# SPECTROPHOTOMETRY

## Absorption Measurements

## & their

## Application to Quantitative Analysis

A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge of chemical substances comes from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample.

Suppose you look at two solutions of the same substance, one a deeper color than the other. Your common sense tells you that the darker colored one is the more concentrated. In other words, as the color of the solution deepens,you infer that its concentration also increases. This is an underlying principle of spectrophotometry: the intensity of color is a measure of the amount of a material in solution.

A second principle of spectrophotometry is that every substance absorbs or transmits certain wavelengths of radiant energy but not other wavelengths. For example, chlorophyll always absorbs red and violet light, while it transmits yellow, green, and blue wavelengths. The transmitted and reflected wavelengths appear green—the color your eye "sees." The light energy absorbed or transmitted must match exactly the energy required to cause an electronic transition (a movement of an electron from one quantum level to another) in the substance under consideration. Only certain wavelength photons satisfy this energy condition. Thus, the absorption or transmission of specific wavelengths is characteristic for a substance, and a spectral analysis serves as a "fingerprint" of the compound.

In recent years spectrophotometric methods have become the most frequently used and important methods of quantitative analysis. They are applicable to many industrial and clinical problems involving the quantitative determination of compounds that are colored or that react to form a colored product.

### LIGHT AND THE PERCEPTION OF COLOR

Light is a form of electromagnetic radiation. When it falls on a substance, three things can happen:

- the light can be reflected by the substance
- it can be absorbed by the substance
- certain wavelengths can be absorbed and the remainder transmitted or reflected

Since reflection of light is of minimal interest in spectrophotometry,we will ignore it and turn to the absorbance and transmittance of light.

The color we see in a sample of solution is due to the selective absorption

of certain wavelengths of visible light and transmittance of the remaining wavelengths. If a sample absorbs all wavelengths in the visible region of the spectrum, it will appear black; if it absorbs none of them, it will appear white or colorless. We see the various colors when particular wavelengths of radiant energy strike our eyes. For example, the wavelength we perceive as green is 0.0000195 inches or, expressed more scientifically, 495 nanometers.

Suppose we shine a beam of white light at a substance that absorbs blue light. Since the blue component of the white light gets absorbed by the substance, the light that is transmitted is mostly yellow, the complementary color of blue. This yellow light reaches our eyes, and we "see" the substance as a yellow colored substance. The table below gives pairs of complementary colors and the corresponding wavelength ranges.

| *Wavelength (nm)* | *Color Absorbed* | *Color Observed* |
|---|---|---|
| 400 | violet | yellow-green |
| 435 | blue | yellow |
| 495 | green | purple |
| 560 | yellow | blue |
| 650 | orange | greenish blue |
| 800 | red | bluish green |

You should remember, of course, that the visible range is only a very small part of the electromagnetic spectrum. Ultraviolet and infrared spectrophotometric methods are suitable for many colorless substances that absorb strongly in the UV or IR spectral regions.

## TRANSMITTANCE, ABSORBANCE, AND THE BEER-LAMBERT LAW

We define **transmittance** as the ratio of the amount of light transmitted to the amount of light that initially fell on the surface.


$$\text{Transmittance} = \frac{P}{P_0} = \frac{\text{intensity of transmitted light}}{\text{intensity of incident light}}$$

**Absorbance** is defined as the negative logarithm of the transmittance, and you will note that absorbance and transmittance bear an inverse relationship.

$$\text{Absorbance} = -\log T = -\log P/P_0$$

Going back to our example of chlorophyll, if you have two colored solutions, you may deduce that the darker colored green solution appears darker because it absorbs more of the light falling on it. Because the darker solution is also the more concentrated one, you can also say that the more concentrated one absorbs more of the light. That is, *the absorbance increases as concentra-*

*tion increases*.

Next, suppose that there are two test tubes, both containing the same solution at the same concentration. The only difference is that one of the test tubes is thicker than the other.


We shine light of the same intensity ($P_O$) on both containers. In the first case the light has to travel through only a short distance, whereas in the second case it has to pass through a much longer length of the sample. We might deduce that in the second case more of the light will be absorbed or cut off, since the path length is longer. In other words, absorbance increases as path-length increases.

The two observations described above (those dealing with the relationship between absorbance and concentration and absorbance and path length) constitute the **BEER-LAMBERT LAW**.

Beer-Lambert Law

Absorbance $\propto$ path length ($\ell$) • concentration

$$A = \varepsilon \bullet \ell \bullet c$$

where

- A is a dimensionless number.
- $\varepsilon$ the proportionality constant, is called the molar extinction coefficient or molar absorptivity. It is a constant for a given substance, provided the temperature and wavelength are constant. It has units of liter/mol • cm.
- $\ell$ and c have the usual units of length (cm) and concentration (mol/liter).

The quantitative measurement of light absorption as a function of wavelength can establish both the identity and the concentration of a substance in solution. The spectrophotometer is an instrument that separates electromagnetic radiation into its component wavelengths and selectively measures the intensity of radiation after passing through a sample. In this laboratory assignment you will use what is affectionately known as a "Spec 20" or Spectronic 20. Its operation is described below.

## SPECTROPHOTOMETRIC ANALYSIS

This section of the introductory material outlines a general approach for spectrophotometric analysis, first finding the absorption spectrum of "finger prints" of a substance and then determining its concentration.

### 1. Plotting Absorption Spectra

Recall that the extinction coefficient for any given substance is a constant only so long as the wavelength of light is constant. You will see that the absorbance changes with wavelength.

The plot of a sample's absorbance of light at various wavelengths is called its **absorption spectrum**. (The abscissa or horizontal axis may be expressed in terms of wavelength and the ordinate or vertical axis in terms of absorbancy.) The plot below gives the absorption spectrum of potassium permanganate ($KMnO_4$), a purple colored solution, at two different concentrations. Curves 1 and 2 represent the absorption spectra measured under the same conditions except that curve 1 represents a more concentrated solution than curve 2. Note the similar shapes of the curves.

## 2. Choice of Wavelength

According to the Beer-Lambert Law absorbance is proportional to concentration at each wavelength. Theoretically we could choose any wavelength for

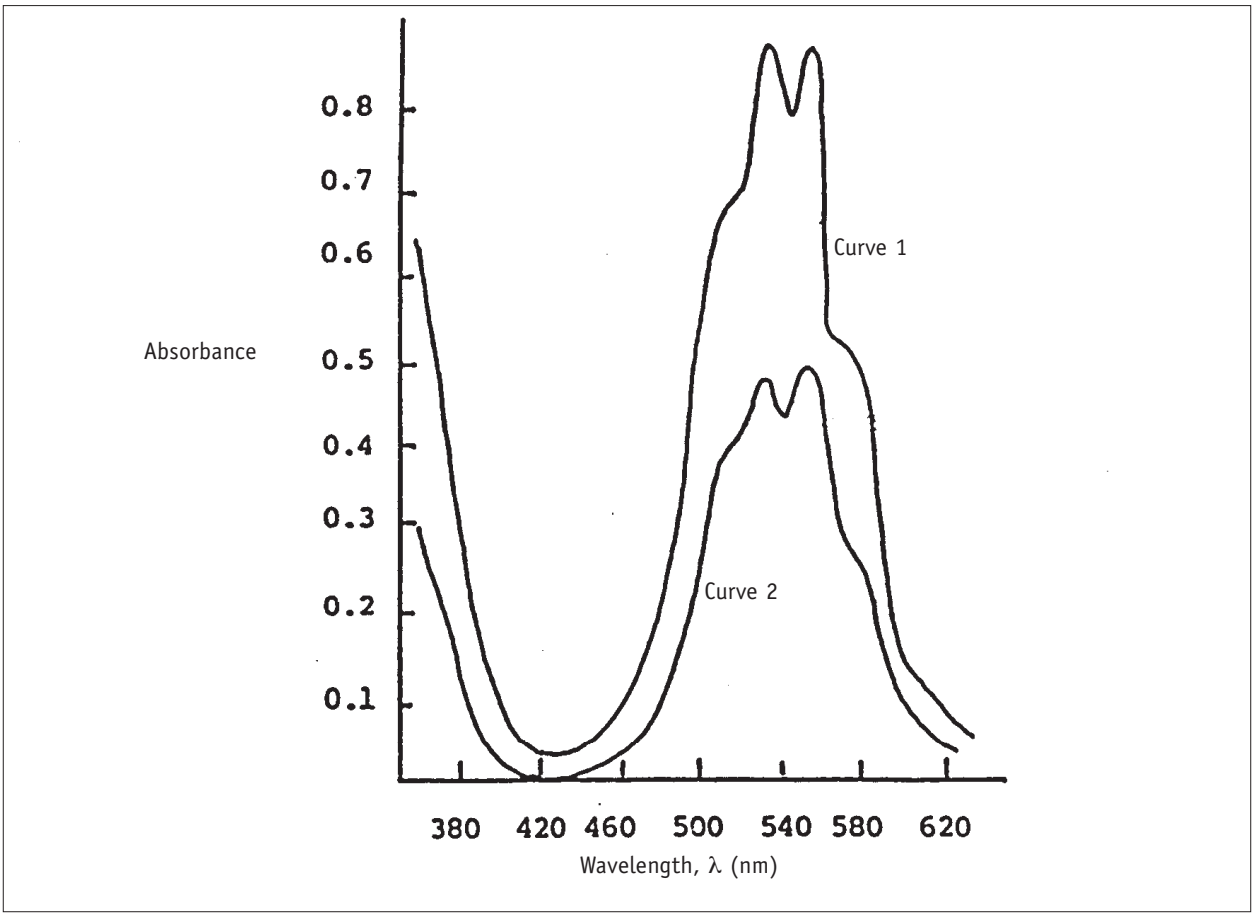



**FIGURE** The absorption spectrum of solutions of potassium permanganate ($KMnO_4$) at two different concentrations. The solution for curve 1 has a *higher* concentration than that for curve 2.

quantitative estimations of concentration. However, the magnitude of the absorbancy is important, especially when you are trying to detect very small amounts of material. In the spectra above note that the distance between curves 1 and 2 is at a maximum at 525 nm, and at this wavelength the change in absorbance is greatest for a given change in concentration. That is, the measurement of concentration as a function of concentration is most sensitive at this wavelength. For this reason *we generally select the wavelength of maximum absorbance for a given sample and use it in our absorbance measurements*.

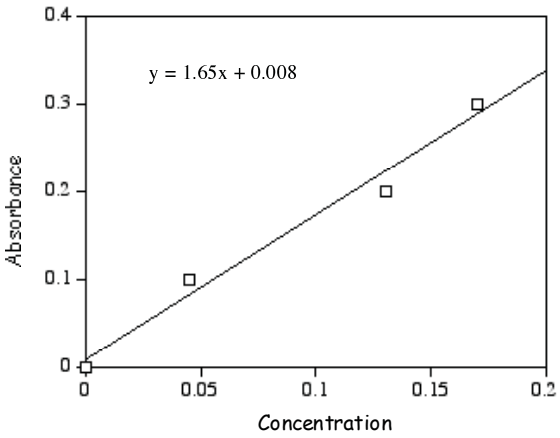
Suppose instead that we had chosen a wavelength of 500 nm for our measurement, this wavelength being on one of the steep portions of the curve. Examination of the curve shows that even a small fluctuation in the wavelength will cause a large error in the absorbance. Most spectrophotometers show a slight fluctuation in the wavelength, so errors in absorbance will be magnified if we select a wavelength such as 500 nm in our preceding example.

## 3. Plotting Calibration Graphs

Once we have chosen the correct wavelength, the next step is to construct a **calibration curve** or **calibration plot**. This consists of a plot of absorbance versus concentration for a series of standard solutions whose concentrations are accurately known.

Because calibration curves are used in reading off the unknown concentrations, their accuracy is of absolute importance. Therefore, make the standard solutions as accurately as possible and measure their absorbances carefully. Each standard solution should be prepared in identically the same fashion, the only difference between them being their concentrations.

When drawing the calibration graphs, take care not to lose any of the accuracy of the experimental data by choosing axes that are too small. Choose axes to represent the accuracy possible in reading the instrument. For example, if it is possible to read absorbance correct to the second decimal place, say 0.47, then construct the absorbance axis so that 0.47 can be located accurately on it.




Slope of the best straight line through the data points in the calibration plot is 1.65. Plot intercept is 0.008.

$$\text{Slope} = \frac{\Delta(\text{absorbance})}{\Delta(\text{concentration})} = 1.65$$

Equation of straight line:

Absorbance = 1.65 (Concentration) + 0.008

To find an unknown concentration for a sample, subtract the intercept from the absorbance reading and divide the result by the slope. Here the equation would be

$$\text{Concentration} = \frac{\text{Absorbance} - 0.008}{1.65}$$

You will find in your experiment that the calibration plot generally does not pass through the origin, but it should be quite close. Thus, the value you see for the intercept (b) in the equation y = mx + b will be small.

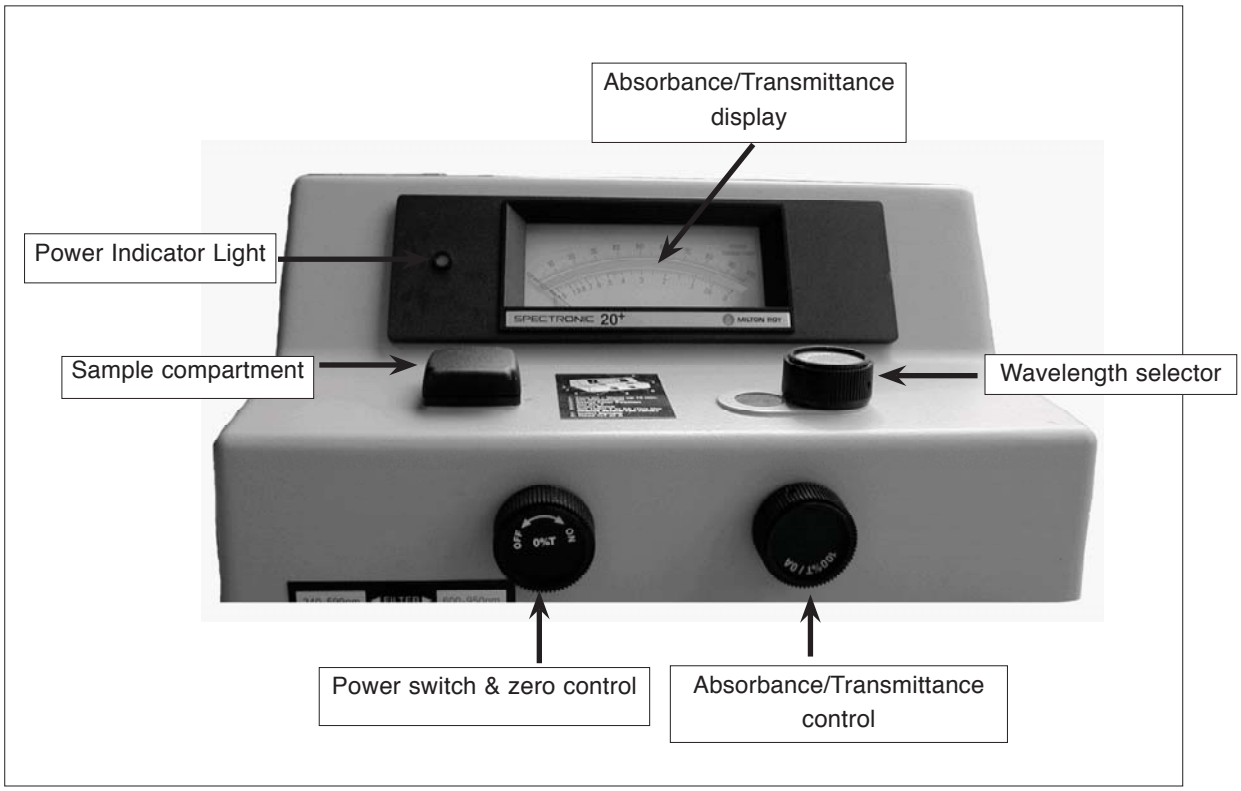
The Beer-Lambert Law ($A = \varepsilon \ell c$) implies that when concentration is equal to zero ($c = 0$), absorbance must also be zero ($A = 0$). In other words, the calibration line must pass through the origin.

A major source of error in spectrophotometric analysis is applying the Beer-Lambert Law at inappropriate concentrations. The Beer-Lambert Law is strictly applicable only for dilute solutions. It becomes less and less accurate as the concentration of the solution increases.

Once you have the calibration curve set up, you can measure the absorbance of any unknown solution at the same wavelength and read off its concentration from the graph or calculate from the slope.

## USE OF THE SPECTRONIC 20

All spectrophotometers have the following fundamental parts: a source of radiant energy, a prism or grating to isolate radiant energy to narrow wavelength regions, a device for holding the sample, and a photoelectric cell for measuring light intensity. The "Spectronic 20" contains an incandescent white lamp as the light source and a system of lenses that focus the beam onto a grating. The grating splits up the light into its composite colors, just as a prism does. A wavelength selector adjusts the position of the grating so that only one wavelength of light is focused on the sample. This is necessary because the Beer Lambert Law is strictly applicable only when we use monochromatic (a single wavelength) light. The light passes through the sample and falls on a phototube that records the intensity of the light. This is electronically converted to absorbance and displayed on a scale outside the instrument.

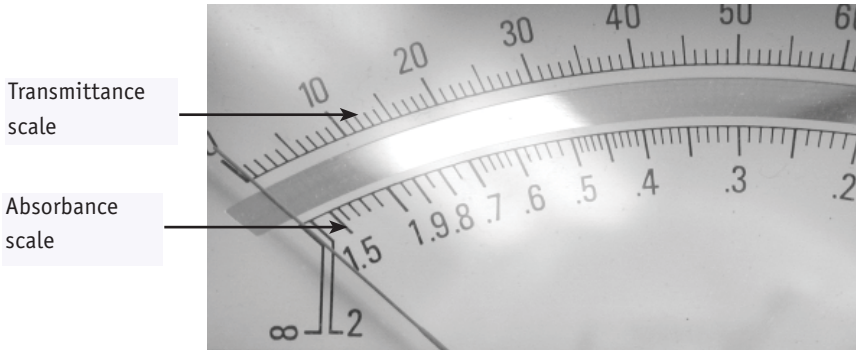



A Spectronic 20 laboratory spectrophotometer.

*The Spectronic 20 is an expensive and sensitive instrument; it should be treated with care. Your laboratory instructor will demonstrate how to use it, but an abbreviated set of instructions follows.*

## OPERATING INSTRUCTIONS FOR SPECTRONIC 20

1. Let the instrument "warm up" for at least 15 minutes.
2. Set the desired wavelength with the wavelength control.
3. Set % transmittance (top scale) at zero, or the absorbance (bottom scale) at infinity using the amplifier control, A.




4. Place a test tube at least half filled with water or the appropriate solvent in the sample holder (sometimes called the blank). Be sure that the sample holder is in place by pressing down on it. The top of the holder must be shut whenever you adjust the instrument or take readings, to prevent stray light from entering the instrument.
5. Set the needle at zero absorbance (or 100% transmittance ) using the light control.
6. Remove the blank. The needle should swing to infinite absorbance (or zero % transmittance). If it does not, repeat steps 3 through 6.
7. The instrument is now correctly calibrated. To take a reading, place a test tube containing your sample in the machine and close the lid; after 10 seconds record the absorbance (and wavelength if necessary).
8. Before taking another reading on the same or different sample, you should again check that the absorbance scale reads infinity with nothing in the sample holder and that it reads zero with the blank sample.