

Perception

Winter Quarter Lab 3: Spectroscopy

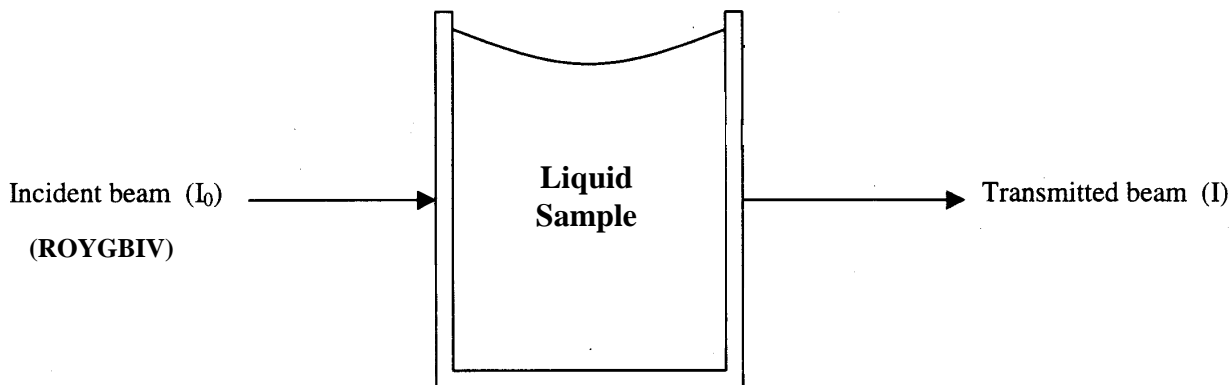
Why is orange juice orange? The simple answer is that one or more components of orange juice absorb selected wavelengths of visible light such that the light passing through or reflecting off the orange juice appears orange; that is, the sample absorbs light whose color is the complement of orange. We can take advantage of this absorption to study molecules, atoms, and ions by exploring their ability to absorb electromagnetic radiation.

Spectrophotometers

A sample's ability to absorb light is measured using a spectrophotometer. The simplest visible spectrophotometer consists of several parts: (a) a place to put the sample; (b) a source of light, typically a tungsten lamp similar to the light bulbs you use at home; (c) a detector for measuring the amount of electromagnetic radiation passing through the sample; (d) a means of dispersing the light so that the wavelengths are spread out in space, typically a prism or a diffraction grating, so that sample's interaction with the light can be analyzed wavelength-by-wavelength; and (e) a signal processor, such as a meter or computer, for manipulating and displaying the resulting measurements. We have two types of spectrophotometers that we will use in this week's lab. Although the model is different, they perform the same function.

Transmittance vs. Absorbance

When light falls on any surface, it can be absorbed or transmitted (although a small fraction of the rays may also be reflected). These processes are shown in the diagram below.



At any wavelength, the fraction of light absorbed by the sample is defined as its transmittance, T

$$T = \frac{P_T}{P_0}$$

where P_T is the intensity of light transmitted through the sample and P_0 is the intensity of light from the source. Frequently, the transmittance is expressed as a percentage, %T, where

$$\%T = T \times 100$$

A little thought should convince you that transmittance must fall within the range of 0 to 1 and the percent transmittance must fall within the range of 0 to 100%. For reasons that will soon be evident, the amount of light absorbed by the sample is more commonly expressed using absorbance, A , where

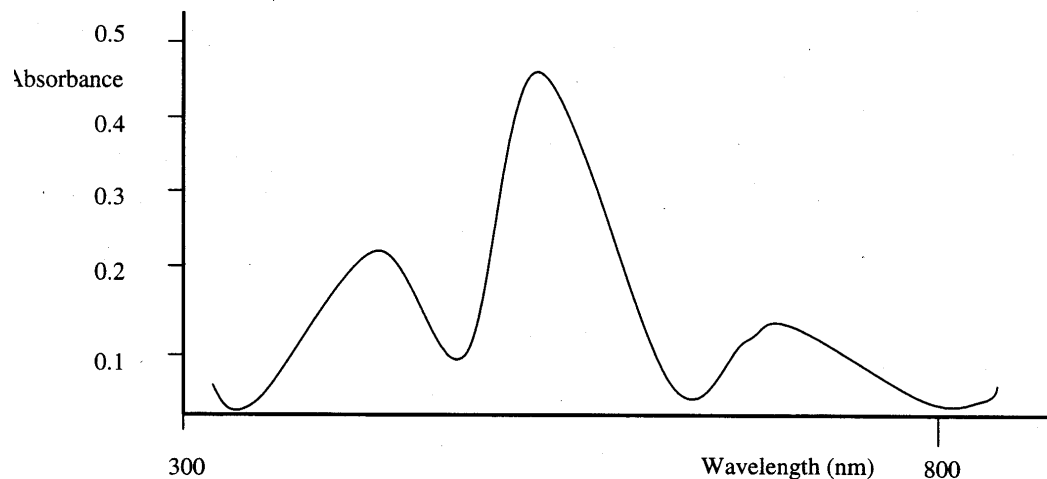
$$A = -\log [I/I_0]$$

Where I_0 = incident beam of light and I = transmitted beam after passing through sample.

Don't worry about the math here! The spectrophotometer will do the calculations for you.

Visible Absorbance Spectra

The spectrophotometer monitors simultaneously the absorbance and transmittance of light at 1024 different wavelengths. A plot of absorbance as a function of wavelength is called an absorbance spectrum, a typical example of which is shown here.



In this lab, using the spectrophotometer, we will record absorption spectra of four colored food dyes and four "unknown colored solutions" (made from combinations of the original food dyes). An absorption spectrum is a graph of absorbance versus wavelength. The spectrum tells us what wavelengths of light are absorbed by a given sample.

General Protocol:

1. You will first need to 'zero' the spectrophotometer by recording the absorbance of water (the solvent which the food dye is dissolved in). (Why do you think this step is crucial?) This is called a **blank spectrum (I_0)**. Obtain a clean, plastic cuvette and fill it with distilled water (DI H_2O). Place it in the spectrophotometer and zero the machine according to the directions given by Shane and/or Nancy. The spec will store the blank information in memory so that you will only need to do this once.
2. Once the spec has been 'zeroed' you may begin measuring the absorbance of the four food dye samples and the four unknown samples. To do this, rinse the cuvette with DI H_2O and fill it with one of the samples. Place the cuvette with the sample in the spec and follow the directions given during the pre-lab session to measure the absorbance of the sample. This is called the **sample spectrum (I)**. The instrument uses the **sample spectrum** and the **blank spectrum** to generate the **absorption spectrum** of the sample. The data for each sample will be exported and saved on the network for later analysis. Because we are using two different model spectrophotometers, the specific instructions to operate each will be passed out during the lab session.
3. Repeat step 2 for each sample (all four food color dyes (red, blue, yellow, and green) and all four unknown samples (1, 2, 3, 4)). Between samples, thoroughly rinse the cuvette with DI H_2O .
4. Once you have run all eight samples through the spec and have saved the data, proceed to the computer lab to complete the analysis. Nancy will do a brief run through on how to import your data into Excel and how to generate the spectrum for each sample.

Analysis:

1. Plot the absorbance spectra for each of the FOUR food dye samples. You may plot them as individual spectra or as a composite graph showing all four samples.
2. By hand or using the Excel program, create a table for with a row for each of the eight samples that contains the following information:

Sample #	Color observed (naked eye)	Absorbance of Peak(s)	Wavelength of Peak(s)
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3. Based on your absorption spectra graphs and your knowledge of light and color, decide which wavelengths are transmitted

4. In your report, consider the wavelengths that are transmitted and the color (naked eye observation) for each sample and explain how the transmission patterns of each of these four samples produce the observed color of the dyed liquid. The information below should help you.

Visible wavelengths in the electromagnetic spectrum roughly range from 390 nm to 780 nm with the following rather arbitrary subdivisions:

violet	390 - 430 nm
blue	430 - 470 nm
blue-green	470 - 500 nm
green	500 - 530 nm
yellow-green	530 - 560 nm
yellow	560 - 590 nm
orange	590 - 620 nm
red	620 - 780 nm

5. Plot the spectra of the four unknown samples (either individually or as a composite).
6. Using the table you generated above in step 2 and the spectra, determine the composition of the unknown samples. That is, which of the four original food dye samples were used in combination to generate each of the unknown samples? Do any of the unknowns seem to be composed of dyes other than the four original food dye samples? How can you tell?