UV-Vis Spectroscopy

Purpose: To become familiar with UV/Vis instrumentation. The spectral response of a photosensitive detector will be determined and then operating procedures and parameters for the Varian Cary 1 and HP 8452 UV/Vis spectrophotometers will be discussed. Three samples will be run on each: Holmium oxide (wavelength calibration and resolution), vapor state benzene (resolution and vibrational fine structure), and alkaline potassium dichromate (S/N and detection limit).


Procedures: When working on each spectrometer, pay particular attention to the following specifications for the instruments where applicable:

- Single beam of double beam design
- Kinds of light sources and their range of output wavelengths
- Total wavelength range of the instrument
- Detector type and specifications
- Absorbance range of the instrument
- Dispersion element(s)

**Spectronic 20 (spectrophotometer):**
- Turn on the instrument with the zero adjust/on-off control and allow about 5 min warm-up time.
- With no sample tube in place and the cover closed, turn the zero adjust knob to bring the meter to 0% transmittance.
- Insert into the sample holder a test tube filled with water. Rotate the 100% transmittance control until the meter needle reads near midscale.
- Adjust the wavelength control until a maximum transmittance reading is achieved. Adjust the 100% transmittance control if necessary, to keep the meter needle on scale.
- At λ of maximum transmittance, dial the instrument to 100% transmittance.
- Without changing any controls, change the wavelength in intervals of 20 nm and record the corresponding transmittance reading.
- Plot the results of transmittance vs. wavelength.

These results represent the overall response of the spectrometer – phototube, emissivity of light source, and intensity diffracted by the grating, each of which is a function of wavelength.

**Varian Cary 1 (double-dispersion spectrophotometer):**

**Wavelength accuracy** is conveniently checked by recording the spectrum of holmium oxide glass filter. The following calibration peaks are used for holmium oxide:

333.8, 360.8, 385.8, 418.5, 446.0, 453.4, 536.4, 637.5nm
• Run holmium oxide sample and compare the spectrum with the calibration peak positions given. Pick one peak in the visible region and one in the UV region, and report the % error of the peak positions of the instrument.
• Repeat the measurements at 2 other slit widths. Note the changes in absorbance, peak width (FWHM), and peak position, if any.
• Holmium oxide filters are also used to detect stray light in the instrument. Holmium oxide is completely opaque (T = 0) below 225 nm.

**Photometric accuracy** of the instrument can be determined by using potassium dichromate solution in basic media, which has very high molar absorptivity.
• Run a sample of the stock solution (5x10^{-6} M K_2CrO_4 in 0.05 M KOH) at optimized condition.
• Using DI water as blank, measure the absorbance of the blank at \( \lambda_{\text{max}} \) of the potassium dichromate sample at least 10 times

**Resolution** of the spectrometer can be determined by collecting spectra of benzene vapor samples:
• Run benzene vapor sample with default parameters.
• Optimize the condition by systematically varying the slit width and scanning speed over the spectrum range of your interest. Overlay the spectra if possible to compare the effect of each parameter.

**HP 8452 (diode-array spectrometer):**
• Repeat the measurements of holmium oxide, benzene vapor, and potassium dichromate on this instrument.

**Data Treatment:** Record all observations in your notebook. Plot the spectral response data from Spec 20. Compare the calibration peaks of the holmium oxide sample with the wavelength readout and report any calibration error for each spectrometer. Note the absorbance (A) of these peaks at different slit width settings and explain your observations. Comment on the benzene vapor spectra at various slit widths on both spectrometers. After recording the concentration data from the potassium chromate flask, calculate the molar absorptivity (\( \varepsilon \)) of the sample at one \( \lambda_{\text{max}} \) in the visible and one in the UV region. Estimate the S/N ratio at both of these wavelengths. Estimate the lower limit of detection for the potassium chromate in the visible region. Compare the performance of the diode-array and the double-dispersing spectrometers by focusing on wavelength accuracy, photometric accuracy, and resolution of the instruments.

**Appendix 1. How to clean cuvette:**
• Rinse the tube with distilled water a few times
• Add about 1 mL of the solution to be measured. Tilt and turn the cuvette so that the solution has contact with all the surfaces. Discard this solution and repeat this rinse once more.
• Fill the cuvette about 3/4 full of the solution you wish to test.
• Wipe the outside of the cuvette with a lint-free, soft tissue (a Kimwipe) to remove any moisture or fingerprints from the outside surface.
Appendix 2. Diagrams of UV-Vis instruments

Fig. 1 The Spectronic 20 optical diagram

Fig. 2 A multichannel diode array spectrometer

Fig. 3 A double-dispersion spectrometer