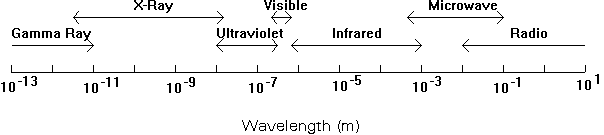
**LIGHT-SCATTERING AND MOLECULAR SPECTROPHOTOMETRY**

**A. Introduction**

Light or electromagnetic radiation can be characterized by wave-like behavior. The wavelength, , is the distance between the crest of one wave and the crest of the next one. The frequency, , is the number of complete waves that pass a reference point in a second.



The human eye perceives light of different wavelengths as being comprised of separate colors. When a substance absorbs light of a certain wavelength (color), it depletes the transmitted light in precisely those wavelengths that are absorbed. For this reason, a solution that appears blue to the human eye does not absorb blue light. Rather, it is blue because it absorbed orange light, and allows all other wavelengths to pass unhindered (giving a bluish color to the transmitted light; see Table 17.1).

Double bonds are strong absorbers of light, especially when they are arranged in an alternating pattern with carbon-carbon single bonds. This alternating pattern of single bond followed by double bond followed by single bond followed by double bond is called a conjugated double bond system. It has a property known as resonance, which makes it absorb light of high wavelengths (i.e., low energy). The molar absorptivity and wavelength of maximim absorbance generally increases with increasing numbers of conjugated double bonds. This is illustrated by the series of polynuclear aromatic hydrocarbons, below.

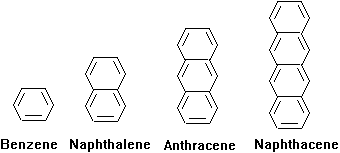


Table 17.1

Relationship Between Color and Absorbance

|  |  |  |
| --- | --- | --- |
| Wavelength of absorbance maximum (nm) | **Color Absorbed** | **Color Remaining** |
| 380-420 | Violet | Green-yellow |
| 420-440 | Violet-blue | Yellow |
| 440-470 | Blue | Orange |
| 470-500 | Blue-green | Red |
| 500-520 | Green | Purple |
| 520-550 | Yellow-green | Violet |
| 550-580 | Yellow | Violet-blue |
| 580-620 | Orange | Blue |
| 620-680 | Red | Blue-green |
| 680-780 | Purple | Green |

When a solution is placed in a path of light, a certain amount of that light will pass through it unaffected. The fraction of the incident light that reaches the other side of a liquid sample will be dependent on the pathlength through the sample, x, and the ability of the sample components to block or otherwise impede the passage of light, , called the extinction coefficient. This relationship is given by Lambert's Law:

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where:

Io = the intensity or radiant power of the incident light

I = the intensity or radiant power of the light after passing through a though a thickness, x, of the liquid sample

The blockage or impedance of light may be due to scattering or absorption. Light scattering occurs when small particles deflect the light so that it does not reach the other side of the sample. Absorbance is the process by which a constituent (usually dissolved) absorbs the light energy and releases it as heat or stores it as bond energy. Thus, the extinction coefficient in equation 17.3 and 17.4 is composed of two components, the scattering coefficient () and the absorption coefficient ().

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#### a. Spectrophotometers

Sample absorbances are measured with a spectrophotometer. This is an instrument composed of a light source, a wavelength selector (monochromator), a sample compartment, and a detector. The type of light source will depend on the desired wavelength. Light in the visible range (350nm-800nm) is best supplied by a tungsten filament lamp. Ultraviolet (UV) light (190nm-350nm) requires a deuterium lamp. The wavelength selector splits the light spectrum into its component colors and selects a narrow band of this spectrum. Either a prism or a grating may be used as a dispersing element to split the light. The spectrum is generally projected on an opaque wall containing a slit. The physical width of the slit determines the band width of the selected light. The wavelength of the selected light may be adjusted by changing the angle of the dispersing element, so that the desired wavelength passes through the slit. The selected light passes into the sample compartment, through the sample (and cell) and to the detector (photomultiplier). By measuring transmittance with (I) and without (Io) the sample present, absorbance can be determined. This is how a single beam spectrophotometer operates.

A more accurate and convenient scheme is embodied in the double beam spectrophotometer. This instrument has a chopper motor which alternately deflects the light beam through a reference cell and the sample cell. This is done many times per second, and the average ratio between the two readings gives the transmittance. This is a more accurate method, because it minimizes variabilities due to rapidly changing lamp output or momentary stray light in the detector compartment. In effect, the analyst is continually monitoring and adjusting for changes in the lamp output as measured by the detector.

In the Environmental Engineering teaching laboratory we have several spectrophotometers. One is a Perkin-Elmer Model 111 Ultraviolet-Visible Spectrophotometer. This is a typical single beam instrument with a spectral range of 200-900 nm. It uses a diffraction grating with fixed bandpass of 2.0 nm. The wavelength accuracy is ±0.5 nm and the precision is ±0.1 nm. The photometric linearity is -0.005 absorbance units at a reading of 0.400 absorbance units. The photometric reproducibility is better than 0.01% Transmittance, and stray light is less than 0.5% T at 220 nm. This instrument also has a stability characterized by a drift of less than 0.5% T per 5 min after a 15 min warm-up period. As with most UV-Vis spectrophotometers, the PE 111 uses a Tungsten lamp in the visible region (900-340 nm) and a Deuterium lamp in the ultraviolet region (340-200 nm).

For best operation, spectrophotometers should be installed in air conditioned rooms, free from dust, corrosive fumes, vibrations, and large changes in temperature and humidity. Instructions for calibrating and operating the PE 111 are as follows:

1. With the operation switch in the off position, verify that the meter mechanical zero is correct.

2. Select the proper lamp for the desired wavelength by adjusting the selector lever to either VISIBLE or ULTRAVIOLET.

3. Adjust the wavelength knob to the correct value.

4. Turn on the appropriate lamp. If the deuterium lamp is chosen, the #1 switch must be turned on first, then 20-30 sec later the #2 switch is turned on.

5. Turn the operation switch to "on".

6. Open the cell compartment and insert both filled sample and reference cells in the cell holder. The reference cell belongs in the position #1.

7. Place the operation switch to the "meter" position.

8. With the cell compartment cover open (this automatically closes the shutter), adjust the meter to infinite absorbance (0 transmittance) with the zero adjusting knob.

9. Close the cell compartment cover, and place the reference cell in the light path by adjusting the cell positioning knob. Adjust the meter to 0 absorbance (100% transmittance) with the zero adjusting knob.

10. Pull out the cell positioning knob and read the absorbance of the sample.

#### Spectrophotometric cells

Cells (or cuvettes) are supplied with varying light paths and different qualities of glass. Most spectrophotometric work is conducted with standard rectangular cells of 1 cm path length. These are square in cross-section and about 4 times as high as they are wide. They generally have a pathlength tolerance of -0.01 mm. In addition, rectangular cells of 0.1, 0.5 and 4 cm are commercially available. For more dilute solutions, cylindrical cells of 5 and 10 cm are available. These have two filler necks into which fit fluoropolymer stoppers. The ability to seal these cells is an attractive feature for volatile or hazardous samples. The cylindrical cells may be supplied with fluoropolymer covers, but these do not make a very good seal. Rectangular cells with extra thick side walls are also available for the analysis of small volumes of liquid. In addition, various types of flow through and jacketed cells are available for kinetic studies, continuous monitoring, and temperature sensitive work.

Cells for absorption spectrophotometry have opposing optically polished windows with frosted glass side walls. Be sure to orient the cells so that the optic windows are perpendicular to the light path. Fluorescence cells are made with all (or adjacent) optically polished windows.

Cells should be cleaned like other laboratory glassware. If detergents prove ineffective, they may be soaked in chromic acid cleaning solution. Note that the windows and side walls are fused forming an acid-proof seal. In addition, quartz cells may also be cleaned by immersing in concentrated nitric acid followed by ultrasonic agitation or 10-15 minutes of boiling in water.

Table 17.2 Spectrophotometric Cells

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Window | Wavelength Range (nm) | Letter Code | Lot #s | Color Code |
| Optical Glass | 360 - 1000 | OG |  | yellow |
| Near-UV Glass or Special Optical Glass | 300 - 1000 | OS or SG | 180's | green |
| Standard Silica | 220 - 2500 |  |  |  |
| Supracil Quartz or Quartz UV | 165 - 2600, 2850 - 3600 | QS or UV | 280's | blue |
| Infracil Quartz or Quartz IR | 220 - 3600 | QI or IR | 300's | red |

  Most spectrophotometric cells sold in the US are marked with a series of letters and numbers. Many are marked with and upper case "SCC" (Scientific Cell Company) and number indicating the path length (e.g., 1.000 for 1 cm) followed by a two letter code, a colored dot or a lot number. These markings will identify the type of glass used in the optical windows (see table below). Cells should always be matched with the same markings including lot number. This is especially important when running absorbance scans. It is of highest importance to choose a cell which is useable for the wavelength chosen. Although any of the cells listed below may be used in the visible range, the optical glass cells are preferred because of their lower cost. For near-UV and UV work, near-UV glass, Supracil quartz or Infracil quartz should be employed depending on the wavelength chosen. In general, the lower the minimum wavelength, the more expensive the cell. For work in the infrared region, Infracil quartz is required.