at different wavelengths. The light control decreases high intensities by blocking some of the light with the slotted metal. Inserting the sample mechanically pushes a lever that lifts the occluder noted on the diagram. The occluder is a piece of metal that either blocks or is lifted out of the way of the light beam, depending on a sample being present. When the sample is present, the light beam reaches the measuring photo tube, and the intensity is displayed on the meter.

The absorption spectrum of a substance is commonly observed while the substance is dissolved in a solvent. Since the solvent carrying the substance might absorb energy at the wavelengths used, the machine is first “zeroed” for the solvent. This is accomplished by placing a sample of the pure solvent in the machine and adjusting the absorbance to zero. The solvent is then removed and the dilute solution put into the machine. Any reading on the absorption dial

must be due to the solute, since the solvent has been "zeroed out".

Absorption Spectra

Electron excitation from the lower to higher energy states is the cause of the absorption of energy when dealing with the portion of the electromagnetic radiation used in these machines. Since we are using ions in solution, not isolated atoms, the absorption observed is a broad band pattern rather than a simple line spectrum. In the sample absorption spectrum shown below, you see a broad peak over 100 nm wide in the blue to yellow portions of the visible portion of the spectrum. The reason for this is that the atoms in the hydrated ions vibrate back and forth with respect to each other, and in each vibrational position, there is a complete set of electronic energy levels. The energy levels for the electrons continually change as the internuclear distances in the molecule change. Under very high resolution, the broad bands begin to show some details of the shifting energy levels. If the small section of the graph below shown with the rectangle at the 520 nanometers portion of the spectrum were examined with a high resolution (and high cost) spectrophotometer, the result would look like this:



Note that the range of wavelengths on the x-axis goes from 520 to 527 nm, a 20-fold amplification over the graph below. The absorbances are amplified as well. Under still greater resolution, there would be peaks within the peaks that would ultimately resolve into a series of lines, each corresponding to an energy absorption of an electron from one energy state in a particular vibrational mode of the molecule to another energy state within that same vibrational mode.

The Beer-Lambert Law

This law relates the absorbance, i.e., the "amount" of light absorbed by a solution, to the concentration of the substance responsible for producing the color in the solution. For example, a darkly colored solution has a stronger intensity than a lightly colored substance in the visible light region. The relationship between absorbance, A, and concentration, C, follows the Beer-Lambert Law, as given below:

$$A_\lambda = E_m.C.L$$

Where "L" is the path length through the cuvette holding the solution, and E_m is the *molar extinction coefficient*, a constant determined from a standard of known concentration. The value of E_m will vary with the wavelength, reflecting the change in absorbance. Typically, the

path length is given in units of centimeters, and many cuvettes are made to be exactly 1.00 cm wide. In this experiment you will determine the molar extinction coefficient and visible spectrum for three ionic solutions by measuring their absorbance corresponding to the wavelength range of 340 to 600 nm.

EXPERIMENTAL PROCEDURE

Instructions for operating the Genesys-20 spectrophotometer are shown on the last page of this experiment.

1) Turn on the spectrophotometer.
2) Fill each cuvette about two-third the height with one of the following:
   H_2O (deionized)
   $KMnO_4$ solution (0.0050 M)
   K_2CrO_4 solution (0.0050 M)
   $CoCl_2$ solution (0.18 M)
3) Gently tap each cuvette to remove bubbles. Wipe the outside of each cuvette with a KimWipe™ to clean off fingerprints and other light-path obstructions. The colors of the solutions are due to the MnO_4^-(aq), CrO_4^{2-}(aq), and Co^{2+}(aq) ions.
4) Set the spectrophotometer to the tested wavelength by pressing the nm ▲ or nm ▼ icon. Check the left side on the display to see if the correct wavelength is set.
5) Insert the H_2O cuvette into the sample holder. Close the lid.
6) Gently press on the key "0 ABS/100 T" to set the absorbance to zero. You should see 0.000 A at the center of the display.
7) Remove the H_2O cuvette and insert the cuvette containing tested solutions. Do NOT adjust any controls during these measurements. Read and record the absorbance value at the center of the display.

Notes: - Cuvettes have optical consistency, so that the amount of light transmitted through them is uniform from one to another, as long as the mark on the top of the cuvette is in line with the raised line on the cuvette holder. *Line up the etched line or letter on the cuvettes with the raised line on the sample holder each time you insert a cuvette.*

- Directions number 4 to 7 are repeated throughout the experiment each time the wavelength is changed. Follow them exactly.

8) Turn off the spectrophotometer.
9) Pour the solutions from the cuvettes into the waste container in the fume hood. Rinse and drain the cuvettes. Do NOT use brushes on cuvettes.

The Photospectrometer

Name _____ Date _____

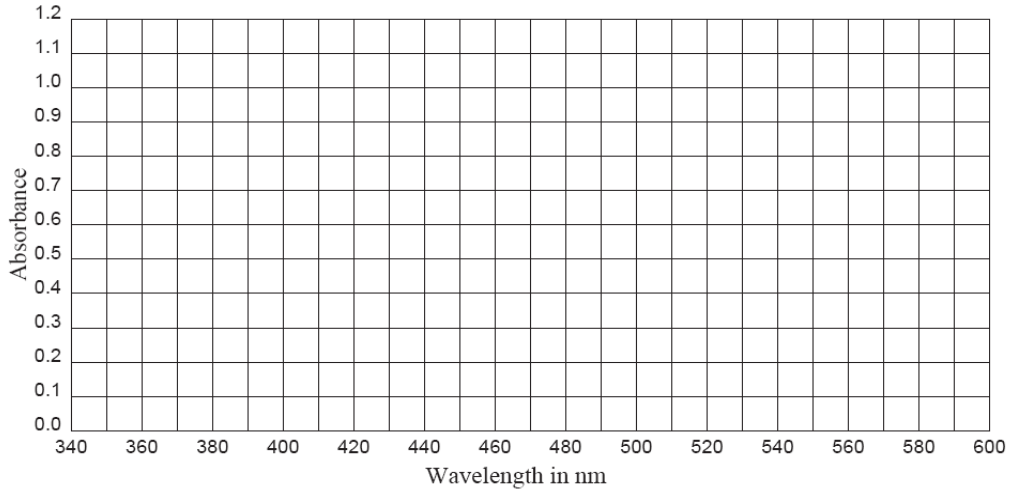
Partner's Name _____

DATA

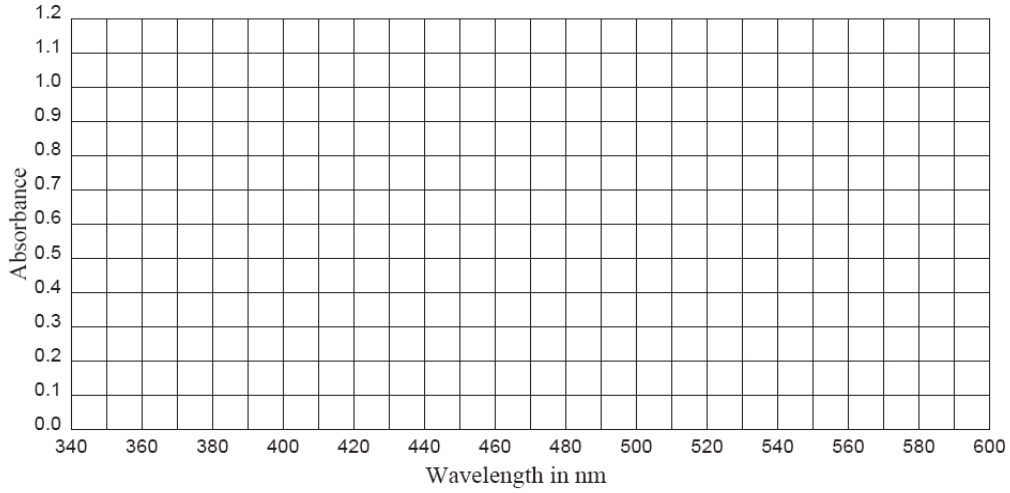
	Absorption		
Wavelength (nm)	**Co^{2+} (aq)**	**CrO_4^{2-} (aq)**	***MnO_4^- (aq)***
340			
360			
380			
400			
420			
440			
460			
480			
500			
520			
540			
560			
580			
600			

Name_____ Grade_____ Date _____

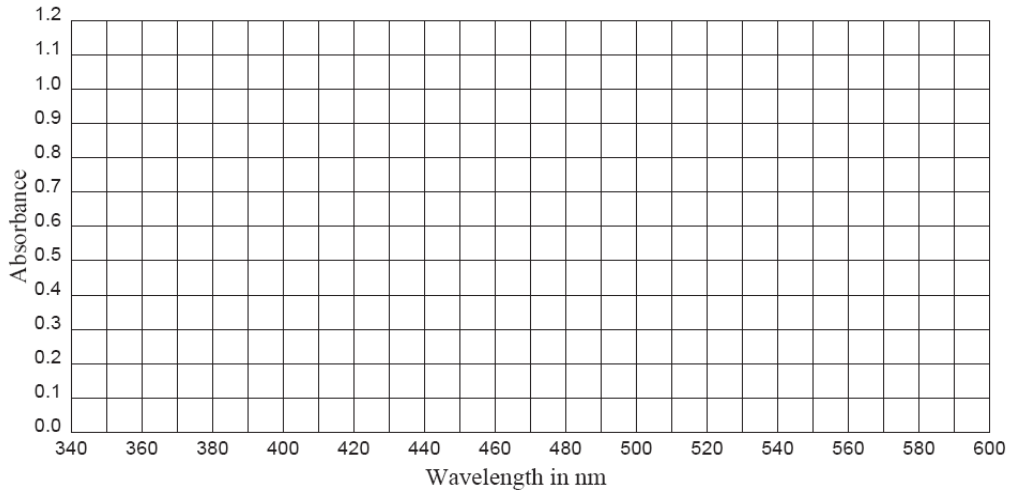
Co^{2+}



CrO_4^{2-}



MnO_4^-



Name _____ Date _____ Grade_____

Post-Lab Questions and Exercises

(All questions must be answered during the lab and submitted with your lab report at the end of the lab period).

Please answer the following questions and show all work and units. Express all answers to the correct number of significant digits.

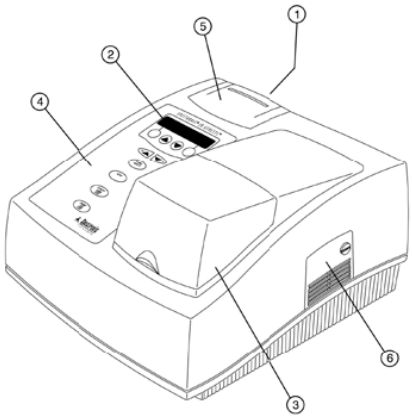
Answer the question after constructing graphs and noting the colors of the absorption bands on them.

1) Compare how the solutions look to your eyes versus the colors you have noted in the absorption band for each substance.

2) Does each solution look like the colors in the absorption band or does it look like some other color?

3) If the solution looks like some other color, how is that color related to the spectrum and to the absorption band? Explain in detail for each ion.

4) Using the Beer-Lambert Law, calculate the E_m value for each solution at its maximum absorbance.

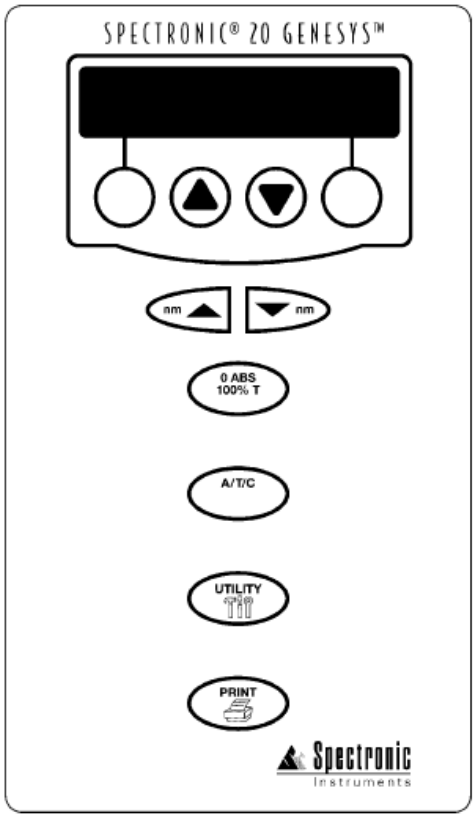


GENESYS™ 20 Spectrophotometer

1. On / Off switch
2. LCD display
3. Sample compartment hood
4. Keyboard
5. ----
6. Lamp compartment door

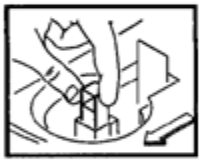
The power switch is located on the bottom left in the back of the instrument. When you turn on your GENESYS^{TM} 20 spectrophotometer, it performs its power-on sequence. This sequence includes checking the software revision, initializing the filter wheel and the monochromator. The power-up sequence takes about two minutes to complete. Allow the instrument to warm up for 10 minutes before using it.

Keyboard Layout of Genesys^{TM} 20 Spectrophotometer



Absorbance and %Transmittance Measurements

Be sure the cell holder is empty before turning on the instrument.

1. Select wavelength using Wavelength Control	
2. 	Press **A/T/C** to select the absorbance or % transmittance mode. The current mode appears on the display
3. 	Press **nm ▲** or **nm ▼** to select the wavelength. **Note:** Holding either key will cause the wavelength to change more quickly.
4. 	Insert your blank into the cell holder and close the sample door. **NOTE:** Position the cell so the light (indicated by the arrow in drawing) passes through the clear walls.
5. 	Press **0 ABS/100%T** to set the blank to 0 A or 100%T.
6. 	Remove your blank and insert your sample into the cell holder. The sample measurement appears on the LCD display.