APPENDIX B

Use of the Bausch & Lomb Spectronic 20 Colorimeter

The Bausch & Lomb Spectronic 20 colorimeter is an extremely versatile instrument that is useful for the spectrophotometric or colorimetric determinations of solutions.

The optical system is shown in Fig. B-1. White light is focused by a lens (1) onto an entrance slit (2), where it is collected by a second lens (3) and refocused on the exit slit (4) after being reflected and dispersed by a diffraction grating (5). Rotation of this grating by a cam (6) enables one to select various wavelengths of light in a range from 375–625 nm. The addition of a filter (9) can extend the usable wavelength to 950 nm. After the light passes the exit slit, it goes through the sample being measured (7) and is picked up by a phototube (8). A dial indicates the amount of light absorbed by the sample.

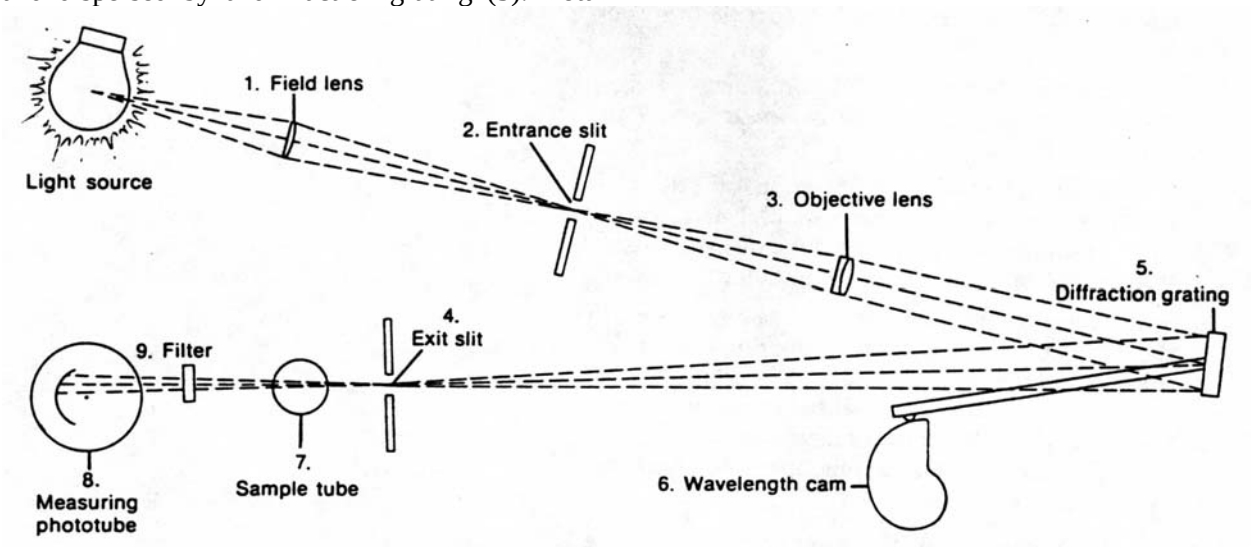

FIG. B-1. Optical system of the Spectronic 20 colorimeter.

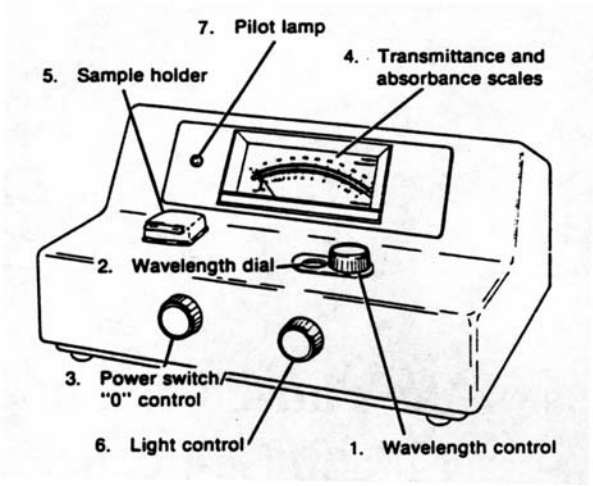

FIG. B-2. Controls on the Spectronic 20 colorimeter.

A. COLORIMETRY

Directions for colorimetric use are as follows:

1. Rotate the wavelength control (1) shown in Fig. B-2 until the desired wavelength is shown on the wavelength dial (2). The wavelength for a given substance can be found by referring to the literature or by determining it experimentally.

2. Turn the instrument on by rotating the "0" control (3) in a clockwise direction. Allow 5 minutes for the instrument to warm up.

3. Adjust the "0" control with the cover of the sample holder closed (5) until the needle is at 0 on the transmittance scale (4).

4. Place a colorimeter tube containing water or another solvent in the sample holder, and close the cover.

5. Rotate the light control (6) so that the needle is at 100 on the transmittance scale (0.0 absorbance). This control regulates the amount of light passing through the second slit to the phototube.

6. The unknown samples may then be placed in the tube holder, and the percent transmittance or the absorbance can be read. The needle should always return to 0 when the tube is removed. Check the 0% and 100% transmittance occasionally with the solvent tube in the sample holder to make certain the unit is calibrated.

Note: Always check the wavelength scale to be certain that the desired wavelength is being used.

The colorimetric measurements made with this apparatus employ standard matched tubes. They are selected so that variation in light transmitted through the tubes due to slight differences in diameter and wall thickness is minimal. You will be issued a set of such matched tubes. *They are to be used only for colorimetry.* The matched tubes must be handled carefully so as not to etch or scratch the surfaces exposed to the light beam. Obviously, the tubes will no longer be "matched" if scratched or etched, because such defects will cause the absorption and scattering of light.

B. SPECTROPHOTOMETRY

The method of operation for spectrophotometry is essentially the same as for colorimetry. The main difference is that the wavelength is reset for each reading, and thus a blank, or solvent, control must be readjusted at each new wavelength setting.

This procedure can be used when no information is available to determine the proper operating wavelength for an unknown substance. To do this, plot an absorption curve (absorbance versus different wavelengths) of the unknown substance (Fig. B-3). An operating wavelength may then be chosen according to the following:

1. Choose the wavelength at which the substance maximally absorbs the light (the minimum transmittance), because the greatest sensitivity will be

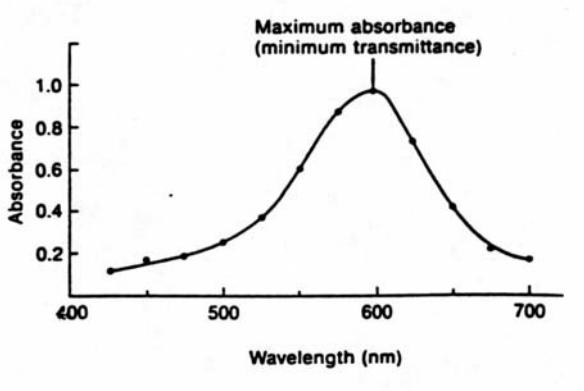

FIG. B-3. Absorption spectrum of an unknown substance.

obtained at this wavelength.

2. Do not choose wavelengths on the slope, because a small error in wavelength will cause a large error in reading.

REFERENCES

Karp, G. 1984. *Cell Biology*. 2d ed. McGraw-Hill.

Schleif, R. F., and P. C. Wensink. 1982. *Practical Methods in Molecular Biology*. Springer-Verlag.