Spectrophotometry

Barbara Sawrey and Gabriele Wienhausen. Science Media: San Diego, 1997. 1–10 copies, $99 each; 11–20 copies, $69 each; 21+ copies, $49 each. (NOTE: CD operates with both Mac and PC.)

Spectrophotometry is an interactive CD-ROM which introduces the basics of UV–visible spectrophotometry with some mention of infrared and other forms of spectrophotometry. A Macintosh System 7.5 or higher, CPU 68040 or Power PC processor, 6 megabytes of free RAM, 2.6 megabytes of free disk space, and 4X CD-ROM or faster are required.

The CD is divided into six sections: Introduction, Background, A Detailed Look, The Spectrophotometer, Practice, and Quantitative Spectrophotometry. The introduction answers questions about why substances are colored, why substances only absorb certain wavelengths of radiation, and how spectrophotometry is applied. One troubling problem in this section is the interchangeable use of the terms transmission and reflection as if they were the same process.

The section on background covers the nature of light, both its wave and particle properties, and the electromagnetic spectrum. To introduce the concept of waves, there is a video clip showing breaking ocean waves complete with surfer. There is also an animated oscilloscope showing a waveform. Following this is a screen illustrating wavelength, amplitude, speed, and frequency. Another screen shows the relation between wavelength, energy, and frequency for electromagnetic radiation from gamma rays through air navigation. For the particle nature of electromagnetic radiation, the relationship between energy, Plank's constant, and frequency is introduced.

In the section entitled "A Detailed Look", mathematical relationships between wavelength, frequency, and energy are presented. An interactive screen allows the user to scroll through the visible portion of the electromagnetic spectrum to explore the relation of wavelength, frequency, and energy to the various colors. Another interactive screen relates intensity of radiation to the low- and high-beam headlights of a car. To illustrate the interaction between light and matter, the authors use chlorophyll-*a* as an example, showing a rainbow of visible radiation passing through the sample with the blues and reds absorbed and the portion of the radiation centered on green being transmitted.

To demonstrate the effect of radiation on vibrational states, three photons of different energies may be fired at a water molecule, allowing the animated symmetric and asymmetric stretches and scissoring of water to be viewed. Even though rotational effects are mentioned, no example is given.

To demonstrate changes in electronic energy, photons with particular wavelengths are fired at what must be a hydrogen atom, on the basis of the wavelengths specified. The electron moves to higher energy levels upon absorption and gives off radiation of the appropriate wavelength as it returns to lower energy states. This particular animation is troublesome. In two cases the electron is excited to higher energy states and then excited again by a second photon to an even higher energy state—a highly unlikely occurrence. A second problem with this example is that excitation of a hydrogen has nothing to do with the absorption of a chlorophyll-*a* molecule. The interaction of radiation with molecules is mentioned briefly, but no illustrations are provided.

The next screen shows the visible and infrared absorption spectra of chlorophyll-*a*. A major problem with the infrared spectrum is that it is either a mislabeled transmission spectrum or it is displayed upside down as an absorption spectrum; furthermore, it is displayed with increasing wavenumbers instead of the more traditional increasing wavelength. Toward the end of this section the viewer is asked why the visible spectrum of chlorophyll-*a* is a continuous (band) spectrum and not a line spectrum. Given that the only example presented is a hydrogen atom, it would be impossible for a novice user to adequately answer the question.

The final topic covered in A More Detailed Look is types of spectrophotometry. This is simply a list of IR, Raman, UV, and visible spectroscopy. Also mentioned are the application of emission and absorption phenomena. The wavelength region for the UV is given as 20–400 nm, instead of the more commonly used 200 nm lower limit. It is not clear why the vacuum UV was included. The visible region of the spectrum is given as 360–720 nm with no explanation for the 40-nm overlap with the UV.

The next section is The Spectrophotometer. This well-conceived section takes the user through the common components of a spectrophotometer with a brief but very adequate explanation of each. Included in the list of components are lamps, monochromators, cuvettes, phototubes, and readouts. There is a good explanation of prisms and gratings, and the segment on cuvettes includes a graphic of 1-, 3-, and 10-mL cuvettes, a mention of quartz and glass cuvettes, and some practical tips about filling and placing cuvettes into the spectrophotometer. This section also includes pictures, descriptions, and specifications of seven common, mostly single-beam, spectrophotometers manufactured by five different companies.

The final portion of this section illustrates seven steps for the operation of a single-beam spectrophotometer: turn on power, turn on lamps (provides the deuterium and tungsten lamps), wait for warm-up, set 0% T, select cuvettes, select the proper wavelength, set 100% T, load sample, and read and record. As each step is selected, a short but complete description of the process is provided, and the appropriate control is exploded from the spectrophotometer.

The Practice section is the best, especially if learning how to run a single-beam spectrophotometer is important. The user is provided with a view of a spectrophotometer, a cuvette containing a blank, and another cuvette containing a default sample of chlorophyll-*a*. Spectral data for chlorophyll-*a* can be obtained by using the Select Sample button. Here one of several compounds may be selected and each of their absorption spectra may be viewed. There is a problem with β-carotene in that spectral data are given between 400 and 500 nm, but the simulation allows only 200–325-nm wavelengths to be selected.

Help balloons are available, allowing the user to move from control to control and discover the use for each. The help balloons may also be turned off. When the power is turned on, the cover of the spectrophotometer is removed, allowing a view of the light path and important parts of the spectrophotometer, including a monochromator that moves as the wavelength is changed and user-selectable deuterium and tungsten lamps. When the tungsten lamp is selected, the monochromator is illuminated by white light, followed by a rainbow of colors exiting the monochromator, which change to only blue, green, yellow, or red after passing through a slit.

To obtain a reading, the user must turn on the proper lamp, selected a wavelength compatible with that lamp, set 0% T (−0.9999 A), insert the blank and adjust 100% T (0.000 A), and insert the sample to make the final reading. If the wavelength is changed, the percent transmission or absorbance will change depending on the relation of the wavelength chosen to maximize. The entire process is interactive and very well done. If more practice is required, additional samples may be selected.

The final section on Quantitative Spectrophotometry, subtitled “The abc’s of Beer’s Law”, explains the derivation and use of Beer’s law. In the background portion the user can place cuvettes containing various concentrations of a sample in front of an arrow depicting the incident radiation, I_0, and see the effect on the transmitted radiation, I. A similar screen allows the user to see the effect of path length on transmission while holding the concentration constant. To show the effect of wavelength, a cuvette of tetraamminecopper(II) may be placed in the path of a representation of the spectral colors from red to violet. The transmitted spectrum shows green and blue being transmitted almost unchanged while the remaining colors show significant attenuation.

The user may also choose to see the derivation of Beer’s law. If this option is selected, the effect of concentration is developed by selecting cuvettes containing various concentrations of a solution. As each cuvette is selected a point is placed on a graph of concentration versus percent transmission. The exponential form of the relationship $T = 10^{-kc}$ is then discussed. A similar approach is used to develop the mathematical relationship between path length and transmission, $T = 10^{-kh}$. The derivation continues step by step until the classical form of Beer’s Law, $A = abc$, is obtained. Whether or not the user undertakes the complete derivation of Beer’s law, it is displayed in another section, and each term, A, a, b, and c, is defined and explained. A working curve is employed showing where each term is applied: A and c as the axes and ab as the slope.

The next topic is Applications, where two example problems are presented. The first shows how the concentration of a solution can be calculated if the absorptivity, a, is known. The other example presumes to determine the concentration using a standard curve. There are two problems with the latter example. A standard curve is not used to determine the concentration. Only one standard is employed and then a direct proportionality between absorbance and concentration is used to solve for the unknown concentration. The other problem with the example is that the absorbance for the standard is given as 24.9% and is used in the subsequent calculation without change. Furthermore, the percent composition of the analyte is used and then converted with minimal explanation to mg/mL. In the derivation of Beer’s law, mol/L was used exclusively as concentration units. This example will cause considerable confusion for users who have carefully followed earlier explanations.

Also included on the CD-ROM is an online dictionary, containing the definitions of a variety of science (chemistry, biochemistry, biology) words. The pronunciation of each word can be heard if the user wishes. There is no explanation of the use or need of the dictionary. All the important terms needed in the material describing spectrophotometry are clearly defined as they are introduced. Currently the dictionary is an add-on with little use. Perhaps it is intended to be more complete as time goes on and to be used in a wide variety of additional CD lessons.

On balance, *Spectrophotometry* is a very useful tool for introducing the subject to students or others who need an understanding of the interaction of matter and light, Beer’s law, and the components and operation of a single-beam spectrophotometer. The program is nicely organized, the graphics and text are clear and helpful, and navigation is quite intuitive. The section on the use of a single-beam spectrophotometer will be very useful to teach novice users basic operating skills before they encounter a spectrophotometer in a laboratory setting. As potentially useful and visually appealing as *Spectrophotometry* is, however, a number of errors need to be remedied in subsequent editions.

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