Chem 321 Lecture 19 - Spectrophotometry

11/5/13

Student Learning Objectives

UV-VIS Spectrophotometers

The basic components of a **spectrophotometer** are shown in Figure 12.6.

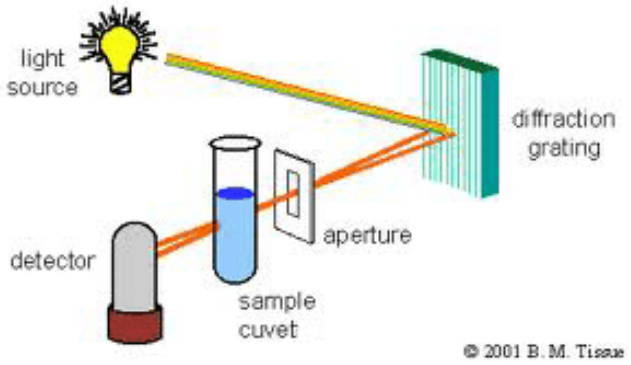

Figure 12.6 Schematic diagram of a single-beam spectrophotometer

The radiation source depends on which region of the electromagnetic spectrum is being used. Visible (VIS) radiation (~400-750 nm) is usually provided by a **tungsten lamp**. This consists of a heated filament of tungsten that produces continuous radiation throughout the visible and near-IR regions. Ultraviolet (UV) radiation is usually provided by a **deuterium lamp**. An electric discharge in a low pressure sample of deuterium produces continuous emissions from about 200-400 nm. The emission profiles of these two radiation sources are shown in Figure 12.7. Both lamps are employed in a UV-VIS spectrophotometer and a switch is made around 360 nm to the lamp with the greater emission intensity.

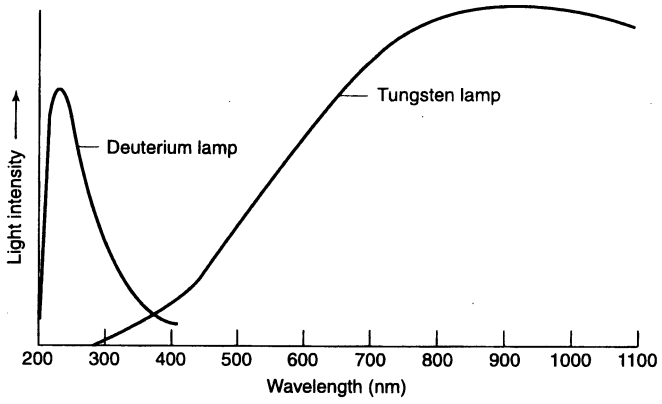

Figure 12.7 Emission spectrum for a tungsten lamp and a deuterium lamp

Since these sources are continuous, a small range of wavelengths must be selected for passage through the sample in order for Beer's law to apply. This is accomplished by the **monochromator**. As diagramed in Figure 12.8, continuous radiation from the source enters the monochromator through a slit and the radiation reflects off a diffraction grating where it is dispersed (spread out). After focusing, only a small band of radiation emerges from the exit slit of the monochromator. By varying the orientation of the diffraction grating with respect to the incoming radiation, a different wavelength can be selected for passage through the monochromator. When you set the wavelength on a spectrophotometer you are altering the orientation of the grating and selecting a small band of radiation centered on this wavelength for passage through the sample.

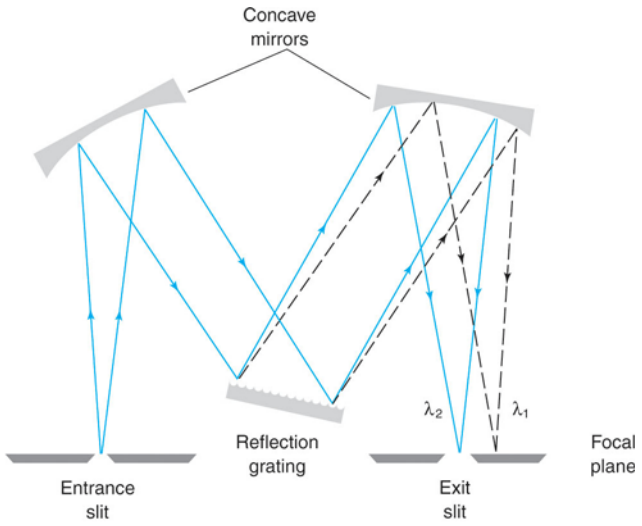

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Figure 12.8 Diagram of the light path through a diffraction grating monochromator

The sample is held in the light path by a sample cell, or **cuvet**. For work in the visible, cuvets are made of glass. However, glass absorbs radiation in the UV, so for measurements in the ultraviolet the cuvet is made of quartz (crystalline silicon dioxide). Cuvets take many different forms, including microcuvets that require less than 0.1 mL of sample and flow-thorough cells that permits continuous absorbance measurements of material eluting from a chromatography column. A sample pathlength of 1.0 cm is common for standard cuvets, however, do not assume a pathlength; measure it before using a cuvet. Cuvets cost about $50 for a pair of matched glass cells to around $200 for a pair made of quartz. They can crack easily, even from toppling over on the hard lab bench. Always handle the cuvet with the frosted sides, not the optically clear faces, to avoid getting fingerprints and scratches on the clear sides. Avoid the use of harsh

cleansers. Never use a brush to clean a cuvet. Rinsing with a mild soap solution and deionized water works well. Gently wipe the optically clear sides with a Kimwipe before measurements.

A variety of detectors are employed in spectrophotometers, depending upon the radiation being monitored and the sophistication of the instrument. In each case, the detector must produce an electrical signal when struck by radiation. A very common and sensitive detector is the photomultiplier tube, or PMT (Fig. 12.9).

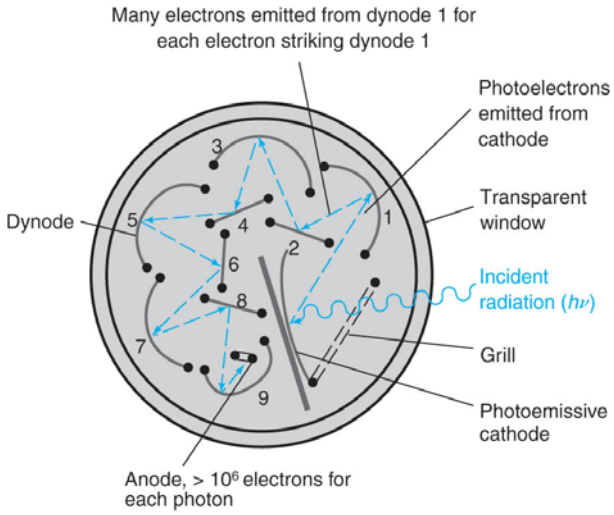

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Figure 12.9 Diagram of a photomultiplier tube

Radiation reaching the PMT first strikes a surface called the photocathode where electrons are ejected by the incident photons. These ejected electrons are drawn to another surface (dynode) maintained at a slightly more positive potential, where each incident electron ejects 2 or more electrons. This process is repeated with a total of 8-10 dynode surfaces and results in a significant number of electrons being collected as the electrical signal. Photomultiplier tubes are generally used as detectors for UV and VIS radiation. The efficiency of such detectors varies with the wavelength of the incident radiation.

In order to determine the appropriate wavelength at which to measure the absorbance of the iron-phenanthroline complex in your standard and unknown solutions, you will first obtain the absorption spectrum for the iron-phenanthroline complex using a **diode array spectrophotometer** (Fig. 12.10). In this type of

instrument all of the radiation from the light source passes through the sample and then is dispersed by a diffraction grating onto an array of photodiodes. Each photodiode detects the transmitted light intensity associated with a small band of wavelengths. Consequently, the sample absorbance is measured simultaneously over a wide range of wavelengths and an absorption spectrum is acquired in just seconds.

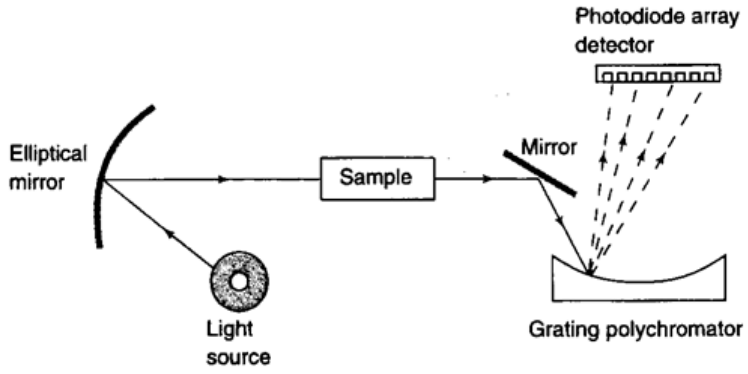

Figure 12.10 Schematic diagram of a diode array spectrophotometer

Note that in both a single-beam and diode array spectrophotometer, the instrument never measures the intensity of light incident on the sample (P_0), only the intensity of light reaching the detector. In order to obtain the sample absorbance (recall $A = -\log P/P_0$), the intensity of light that passes through a blank solution is first measured (P_{blank}) and this value is taken as P_0. Then the intensity of light that passes through the sample (P_{sample}) is measured. The instrument then computes the sample absorbance from $-\log (P_{sample}/P_{blank})$.

Exercises for Spectrophotometry