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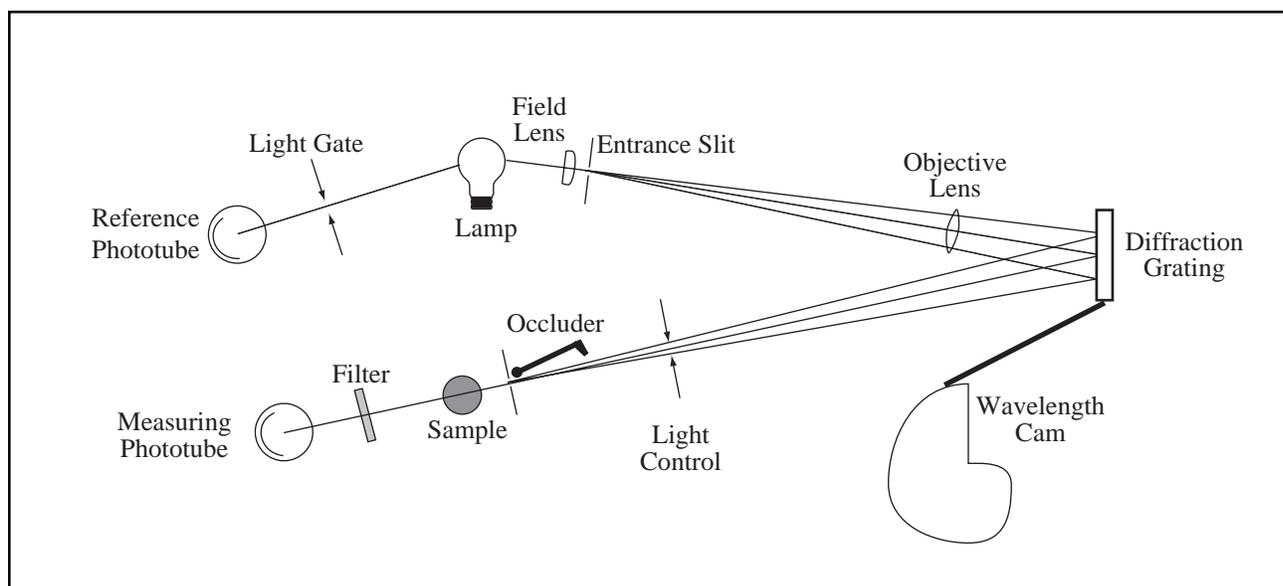
# The Spectrophotometer

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## 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 measure 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, low cost UV/VIS spectrophotometer. An image of the machine is shown on page 54. 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 set the needle to the left zero using the left knob. The needle may drift for 15 minutes or so, and minor adjustments will need to be made during this time. This is called "zeroing" 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 to the right. 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. The right front knob controls this function. To use this knob, a sample must be in the sample holder. 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.

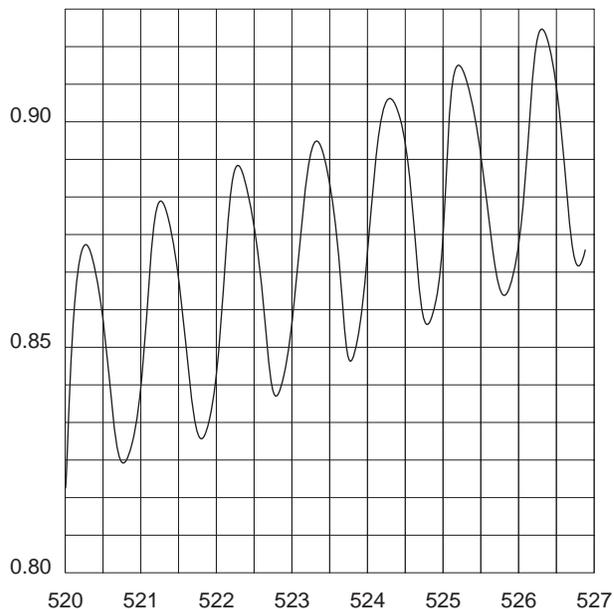
The absorption spectrum of a substance is commonly observed while the substance is dissolved in a solvent. Since the solvent carrying the substance might absorb energy at the wavelengths used, the machine is first “zeroed” for the solvent. This is accomplished by placing a sample of the pure solvent in the machine and adjusting the absorbance control knob 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.

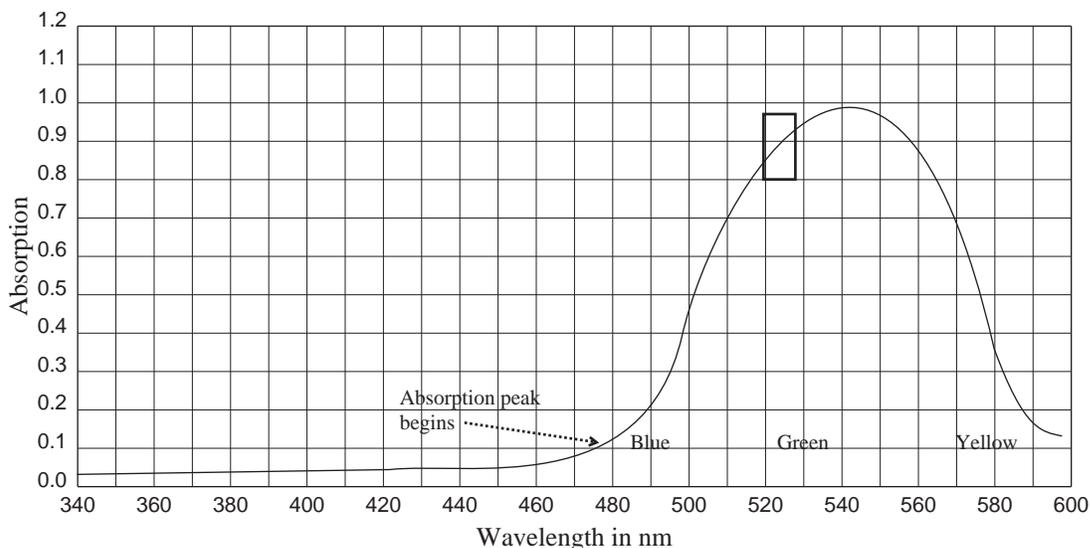
Electron excitation from the lower to higher energy states is the cause of the absorption of energy when dealing with the portion of the electromagnetic radiation used in these machines. Since we are using ions in solution, not isolated atoms, the absorption observed is a broad band pattern rather than a simple line spectrum. In the sample absorption spectrum shown below, you see a broad peak over 200 nm wide in the blue to yellow portions of the visible 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 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.



## EQUIPMENT

Special supplies:

- 4 cuvetts

From your drawer:

- 150 ml beaker to hold cuvetts

## EXPERIMENT

See the diagram of the instrument and a brief summary of operating procedures on page 54. Work in groups of 2 or 3. **Steps 1 and 6 are very important.**

### Spec 20 setup:

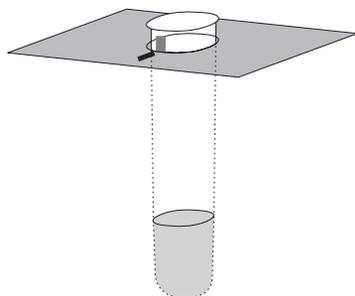
1. Turn the machine on as you enter the lab. Use the left front knob. Make sure the sample holder is empty and the lid is closed. It will take 15 minutes for the machine to warm up. After it warms up, do the following:

**Analog Spec 20:** Use the **left** front knob to set the pointer to the **left** zero on the dial.

**Digital Spec 20:** Make sure the lever on the front left of the machine is turned to the left. Turn the **left** front knob until 0 is displayed. Press the mode button to display A

*Once the machine is warmed up and the needle is set to the left zero, do not touch the left front dial.*

2. Fill each cuvet about 3 cm deep with one of the following:  
H<sub>2</sub>O (deionized)  
KMnO<sub>4</sub> solution  
K<sub>2</sub>CrO<sub>4</sub> solution  
CoCl<sub>2</sub> solution
3. Gently tap each cuvet to remove bubbles. Wipe the outside of each cuvet with a KimWipe™ to clean off fingerprints and other light-path obstructions. The colors of the solutions are due to the MnO<sub>4</sub><sup>-</sup>(aq), CrO<sub>4</sub><sup>2-</sup>(aq), and Co<sup>2+</sup>(aq) ions.
4. Set the wavelength control dial, which is the upper right dial on the Spec 20, to 340 nm.
5. Insert the H<sub>2</sub>O cuvet into the sample holder as shown:



Cuvets 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 cuvet is in line with the raised line on the cuvet holder. *Line up the etched line or letter on the cuvetts with the raised line on the sample holder each time you insert a cuvet.*

**Directions number 6, 7, and 8 are repeated throughout the experiment each time the wavelength is changed. Follow them exactly.**

6. **Analog Spec 20:** Adjust the **right** hand knob to bring the needle to the right hand zero on the dial.  
**Digital Spec 20:** Turn the **right** front knob until 0A shows on the display. This may take several full turns.
7. Remove the water cuvet and insert each of the other cuvetts, reading and recording the absorption value for each sample. Do not adjust any controls during these readings.  
Record the absorbance on the data sheet.  
(Note for **Analog Spec 20:** Make sure you read the absorption, not the transmission side of the dial. The number of significant digits that you can read depends on the portion of the scale you are reading. It will be two or three digits.)
8. Now adjust the wavelength to 360 nm. Insert the water cuvet. Again adjust the front right hand knob to bring absorbance to zero. Remove the water cuvet and insert each of the other cuvetts as before, reading and recording the absorption values. Repeat this procedure for each of the wavelengths listed on the data sheet.
9. Pour the liquid from the cuvetts into the waste container in the fume hood. Rinse and drain the cuvetts. Do not use brushes on the cuvetts. Turn off the Spec 20.

## GRAPHING THE DATA

Graph the absorption versus the wavelength for each substance using the grids provided on page 53. Wavelengths where the absorption values rise above and then fall back to zero are of interest. Each region where this occurs is called a peak. Since the span of wavelengths used in this experiment is quite narrow, you will only see one peak or part of a peak for each substance. Use the spectral charts in the lab to label peaks with the names of the colors absorbed as shown in the sample graph on the page 50.

### DATA

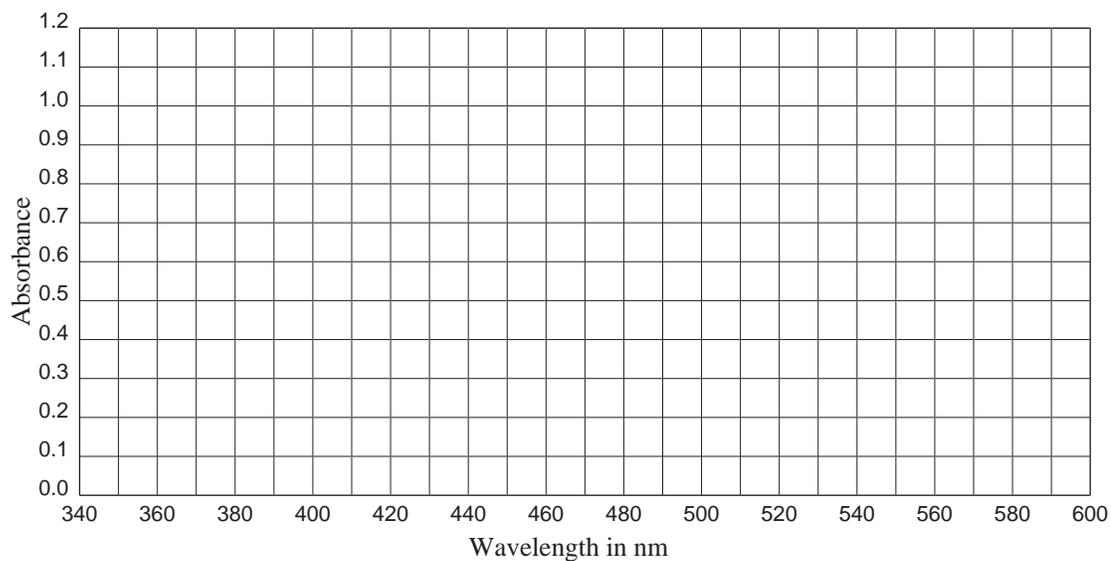
Wavelength/nm	Absorption		
	$\text{Co}^{2+}(\text{aq})$	$\text{CrO}_4^{2-}(\text{aq})$	$\text{MnO}_4^{-}(\text{aq})$
340			
360			
380			
400			
420			
440			
460			
480			
500			
520			
540			
560			
580			
600			

### QUESTION

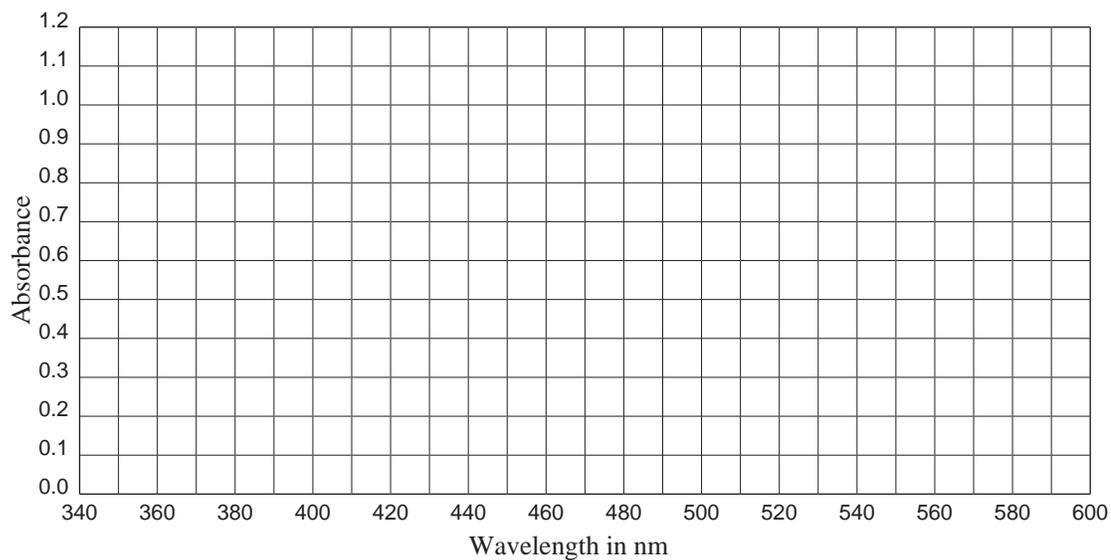
Answer the question after constructing graphs and noting the colors of the absorption bands on them.

Compare how the solutions look to your eyes versus the colors you have noted in the absorption band for each substance. Does each solution look like the colors in the absorption band or does it look like some other color? If some other color, how is that color related to the spectrum and to the absorption band? Explain in detail for each ion. Your textbook has a diagram of an artist's color wheel on page 1026 that will help you to visualize this.

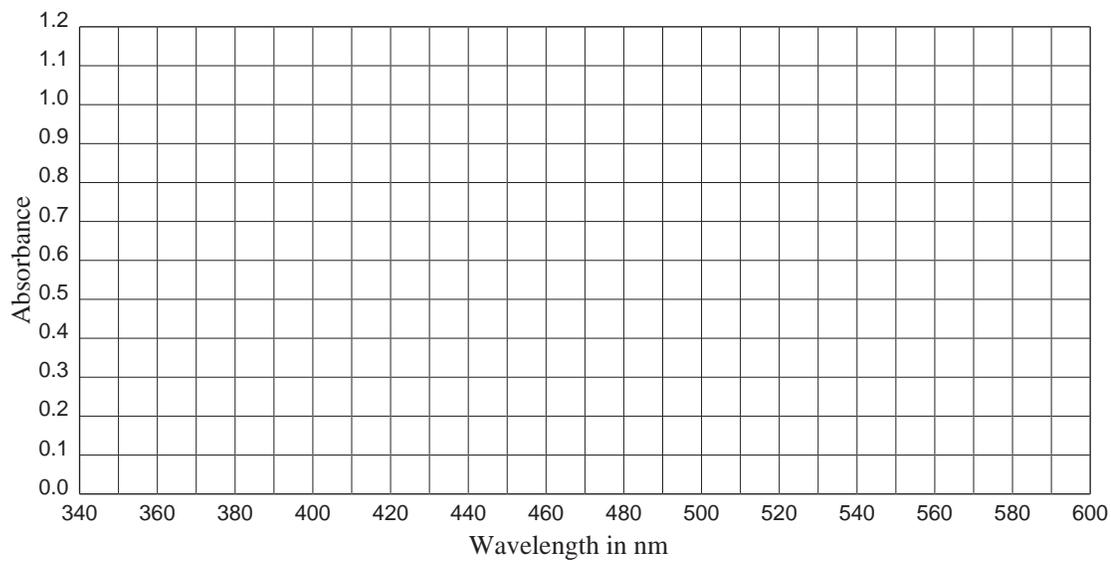
$\text{Co}^{2+}$



$\text{CrO}_4^{2-}$



$\text{MnO}_4^-$





**SPECTRONIC 20 Spectrophotometer**

### **SAMPLE MEASUREMENT**

The sequence for sample measurement is:

- a. Select wavelength using Wavelength Control.
- b. With sample compartment empty and cover closed, adjust Zero Control so that the meter reads zero (on the left side of the dial).
- c. Insert reference blank into the sample compartment and set Absorbance Control to zero (on the right side of the dial).
- d. Insert unknown sample into the sample compartment and read measurement from meter in percent transmittance or absorbance.

For newer digital models, follow instructions on the right side of the spectrophotometer.