Production of an Isosbestic Point by UV-VIS Absorption Spectrophotometry

Part I: Calibration and Use of UV-VIS Spectrophotometers

Instrumentation

Beckman DB-G Spectrophotometer
Perkin-Elmer Lambda 3B Spectrophotometer
Ocean Optics USB2000 Spectrophotometer

Objective

In order to make meaningful measurements and to be confident in the quality of the data obtained, an experimentalist must ensure that the instrument is calibrated and operating properly. The objective of the following exercises is to acquaint you with some basic procedures for evaluating the performance of UV-VIS spectrophotometers. Although these procedures need not be performed every time the spectrophotometer is used, they should be performed on a routine basis to keep track of instrument performance over time or whenever data quality appears to be degrading.

Cuvette Care

The quartz cuvettes you will be using are expensive (~$350 for a matched set) so be careful when handling the cuvettes. Keep them in the cuvette rack between measurements and return them cleaned to the storage box when you are through for the day. Clean the outer optical faces with lens paper (not kimwipes or paper towels). Rinse the inside with water and dry with a Q-tip.

<u>Baseline Linearity</u>

Instruments: Perkin-Elmer Lambda 3B Spectrophotometer
Ocean Optics USB2000 Spectrophotometer

1. Set the absorbance range at 0 to 1 AU.
2. Set the recorder baseline to ~3/4 full scale.
3. With no cells in the light path, scan from 800 to 200 nm.
4. Repeat step 3 with the absorbance range set for 0 to 0.1 AU.
5. Repeat step 3 with the absorbance range set for 0 to 0.01 AU.

Report baseline linearity as ± AU deviation from zero.

Matching of the Cuvettes

Differences in path length or other optical imperfections between two cuvettes do not lead to appreciable differences in absorbance measurements as long as one cuvette is dedicated for the reference and the other for the sample. However if the two cuvettes are interchanged during use differences in absorbance readings may be noticed.

Instrument: Perkin-Elmer Lambda 3B Spectrophotometer
Solutions: 50 mg/L $K_2Cr_2O_7$ in 0.10 M HCl

The cuvettes in the box labeled 'matched cuvettes' were purchased as a matched set. Fill one with diH_2O and place in the reference compartment. Fill the other with the $K_2Cr_2O_7$ solution. Record the absorbance at 257 nm. Now switch the reference and sample solutions and their respective cuvettes. Record the absorbance. How well matched are the cuvettes? Replace one of the cuvettes with one from the box labeled 'unmatched cuvettes'. Record the absorbance. Again switch the reference and sample solutions and their respective cuvettes. Record the absorbance. How well matched are the cuvettes?

Calibration of the Wavelength Scale

A quick check of the wavelength accuracy of a spectrophotometer can be performed by observing the strong emission band at 656.1 nm produced by the D_2 lamp.

Instrument: Perkin-Elmer Lambda 3B Spectrophotometer

With the reference and sample light paths unobstructed, put the instrument in wavelength calibration mode. Scan from 660 nm to 650 nm at 15 nm/min. Record the wavelength at maximum transmission. Do this at least three times and average the results. Report the value as ± x nm from 656.1 nm.

Instrument: Ocean Optics USB2000 Spectrophotometer

With diH_2O as the sample, put the instrument in scope mode and observe the sharp emission peak at 656.1 nm.

A more rigorous check of wavelength accuracy is performed using a holmium oxide filter. Holmium oxide filters provide sharp, discrete, highly reproducible absorption bands.
Instrument: Perkin-Elmer Lambda 3B Spectrophotometer

Place the holmium oxide filter in the sample compartment. Set the ordinate limits to 0 to 2. With air as the reference, scan from 580 nm to 400 nm at 60 nm/min. Compare the peak wavelengths to the reference spectrum. Report your values as ± x nm from the actual.

Instrument: Ocean Optics USB2000 Spectrophotometer

Obtain the full holmium oxide spectrum and compare the peak values to the reference spectrum.

Calibration of the Absorbance Scale

The most accurate method of calibrating the absorbance scale is to use neutral density filters designed to give a specified absorbance/transmission value at a specified wavelength. Locate the neutral density filters in the spectronics standards box and test both the PE and OO instruments against all four. For the PE instrument, use air as the reference. Report your readings as ± x % from the actual.

Measurement of Stray Light

The most convenient method of measuring stray light is to absorb all the source radiation within a given monochrometer bandpass. Any remaining light transmitted is reported as stray light. Locate the SRE (stray radiant energy) filters in the spectronics standards box and test both the PE and OO instruments against all three. For the PE instrument, use air as the reference. Report your readings as % residual transmission.

Effect of Slit Width

Instrument: Beckman DB-G

Place 2-3 drops of benzene in the sample cuvette, insert the stopper and let stand for 1 minute. Set the slit width at 2 mm. Slowly scan from 280 to 220 nm vs. an empty cuvette. Repeat the scan for 1.0, 0.5, and 0.10 mm slit widths. What effect does the slit width have on the resolution of the doublet?

Part II: The Isosbestic Point of an Acid-Base Indicator

Reference
Harris, D.C. *Quantitative Analysis* 4th ed. pg. 527. (available in 408)

Objective
Determine the isosbestic point of an acid-base indicator. You may practice with methyl red (the example in Harris) but you must choose a different indicator for your final report.

Instrumentation
Ocean Optics USB2000 UV-VIS Spectrophotometer

Criteria

All absorbances must be less than 1 AU.

A minimum of six individual spectra; three (or more) below your indicators' pH transition and three (or more) above.

Spectra should be more or less evenly spaced.

All spectra should be plotted on the same graph.

Notes

Since indicators are themselves weak acids/bases, your buffer strength should be at least 100 mM.

Depending on your indicators' pH transition point, it may be necessary to use several different buffers to achieve your desired pH range.

A list of available buffers is posted in 408.

Report
Include in your report:

All data collected from part I

A plot of your spectra from part II. Clearly label all spectra.

Compare your experimental isosbestic point with the literature value. What are possible sources of error?