Procedure for use of Spectronic 20D Spectrophotometers

General Technique

Successful use of the spectrophotometer depends on the consistent use of correct laboratory procedures and analytical techniques. To minimize problems, follow these guidelines:

- Keep all solutions free of bubbles.
- Make sure that all sample holders are at least half full.
- Use the same cuvette for both sample and blank measurements.
- Make sure that the mark (fiducial line) on the test tube aligns with the mark on the adapter toward the front of the instrument.
- During extended operation at a fixed wavelength, check from time to time for 100%T drift.
- Use clean test tubes and do not touch the test tubes below the fiducial line.

Flashing Display

A flashing display indicates that the reading is out of range and the 100%T/0 A control must be adjusted. This adjustment controls an optical occluder which regulates the amount of light passing through the sample.

In 100%T mode, a reading greater than 200%T will cause the display to flash.

- If the flashing reading is –1999, turn the 100%T/0A control clockwise until the display operates normally.
- If the flashing reading is +1999, turn the 100%T/0A control counterclockwise until the display operates normally.

In absorbance mode, a reading greater than 2A will cause the display to flash.

- If the flashing reading is –1999, turn the 100%T/0A control counterclockwise until the display operates normally.
- If the flashing reading is +1999, turn the 100%T/0A control clockwise until the display operates normally.

It may require several complete turns of the 100%T control to return to the proper range.

Change in wavelength

It is important to insert the blank and reset the display to 100% or 0.0 A every time the wavelength is changed.

Taking measurements of Transmittance or Absorbance

Tip: The basic steps for taking measurements are highlighted in **bold text** in the following instructions.

1. **Turn on** the instrument by turning the Power Switch (knob on the left side of instrument) clockwise. Allow the spectrophotometer to warm up for at least 15 minutes to stabilize.
2. After the warmup period, **set the desired wavelength** with the Wavelength Control Knob.
3. **Set the filter lever** to the appropriate position for the selected wavelength.
4. With the mode **set to transmittance, adjust the display** to 0%T with the Zero Control (knob on the front left side of the instrument). Make sure that the sample compartment is empty and the cover is closed.
5. **Set the display mode** to TRANSMITTANCE or ABSORBANCE by pressing the MODE control key until the appropriate LED is lit.
6. Fill a clean cell with water (or another blank solution) and wipe the cell with a tissue to remove liquid droplets, dust and fingerprints.
7. **Place the cell in the sample compartment** and align the guide mark on the cell with the guide mark at the front of the sample compartment. Press the cell firmly into the sample compartment and close the lid.
8. **Adjust the display to 100%T or 0.0A** with the Transmittance/Absorbance Control (knob on the right side of the instrument).
9. Remove the cell from the sample compartment and empty the water.
10. Rinse the cell twice with small volumes of the solution to be measured and fill it with the solution.
11. Wipe the cell with a tissue and **insert the cell into the sample compartment**. Align the guide marks and close the lid.
12. **Read the appropriate value (%T or A)** from the display.
13. Remove the cell from the sample compartment and repeat steps 10 through 12 for any remaining sample solutions.
14. When all measurements are completed, turn off the spectrophotometer by turning the Power Switch counterclockwise until it clicks.